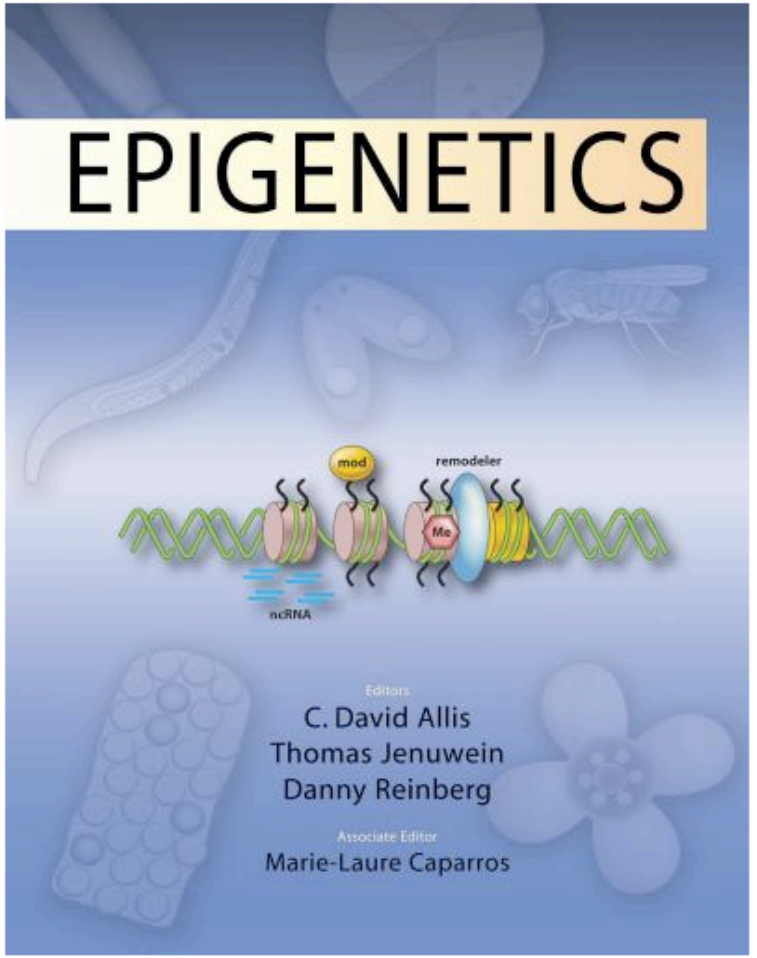
Gene Expression GE4055

- Lecture 1: Introduction to Epigenetics
- Lecture 2: Histone modifications
- Lecture 3: Polycomb and Trithorax proteins
- Lecture 4: DNA Methylation
- Lecture 5: Non-Coding RNAs
- Lecture 6: Epigenetics in development/differentiation
- Lecture 7: Epigenetic reprogramming
- Lecture 8: Epigenetics in cancer and other diseases



How is a stable “Epigenetic state” established?

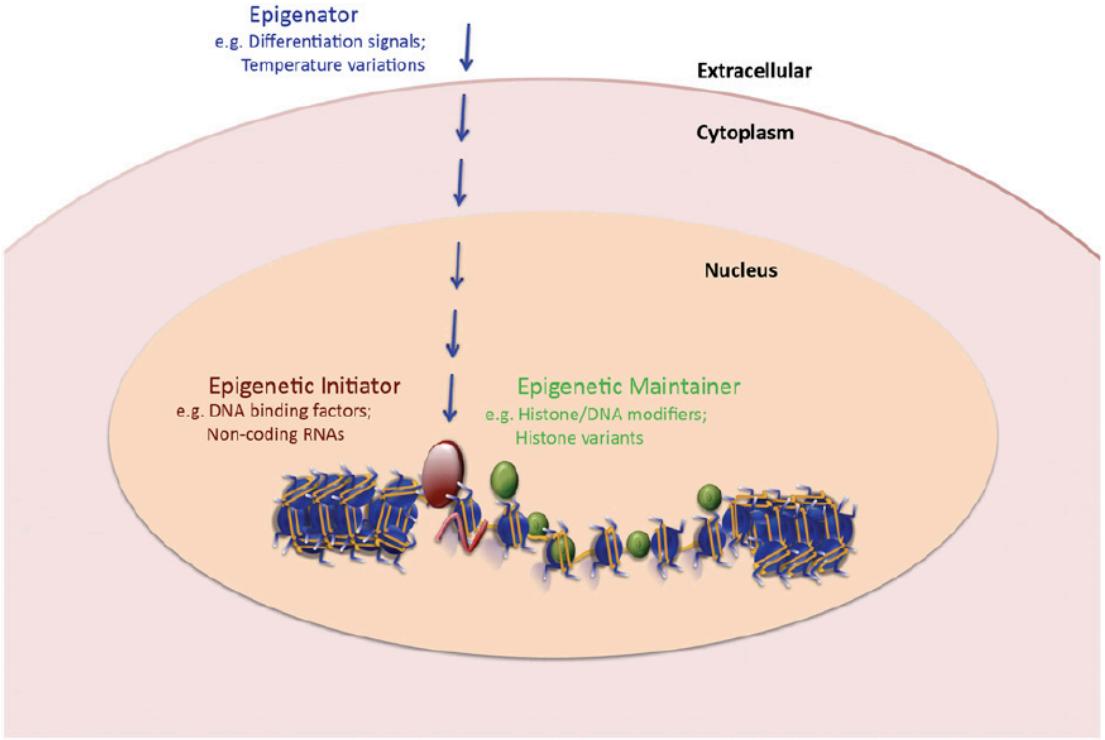


Figure 1. The epigenetic pathway. Three categories of signals are proposed to operate in the establishment of a stably heritable epigenetic state. An extracellular signal referred to as the “Epigenator” (shown in blue) originates from the environment and can trigger the start of the epigenetic pathway. The “Epigenetic Initiator” (shown in red) receives the signal from the “Epigenator” and is capable of determining the precise chromatin location and/or DNA environment for the establishment of the epigenetic pathway. The “Epigenetic Maintainer” (shown in green) functions to sustain the chromatin environment in the initial and succeeding generations. Persistence of the chromatin milieu may require cooperation between the Initiator and the Maintainer. Examples for each category are shown *below* each heading. Chromatin is depicted in blue.

