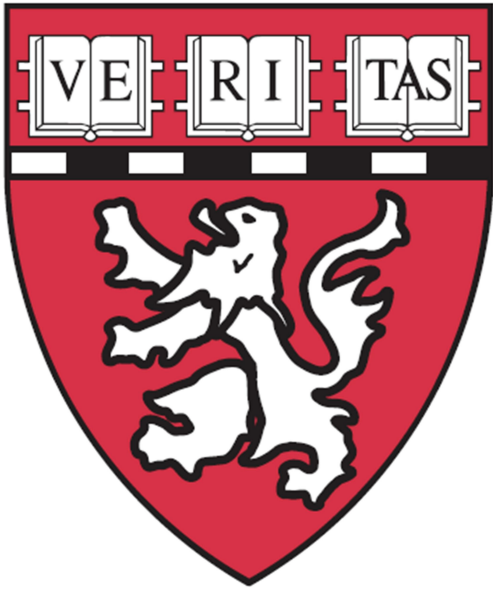
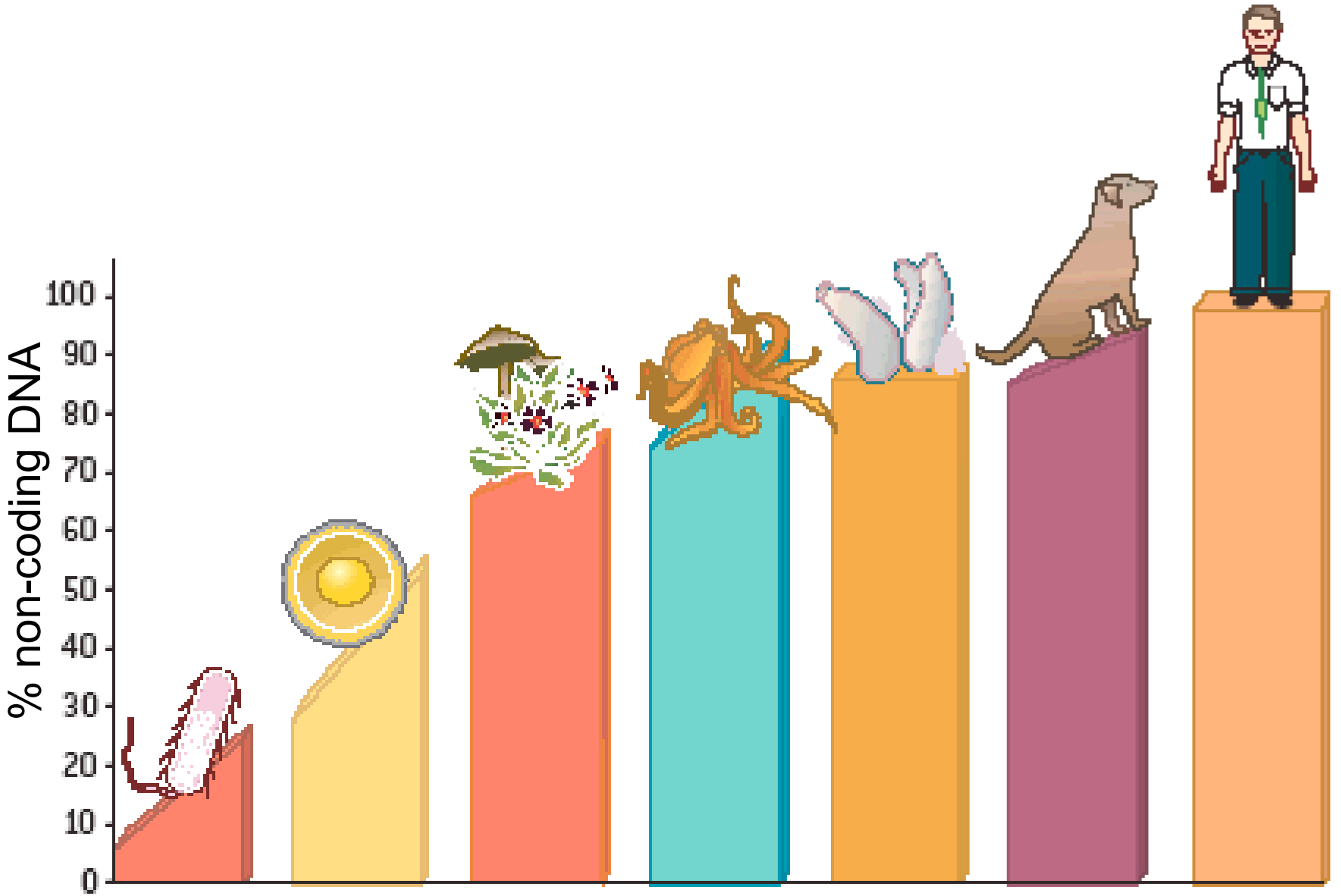
Probing the function of non-coding sequences in human and mouse using ChIP-seq and RNA-seq

Martin Hemberg

University of Michigan
March 9, 2011



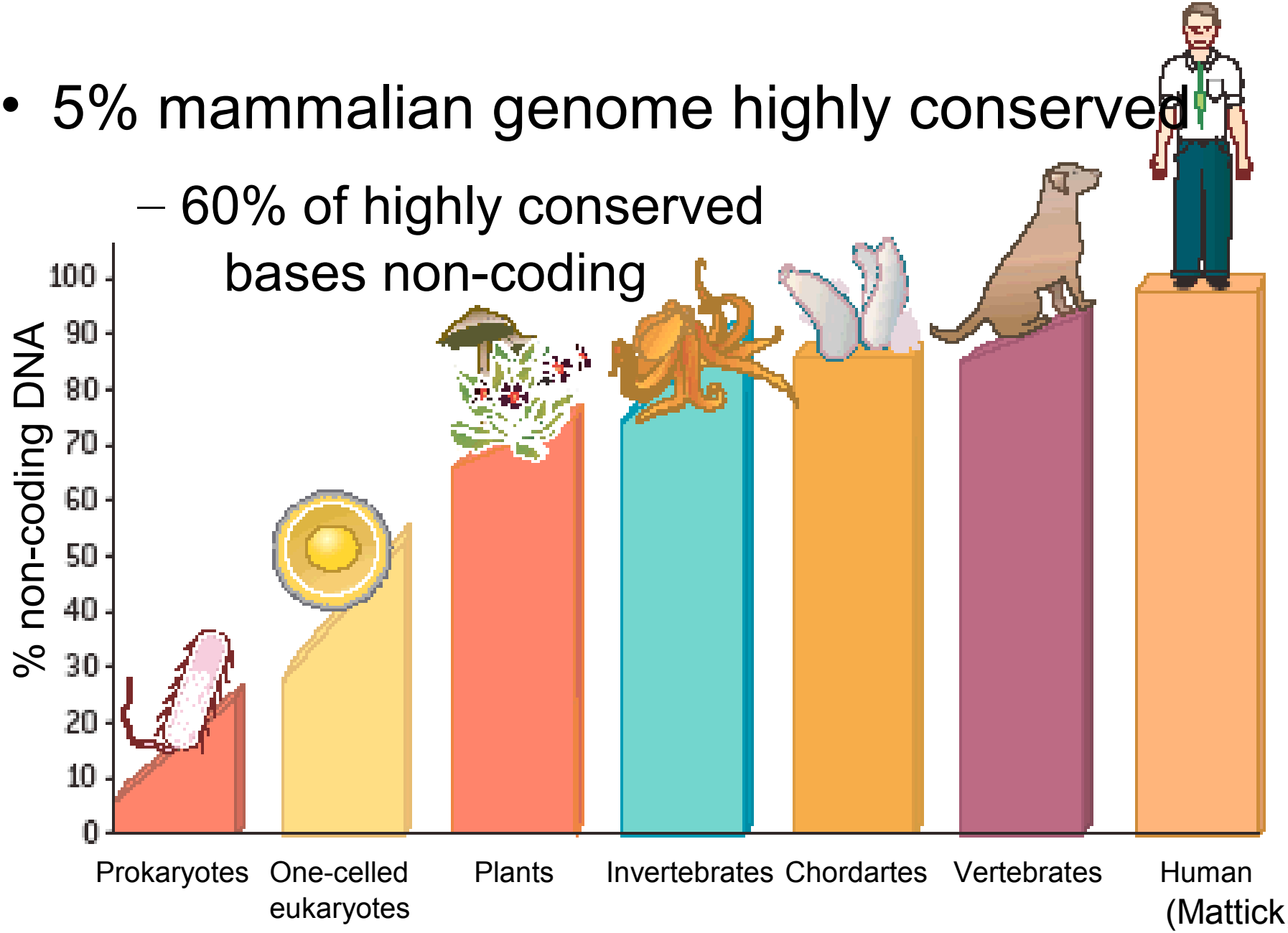
Most of the genome is **not** protein-coding



(Mattick, 2004)

Most of the genome is **not** protein-coding

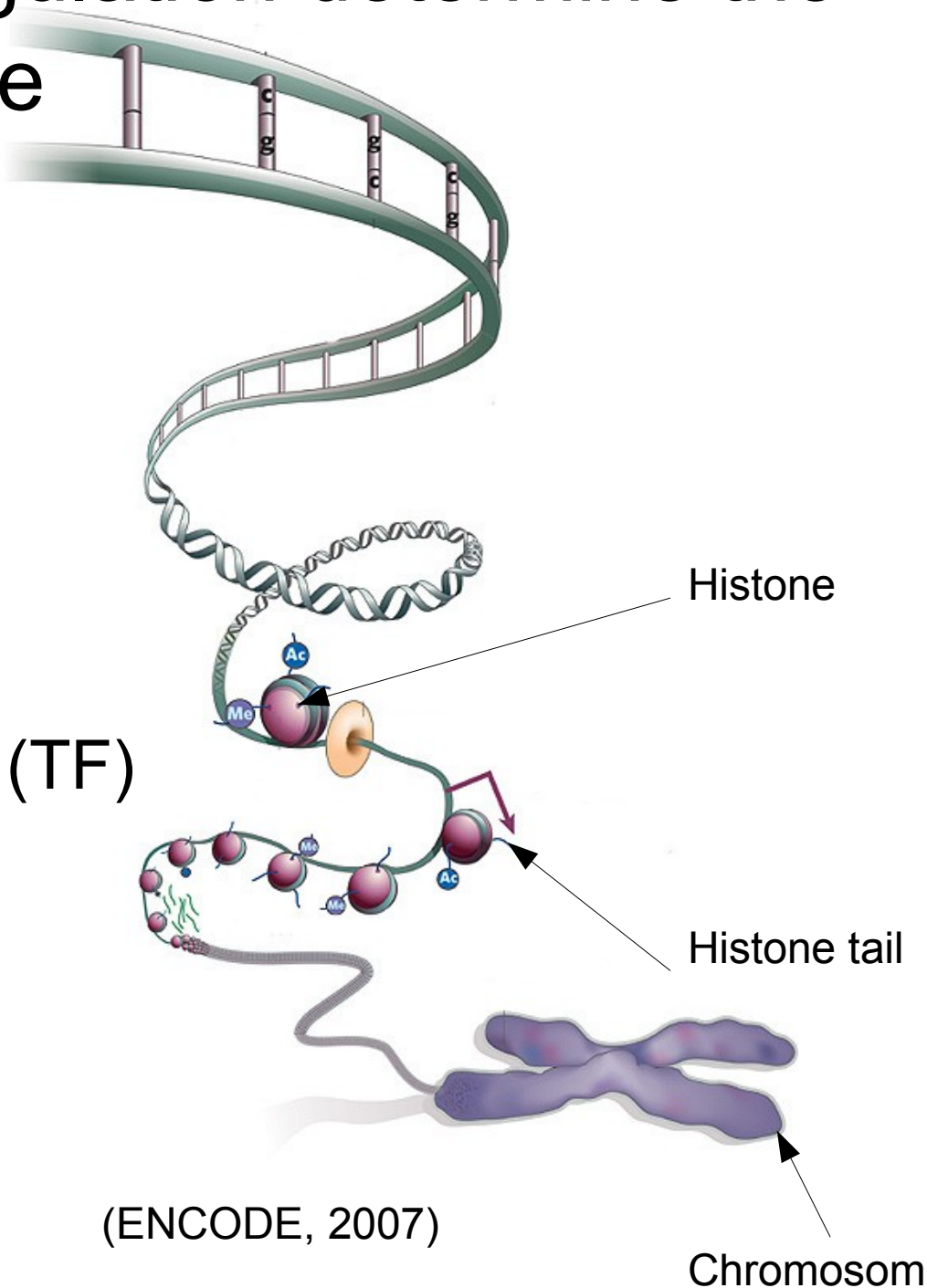
- 5% mammalian genome highly conserved
  - 60% of highly conserved bases non-coding



(Mattick, 2004)

Additional layers of regulation determine the function of the genome

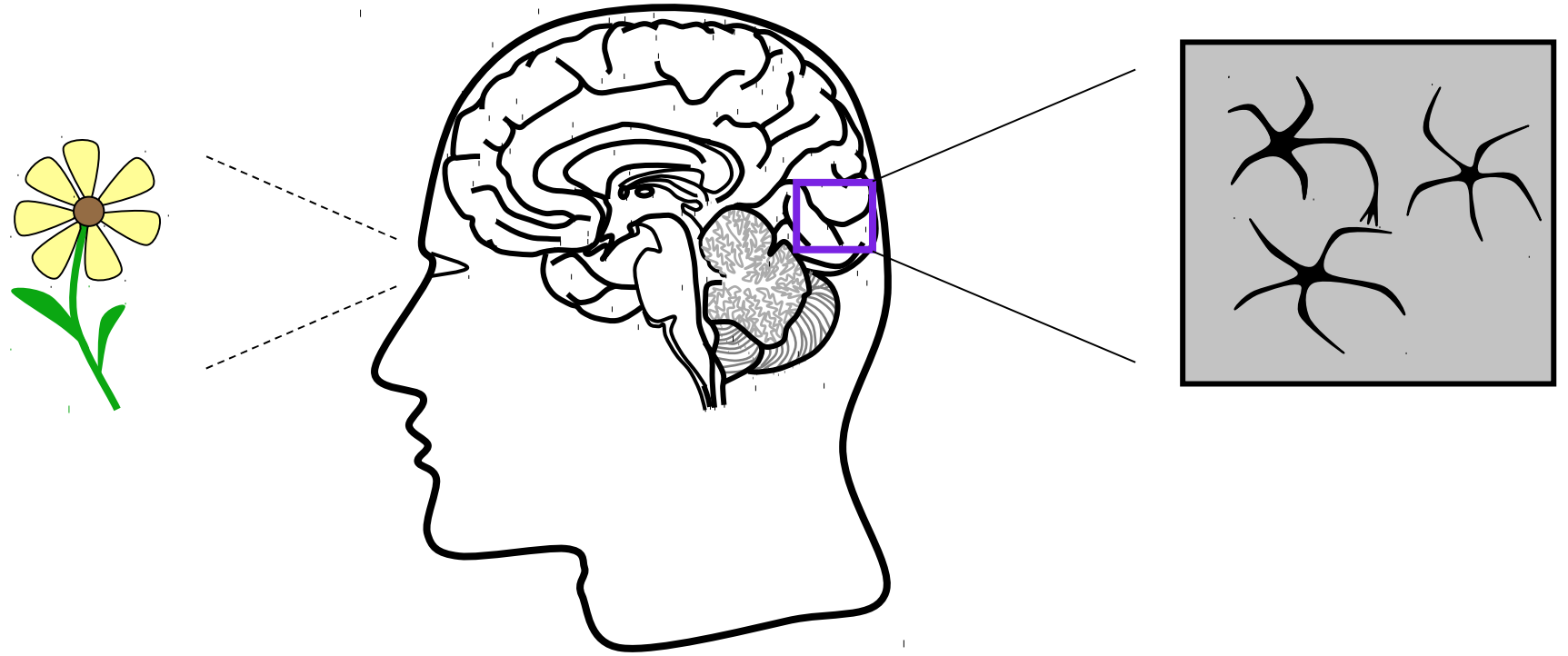
- Cell-type specific
  - DNA methylation
  - Post-translational modification of histone tails
  - Transcription factor (TF) binding



(ENCODE, 2007)

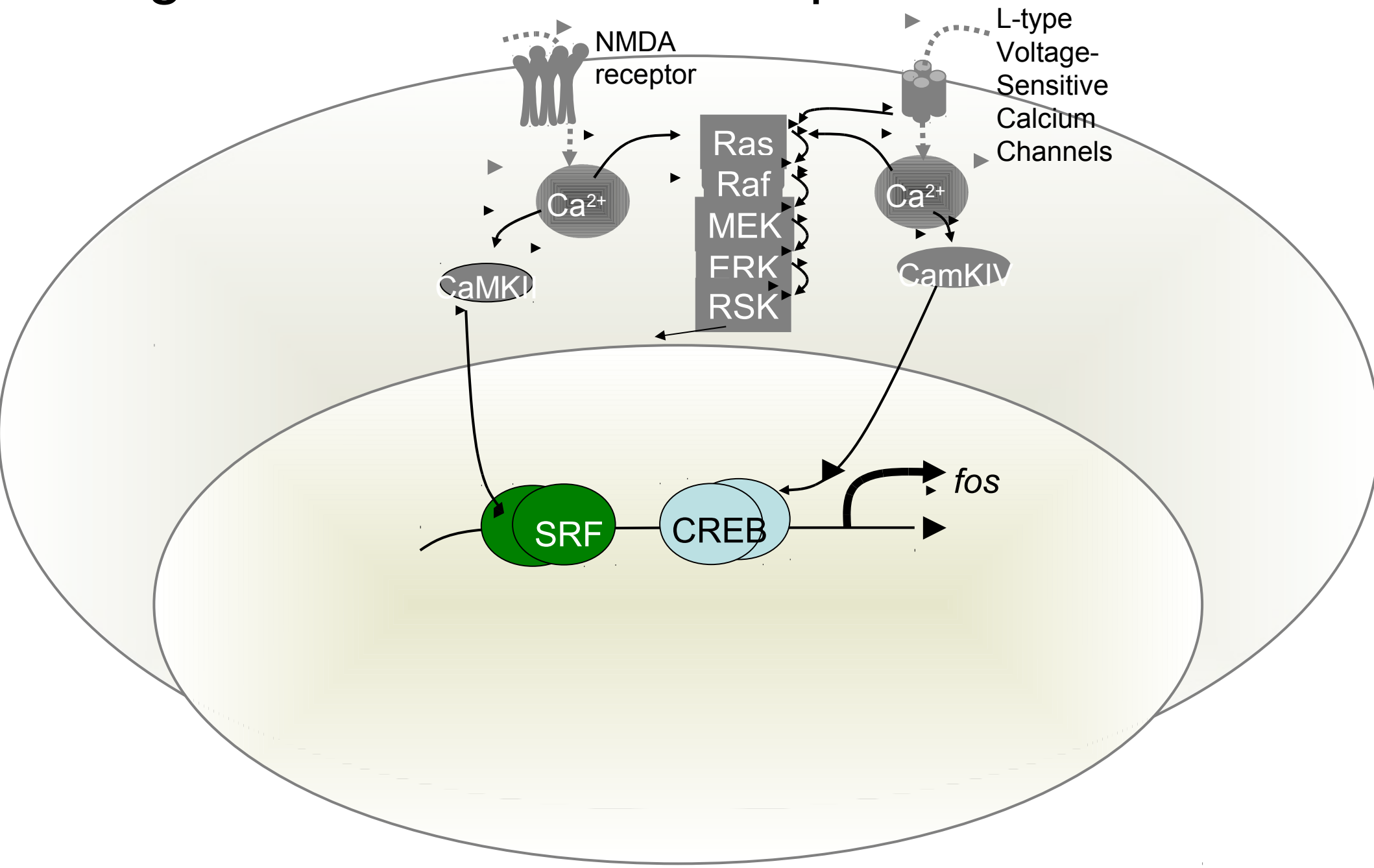
Activity dependent gene expression

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

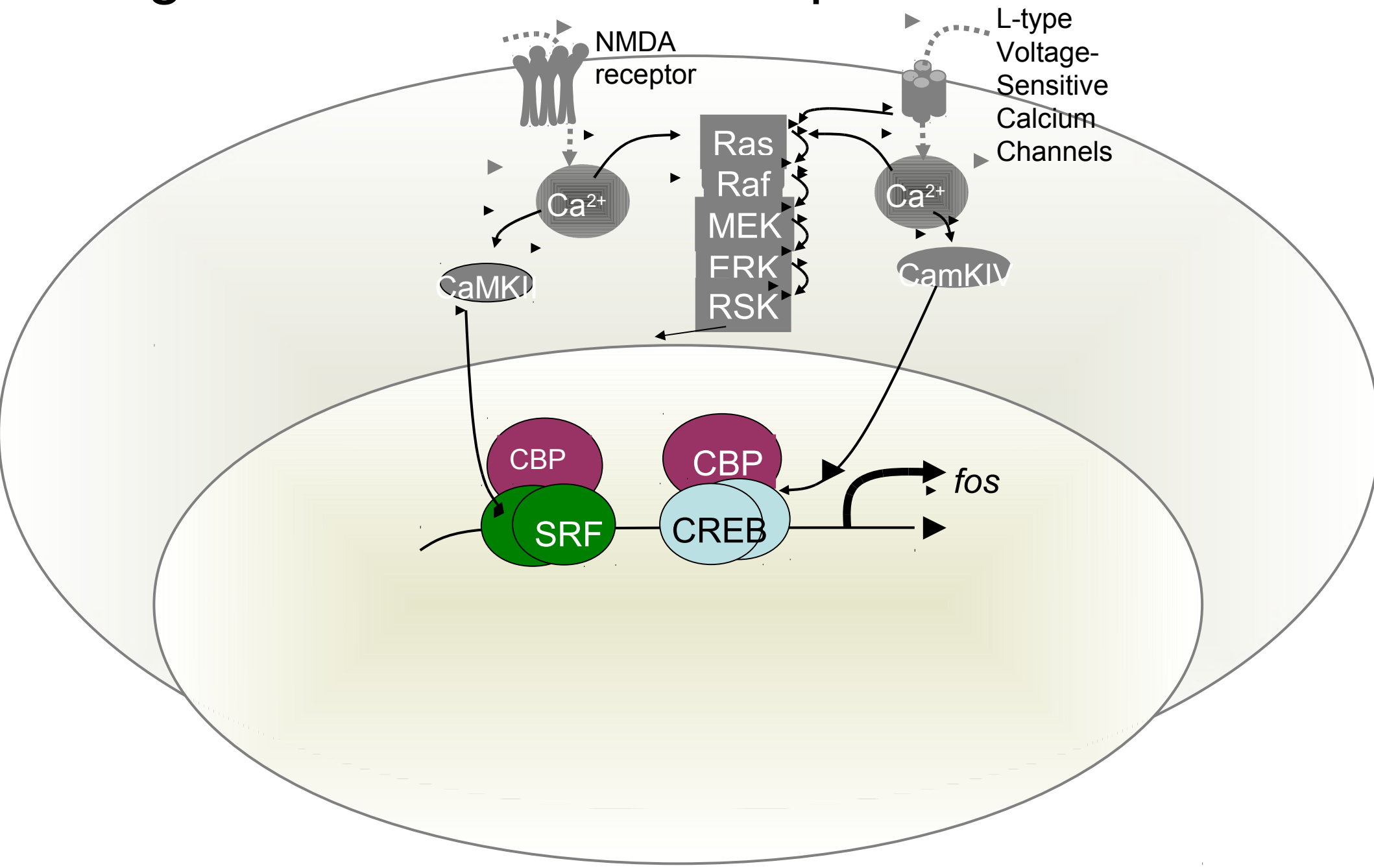


Hubel & Wiesel, 1970's

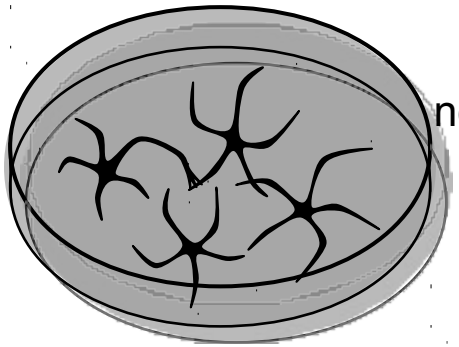
Regulation of c-fos transcription



Regulation of c-fos transcription



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

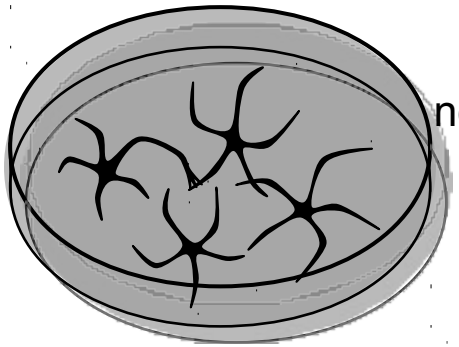


mouse cortical neurons

neuronal activation via potassium chloride (KCl) depolarization

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

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



mouse cortical neurons

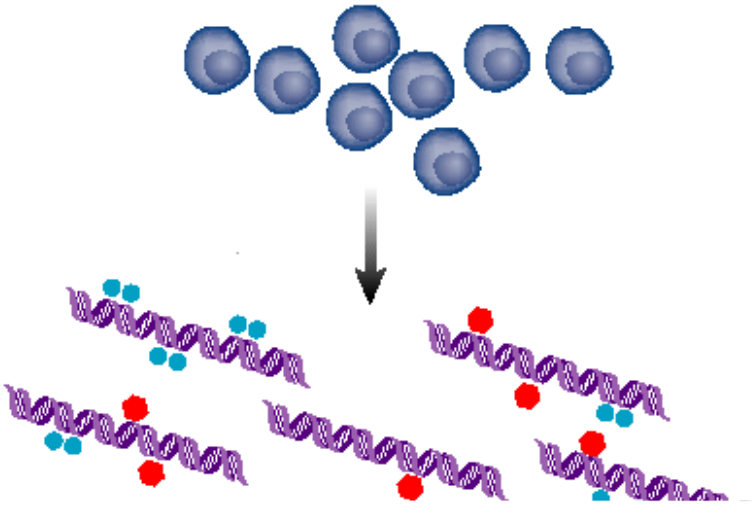
neuronal activation via potassium chloride (KCl) depolarization

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

Jesse Gray
Tae-Kyung Kim
Greenberg Lab

Chromatin immunoprecipitation and sequencing (ChIP-Seq) finds TF binding sites *in vivo*

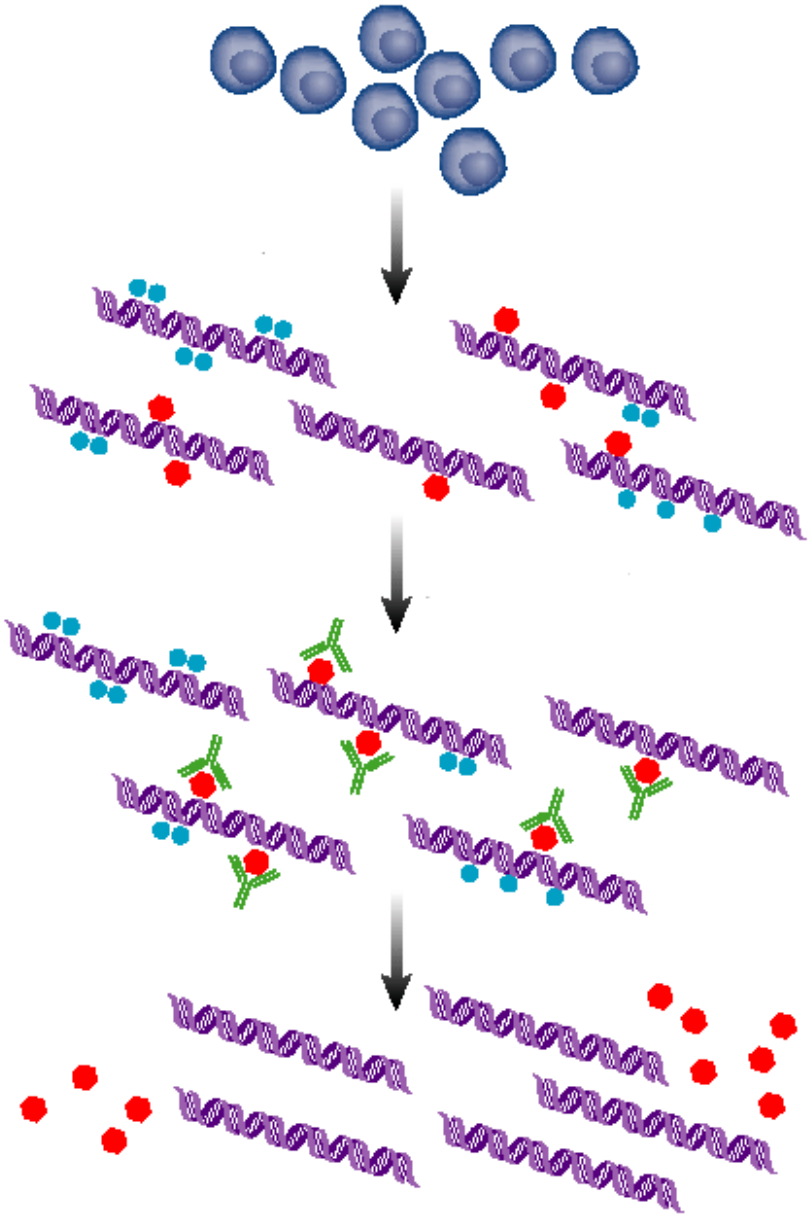
- Cross-link TF
- Fragment DNA



(Mardis, 2007)

