Chromatin dynamics

A tale about histone tails

Nir Friedman
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Acknowledgments

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Assaf Weiner

Alon Applebaim

Ayelet Rahat
Histone Marks

H2A

SGRGKGQGGKARAKAKSRR

1  5  9  13  15

LPKKTESHKA

118

H2B

PEPAKAPAPKGGSKKAVTKAQKKD

5  12  14  20  23  24

VTKYTSS

120

H3

ARTKYTARKSTGGKAPRKLATKAARKSAPATGGVVK

2  4  8  9  11  14  20  25  30

H4

SGRGKGGKGLGGGAKRHRKVL

1  3  5  8  12  16  20

Acetylation  Me Methylation  P Phosphorylation  Ub Ubiquitination

H2A

H2B

H3

H4
Histone Marks

50-100 known histone marks

Highly conserved pathways (modifiers / readers)

Active substrate of DNA-based processes
Part-I

Chromatin State Dynamics During Blood Formation

David Lara-Astiaso¹,*, Assaf Weiner²,³*, ..., Nir Friedman²,³,†, Ido Amit¹,†
Chromatin in Development

Open and permissive chromatin

Pluripotent cells

Compact chromatin state

Differentiated cells
Chromatin Annotates the Genome

Enhancer: where? Active?  Promoter: Active?

K4me1

K4me1  K4me2

Enhancer

K4me3

K4me1  K4me2  K27Ac

Active

POISED

ACTIVE

Model System: Hematopoiesis

Multipotent Stem Cells

Oligopotent cells (generate a specific lineage)

Challenge:
Mapping chromatin states in hematopoietic progenitor populations with current technologies is practically impossible
Chromatin indexing ChIP (iChIP)
Chromatin Dynamics During Hematopoiesis

- mRNA
- H3K4me3 (promoters)
- H3K4me1 (enhancers)
- H3K4me2 (enhancers)
- H3K27ac (active PolII)
- ATAC-seq (accessibility)
Research Questions

Dynamics of change?

Gradual closing

Opening

Transient
Maintained Potential
Open in Differentiated Cells
Open in Lineage Precursor
Comprehensive Enhancer Catalog

In total we define 48,415 enhancers

Unified set of “peaks”
40% Transient Dynamics
Research Questions

Dynamics of change?

Transient changes (40%)

Gradual or abrupt?
Establishment at Linage Commitment
Research Questions

Dynamics of change?

Transient changes (40%)

Gradual or abrupt?

Abrupt at lineage commitment

Function: are they active?
Stepwise Enhancer Activation

RNA expression (A.U)

Active enhancers (H3K27ac)
Enhancer Establishment Precede Expression

RNA expression correlation matrix

LT-HSC  HSC  MPP  CLP  CMP  GMP  Mφ  Gn  Mo  B  CD4  CD8  NK  MEP  EryA  EryB

0 0.5 1
Enhancer Establishment Precede Expression

H3K4me1 correlation matrix
Research Questions

Dynamics of change?

Gradual or abrupt?

Function: are they active?

marked in progenitors
activated in differentiated cells

Mechanisms?
Enhancer Establishment Correspond to Open Regions

A Gypa

F7–F10

H3K4me1 & ATAC-seq coverage

K4me1 coverage (log)

ATAC peak

K4me1 signal

ATAC-seq coverage (log)

CMP

NK cells

MPP

CMP

GMP

Gn

B

CD4

NK

MEP

EryA
Moving from lineage specific enhancers to the TF regulating the chromatin landscape in hematopoiesis

<table>
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<tr>
<th>Sequence</th>
<th>TF candidate</th>
<th>TF expression profile</th>
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Predicting enhancer activity
Establishment of cell type specific enhancers is regulated by a combination of known and novel lineage specific TF
Conclusions (Part I)

- iChIP–seq: histone mapping in low cell numbers
- Comprehensive histone modifications map in blood formation
- Committed progenitors:
  - Enhancer landscape similar to differentiated cells
  - Expression profiles similar to other progenitors
- A map of the TF involved in setting and maintaining enhancers
Part II

High resolution chromatin dynamics during a yeast stress response

Assaf Weiner$^{1,2,*}$, Tsung-Han Hsieh$^{3,*}$, Alon Appleboim$^{1,2,*}$, Hsiuyi Chen$^3$, Ayelet Rahat$^{1,2}$, Ido Amit$^4$, Oliver J. Rando$^{3,*}$, Nir Friedman$^{1,2,*}$

