Elongation rates and H2B mono-ubiquitination

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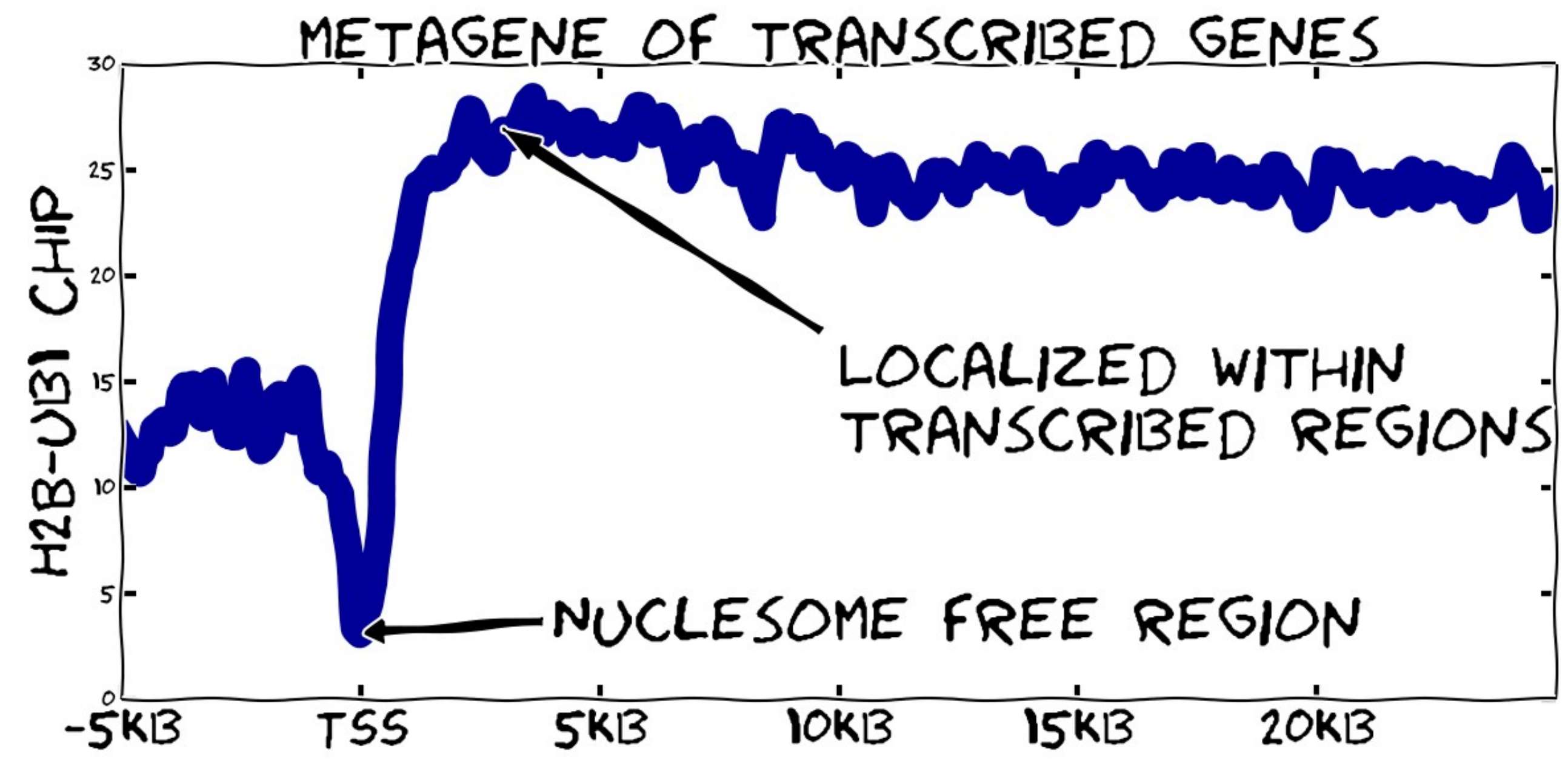
BACKGROUND