Polycombs, Histone modifying enzymes, DNMTs and ncRNAs are essential for development

Modifier	Function	Mutant Phenotype	Maternally Inherited	ES Cell Derivation	Reference
Polycomb					
Eed	PRC2/3 complex	Defective gastrulation; failure to maintain inactive X in trophoblast cells	yes	yes	Shumacher et al. (1996)
Suz12	PRC2/3 complex	Early postimplantation lethality; gastrulation defects	yes	ND	Pasini et al. (2004)
YY1	PRC2/3 interaction	Defects in epiblast cell growth/survival; peri-implantation lethality	yes	no	Donohoe et al. (1999)
Ring1b/ Rnf2	Ubiquitin ligase PRC1 complex	Gastrulation defects; lethality by E9.5	yes	ES viable	Voncken et al. (2003)
DNA Methylation					
Dnmt1	DNA MTase	Genome-wide demethylation; developmental arrest at E8.5	yes	yes	Li et al. (1992)
Dnmt3a	DNA MTase	Malfunction of gut; spermatogenesis defects; postnatal lethality (~4 weeks of age)	yes	yes	Okano et al. (1999)
Dnmt3b	DNA MTase	Demethylation of minor satellite DNA; mild neural tube defects; embryonic lethality at E14.5–E18.5	yes	yes	Okano et al. (1999)
Dnmt3L	DNA MTase (no enzymatic function)	Failure to establish maternal methylation imprints in oocytes; male sterility due to spermatogenesis defects	yes	ND	Bourc'his et al. (2001); Hata et al. (2002)
MBD Proteins					
Mbd3	Chromatin-remodeling NuRD complex	Normal implantation; developmental arrest at E6.5 or earlier	yes	no (ES viable)	Hendrich et al. (2001); Kaji et al. (2006)

Histone modifying enzymes, DNMTs, Polycombs and ncRNAs are essential for development

Modifier	Function	Mutant Phenotype	Maternally Inherited	ES Cell Derivation	Reference
Glp/Ehmt1	HMTase	Severe growth retardation and lethality at E9.5; reduction of H3K9me1 and H3K9me2 in embryos	ND	yes	Tachibana et al. (2005)
G9a/Ehmt2	HMTase	Loss of H3K9 methylation in euchromatin; developmental and growth arrest at E8.5	yes	yes	Tachibana et al. (2002)
Eset/ SETDB1	HMTase	Peri-implantation lethality (between E3.5 and E5.5); defects in ICM outgrowth	yes	no	Dodge et al. (2004)
Suv39h1 Suv39h2	HMTase	Double knockout shows loss of H3K9 methylation in heterochromatin; polyploidy in MEF cells; chromosome pairing defects during spermatogenesis; male sterility and death of some double-mutant embryos at E14.5	ND	yes	Peters et al. (2001)
Ezh2/ Enx-1	HMTase PRC2 complex	Growth defect of the primitive ectoderm; peri-implantation lethality	yes	no	O'Carroll et al. (2001)
Mll/All-1	HMTase	Skeletal abnormalities; Hox gene misregulation (loss of H3K4me1 and aberrant DNA methylation); other morphogenetic defects by E10.5; embryonic lethality; truncation in exon 5 leads to early developmental arrest prior to two-cell stage	ND	ES viable (defective gene expression)	Glaser et al. (2006); Yagi et al. (1998); Yu et al. (1995)
Meisetz	HMTase	Meiotic defect causing sterility	no	ND	Hayashi et al. (2005)
PRMT1	Arg MTase	Early postimplantation lethality before gastrulation	ND	yes	Pawlak et al. (2000)
Blimp1/ PRDM1	PR/SET domain protein	Patterning defects; loss of germ cell precursors	no	yes	Ohinata et al. (2005); Vincent et al. (2005)
Gcn5	HAT	Lethal at E7.5–E8.5; patterning defects	yes	ND	Xu et al. (2000)
HDAC1	HDAC	Defects in proliferation; delayed development; embryonic lethality by E10.5	yes	yes (ES cells defective)	Lagger et al. (2002)

Histone modifying enzymes, DNMTs, Polycombs and ncRNAs are essential for development

Modifier	Function	Mutant Phenotype	Maternally Inherited	ES Cell Derivation	Reference
Chromatin-Remodeling/Histone Chaperones					
Brg1	SWI/SNF	Growth defects of primitive ectoderm and trophectoderm; peri-implantation lethality; oocyte depletion causes zygotic arrest	yes	no	Bultman et al. (2000); Bultman et al. (2006)
Snf5/Ini1/ Smarcb1	SWI/SNF	Peri-implantation lethality	ND	no	Klochendler-Yeivin et al. (2000)
Lsh/Hells/ PASG	SWI/SNF	Global demethylation of genomic DNA at E13.5; role in meiotic chromosome synapsis and retrotransposon silencing in female germline; postnatal lethality	yes	ND	Geiman and Muegge (2000); Sun et al. (2004); De La Fuente et al., 2006
Srg3/ Smarcc1	SWI/SNF	Lethality around implantation; defective ICM outgrowth	ND	no	Kim et al. (2001)
ATRX	SWI/SNF	Male-specific embryonic lethality by E9.5 due to defect in formation of extraembryonic trophoblast and X inactivation	yes	ND	Garrick et al. (2006)
CAF-1	Histone chaperone	Early preimplantation lethality; arrest at 16-cell stage; defects in constitutive heterochromatin	yes	no	Houlard et al. (2006)
HIRA	Histone chaperone	Gastrulation defects; embryonic lethality by E10.5	yes	yes	Roberts et al. (2002)
Nasp	Histone chaperone	Preimplantation lethality at blastocyst stage	yes	no	Richardson et al. (2006)
Npm2	Histone chaperone	Defective nucleolar structure; loss of heterochromatin and acetylated histone H3; early preimplantation lethality (most embryos arrested at two-cell stage)	yes	no	Burns et al. (2003)
miRNA Metabolism					
Ago2	miRNA processing	Lethal at E9.5	ND	ND	Liu et al. (2004)
Dicer	miRNA processing	Postimplantation lethality before gastrulation	yes	no (ES viable)	Bernstein et al. (2003)

