

# Genome-wide profiling of histone variants in *Drosophila* and *Caenorhabditis*

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# Specialized chromatin functions of core histone variants

H3

H3

cid (centromeres)

H3.3

(replacement)

Major histones  
Variants

H2A

H2A

H2A.X

(DNA repair)

H2A.Z

(insulation)

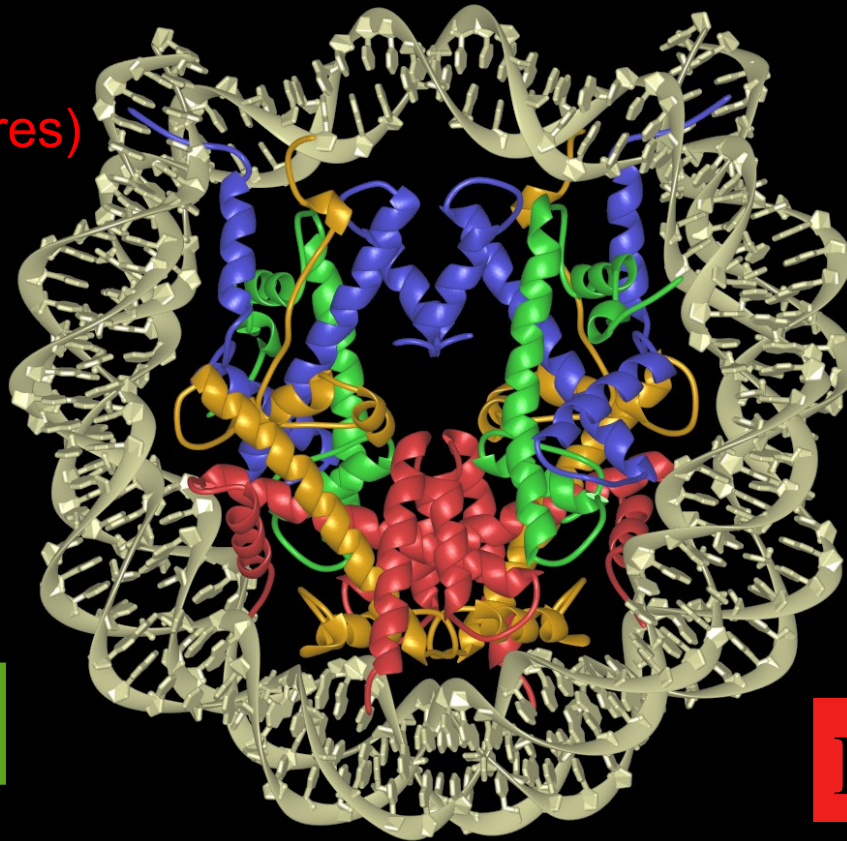
H2AV

H4

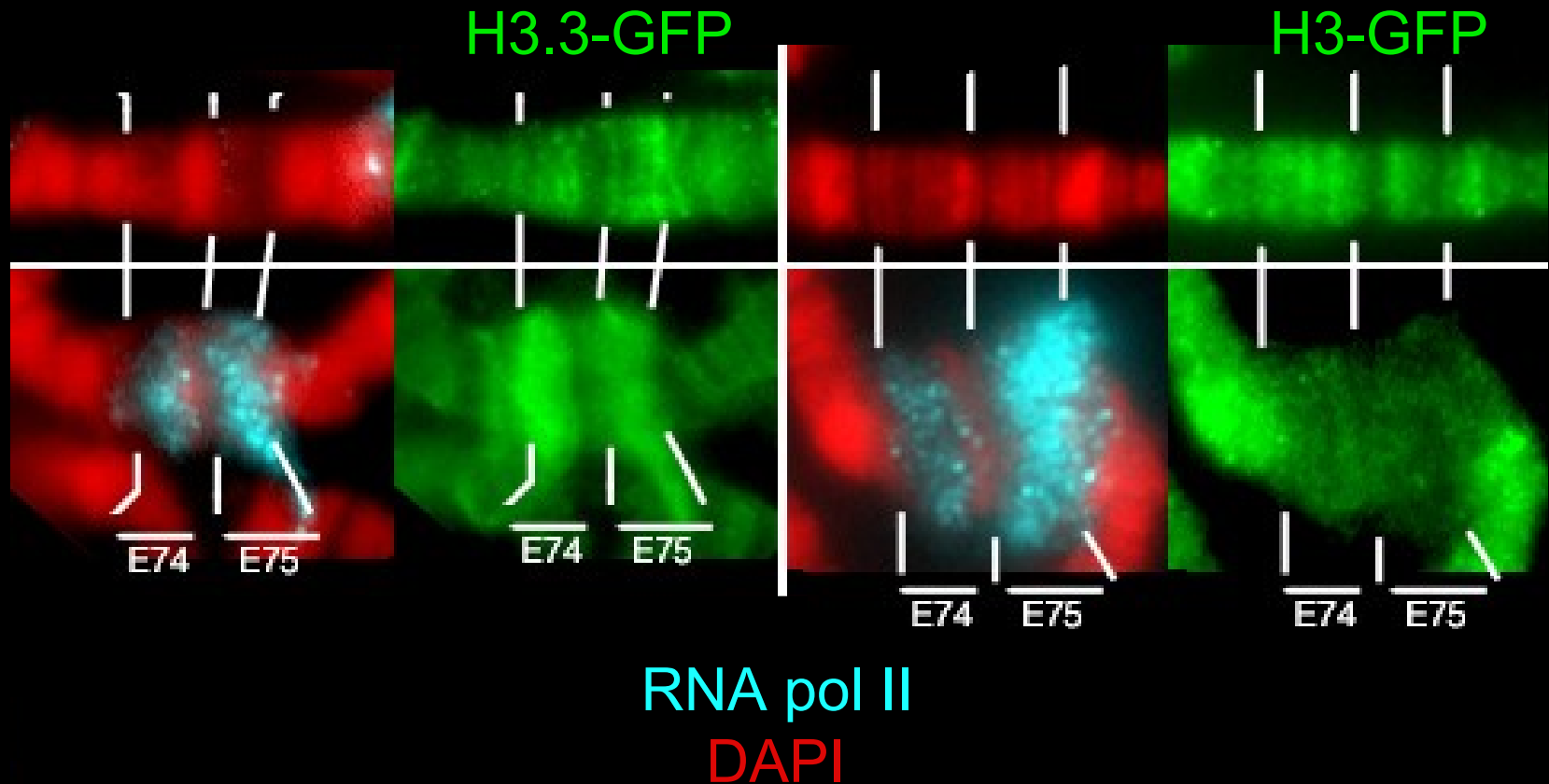
H4

H2B

H2B