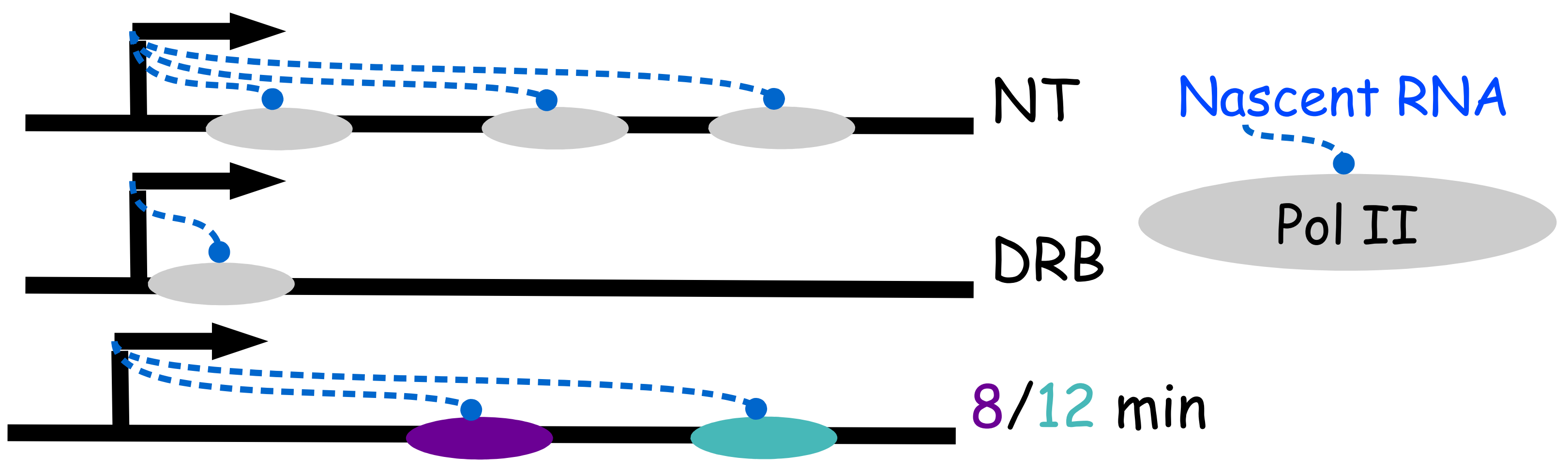
Histone modifications have important roles in regulating molecular and cellular functions. Monoubiquitylation of histone H2B on K120 (H2B-ub1) localized within transcribed regions and correlates with gene expression and elongation rate.

Here we measure genome-wide elongation rates in H2Bub1 depleted cells (knockdown of RNF20 ubiquitin ligase) and try understand how H2B-ub1 affects transcription and elongation.



Bonnet et al.

EXPERIMENT OVERVIEW



Genome-wide *in vivo* measurements of nascent RNA in si-RNF20 and lacZ using 4sUDRB-seq within 8/12 minutes after DRB removal

4SU-DRB-seq
(Fuchs et al.)