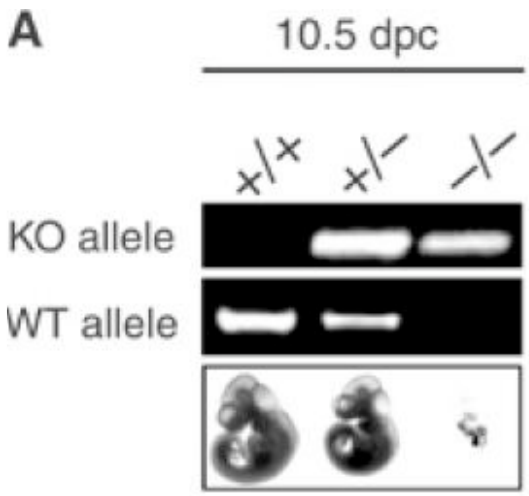
Just one example: The Polycomb Suz12 -/-

The EMBO Journal (2004) 23, 4061–4071 | © 2004 European Molecular Biology Organization | All Rights Reserved 0261-4189/04
www.embojournal.org

Suz12 is essential for mouse development and for EZH2 histone methyltransferase activity

Diego Pasini[1], Adrian P Bracken[1], Michael R Jensen[1], Eros Lazzerini Denchi[1] and Kristian Helin[1,2,*]

[1]European Institute of Oncology, Milan, Italy and [2]Biotech Research and Innovation Centre, Copenhagen, Denmark



MOLECULAR AND CELLULAR BIOLOGY, May 2007, p. 3769–3779 Vol. 27, No. 10
0270-7306/07/$08.00+0 doi:10.1128/MCB.01432-06

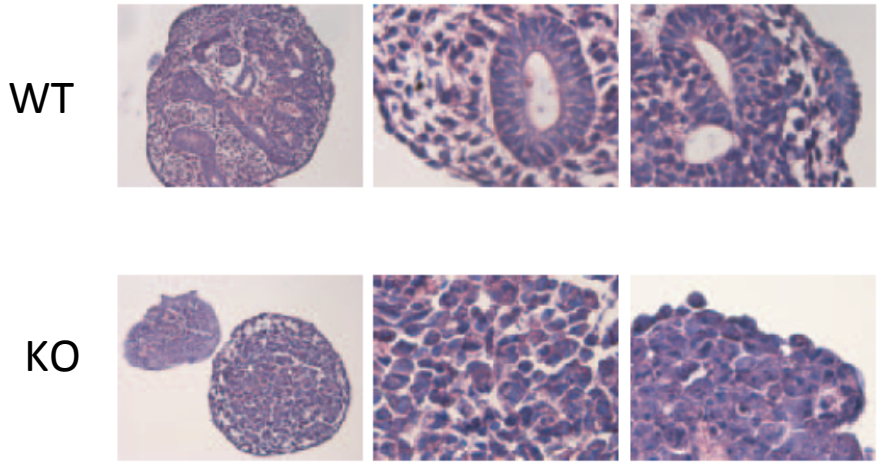
The Polycomb Group Protein Suz12 Is Required for Embryonic Stem Cell Differentiation▽†

Diego Pasini,[1] Adrian P. Bracken,[1] Jacob B. Hansen,[2] Manuela Capillo,[3,4] and Kristian Helin[1]*

