Maintaining the Silenced State of Master Regulatory Genes During Development

Prof. Bob Kingston
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Most cells

Homeotic Gene (Master regulator)

Some cells

Cell division

Early homeotic gene expression patterns are transmitted through development
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The polycomb group is responsible for stable repression of homeotic genes

Early Embryogenesis

Late Embryogenesis

Polycomb group mutants fail to maintain repression of homeotic genes

Transcription levels of developmentally important genes are established by one set of mechanisms and maintained by different mechanisms

Genetic analysis has identified numerous genes required for maintenance of transcription patterns

These gene products form several complexes that make transcription patterns mitotically heritable - "transcriptional memory"
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Polycomb-group genes

<table>
<thead>
<tr>
<th>Fly</th>
<th>Mammal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycomb (Pc)</td>
<td>hPc1, hPc2 (M33), hPc3</td>
</tr>
<tr>
<td>Posterior sex combs (Psc)</td>
<td>bmi-1, mel-18</td>
</tr>
<tr>
<td>polyhomeotic (ph-p and ph-d)</td>
<td>PHH1 (Ras28), hPn2</td>
</tr>
<tr>
<td>Polycomb-like</td>
<td>M96</td>
</tr>
<tr>
<td>Additional sex combs</td>
<td>hASX</td>
</tr>
<tr>
<td>Enhancer of zest (E(z))</td>
<td>EZH1, EZH2</td>
</tr>
<tr>
<td>extra sex combs (esc)</td>
<td>Eed</td>
</tr>
<tr>
<td>Su(z)12</td>
<td>Suz12</td>
</tr>
<tr>
<td>Multi Sex Combs</td>
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<tr>
<td>Pleiohomeotic</td>
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<tr>
<td>Sex combs on midleg (Scm)</td>
<td>SCML1, SCML2</td>
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<tr>
<td>Sex comb extra</td>
<td></td>
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<tr>
<td>super sex comb</td>
<td></td>
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<tr>
<td>Cramped</td>
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</tr>
</tbody>
</table>

Core functional PcG complexes

mammalian names

- PRC2
  - SUZ12
  - EZH2
  - EED
  - bmi-1
- PRC1
  - PC
  - RING1B
  - PH
  - BMI-1
- Histone
  - H3, K27

Polycamb-group targets include large regions of the genome - e.g., HOX
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Outline:
I. Methylation by PRC2, what is its role?
II. Targeting – How are the PcG complexes that maintain silencing targeted to the right places in the genome?
III. Mode of repression – How are genes silenced?
IV. Epigenetic memory – How is the silenced state maintained during replication and mitosis?

PRC2 complexes and alternative subunits in drosophila and humans

Key activity: H3-K27 methylation

PRC2 core subunits in humans

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Are histones major carriers of epigenetic information?

Prominent modifications that can occur

Marking of the ‘epigenome’ via differential H3 methylation

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Not all histone methyl marks, binding effectors, are equal

H3

α-H3 K9 (Me)_3

α-H3 K27 (Me)_3

'always' off

'regulated' off

α-HP1

α-Pc

 Merge

Merge

Chromodoms bind H3 tail marks

A

HP1

PC

B

CD

CSD

206

Me-rich

206

350

Me-rich

HP1/H3-Me3K9

H3 tail peptides insert as β-strands

Me groups are 'caged' by 3 aromatic residues

Differential chromodomain binding to methyl (Lys9) vs. methyl (Lys27) H3 peptides

HP1

K9Me3

K27Me3

Pc

K27Me3

K9Me3

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Drosophila PREs are bound by PHO (YY1 in humans)
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Targeting - how are the PcG complexes that maintain silencing targeted to the right places in the genome?