DRB
Blocks transcription
in vivo

Biotinylated 4sU
Marks nascent RNA

Conditions

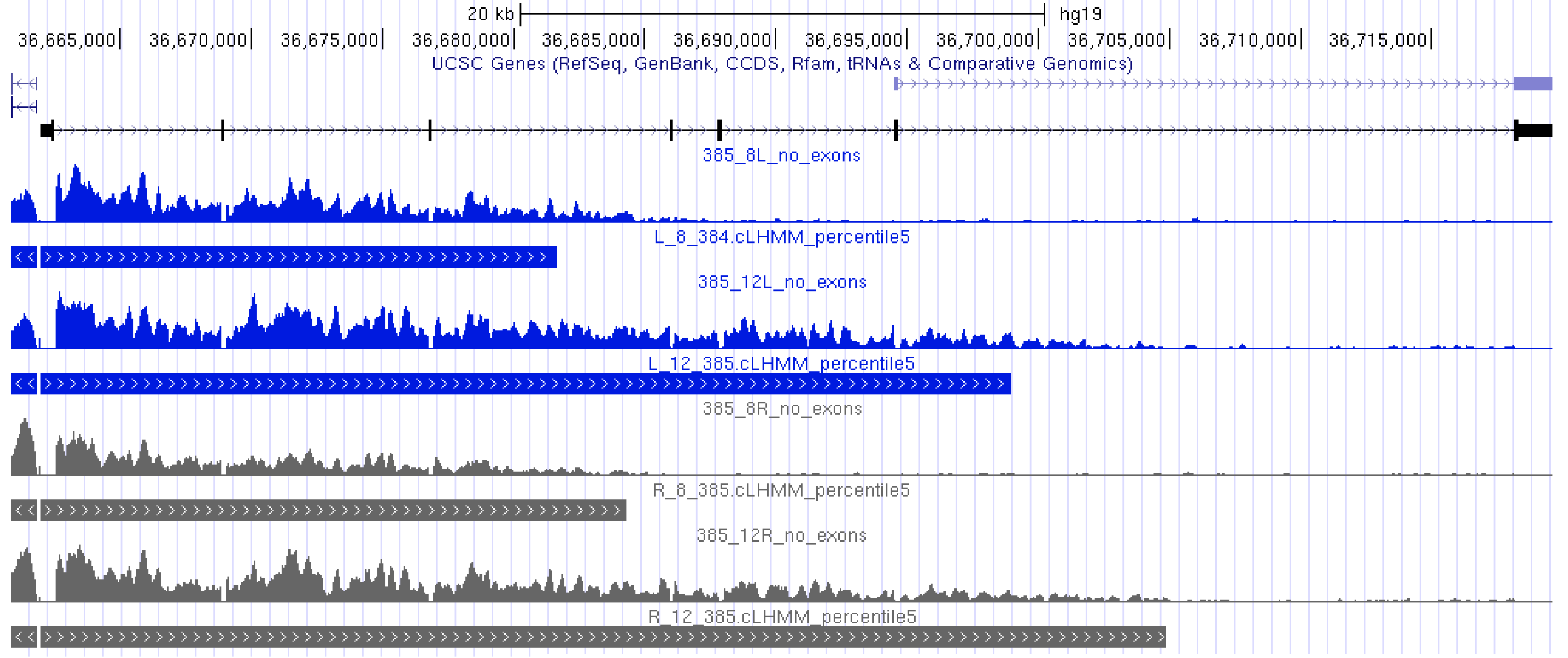
siLacZ