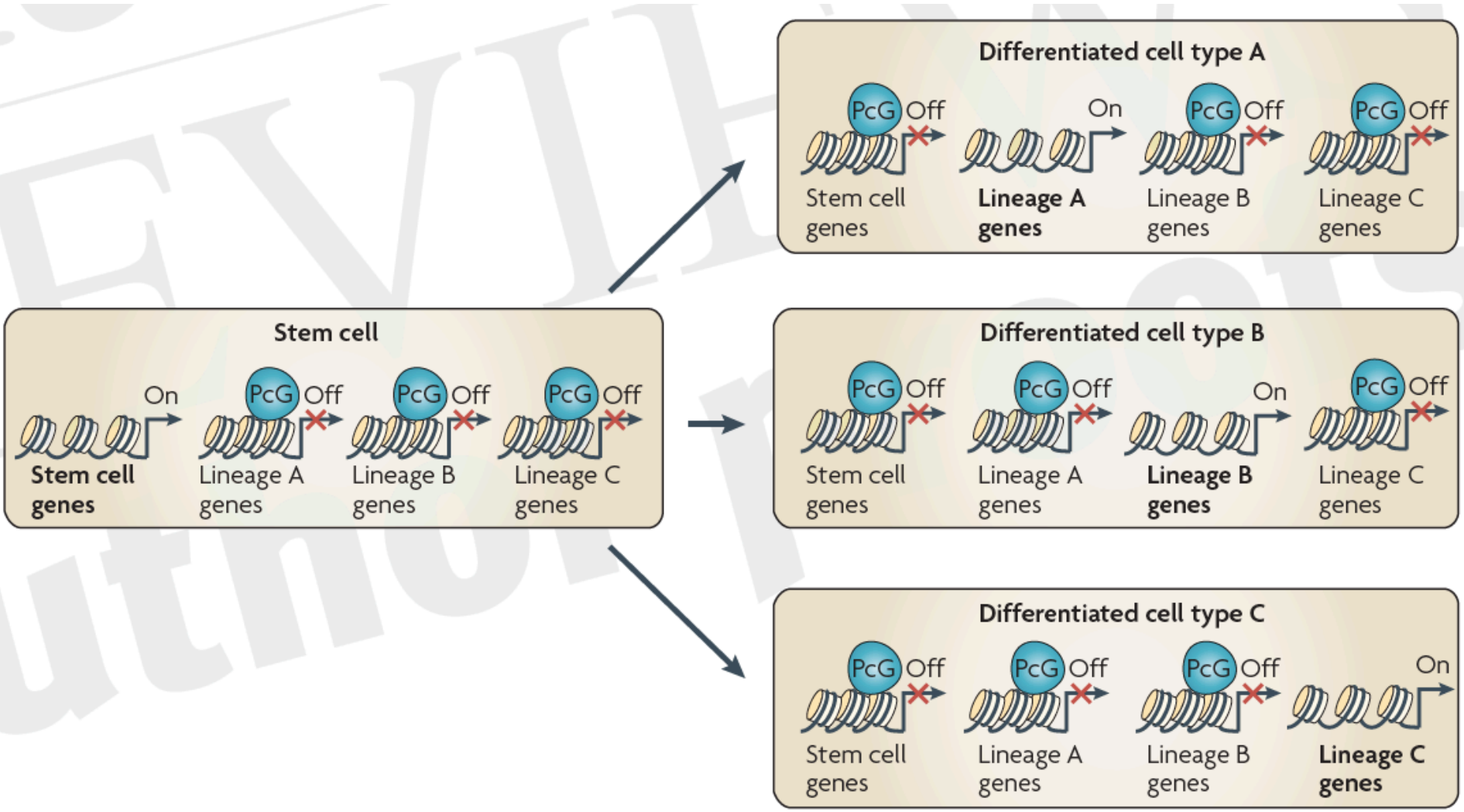
Centre for Epigenetics and BRIC, University of Copenhagen, Ole Maaløes Vej 5, 2200 Copenhagen, Denmark[1]; Department of Biomedical Sciences, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen, Denmark[2]; Department of Experimental Oncology, European Institute of Oncology, Via Ripamonti 435, 20141 Milan, Italy[3]; and Institute of Molecular Oncology of the Italian Foundation for Cancer Research, Via Adamello 16, 20139 Milan, Italy[4]

Received 3 August 2006/Returned for modification 10 October 2006/Accepted 22 February 2007

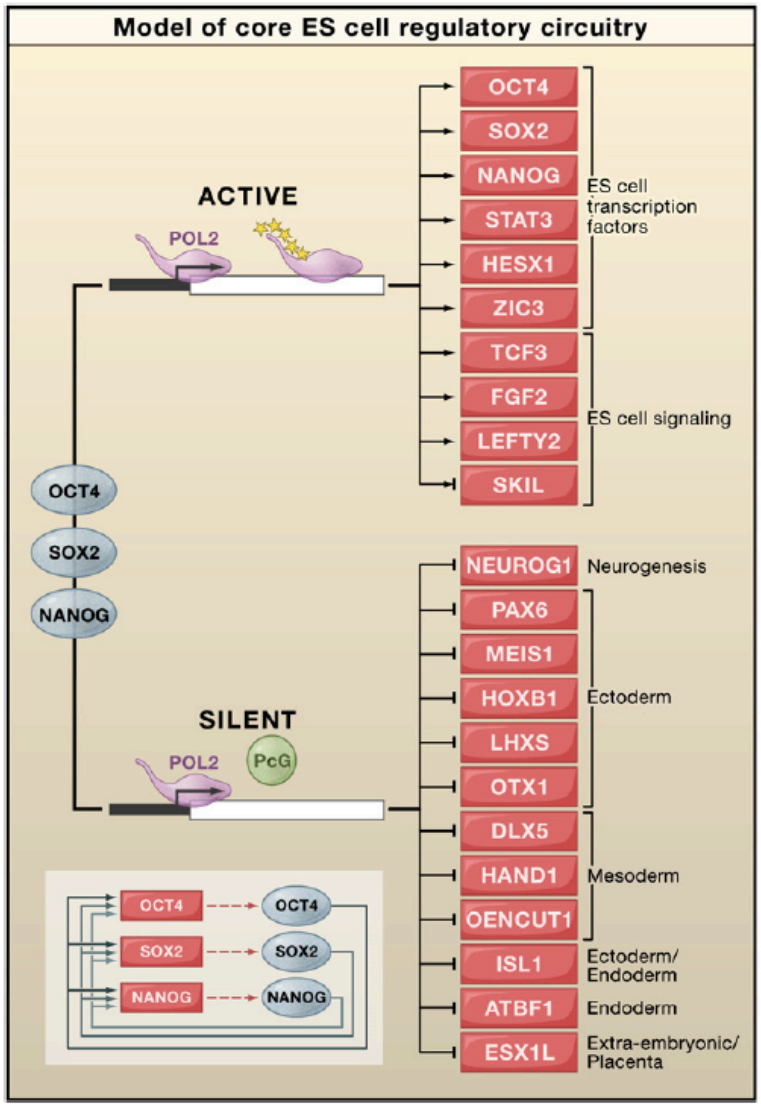
Polycomb group (PcG) proteins form multiprotein complexes, called Polycomb repressive complexes (PRCs). PRC2 contains the PcG proteins EZH2, SUZ12, and EED and represses transcription through methylation of lysine (K) 27 of histone H3 (H3). Suz12 is essential for PRC2 activity and its inactivation results in early lethality of mouse embryos. Here, we demonstrate that *Suz12*$^{-/-}$ mouse embryonic stem (ES) cells can be established and expanded in tissue culture. The *Suz12*$^{-/-}$ ES cells are characterized by global loss of H3K27 trimethylation (H3K27me3) and higher expression levels of differentiation-specific genes. Moreover, *Suz12*$^{-/-}$ ES cells are impaired in proper differentiation, resulting in a lack of repression of ES cell markers as well as activation of differentiation-specific genes. Finally, we demonstrate that the PcGs are actively recruited to several genes during ES cell differentiation, which despite an increase in H3K27me3 levels is not always sufficient to prevent transcriptional activation. In summary, we demonstrate that Suz12 is required for the establishment of specific expression programs required for ES cell differentiation. Furthermore, we provide evidence that PcGs have different mechanisms to regulate transcription during cellular differentiation.