Chromatin immunoprecipitation and sequencing (ChIP-Seq) finds TF binding sites *in vivo*

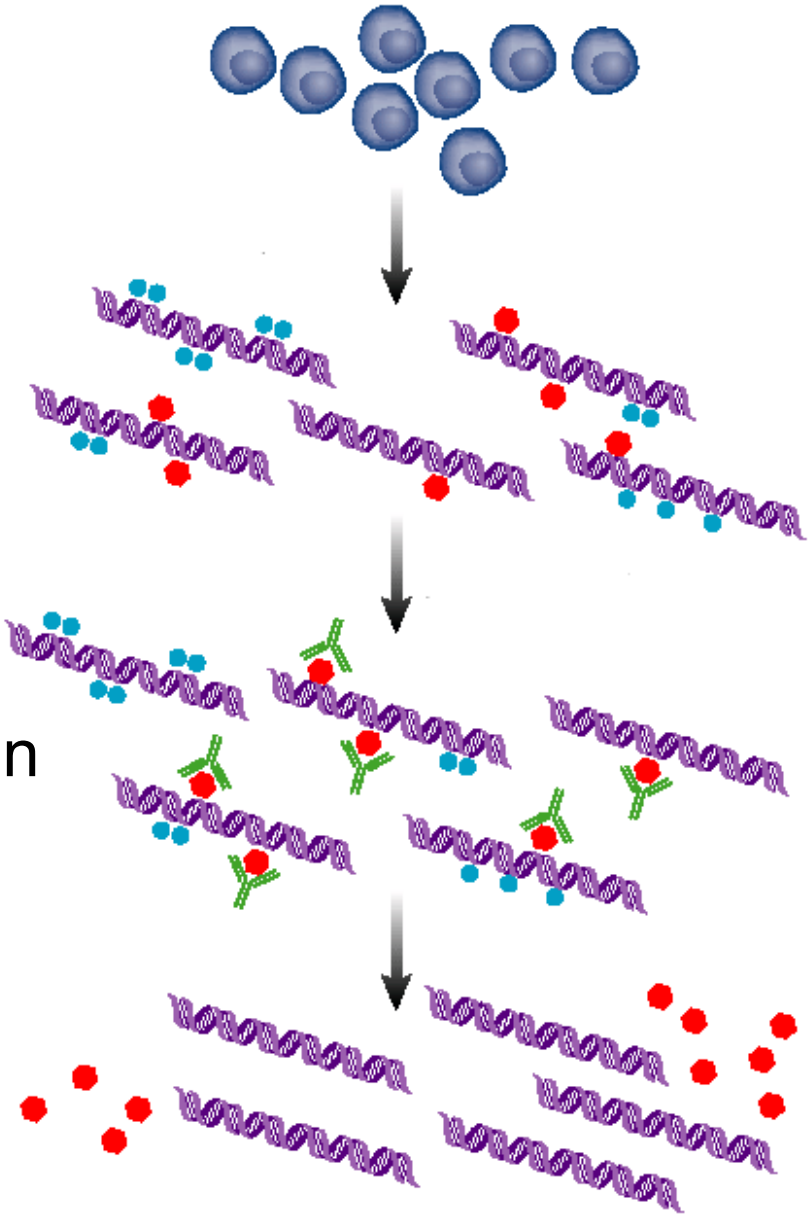
- Cross-link TF
- Fragment DNA
- Extract with antibody
- Reverse crosslink
- Sequence fragments



(Mardis, 2007)

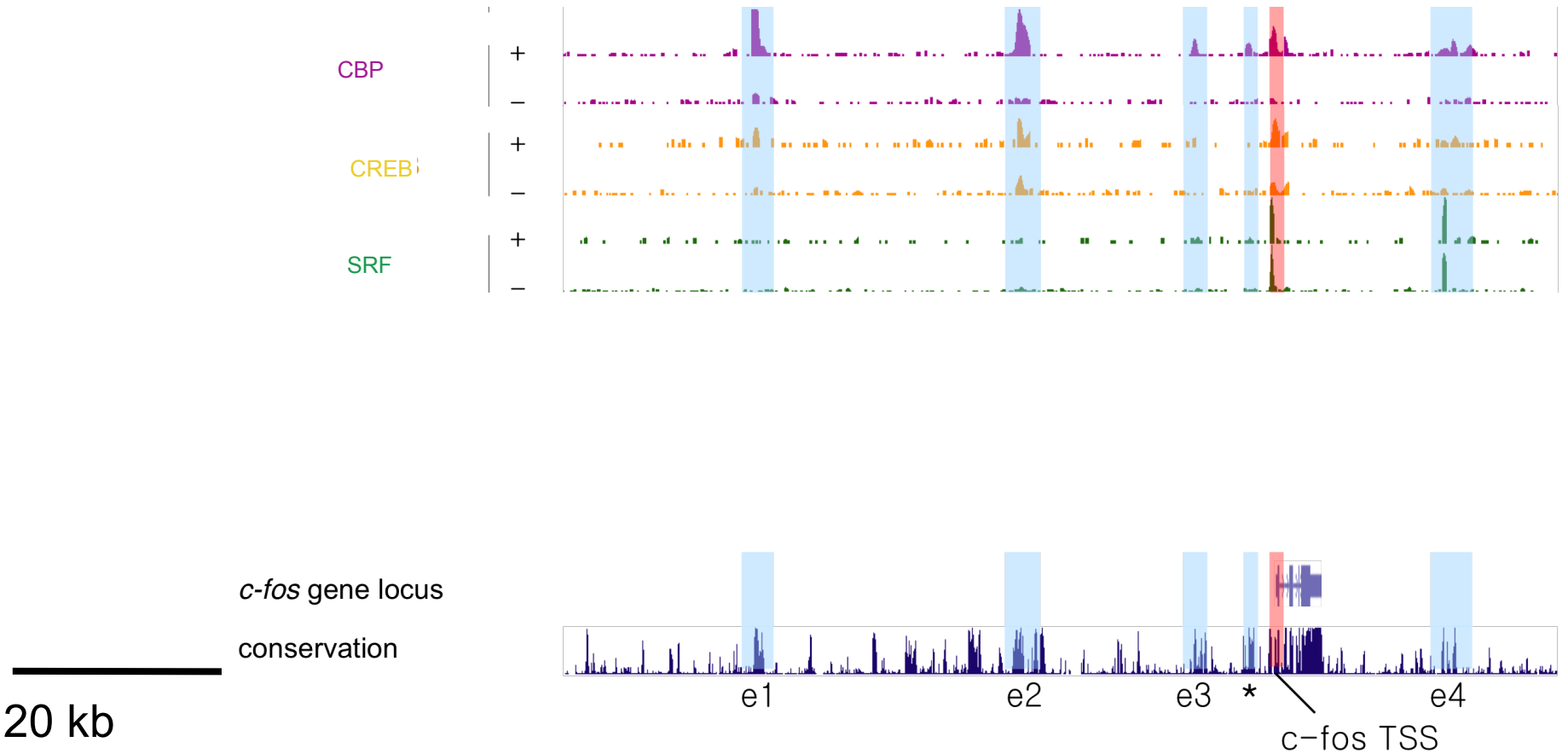
Chromatin immunoprecipitation and sequencing (ChIP-Seq) finds TF binding sites *in vivo*

- Cross-link TF
- Fragment DNA
- Extract with antibody
- Reverse crosslink
- Sequence fragments
  - Before and after KCl stimulation
  - CREB, SRFCBP, RNAPII
    H3K4me3, H3K4me1
  - Input



(Mardis, 2007)

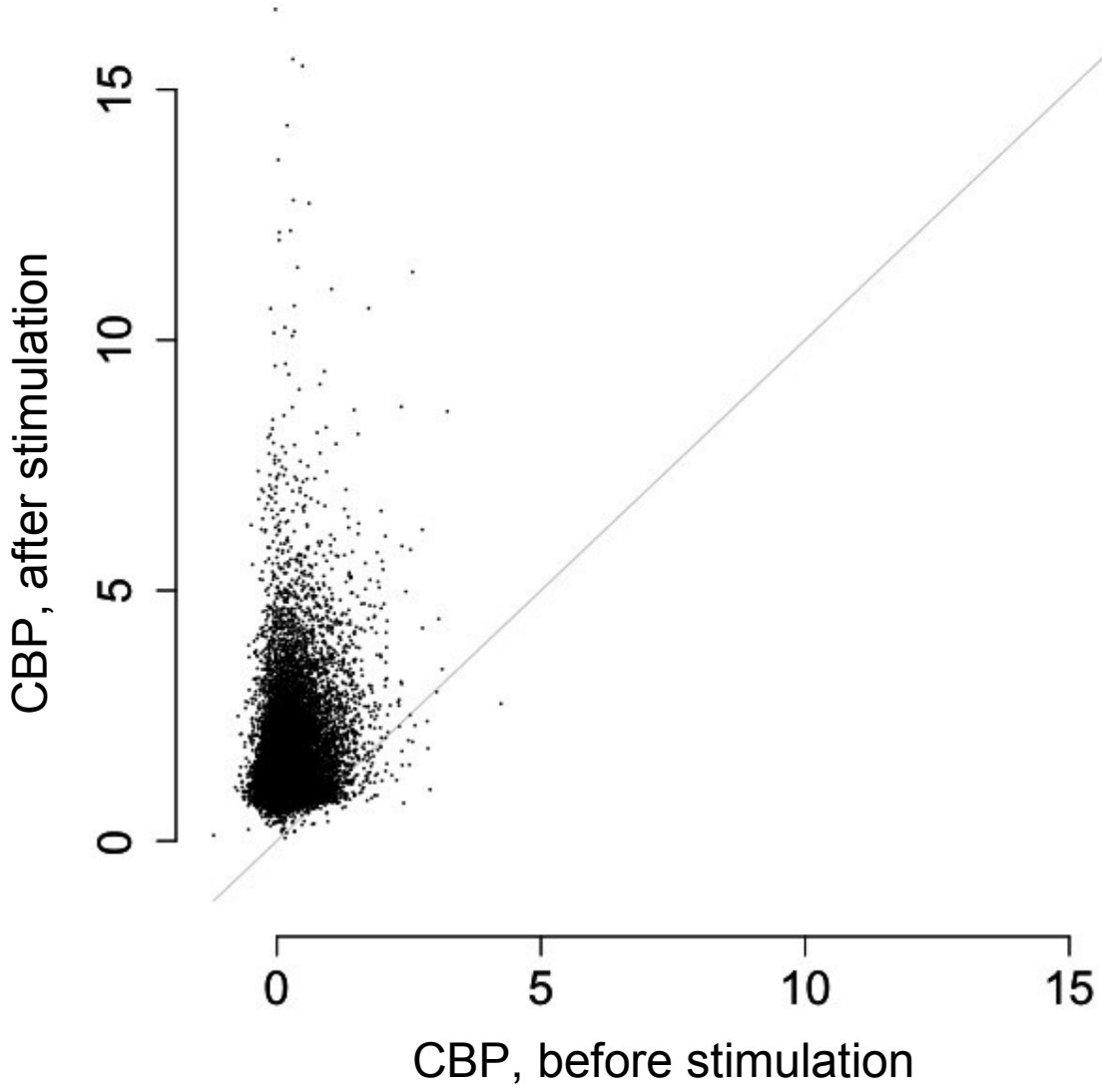
CBP binding depends strongly on activity at the *fos* promoter and flanking loci



Identifying regions with larger than expected number of ChIP-Seq reads

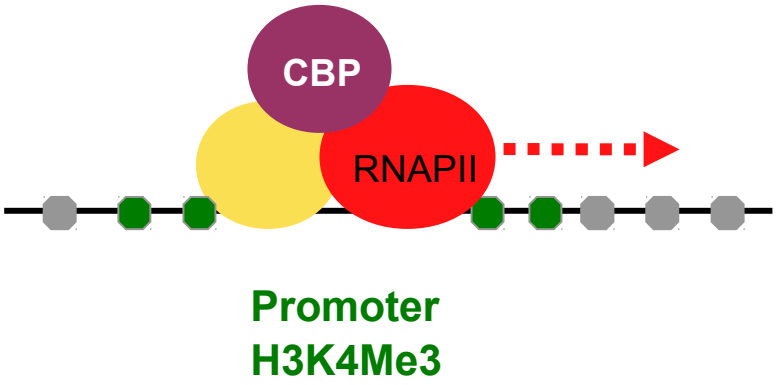
- Assume both ChIP and input ~ Poisson(λ_c)
  - ~1 read/100 bps
  - Sliding window of size 240 bps
- Z = #ChIP reads - #Input reads ~ Skellam(λ_c,λ_i)
- False Detection Ratio (FDR) determines cut-off
  - Controls for expected number of false positives

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

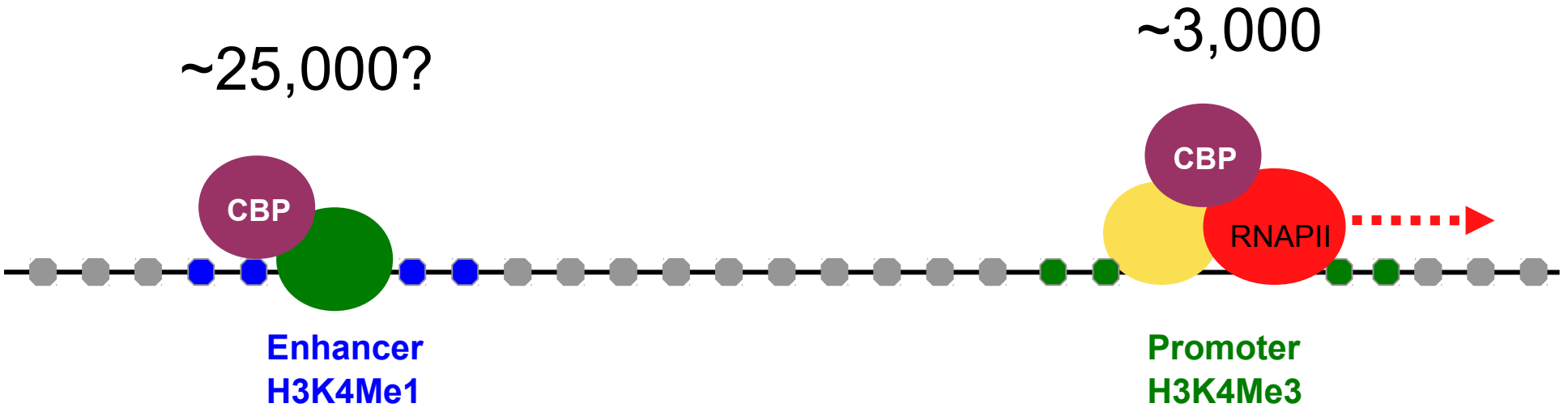


Only ~3000 CBP peaks at promoters

~3,000

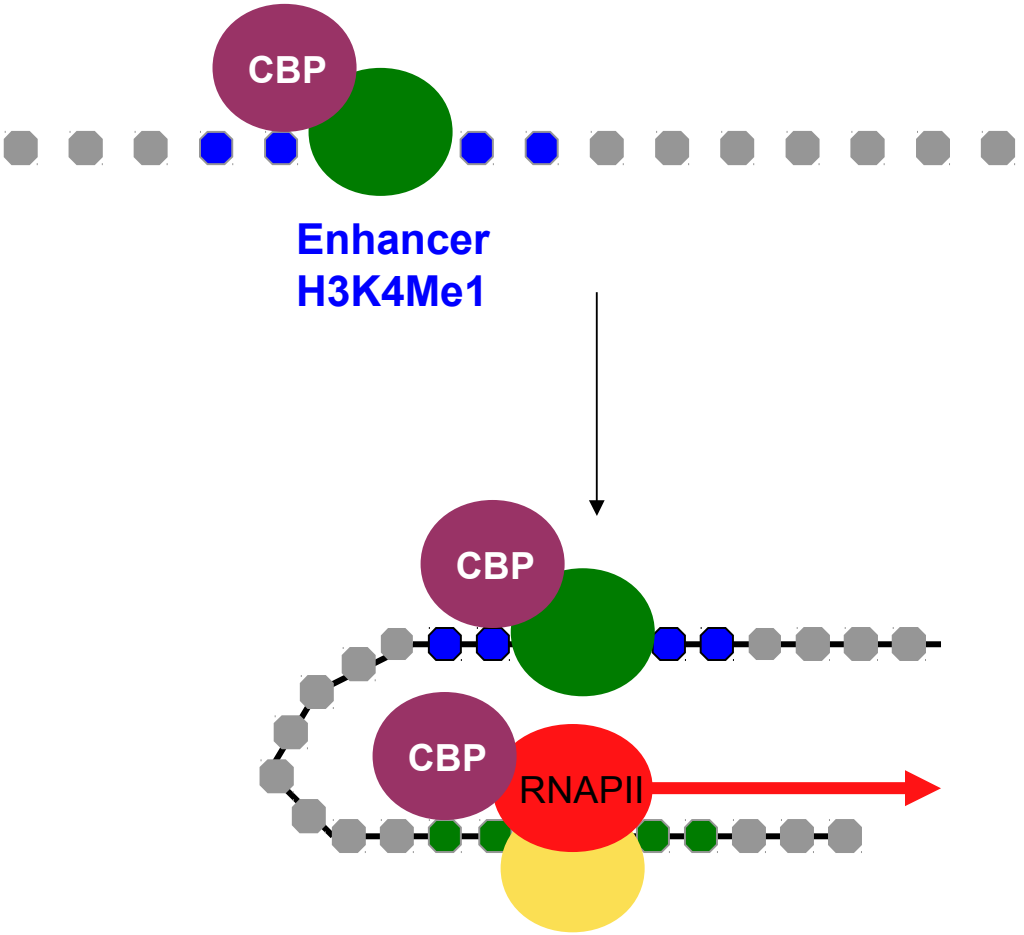


CBP hypothesized to bind at enhancers



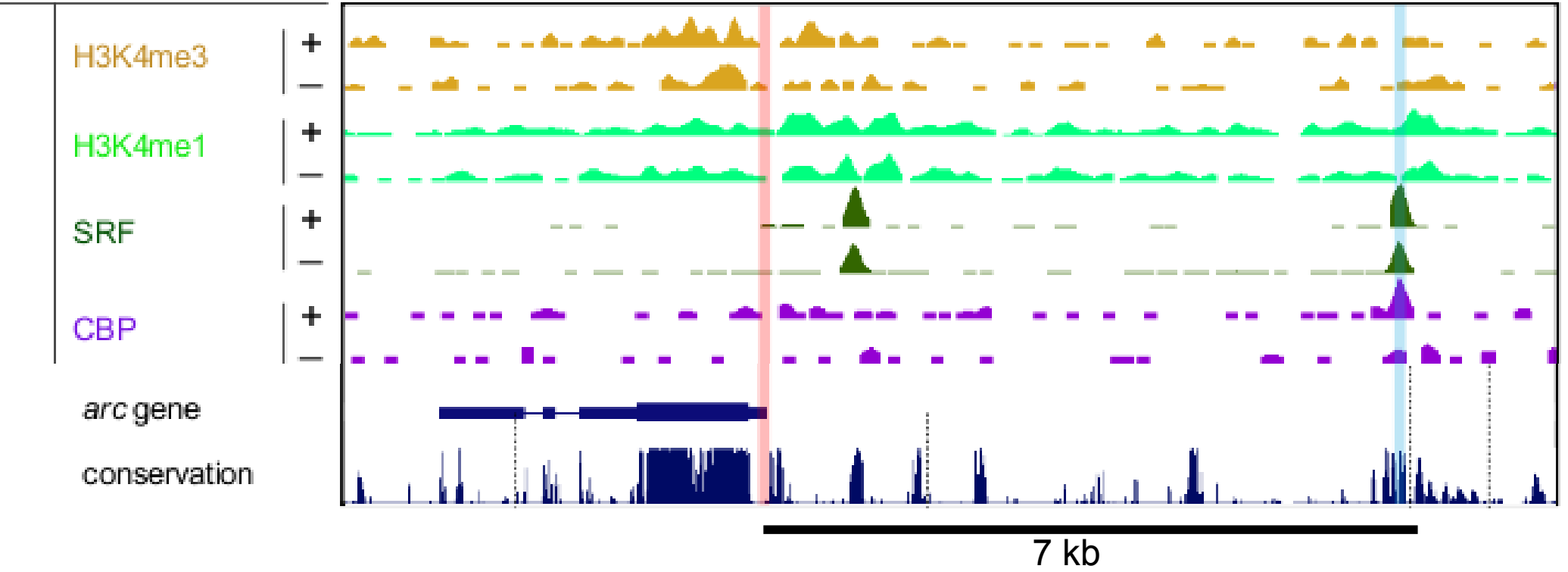
Enhancers are distal TF binding sites

- Various mechanisms for interaction with promoters suggested
- Marked by high levels of H3K4me1

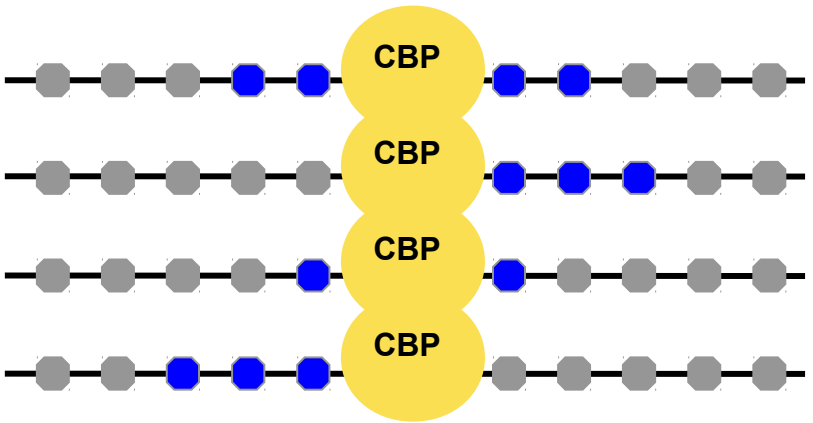


ENCODE, 2007
Heintzman et al, 2007
Roh et al, 2005
Visel et al, 2009

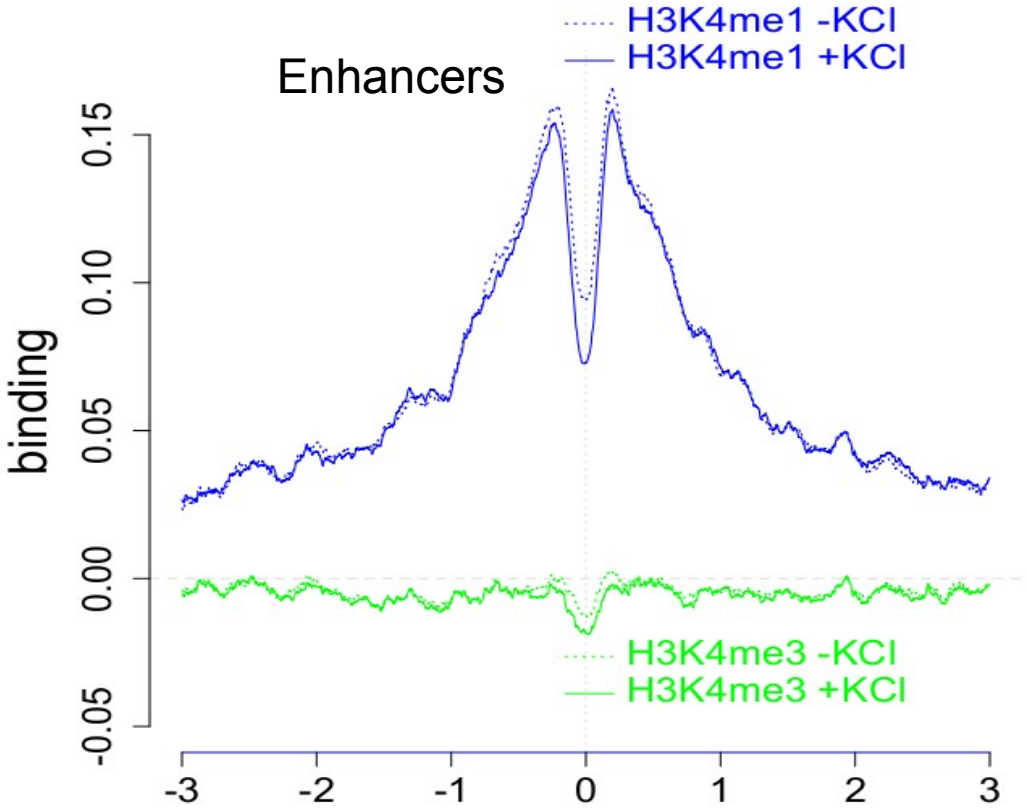
Distal CBP peaks have high levels of H3K4me1 but not H3K4me3

