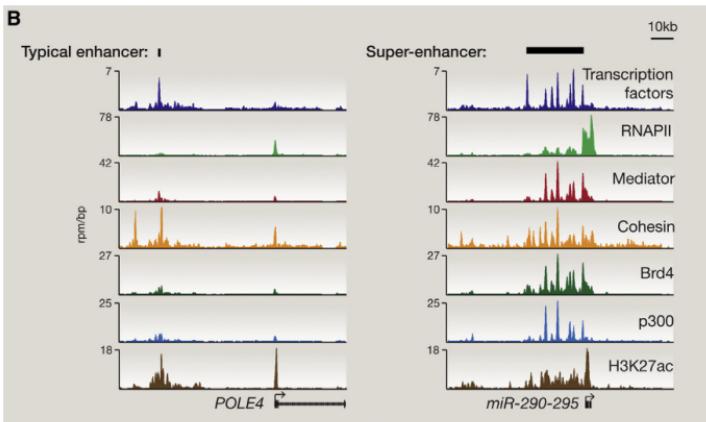
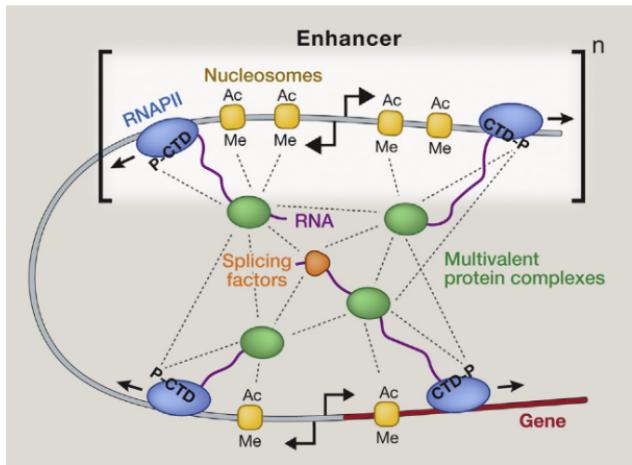


# Research Update

Clayton W. Seitz

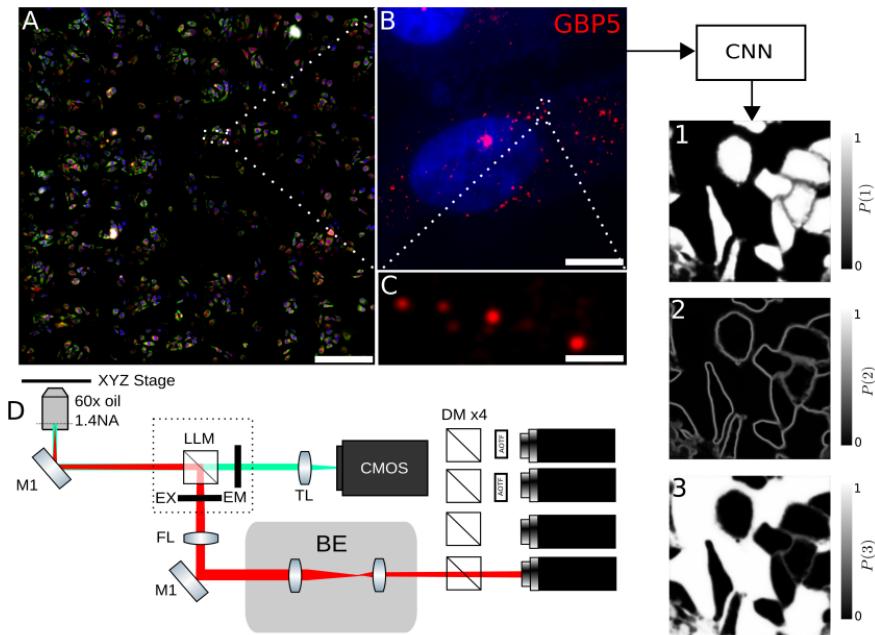
April 9, 2023

# Recap: A modern view of transcriptional control



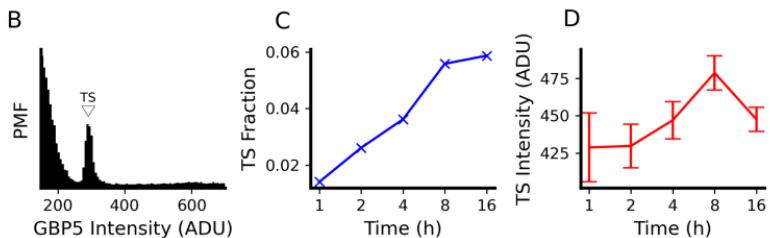
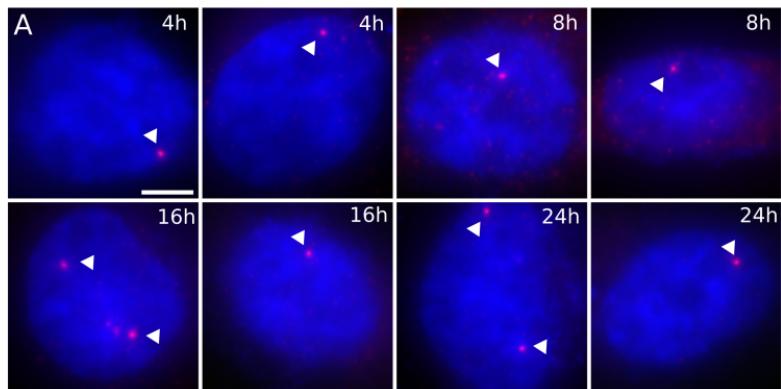
Hnisz et al. A phase separation model of transcriptional control.  
Cell 2017

