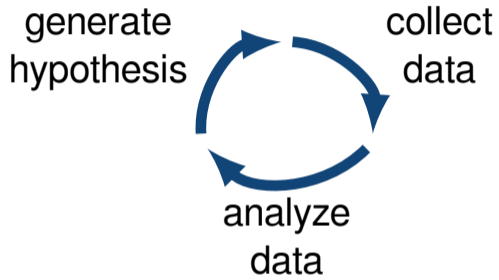


# Genomic analyses of transcription elongation factors and intragenic transcription

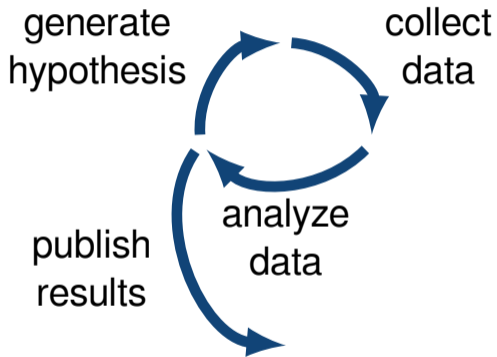
James Chuang

June 19, 2019

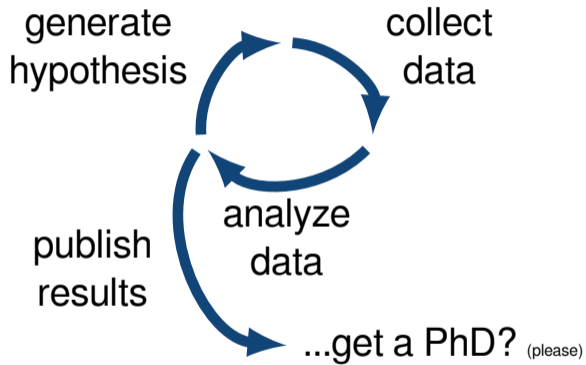
# The scientific process

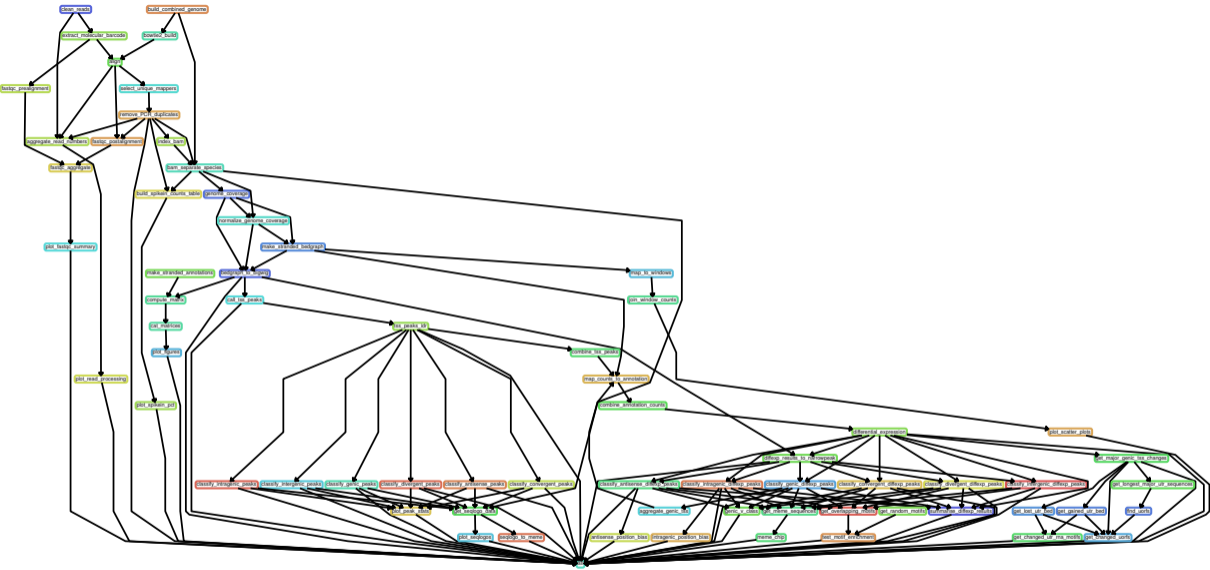


# The scientific process



# The scientific process



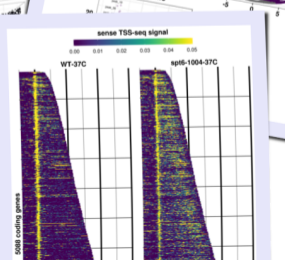
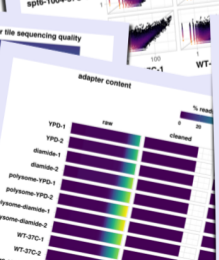
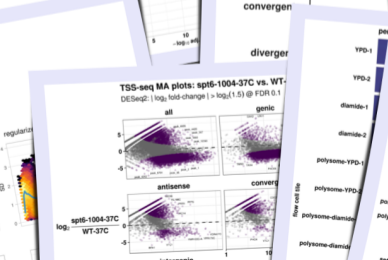
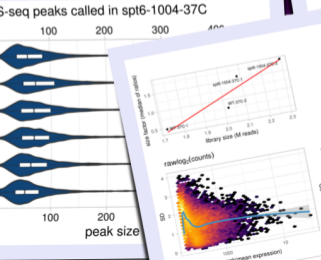
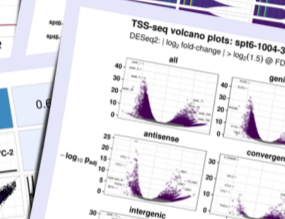
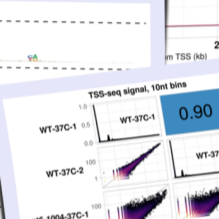
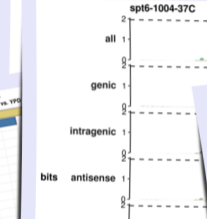
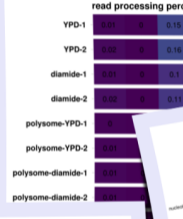
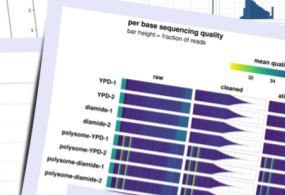
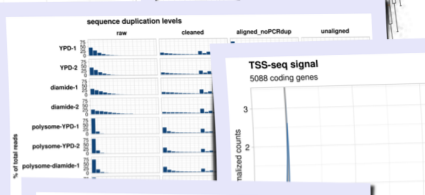
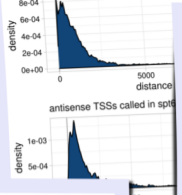
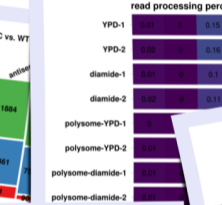
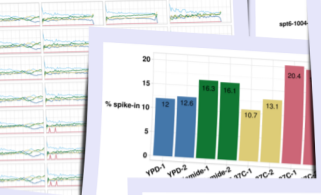




*# an example Snakemake rule:*

```
rule foobar:  
    input:  'input.txt'  
    output: 'output.txt'  
    script: 'make_output_from_input.py'  
    conda:  'environment.yaml'
```





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

## Contact person(s)

Winston, Fred

## 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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## Publication date:

September 7, 2018

## DOI:

DOI: [10.5281/zenodo.1409826](https://doi.org/10.5281/zenodo.1409826)

## Keyword(s):

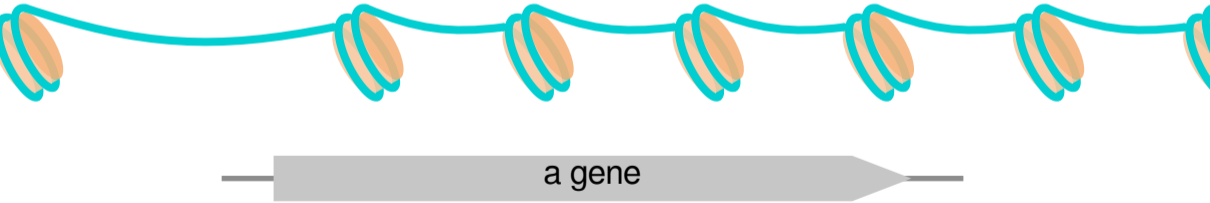
Spt6 transcription chromatin genomics  
intragenic transcription TSS-seq CHIP-nexus NET-seq  
MNase-seq

## Published in:

Molecular Cell.