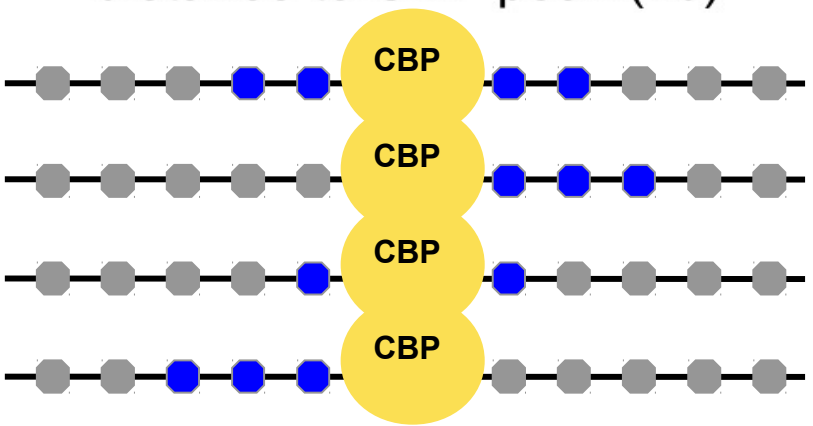


Aligning CBP peaks to calculate average binding profiles

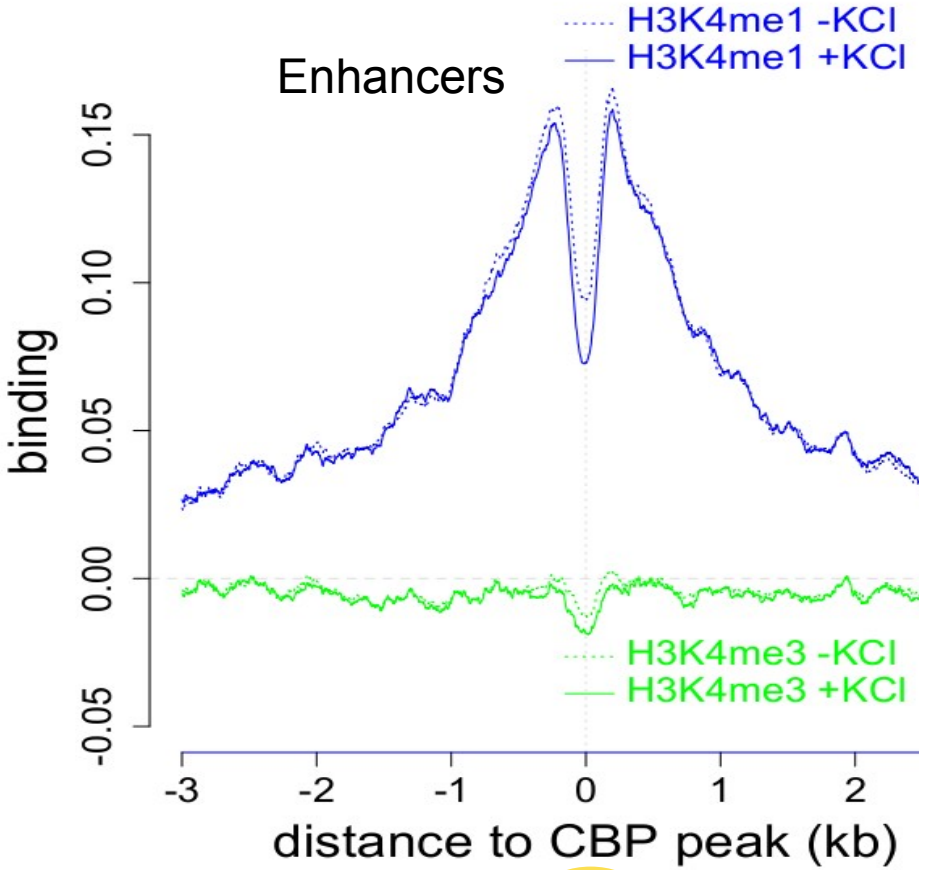


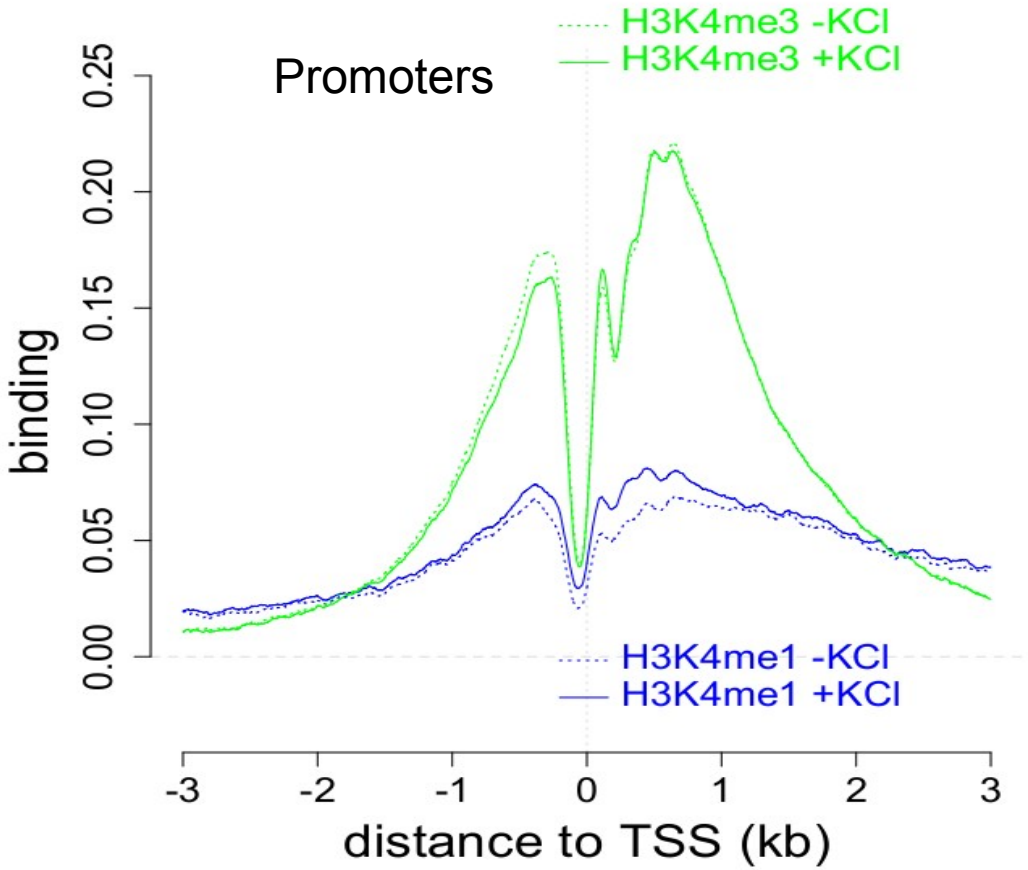
Most CBP peaks have high levels of H3K4me1 but not H3K4me3

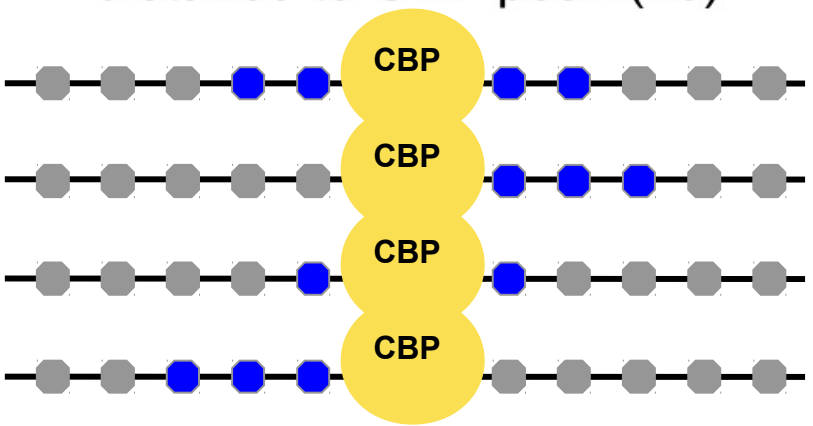




Transcription start sites (TSSs) have high levels of H3K4me1 and H3K4me3





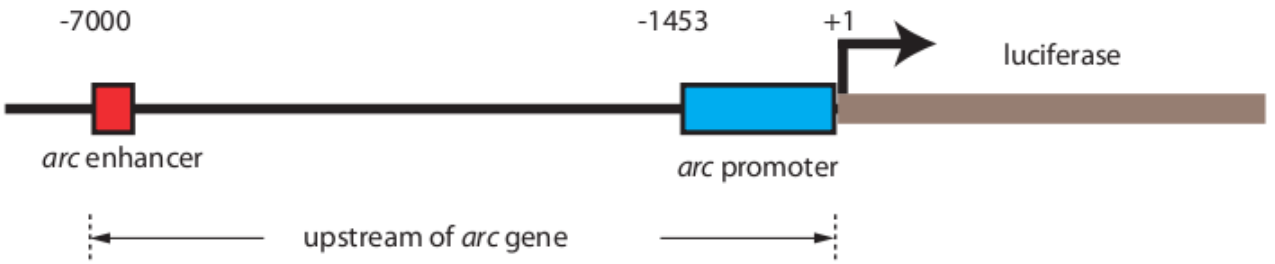


Identifying 5130 activity regulated enhancers

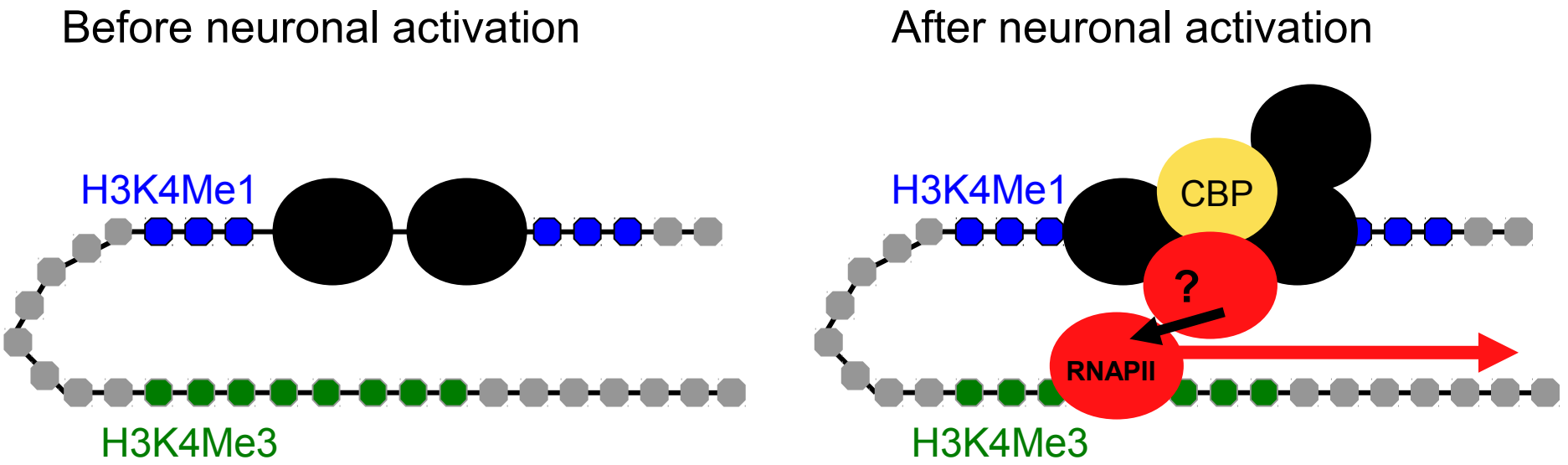
- CBP peak
- High levels of flanking H3K4me1
- Low levels of H3K4me3
- >1 kb from annotated promoter

Identifying 5130 activity regulated enhancers

- CBP peak
- High levels of flanking H3K4me1
- Low levels of H3K4me3
- >1 kb from annotated promoter
  - 8/8 validated in luciferase assay
  - ~7000 intragenic enhancers

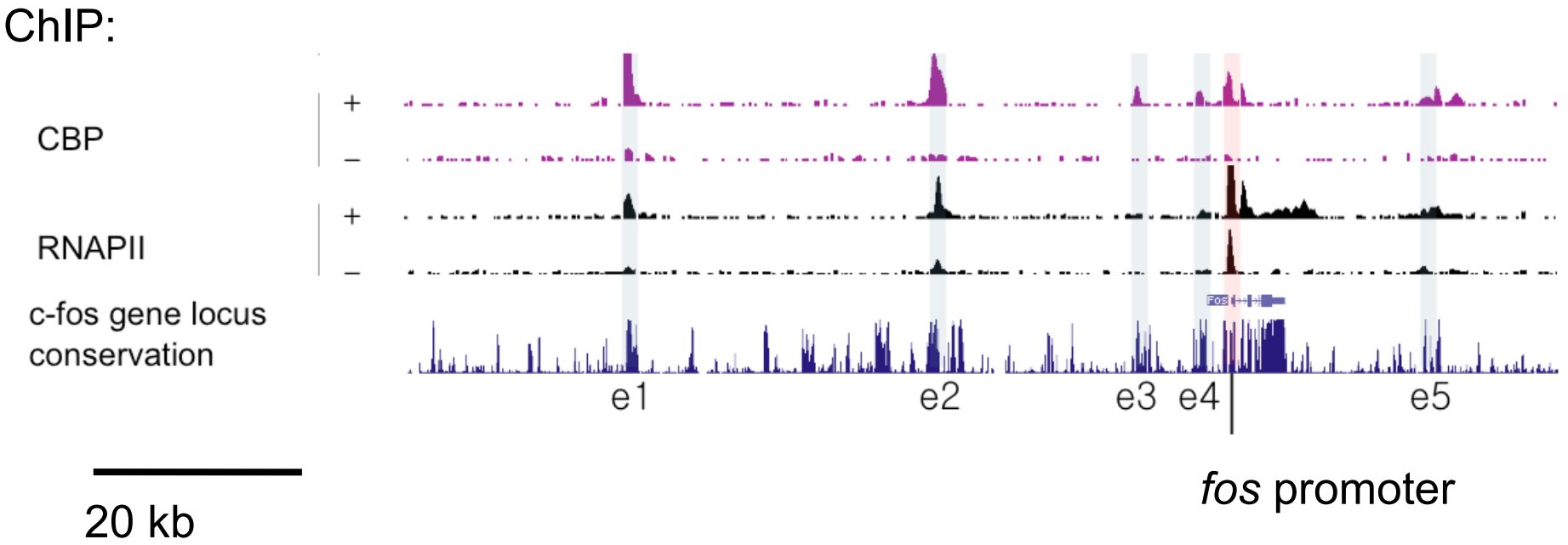


Properties of activity regulated enhancers

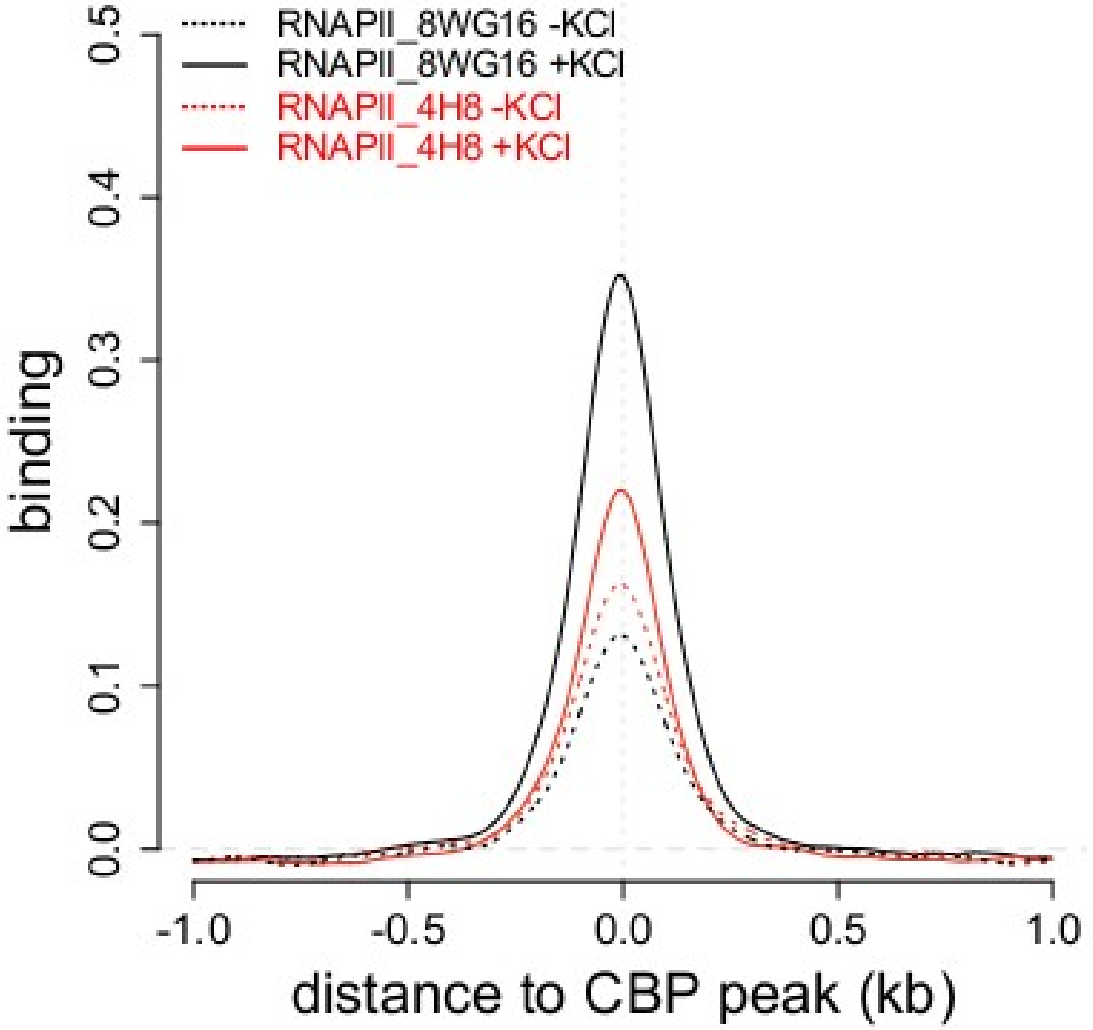


- Does RNAPII bind at enhancers?

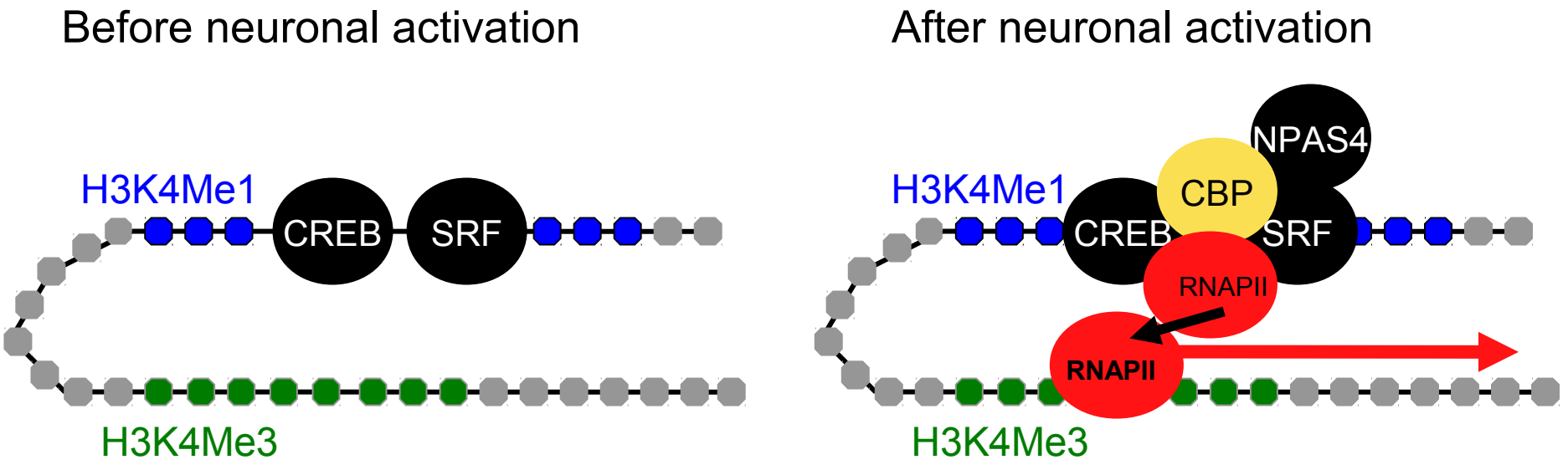
RNAPII is recruited to CBP binding sites at the *fos* locus



RNAPII is recruited at all enhancers



Properties of activity regulated enhancers



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

RNA-Seq reveals which parts of the genome are transcribed

- Fragment
- RNA → cDNA
- 35 bp reads mapped to genome

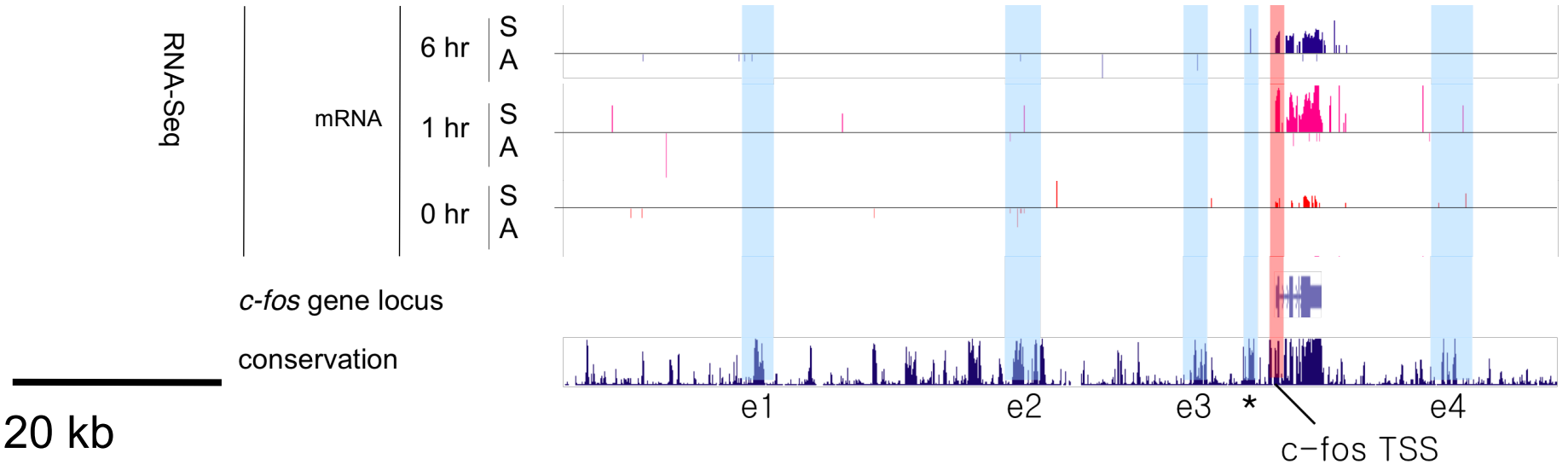
(Wang et al, 2009)

RNA-Seq reveals which parts of the genome are transcribed

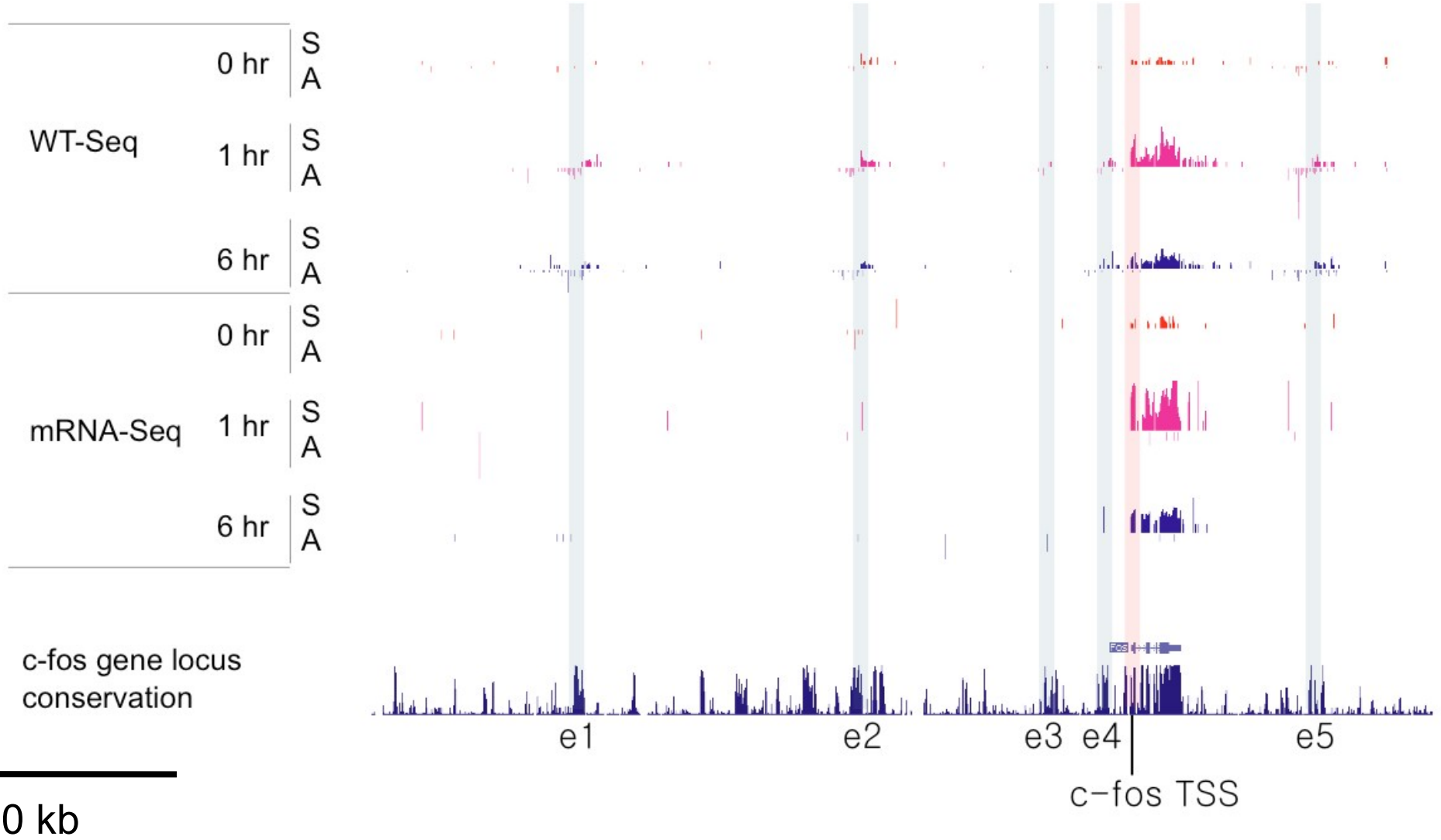
- Fragment
- RNA → cDNA
- 35 bp reads mapped to genome
  - Before and after KCl
  - Total RNA and polyA+

(Wang et al, 2009)

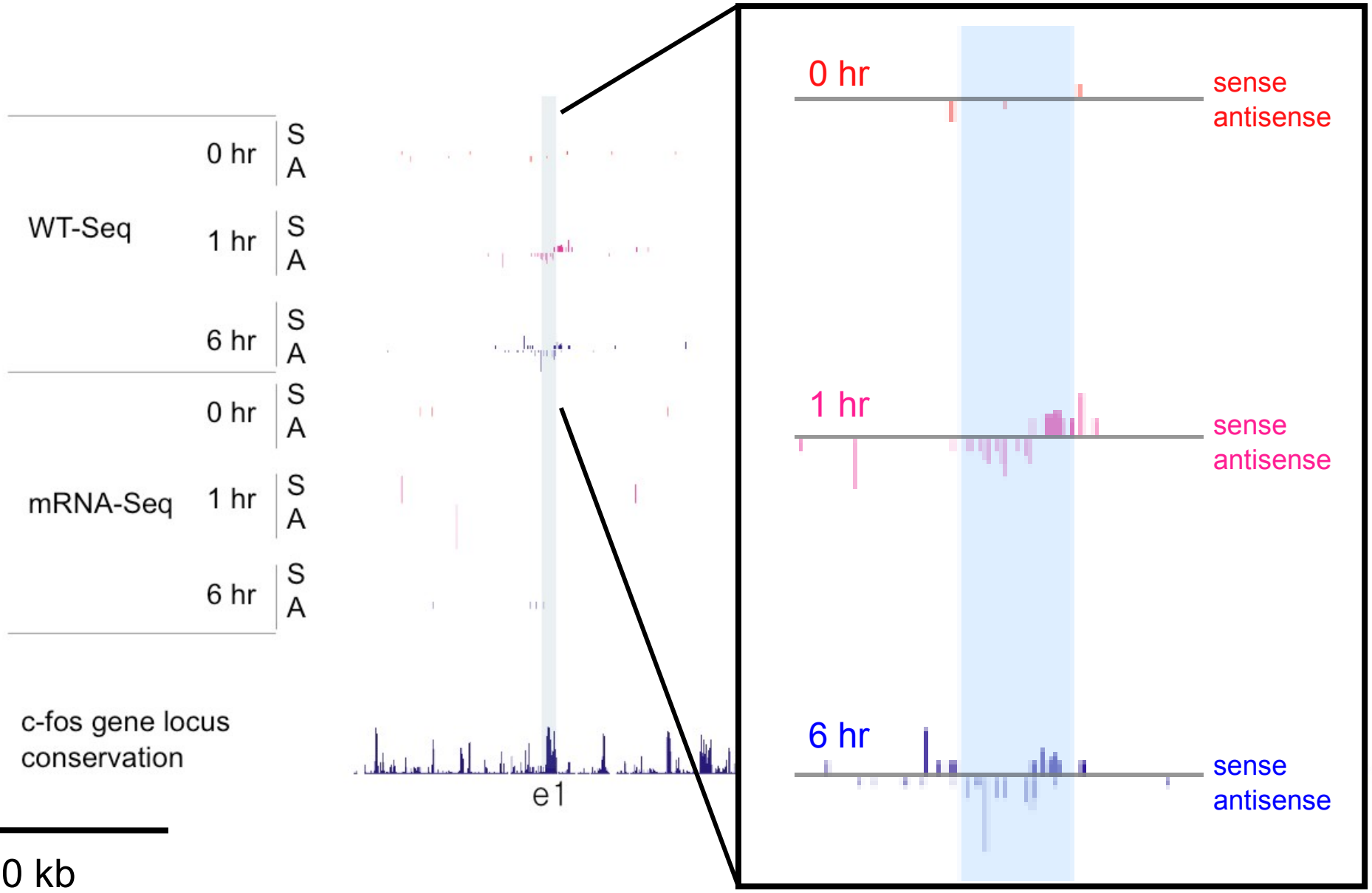
mRNA levels are strongly induced at the *fos* locus



