

# RNA-mediated feedback control of transcriptional condensates

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CSHL Regulatory & Non-Coding RNAs  
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# Current view of eukaryotic transcription

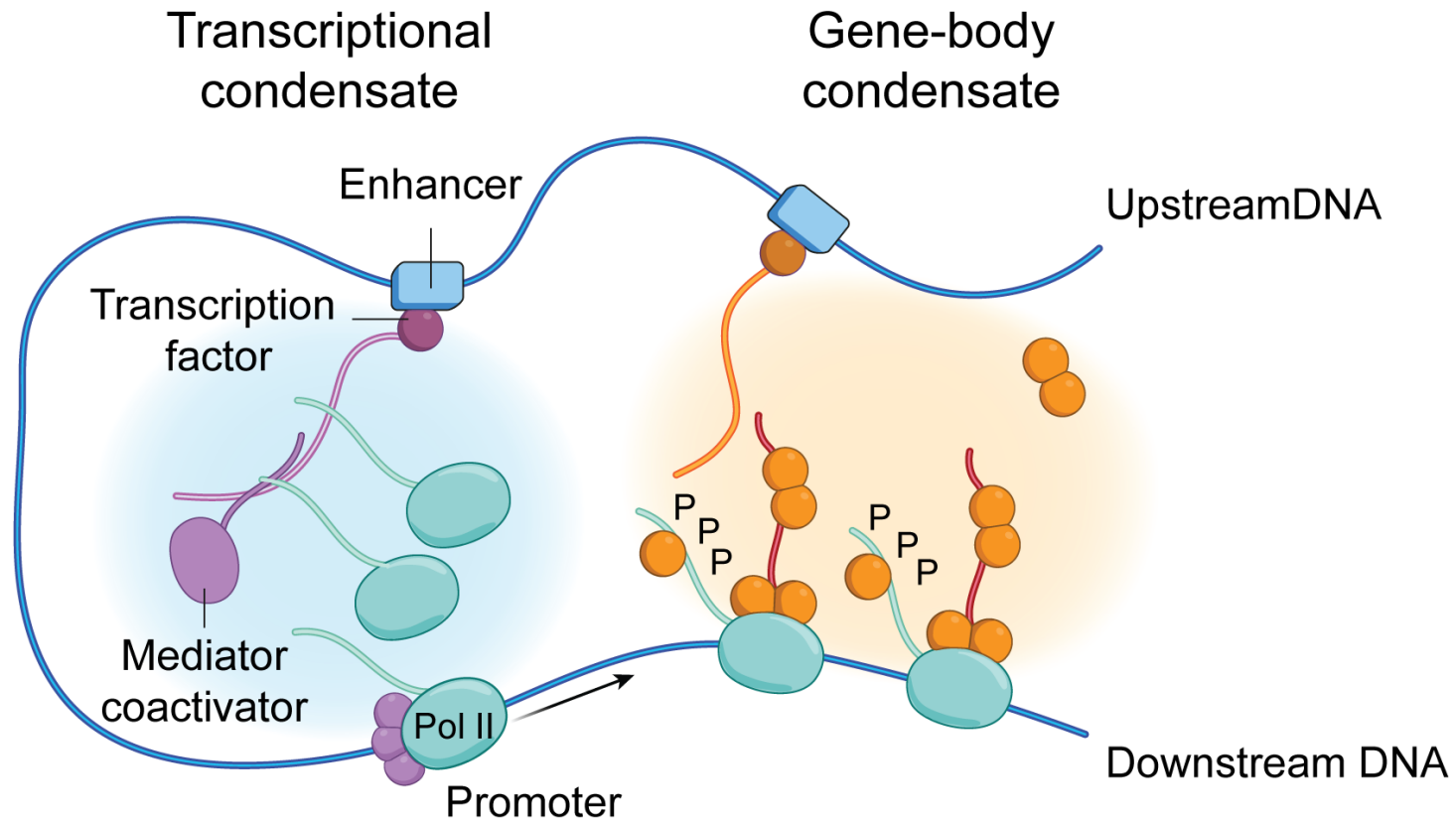


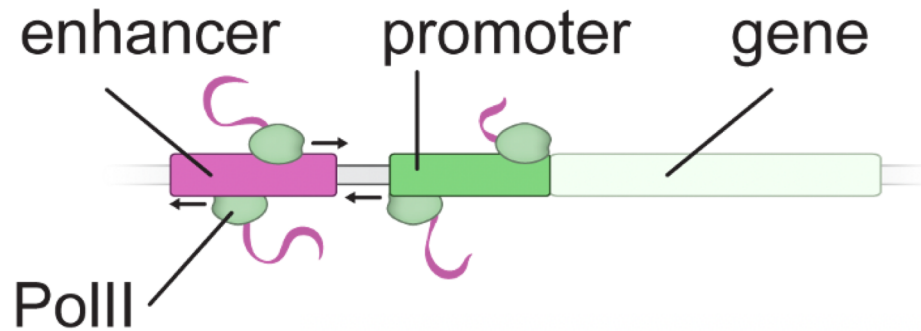
Figure adapted from Cramer, 2019  
Hnisz, Shrinivas et al., 2017  
Boija, Klein et al., 2018  
Sabari, Dall'Agnese et al., 2018  
Cho et al., 2018  
Guo, Manteiga et al., 2019  
Zamudio et al., 2019  
Shrinivas, Sabari et al., 2019