Molecular Cell, in press
Experimental Plan

Mid-log growth

Stress Response

Repression  “Control”  Induction
High-Throughput Mapping of Nucleosome States

Grow to midlog → Sample time course → Fixed cells → mono-nucleosomes → MNase

Sequencing

Multiplexed library

Input, H3K36me3, H3K4me3 → ChIP

High-Throughput Mapping of Nucleosome States

Nucleosomes

H3K79me3

H3K4me3

H2AS129ph
Comprehensive Yeast Chromatin “Abcam-ome”

- All relevant ChIP-grade antibodies from Abcam, Milipore
- ChiP-seq from 36 ABs
- 26 passed QC
~66,000 nucleosomes

Weiner et al. Genome research 2010
Research Questions

• Are there stereotypical modification patterns?
Histone Modifications

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The heatmaps and scatter plots on the right show the distribution and correlation of different histone modifications.
Histone Modifications Follow Stereotypical Patterns

RNA-seq quintiles

- >80
- 60-80
- 40-60
- 20-40
- < 20

Gene start,
Correlates with expression

Acetylation:
H3K/4/9/14/18/23/27/56
Histone Modifications Follow Stereotypical Patterns

Methylation:
- H3K36me3, H3K79me3, H4R3me/me2

Gene body, Correlates with expression
Histone Modifications Follow Stereotypical Patterns

Throughout the gene Anti-correlates with expression
Research Questions

• Are there stereotypical modification patterns?
  • Yes
  • Reflect transcriptional activity level and position along the gene

• Chromatin changes during transcriptional reprogramming?
Modifications Change with Transcription

Number of nucleosomes changing in X modifications or more

- RNA change (2-fold from mid-log)
- PolIII change (top 10% changes from mid-log)
- Change in the given number of modifications or more
Modifications Change with Transcription
Modifications Change with Transcription

Promoter:
- H3 Ac marks

5’ end:
- H3K4me3

Gene body:
- H3K36me3

Promoter / 5’ end:
- H3K4me2
- H2A.Z

Gene body:
- H3K36me2
- H2AK120
- H4K16ac

Correlated

Anti-correlated
Research Questions

• Are there stereotypical modification patterns?
• Chromatin changes during transcriptional reprogramming?
  • Changes coordinate with transcriptional changes
  • Association with transcription maintains steady-state “rules”
• Changes in combinatorial complexity?
Transient Increase in Complexity

- Unexplained percentage
- Percent variance explained
- Principal components
- T = 0
- T = 15
New Combinations?

5’ nucleosomes scatter

H3K4me3 vs H3K18ac
New Combinations?
New Combinations?
Research Questions

• Stereotypical modification patterns?
• Chromatin changes during transcriptional reprogramming?
• Changes in combinatorial complexity?
  • Mild transient increase
• Timing of events?
Timing of Change & Expression

H3K23ac, nucleosome +1

measured

interpolated

expression

early

GLK1

late

early

4' 8' 15' 30' 60'

0' 10' 20'

5' 20' 40' 60'

1413 genes
Choreography of Induced Genes

H3K14ac  H2A129ph  H3K4me3

H2A129ph – H3K14ac  H3K4me3 - H2A129ph

p<10^{-5}  74%
p<10^{-30}  95%
Choreography of Induced Genes
Differences in Repression

Ribosomal Biogenesis

Stress

5' 00"

H3K4/9/14/18/23/27/56ac
H2AK5ac H4R3me2s

1' 20"

H4K5/8/12ac
H4K20me Htz1

1' 55"

H2AS129ph RNA
H3K4me H3K4me3
H3K36me2 H3S10ph

3' 25"

H3K4me2
H3K4me3 H3K36me
H3K36me2
H3K79me H4R3me

8' 35"

H3K79me3
H3K36me3

Ribosomal Proteins

Stress

6' 40"

H4R3me
H4K5/8/12/16ac

2' 25"

H2AK5ac
H2AS129ph Htz1
H3K4/14/18/23/27/56ac
H4R3me

1' 40"

H3K9ac
H4K20me

3' 55"

H3K4me2 H3S10ph
H3K36me2 H3K79me

6' 30"

RNA H3K36me2
Conclusions (Part II)

• Comprehensive modifications map
• Stereotypical patterns, few combinations
• Strong connection with transcription (static and dynamic)
• New (transient) combinations emerge during transcriptional changes
• Hints about timing of events
Parting Thoughts

“Traffic Cops” or “Bystanders”? Do histone modifications play an instructive or largely passive role in transcription?

Necessary Instructive  

Dispensable Permissive
Parting Thoughts
“Traffic Cops” or “Bystanders”?

KO/mutation in ubiquitous marks results in subtle expression defects

Lenstra et al, Mol. Cell 2012