Widespread transcription at thousands of enhancers during activity-dependent gene expression in neurons

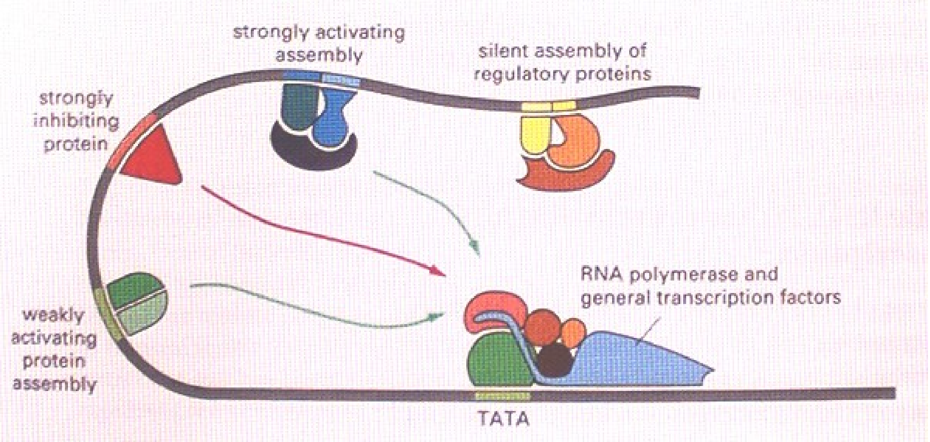
Martin Hemberg
Kreiman Lab
CBS, September 29 2010

Outline

- Background
  - Gene regulation and histones
  - Activity dependent gene expression
- Characterizing and locating enhancers
  - Transcription at enhancers

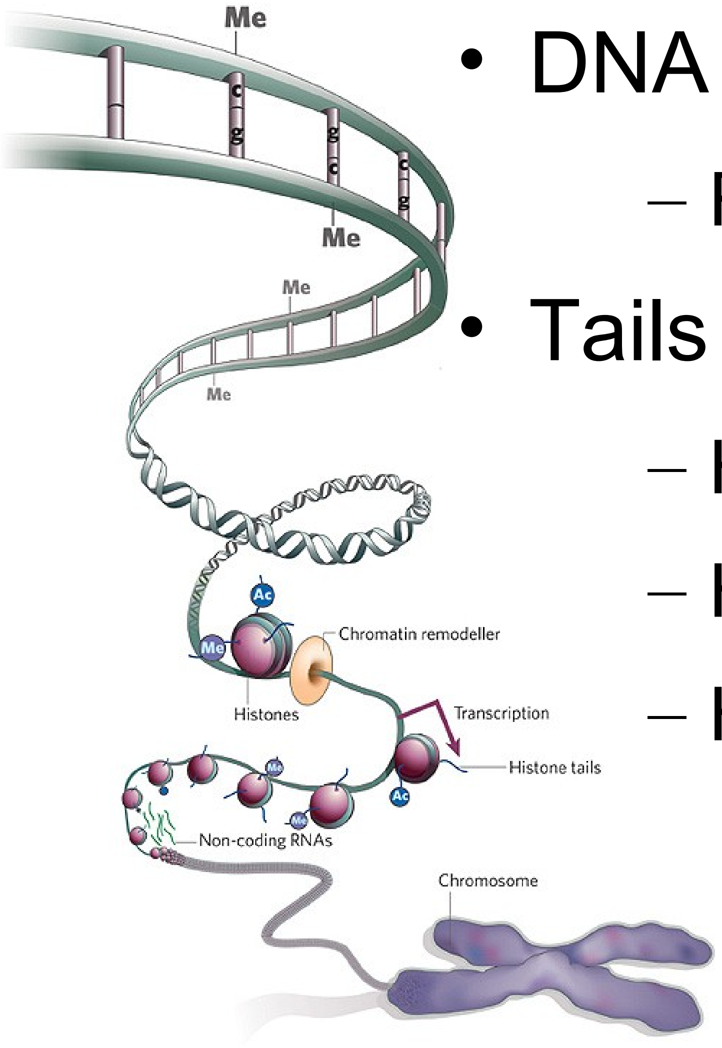
Gene regulation and transcription factors

- Transcription factors (TFs) proteins bind DNA
  - Presence required to turn a gene on or off
  - Predicting binding *very* hard
  - Impact of binding not well understood
  - Bind at specific loci
    - Promoters (proximal)
    - Enhancers (distal)



(Alberts et al, 4th ed)

Histones and their modifications

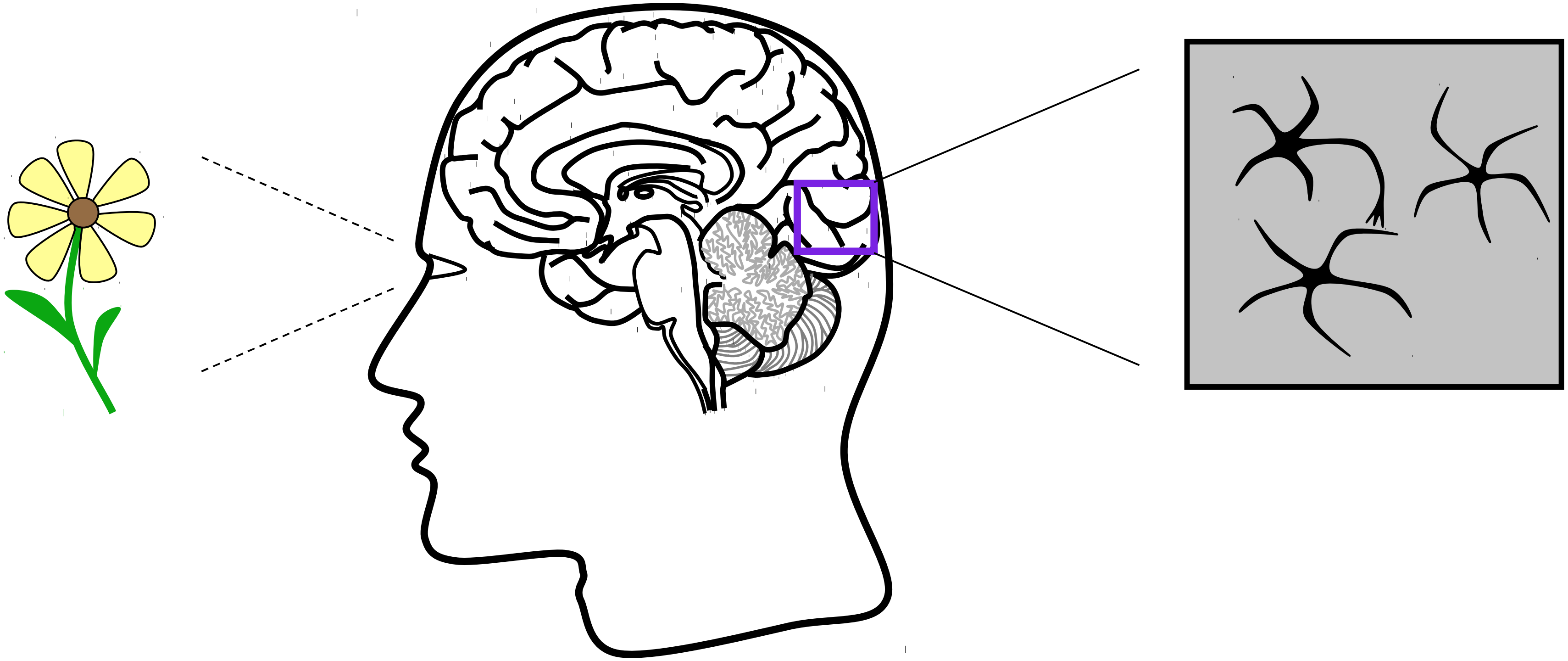


(ENCODE, 2007)

- DNA wrapped around histones
  - Restrict access for TFs
- Tails can be modified (>100)
  - H3K4me1 – open chromatin
  - H3K4me3 – active promoters
  - H3K27me3 – inactive promoters

Activity dependent gene expression

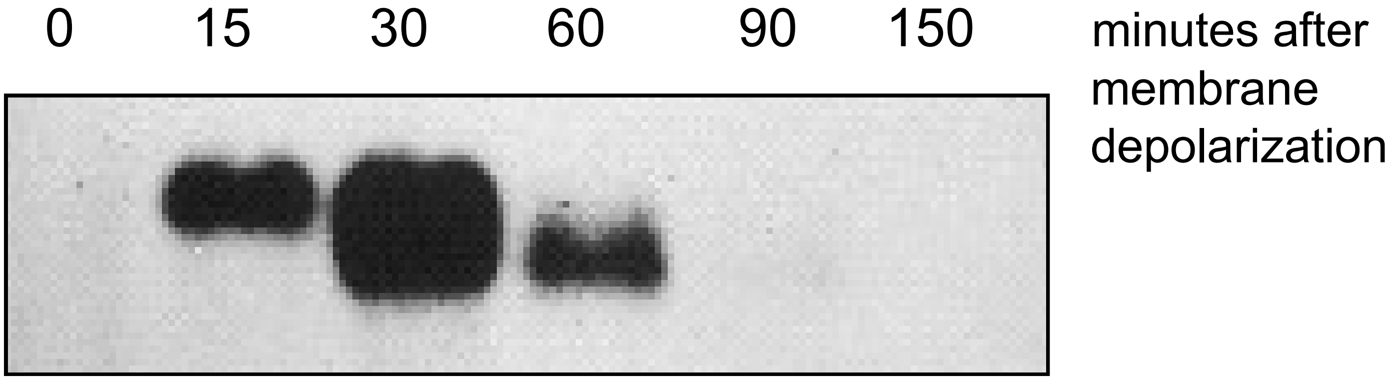
- Sensory experience shapes wiring in the brain
  - Synapses and patterns of neuronal activity changed



Hubel & Wiesel, 1970's

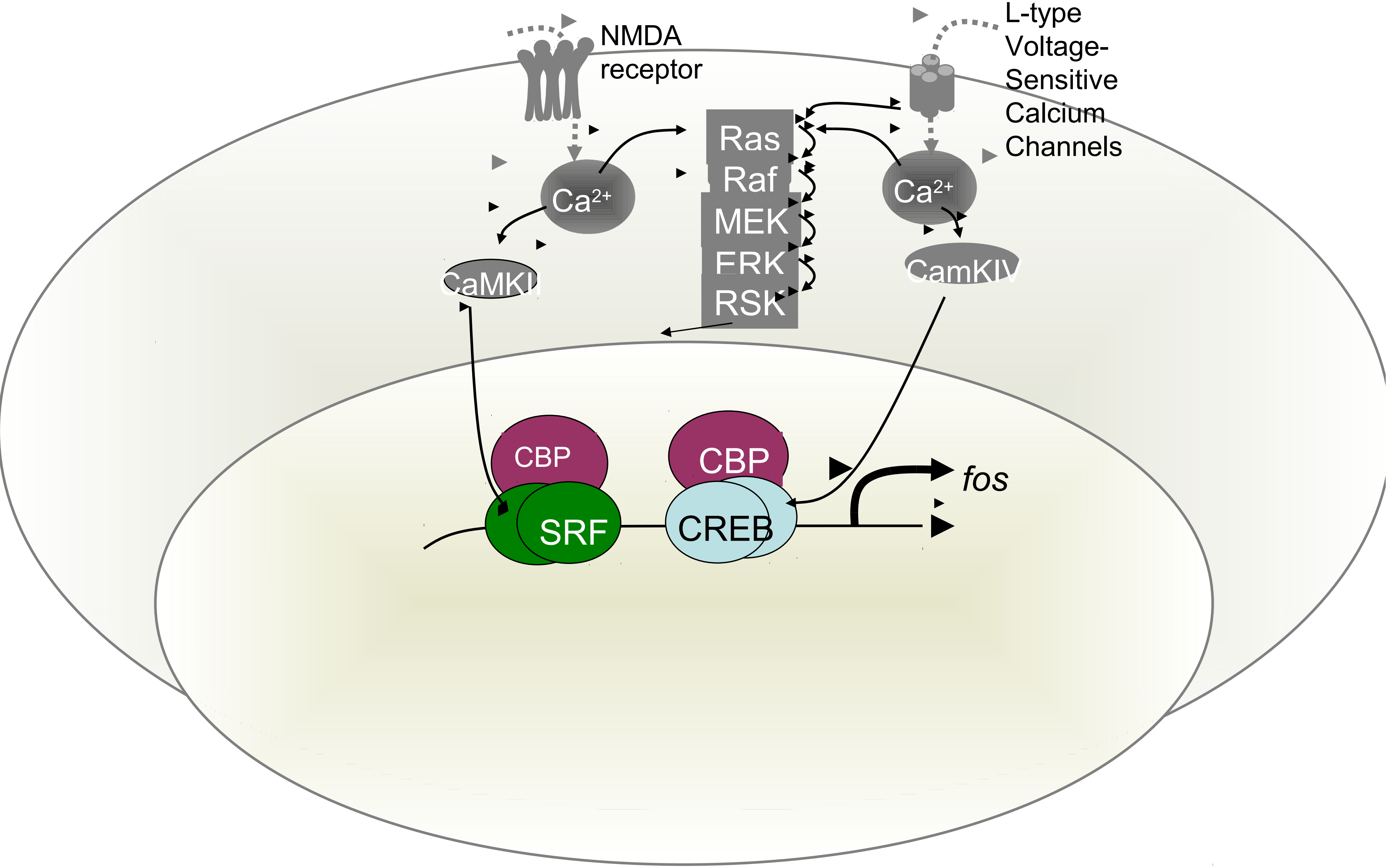
Activity dependent gene expression

- Sensory experience shapes wiring in the brain
  - Synapses and patterns of neuronal activity changed
  - Changes in gene expression in hundreds of genes

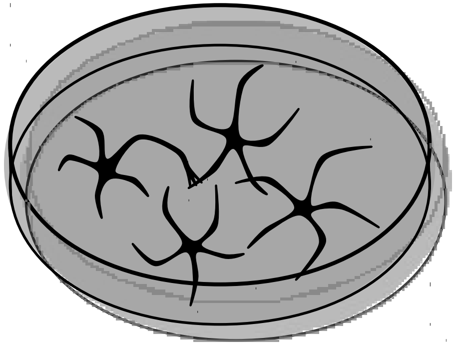


Greenberg et al, 1985

Regulation of c-fos transcription



An experimental system for genome-wide study of activity dependent gene expression