# Transcription-coupled deposition of H3.3

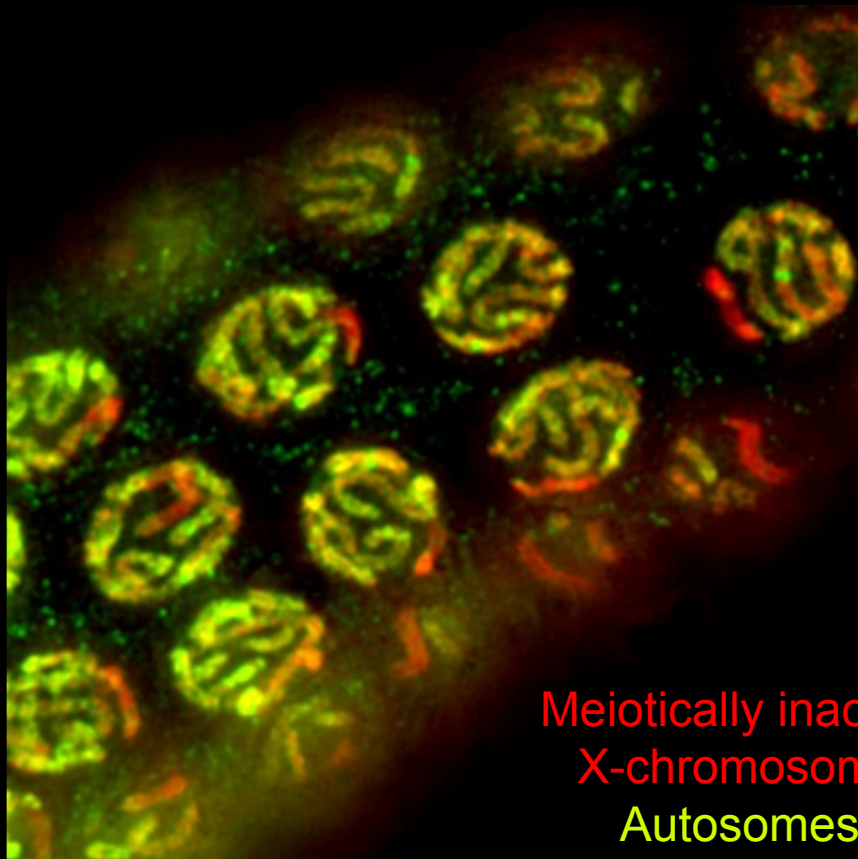


# H3.3 marks active chromatin in *C. elegans*

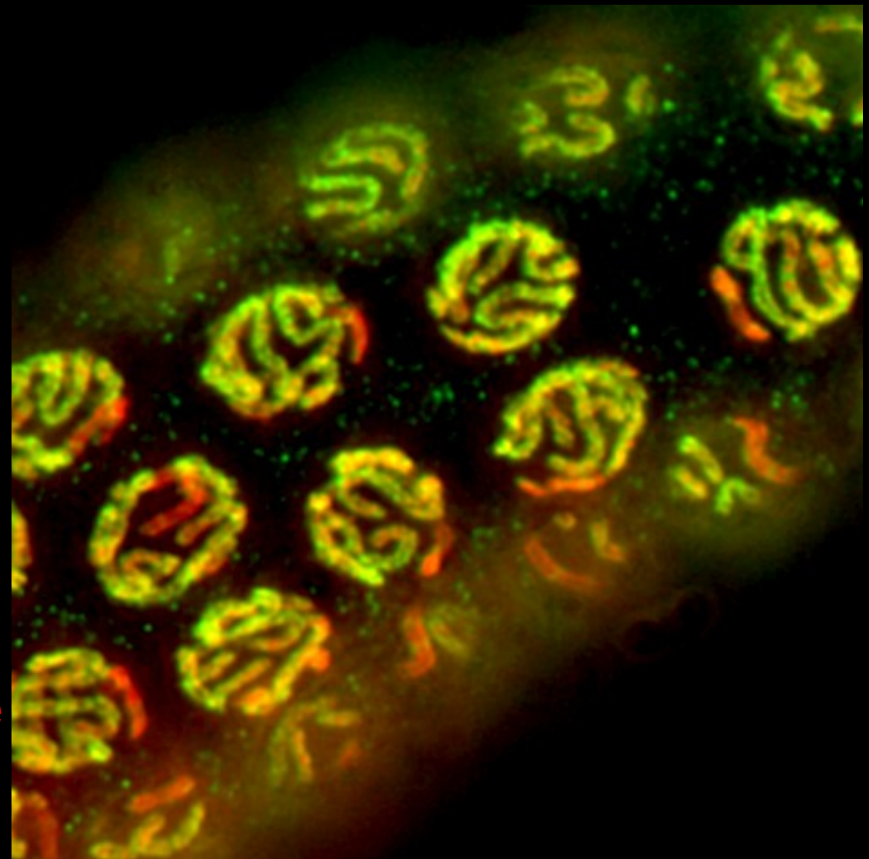
Histone H3.3

DNA

Histone H3K4me2



Meiotically inactive  
X-chromosome  
Autosomes

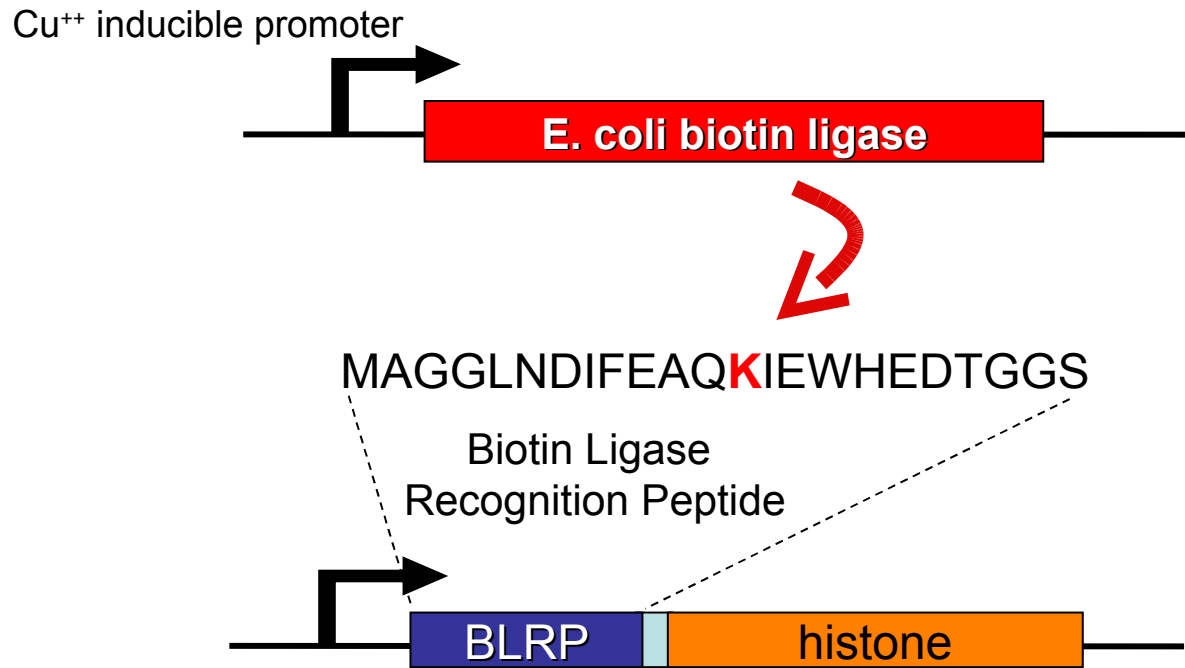


Pachytene stage of gametogenesis

Histone variants index chromatin:  
Little overlap between H3.3 and H2AV  
in polytene chromosomes