Mechanisms known/proposed to be involved in targeting:

- Sequence-specific factors that bind to PREs (PHO, YY1)
- Covalent modification of histones (H3K27 methylation)
- Nucleosome free regions at PREs
- CpG islands recruit PRC2 and PRC1 (Mendenhall et al., PLOS Genetics, 2010)
- Long non-coding RNAs (Xist, HOTAIR)

Mammalian HOX clusters are a key PRC1 target: what happens to nucleosome occupancy?

A region between HOXD11 and HOXD12 is depleted of nucleosomes in human MSCs

Woo et al., Cell, 2010
Human D11.12 has many similarities to fly PREs

1. Depleted of nucleosomes
2. Bordered by high K27 methylation

D11.12 repression requires YY1 sites and a conserved region

Endogenous D11.12 is occupied by PcG proteins and YY1 in the genome of MSCs
Human D11.12 has many similarities to fly PREs
1) Depleted of nucleosomes
2) Bordered by high K27 methylation
   • Confers repression on a reporter
   • YY1 (PHO homolog) sites are important
   • Recruits PRC1 and PRC2 components
   • PRC1 and PRC2 components are required for repressive function
   • Repression is maintained during differentiation; requires PcG function
Proposal: Recruitment of PcG function in humans is similar to what has been seen in flies

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  (Mendenhall et al., PLOS Genetics, 2010)
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Examples of noncoding RNAs that influence chromatin structure

- RNAi-mediated heterochromatin in fission yeast
  e.g. Verte, A. et al. Science 303, 672–676, 2004
- RNA-directed DNA methylation in plants
- RNA-guided gene rearrangement and deletion in ciliates
- lncRNA-directed gene imprinting in mammals
- Dosage compensation in mammals

Dosage compensation in mammals is an lncRNA mediated process


Polycomb Proteins Targeted by a Short Repeat RNA to the Mouse X Chromosome

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Many IncRNAs may act in trans to regulate chromatin

Adapted from: Koziol & Rinn Curr Opin Genet Dev 2010

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PRC1 family complexes are involved in compaction and H2A ubiquitylation

Primary engine of silencing are the complexes in the PRC1 family

PRC1 Complexes that can compact (and ubiquitylate?)

Drosophila

PRC1 Complexes that can ubiquitylate (and compact?)

DRAF (Drosophila)

BCOR (Mouse)

Zhang, Verrijzer, Bardwell, Vidal groups

Prominent modifications that can occur

- Lysine 119 of histone H2A is ubiquitylated
- Knocking out the ability to ubiquitylate in Drosophila and Mouse decreases repression of target genes (Endoh et al., PLOS Genetics, 2012)

PRC1 family complexes are involved in compaction and H2A ubiquitylation

PRC1 Complexes that can compact (and ubiquitylate?)

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PRC1 Complexes that can ubiquitylate (and compact?)

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Nucleosome dynamics

Decreasing nucleosome stability

Nucleosome position might contribute to epigenetic regulation

One simple hypothetical example:

Repressed → Active

Polycomb-Group proteins maintain repressive nucleosome position

Mobilizing nucleosomes using SWI/SNF

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Cellular memory proteins compact nucleosomal structure and block nucleosome movement

Core functional PcG complexes mammalian names

In flies, PSC is required for compaction and mutations that impact compaction have in vivo phenotypes

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The domain in PSC that compacts is not conserved in the mouse PSC homologs

- Psc
- Bmi1

45% Identity

The region that compacts is not conserved in Bmi1, the mouse homolog of PSC

M33 (mouse PC homolog) compacts nucleosomes, Bmi1 (mouse PSC homolog) does not

- Drosophila
- Mouse

No protein

M33

Bmi1

Domain of M33 required for compaction mapped using deletions

- Compaction

1 519

The domain required for compaction has high positive charge and is predicted to be disordered
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**Polycomb compaction mediated by charge**

- PSC: Fly
- CBX2: Mouse
- Mig32: Worm
- CBX6: Frog
- CBX6: Zebrafish

*Grau et al., G&D, 2011*

**Is compaction biologically relevant: data from others**

- Deal, Henikoff and Henikoff, “Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones”, *Science*, 2010
- ‘Nucleosomes turn over faster at sites for trithorax-group than polycomb-group protein binding, suggesting that nucleosome turnover differences underlie their opposing activities and challenging models for epigenetic inheritance that rely on stability of histone marks.’