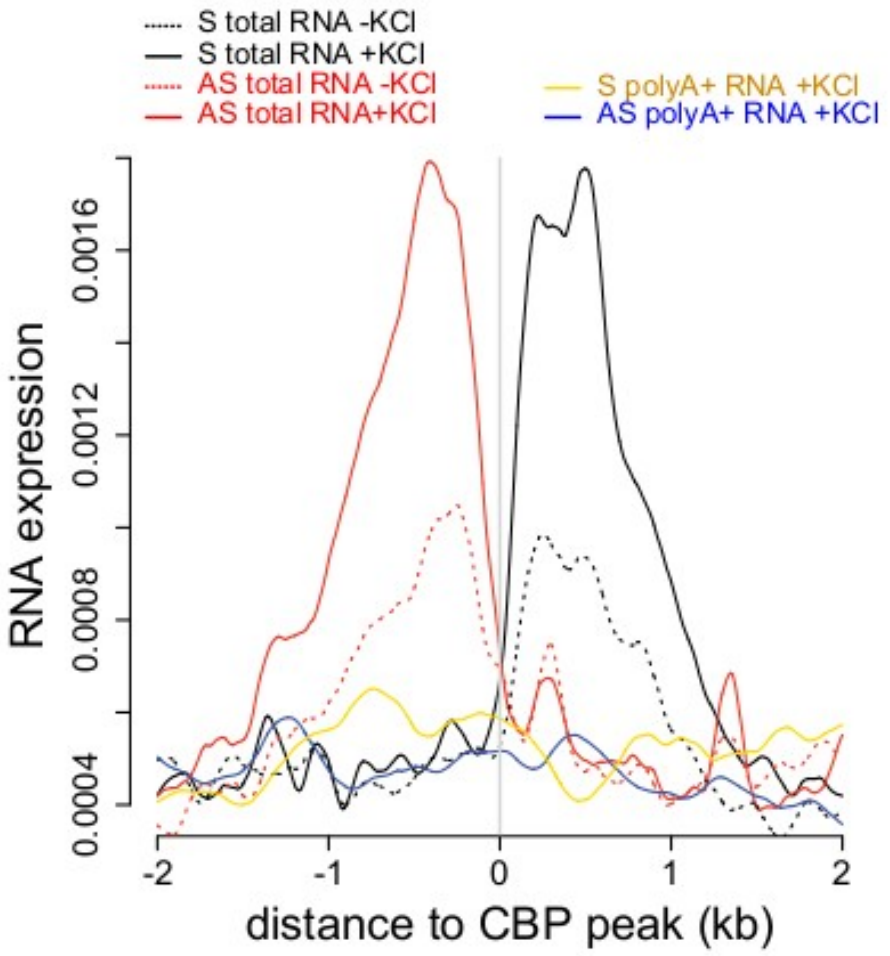
Transcription of enhancer RNA (eRNA) at the *fos* locus



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

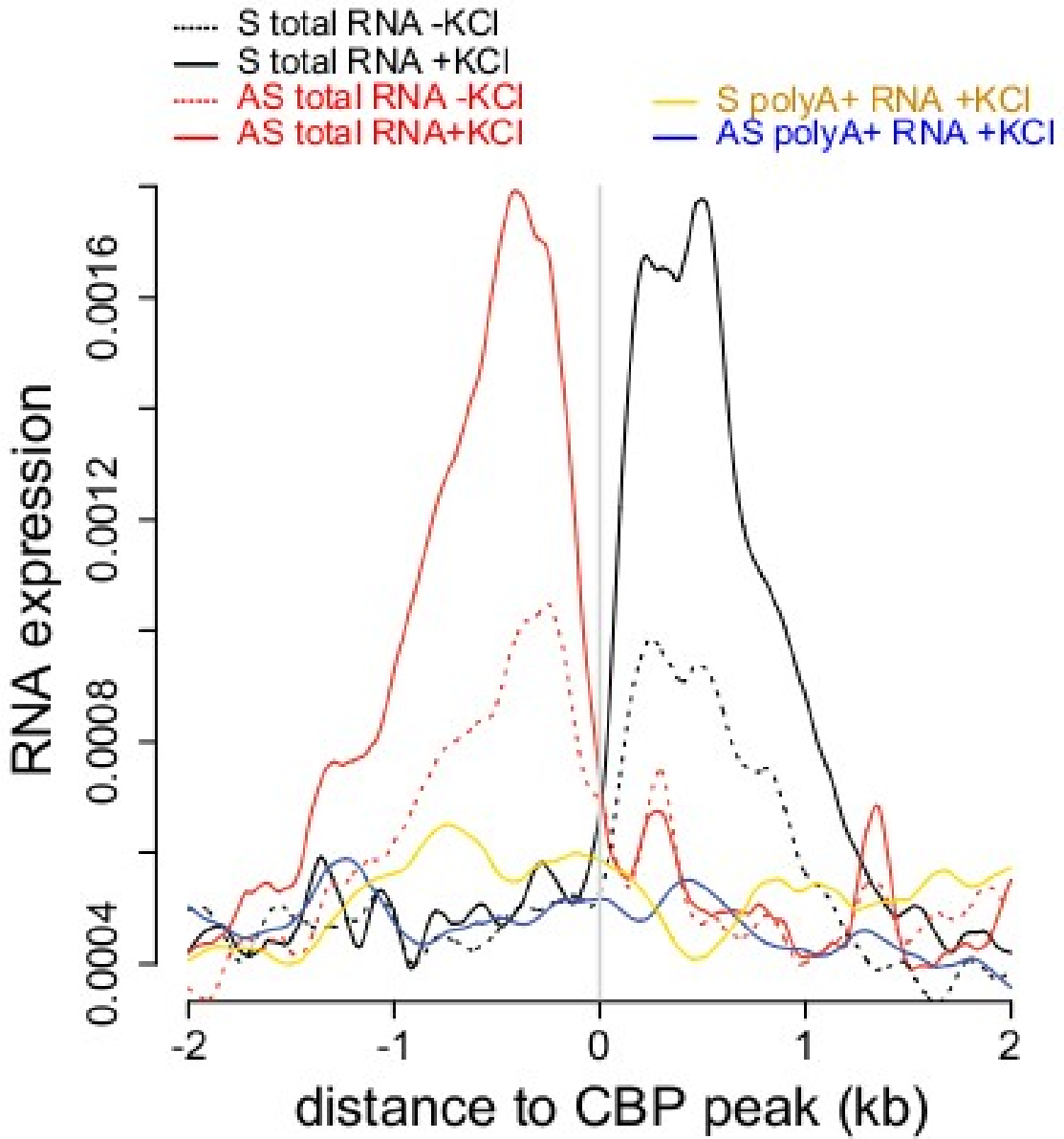


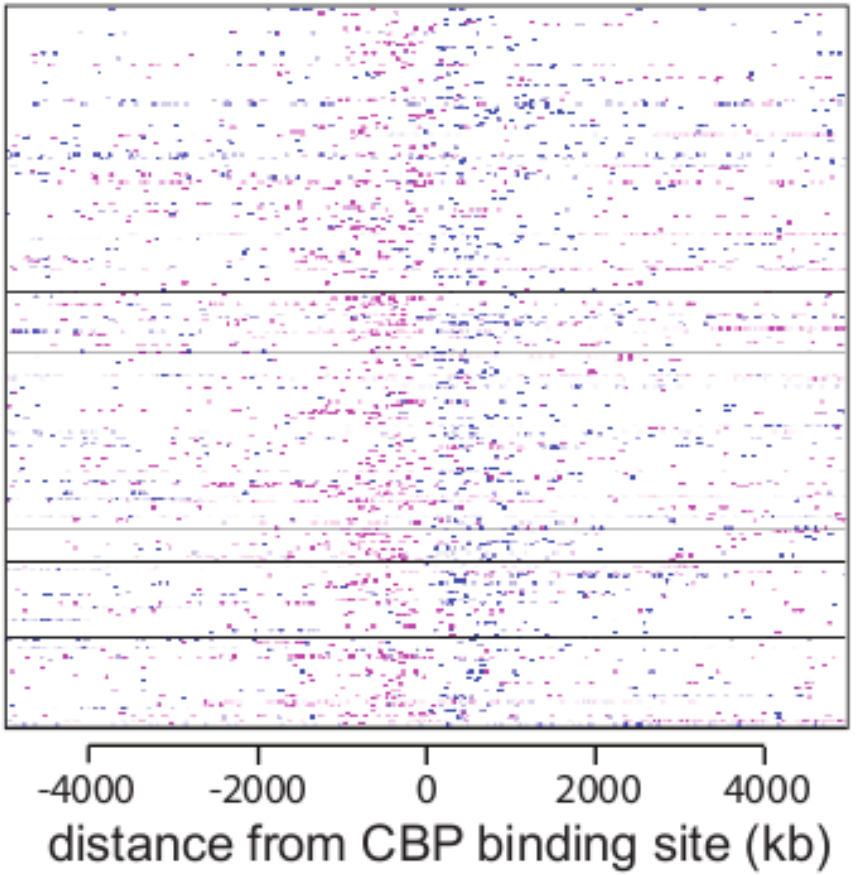
Genome-wide profile of transcription at enhancers



- Low expression
- ~1.5 kb
- Bidirectional
- No polyA-tail
- Not protein-coding

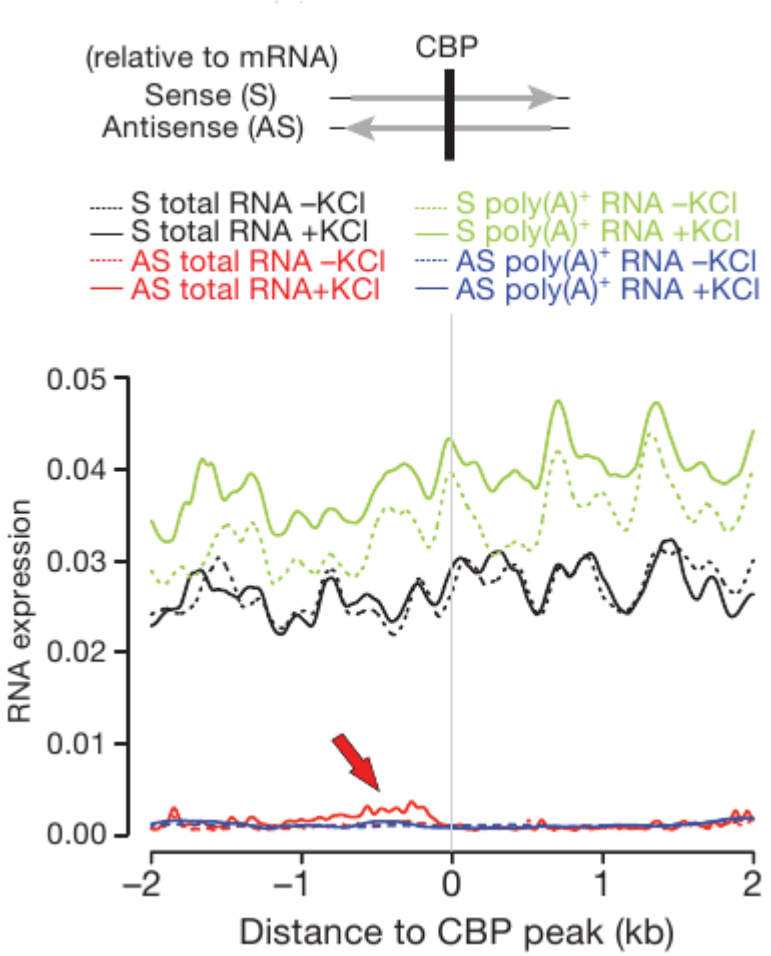
Genome-wide profile of transcription at enhancers



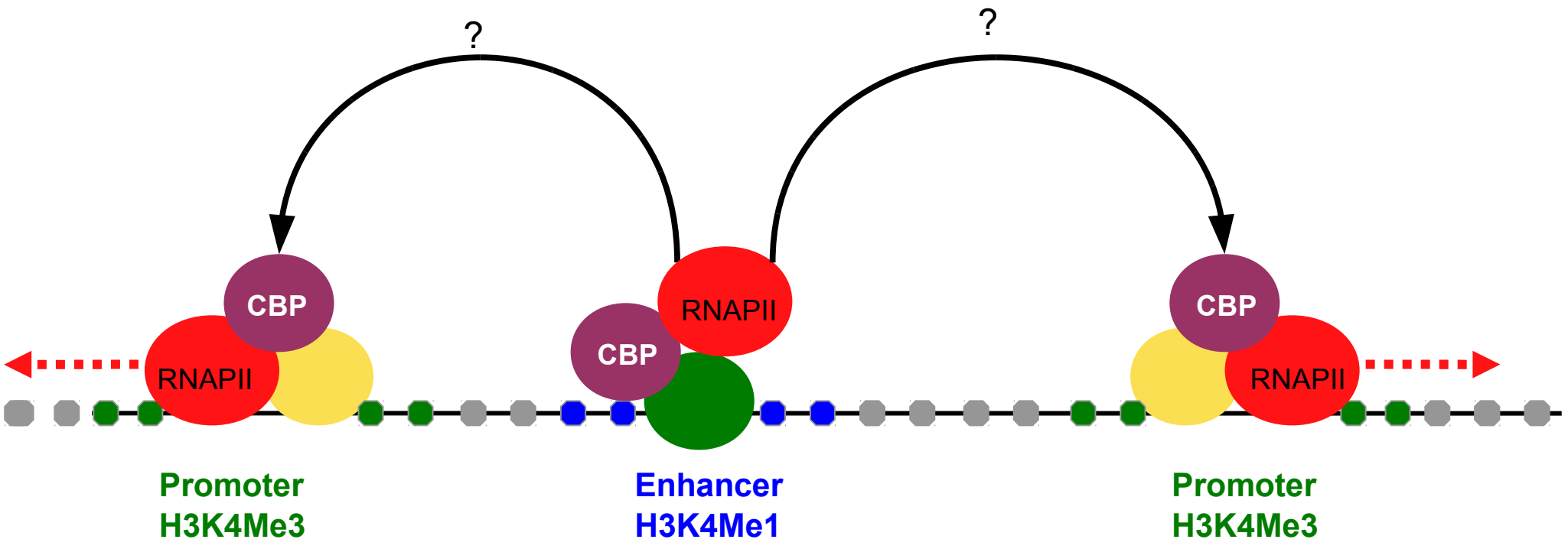


Intragenic enhancers are also transcribed

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

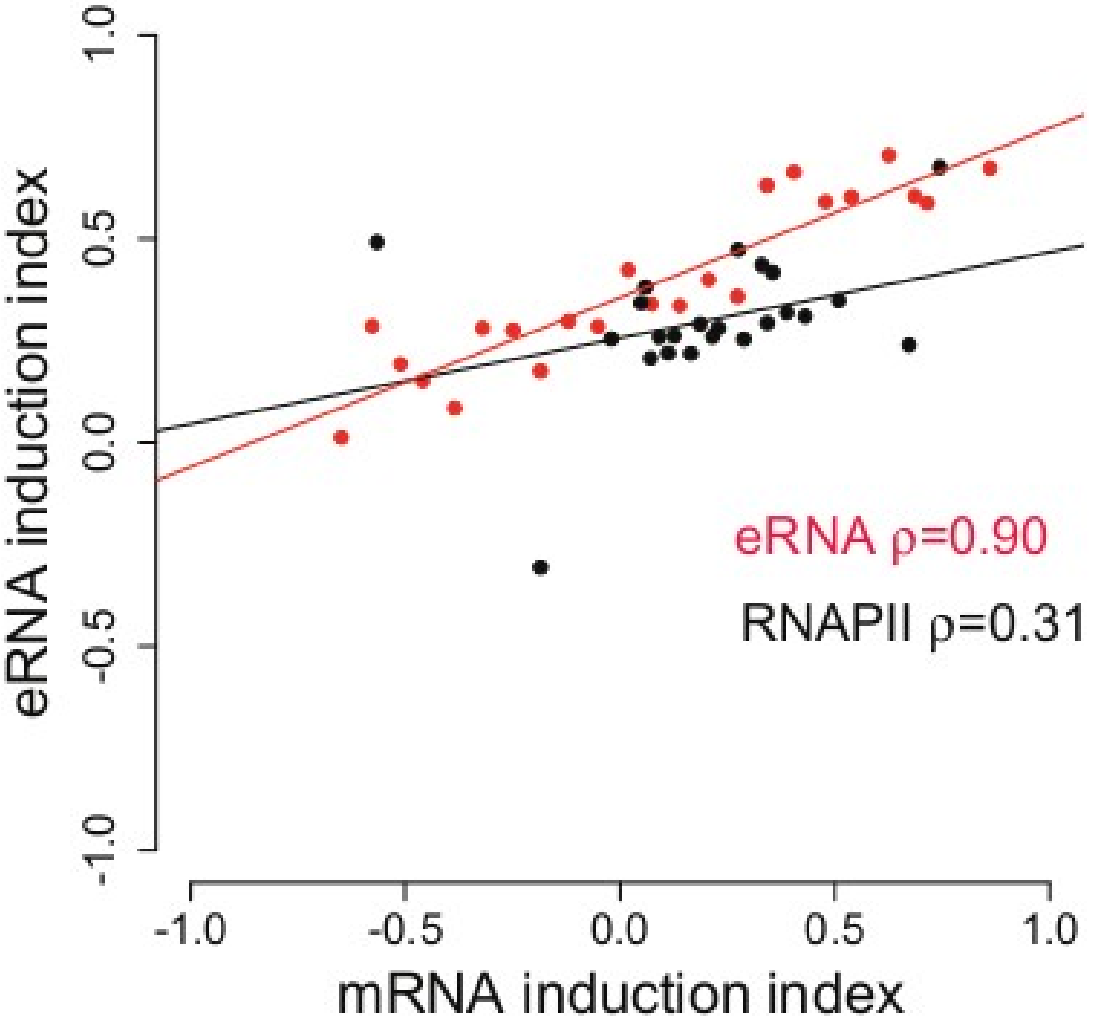


How do eRNA levels relate to mRNA levels?

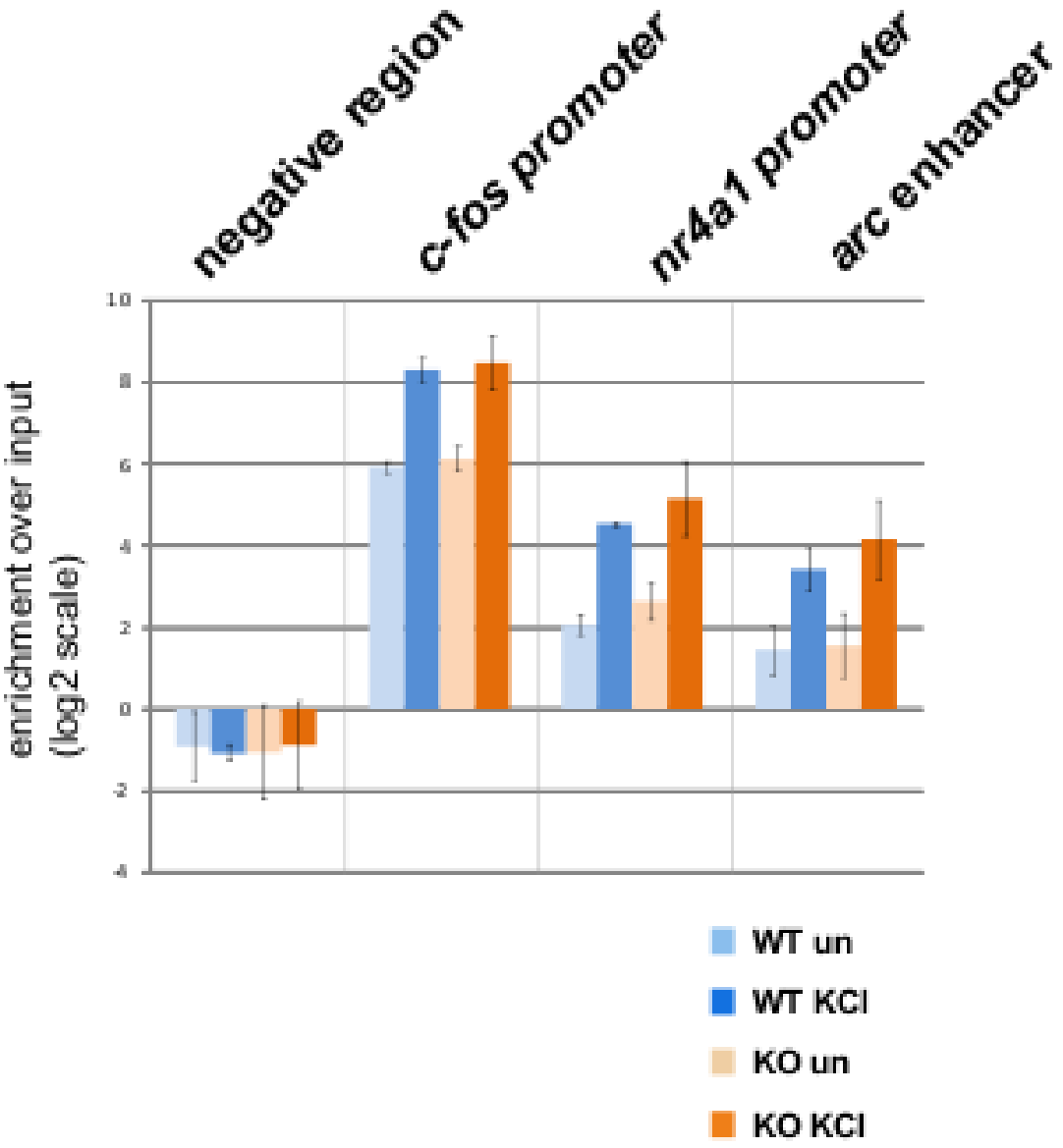


eRNA induction is correlated with induction of nearby mRNAs

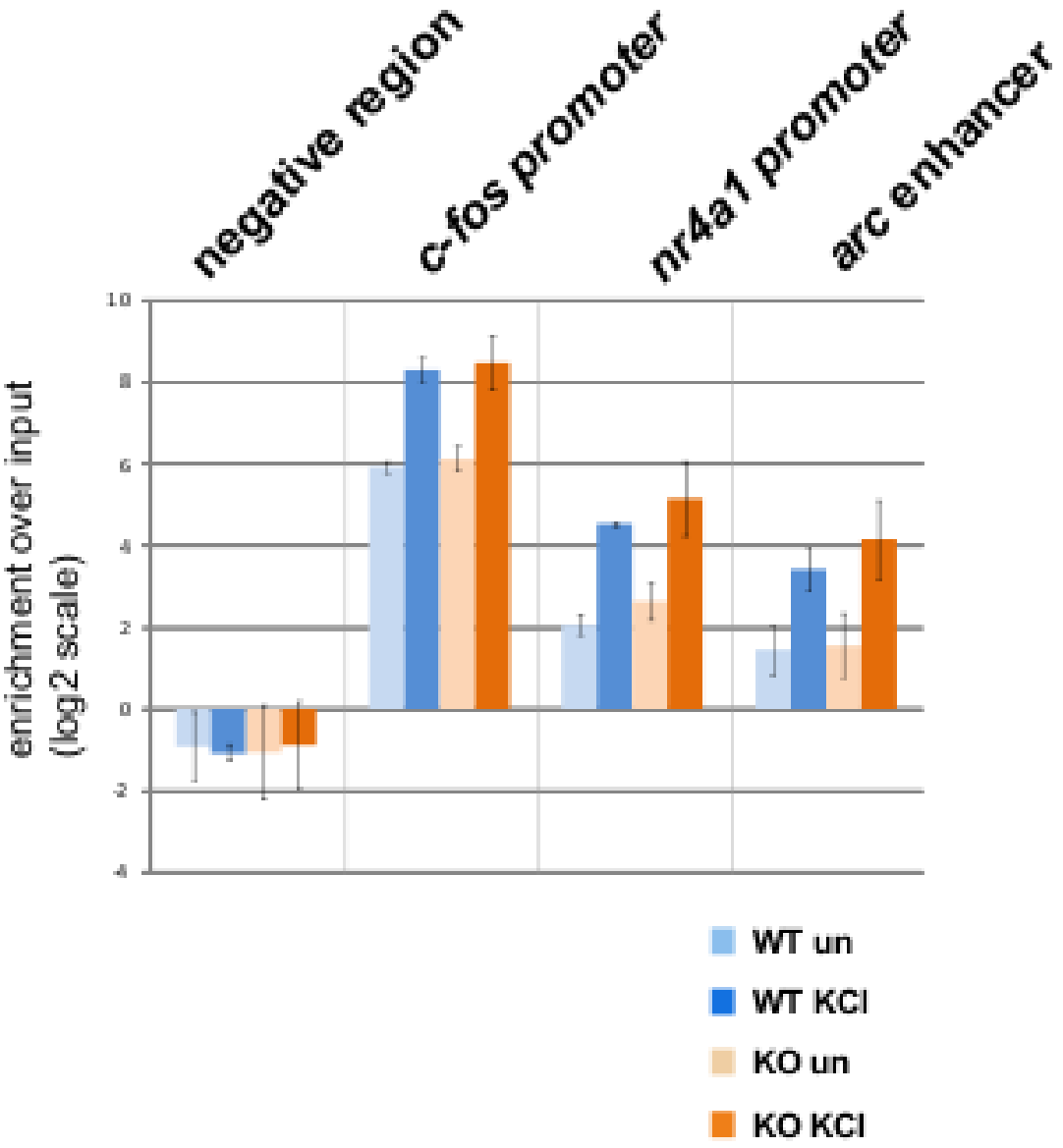
induction index = (KCl^+ – KCl^-)/(KCl^+ + KCl^-)

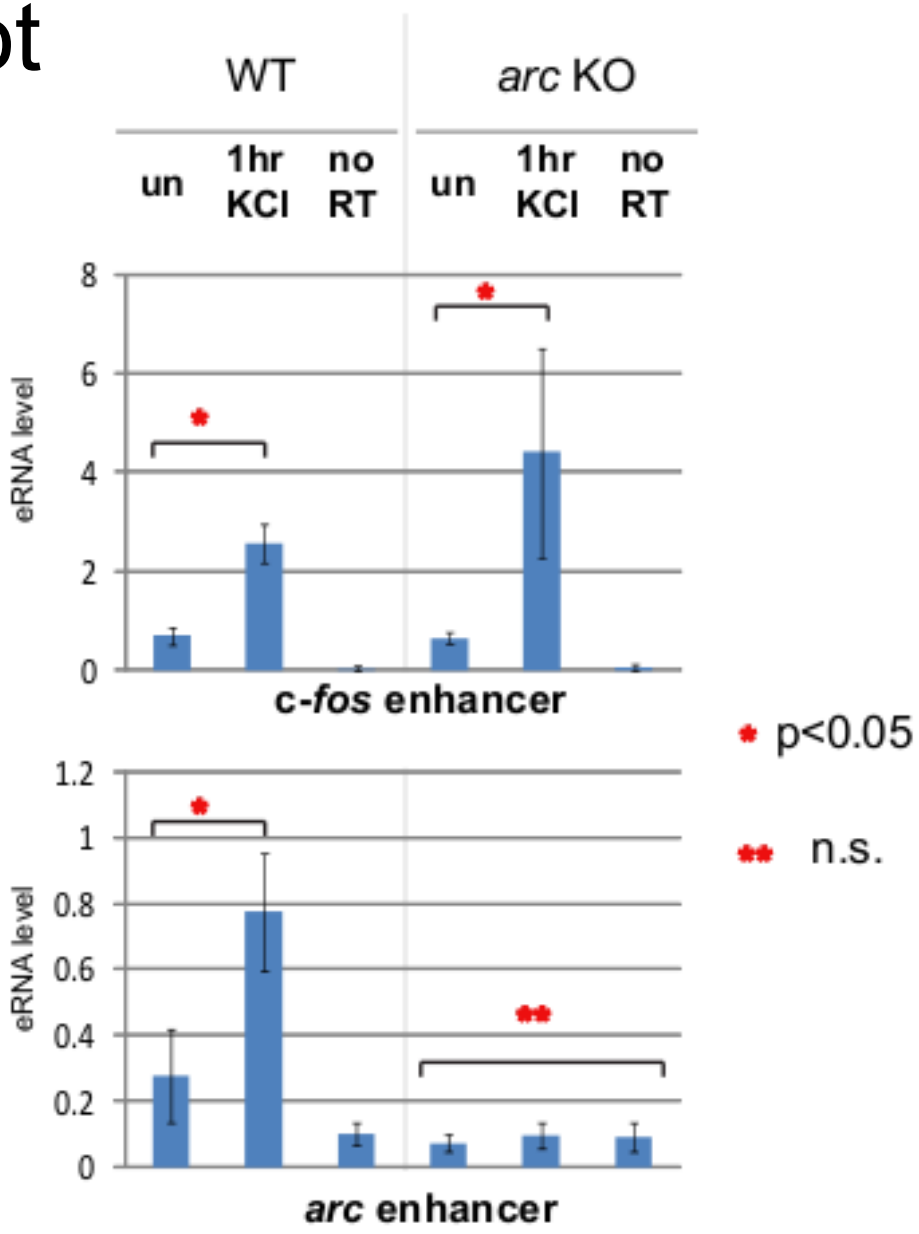


Knock-out experiment confirms that RNAPII recruitment is independent of the promoter