Current model of “Lineage choice” from a Polycomb only perspective



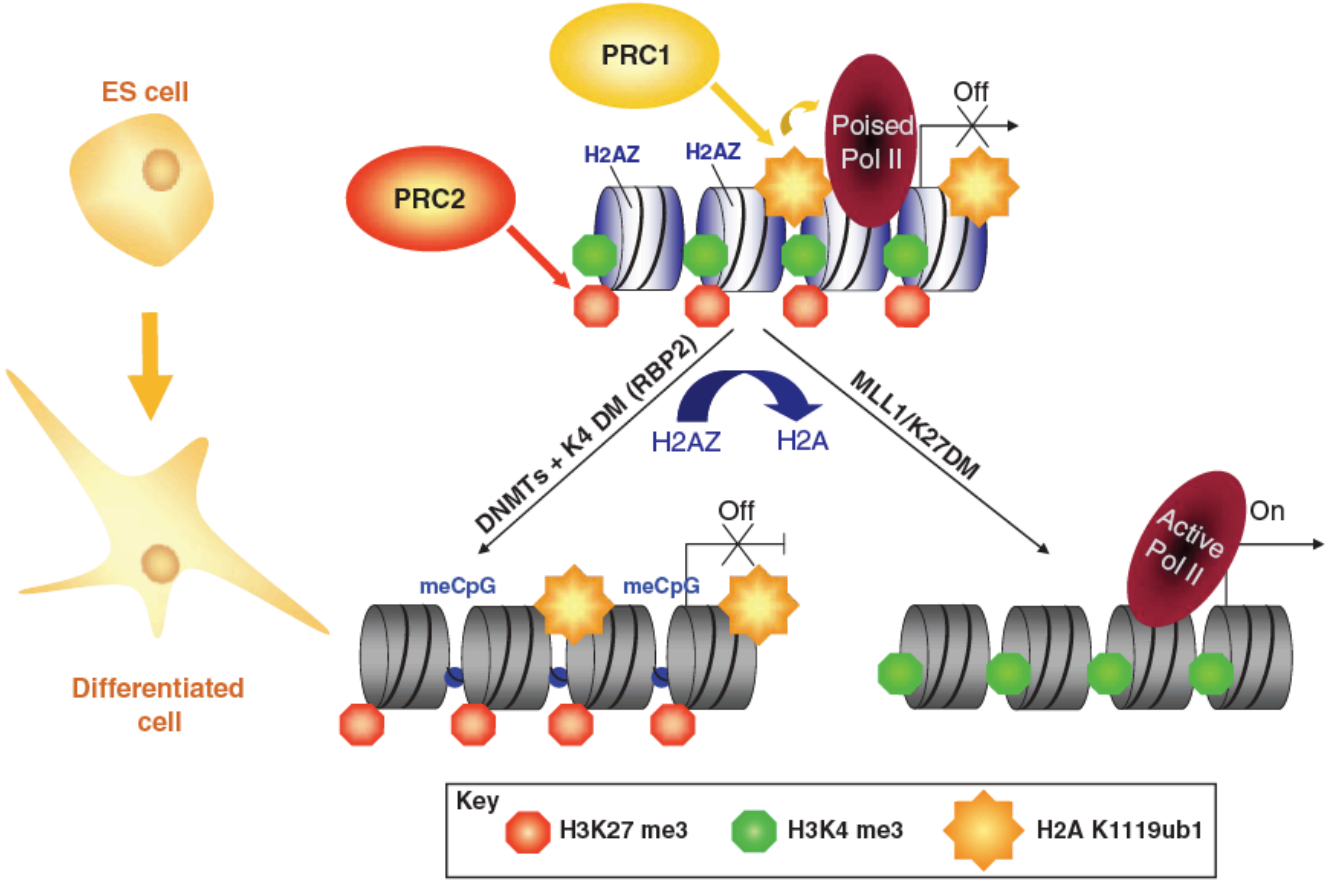
SO what are Polycombs doing during embryonic development?



Silence Differentiation genes in ES cells

Silence ES genes in Differentiated cells

The current model of Polycombs, Trithorax and DNA methylation during lineage commitment during development and differentiation

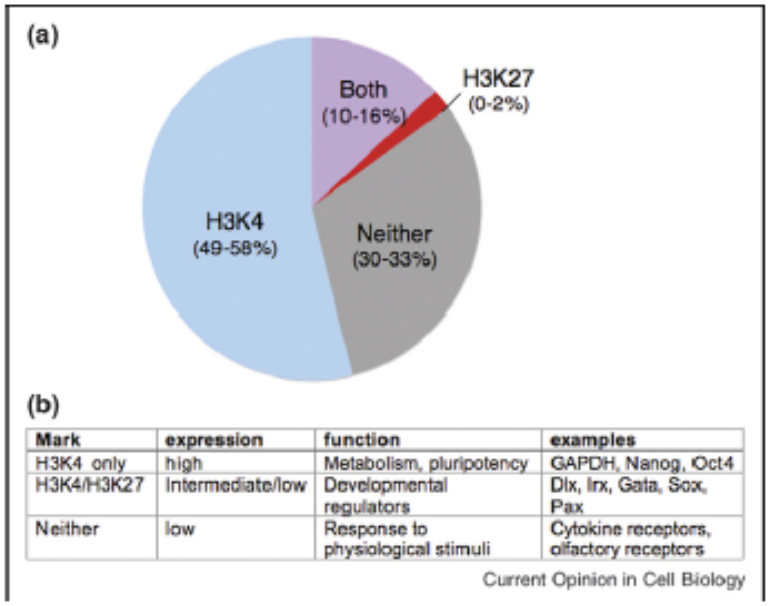


"Bivalency" Bernstein 2006

A Bivalent Chromatin Structure Marks Key Developmental Genes in Embryonic Stem Cells