# *in vivo* biotin tagging for profiling histone variants



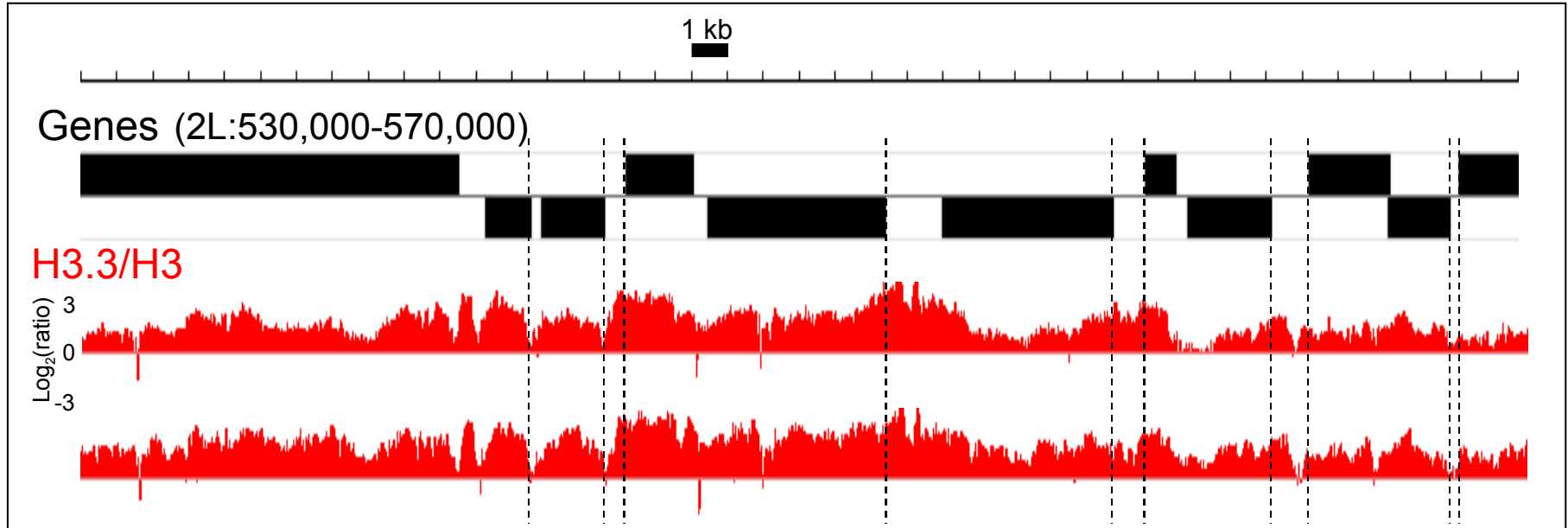
- Log-phase fly S2 cells induced for ~1-3 cell cycles
- MNase digestion of native chromatin
- Streptavidin pull-down vs. input ( $\log_2$ -ratios)
- Hybridize to fly genome tiling arrays (NimbleGen)

# Histone variant landscapes for modENCODE

- Both *D. melanogaster* and *C. elegans*: a common system for comparison
- Biotin-tagging provides ~100% capture efficiency with  $\sim K_d=10^{-15}$  affinity
- Profiling H3.3, H2A.Z (H2Av in fly), nucleosome occupancy and expression
- 3 fly cell lines: Embryonic (S2), Wing disc (Clone 8), Neuronal (Clone 6)
- 2 worm stages (Adults and embryos), 3 tissue-specific promoters driving biotin ligase
- Current platform: NimbleGen 2-color arrays with  $2.1 \times 10^6$  probes
- Functional analysis: RNAi knockdown of histone chaperones, chromatin remodelers, etc.
- Synergies with projects profiling transcription factors and histone modifications
- Nucleosome dynamics provides a “filter” for regulatory site prediction

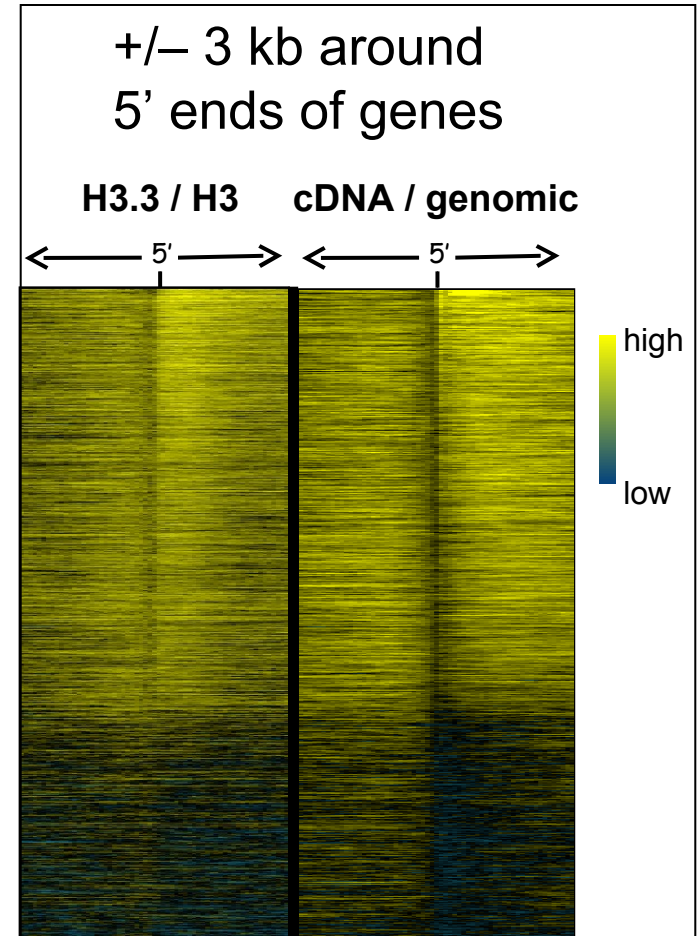
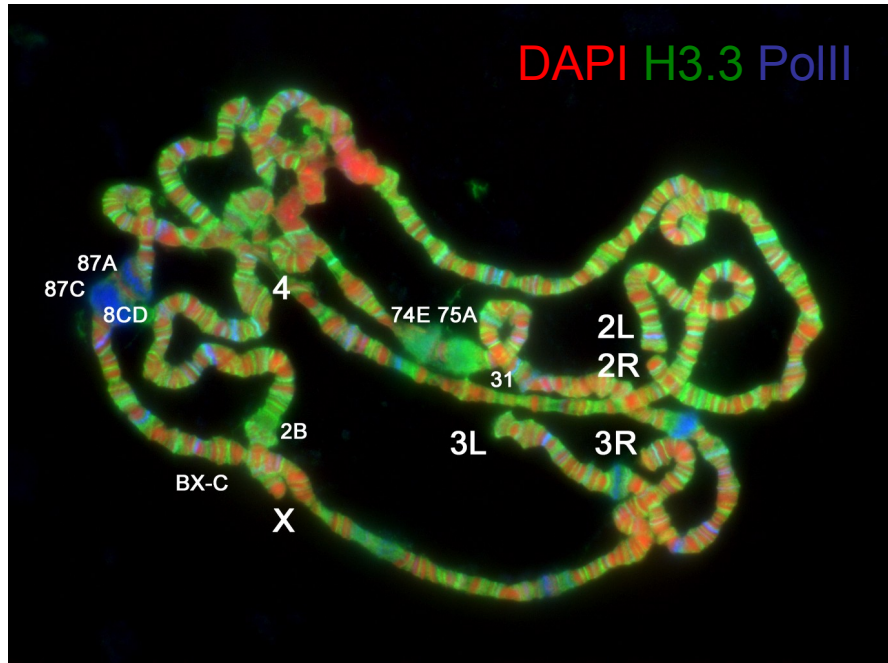
# Histone replacement and nucleosome occupancy profiles