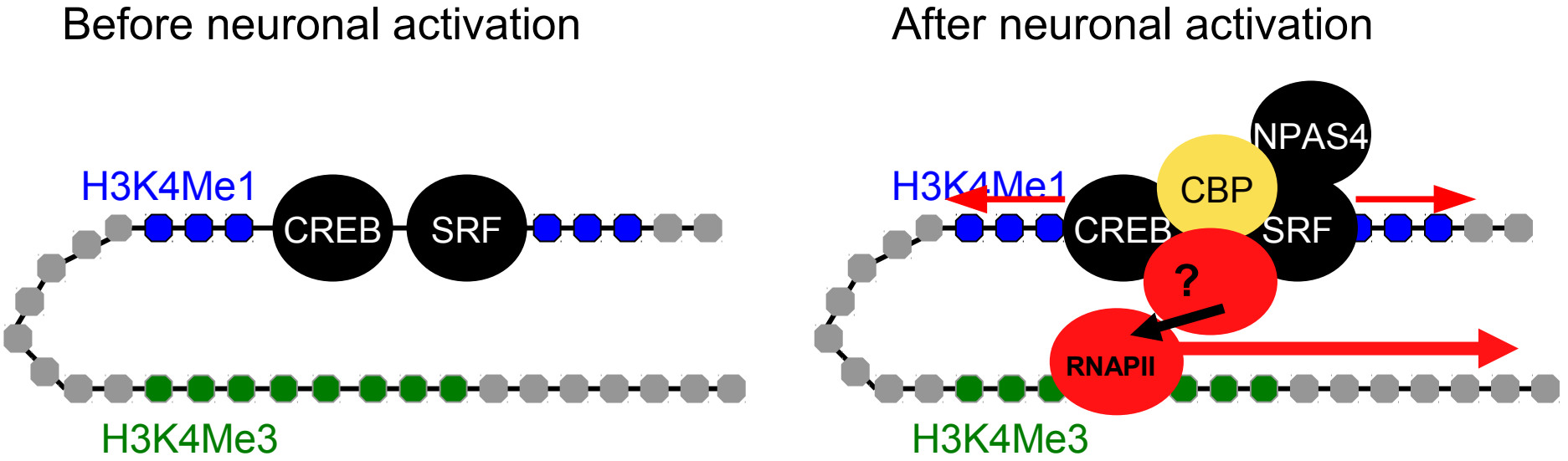


Knock-out experiment confirms that RNAPII recruitment is independent of the promoter but eRNA synthesis is not





Enhancers bind RNAPII independently, but the transcription is promoter-related



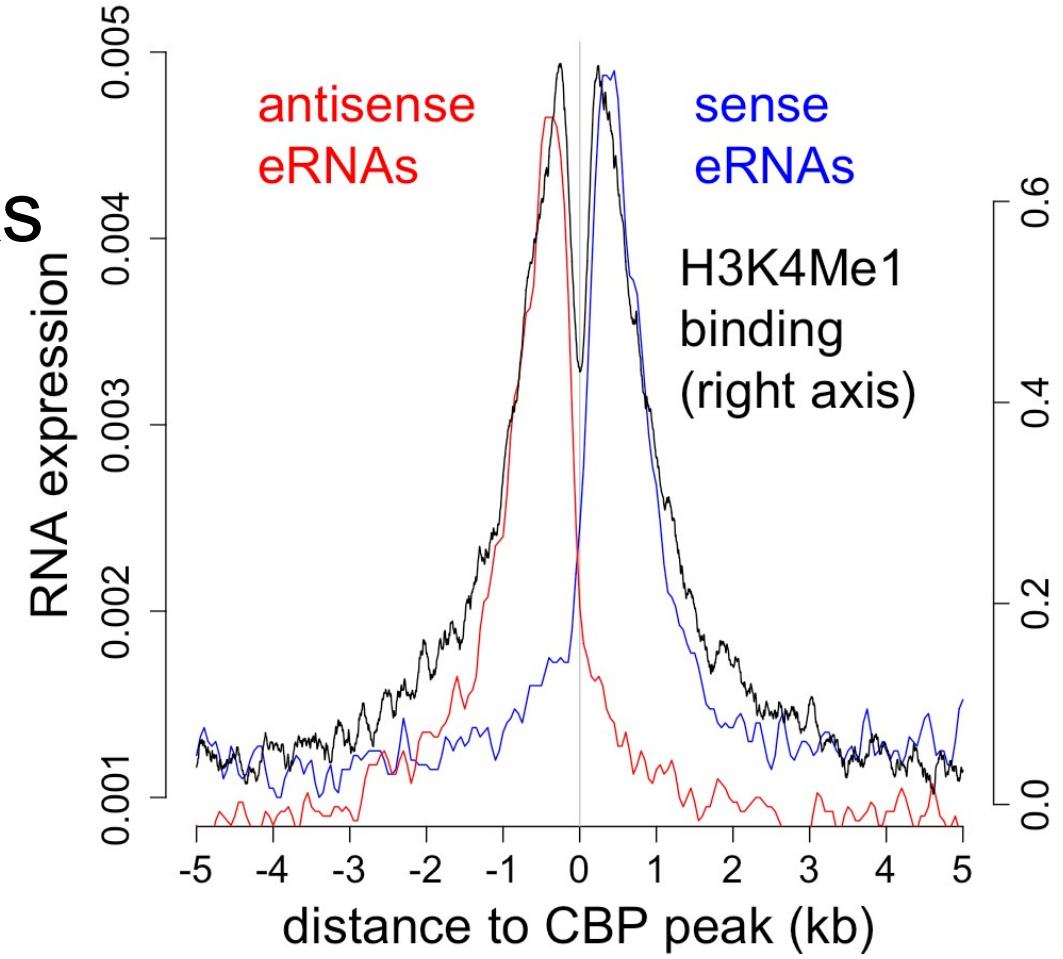
- Does RNAPII bind at enhancers? YES
- Are transcripts produced at enhancers? YES
- Is RNAPII recruitment independent? YES
- Is eRNA production independent? NO

We have not yet been able to determine the function of eRNAs

Science is always wrong. It never solves a problem without creating ten more.

-George Bernard Shaw

- Noise
- Establish histone marks
- Transcript has function



eRNAs have been found in other cell types

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]

Histone H3K27ac separates active from poised enhancers and predicts developmental state

Menno P. Creyghton[a,1], Albert W. Cheng[a,b,1], G. Grant Welstead[a], Tristan Kooistra[c,d], Bryce W. Carey[a,e], Eveline J. Steine[a,e], Jacob Hanna[a], Michael A. Lodato[a,e], Garrett M. Frampton[a,e], Phillip A. Sharp[d,e], Laurie A. Boyer[e], Richard A. Young[a,e], and Rudolf Jaenisch[a,e,2]

OPEN ACCESS Freely available online PLoS BIOLOGY

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]*

LETTER

doi:10.1038/nature09692

A unique chromatin signature uncovers early developmental enhancers in humans

Alvaro Rada-Iglesias[1], Ruchi Bajpai[1], Tomek Swigut[1], Samantha A. Brugmann[1], Ryan A. Flynn[1] & Joanna Wysocka[1,2]

Summary I:Identified ~12,000 activity regulated enhancers that are transcribed

- Histone modification and CBP binding
- Transcription induction correlated with nearby promoter

What is the function of conserved non-coding sequences?

Evolution at Two Levels in Humans and Chimpanzees

Their macromolecules are so alike that regulatory mutations may account for their biological differences.

Mary-Claire King and A. C. Wilson

What is the function of conserved non-coding sequences?

Evolution at Two Levels in Humans and Chimpanzees

Their macromolecules are so alike that regulatory mutations may account for their biological differences.

Mary-Claire King and A. C. Wilson

Large-Scale Transcriptional Activity in Chromosomes 21 and 22

Philipp Kapranov,[1] Simon E. Cawley,[1] Jorg Drenkow,[1]
Stefan Bekiranov,[1] Robert L. Strausberg,[2] Stephen P. A. Fodor,[1]
Thomas R. Gingeras[1]*

OPEN ACCESS Freely available online PLoS BIOLOGY

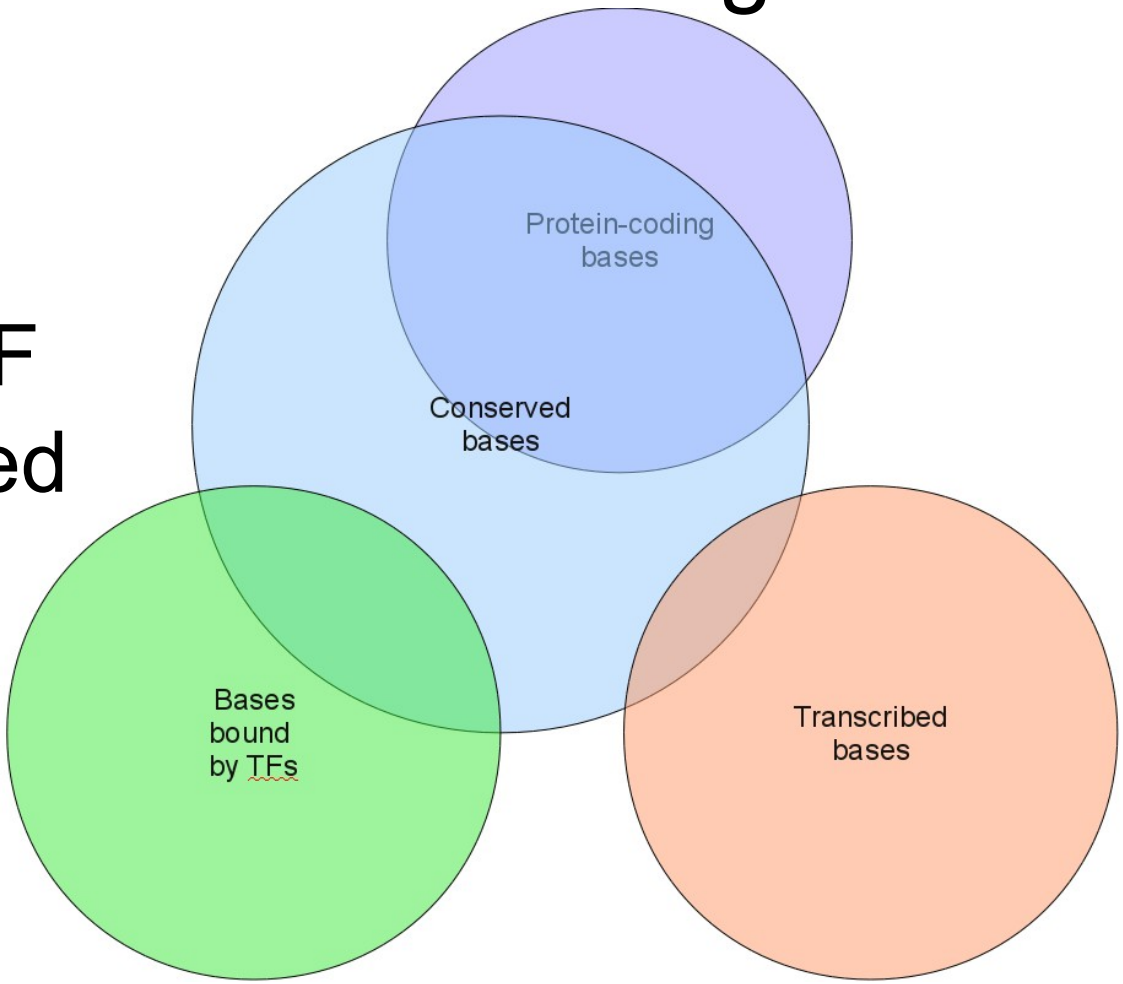
Most "Dark Matter" Transcripts Are Associated With Known Genes

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

What drives the conservation of extragenic regions?

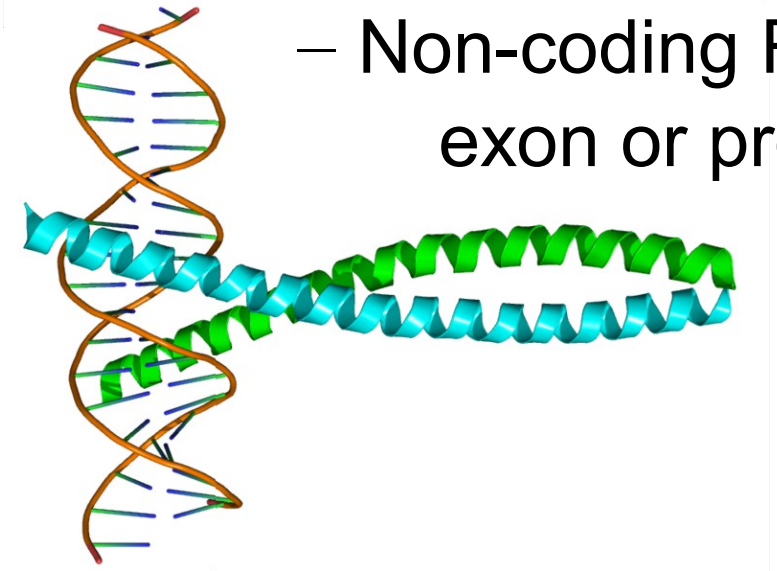
- Compare extragenic transcription and TF binding to conserved bases

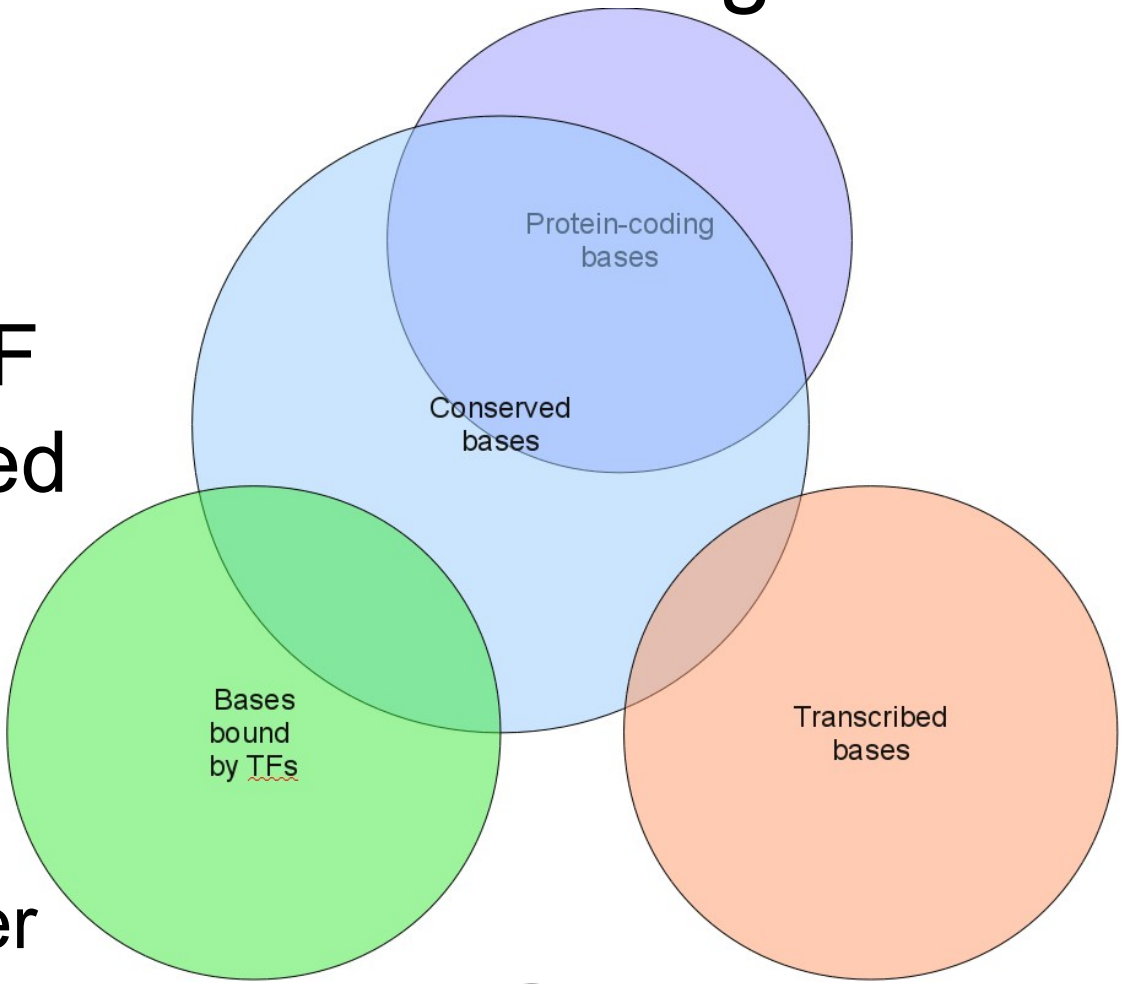


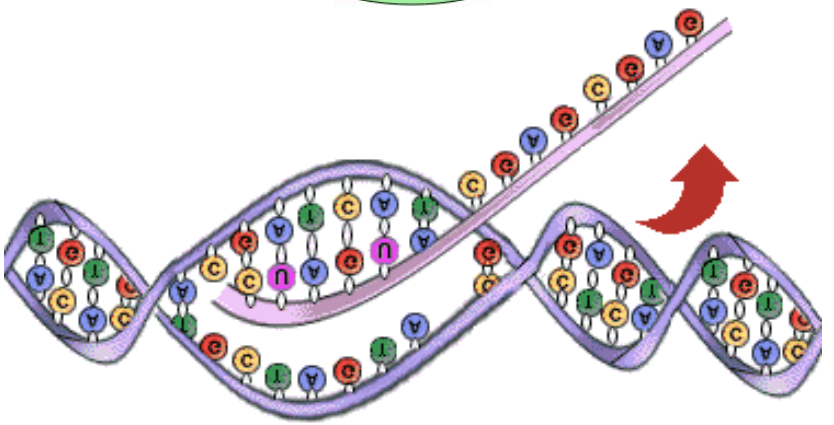
What drives the conservation of extragenic regions?

- Compare extragenic transcription and TF binding to conserved bases
  - TF binding sites
  - Non-coding RNA

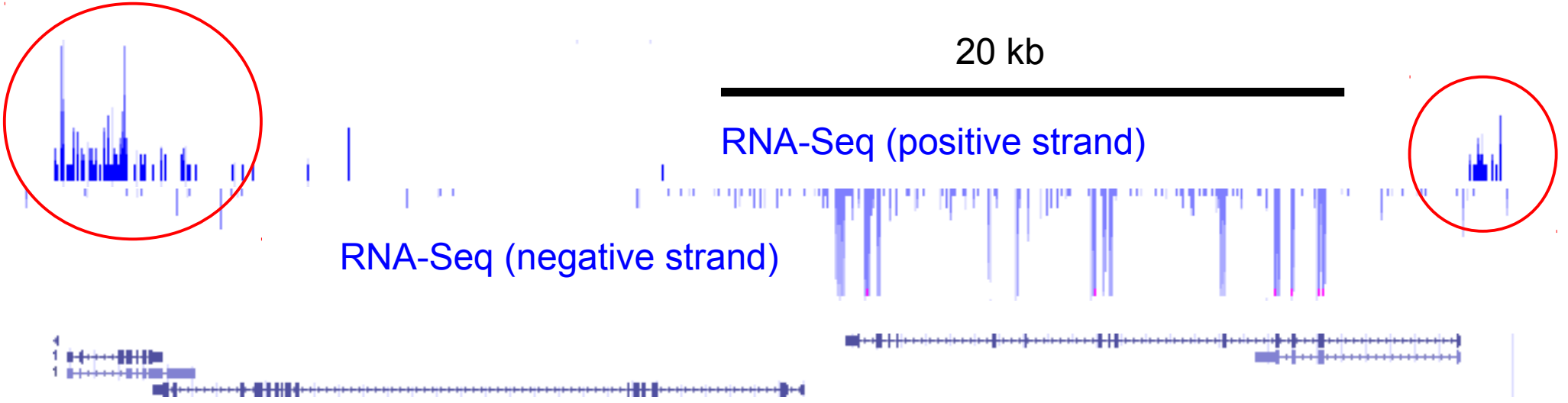
exon or promoter





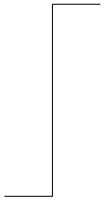


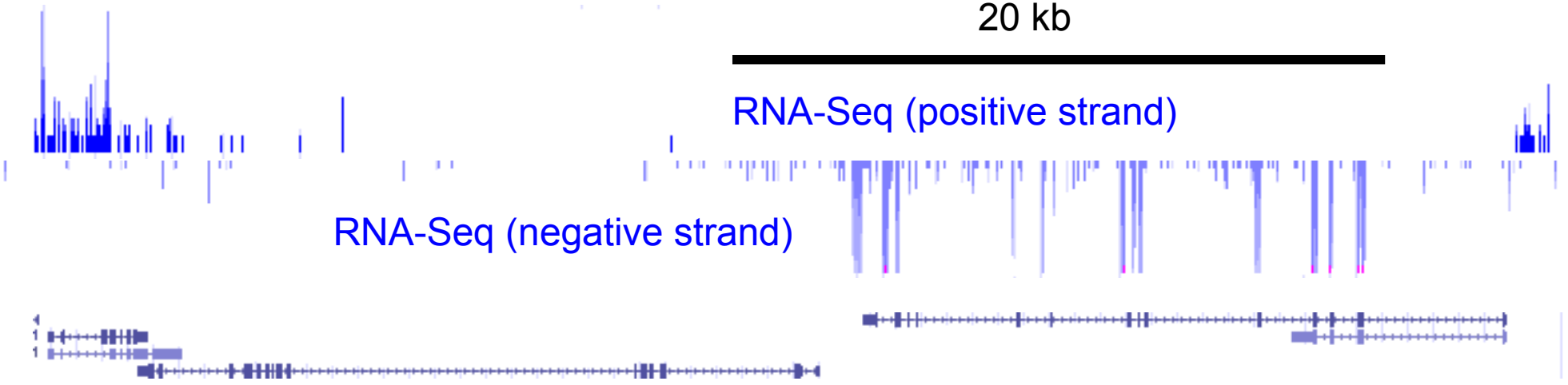
De novo identification of transcribed regions



Using Haar-wavelets to identify transcribed regions (HaTriC)

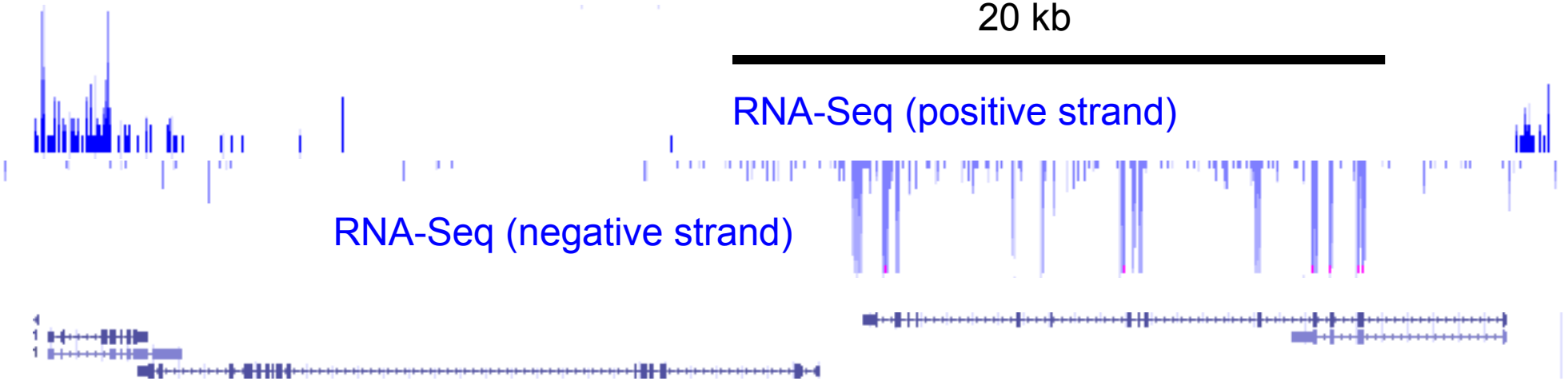
- Find where read-density changes abruptly





Using Haar-wavelets to identify transcribed regions (HaTriC)

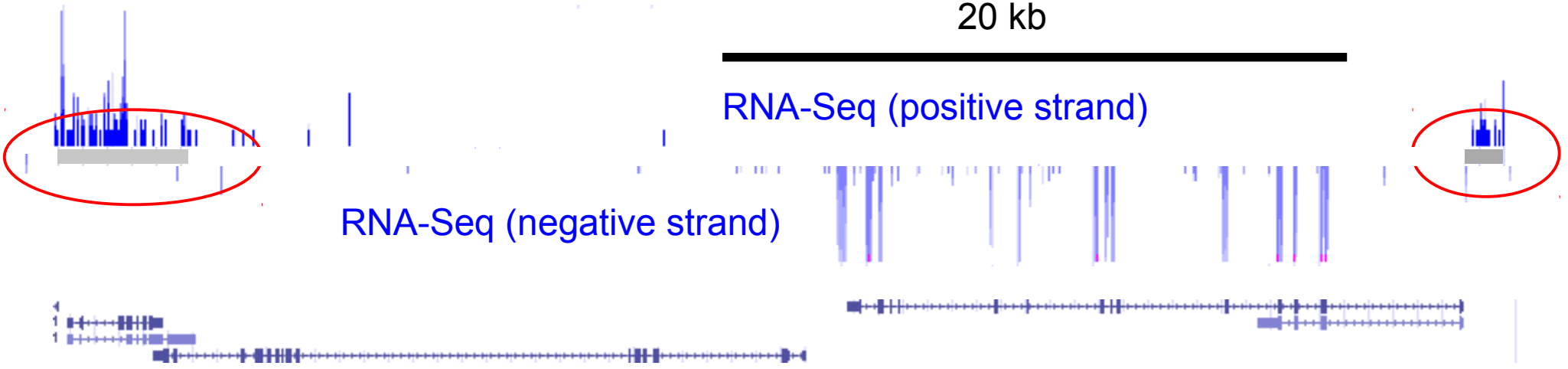
- Find where read-density changes abruptly
  - Consider multiple length scales



Using Haar-wavelets to identify transcribed regions (HaTriC)