control

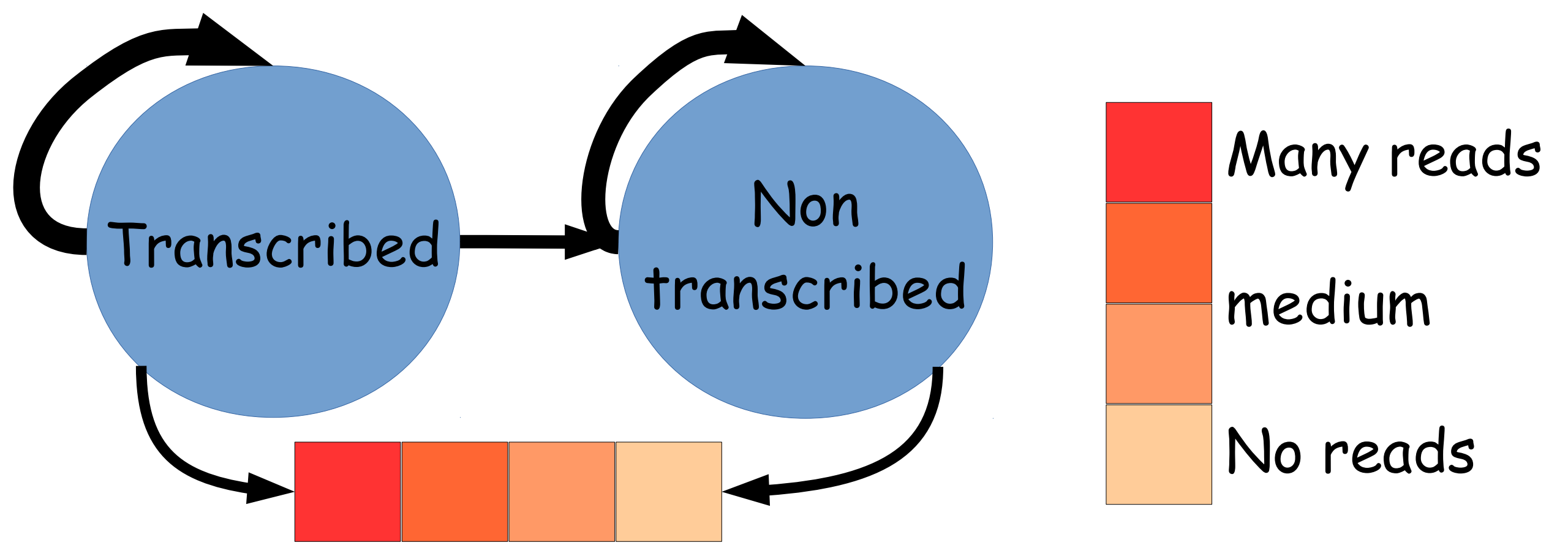
siRNF20
H2Bub1 depletion

Pol II ChIP-seq: position of Pol II