# High-throughput imaging of GBP5 transcripts in single cells



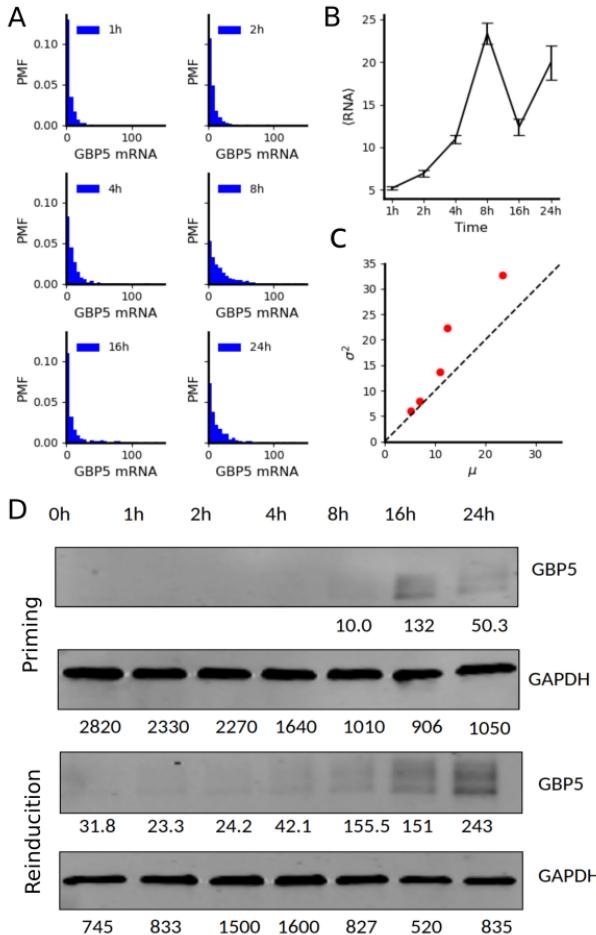
- ▶ (A) Large FOV three channel maximum intensity projection. Scalebar 300um
- ▶ (B) GBP5 mRNAs. Scalebar 10um
- ▶ (C) Diffraction-limited image of GBP5 transcripts hybridized with 48 complementary fluorescent probes
- ▶ (D) 4-color widefield microscope used for fluorescence imaging with a 60X Nikon oil-immersion objective.
- ▶ (1-3) Single cell masks predicted by a convolutional neural network (CNN)

# Identification of GBP5 transcription sites

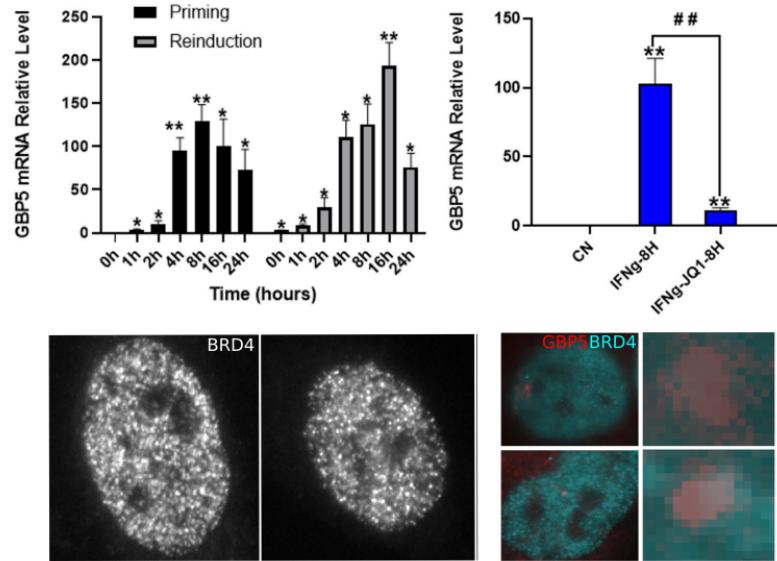


- ▶ (A) Putative transcription sites at 4h, 8h, 16h, and 24h after first exposure of HeLa cells to Interferon-gamma (50 ng/ml)
- ▶ (B) Histogram of peak intensities of single GBP5 transcripts used to determine the criterion for transcription site classification.
- ▶ (C) The fraction of cells with at least one active transcription site as a function of time since first exposure to IFN-gamma
- ▶ (D) Average intensity of putative transcription sites since first interferon exposure. Error bars represent standard errors

# High-throughput imaging of GBP5 transcripts in single cells



# RT-qPCR validation of GBP5 expression and identification of BRD4 as a potential transcription factor



- ▶ Optimized sequential FISH + IF @ 8h after IFN- $\gamma$  exposure
- ▶ Need to do analysis of GBP5 and BRD4 co-localization
- ▶ Most papers use a DNA FISH probe to prove TS unequivocally
- ▶ Can also try the JQ1 inhibitor