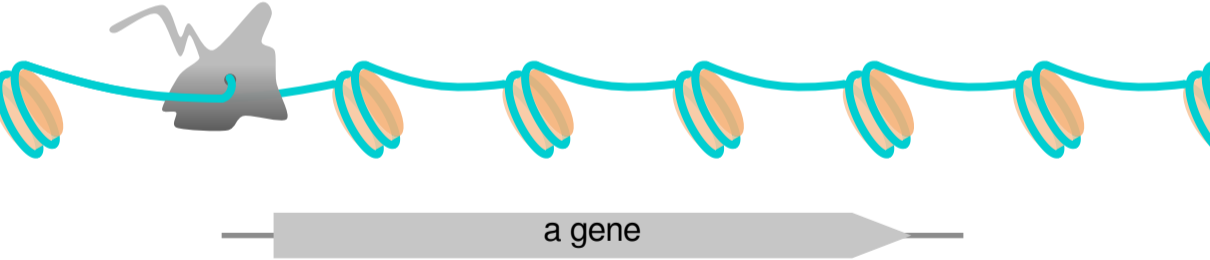
# An introduction to transcription



# An introduction to transcription

RNA polymerase II

initiation

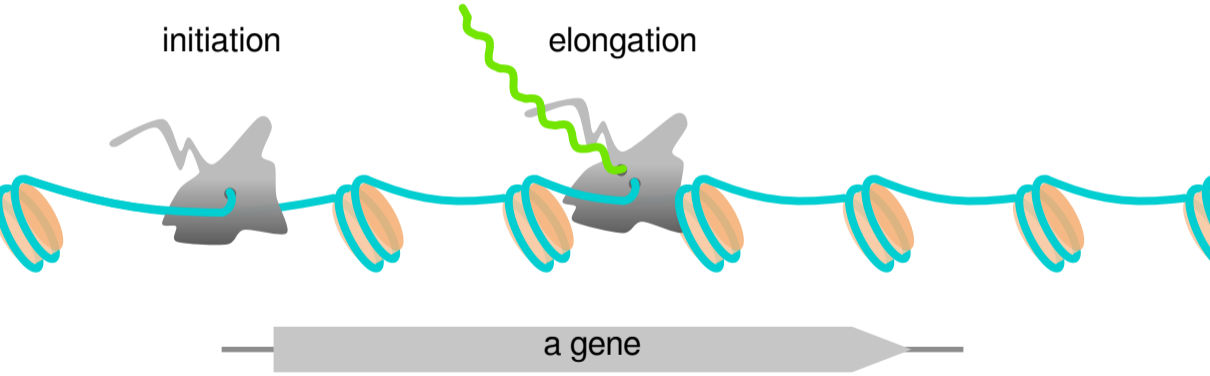


# An introduction to transcription

RNA polymerase II

initiation

elongation



a gene

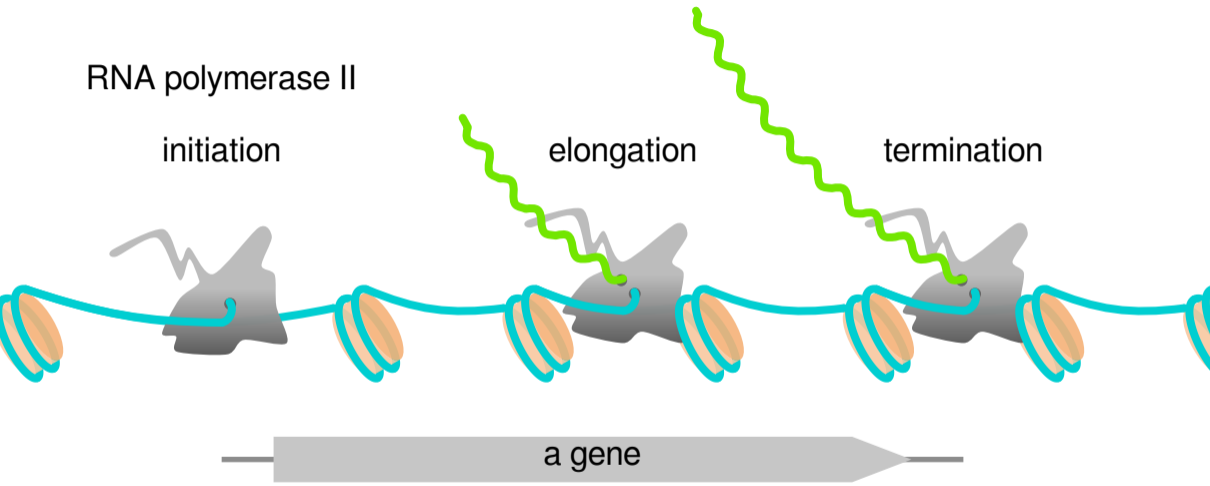
# An introduction to transcription

RNA polymerase II

initiation

elongation

termination



a gene

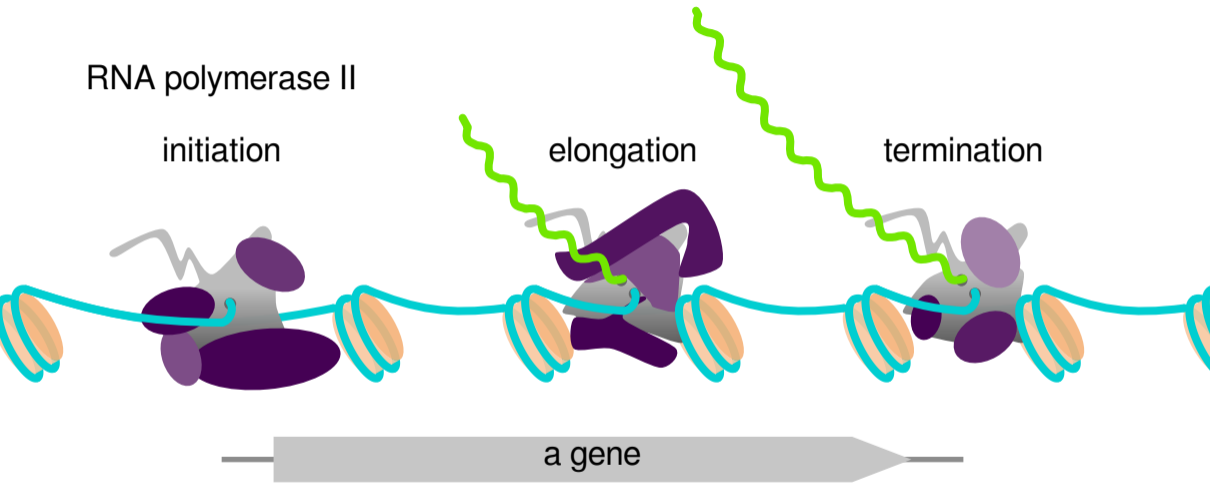
# An introduction to transcription

RNA polymerase II

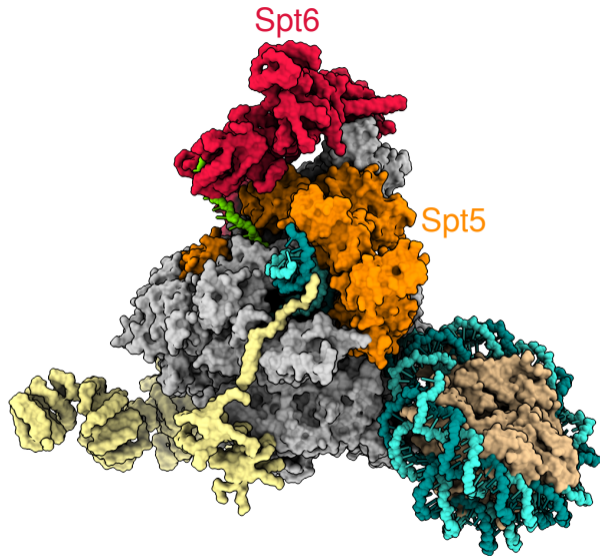
initiation

elongation

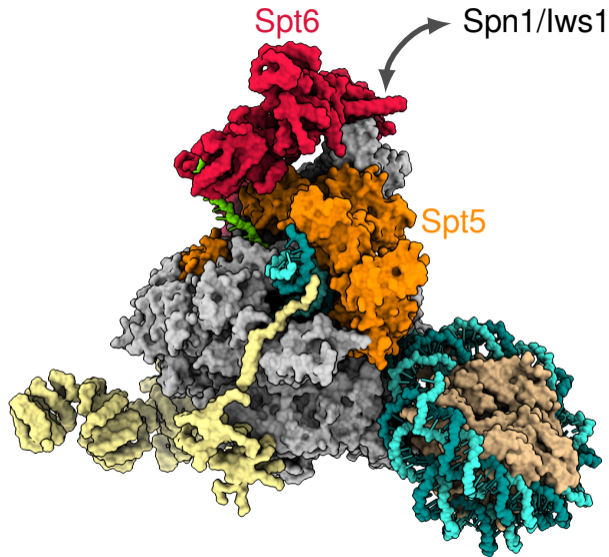
termination



a gene

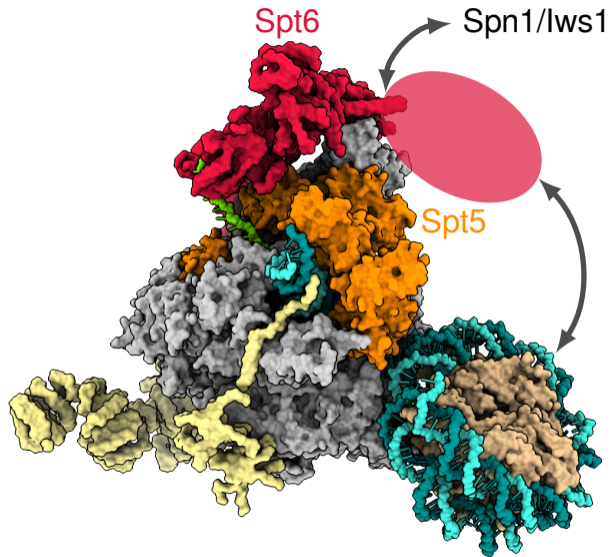


Vos *et al.* (2018). *Nature*  
Farnung *et al.* (2018). *Nat. Commun.*



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

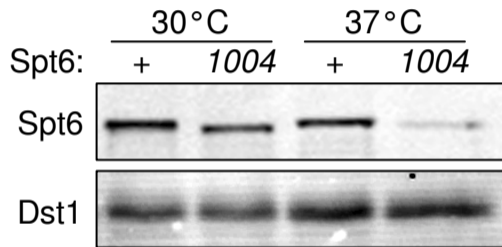
**Steve Doris** TSS-seq and ChIP-nexus

**Olga Viktorovskaya** MNase-seq

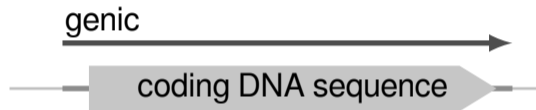
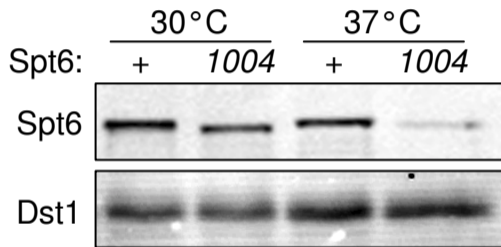
**Magdalena Murawska** NET-seq

**Dan Spatt** Northern, Western, and ChIP experiments

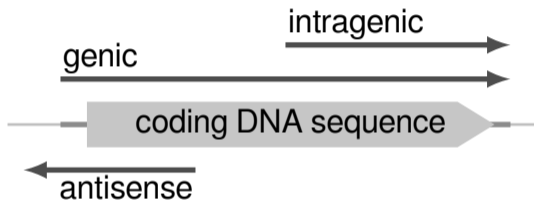
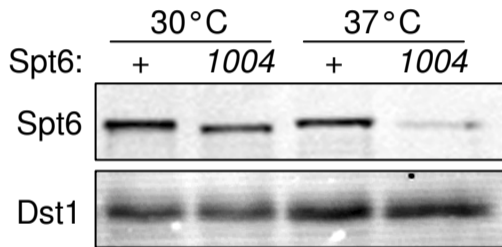
# The *spt6-1004* mutant expresses intragenic transcripts