## Key features of transcriptional condensates

- Concentrates >100 molecules of Mediator and Pol II
- Form and dissolve at relatively short timescales

# Two features of eukaryotic transcription that are conserved but poorly understood

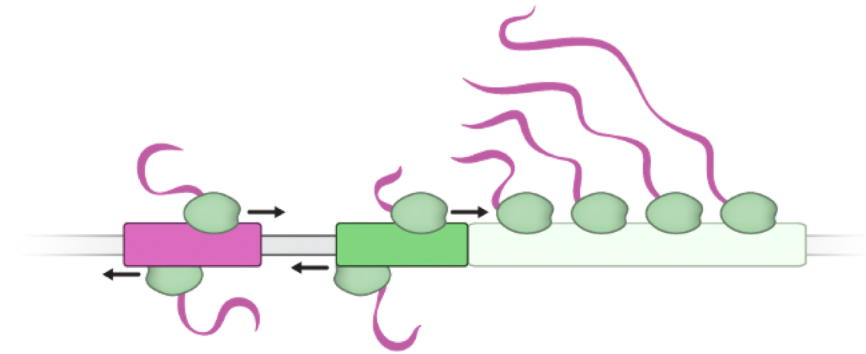
Divergent transcription from regulatory elements



Pol II transcribes regulatory elements, producing low levels of nascent RNA species

Adelman and Lis, 2012; Andersson et al., 2014; Core et al., 2014; Core and Adelman, 2019; Henriques et al., 2018; Jin et al., 2017; Kim et al., 2010; Mikhaylichenko et al., 2018; Pefanis et al., 2015; Scruggs et al., 2015; Seila et al., 2008.

Bursty transcription



Transcription occurs in periodic bursts rather than in a continuous fashion

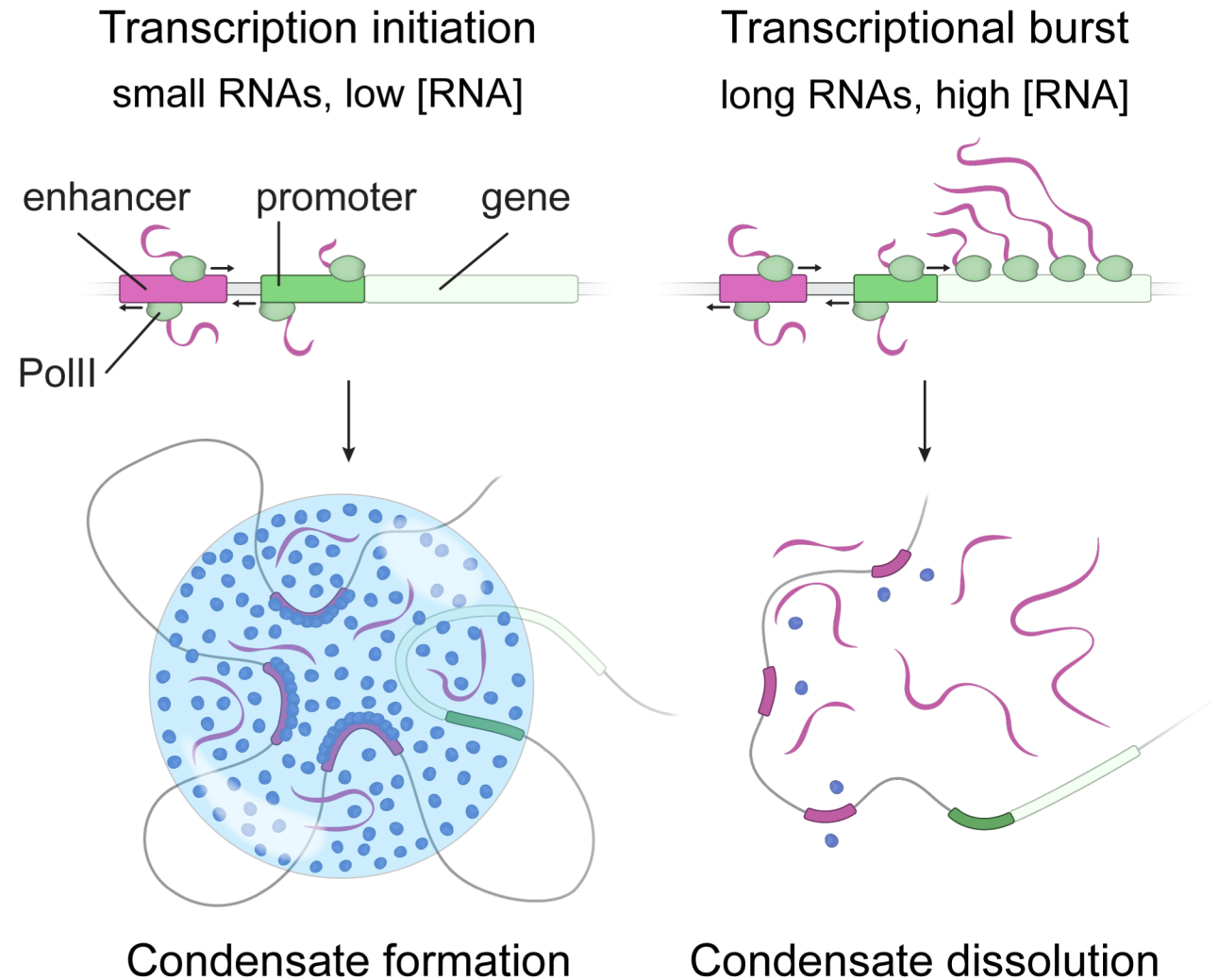
Bahar Halpern et al., 2015; Chubb et al., 2006; Fritsch et al., 2018; Golding et al., 2005; Larson et al., 2011; Lenstra et al., 2015; Pare´ et al., 2009; Raj et al., 2006; Raj and van Oudenaarden, 2008; Rodriguez et al., 2019; Suter et al., 2011; Zenklusen et al., 2008.