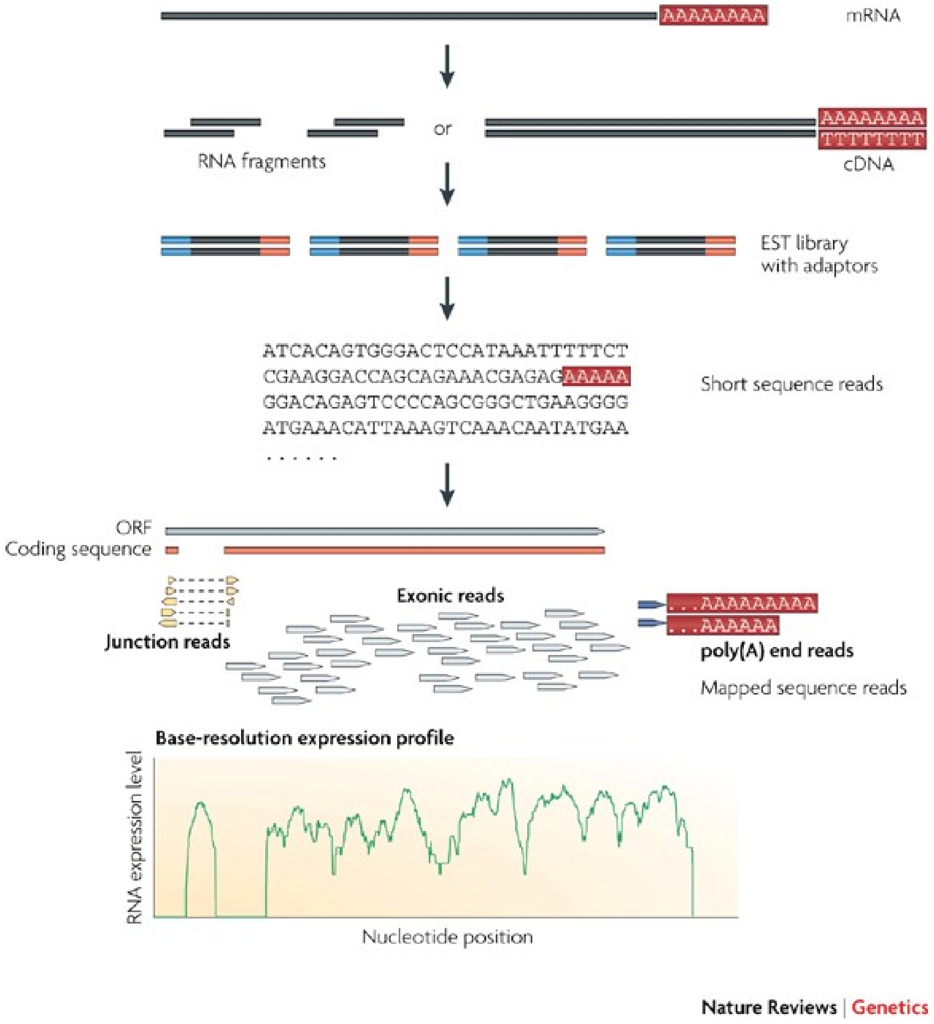
mouse cortical neurons

neuronal activation via KCl depolarization

- KCl	+ KCl
ChIP-Seq RNA-Seq	ChIP-Seq RNA-Seq

RNA-Seq

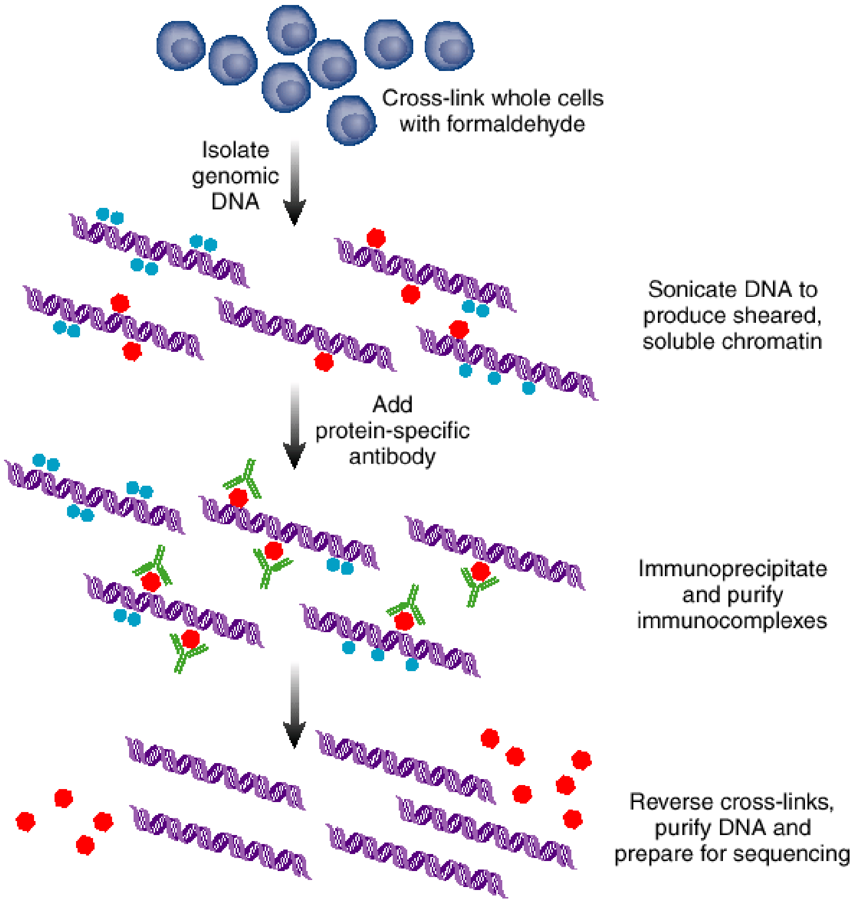
- Unbiased sampling of transcriptome
  - RNA → cDNA
  - Fragmented
  - 35 bp reads mapped to genome
    - 0h, 1h, 6h after KCl
    - ~40M reads
    - Total RNA and polyA+



(Wang et al, 2009)

ChIP-Seq

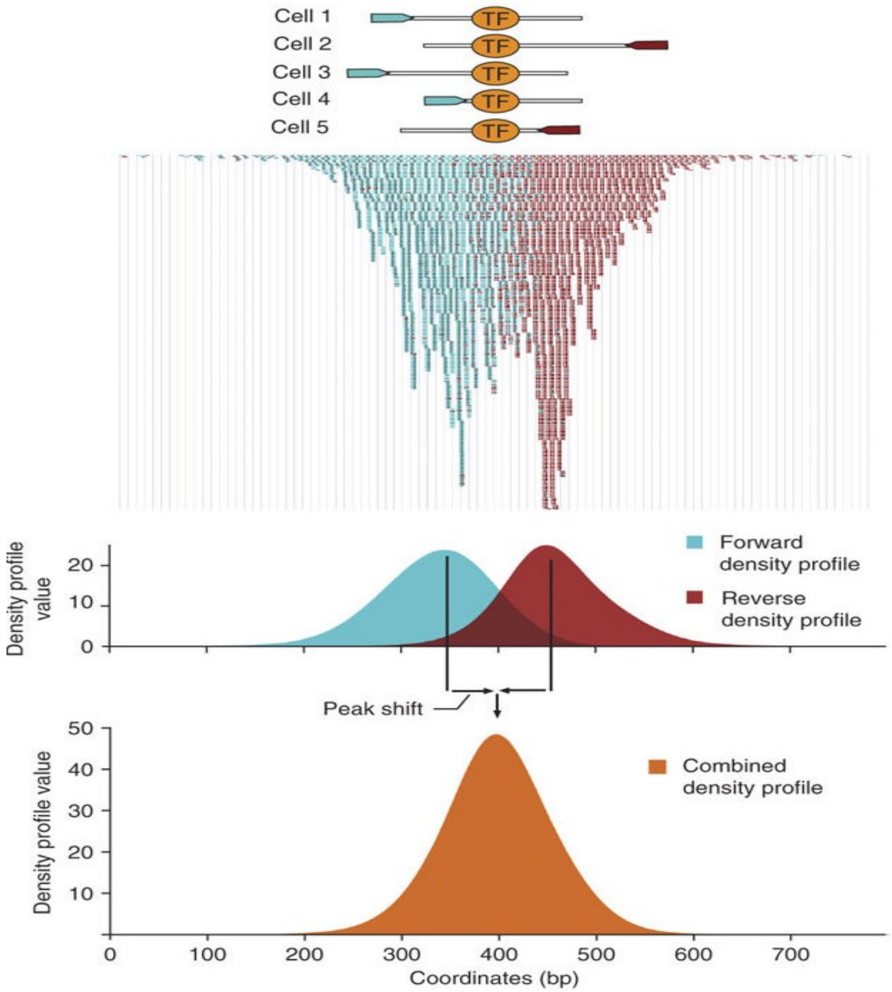
- Find where TFs bind *in vivo*
  - Chromatin immunoprecipitation (ChIP)
    - Cross-link TF
    - Fragment DNA
    - Extract with antibody
    - Reverse crosslink
    - Sequence fragments
      - 0h, 2h after KCl
      - ~10M reads
      - CREB, SRF, CBP, RNAPII
      - H3K4me3, H3K4me1
      - Input



(Mardis, 2007)

Identifying transcription factor binding sites

- Reads correspond to part of fragment
  - Infer length & extend
- Always subtract input
  - No specific antibody
  - Remove spurious peaks



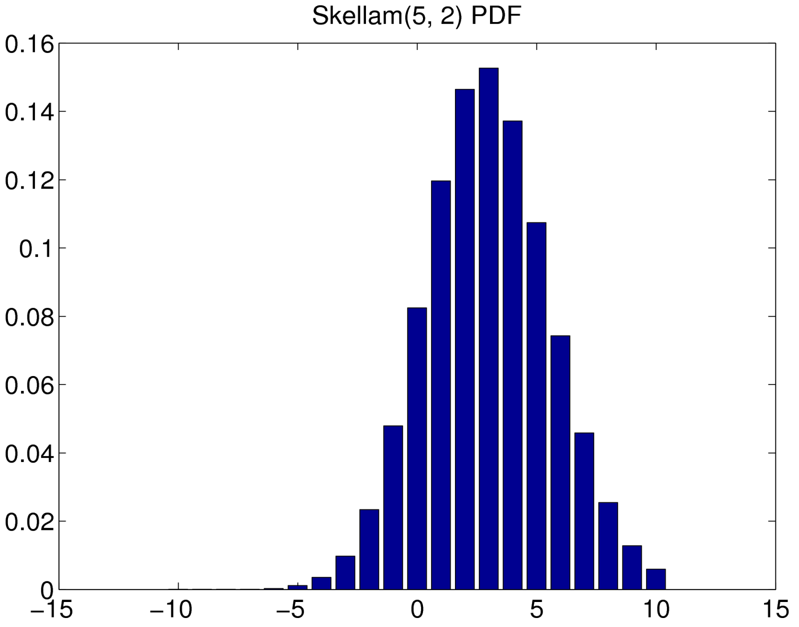
Selecting significant peaks

- Z_i = #ChIP reads - #input reads in window *i*
  - Difference between two Poisson random variables
  - Z_i ~ Skellam(z, k_1, k_2)

$$p(x) = e^{-(\lambda_1+\lambda_2)} (\lambda_1/\lambda_2)^{x/2} I_x(2\sqrt{\lambda_1\lambda_2})$$

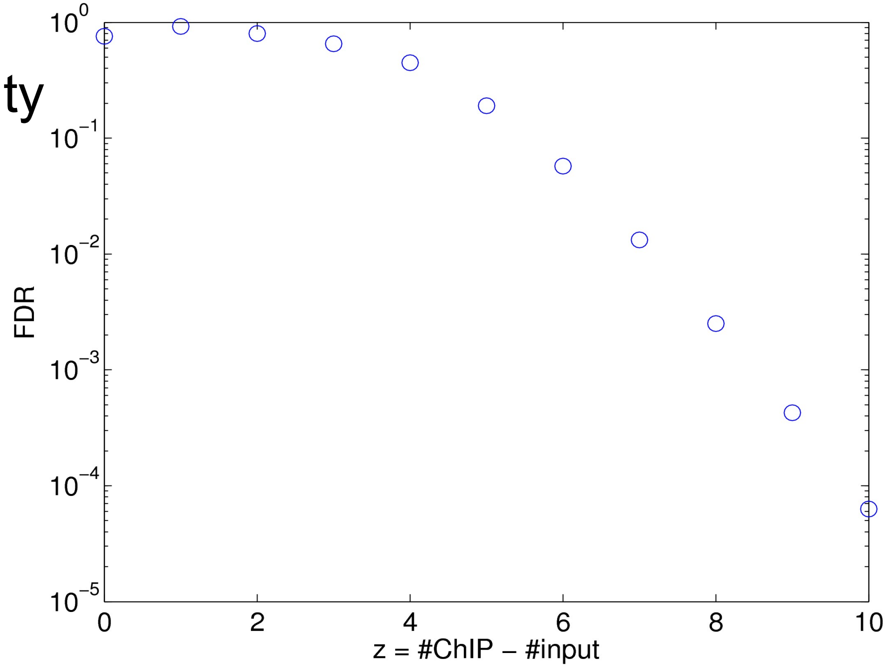
mean = k_1-k_2

variance = k_1+k_2

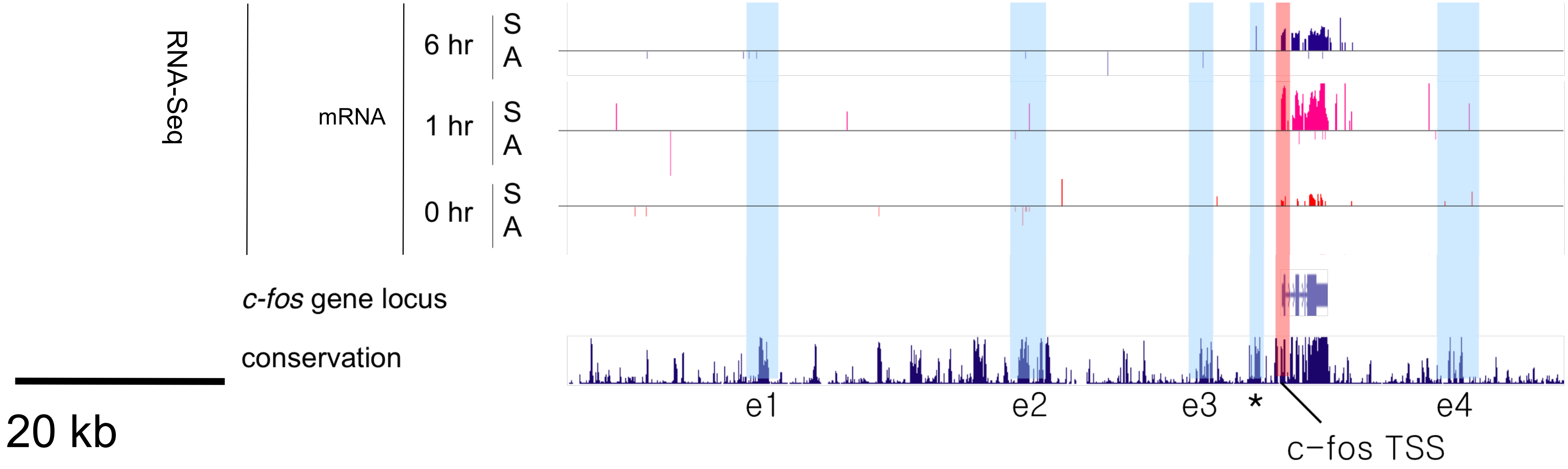


Selecting significant peaks

- Millions of windows need to be tested
  - Use False Detection Ratio (FDR, q)
    - Expected #false positives = q x #peaks
    - FDR(z) = Pr{null | $Z >= z$} = $[1-F_0(z)]/[1 - F(z)]$
      - $F(z)$ empirical CDF
      - $F_0(z)$ CDF null density



Induction and TF binding at the *fos* locus



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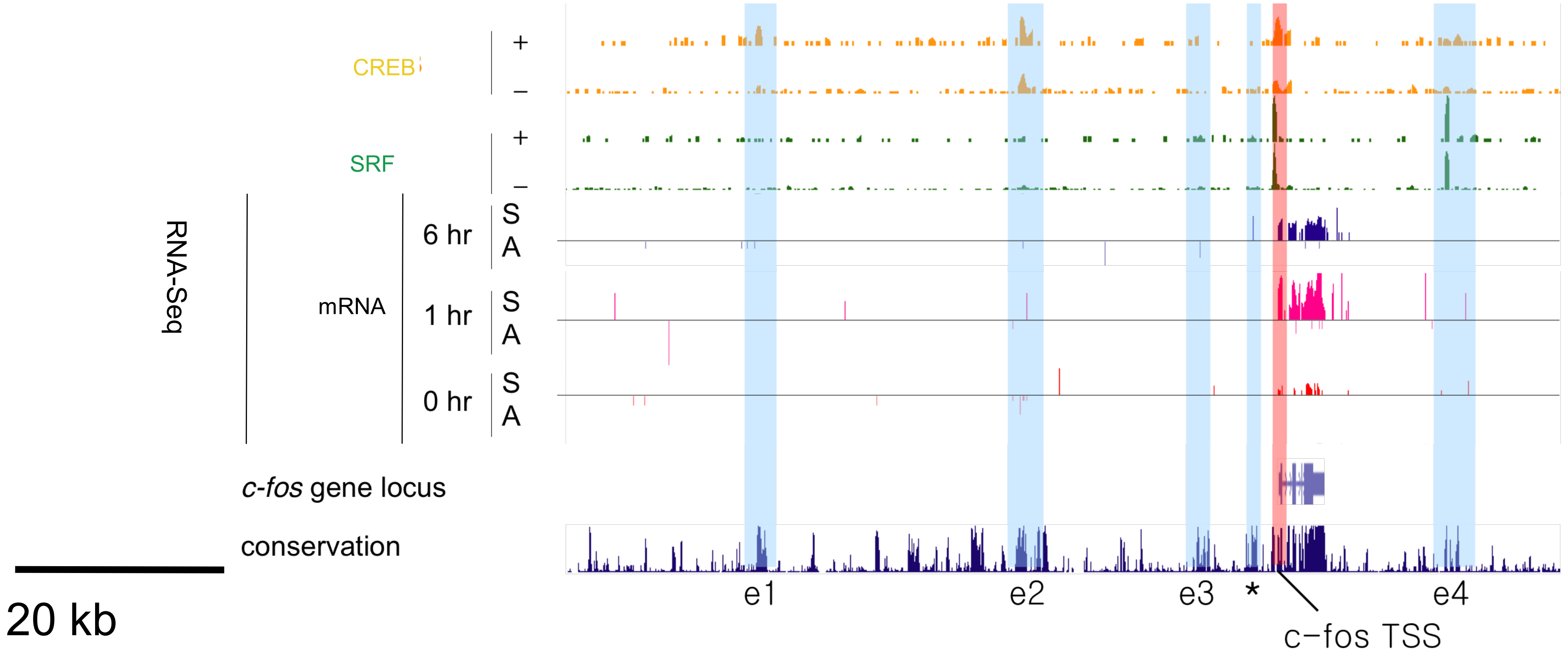