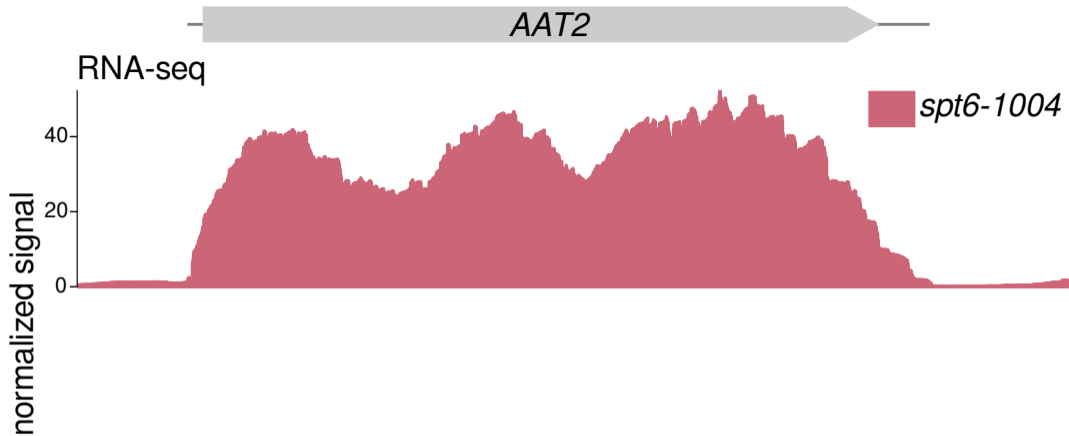


# The *spt6-1004* mutant expresses intragenic transcripts

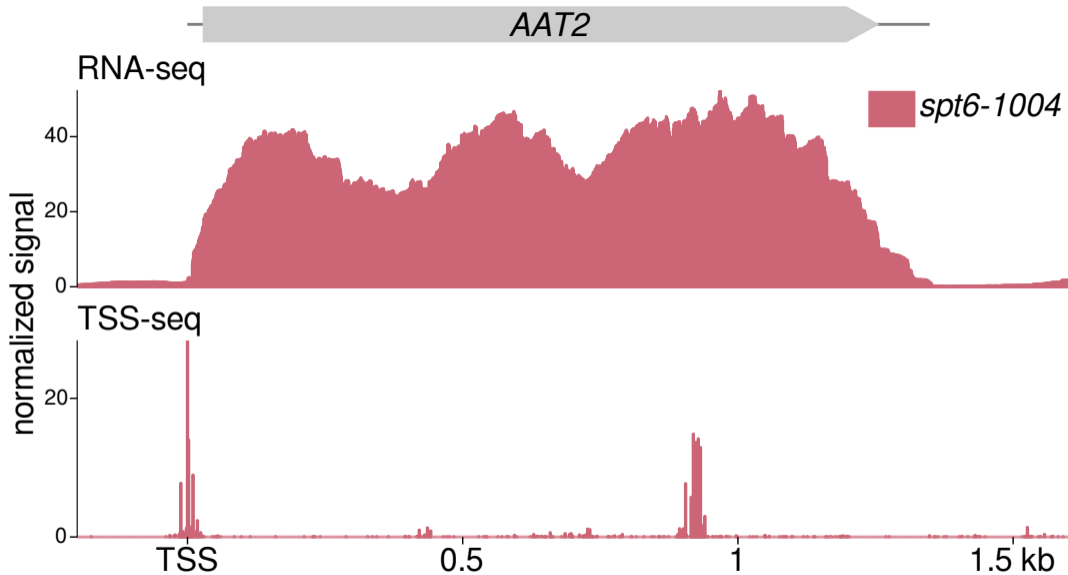


# The *spt6-1004* mutant expresses intragenic transcripts





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



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

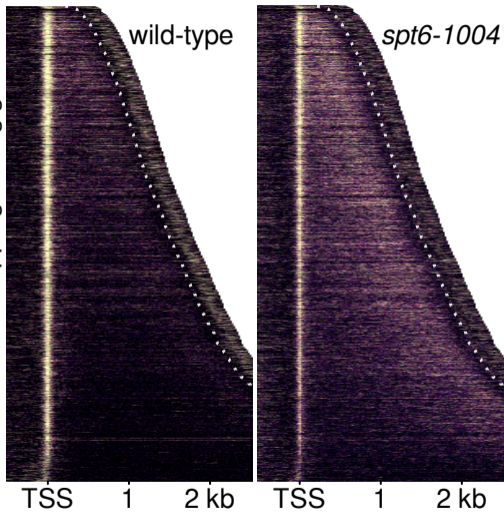
# sense TSS-seq

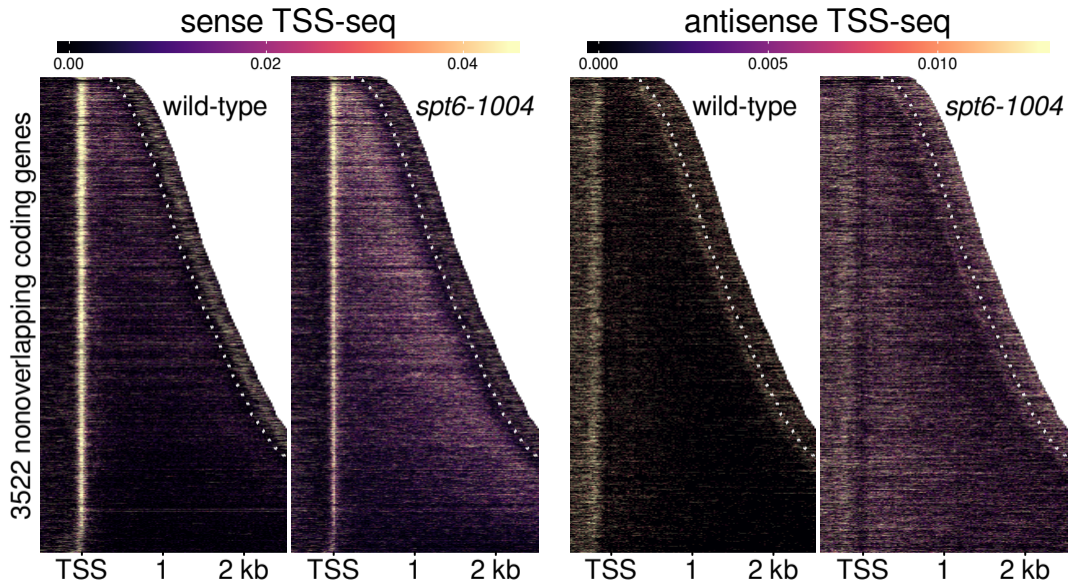


3522 nonoverlapping coding genes

wild-type

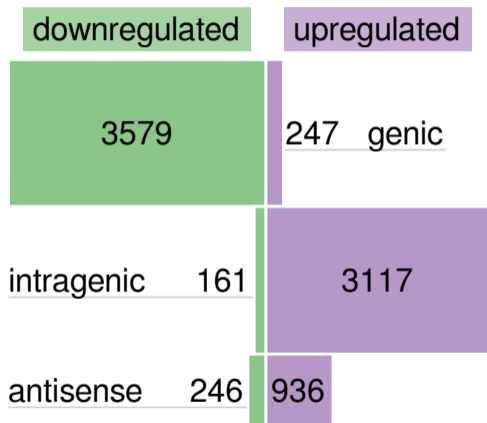
*spt6-1004*



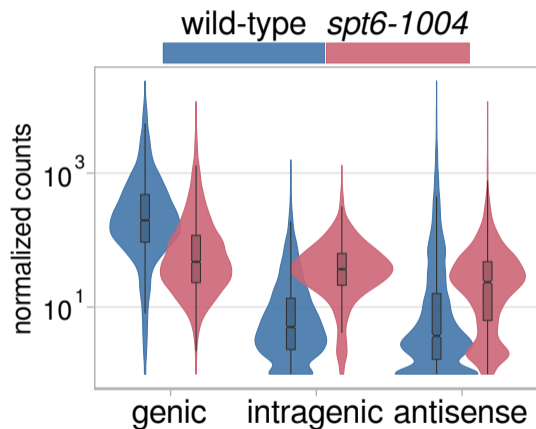
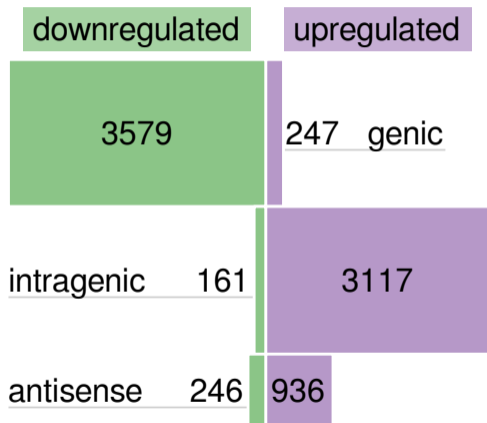




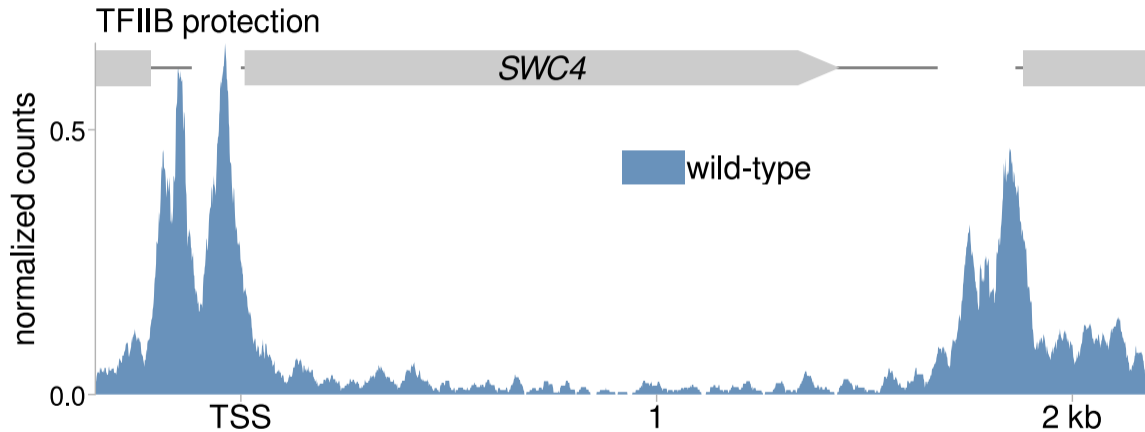
# Genic transcript abundances are reduced in *spt6-1004*



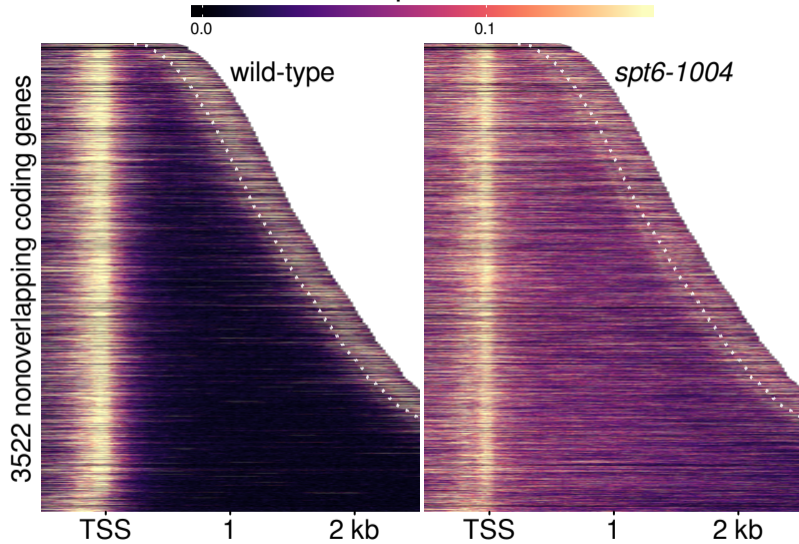
# Genic transcript abundances are reduced in *spt6-1004*



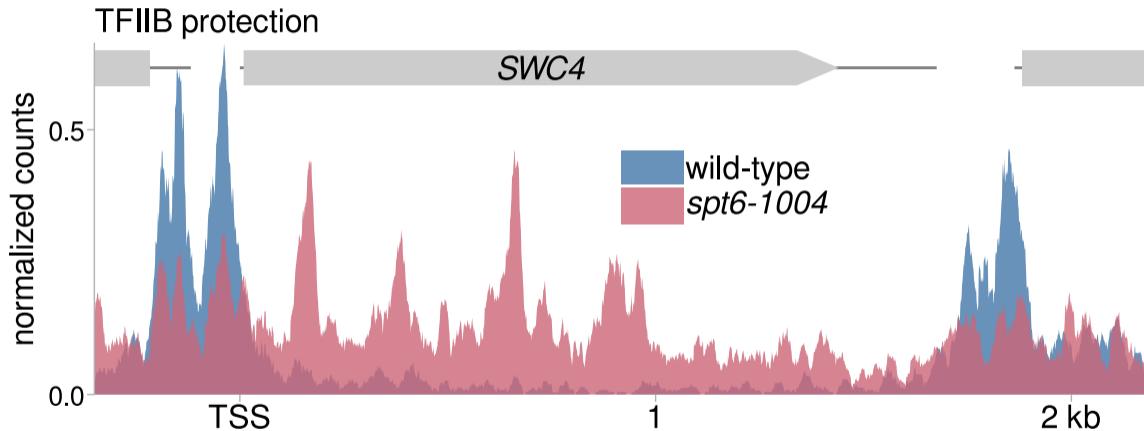
# TFIIB ChIP-nexus measures transcription initiation