# RNA-mediated feedback control of transcriptional condensates?

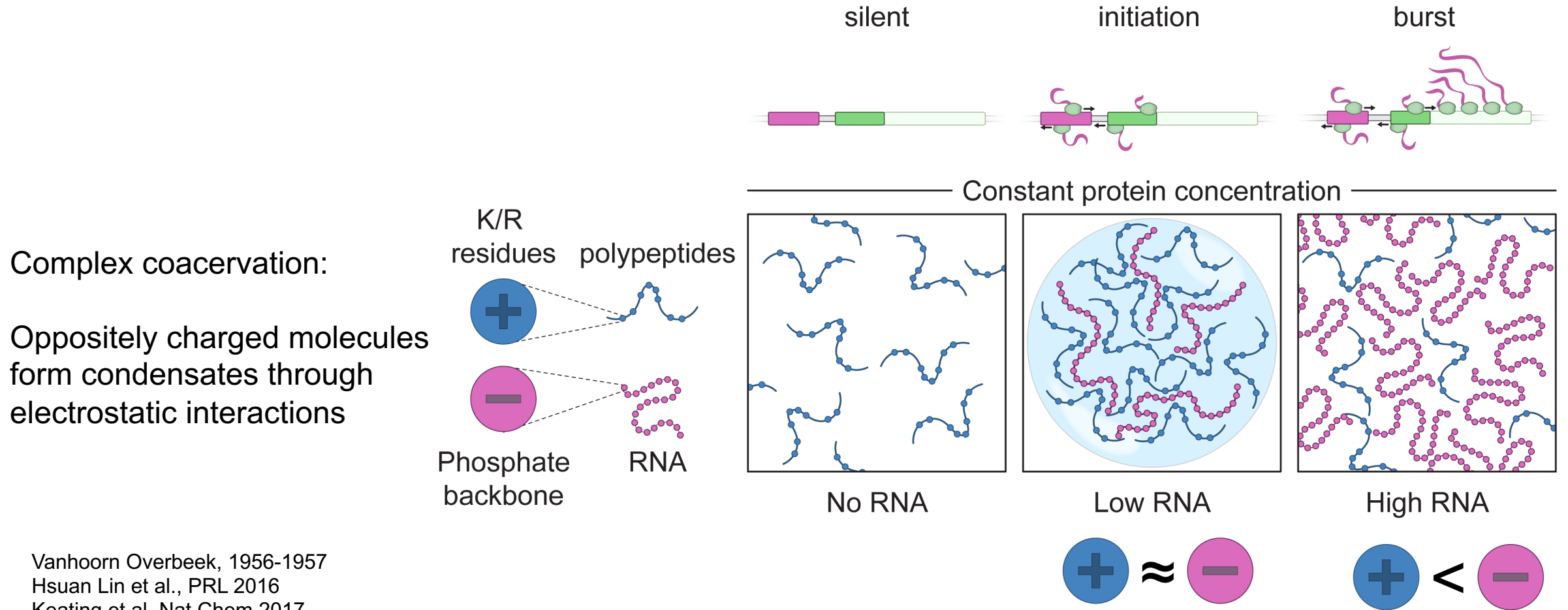
Two features of eukaryotic transcription that are conserved but poorly understood:

- Pol II transcription of regulatory elements produces low levels of nascent RNA species
- Gene transcription occurs in periodic bursts rather than in a continuous fashion

**Hypothesis:** both features may contribute to condensate regulation via an RNA feedback mechanism



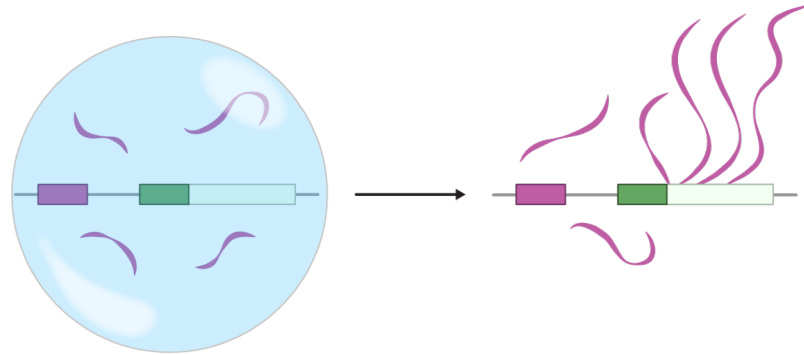
# Inspiration for a model of condensate regulation via RNA feedback