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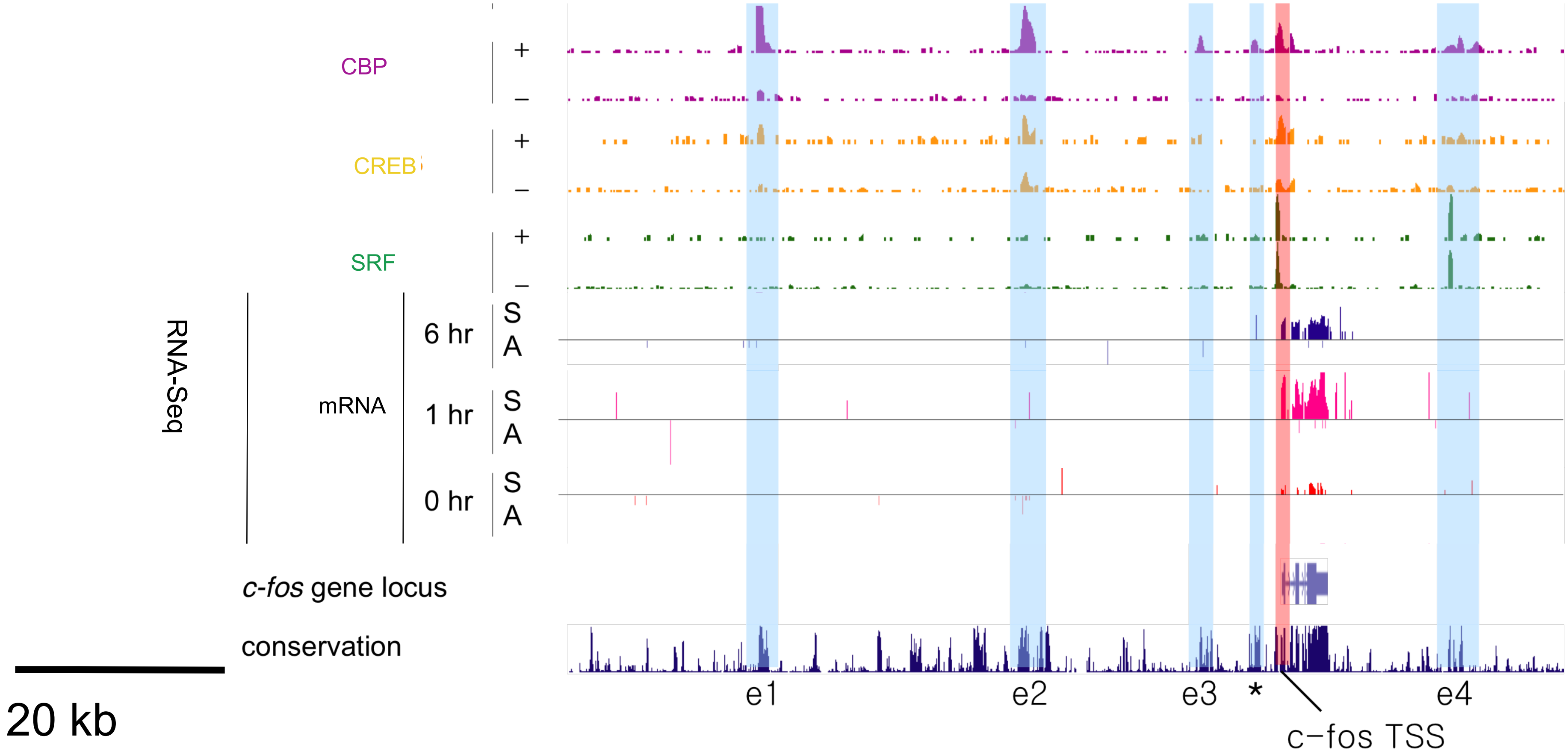
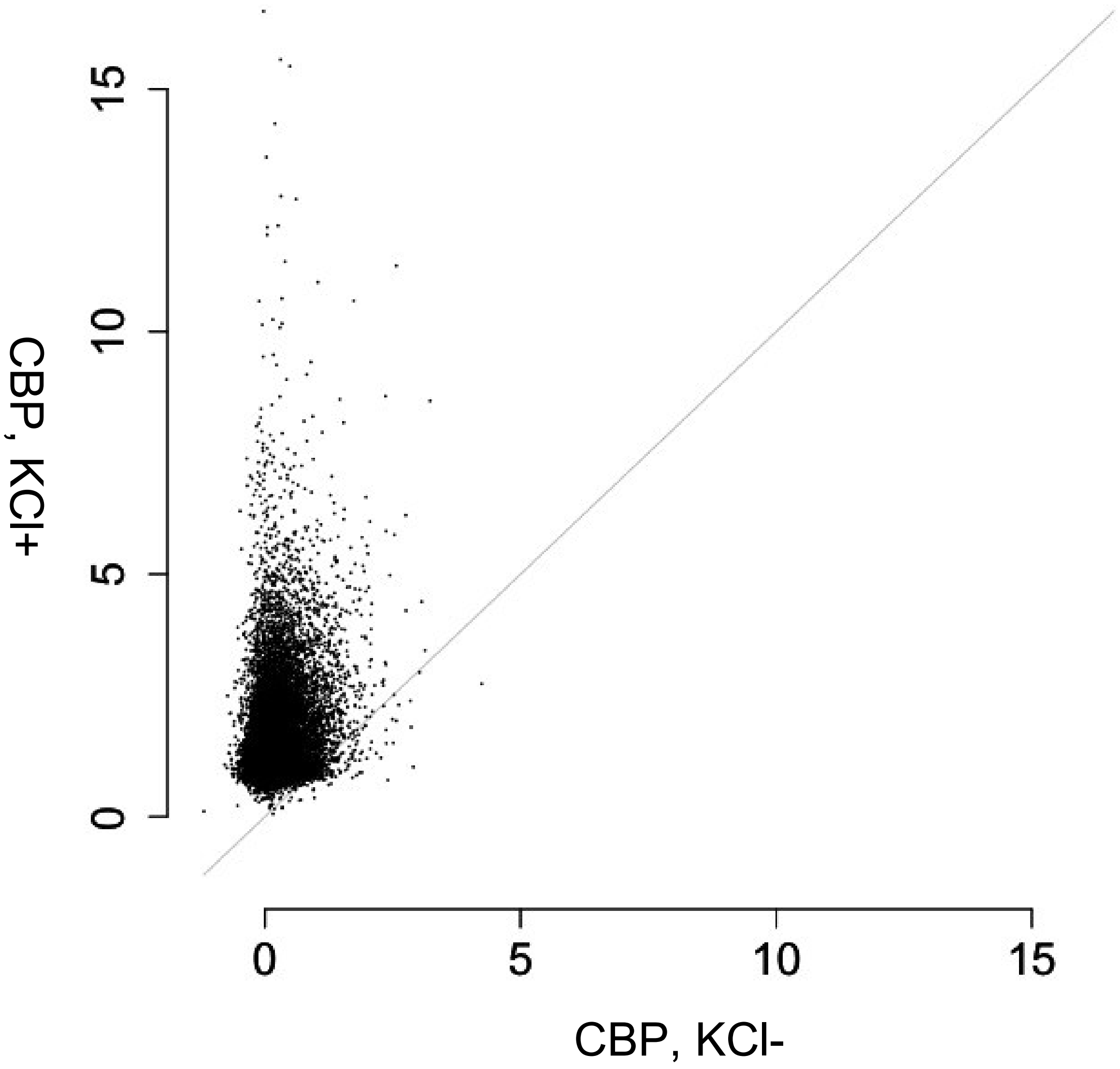
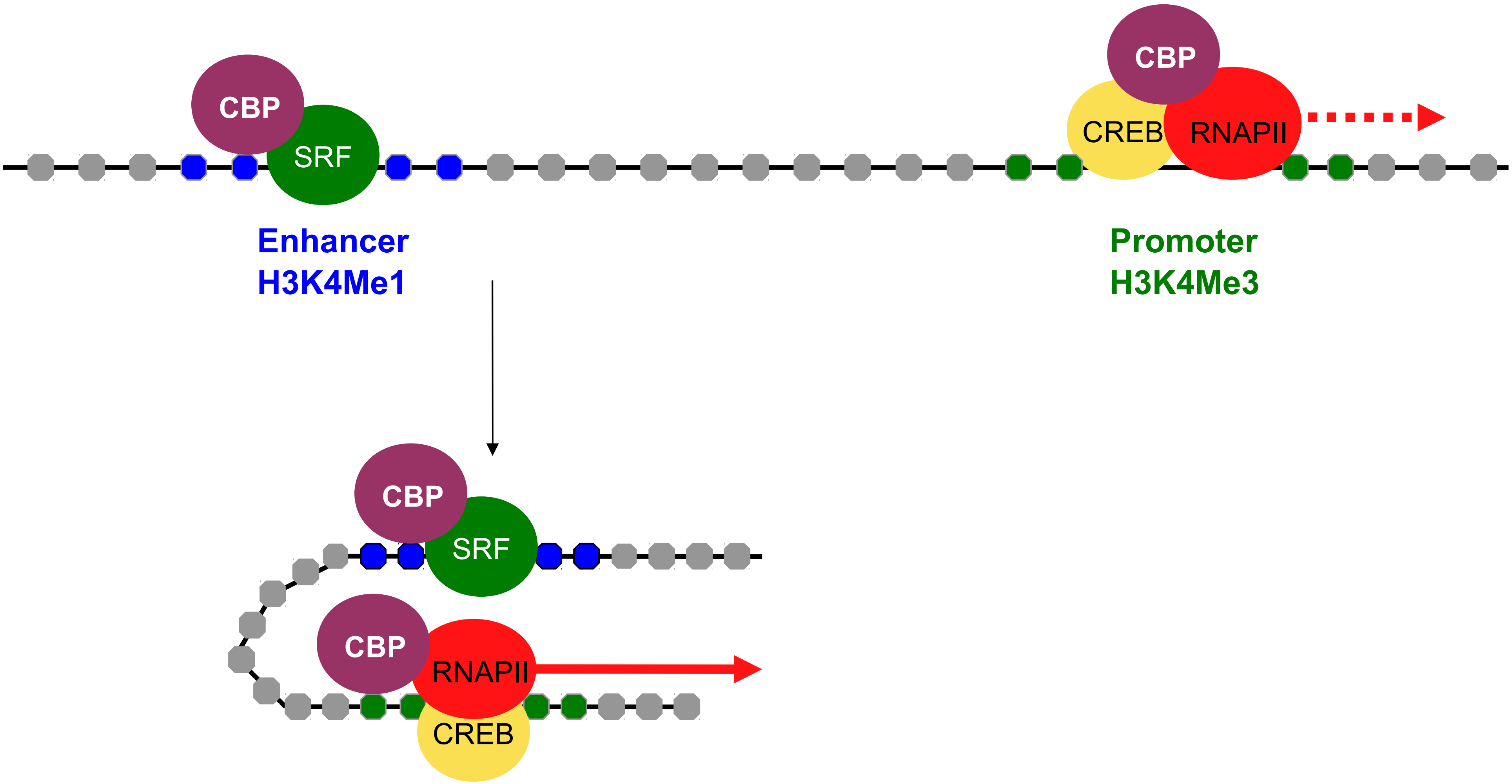


CBP binds in an activity regulated manner to ~28,000 sites throughout the genome



Where does CBP bind?



~25,000?

~3,000

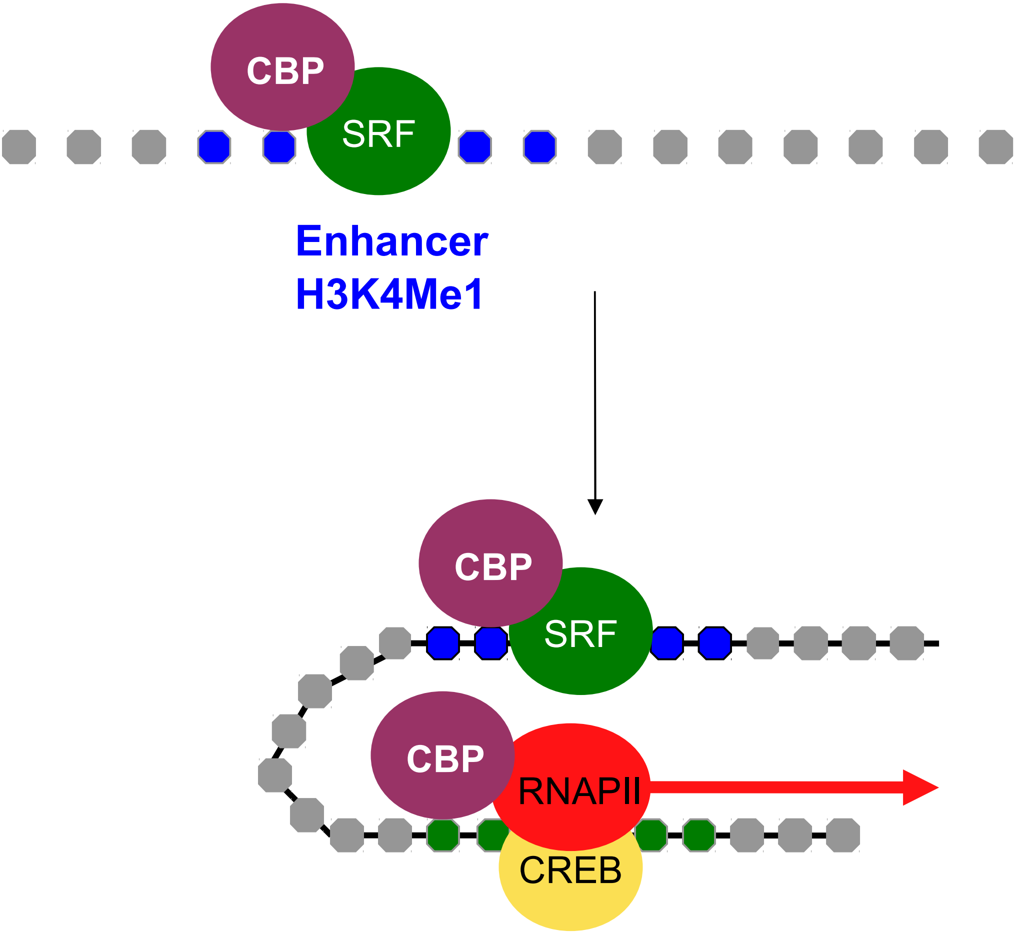
Enhancer
H3K4Me1

Promoter
H3K4Me3

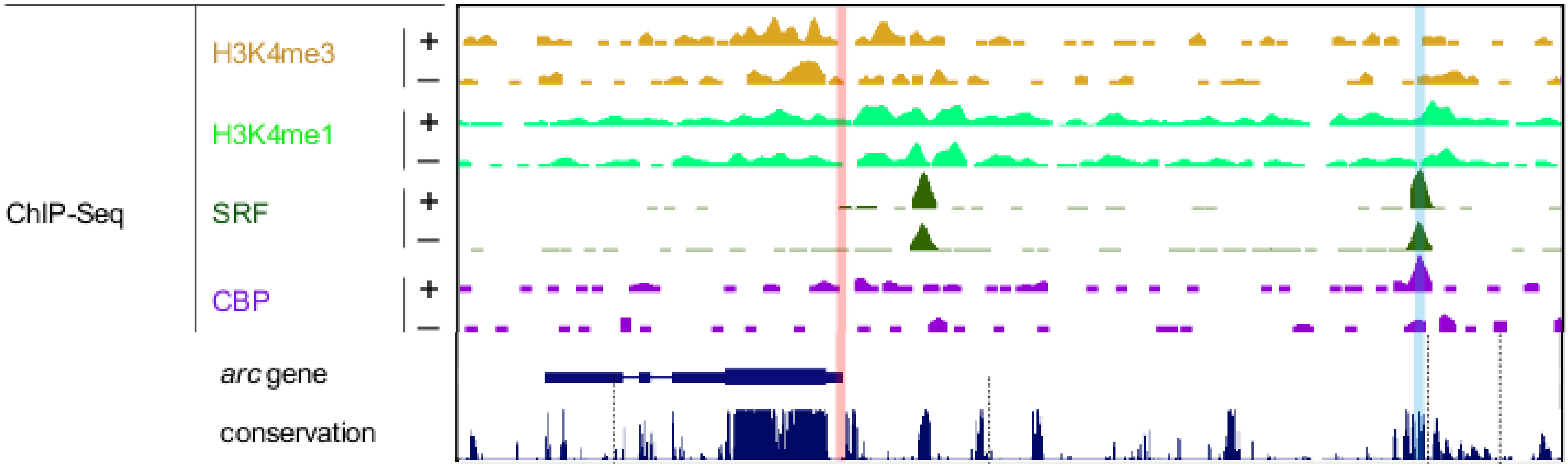
ENCODE, 2007
Heintzman et al, 2007
Roh et al, 2005
Visel et al, 2009

What is an enhancer?