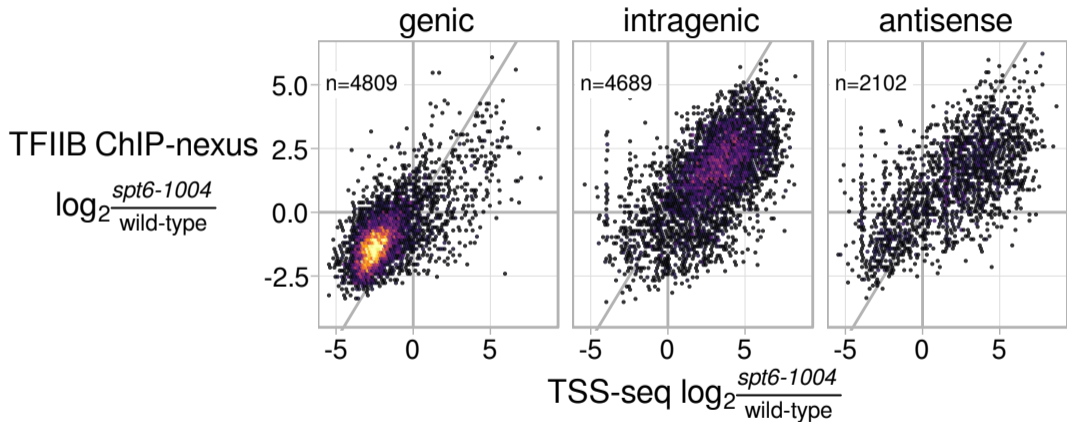
# TFIIB protection



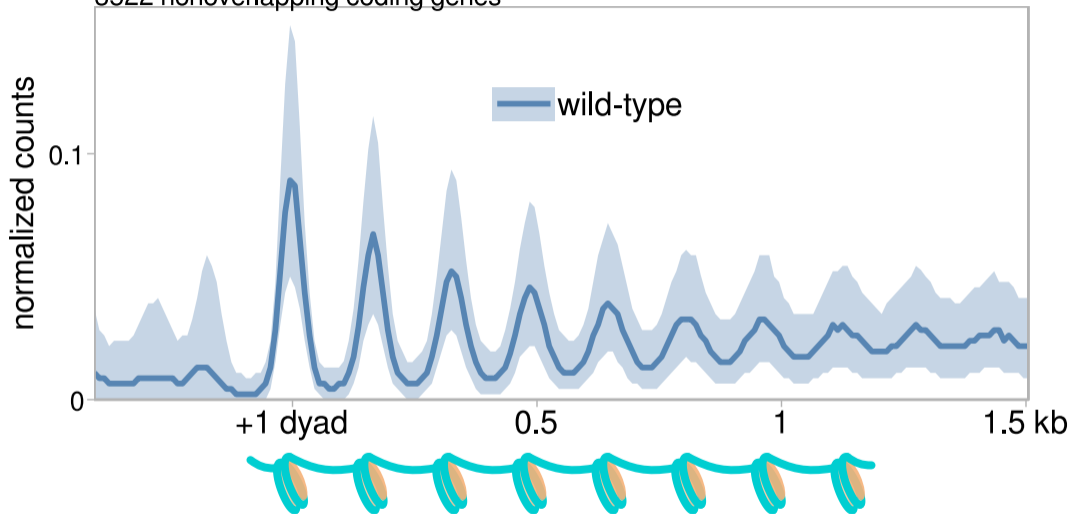
# TFIIB binding changes dramatically in *spt6-1004*



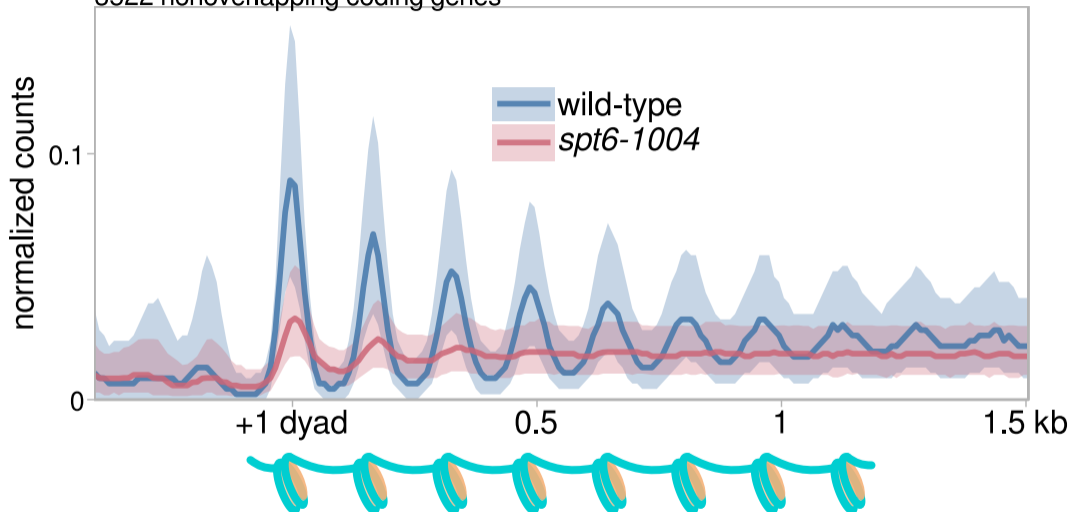
# Transcriptomic changes are mostly explained by changes in initiation



MNase-seq dyad signal  
3522 nonoverlapping coding genes

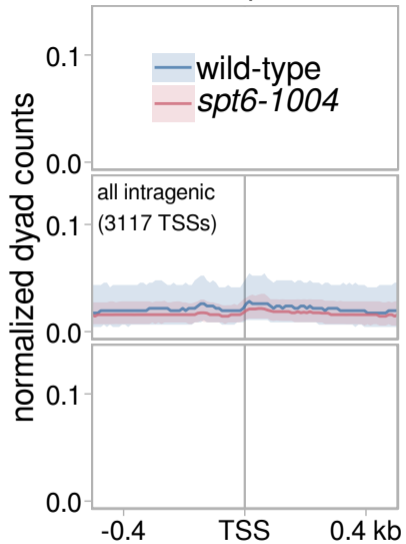


MNase-seq dyad signal  
3522 nonoverlapping coding genes

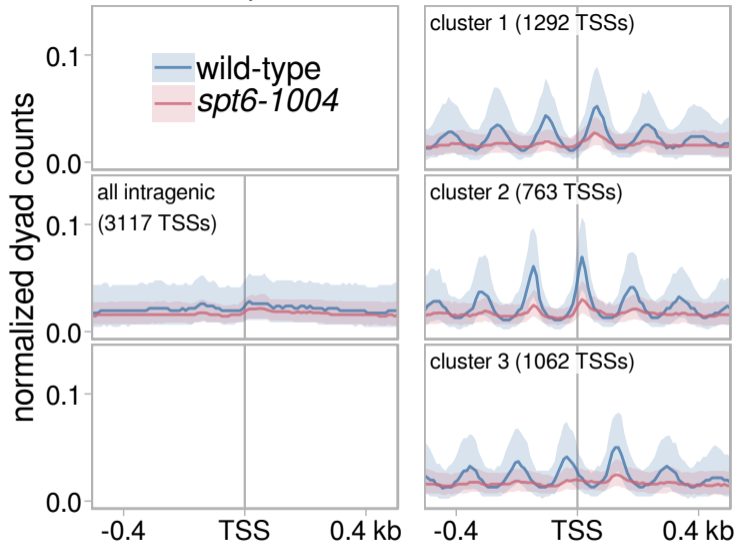




# MNase-seq

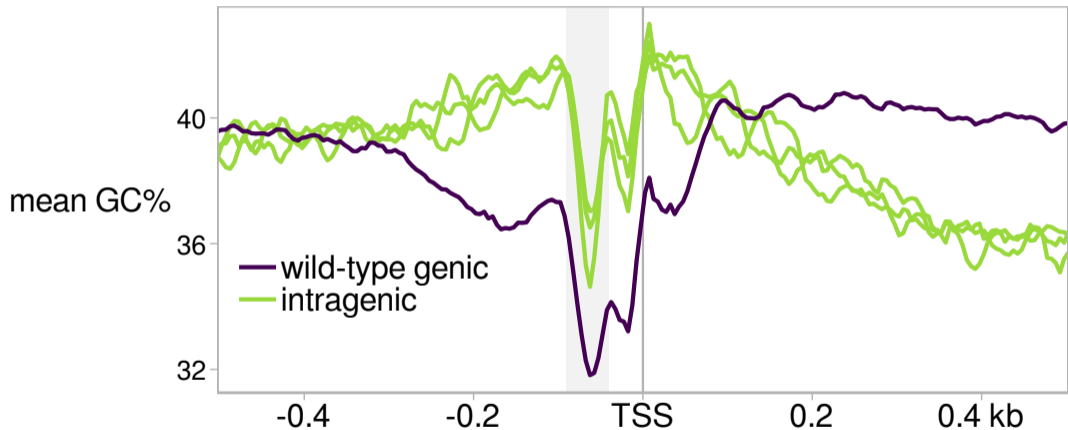


# MNase-seq

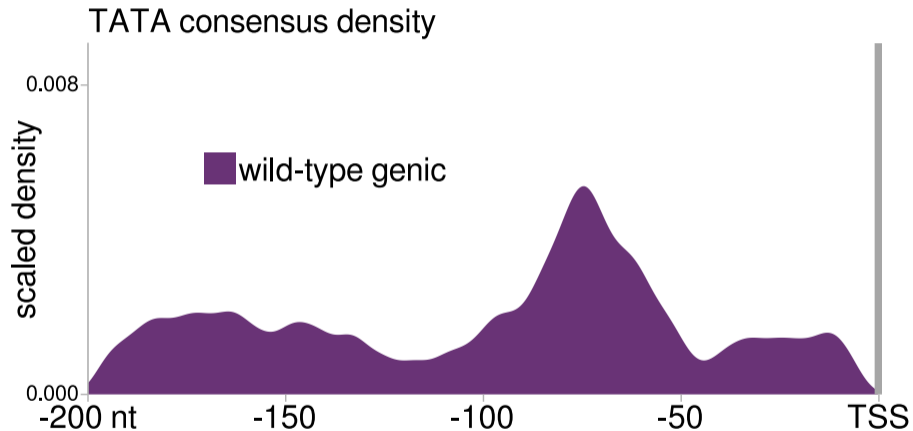




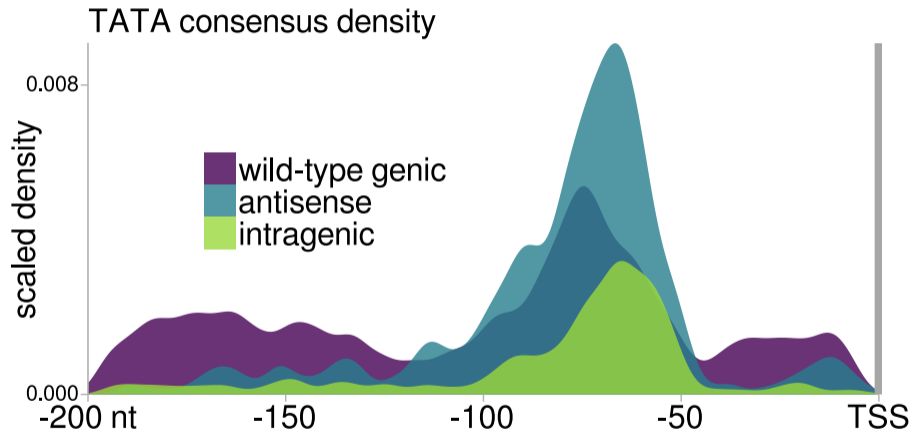
## Intragenic promoters have features of genic promoters



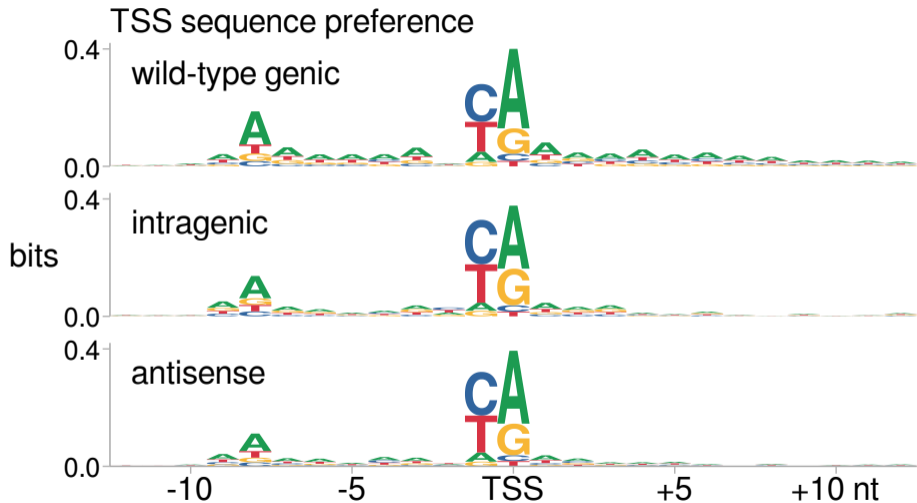
# Intragenic promoters have features of genic promoters