An example of replicate profiles at 20-bp (average) tiling (50mers overlapping 30bp)

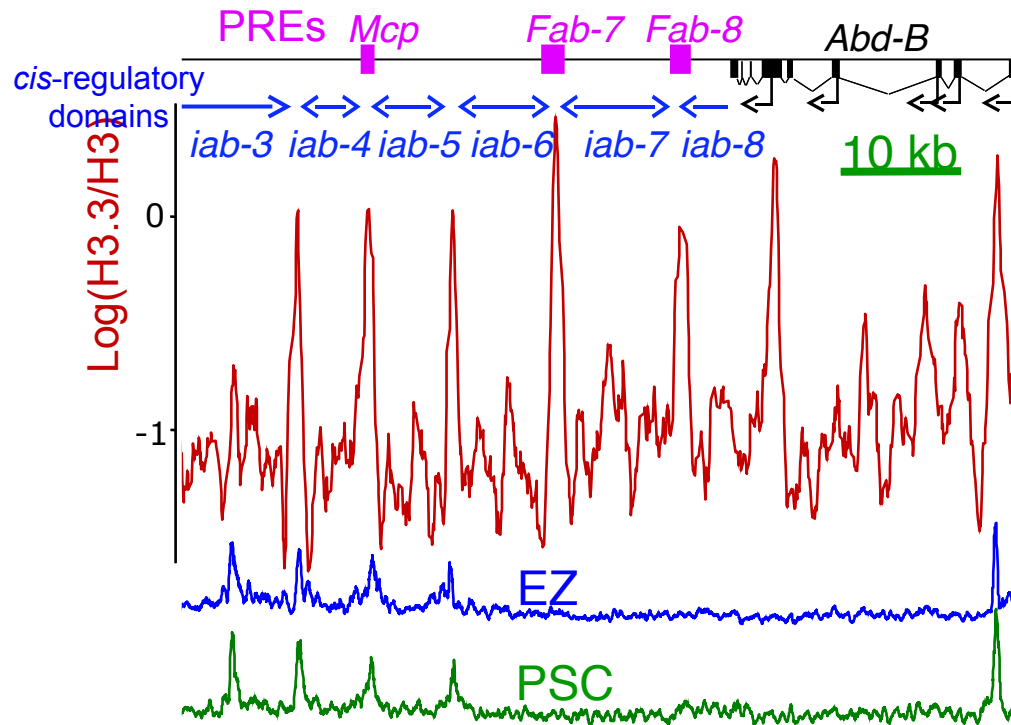




# H3.3 Patterns I: H3.3 enrichment scales with transcription

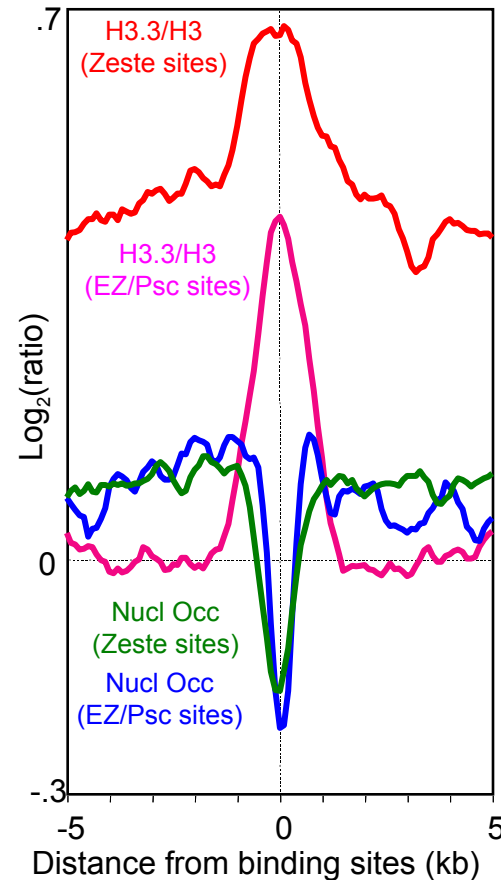


## H3.3 Patterns II: H3.3 marks elements in the *Bithorax* Complex

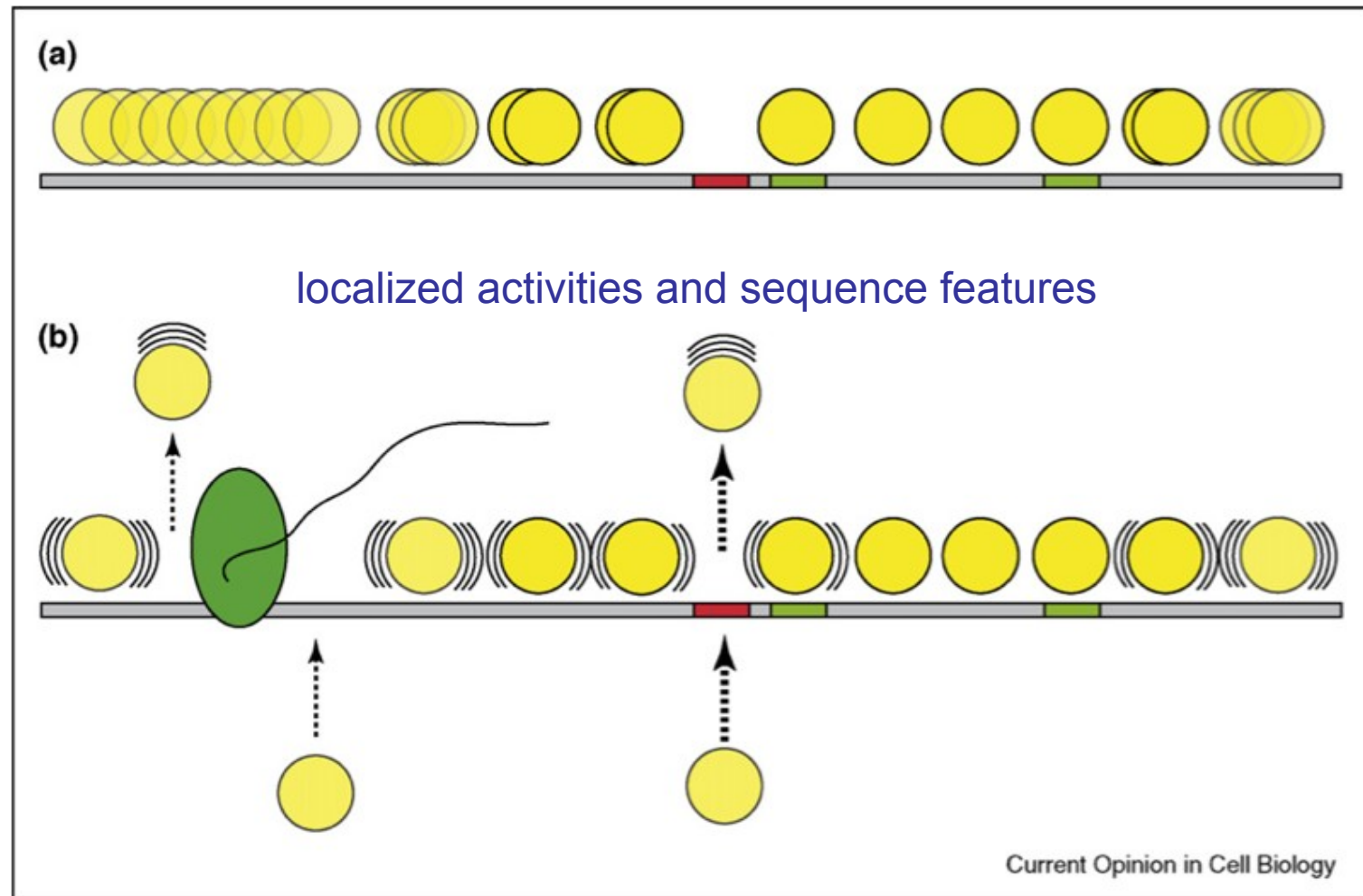