- Enhancers are distal regions where TFs bind
- Various mechanisms for interaction with promoters suggested
- Marked by high levels of H3K4me1



Most CBP peaks have high levels of H3K4me1 but not H3K4me3



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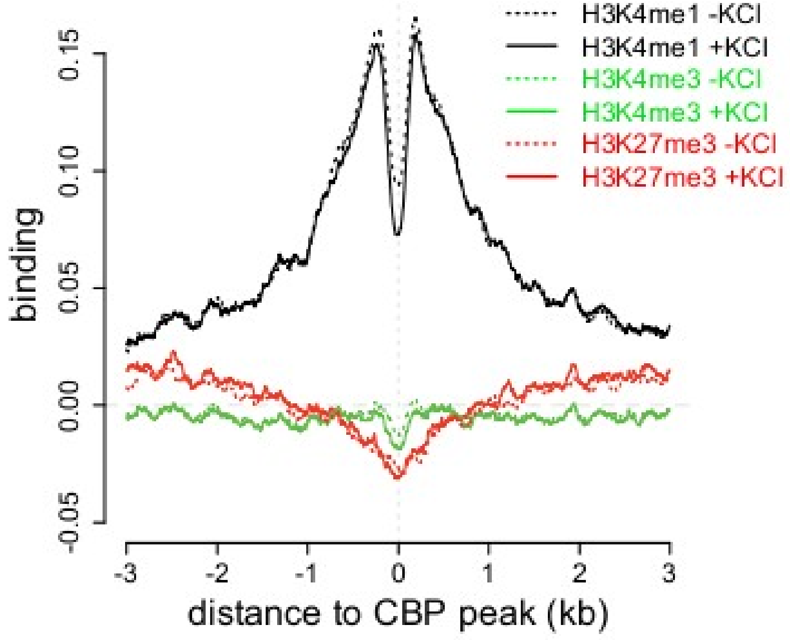
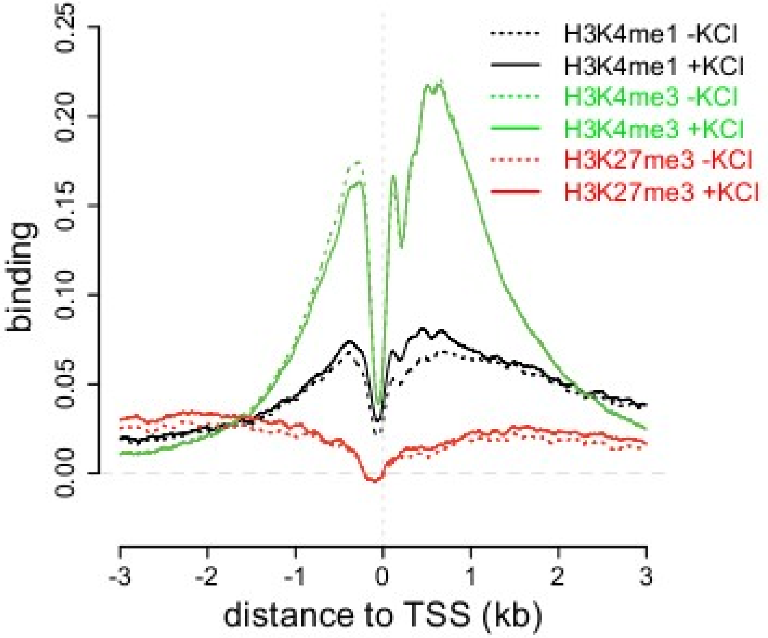
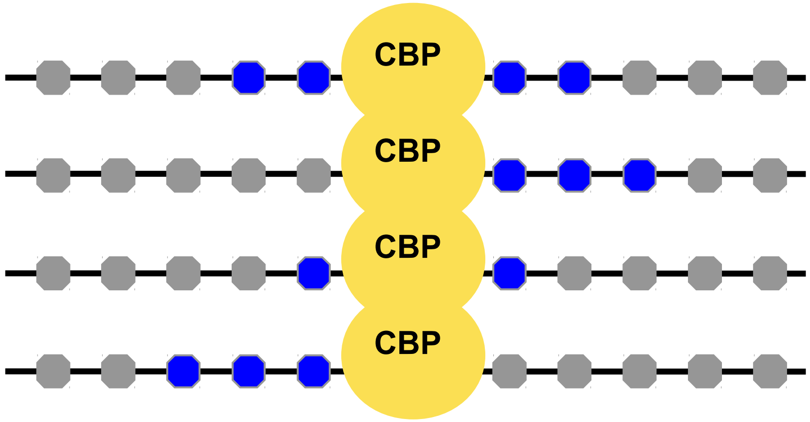
Enhancers



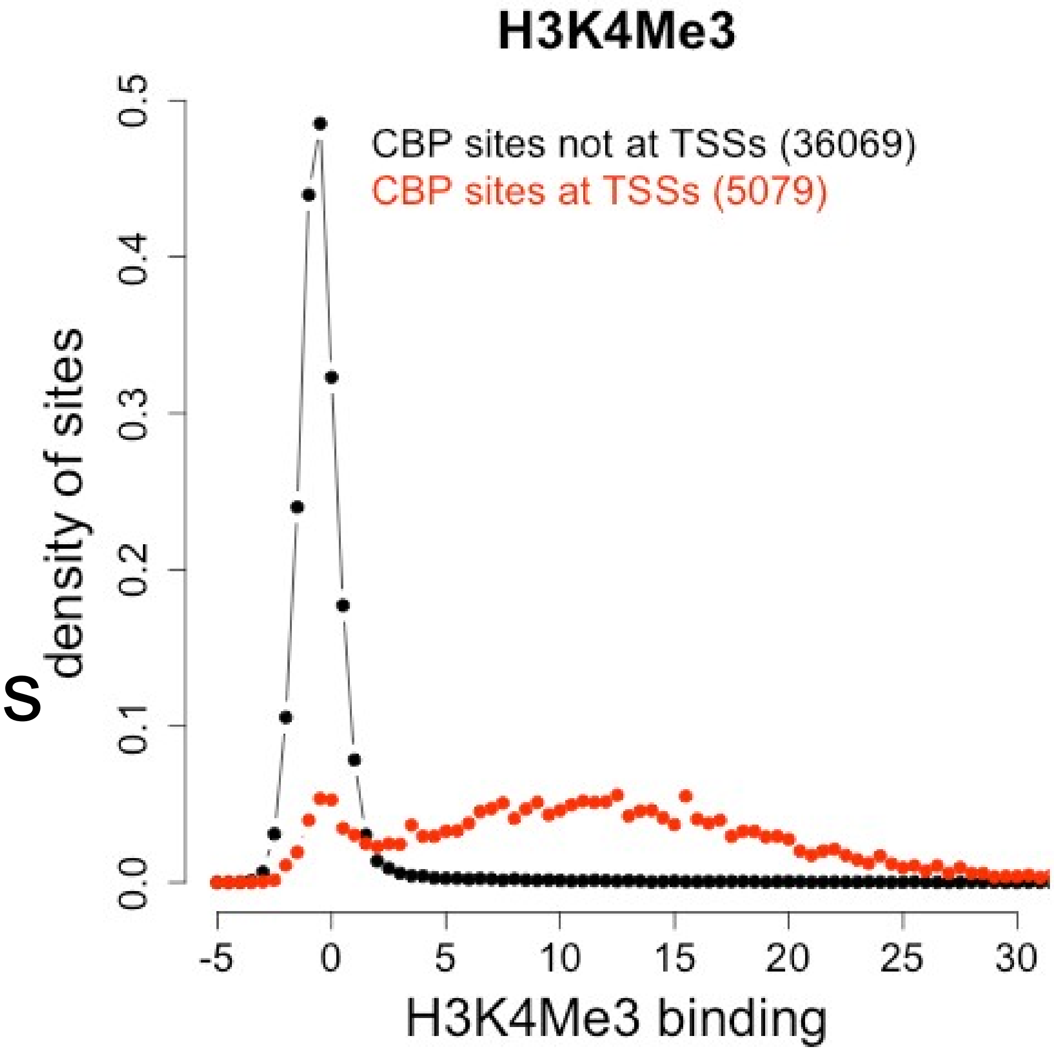
Promoters



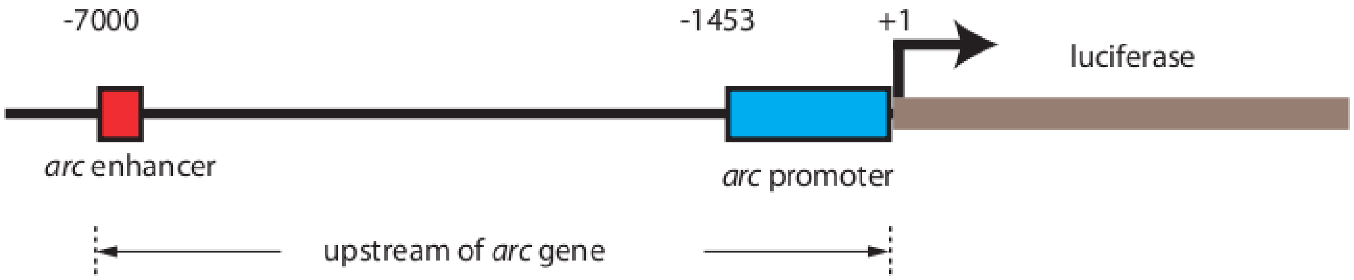


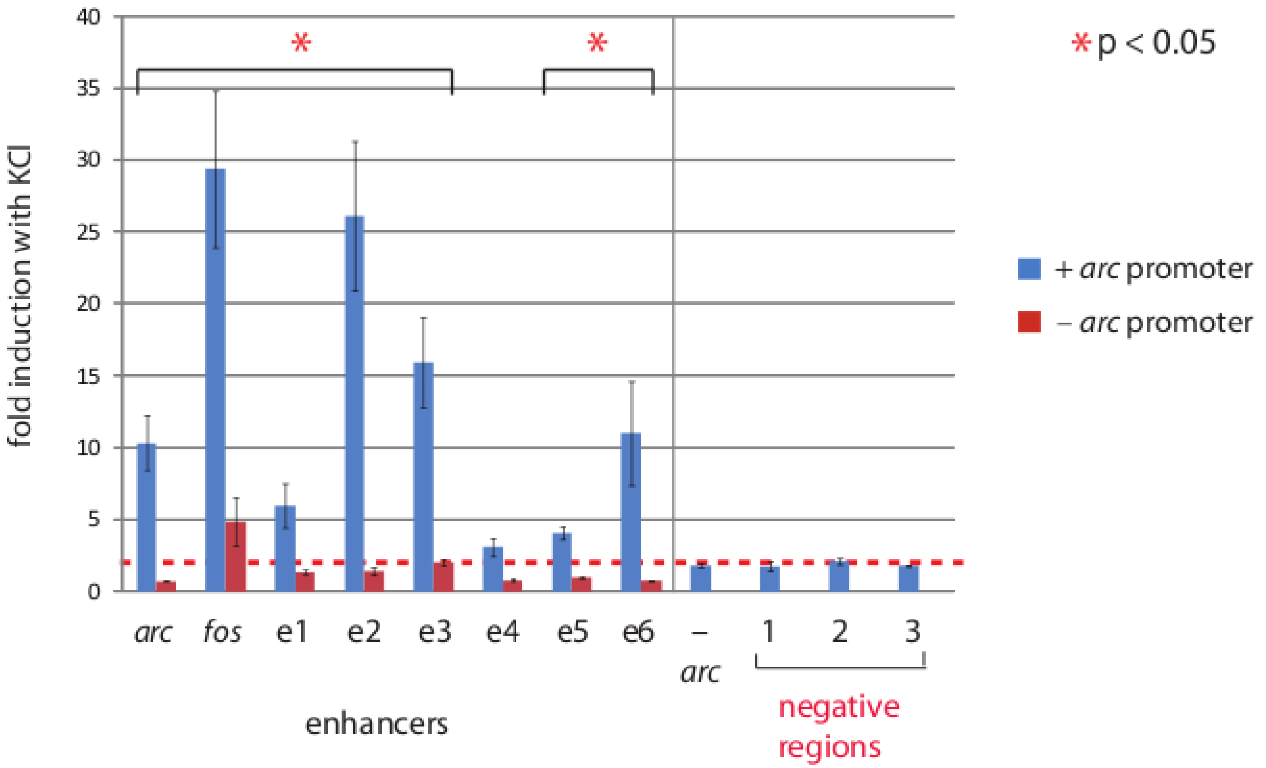
Identifying 5130 activity regulated enhancers

- CBP binding
- High levels of H3K4me1 nearby
- Low levels of H3K4me3
- >1kb from annotated transcription start sites
- No ESTs crossing TSSs starting nearby

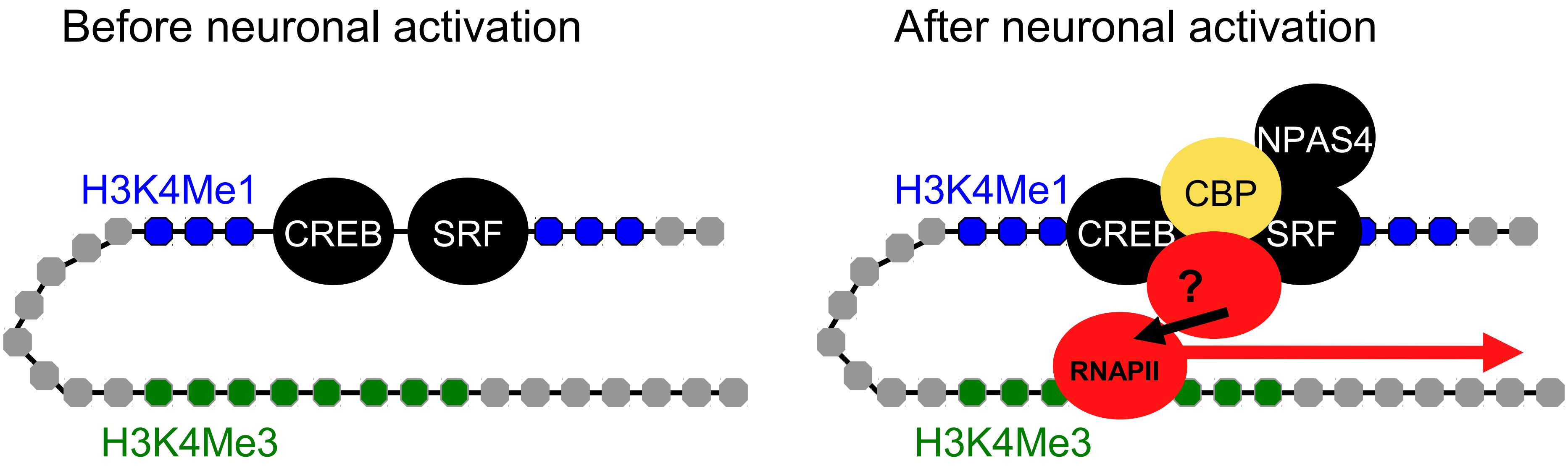


Experimental validation of 8 enhancers using a luciferase assay





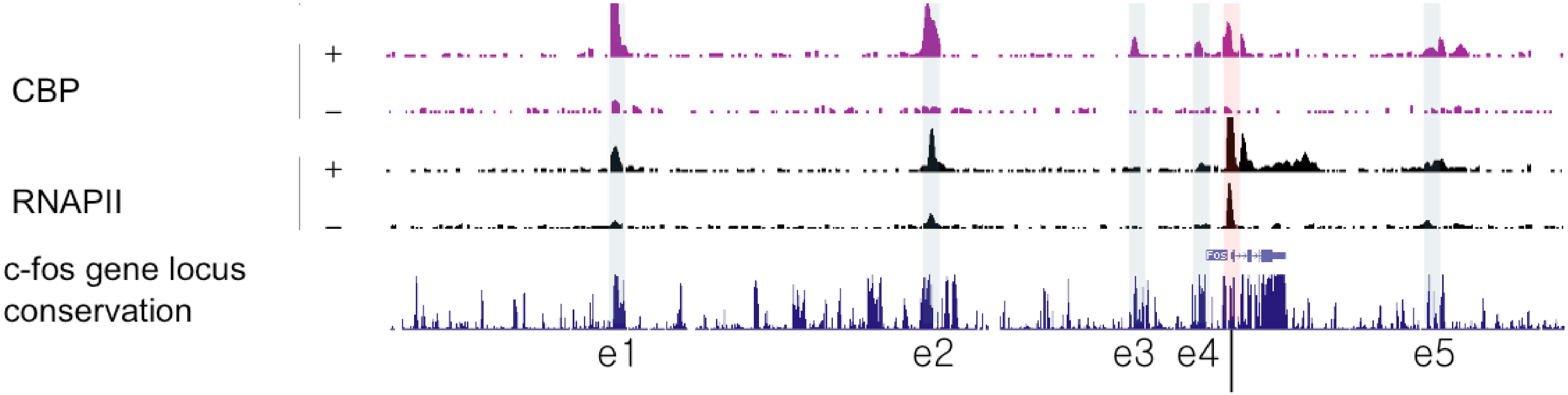
Properties of activity regulated enhancers



- Does RNAPII bind at enhancers?