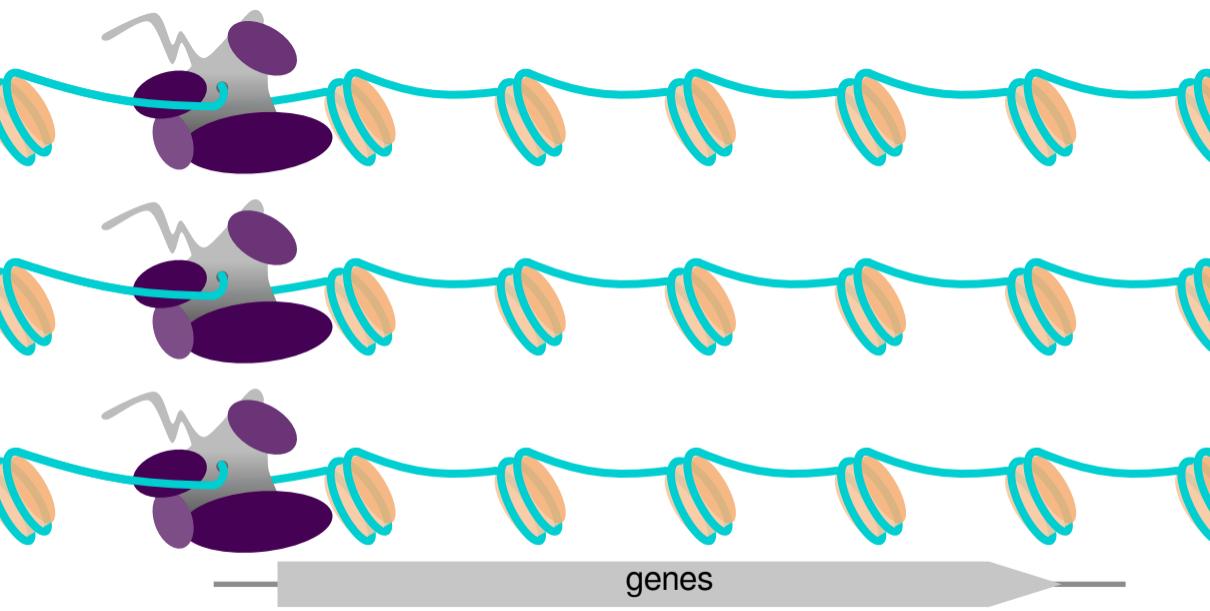


# Intragenic promoters have features of genic promoters

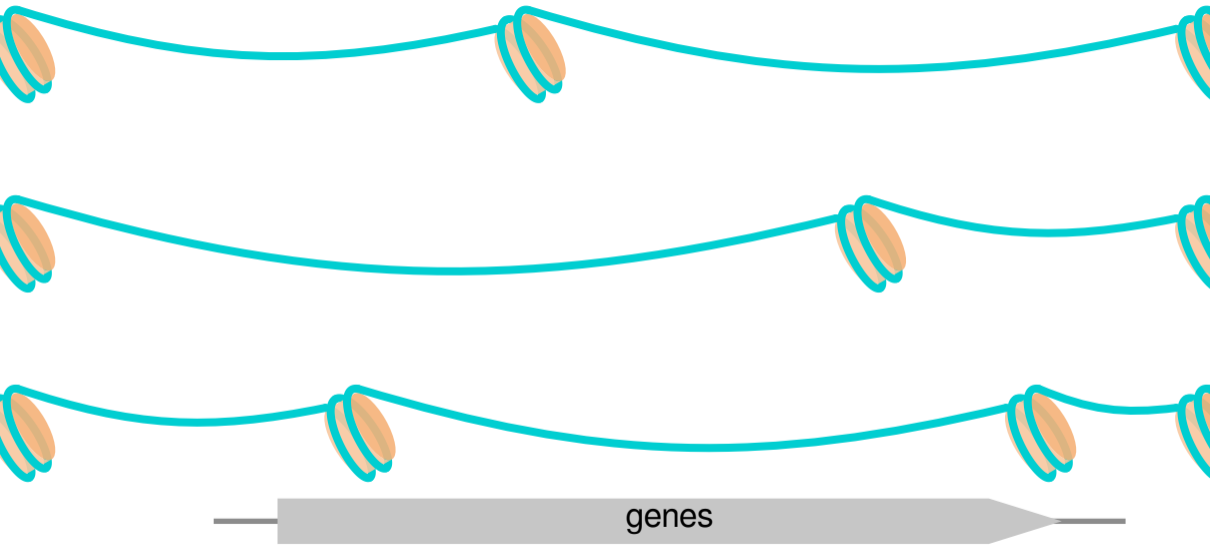


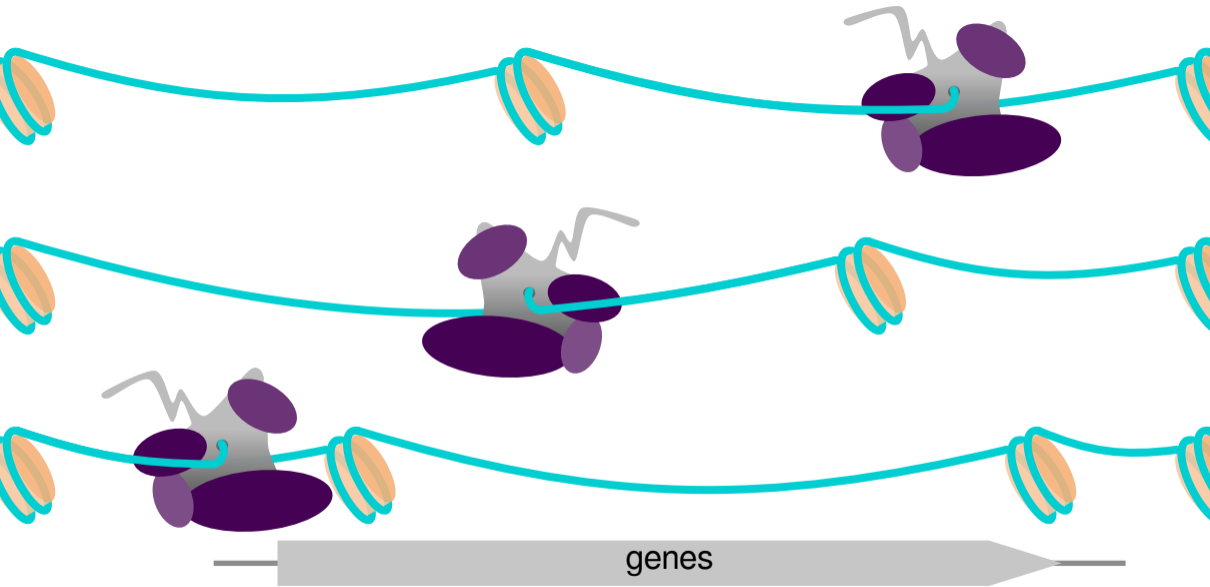
# Intragenic promoters have features of genic promoters

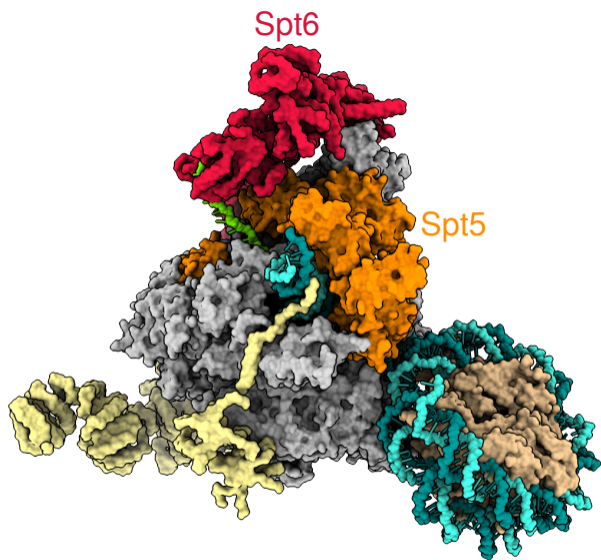




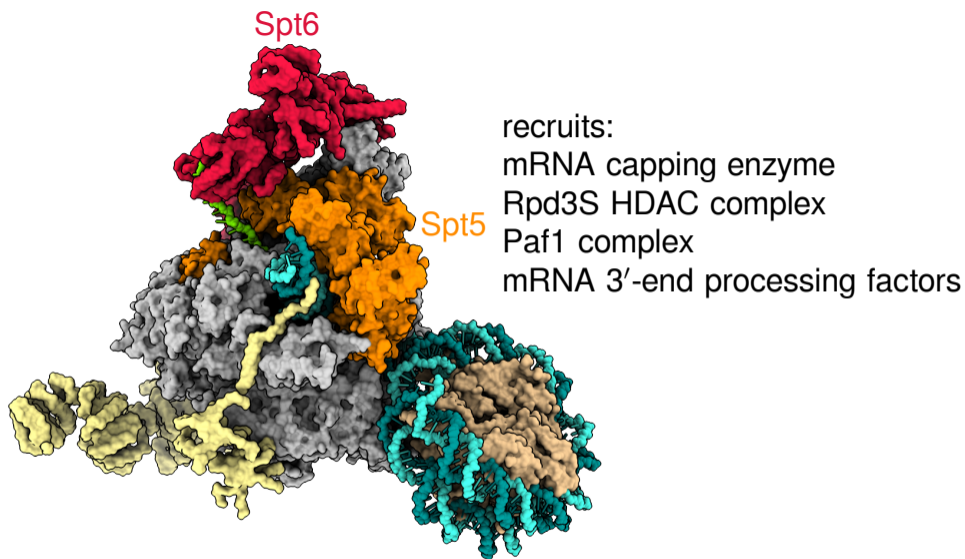








Vos *et al.* (2018). *Nature*  
Farnung *et al.* (2018). *Nat. Commun.*

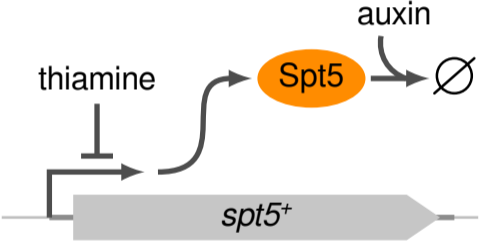


Vos *et al.* (2018). *Nature*  
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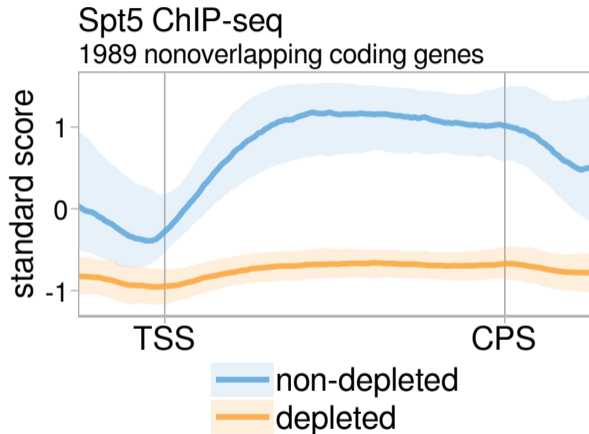
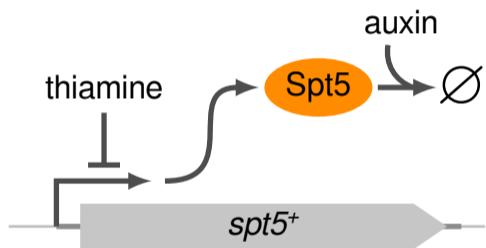
## Spt5 project collaborators