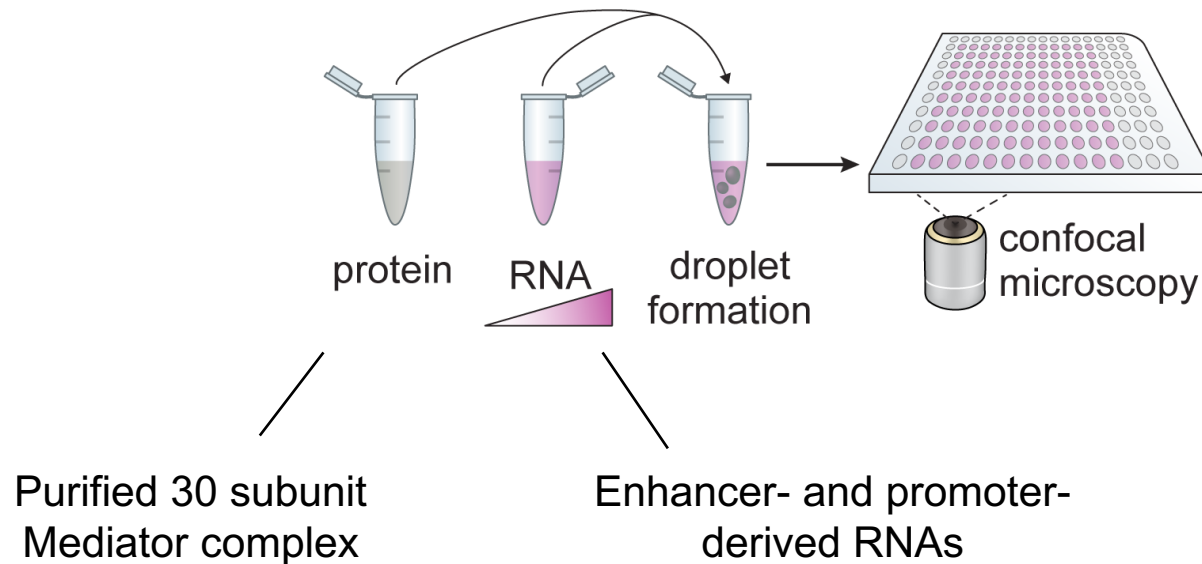
Vanhoorn Overbeek, 1956-1957  
 Hsuan Lin et al., PRL 2016  
 Keating et al, Nat Chem 2017  
 Deniz and colleagues ~ 2017-2019  
 Zhen Gang et al., JCP 2017, Macro 2018  
 Frederickson et al., Macro 2016-18, ACS 2018  
 Tirrel et al., ACS 2016, Macromolecules 2018

# Investigating influence of RNA on transcriptional condensates in vitro

Prediction of the RNA feedback model:



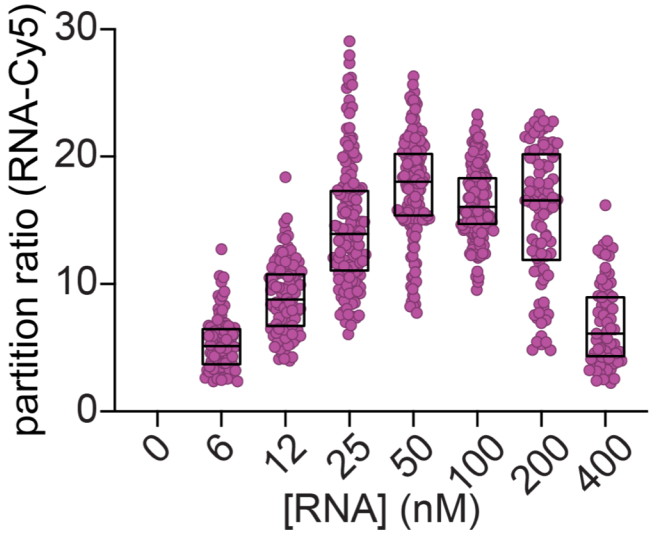
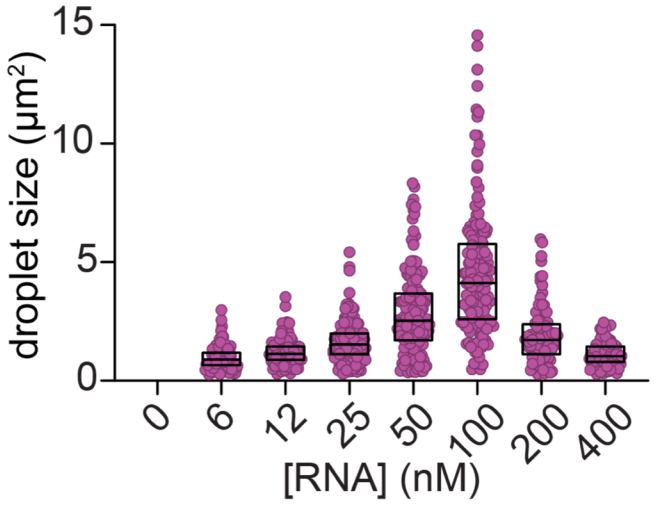
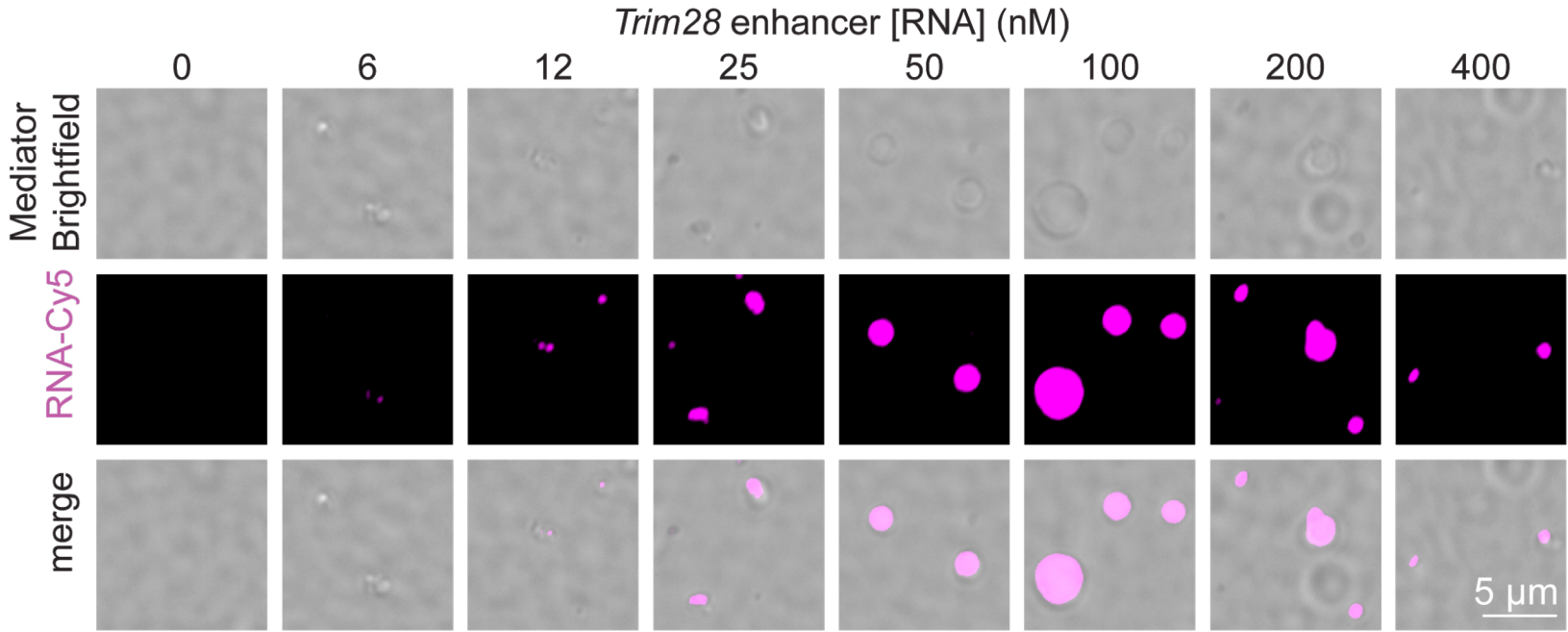
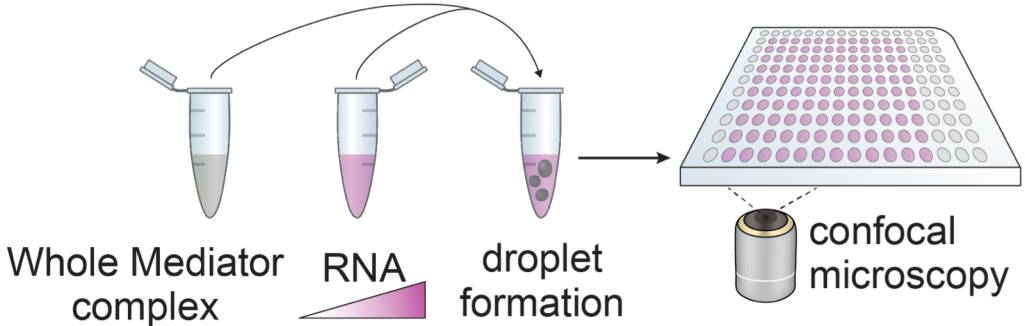
Experiment:



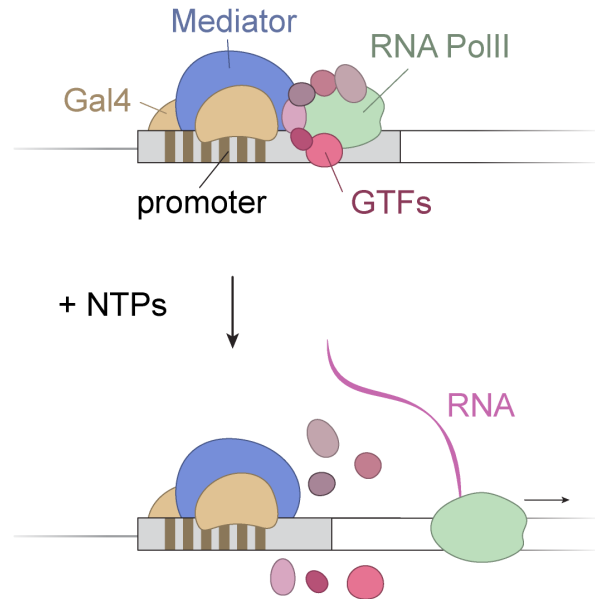
Protein and RNA components studied at approximately physiological concentrations