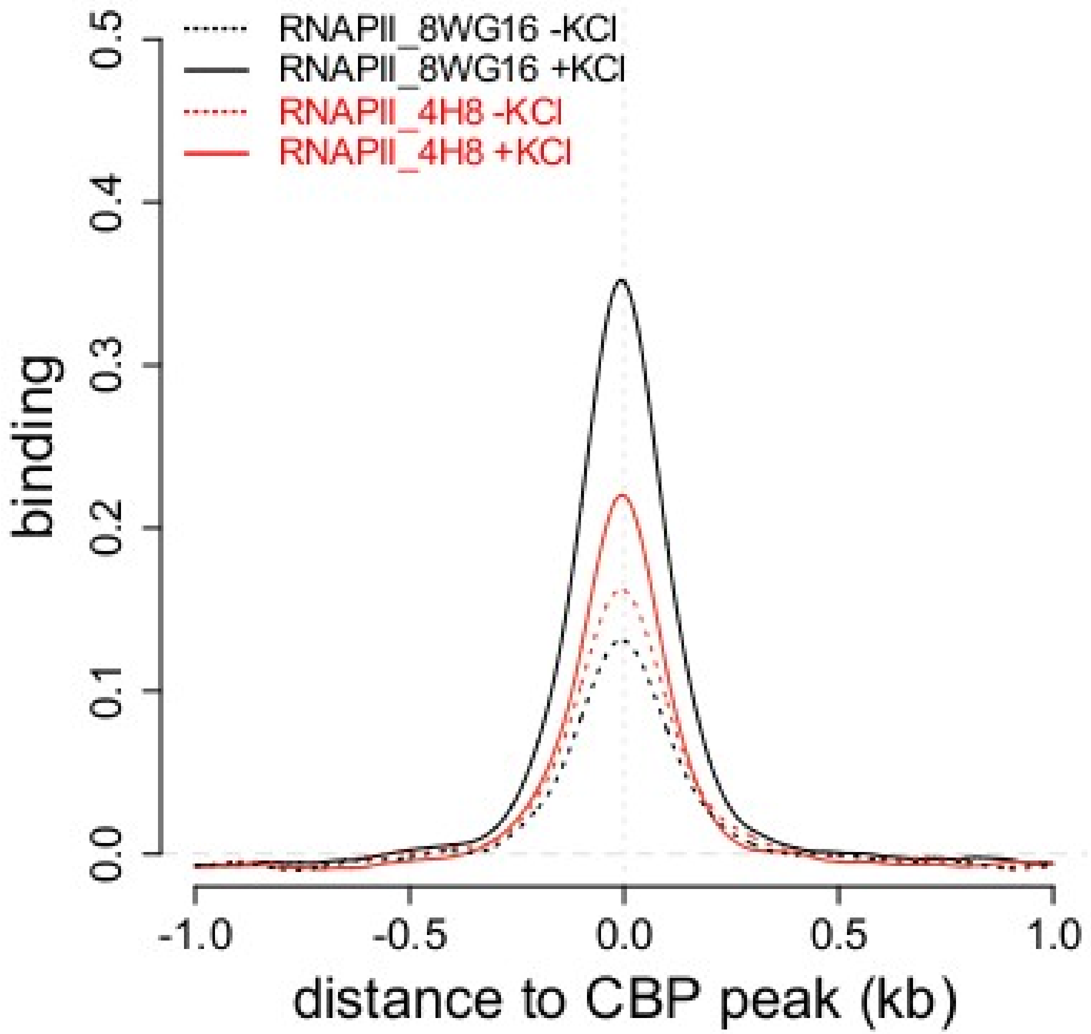
Recruitment of RNAPII at the *fos* locus

ChIP:

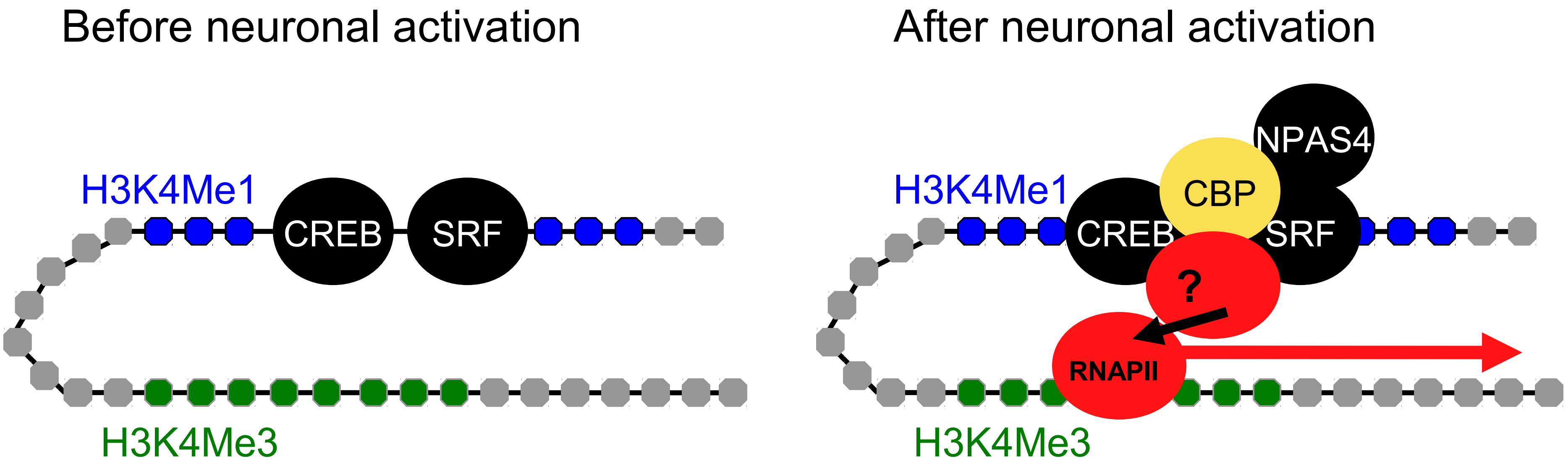


fos promoter

Recruitment of RNAPII for all enhancers

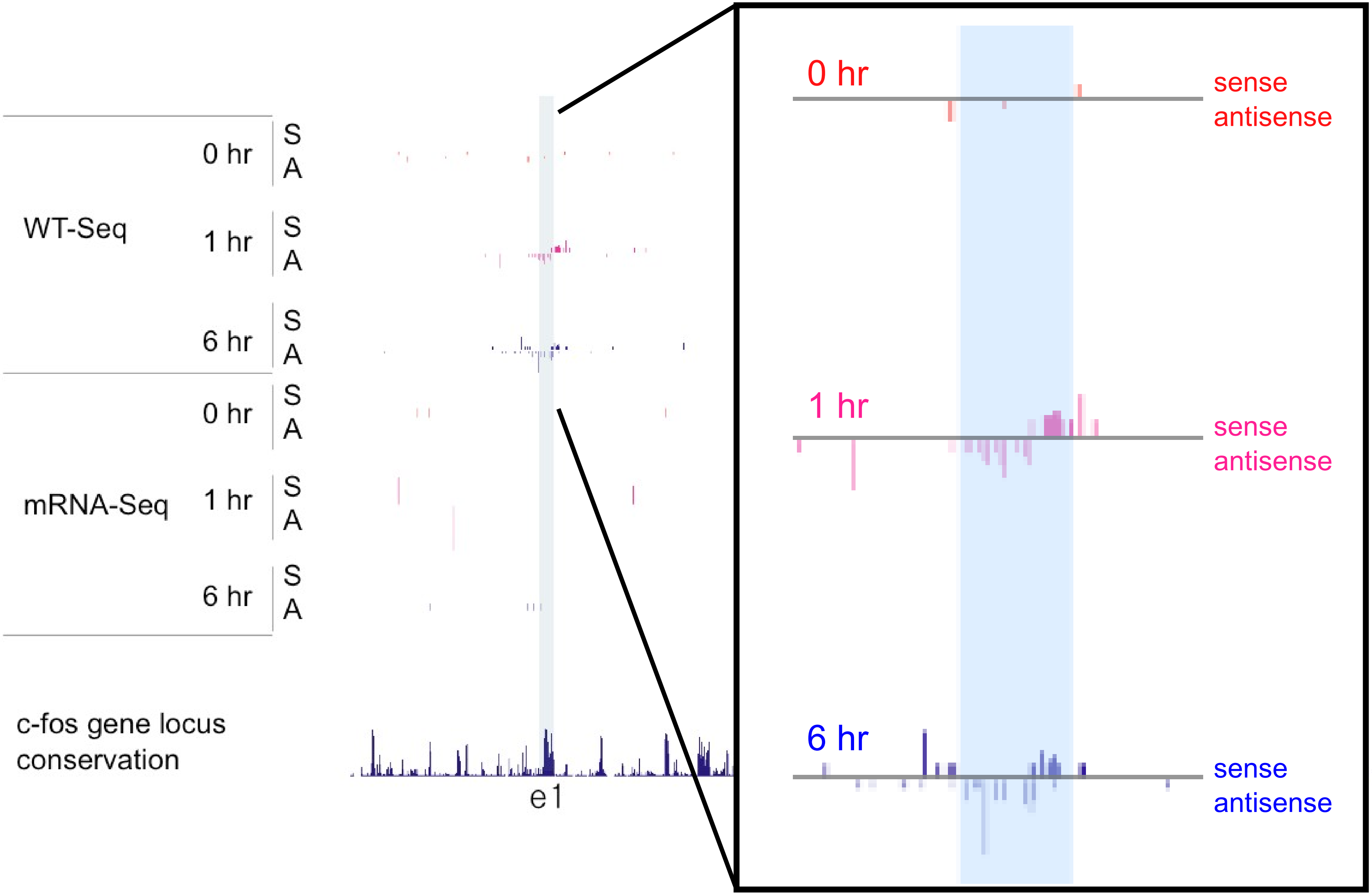


Properties of activity regulated enhancers

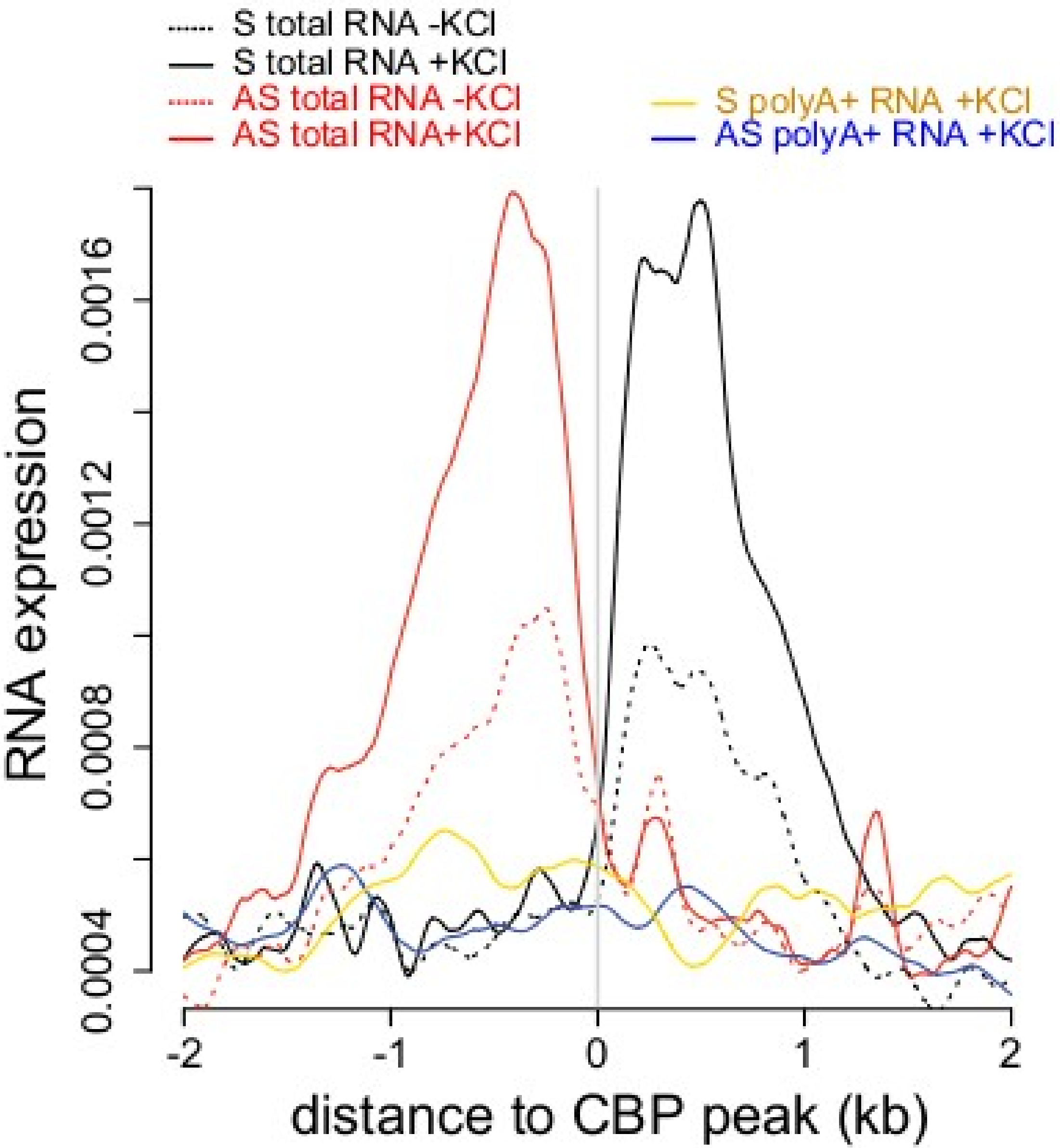


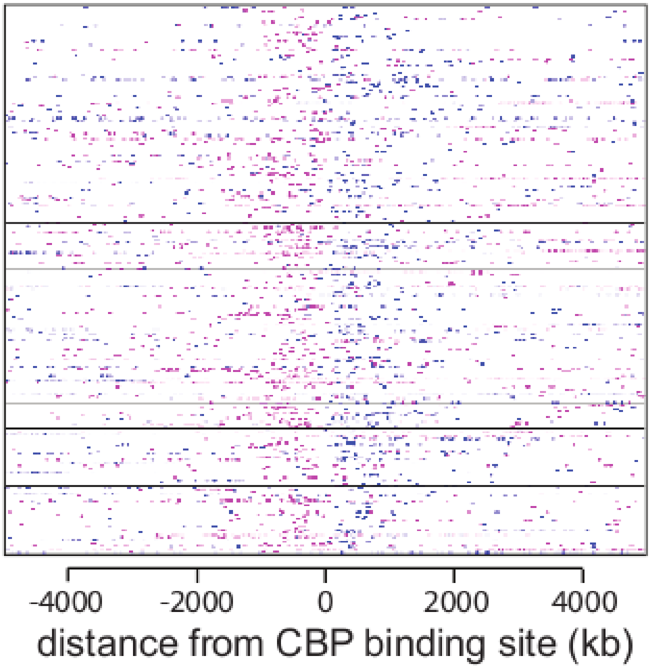
- Does RNAPII bind at enhancers? YES
- Are transcripts produced at enhancers?