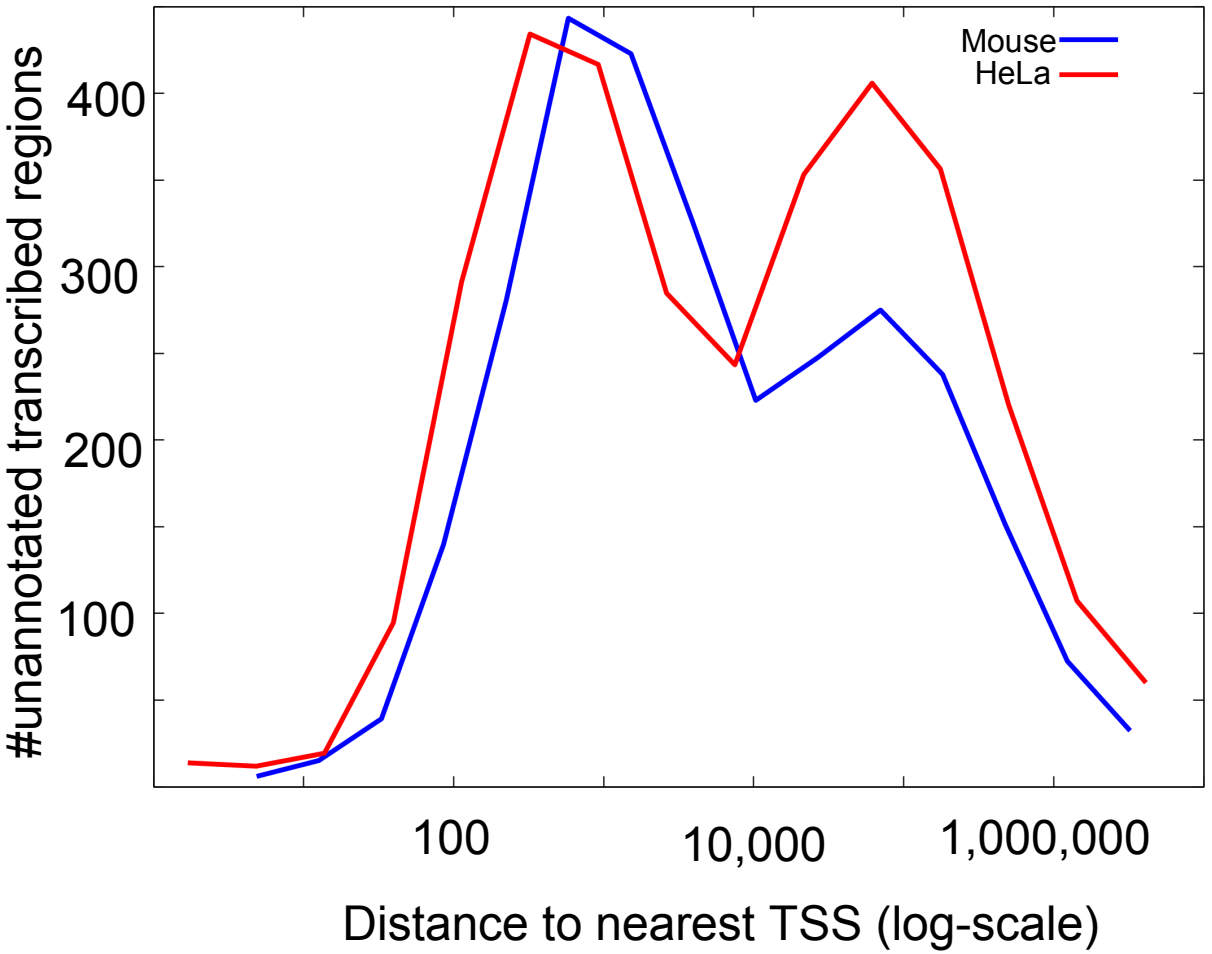
- Find where read-density changes abruptly
  - Consider multiple length scales

- Interleaving regions of high/low density
- Mask expressed regions and repeat



Most unannotated transcribed regions are found near annotated genes

- 80% of active genes correctly identified



Most reads are found in annotated genes

- Genic 88.66%
- Annotated ncRNA 3.87%

Transcribed regions account for 99.54% of all reads

- Genic 88.66%
- Annotated ncRNA 3.87%
- Upstream anti-sense 0.51%
- Anti-sense 0.23%
- Novel 5.28%
- eRNAs 0.04%
- Other regulatory factor binding sites 0.96%

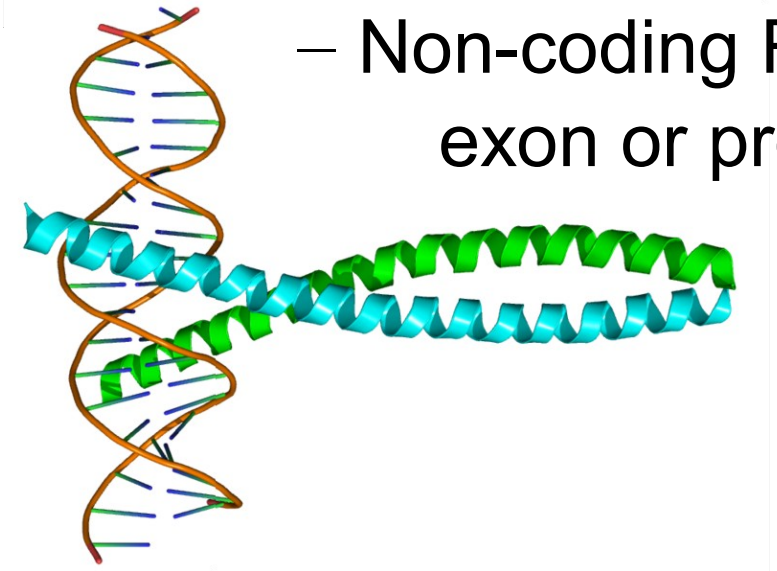
There are many extragenic regions transcribed at very low levels

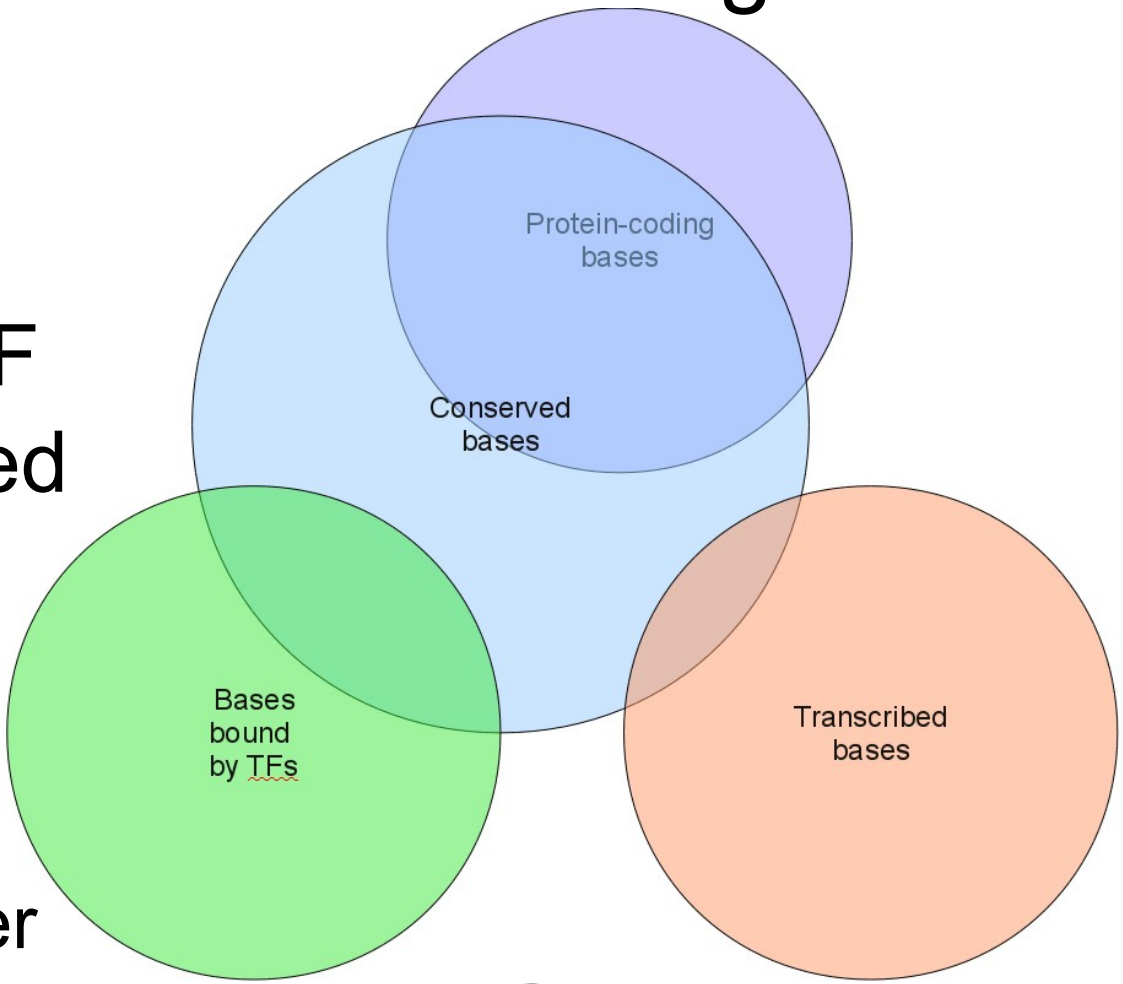
• Genic	15,262
• Annotated ncRNA	4,870
• Upstream anti-sense	5,427
• Anti-sense	1,289
• Novel	251
• eRNAs	1,018
• Other regulatory factor binding sites	1,365

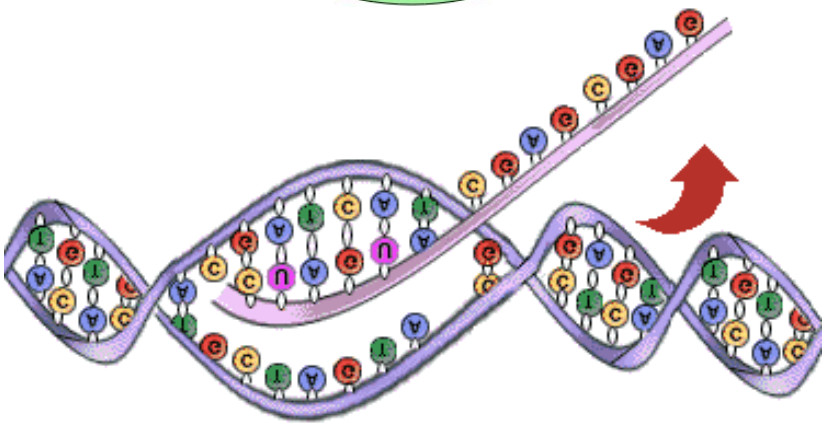
What drives the conservation of extragenic regions?

- Compare extragenic transcription and TF binding to conserved bases
  - TF binding sites
  - Non-coding RNA

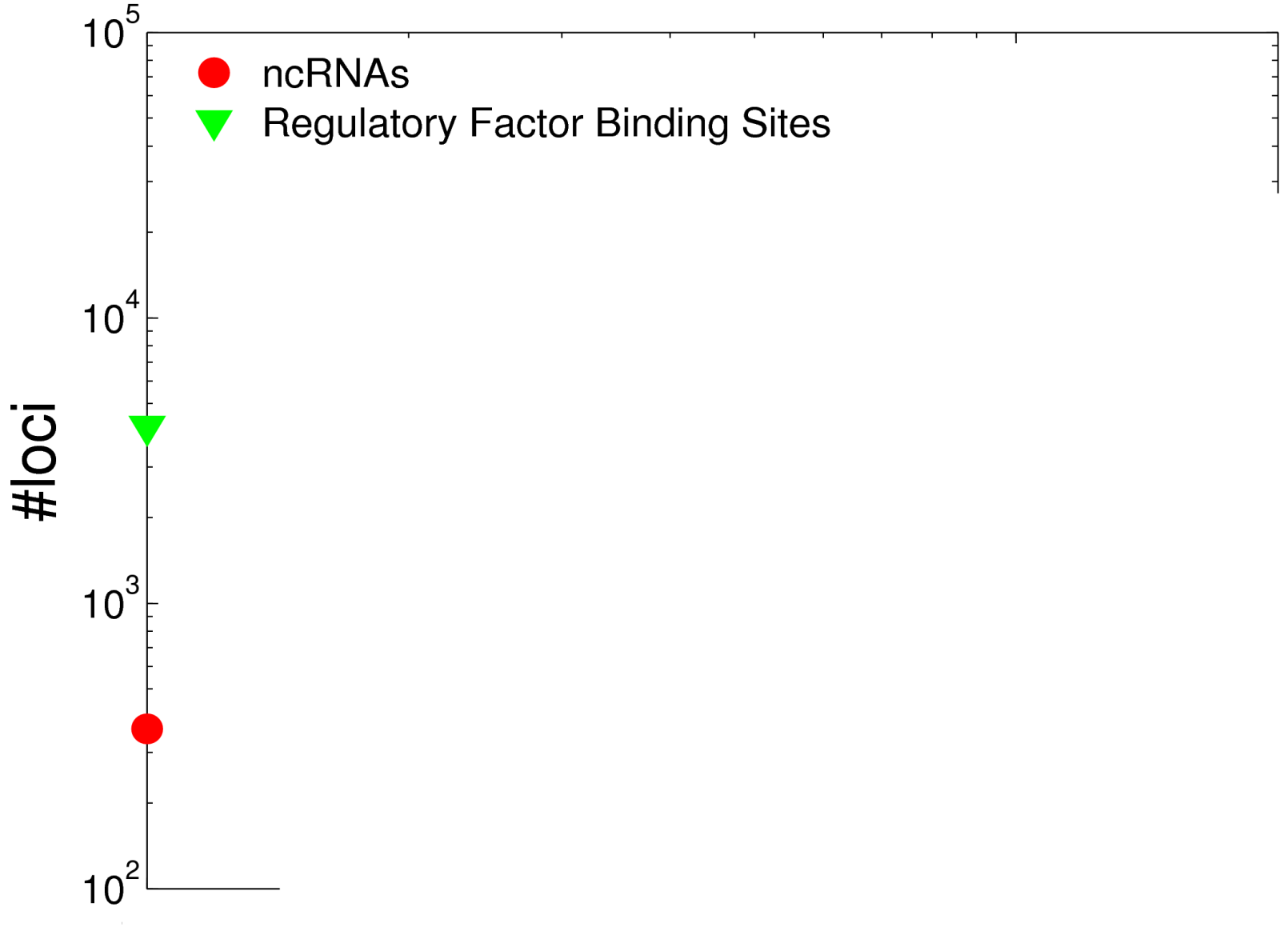
exon or promoter



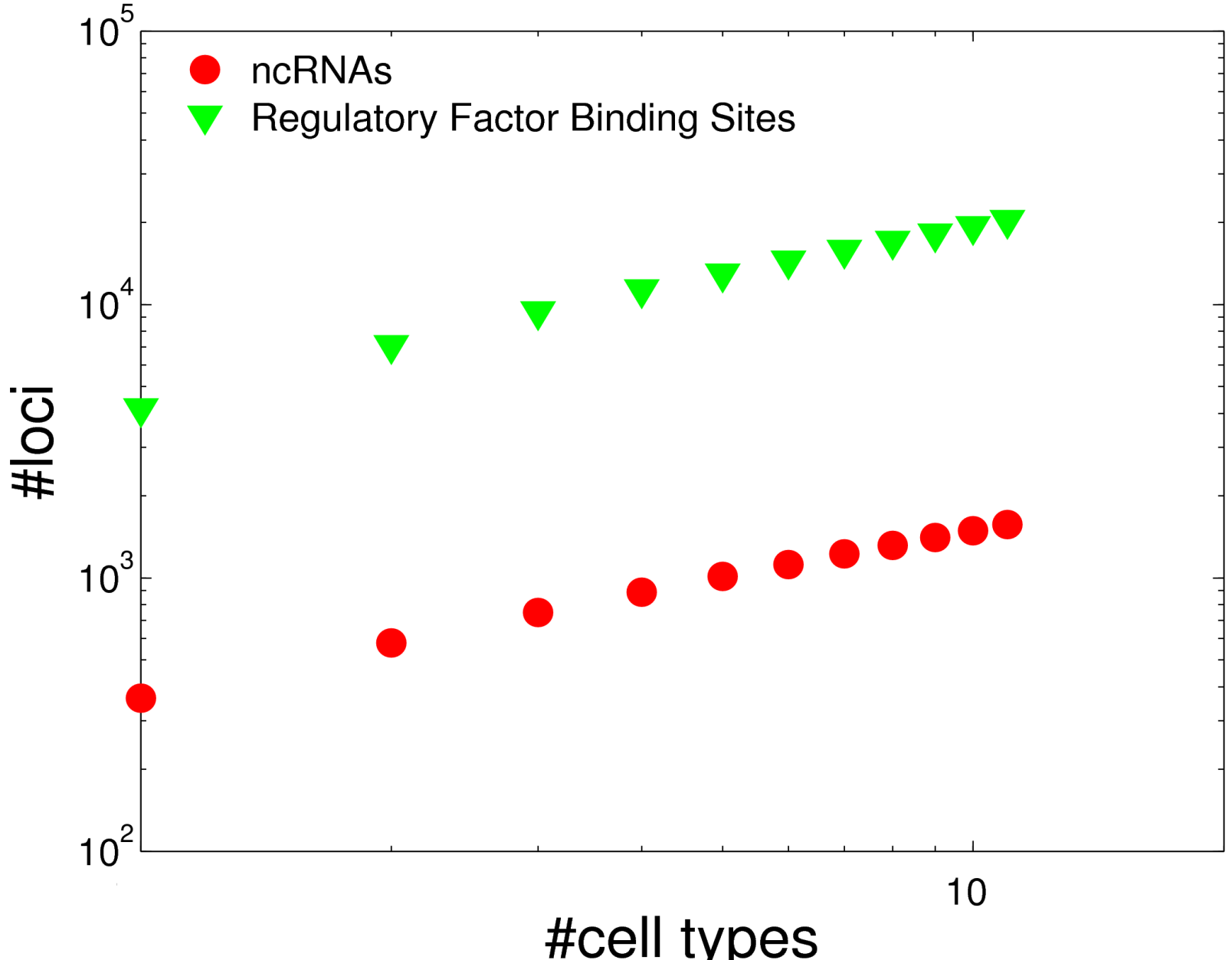




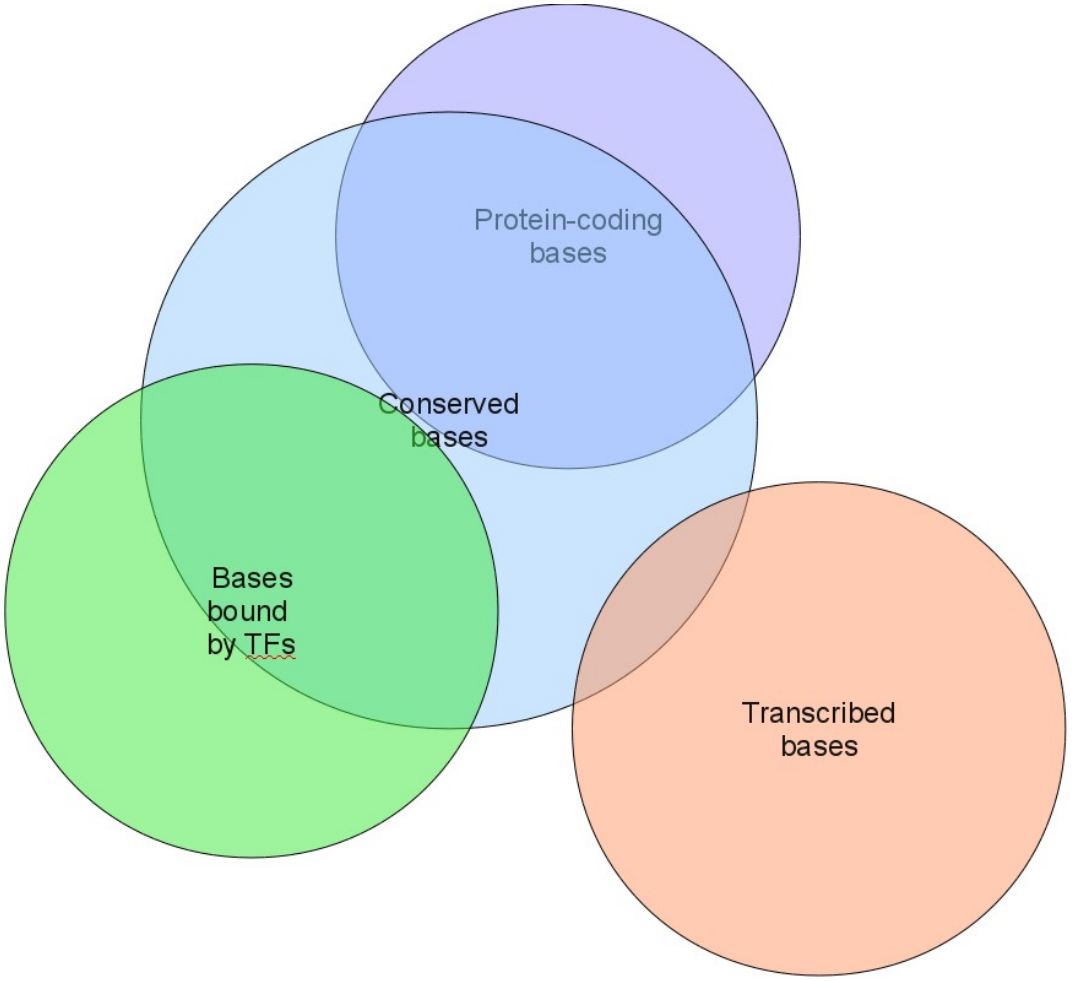
About 80% of conserved bases are transcription factor binding sites



About 80% of conserved bases are transcription factor binding sites

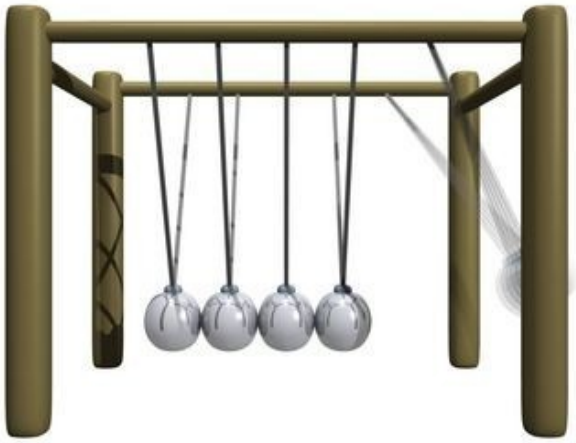


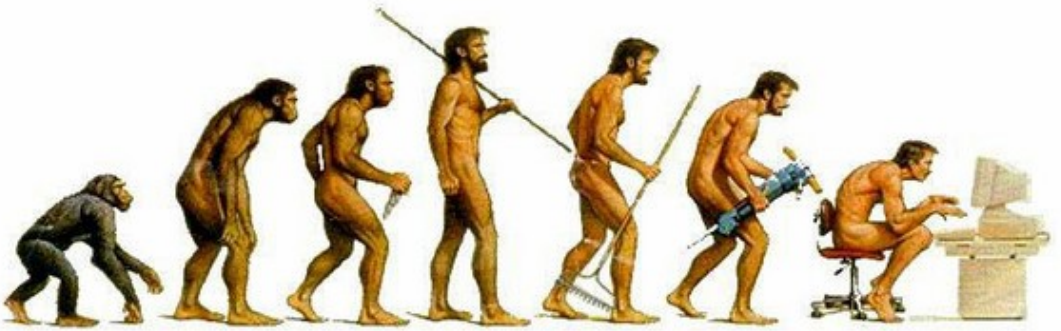
Summary II: *De novo* identification of transcribed regions suggests that most conservation is due to TF binding



Future Work: Organizing principles of the genome

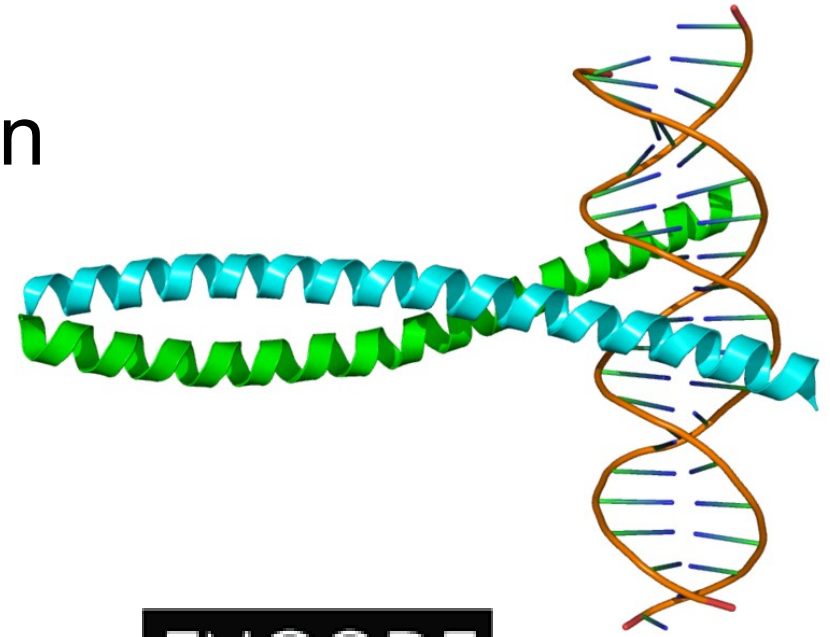
- Systems biology approach to develop biophysical models





What determines the level of 'epigenomic modifications' and how are they read out?

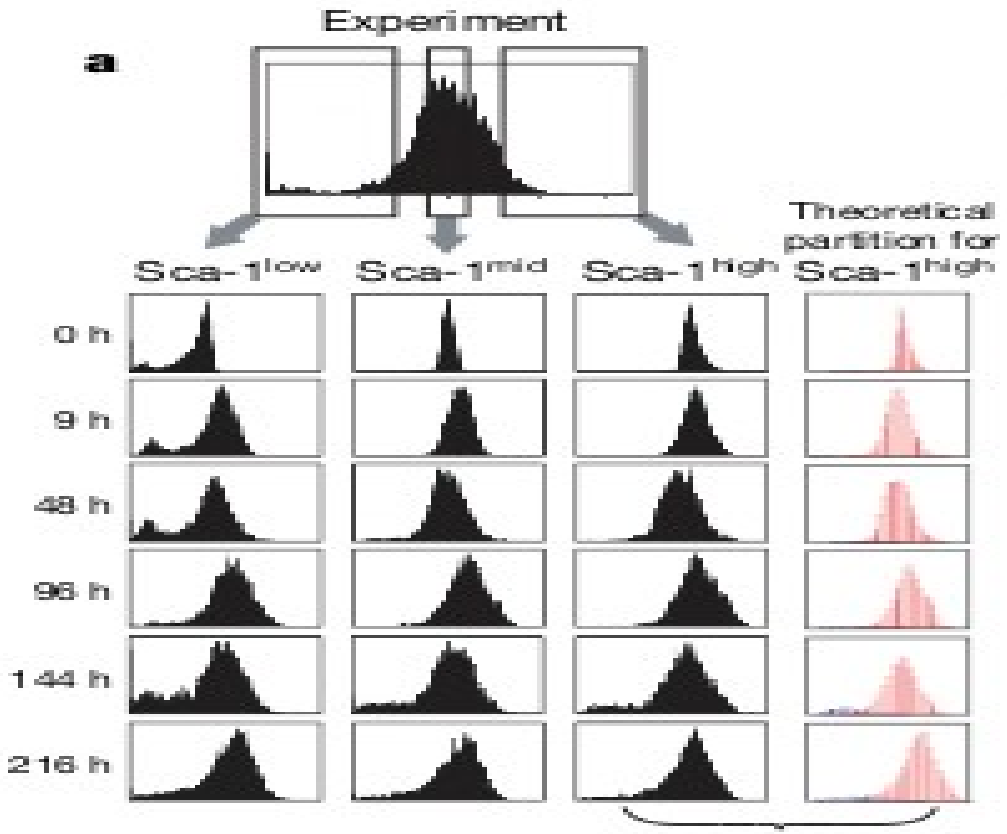
- How can histone modifications be read and written?
- What determines transcription factor binding?
- What determines the level of transcription?



ENCODE

What is the impact on the phenotype from gene expression noise?