[Protein] = 100-1000 nM  
[RNA] = 5-400 nM (~500 nt)  
Physiological salt (60-125 mM)  
No crowder

# RNA enhances formation of Mediator droplets at low RNA levels and dissolves the droplets at high RNA levels

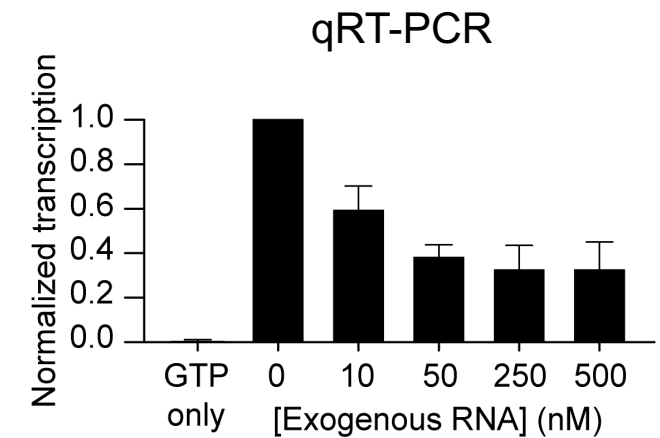
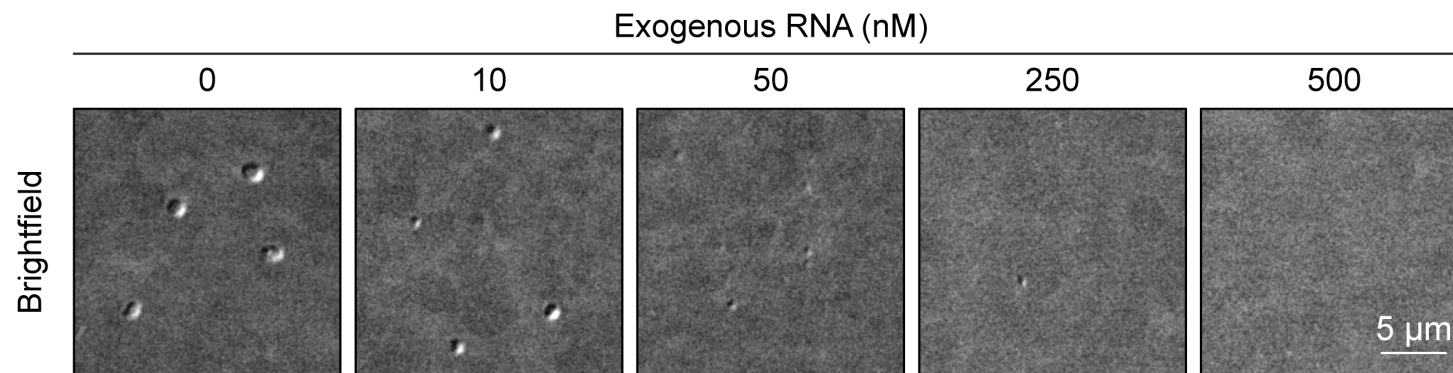
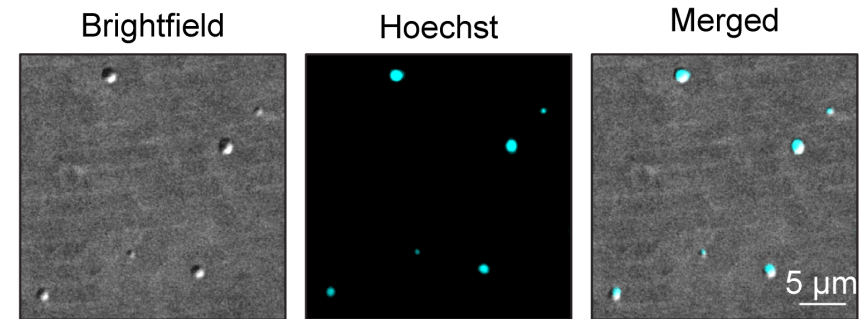


# Increasing the RNA levels in the reconstituted system reduces droplet formation and transcriptional output



Incubate at 30°C  
for 2 hours

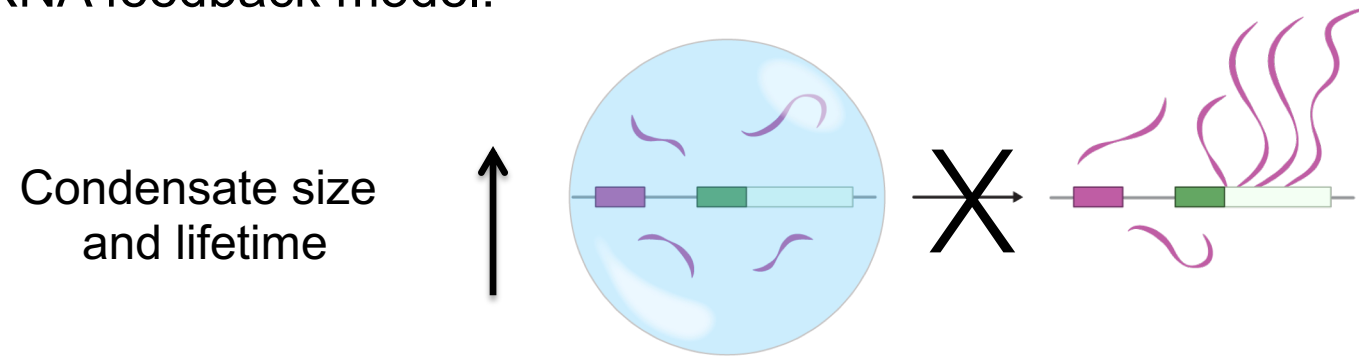
Brightfield  
microscopy  
Quantify RNA  
by qRT-PCR