# H3.3 Patterns III: H3.3 and nucleosome occupancy are anti-correlated

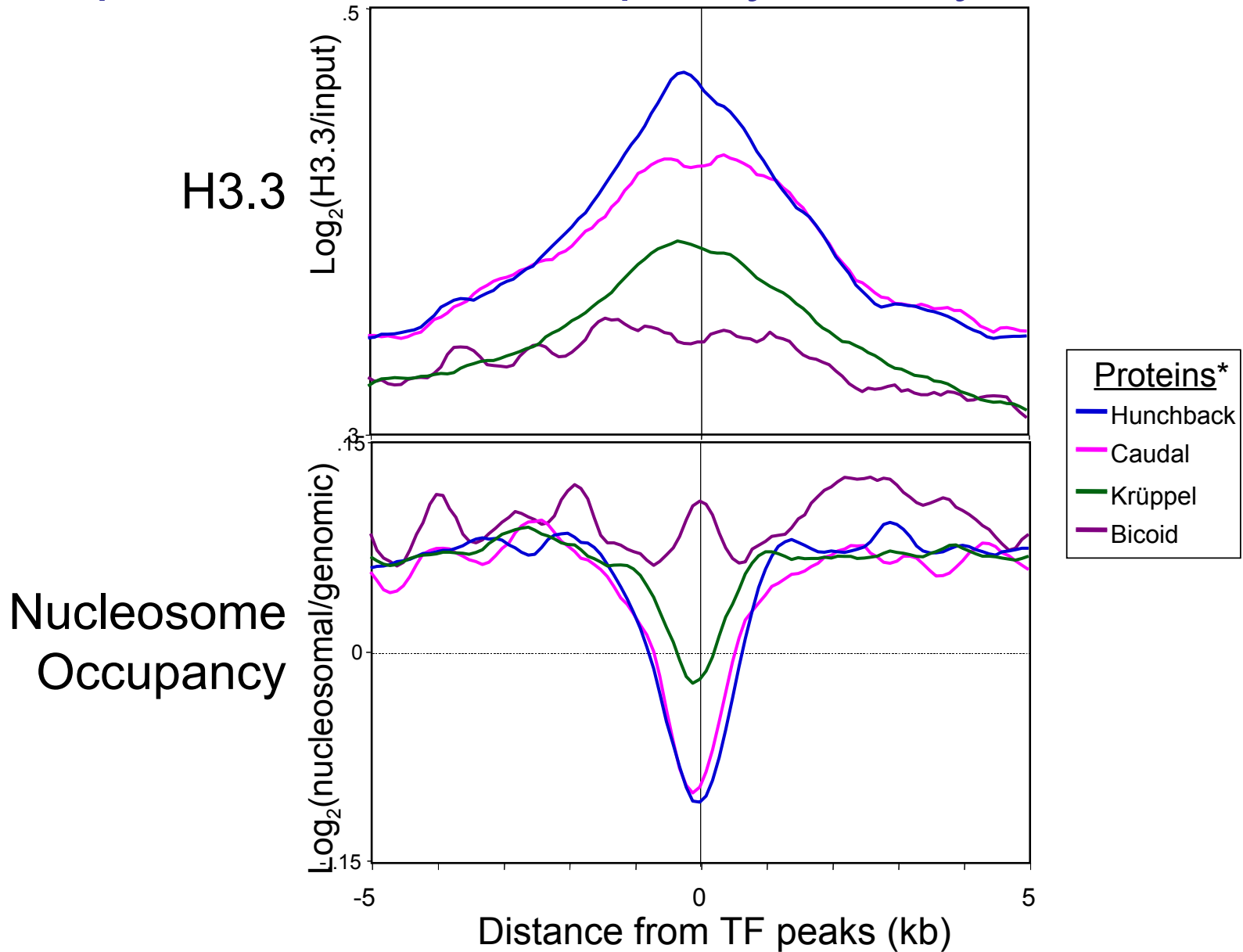
aligned at PcG- and trxG-protein binding sites



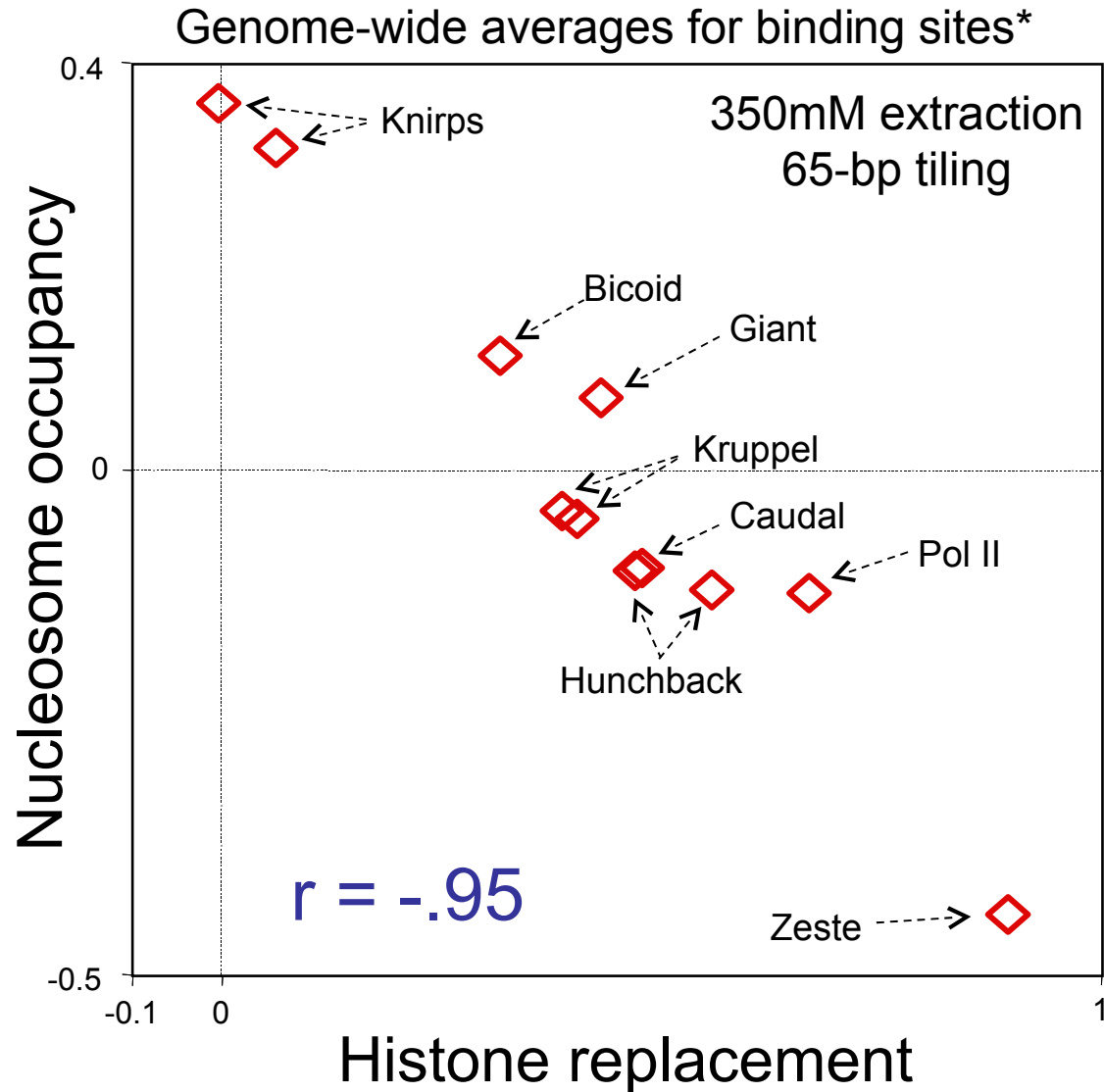
# H3.3 Patterns III: H3.3 and nucleosome occupancy are anti-correlated