Bradley E. Bernstein,[1,2,3,*] Tarjei S. Mikkelsen,[3,4] Xiaohui Xie,[3] Michael Kamal,[3] Dana J. Huebert,[1] James Cuff,[3] Ben Fry,[3] Alex Meissner,[5] Marius Wernig,[5] Kathrin Plath,[5] Rudolf Jaenisch,[5] Alexandre Wagschal,[6] Robert Feil,[6] Stuart L. Schreiber,[3,7] and Eric S. Lander[3,5]

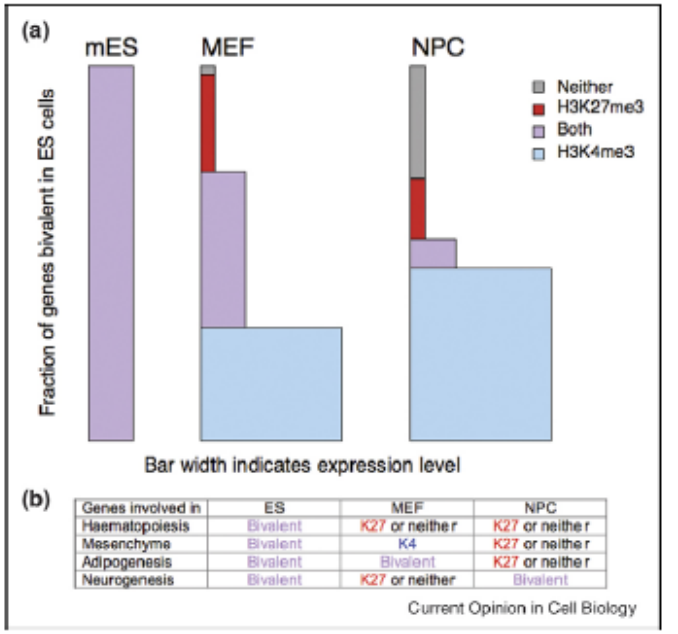
Figure 2



Mark	expression	function	examples
H3K4 only	high	Metabolism, pluripotency	GAPDH, Nanog, Oct4
H3K4/H3K27	Intermediate/low	Developmental regulators	Dlx, Irx, Gata, Sox, Pax
Neither	low	Response to physiological stimuli	Cytokine receptors, olfactory receptors

Bivalent domains in human ES cells. **(a)** Distribution of H3K4me3 and H3K27me3. The percentage of genes that was marked by trimethylation of H3K4 (blue), H3K27 (red), both (purple) or neither (grey), based on two studies [18**,19**] in human ES cells. Of note, sequential ChIP in ES cells has demonstrated that trimethylation of K4 and K27 occur on the same region [18**], though not necessarily on the same nucleosome [19**]. **(b)** Functional distinct groups based on histone modifications. Genes classified by the presence or absence of H3K4me3 and H3K27me3 are associated with different expression levels and gene ontology classes ('function') in both human and mouse ES cells [18**,19**,20**].

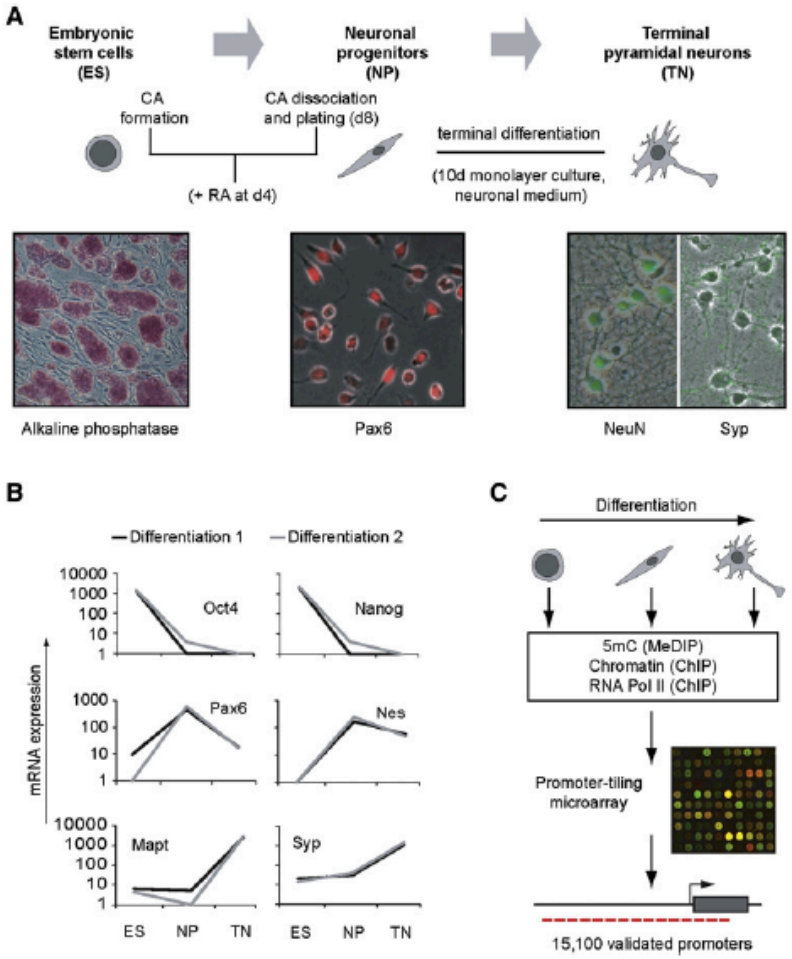
Figure 3



Genes involved in	ES	MEF	NPC
Haematopoiesis	Bivalent	K27 or neither	K27 or neither
Mesenchyme	Bivalent	K4	K27 or neither
Adipogenesis	Bivalent	Bivalent	K27 or neither
Neurogenesis	Bivalent	K27 or neither	Bivalent