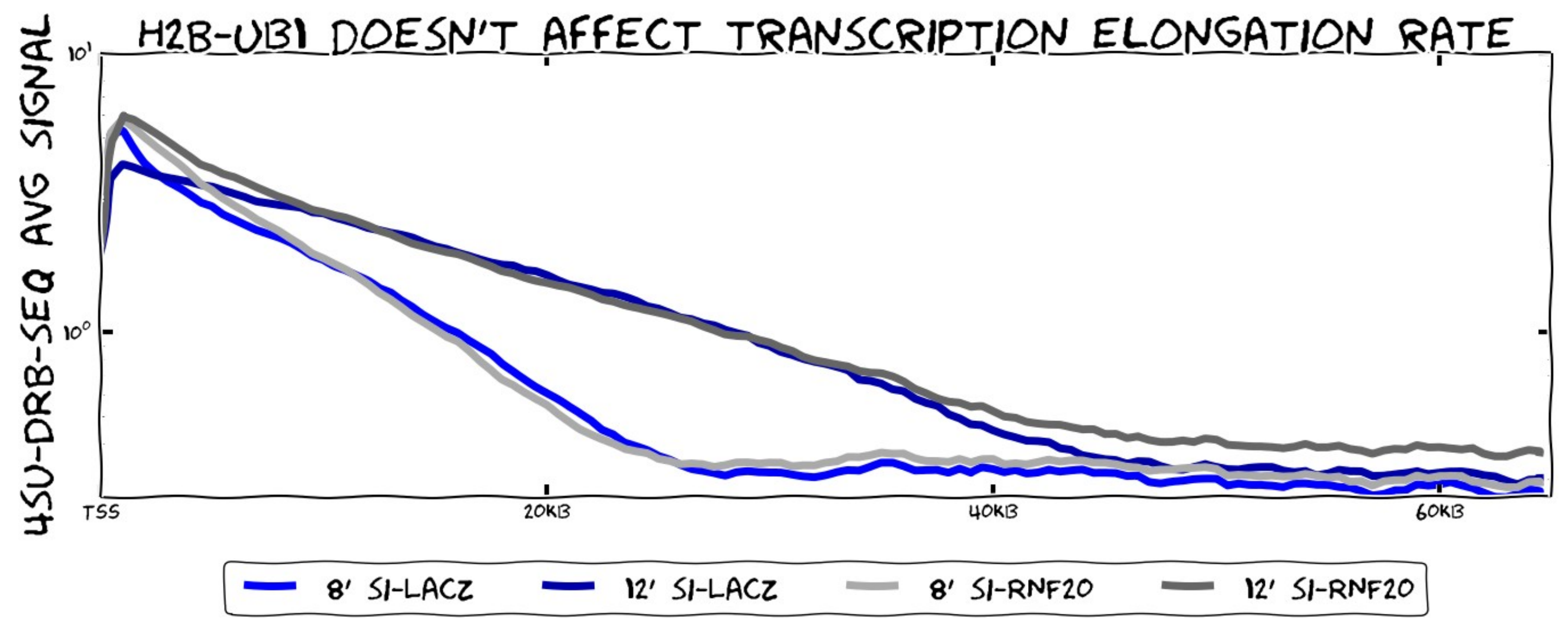
COMPUTATIONAL MODEL

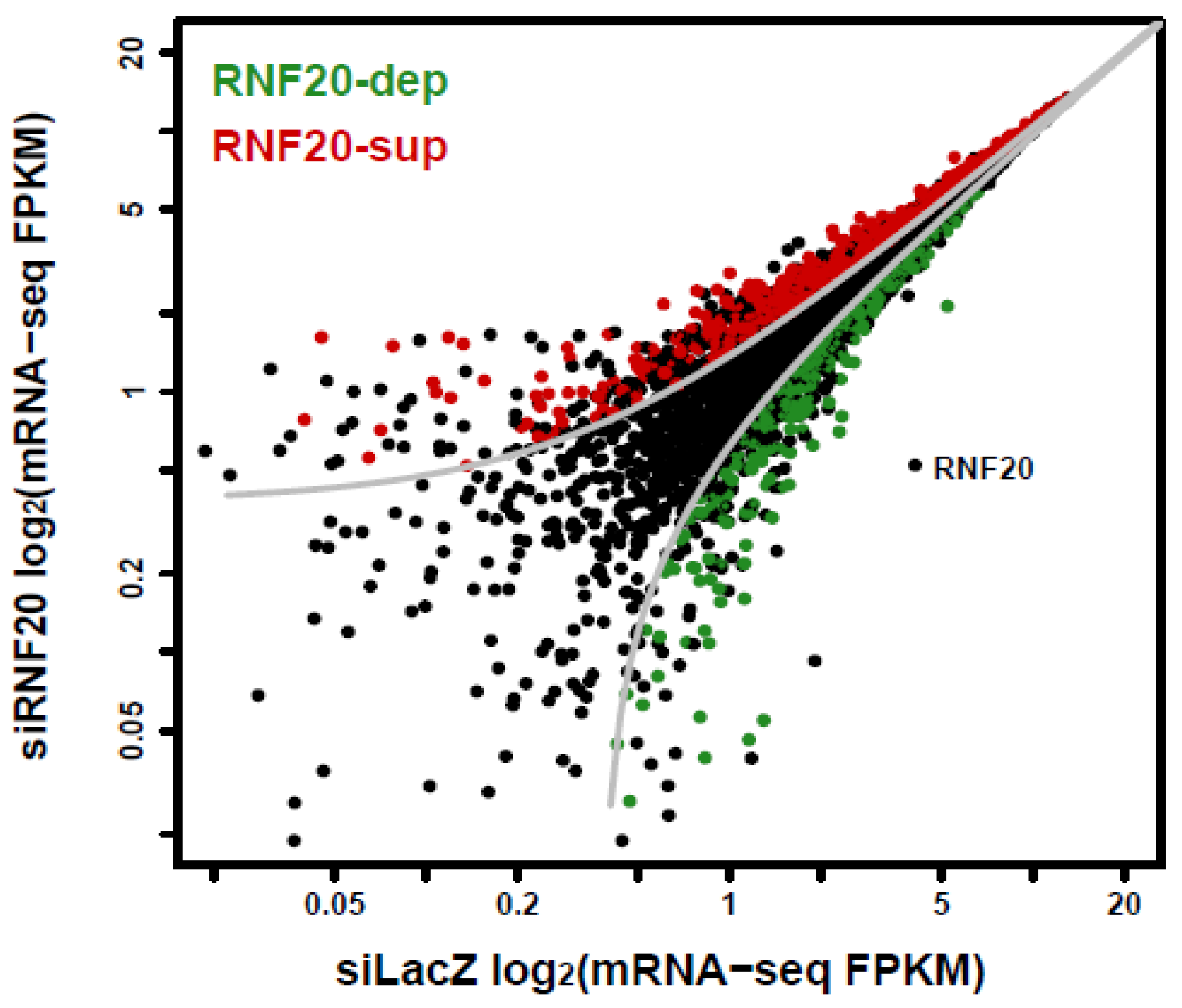
2 state HMM defines the transcribed region