- RNA-Seq for single cells
- Global view of noise in gene expression
  - Pathways
  - Proximity
  - Cell-types
  - Propagation

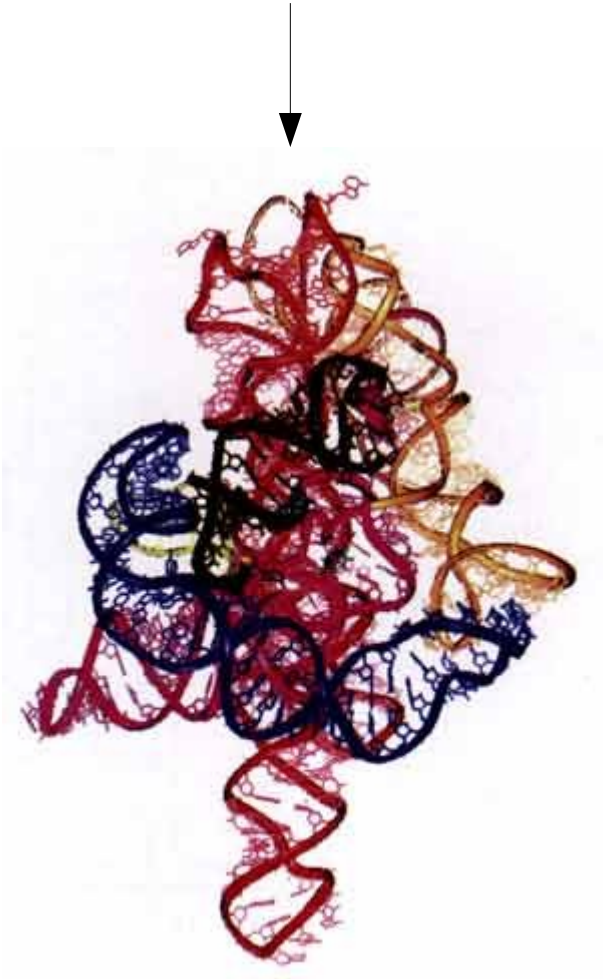


Tracing the Derivation of Embryonic Stem Cells from the Inner Cell Mass by Single-Cell RNA-Seq Analysis

Fuchou Tang,[1,3] Catalin Barbacioru,[2] Siqin Bao,[1] Caroline Lee,[1] Ellen Nordman,[2] Xiaohui Wang,[2] Kaiqin Lao,[2,*] and M. Azim Surani[1,*]

Is there a non-coding genetic code for determining the structure of RNAs?

.....ACGUCCAAAUUCCCUAGGCUCAAGGCAUUCGAUCGGGAUUAUA.....



Acknowledgements

Gabriel Kreiman, **Children's Hospital Boston**
Wui Ip
Enrique Tobis
Michael Greenberg, **Harvard Medical School**
Tae-Kyung Kim
Jesse Gray
Allen Costa
Daniel Bear
David Harmin
Mike Laptewicz
Eirene Markenscoff-Papadimitriou

Molecular Genetics Core
Children's Hospital Boston
Kellie Haley
Josh Davis
Hal Schneider

Life Technologies
Rob David
Jingwei Ni
Scott Kuersten
Gina Costa
Kevin McKernan

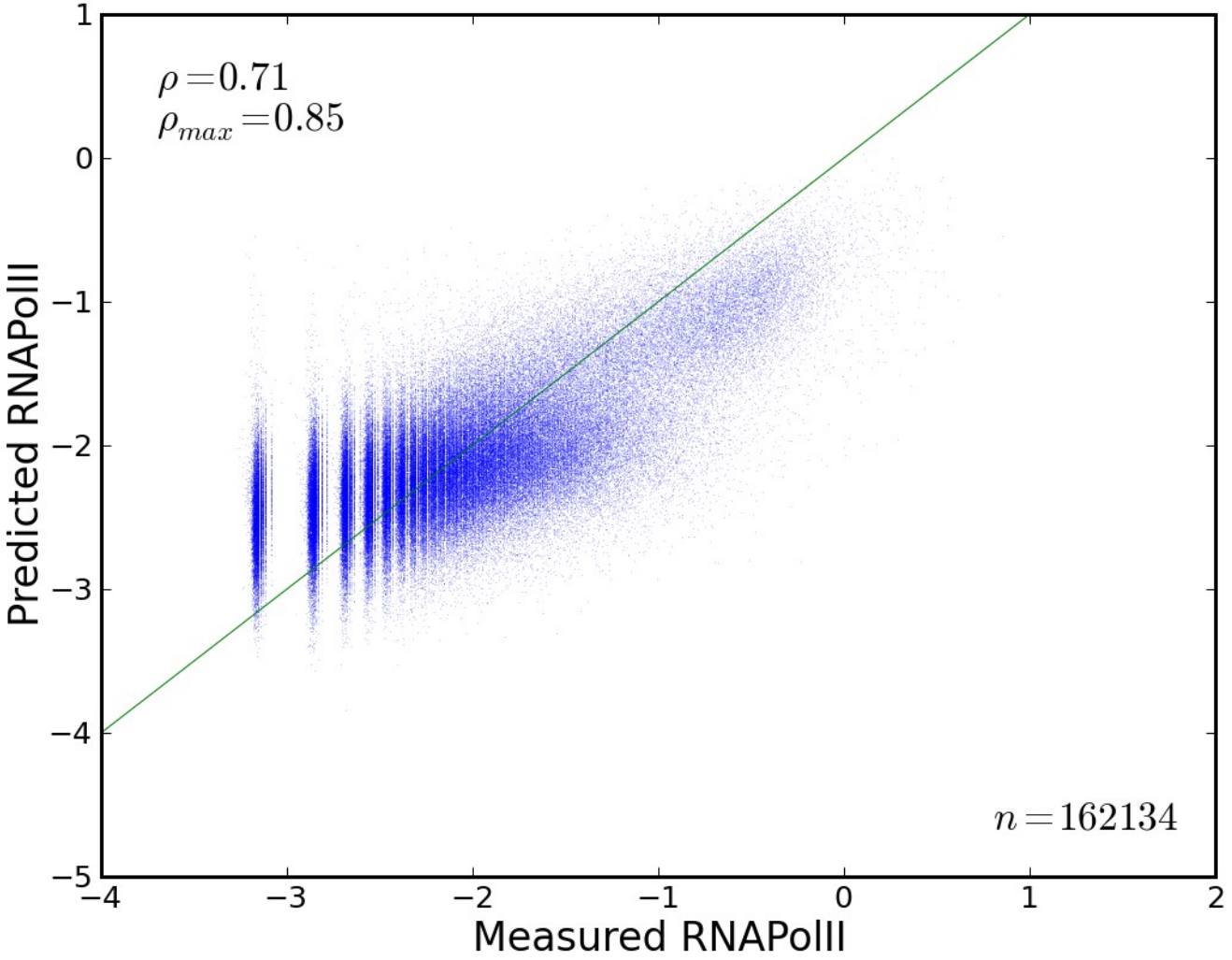
Harvard Medical School
Biopolymer facility
Kristin Waraska
Robert Steen

Johns Hopkins
Jing Wu, Paul Worley Lab

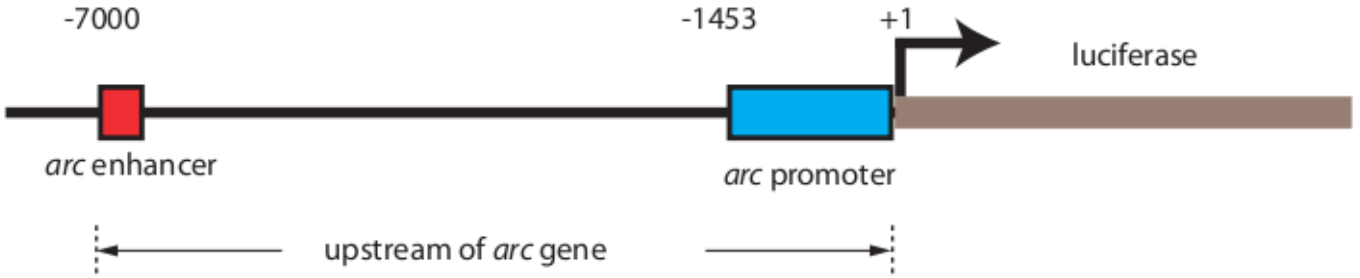
Thank You

?

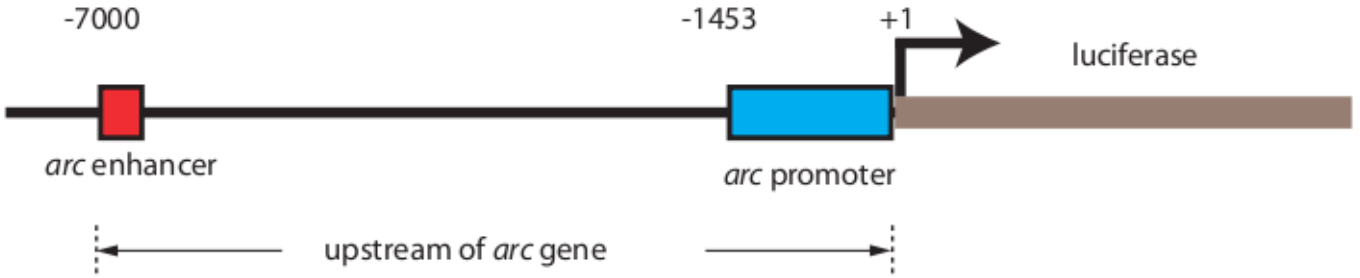
Is there an epigenetic code to determine the cell-type specific function of the sequence?

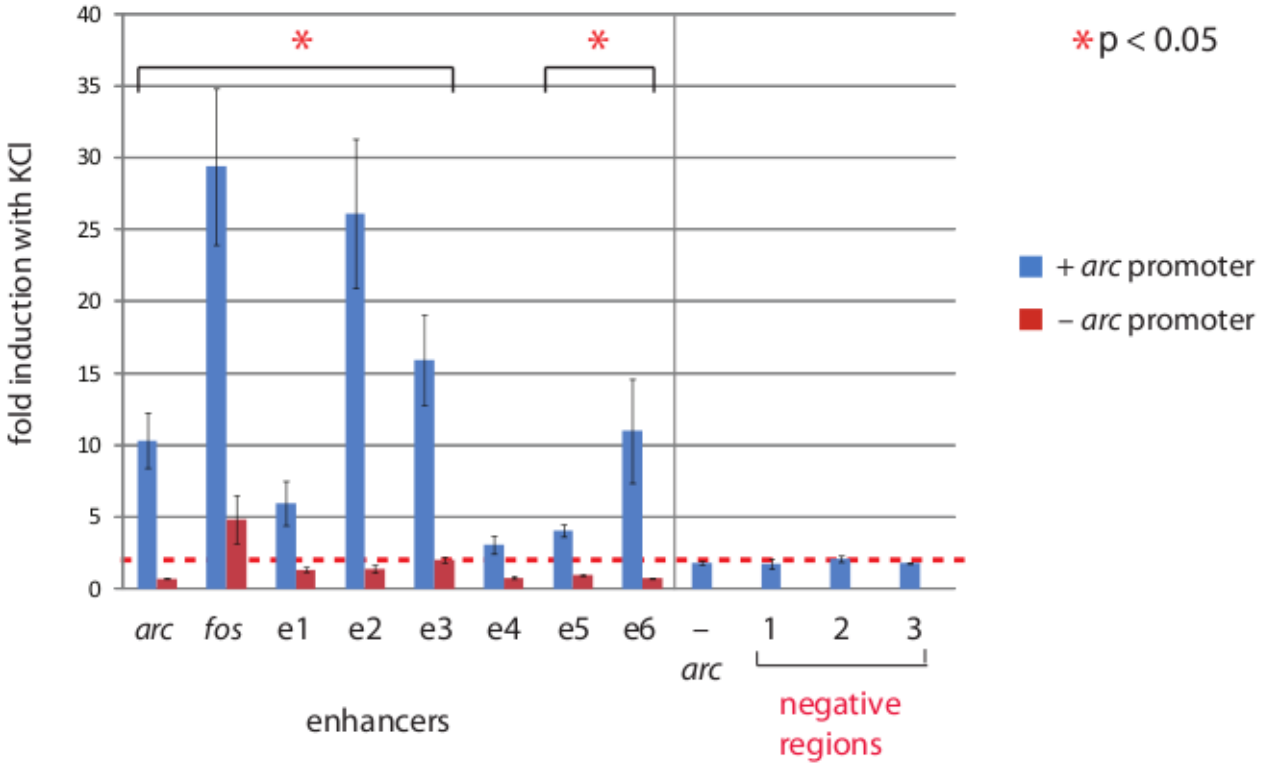


Experimental validation of 8 enhancers using a luciferase assay

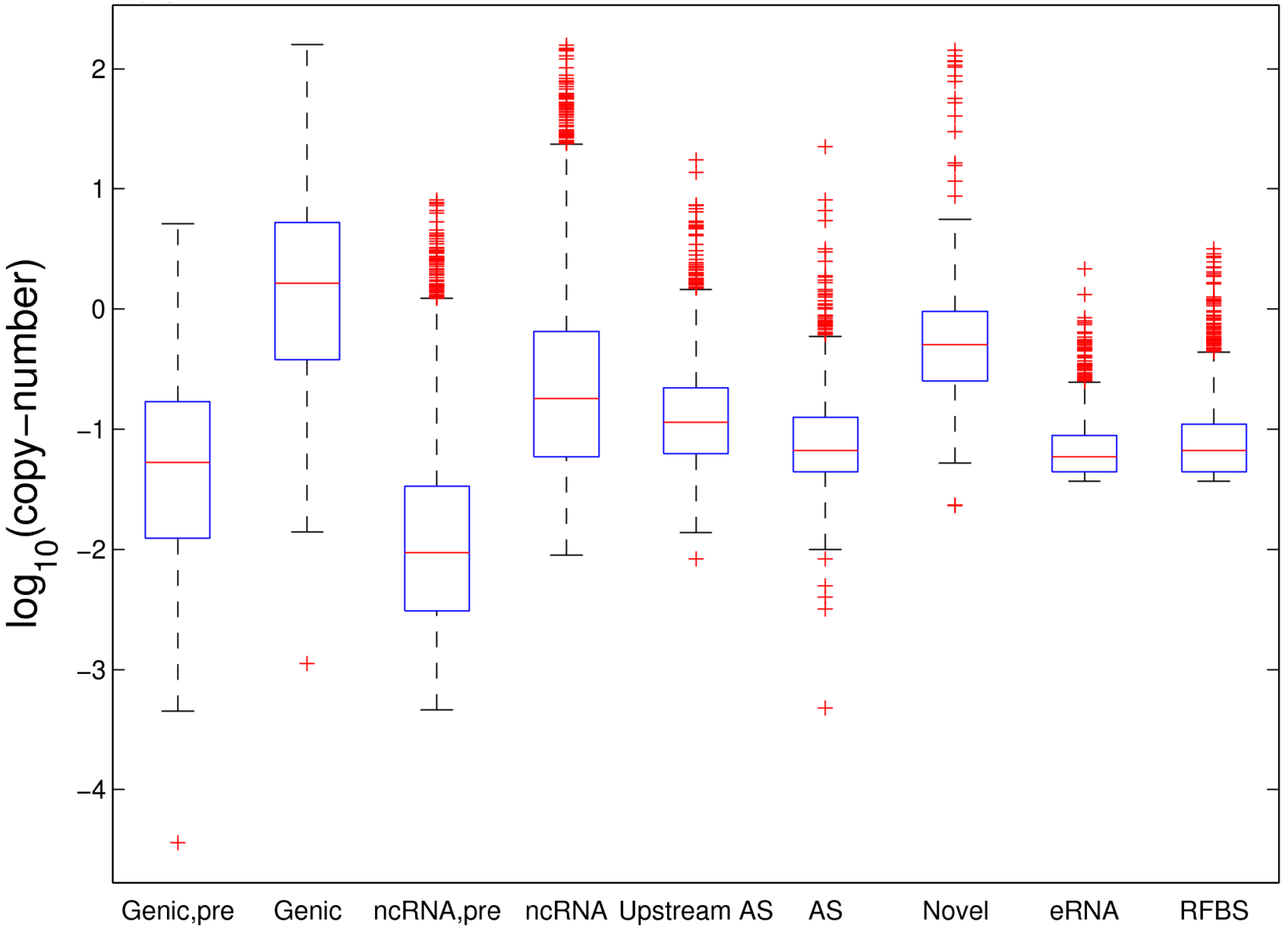


Experimental validation of 8 enhancers using a luciferase assay



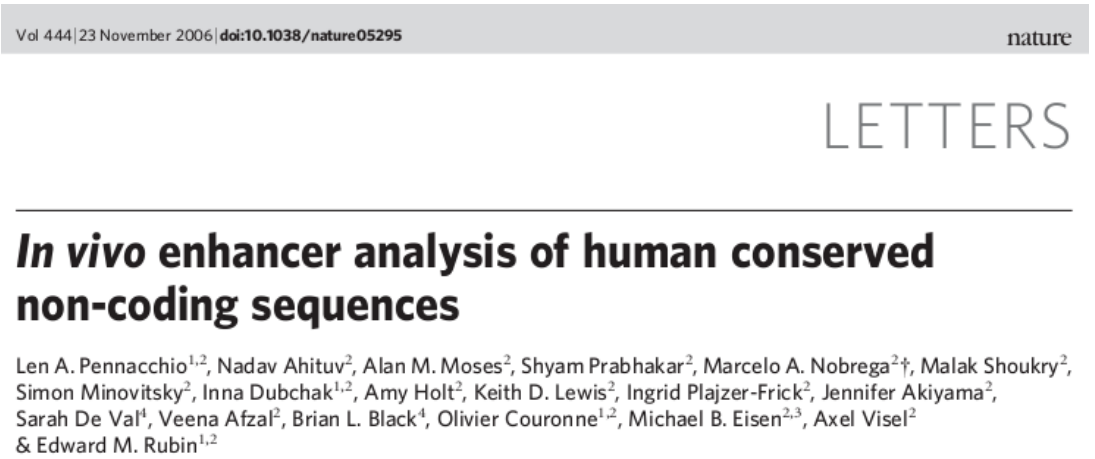


Copy numbers for different categories



Non-coding sequences are important

- Conserved
  - ~60% of highly conserved are non-coding
- Regulatory
  - Enhancers: distal regulatory binding sites
    - ~1/2 million estimated for human and mouse
  - Insulators
  - Silencers



Vol 444|23 November 2006|doi:10.1038/nature05295 nature

LETTERS

In vivo enhancer analysis of human conserved non-coding sequences

Len A. Pennacchio[1,2], Nadav Ahituv[2], Alan M. Moses[2], Shyam Prabhakar[2], Marcelo A. Nobrega[2]†, Malak Shoukry[2], Simon Minovitsky[2], Inna Dubchak[1,2], Amy Holt[2], Keith D. Lewis[2], Ingrid Plajzer-Frick[2], Jennifer Akiyama[2], Sarah De Val[4], Veena Afzal[2], Brian L. Black[4], Olivier Couronne[1,2], Michael B. Eisen[2,3], Axel Visel[2] & Edward M. Rubin[1,2]

Non-coding sequences are important

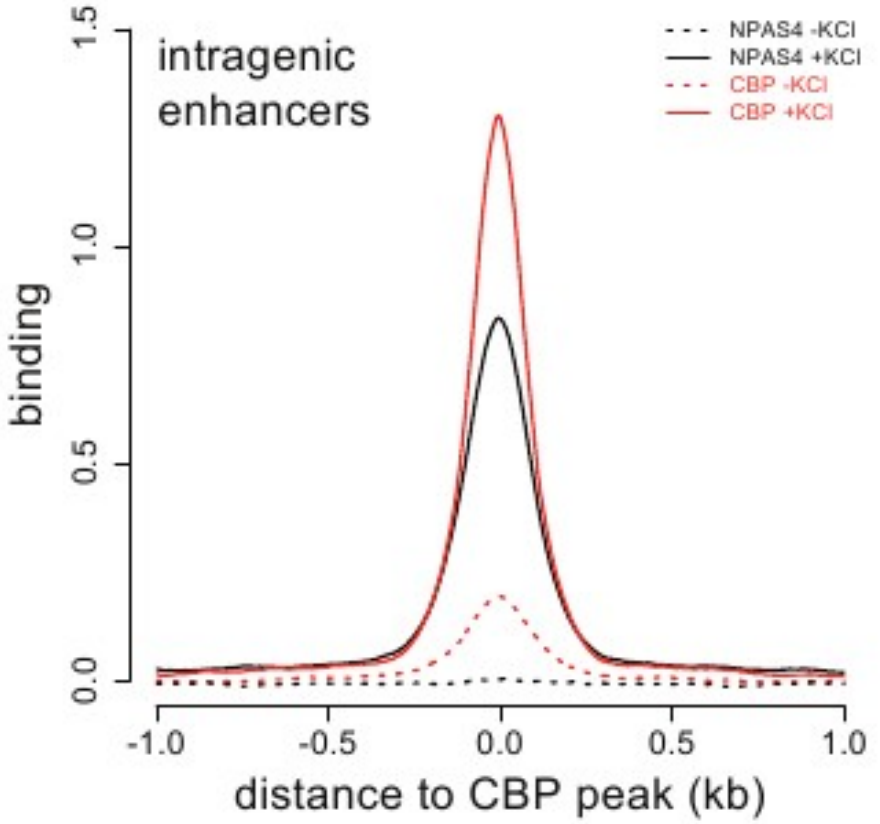
- Conserved
  - ~60% of highly conserved are non-coding
- Regulatory
  - Enhancers: distal regulatory binding sites
- Transcribed
  - Long non-coding RNAs (lncRNAs)
    - Most have unknown function

Functional Demarcation of Active and Silent Chromatin Domains in Human *HOX* Loci by Noncoding RNAs

John L. Rinn,[1] Michael Kertesz,[3,5] Jordon K. Wang,[1,5] Sharon L. Squazzo,[4] Xiao Xu,[1] Samantha A. Brugmann,[2] L. Henry Goodnough,[2] Jill A. Helms,[2] Peggy J. Farnham,[4] Eran Segal,[3,*] and Howard Y. Chang[1,*]