Transcription of enhancer RNA (eRNA) at the *fos* locus



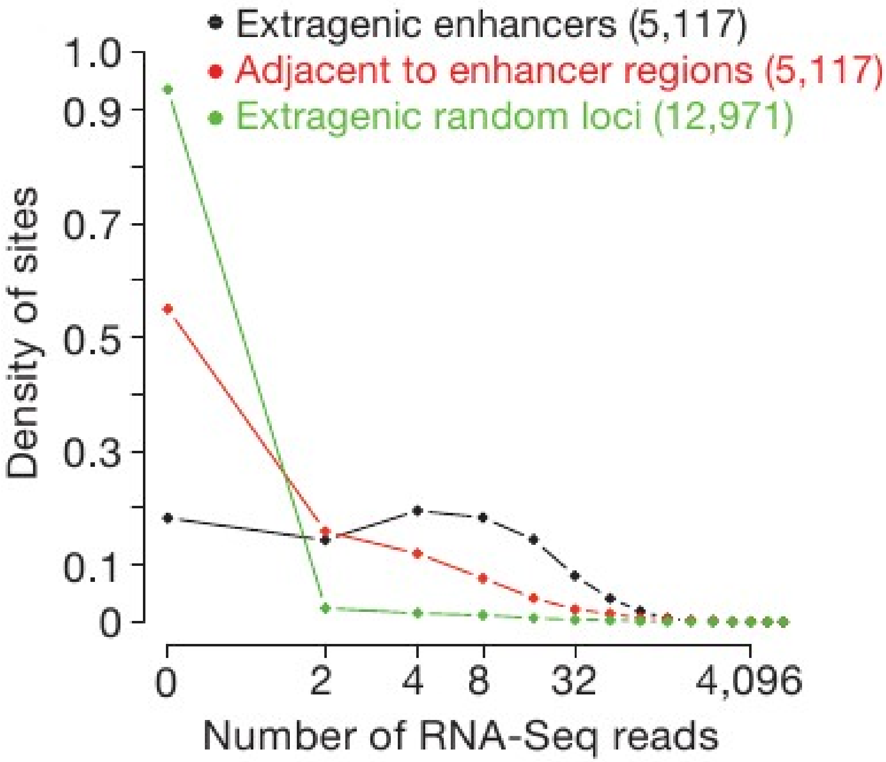
Transcription of eRNA genome-wide





Detecting eRNA

- Look for above background levels of transcription near enhancers
  - Compare (0, 1.5) kb with (2, 3.5) kb
  - Use 7 reads as threshold
    - 44% of enhancers
    - 16% of flanking regions
    - 2% of random regions



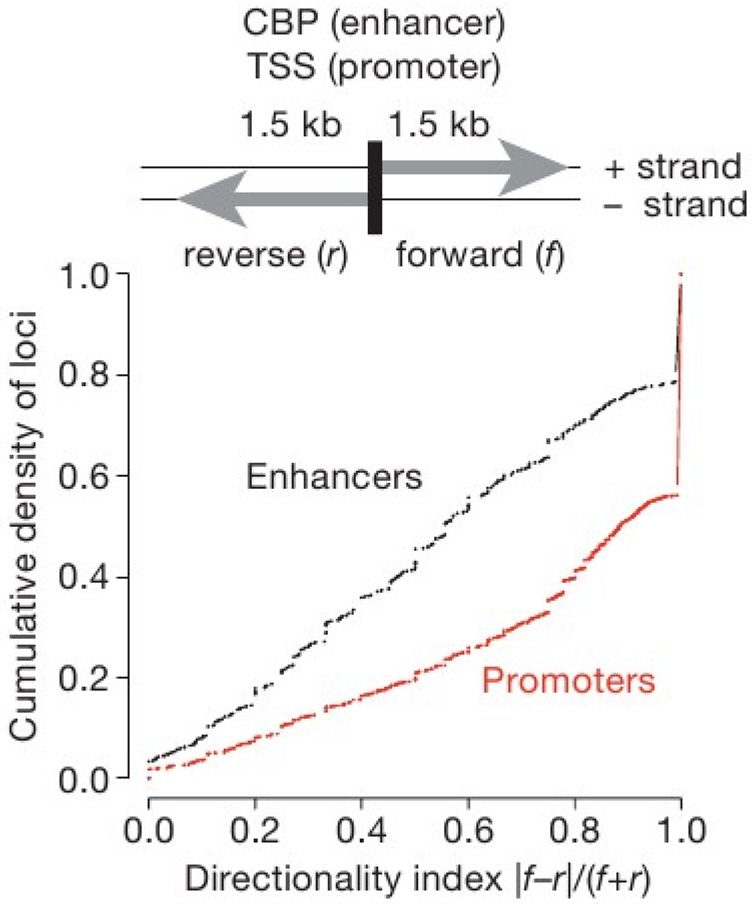
eRNAs are produced at very low levels

- Estimate number of transcripts in the cell

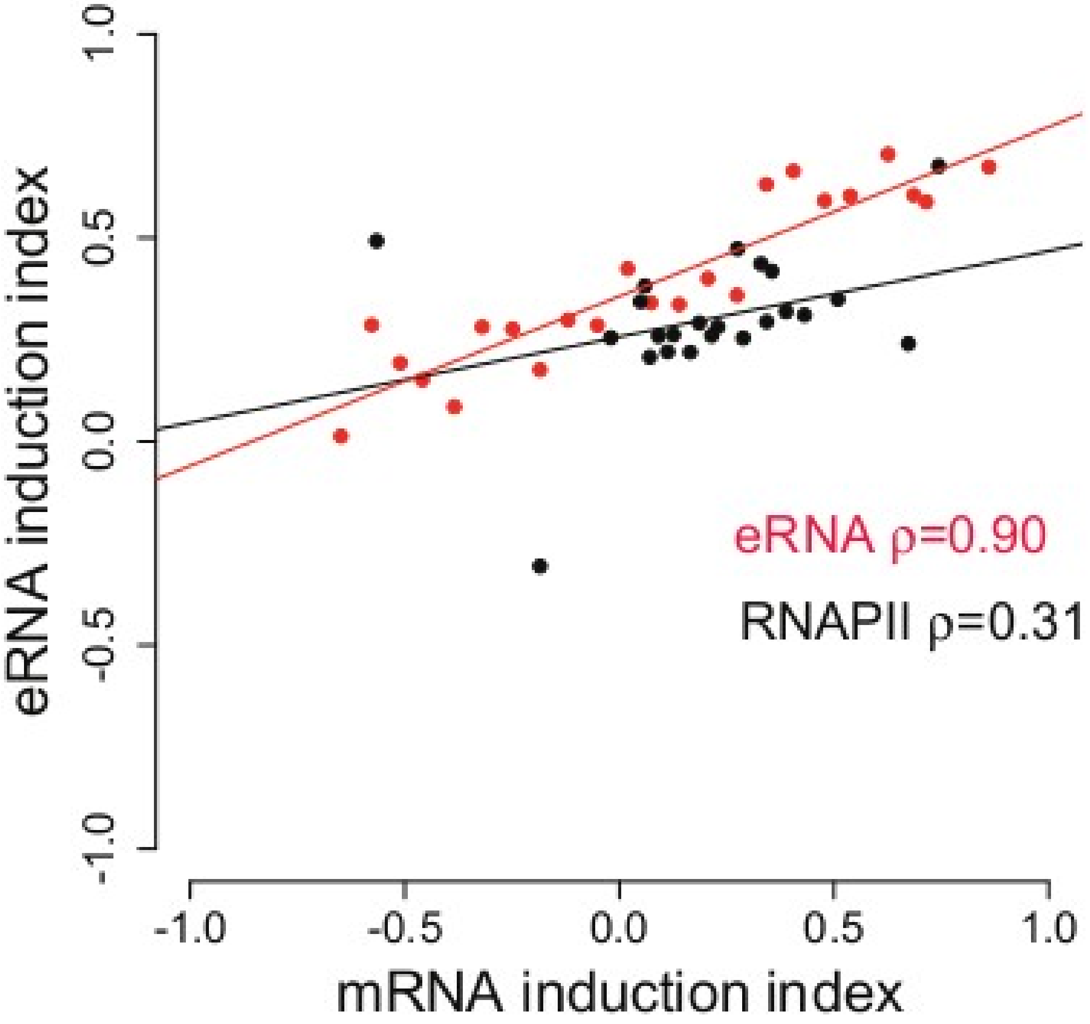
Category	#regions	#copies	reads
– mRNAs	~15,000	~100,000	~.95
– eRNAs	~3,000	~400	.0001

Enhancers are more bidirectional than promoters

- Most promoters produce transcripts in one direction
  - Directionality index
  - Downsample promoters

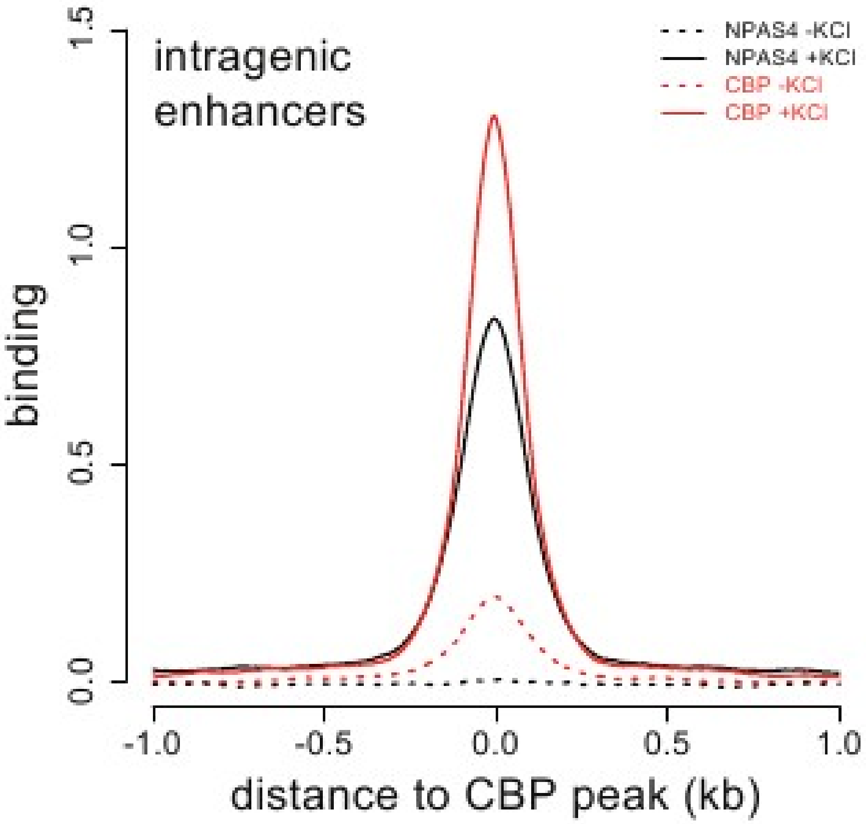


eRNA induction is correlated with induction of nearby mRNAs



Intragenic enhancers

- ~7,000 enhancers overlapping introns
  - No signal detectable on sense strand
  - Significant anti-sense transcription



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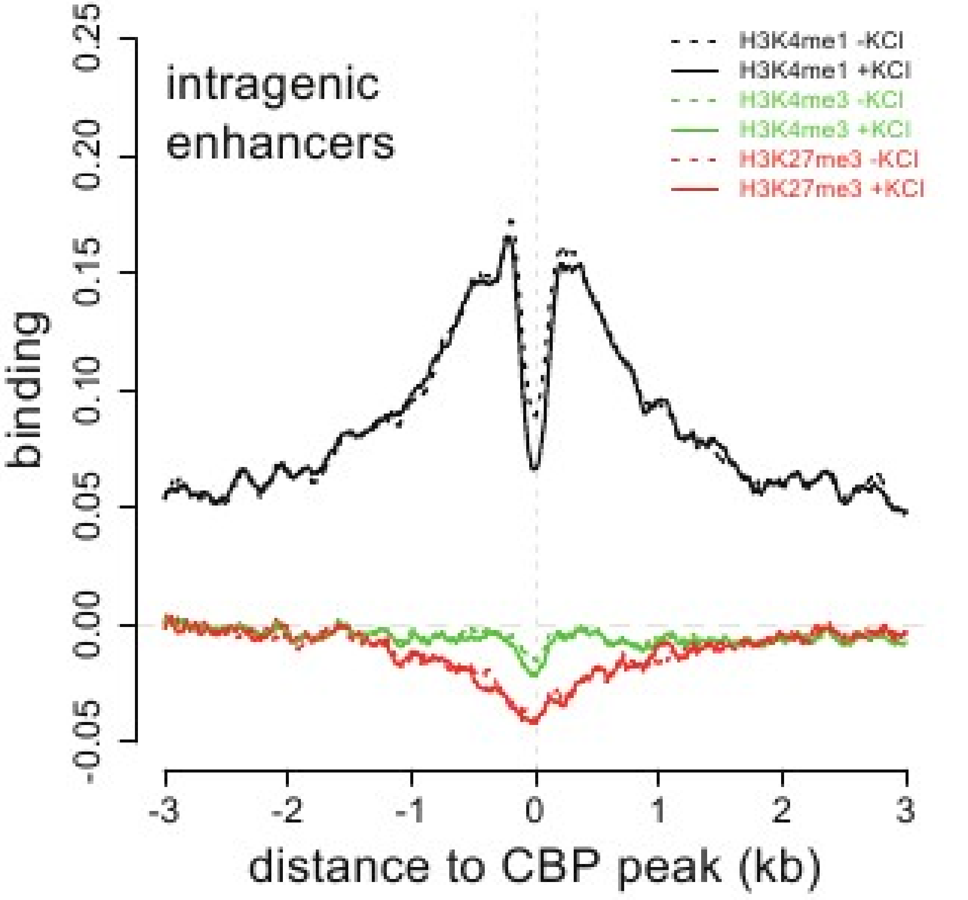