Bivalent domains in committed cells. **(a)** Resolution of bivalent domains in MEFs and NPC. About 22% of CpG-rich promoters are bivalently marked in mouse ES cells. These bivalent domains (purple) have an intermediate expression. In MEFs, 32% of these regions are marked by H3K4me3 alone (blue), 22% by H3K27me3 alone (red) and 3% with neither mark (grey). In NPCs, 46% resolve to H3K4me3, only 8% remain bivalent, and the rest becomes associated with low expression (14% H3K27me3 and 32% with neither mark). Based on Mikkelsen *et al.* [20**]. **(b)** Resolution is linked to developmental potential. Genes that were marked bivalent in mES cells become repressed (H3K27me3 or neither) when these genes are not required for the cell lineage that is committed to, but remain bivalent or become expressed (H3K4me3) if the gene function belongs to the remaining cell fate choices [20**].

Lineage-Specific Polycomb Targets and De Novo DNA Methylation Define Restriction and Potential of Neuronal Progenitors



SUMMARY

Cellular differentiation entails loss of pluripotency and gain of lineage- and cell-type-specific characteristics. Using a murine system that progresses from stem cells to lineage-committed progenitors to terminally differentiated neurons, we analyzed DNA methylation and Polycomb-mediated histone H3 methylation (H3K27me3). We show that several hundred promoters, including pluripotency and germline-specific genes, become DNA methylated in lineage-committed progenitor cells, suggesting that DNA methylation may already repress pluripotency in progenitor cells. Conversely, we detect loss and acquisition of H3K27me3 at additional targets in both progenitor and terminal states. Surprisingly, many neuron-specific genes that become activated upon terminal differentiation are Polycomb targets only in progenitor cells. Moreover, promoters marked by H3K27me3 in stem cells frequently become DNA methylated during differentiation, suggesting context-dependent crosstalk between Polycomb and DNA methylation. These data suggest a model how de novo DNA methylation and dynamic switches in Polycomb targets restrict pluripotency and define the developmental potential of progenitor cells.

Lineage-Specific Polycomb Targets and De Novo DNA Methylation Define Restriction and Potential of Neuronal Progenitors

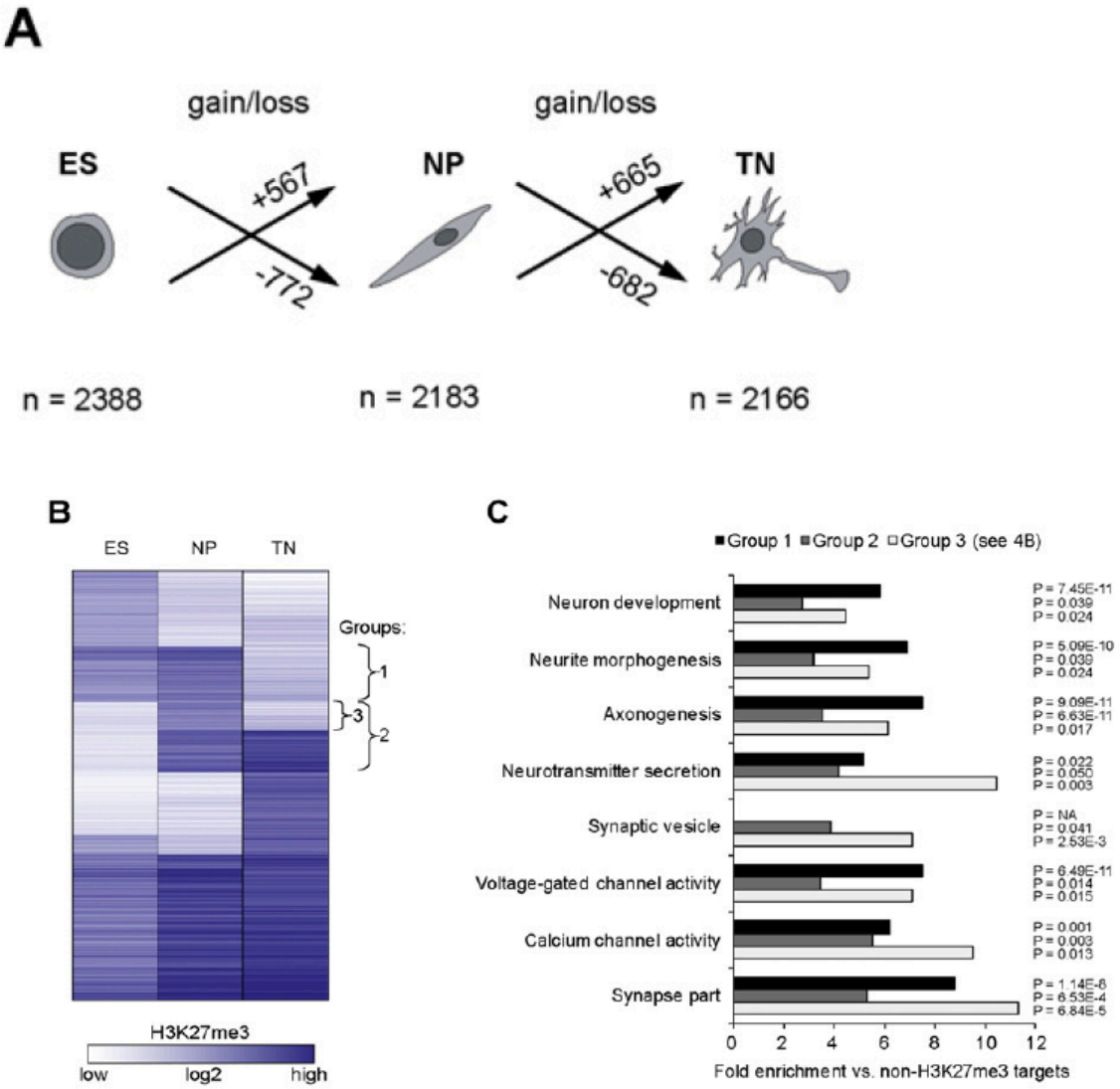


Figure 4. Polycomb Targets Are Highly Dynamic and Stage Specific

(A) Illustration of H3K27me3 target dynamics during neuronal differentiation. Arrows indicate loss (–) and gain (+) of Polycomb targets between the cellular states. "n" indicates the total number of H3K27me3 modified promoters at every individual state.