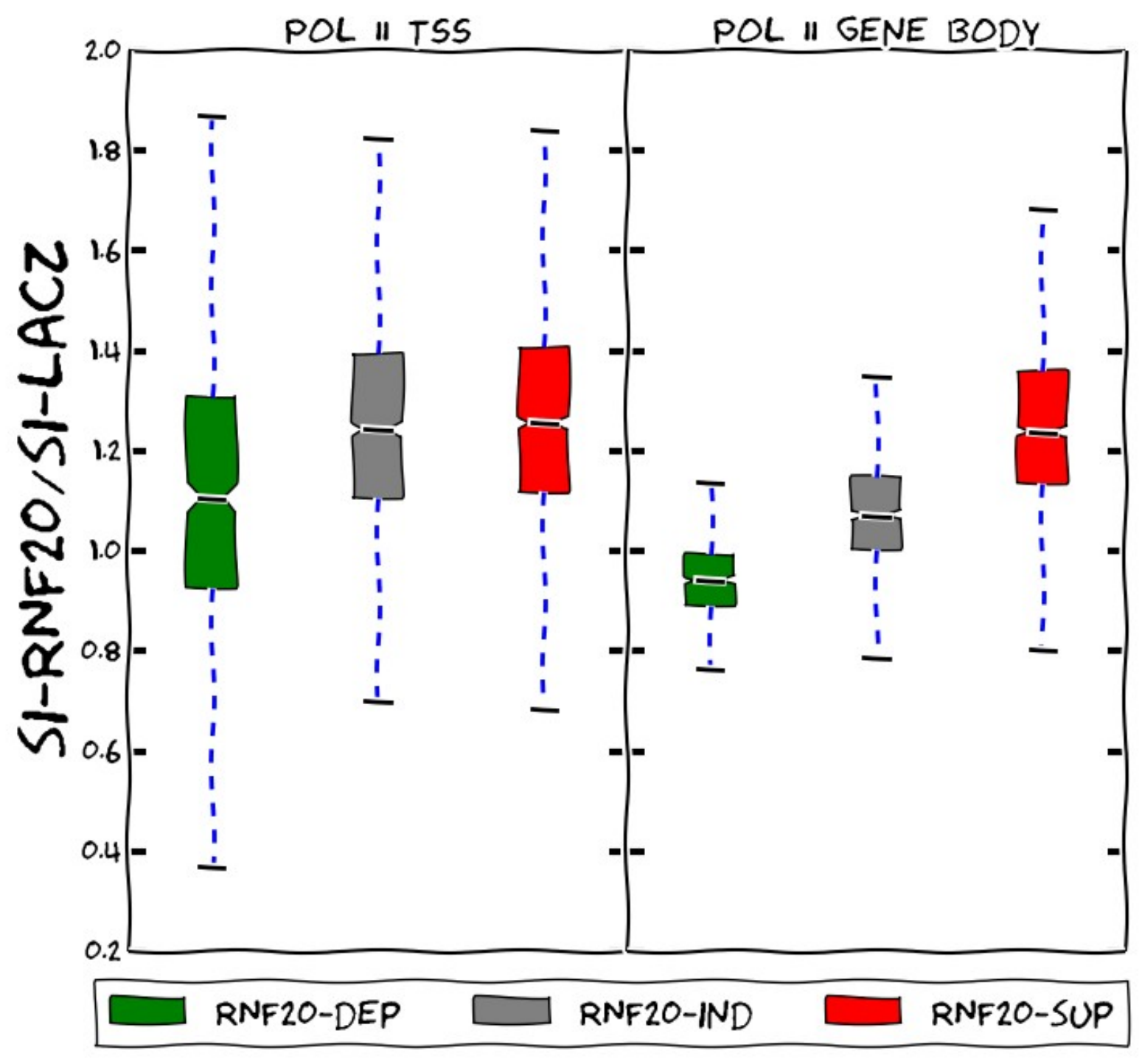




RESULTS

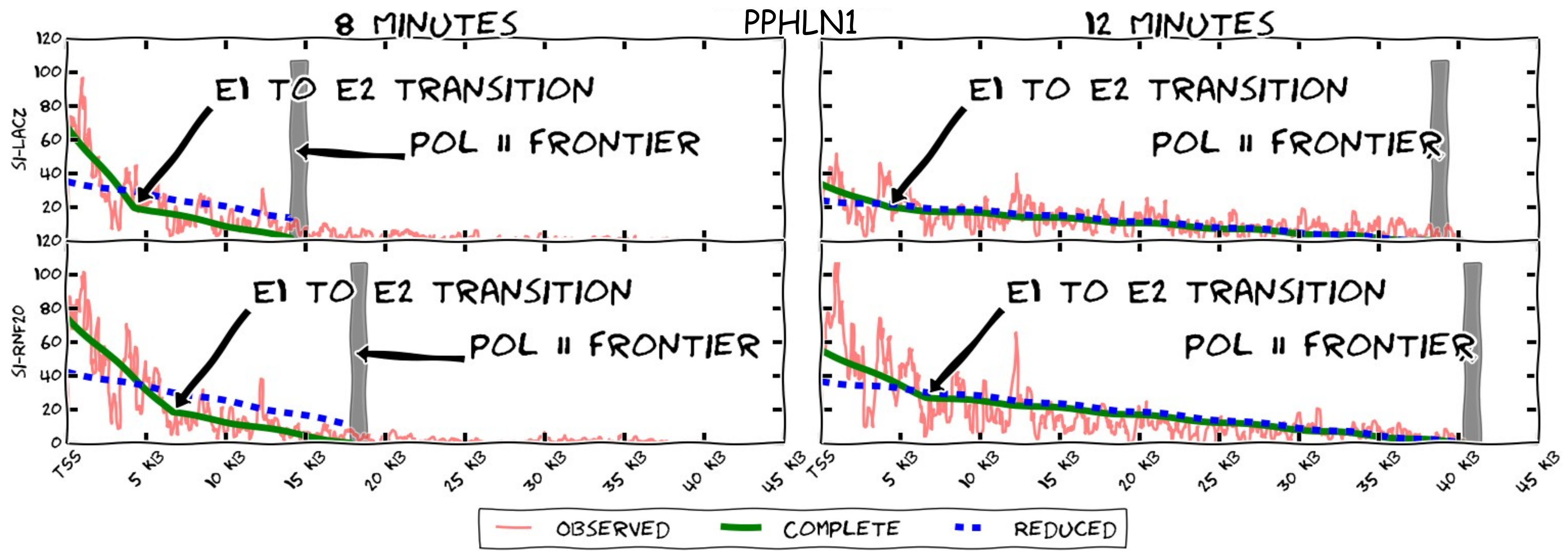




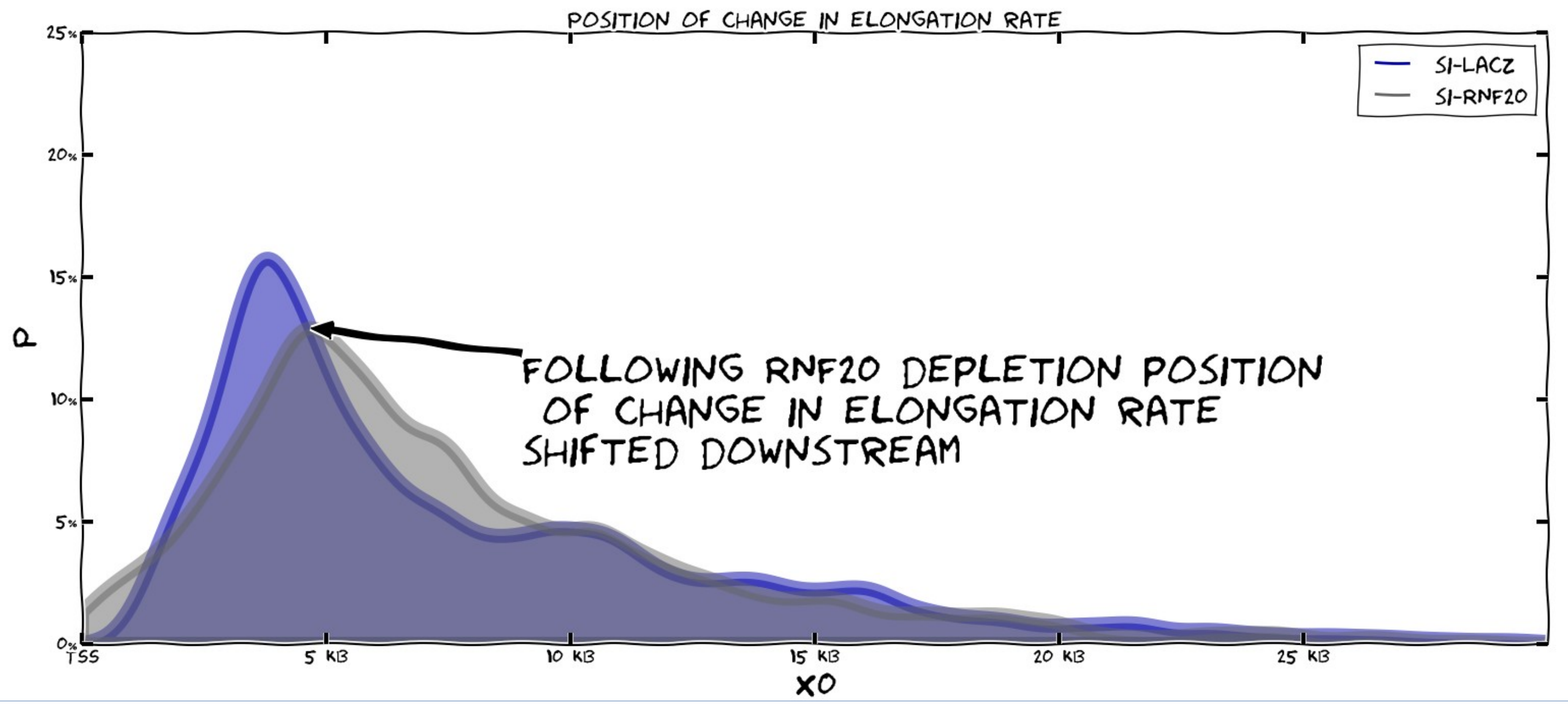


- Up regulated (suppressed) and down regulated (dependent) genes after RNF20 knockdown were defined based on mRNA & nascent RNA.
- In suppressed genes, the increase in PolII only in gene body, may indicate H2Bub1 inhibit the transition from paused state to productive elongation
- Decrease in Pol II (on TSS & gene body) for dependent genes suggest H2Bub1 is needed for Pol II recruitment or shorter re-initiation time of Pol II

HINGE MODEL



Elongation rate and re-initiation time estimation using a hinge model assuming 2 elongation rates and comparing it to a reduced model.



REFERENCES

1.Fuchs et al., Gen. Biol (2014). 4sUDRB-seq: measuring genomewide transcriptional elongation rates and initiation frequencies within cells.
2.Bonnet, et al. Genes & dev. (2014). The SAGA coactivator complex acts on the whole transcribed genome and is required for RNA polymerase II transcription.
3.Fuchs et al. (submitted) Gene body H2B monoubiquitylation regulates gene-selective RNA Polymerase II pause release and is not rate limiting for transcription elongation