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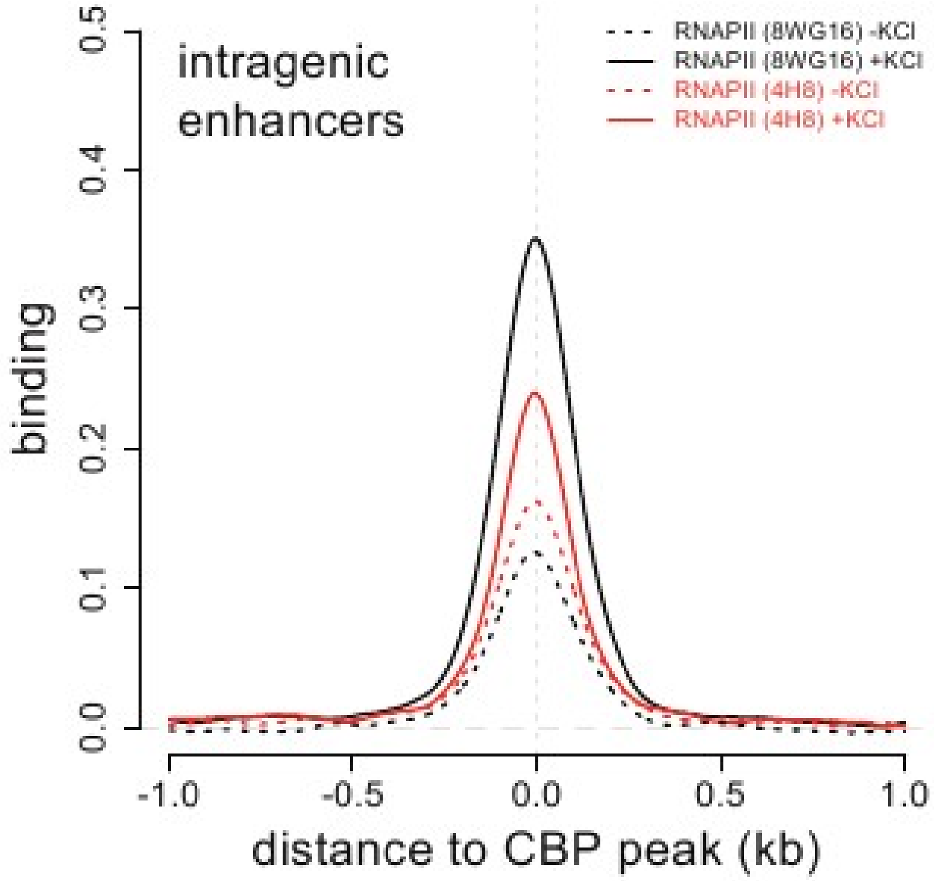

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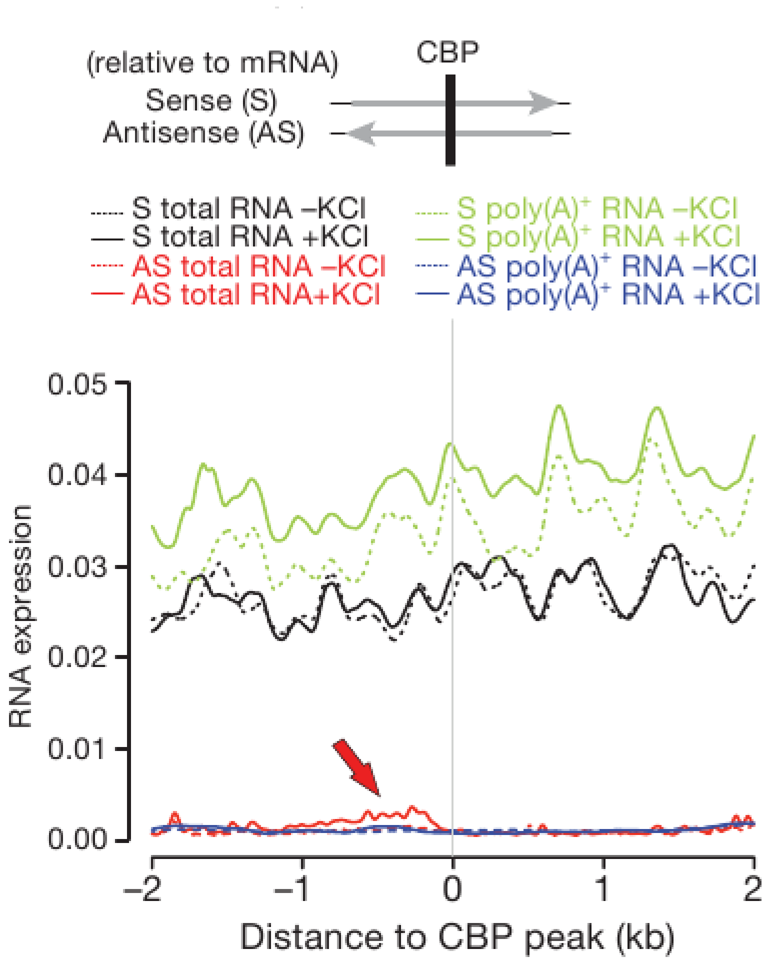
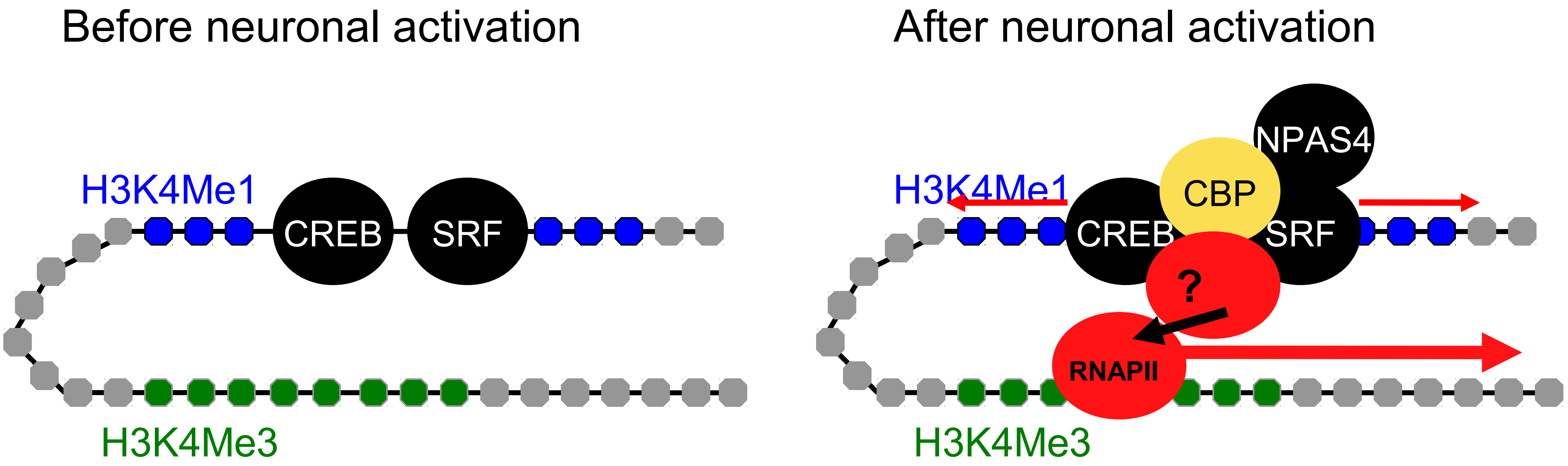
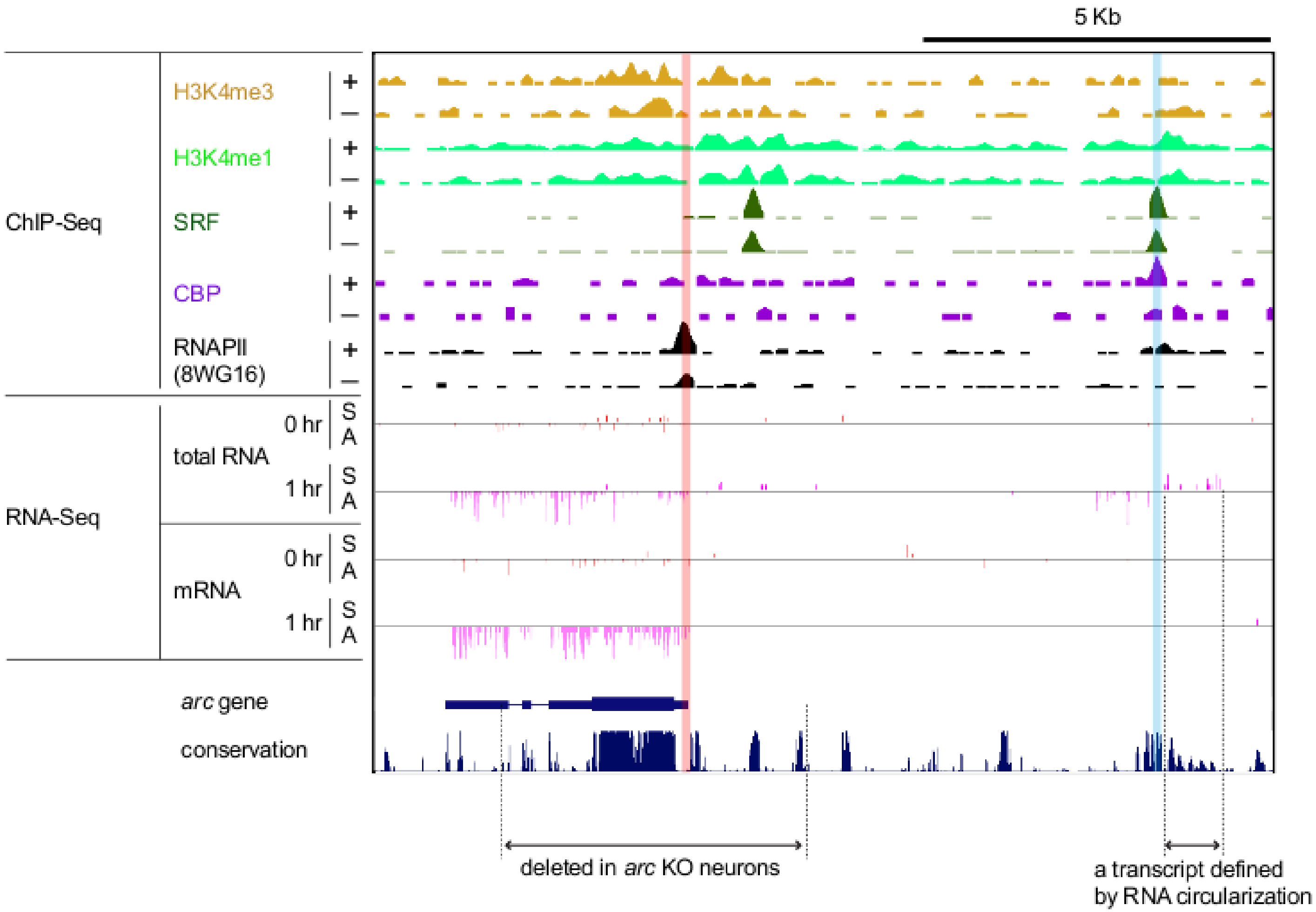


Properties of activity regulated enhancers

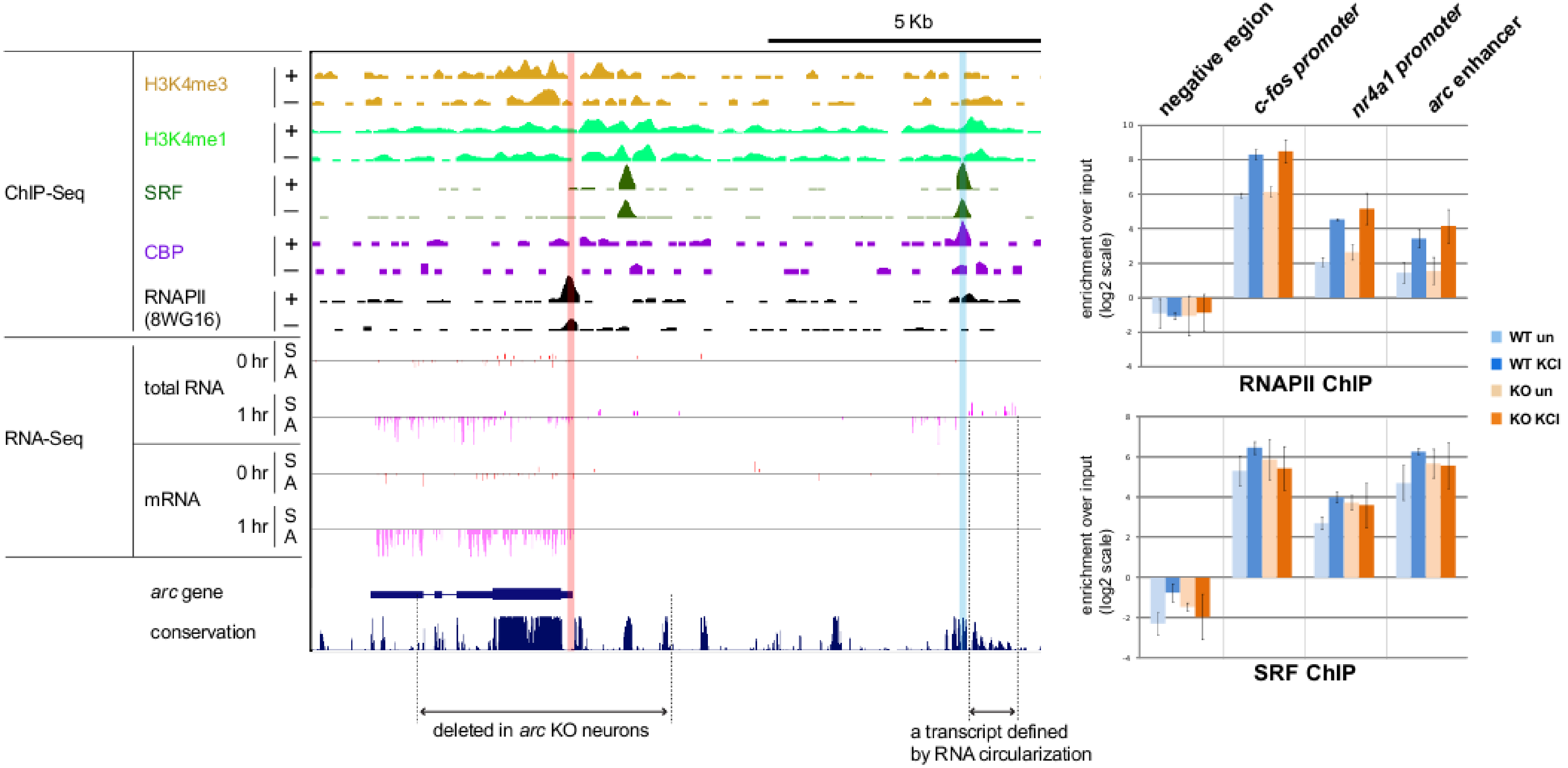


- Does RNAPII bind at enhancers? YES
- Are transcripts produced at enhancers? YES
- Is RNAPII recruitment independent?