# Replacement and occupancy at early TF binding sites

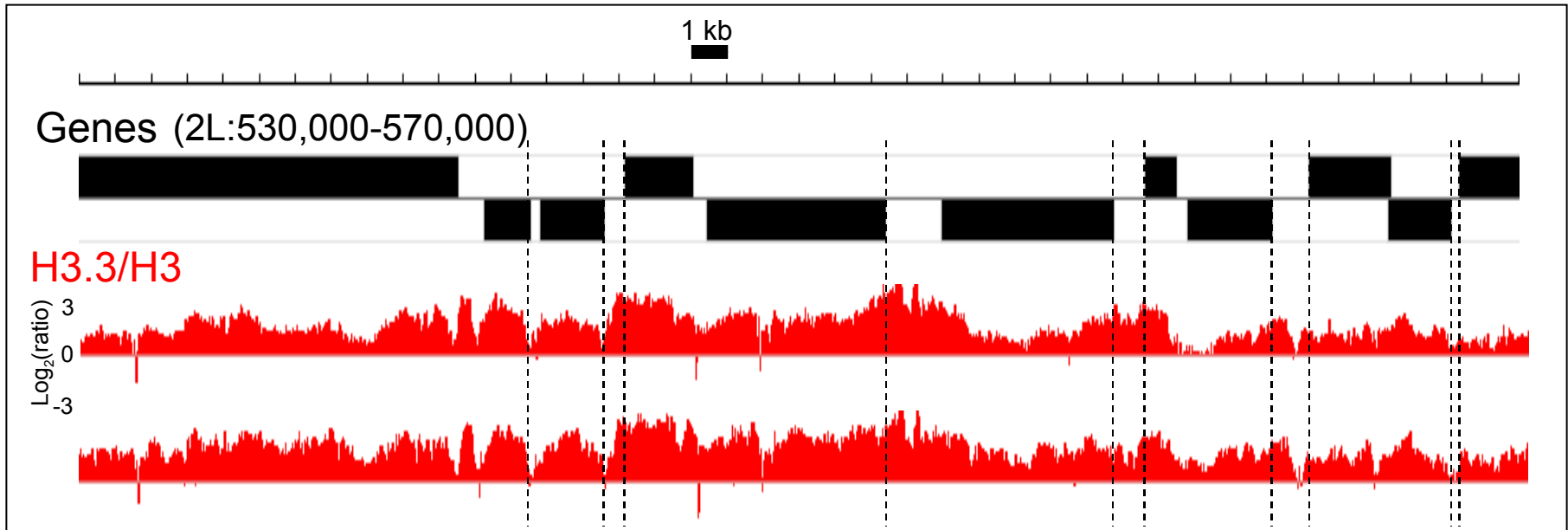
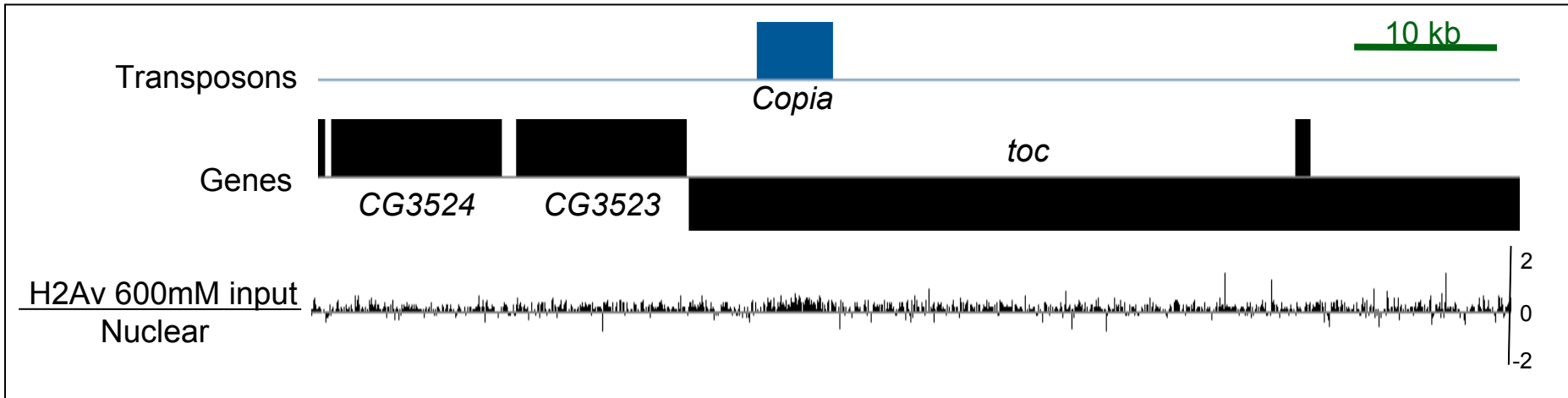


# Replacement anti-correlates with occupancy at regulatory sites



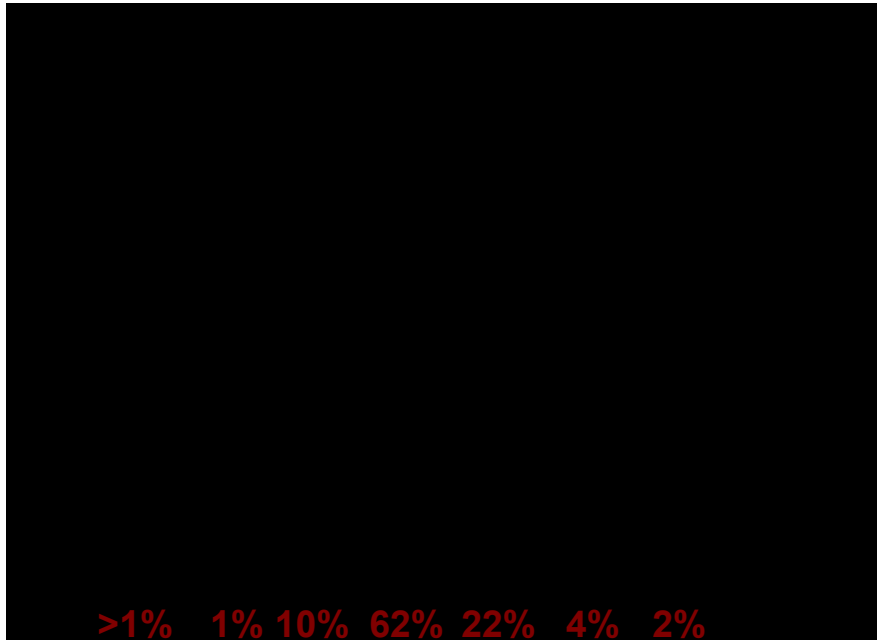
## Sub-dividing Histone variant landscapes I: Salt Fractionation