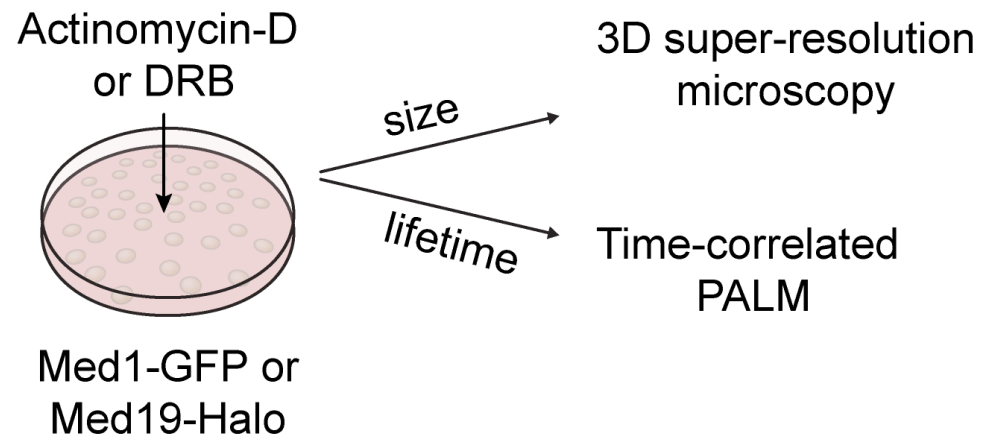


# Investigating influence of RNA on transcriptional condensates in cells

Prediction of the RNA feedback model:

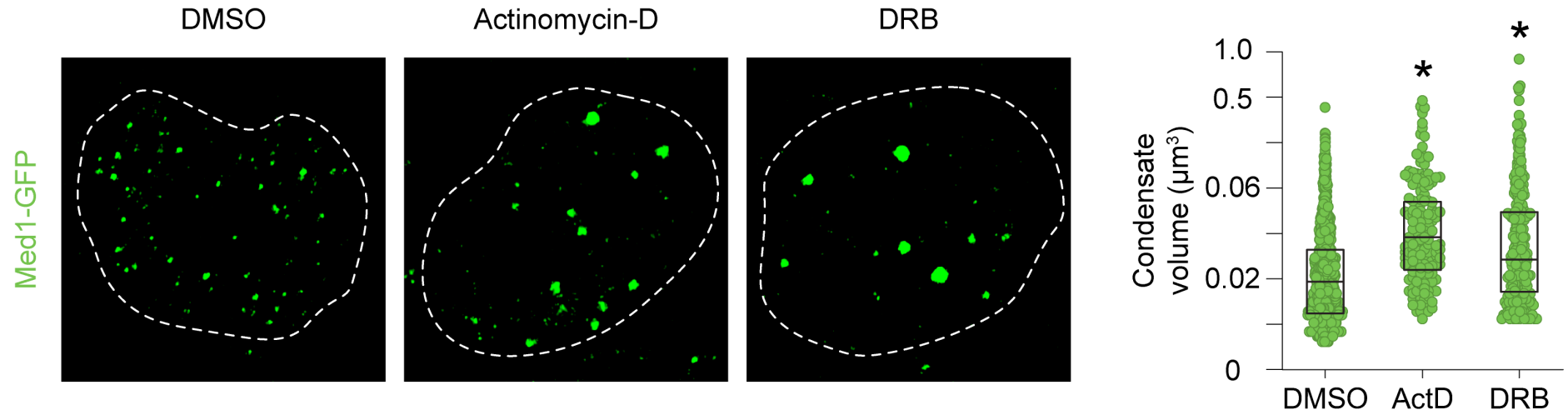


Experiment:

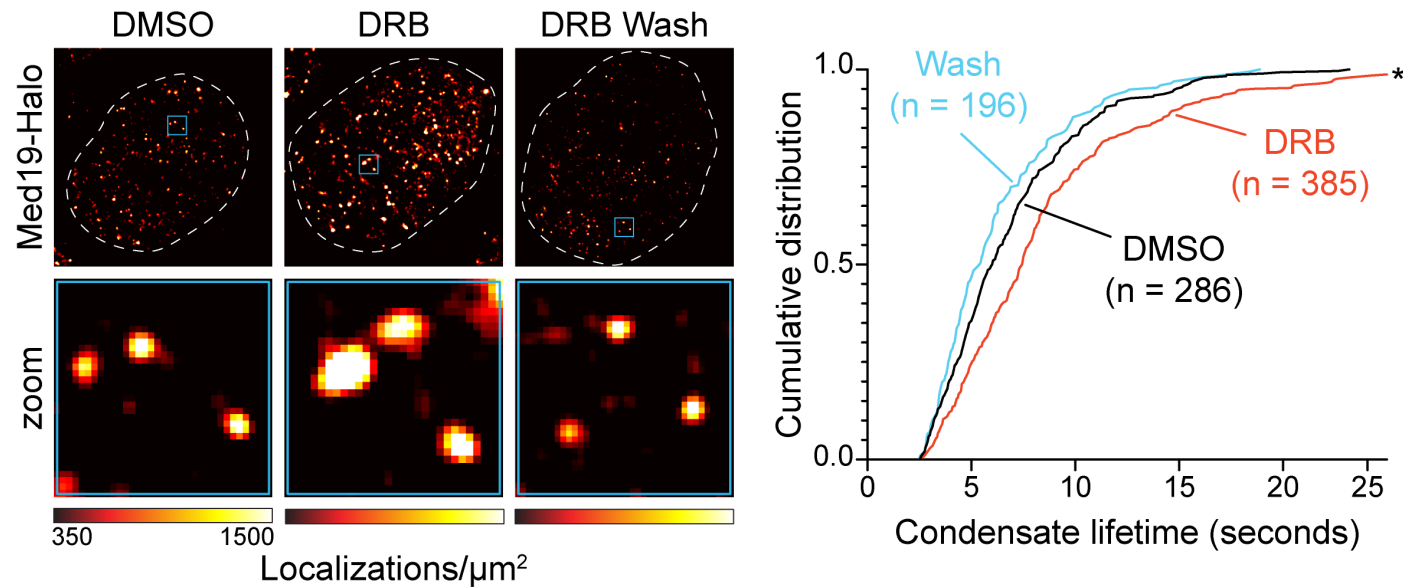


# Inhibition of elongation leads to an increase in condensate size and lifetime

Condensate size:



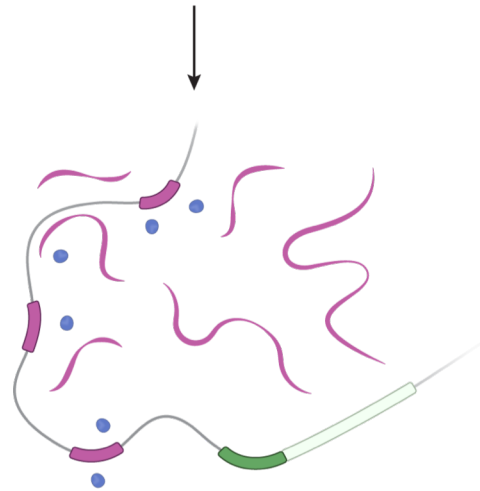
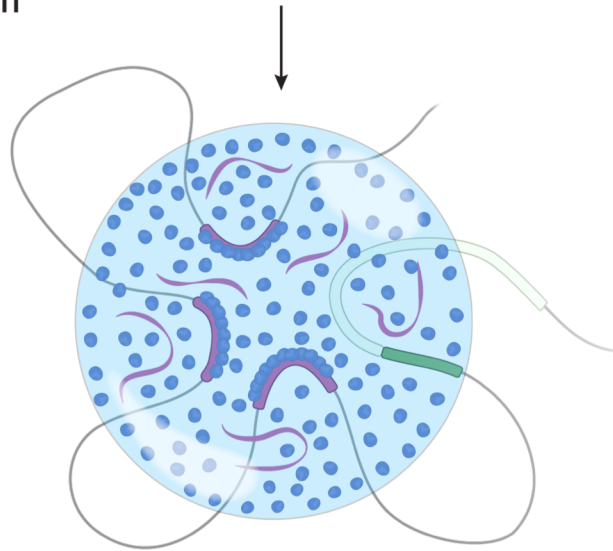
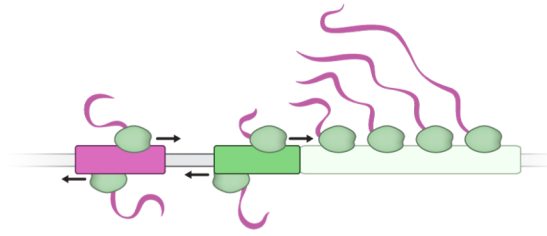
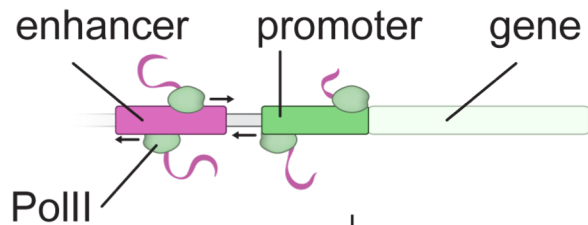
Condensate lifetime:



# RNA-mediated feedback control of transcriptional condensates

Transcription initiation  
small RNAs, low [RNA]

Transcriptional burst  
long RNAs, high [RNA]



Condensate formation

Condensate dissolution

This model provides an explanation for:

- function of enhancer RNA and promoter RNA
- bursty transcription

# Acknowledgements



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## Young Lab

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Nancy Hannett

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## Collaborators

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