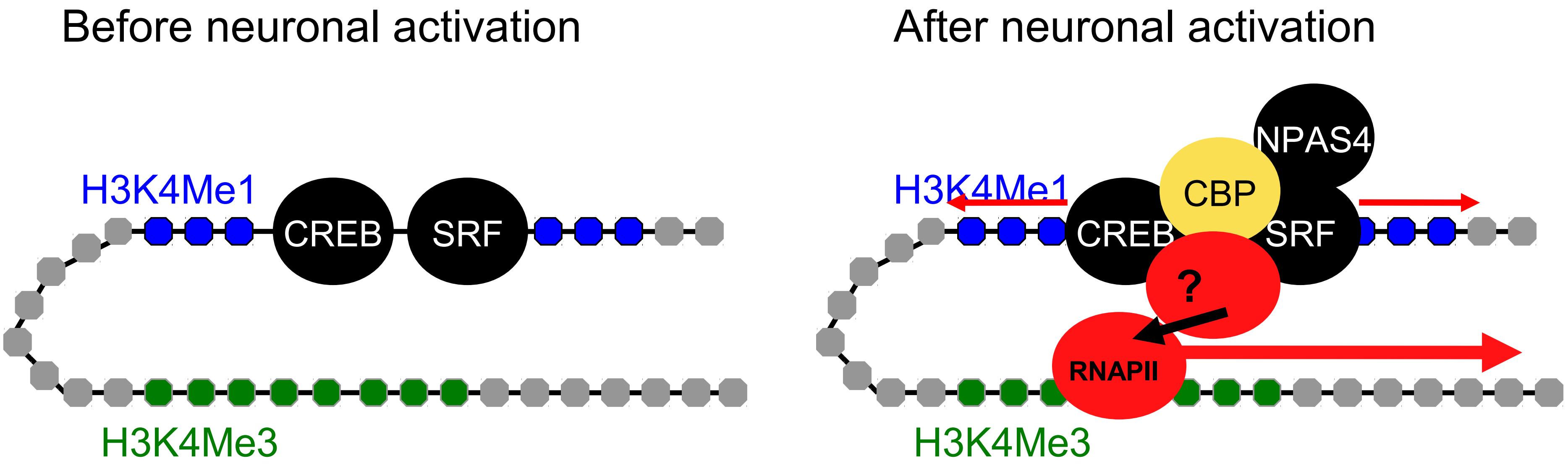
arc locus and enhancer



Recruitment of RNAPII and TFs without promoter



Properties of activity regulated enhancers



- Does RNAPII bind at enhancers? YES
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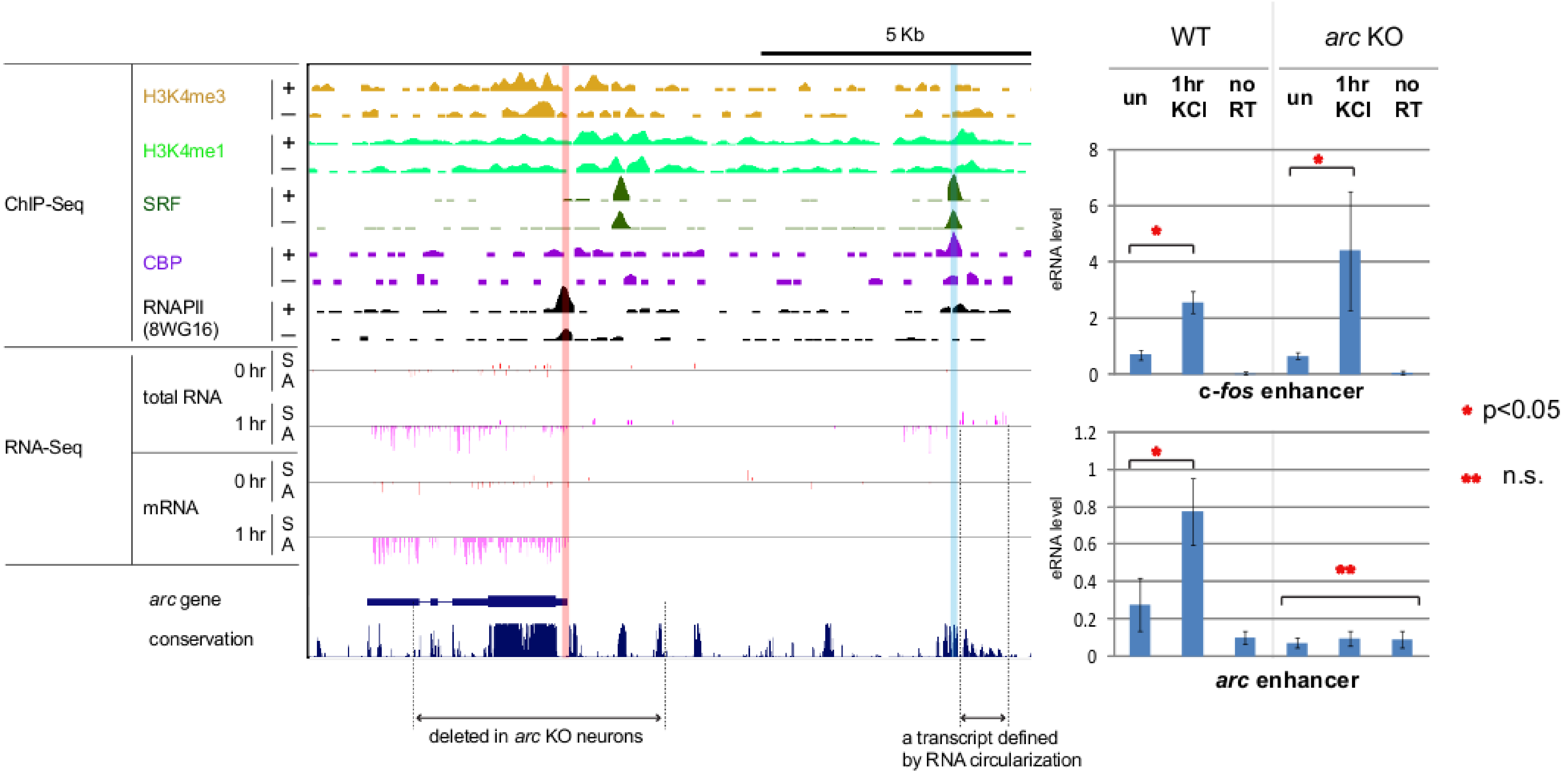
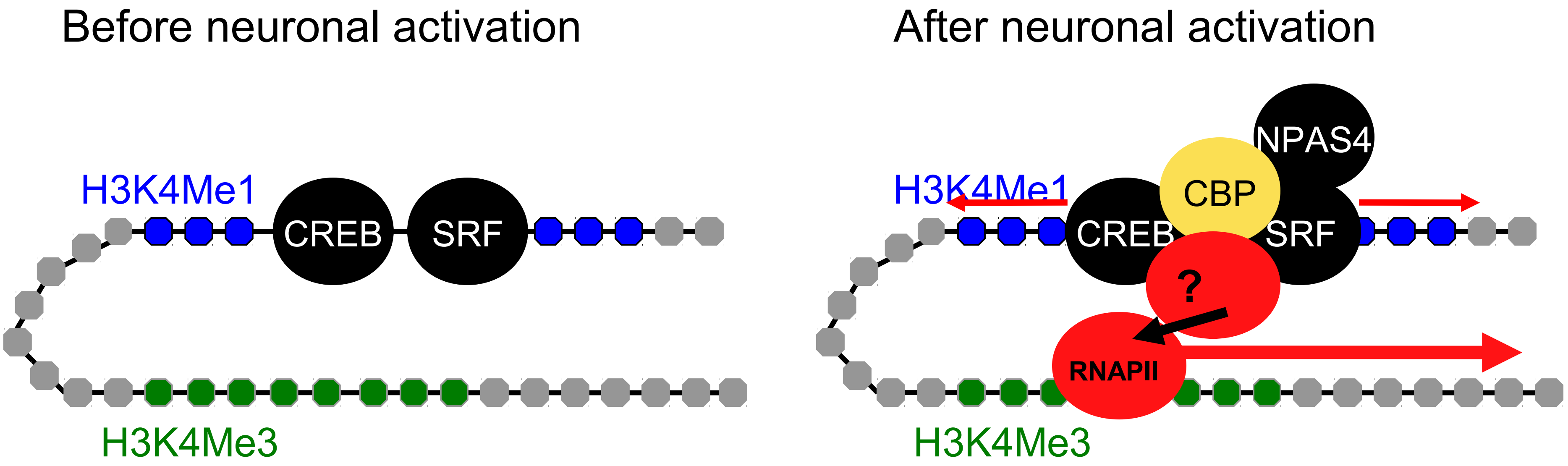
- Is eRNA production independent?

arc promoter required for eRNA



Summary



Does RNAPII bind at enhancers?	YES
Are transcripts produced at enhancers?	YES
Is RNAPII recruitment independent?	YES
Is eRNA production independent?	NO

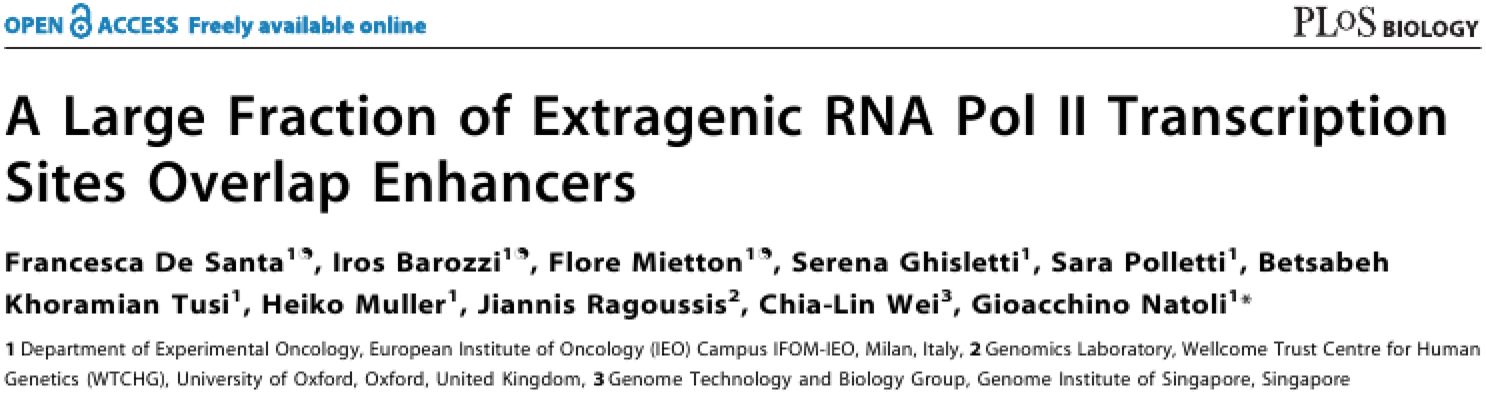
Related work

doi:10.1038/nature09033 nature

ARTICLES

Widespread transcription at neuronal activity-regulated enhancers

Tae-Kyung Kim[1]*†, Martin Hemberg[2]*, Jesse M. Gray[1]*, Allen M. Costa[1], Daniel M. Bear[1], Jing Wu[3], David A. Harmin[1,4], Mike Laptewicz[1], Kellie Barbara-Haley[5], Scott Kuersten[6], Eirene Markenscoff-Papadimitriou[1]†, Dietmar Kuhl[7], Haruhiko Bito[8], Paul F. Worley[3], Gabriel Kreiman[2] & Michael E. Greenberg[1]



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A Large Fraction of Extragenic RNA Pol II Transcription Sites Overlap Enhancers

Francesca De Santa[1¶], Iros Barozzi[1¶], Flore Mietton[1¶], Serena Ghisletti[1], Sara Polletti[1], Betsabeh Khoramian Tusi[1], Heiko Muller[1], Jiannis Ragoussis[2], Chia-Lin Wei[3], Gioacchino Natoli[1]*

1 Department of Experimental Oncology, European Institute of Oncology (IEO) Campus IFOM-IEO, Milan, Italy, **2** Genomics Laboratory, Wellcome Trust Centre for Human Genetics (WTCHG), University of Oxford, Oxford, United Kingdom, **3** Genome Technology and Biology Group, Genome Institute of Singapore, Singapore

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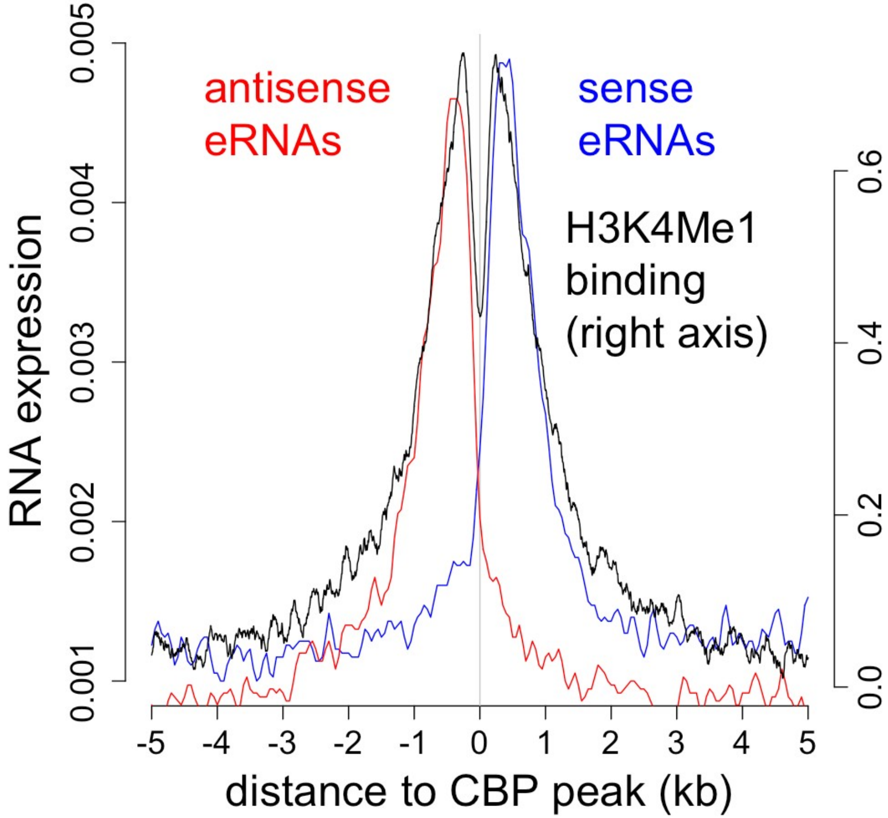
Most "Dark Matter" Transcripts Are Associated With Known Genes

Harm van Bakel[1], Corey Nislow[1,2], Benjamin J. Blencowe[1,2], Timothy R. Hughes[1,2]*

1 Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada, **2** Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada

Conclusions

- Enhancers and promoters more similar than previously thought
- Function of eRNAs still unclear
  - eRNAs may help establish histone marks
  - Read-out for enhancer activity



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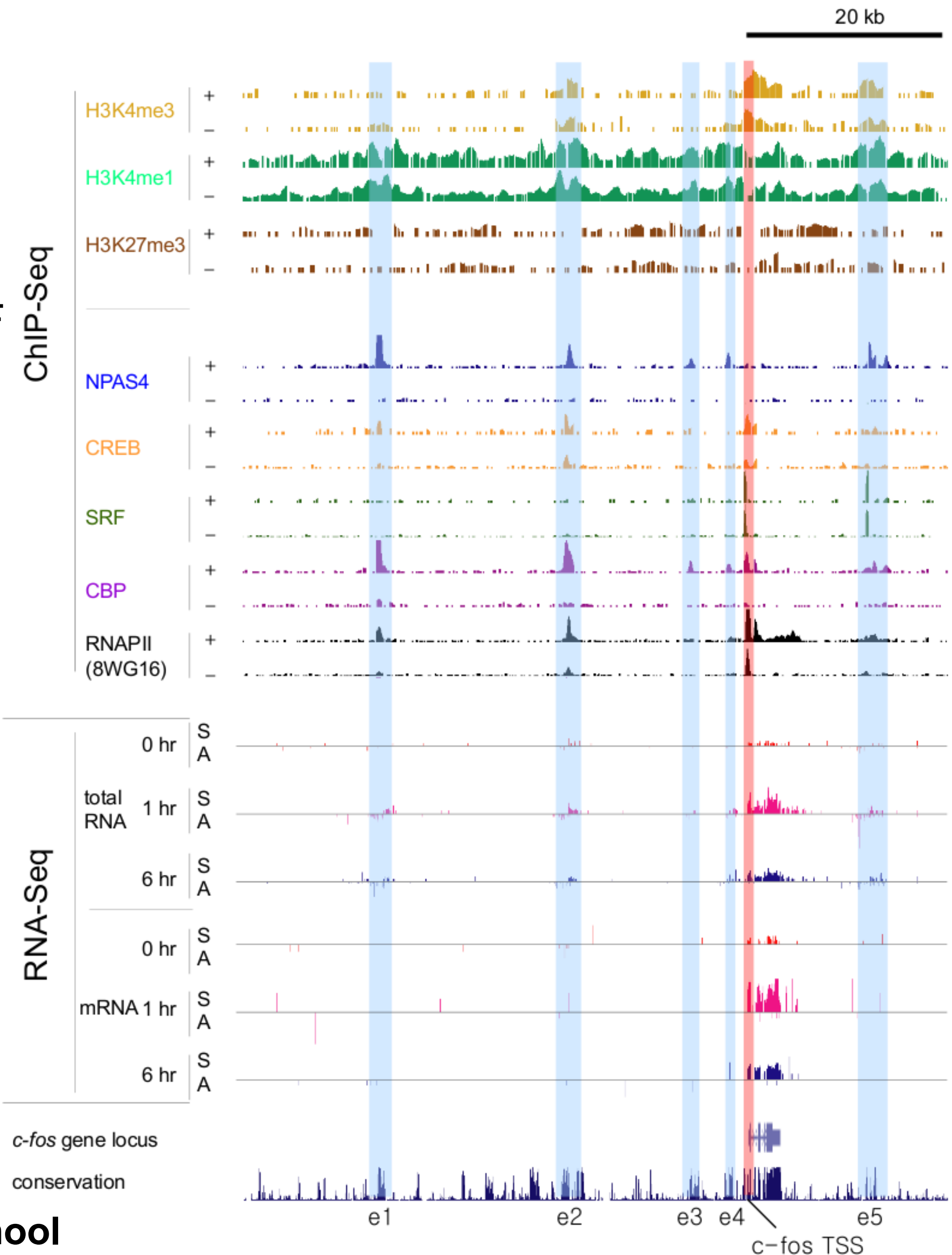

Johns Hopkins
Jing Wu, Paul Worley Lab

Thank You

Recruitment of RNAPII for all enhancers