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

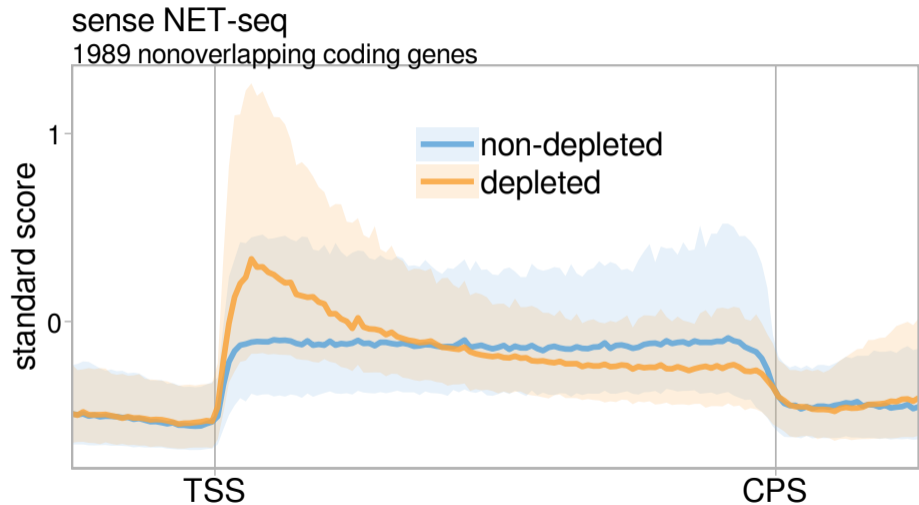
# Spt5 depletion system



# Spt5 depletion system

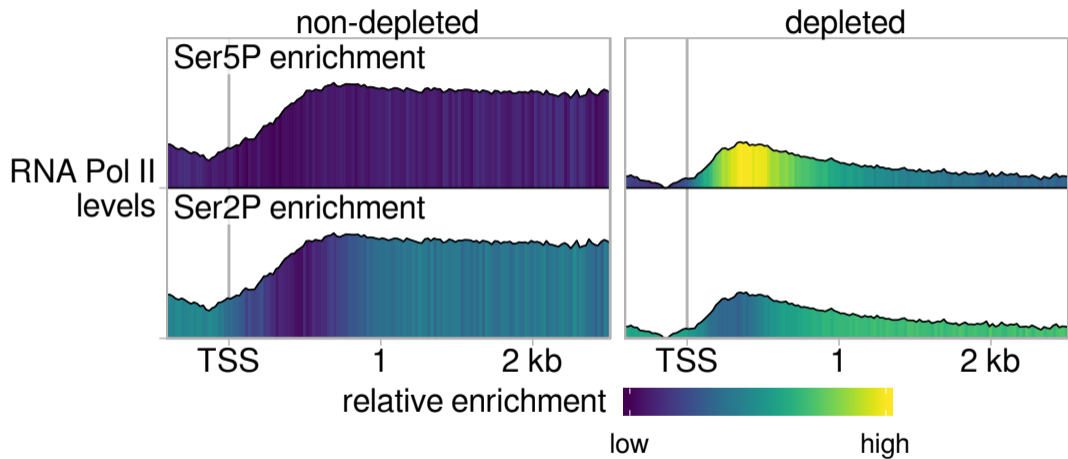


# Elongation defects upon Spt5 depletion

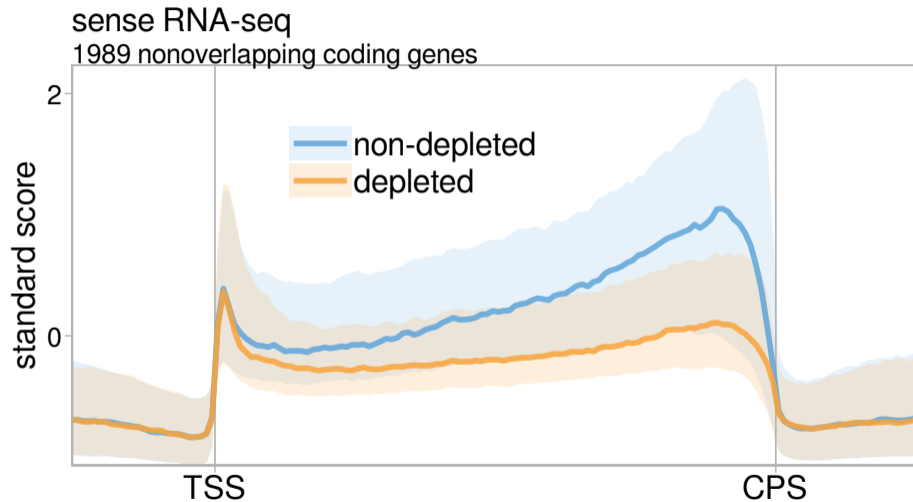




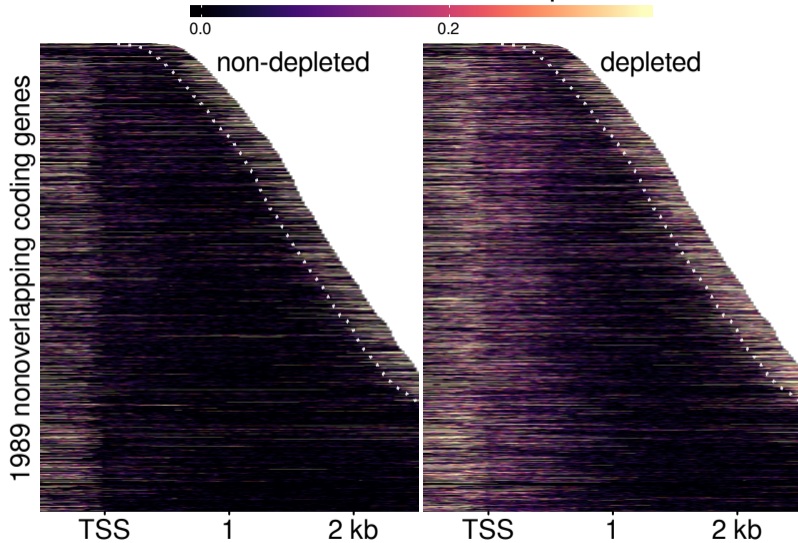
## Trapped Pol II is enriched for CTD serine 5 phosphorylation

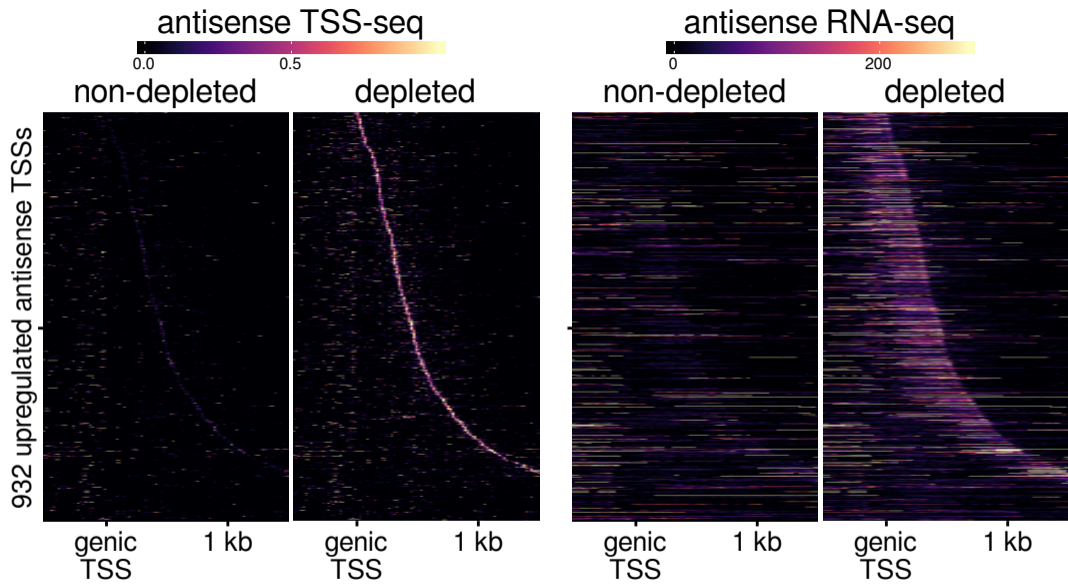


# Evidence for premature termination upon Spt5 depletion

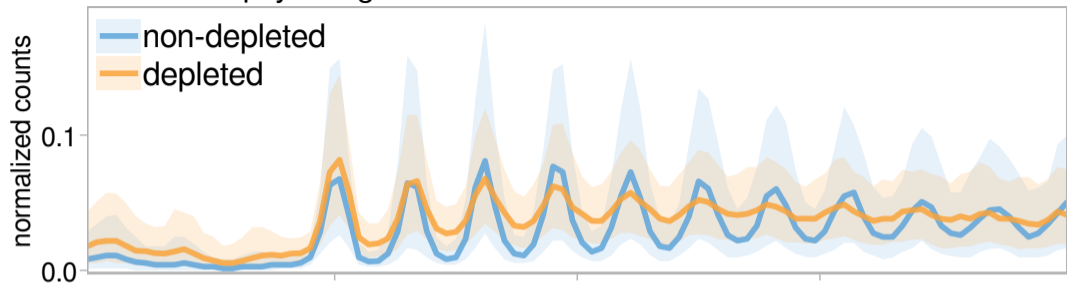


# antisense RNA-seq

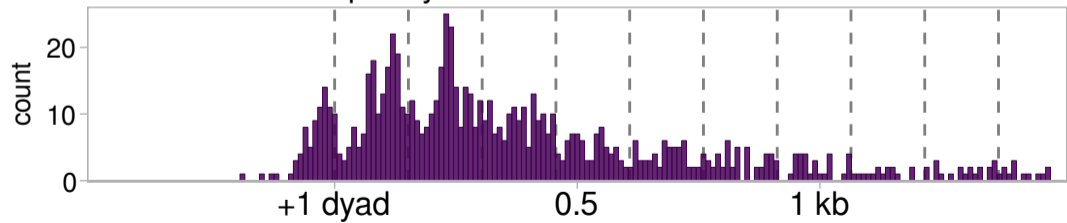




## MNase-seq dyad signal

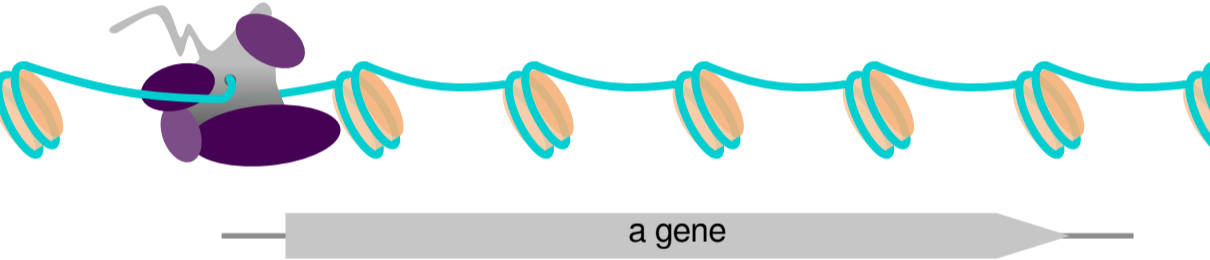


## antisense TSS frequency

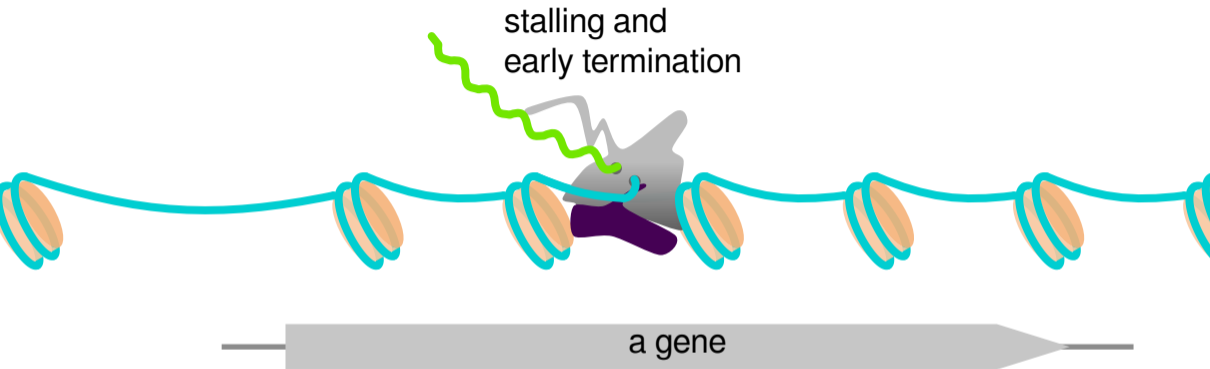


# Transcription upon Spt5 depletion:

initiation

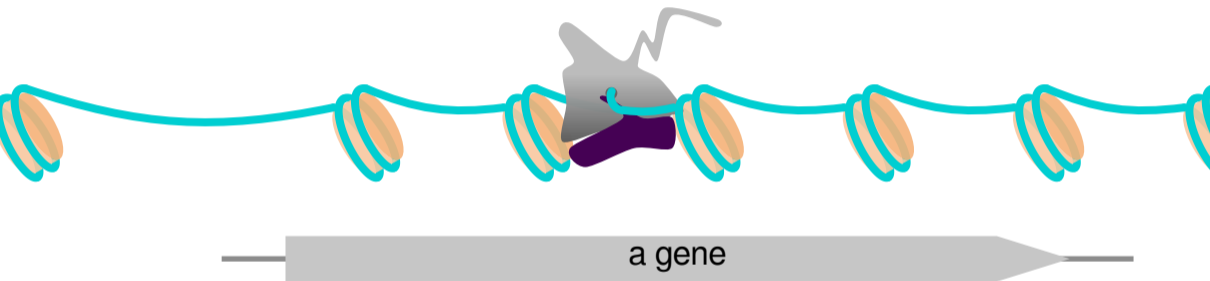


## Transcription upon Spt5 depletion:



Transcription upon Spt5 depletion:

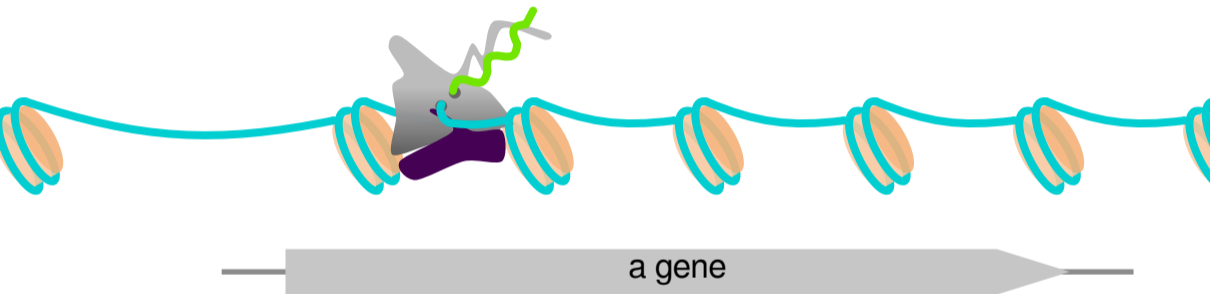
possible antisense re-initiation



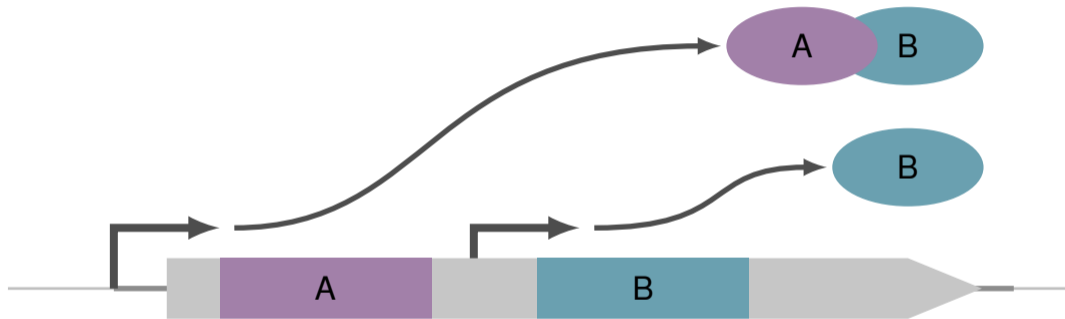


Transcription upon Spt5 depletion:

antisense transcription



## Intragenic transcription in wild-type cells



## project collaborators

**Steve Doris** TSS-seq and ChIP-nexus

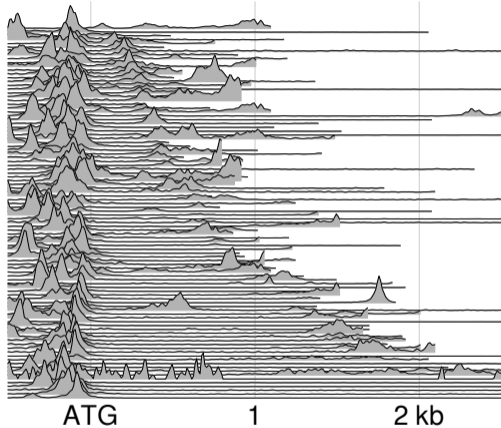
**Dan Spatt** polyribosome fractionation,  
competitive growth assays,  
and Northern blots

**James Warner** Northern blots

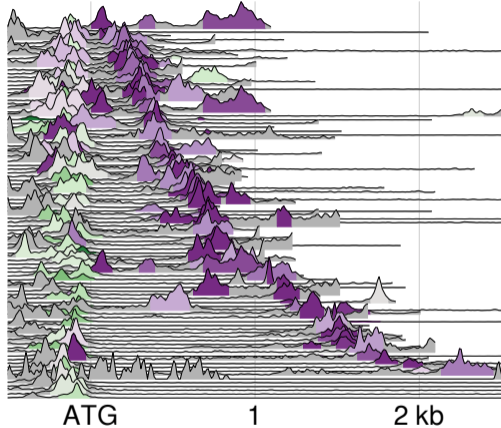
relative TFIIIB protection

unstressed

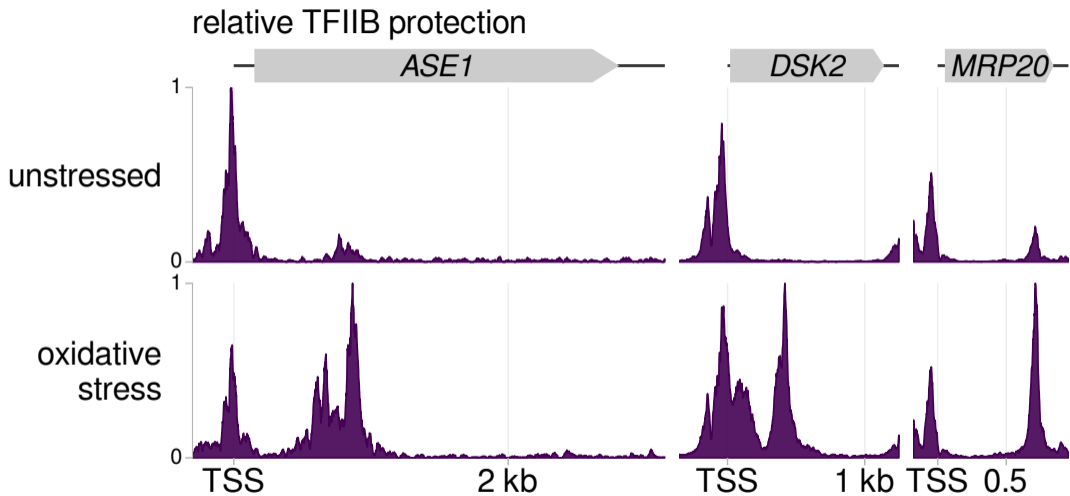
98 induced intragenic peaks



oxidative stress

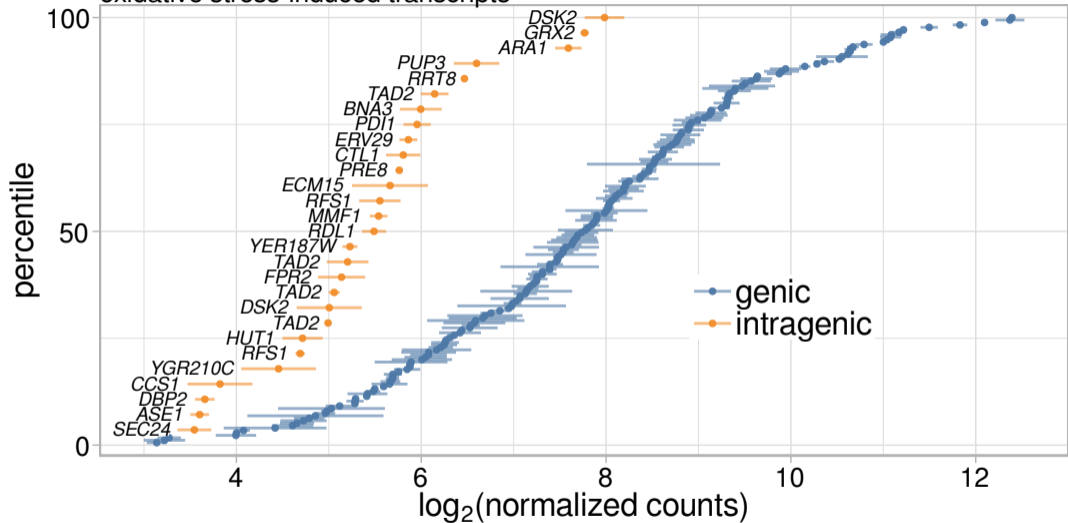


$\log_2 \frac{\text{oxidative stress}}{\text{unstressed}}$    $\leq -2$  -1 0 1  $\geq 2$