# Verifying genomic representation





# Salt fractionation of S2 cell chromatin



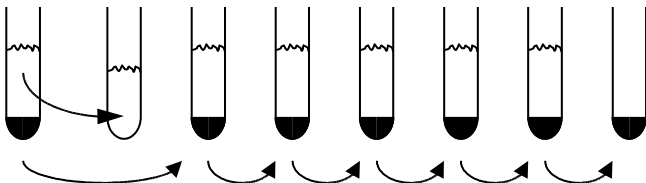
MNase nucle  
 MNase sup  
 15mM  
 150mM  
 350mM  
 600mM  
 600mM O/N  
 Pellet

>1% 1% 10% 62% 22% 4% 2%

Salt fractionation of Avian erythrocyte nuclei

Rocha, Davie, van Holde & Weintraub (1984)  
JBC 259:8558-8563

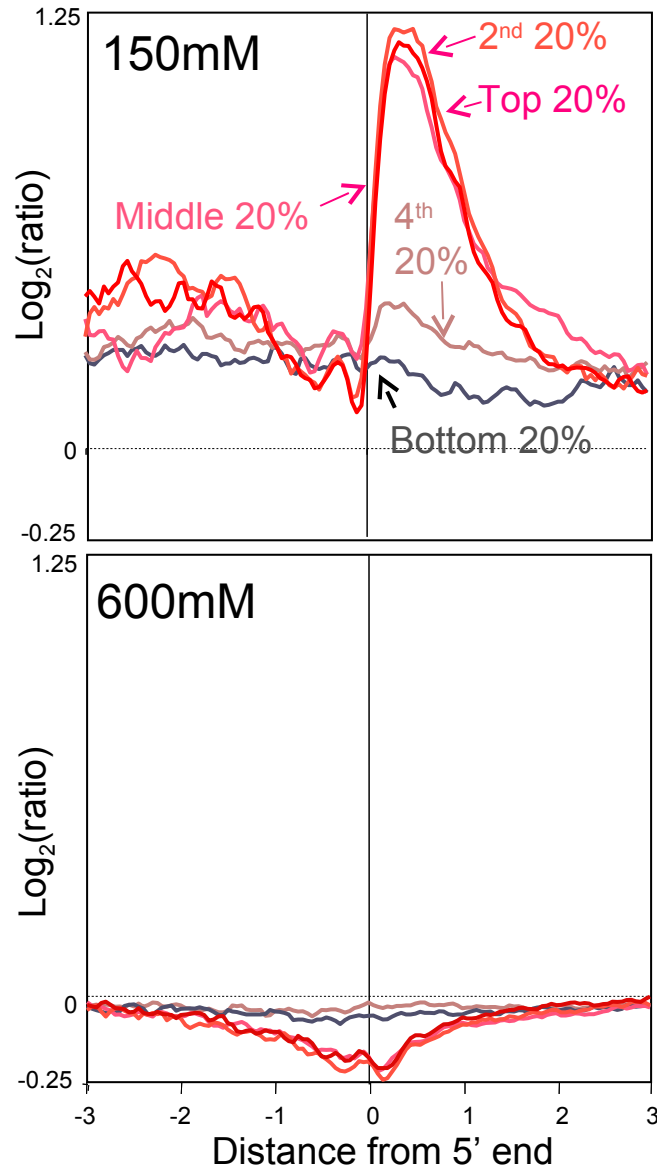
<u>Salt</u>	<u>Active</u>	<u>Inactive</u>	<u>Acetyl-H4</u>
50mM	++++	+	High
100mM	+++	++	
200mM	+++	+++	
400mM	+	++++	Low



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# Preferential extraction of active chromatin recovers H3.3-containing nucleosomes

b-H3.3 / input



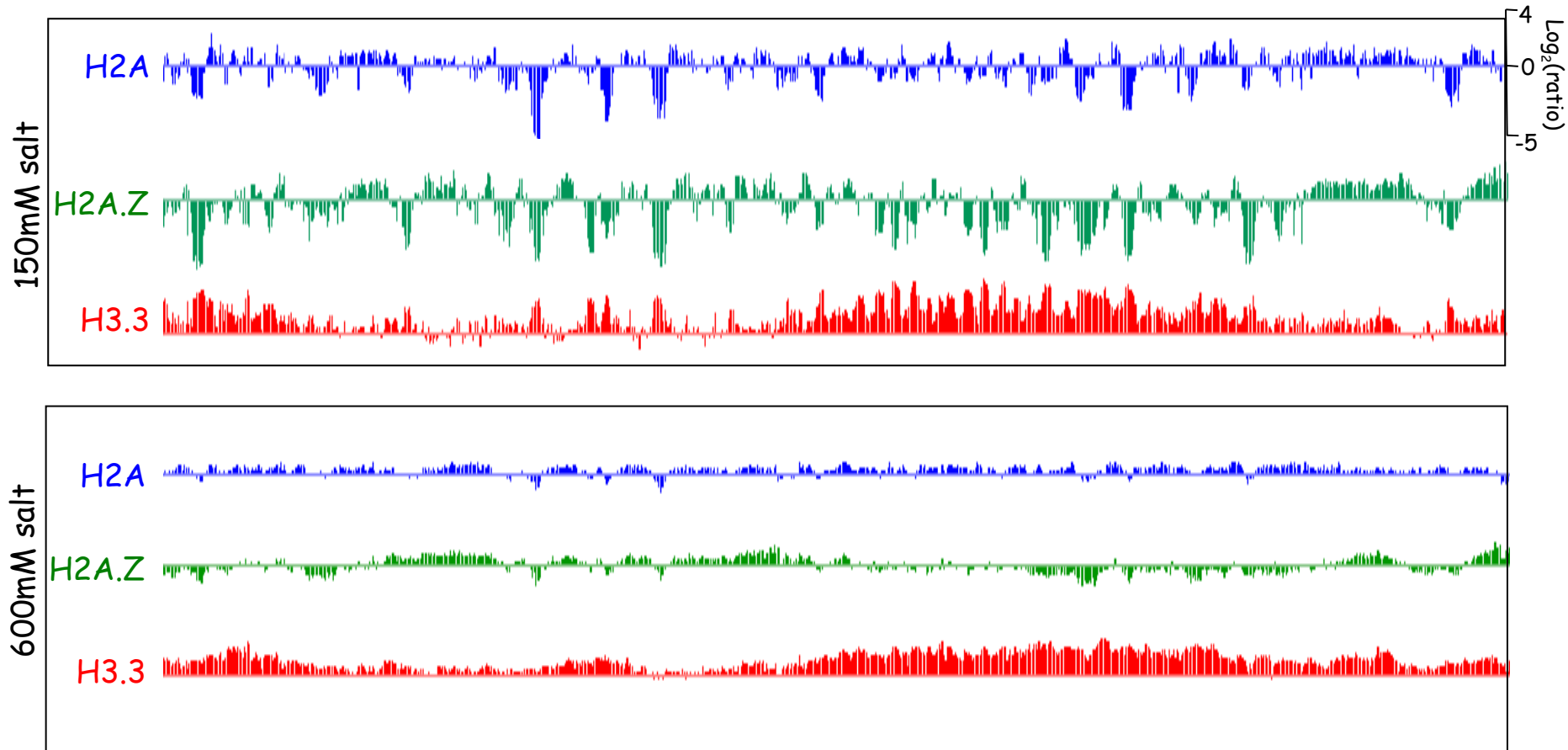
- biotin-H3.3 pulldown
- 9300 fly genes
- 2.1 million  $\geq 50$ mers
- isothermal  $\sim 65$ -bp
- whole genome tiling