**Chromatin compaction as a mechanism for gene silencing**

- Define exactly what these structures are at anatomic level
- Look at mutations that would impact the ability to create these structures
- Determine whether or not those mutations impact the ability to repress genes in vivo
- Generate a crystal structure

*Francis, Kingston, Woodcock, Science, 2004*

*Johnson, Li, Sikorski, Buniewski, Woodcock, Moazed, *Mol Cell*, 2009*
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The SIR complex is central to mating type silencing
- Regulation of mating in budding yeast – paradigm for gene silencing
- Silent mating type loci and telomeres silenced by trimeric SIR complex
  - Deacetylation of H4 K16

The SIR3 BAH domain is central to mating type silencing
- Sir3 alone compacts chromatin arrays
- The BAH domain is key to silencing (numerous genetic studies) and compaction

Sir3 BAH/NCP complex at 3 Å resolution

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Key features of the structure

- Excellent correlation between genetic and physical contacts
- Structural rearrangements in NCP and BAH upon complex formation
- Acetylation of H4 K16 disrupts multiple contacts

Physical and genetic contacts correlate

We have an atomic explanation for over 30 genetically identified mutations (50% of those identified)
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Key features of the structure

- Excellent correlation between genetic and physical contacts
- Structural rearrangements in NCP and BAH upon complex formation
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Folding events in Sir3 BAH/NCP complex

NCP

Apo BAH
Z. Hou et al., Protein Sci, 2006

Folding events in Sir3 BAH/NCP complex (2)

loops that fold in the complex
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Both Sir3 BAH and the NCP alter structure when in complex

Both Sir3 BAH and the NCP alter structure when in complex (2)

Folding events in Sir3 BAH/NCP complex
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Sir3 BAH changes

Sir3
BAH
NCP

Apo BAH
Xu Sternglanz

Speculative model based upon crystal packing interactions

BAH domains bridge adjacent nucleosomes
Same BAH dimer in apo structure

Sternglanz and Xu labs

Summary

Large interaction surface
Good fit between structure and genetics

Folding events in the complex
Molecular basis for K16 interaction

Armache et al., Science

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The nucleosome as a component of the silencing machinery

Mechanisms to regulate compaction/dynamics of nucleosomes:
The silencing protein Sir3 forms a large interface with the nucleosomal histones that can bridge nucleosomes; thus the histones and the BAH domain work in concert to form a regulatory complex that contains both structural components and the regulator.

Polycomb compaction mediated by charge

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Epigenetic memory - how is the silenced state maintained during replication and mitosis?
1) Is methylated H3 K27 a mark that is 'remembered' following replication?
Mechanisms have been found that might allow this

PRC2 core subunits in the EED subunit of PRC2 binds to methylated K27

Thus, if histones that have this mark are divided on the two daughter strands they will promote more K27 methylation
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Epigenetic memory - How is the silenced state maintained during replication and mitosis?

1) Is methylated H3 K27 a mark that is ‘remembered’ following replication?

2) Does PRC1 stay on chromatin during replication?

Yes, both in vitro and in cells PRC1 components remain associated with chromatin during replication

Francis et al., Cell 2009; Lo et al., Mol. Cell 2012
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Epigenetic memory - How is the silenced state maintained during replication and mitosis?

1) Is methylated H3 K27 a mark that is ‘remembered’ following replication?
2) Does PRC1 stay on chromatin during replication?
3) Are repressed regions ‘bookmarked’ by proteins so that PcG components and repression stays in place during mitosis and cell division?

Less is known: PRC1 can stay on chromatin during mitosis in stem cells (Fonseca et al., Genes Dev., 2012)

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