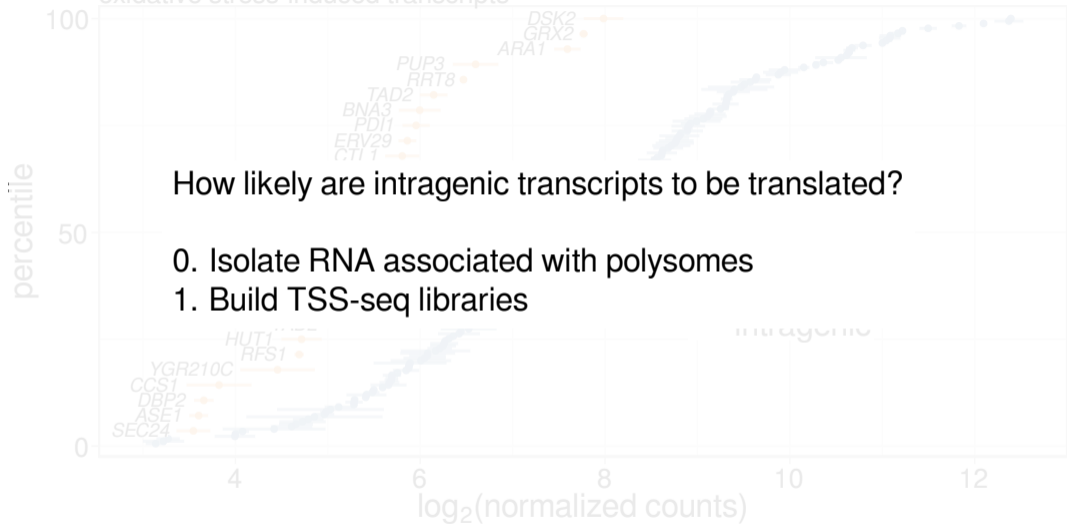


# transcript abundances in oxidative stress

## oxidative stress-induced transcripts

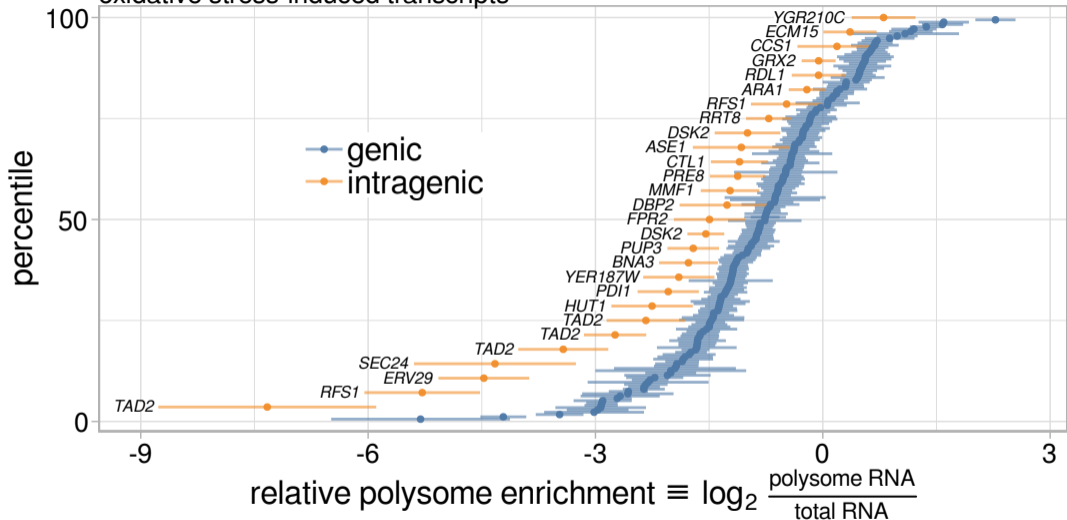


transcript abundances in oxidative stress  
oxidative stress-induced transcripts



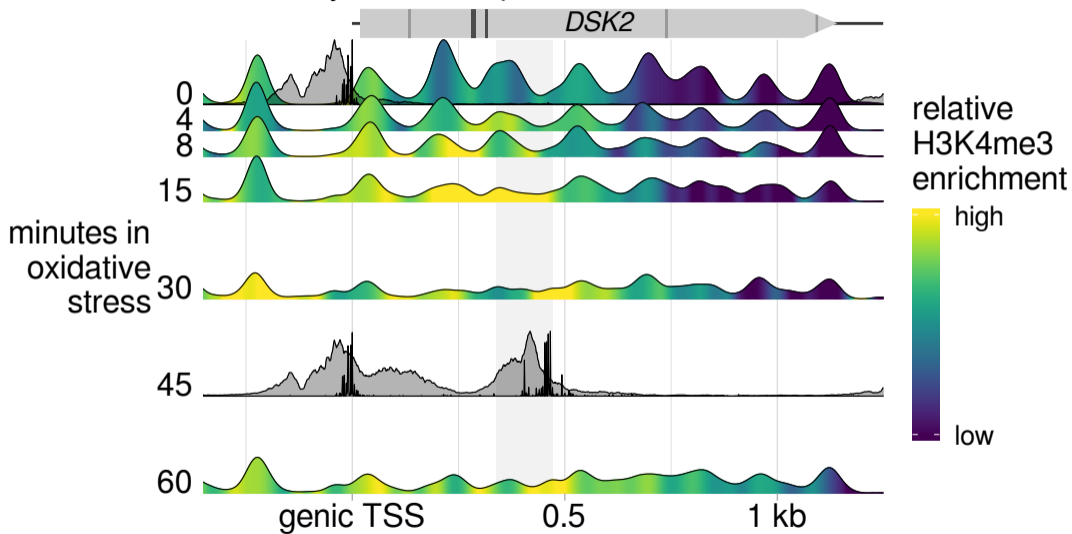
# polysome enrichment in oxidative stress

## oxidative stress-induced transcripts

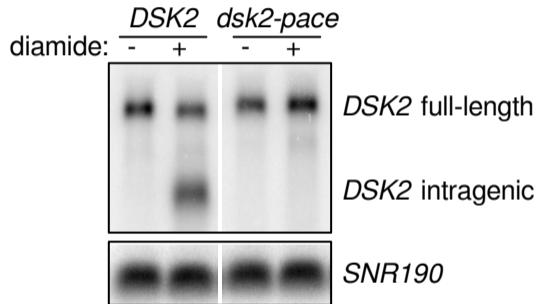




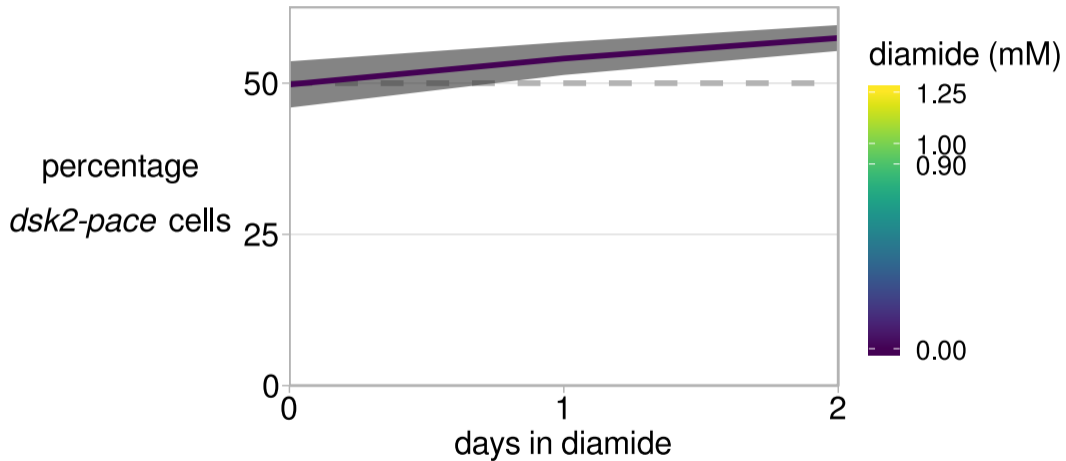
# MNase dyads, TFIIIB protection, and sense TSSs



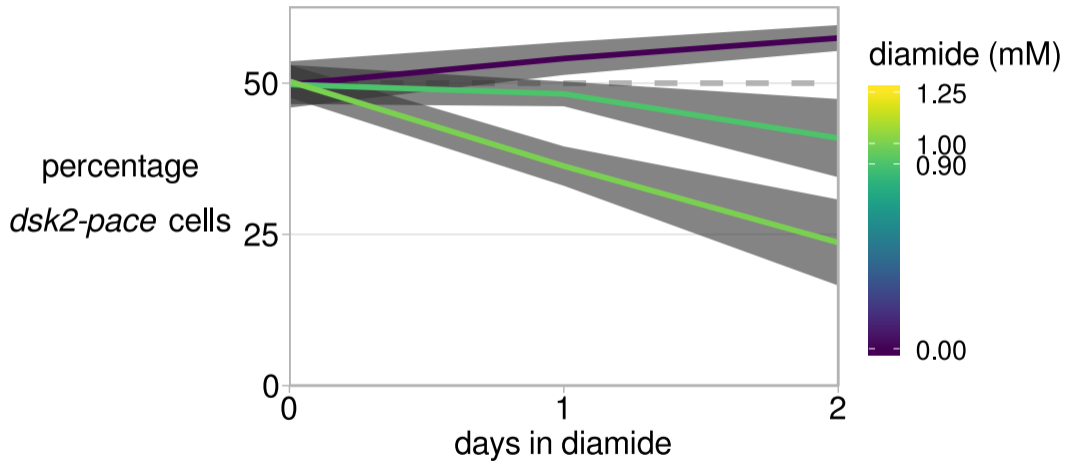
# intragenic *DSK2* expression requires PACE elements



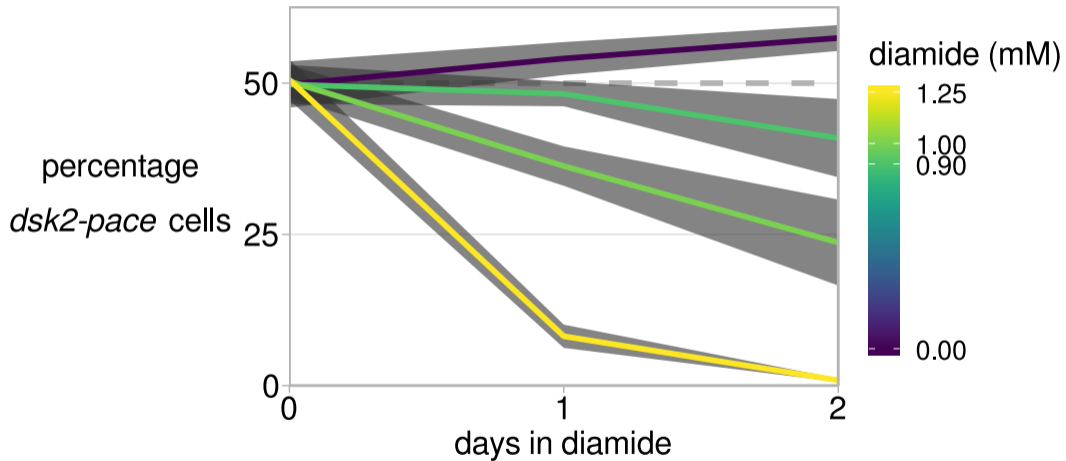
# *dsk2-pace* cells respond poorly to oxidative stress



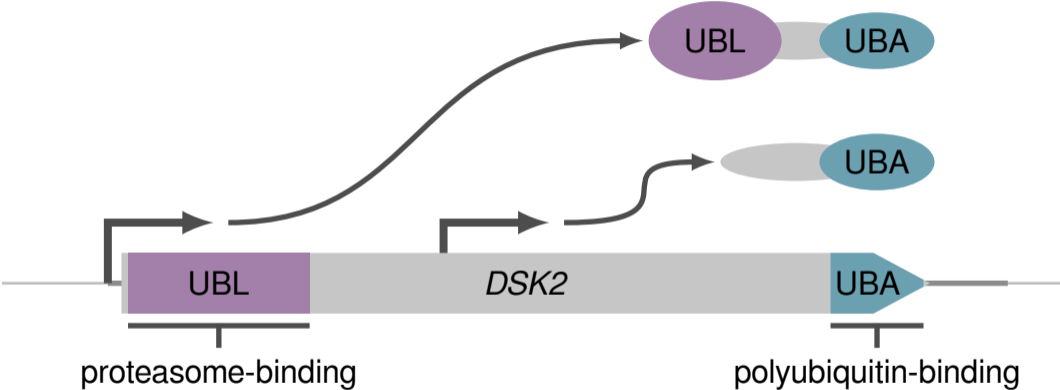
# *dsk2-pace* cells respond poorly to oxidative stress



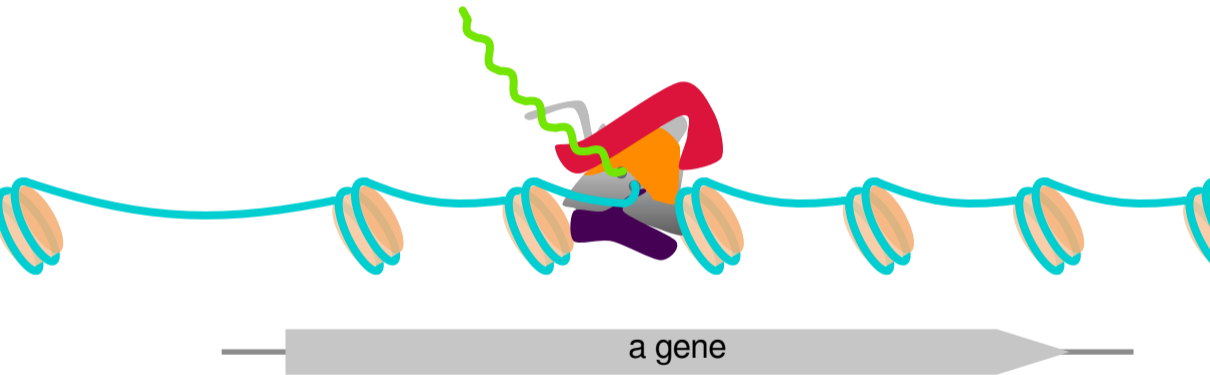
# *dsk2-pace* cells respond poorly to oxidative stress



# One possible mechanism for intragenic *DSK2* function



# Summary



# Acknowledgements

## **Winston lab**

Fred Winston  
Ameet Shetty  
Steve Doris  
Olga Viktorovskaya  
Magdalena Murawska  
Dan Spatt  
Natalia Reim  
Rajaraman Gopalakrishnan  
Francheska Lopez Rivera  
Katie Weiner  
James Warner  
Francis Apolinario  
Mallory Rice

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