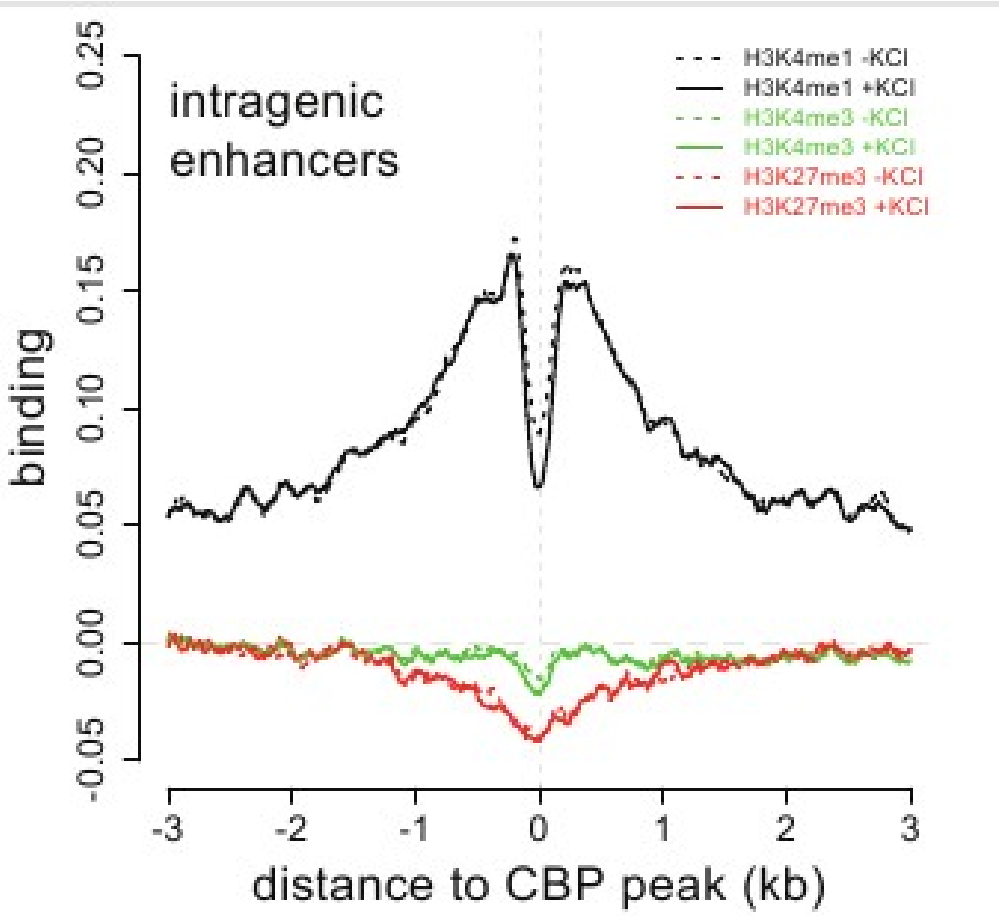
Intragenic enhancers

- ~7,000 enhancers overlapping introns
  - Inducible CBP



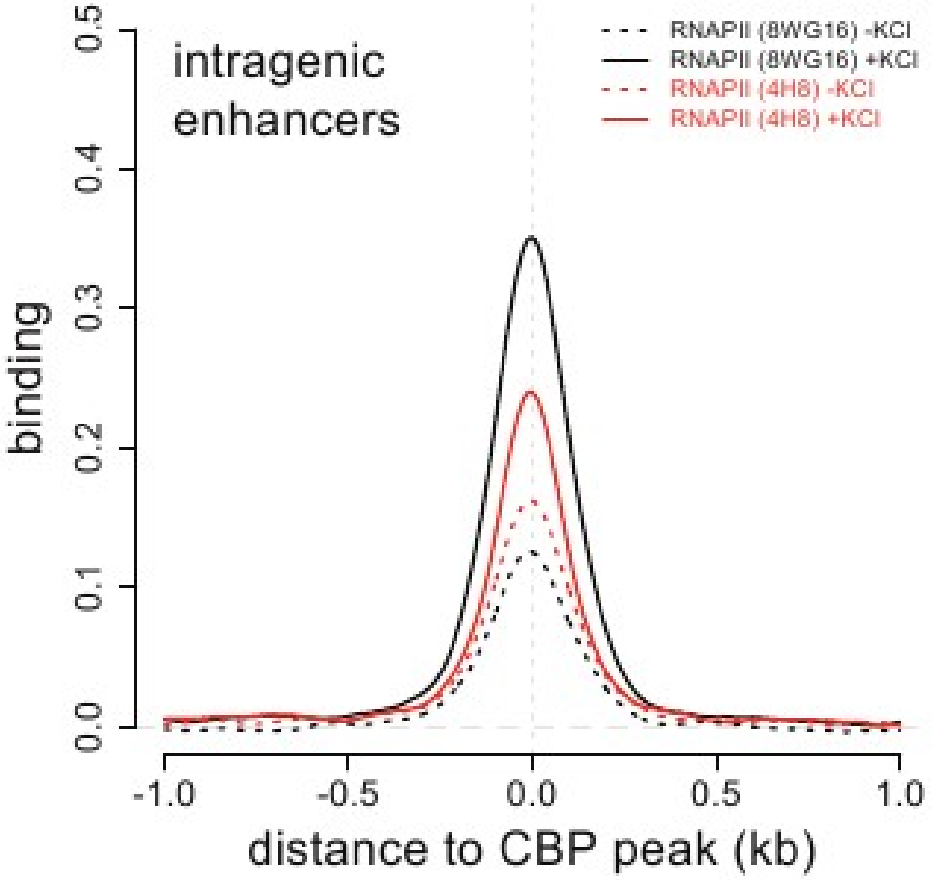
Intragenic enhancers

- ~7,000 enhancers overlapping introns
  - H3K4me1, but no H3K4me3

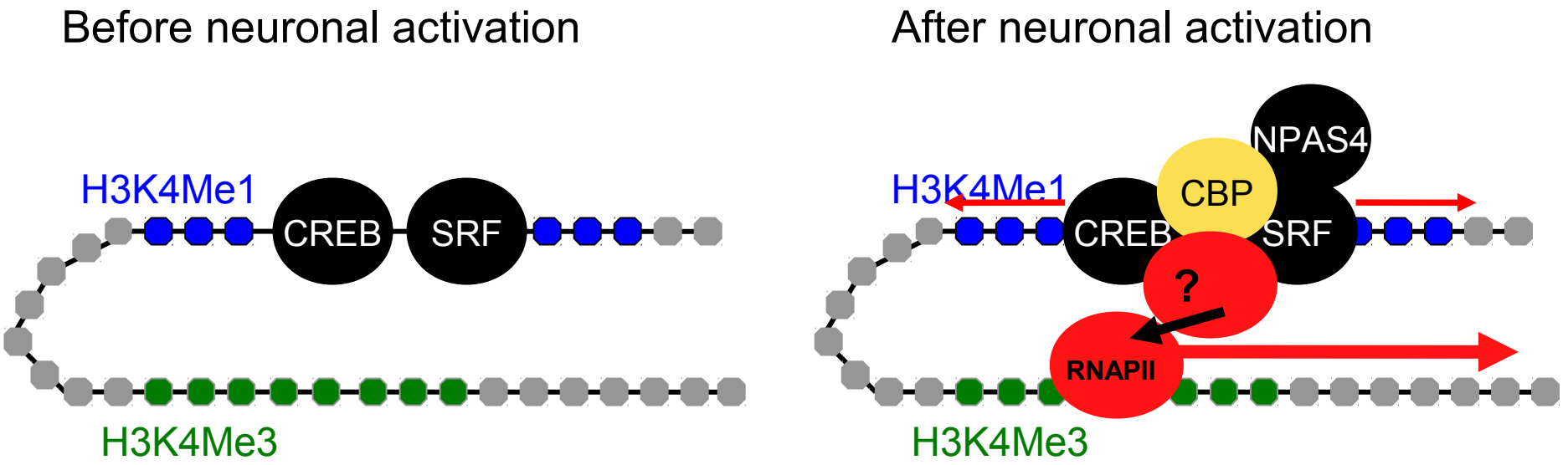


Intragenic enhancers

- ~7,000 enhancers overlapping introns
  - Inducible RNAPII

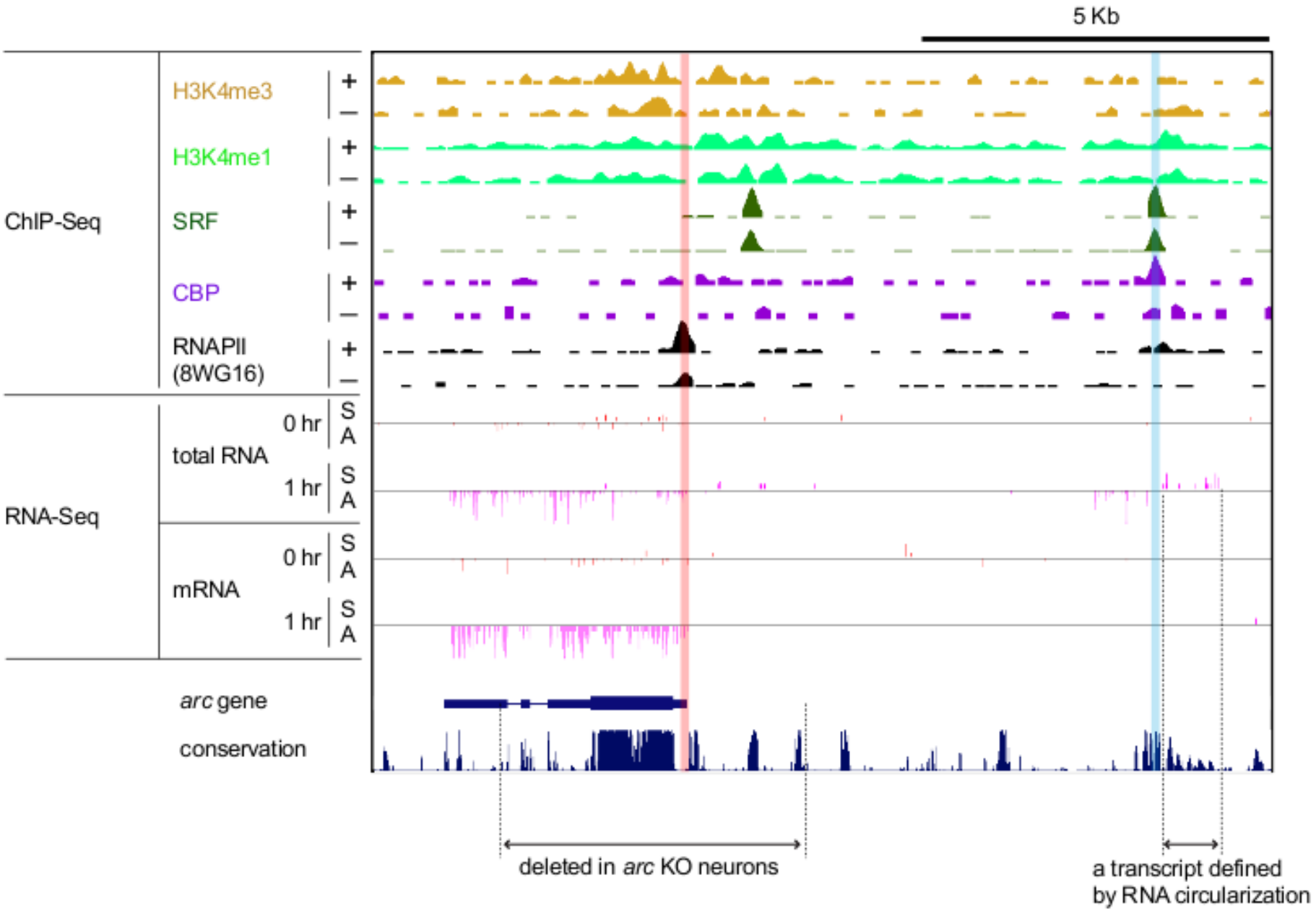


Properties of activity regulated enhancers



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

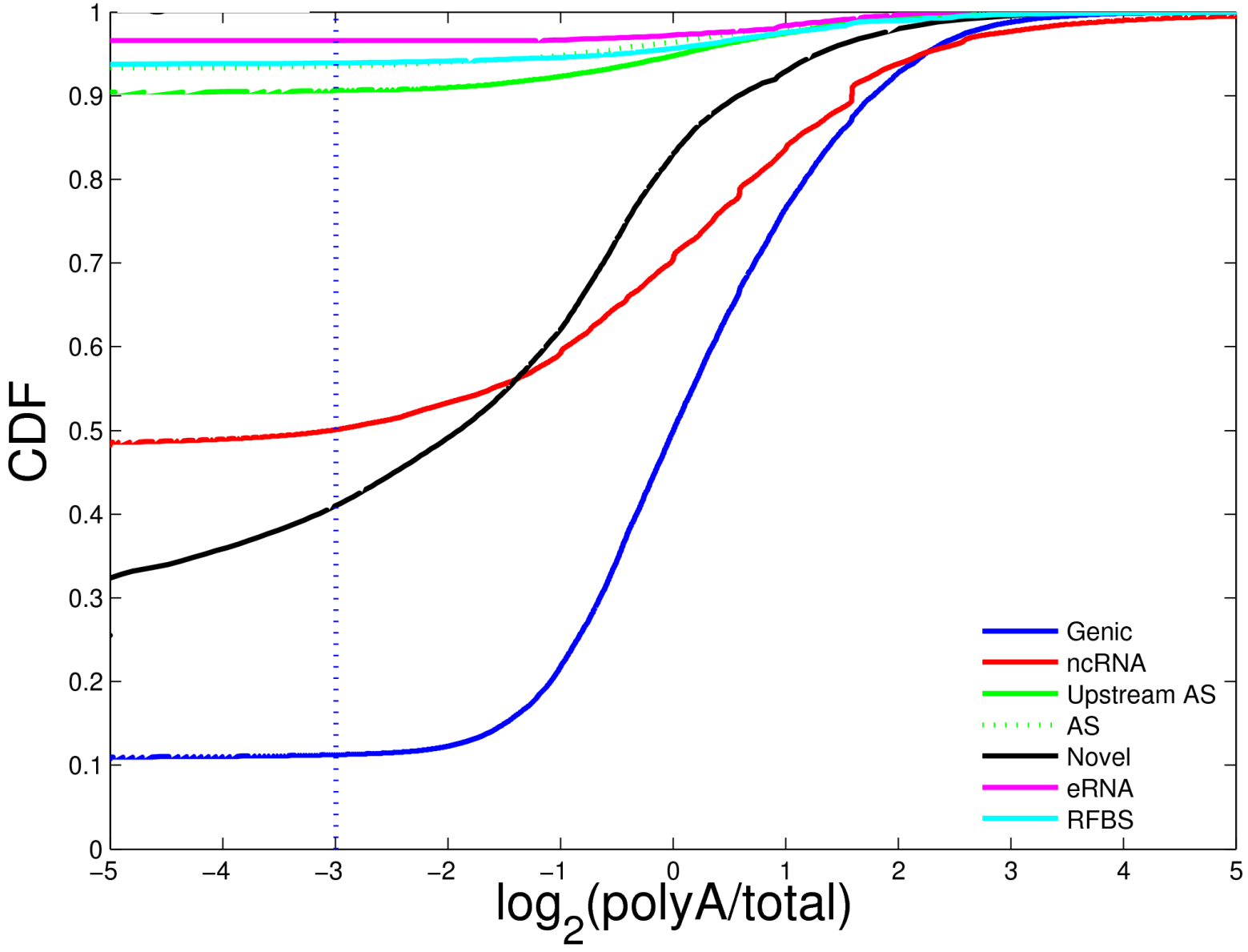
arc locus and enhancer



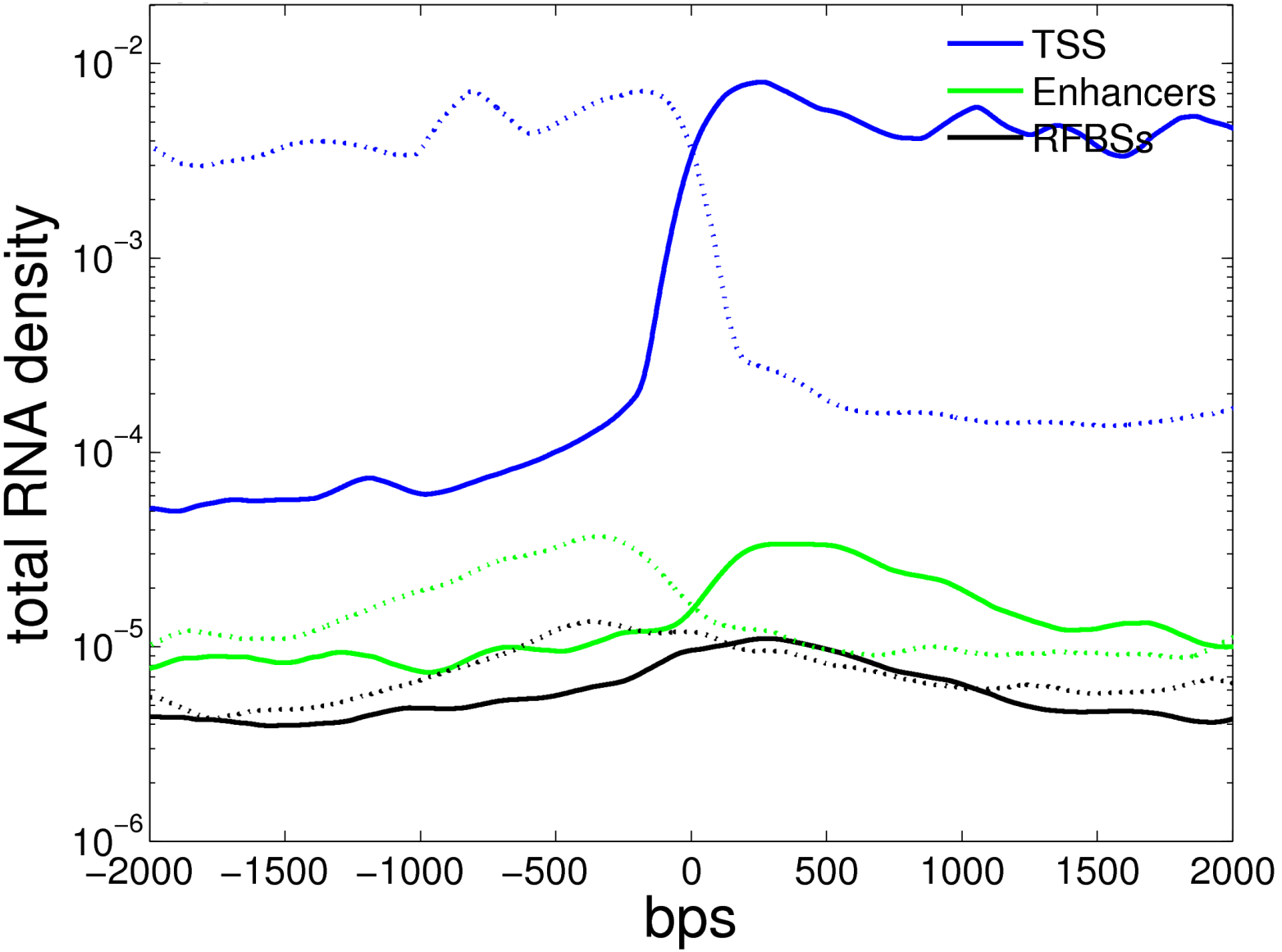
Optimizing the parameters

- Binning, minimum and maximum Haar-wavelet-length
- FDR for choosing break-points and transcribed regions
  - Sweep parameter space and maximize the fraction of regions that have a H3K4me3 peak at their start
    - Running HaTriC on one chr takes only a few minutes

Most ncRNAs are not polyadenylated

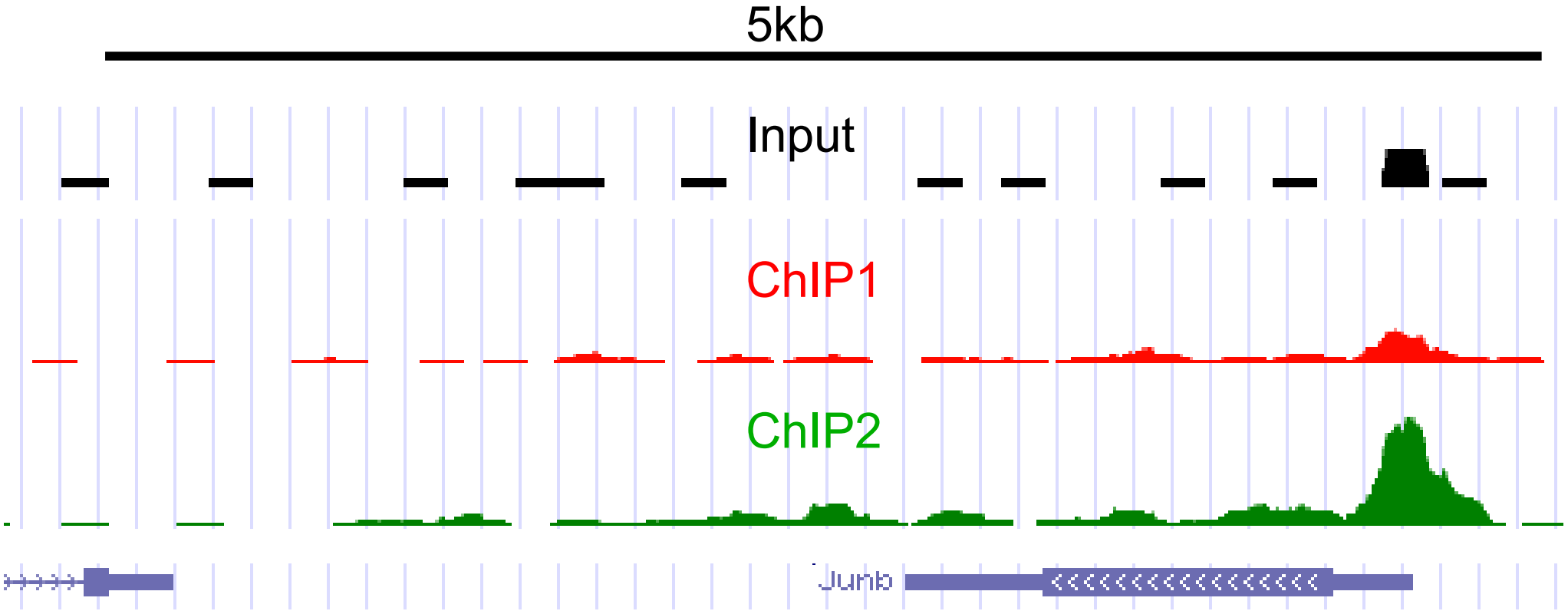


mRNA much more abundant than eRNA



Subtract input to identify significant peaks

- Z_i = #ChIP reads - #input reads in window *i*
- ~1 read/100 bp



Assume ChIP and input Poisson distributed

- Z_i = #ChIP reads - #input reads in window *i*
- ~1 read/100 bp
  - Assume #reads in window P(k) = $\lambda^k \exp(-\lambda)/k!$
    - Difference between two Poisson random variables
    - Z_i ~ Skellam(z, λ_1, λ_2)

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

Use False Detection Ratio (FDR) to correct for multiple hypotheses

- Z_i = #ChIP reads - #input reads in window *i*
- ~1 read/100 bp
  - Assume #reads in window P(k) = $\lambda^k \exp(-\lambda)/k!$
    - Difference between two Poisson random variables
    - Z_i ~ Skellam(z, λ_1, λ_2)

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

- Millions of windows need to be tested
  - FDR - expected fraction of false positives

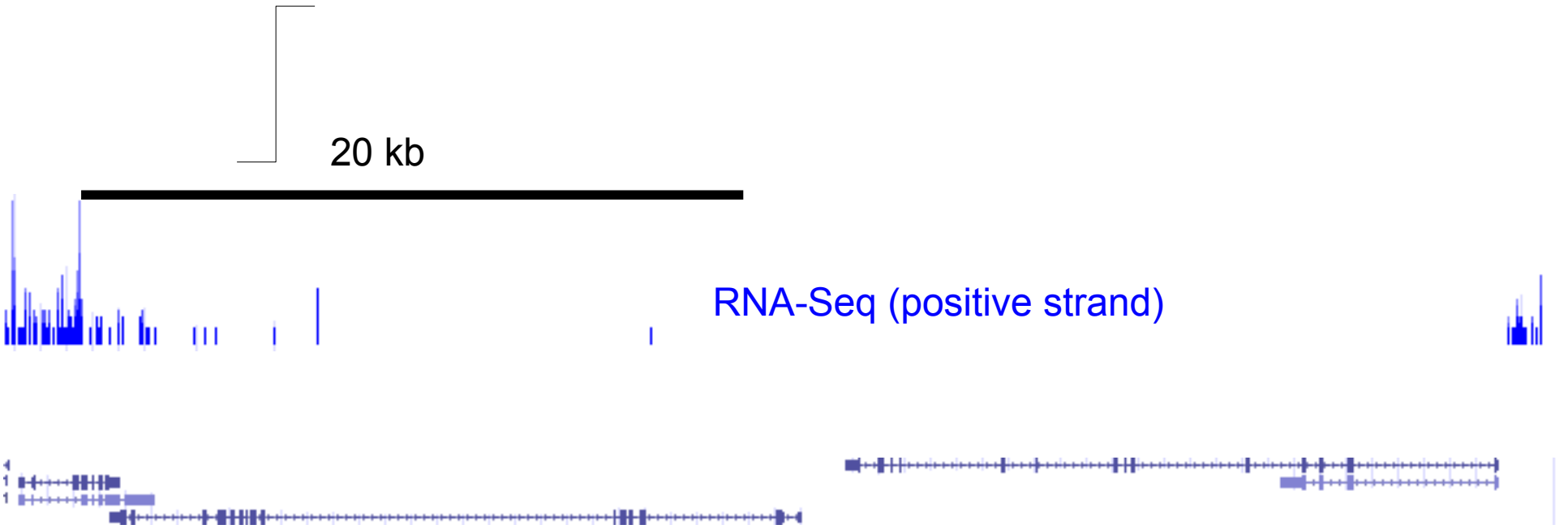
Haar-wavelet Transcript Calling (HaTriC) for *de novo* identification of transcribed regions

```
Calculate_RNA_density_for_128_bp_bins
do
   find_breakpoints
   calculate_region_densities
   determine_cutoff_density
   remove_transcribed_regions
while new_regions_found
```

The Haar-wavelet picks out regions with sharp changes in read density

- Break points correspond to sharp changes in read density

$$h_L(n) = \frac{1}{\sqrt{2^{L+1}}} \left(\sum_{i=n}^{n+2^L-1} \log(1+r_i) - \sum_{n-1}^{i=n-2^L} \log(1+r_i) \right)$$

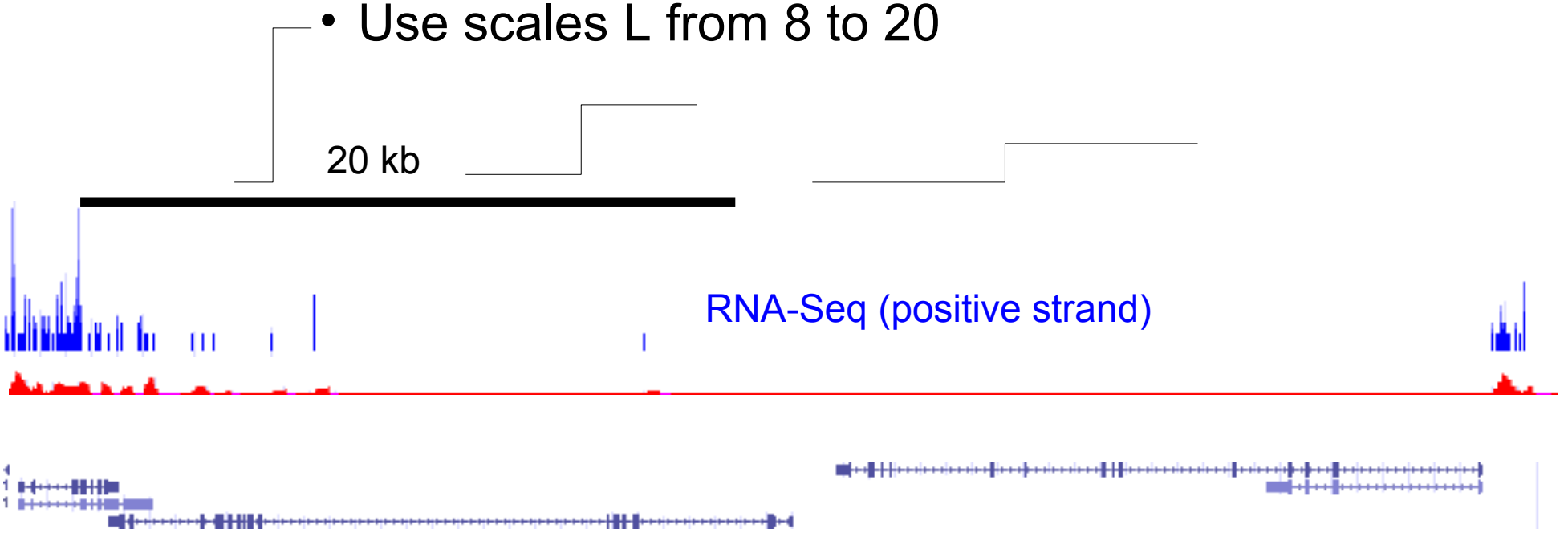


The Haar-wavelet can be scaled to analyze multiple length scales

- Break points correspond to sharp changes in read density

$$h_L(n) = \frac{1}{\sqrt{2^{L+1}}} \left(\sum_{i=n}^{n+2^L-1} \log(1+r_i) - \sum_{n-1}^{i=n-2^L} \log(1+r_i) \right)$$

- Use scales L from 8 to 20

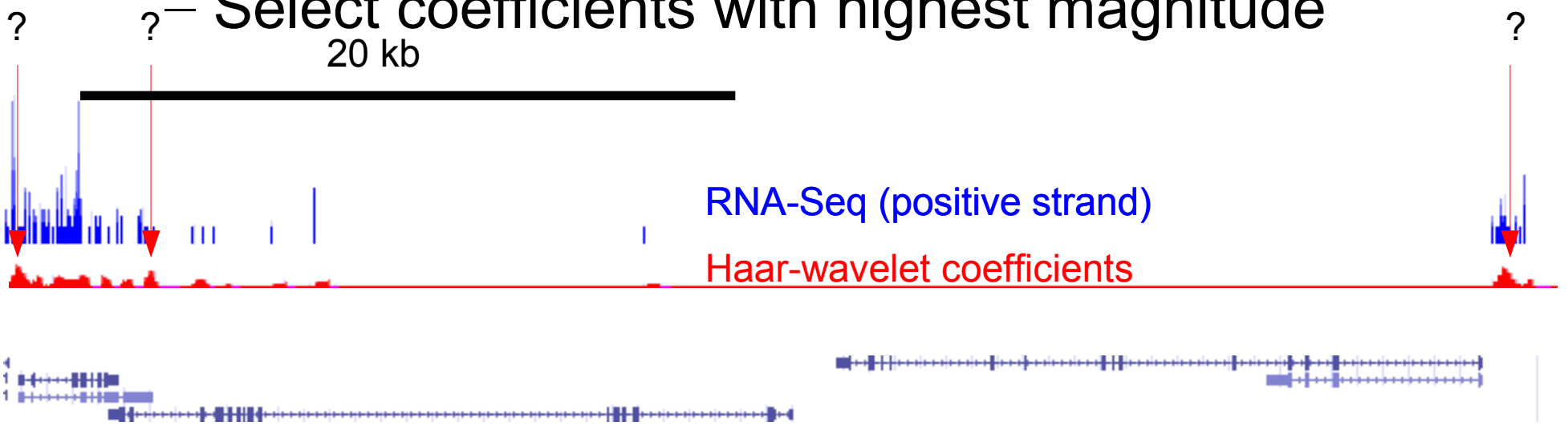


The coefficients with largest magnitude are selected as candidate break points

- Break points correspond to sharp changes in read density

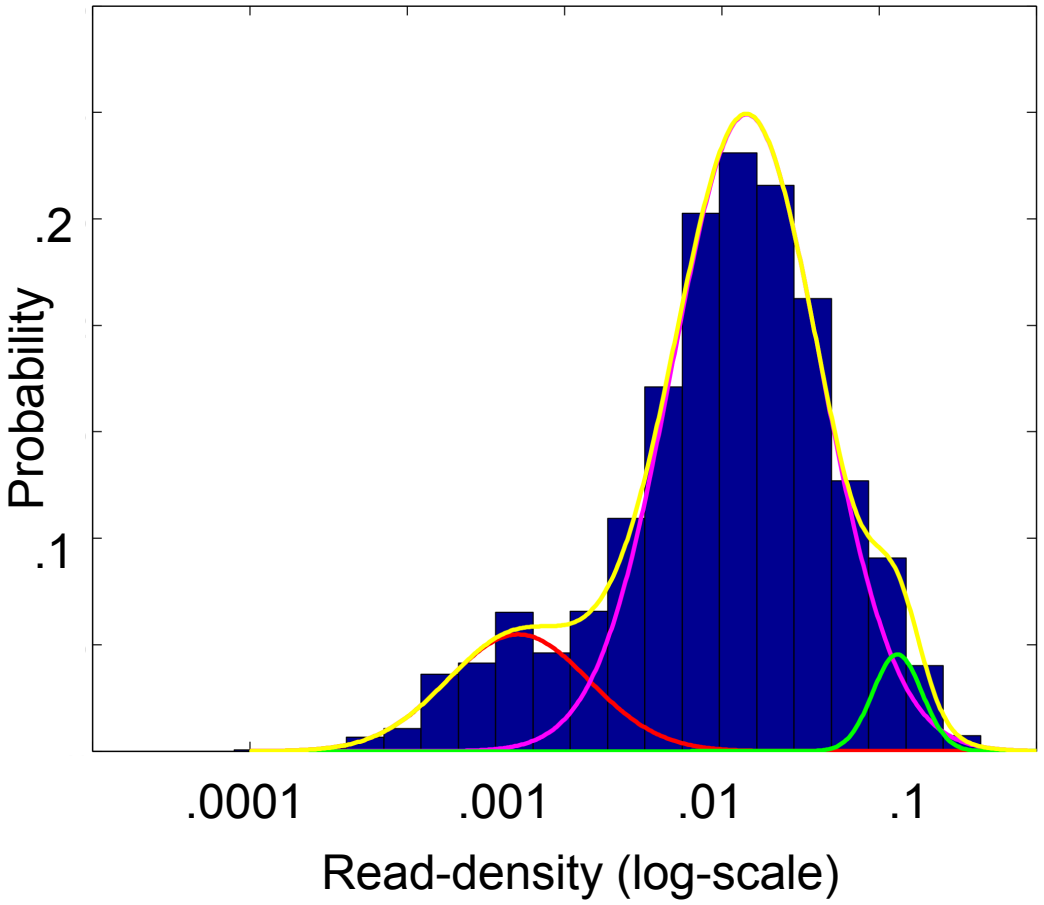
$$h_L(n) = \frac{1}{\sqrt{2^{L+1}}} \left(\sum_{i=n}^{n+2^L-1} \log(1+r_i) - \sum_{n-1}^{i=n-2^L} \log(1+r_i) \right)$$

– Select coefficients with highest magnitude



The density distribution for the regions determined by the break points is bimodal

- Average density between breakpoints
- Keep regions belonging to higher mode



Remove transcribed regions, iterate the process is until no new regions are found

- Allows us to find regions with lower expression levels