# Histone variant profiles from low- and high-salt extracted nucleosomes

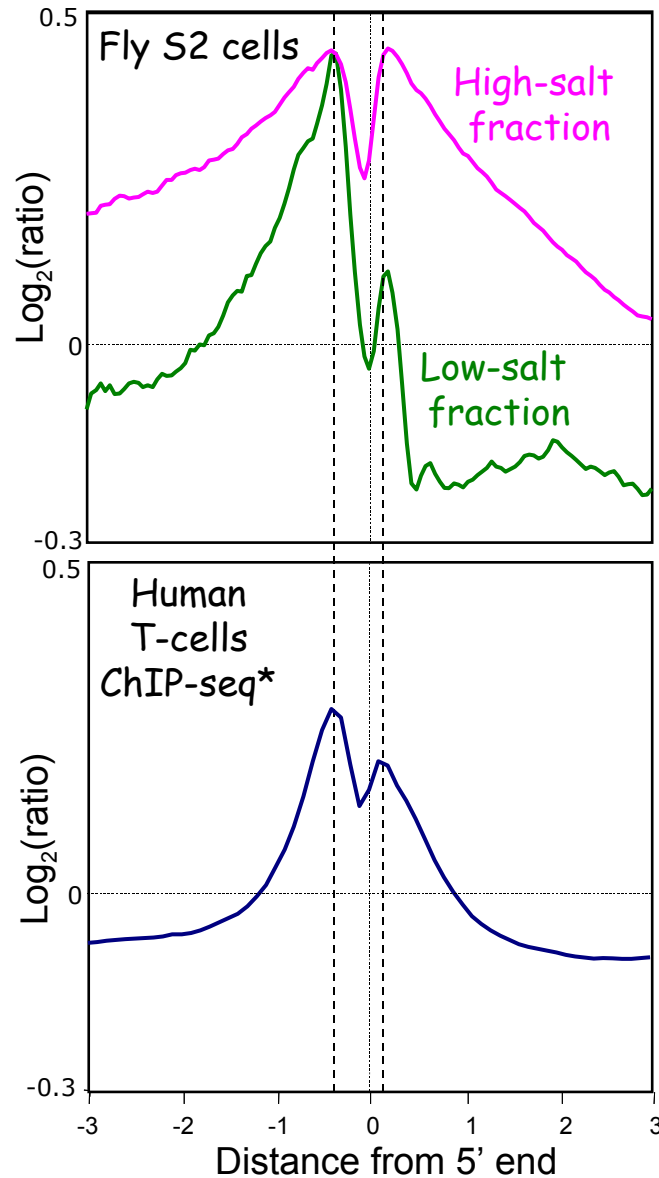
Chr 3R:6179000-6233000

5 kb

Genes



## Similar profiles for fly and human H2A.Z

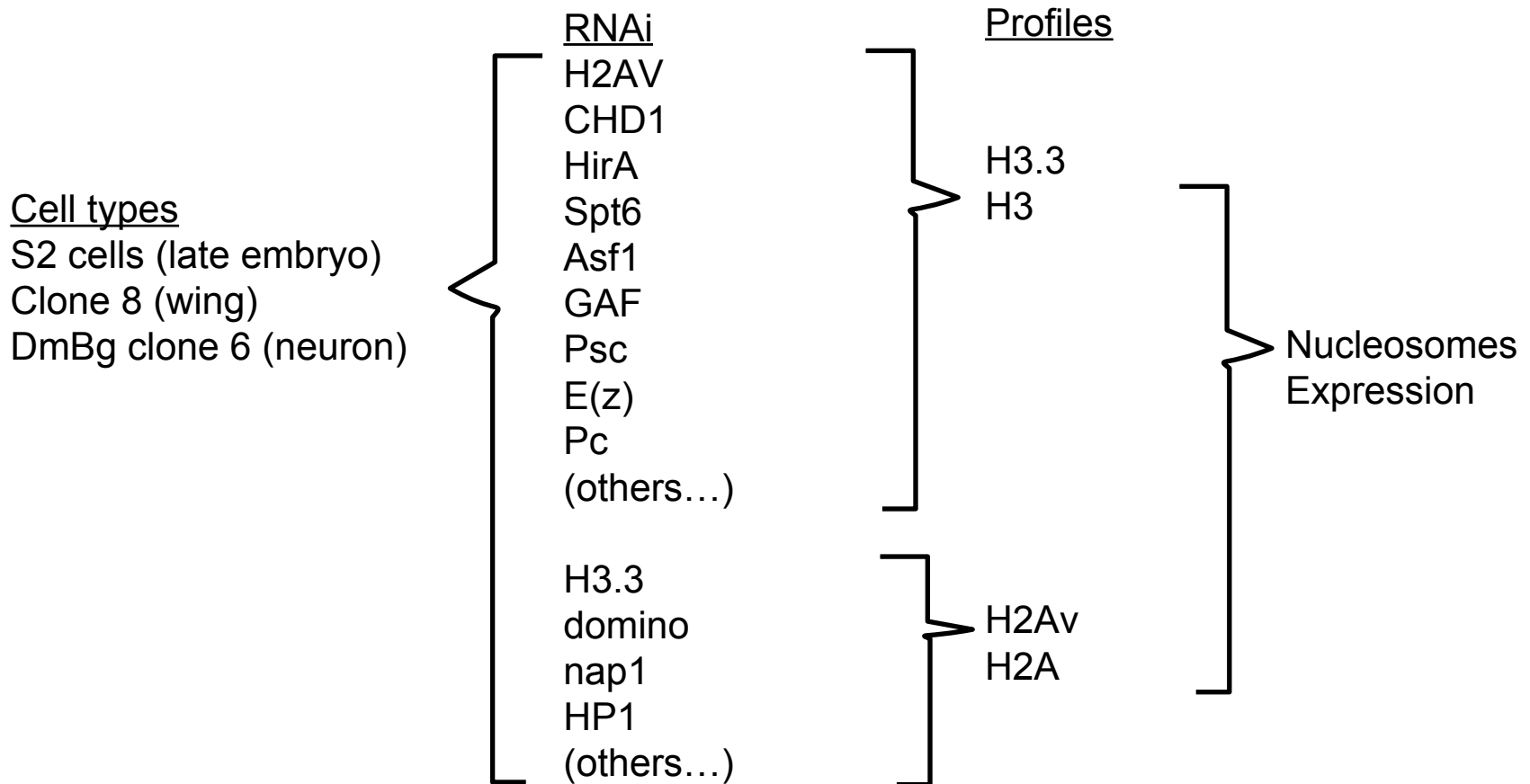


\*Rescaled from Barski, et al. Cell (2007) 129:823

# Sub-dividing Histone variant landscapes II: cell types

## III: assembly systems

*D. melanogaster*



Distinguish sequence, transcription, activities...