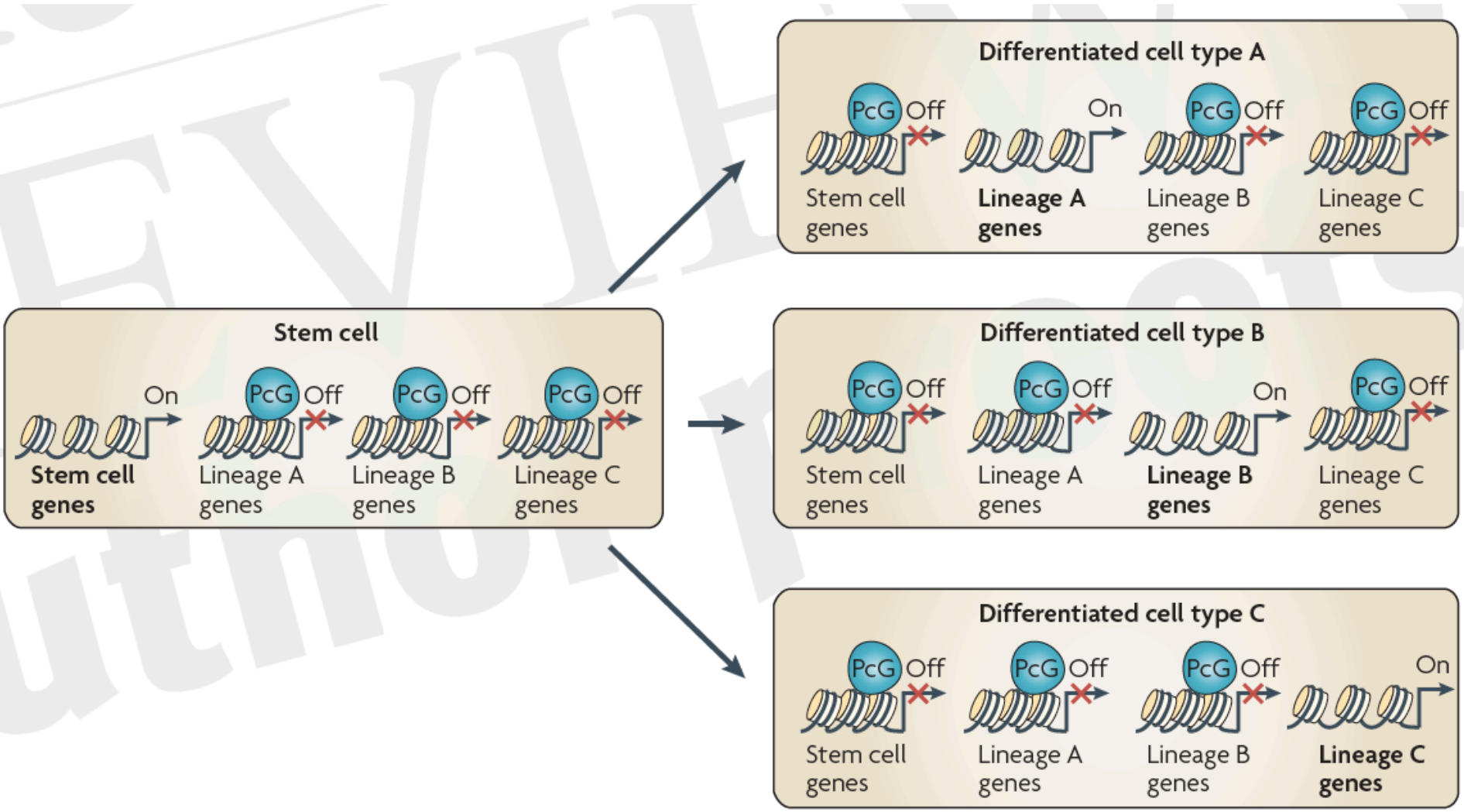
(B) Heatmap for all promoters that are H3K27me3 positive in at least one cell state. Only 43% remain H3K27me3+ throughout the differentiation, while the majority behaves highly plastic (see text).

(C) GO term analysis for genes that lose H3K27me3 in terminal differentiation to TN (Group 1, black), for genes that become Polycomb targets in NP (Group 2, gray), and for Polycomb targets that are specific for NP and lose H3K27me3 during terminal differentiation (Group 3, white). p values are listed next to bars, while NA indicates no significant enrichment in the respective group.

(D) Validation of microarray results for NP-specific H3K27m3 targets by ChIP and real-time PCR. Blue bars represent H3K27me3 enrichments, and red lines indicate Pol II enrichment (left y axis, numbers normalized to an intergenic control). Black lines indicate mRNA levels (Affymetrix, right y axis). *Syt1*, *Sema4f*, *Grid1*, and *Scn1b* are induced upon terminal differentiation and lose H3K27 methylation. *Hes3* and *Adrb2* are not activated and keep H3K27me3. Error bars indicate ± SEM of averages from at least two independent differentiation experiments.

(E) Examples of genes that become repressed in NP (*Zic3*) or TN (*Sall4* and *Uhrf1*) coinciding with a gain of H3K27me3.

Current model of “Lineage choice” from a Polycomb only perspective



How are Polycombs moved around during differentiation?

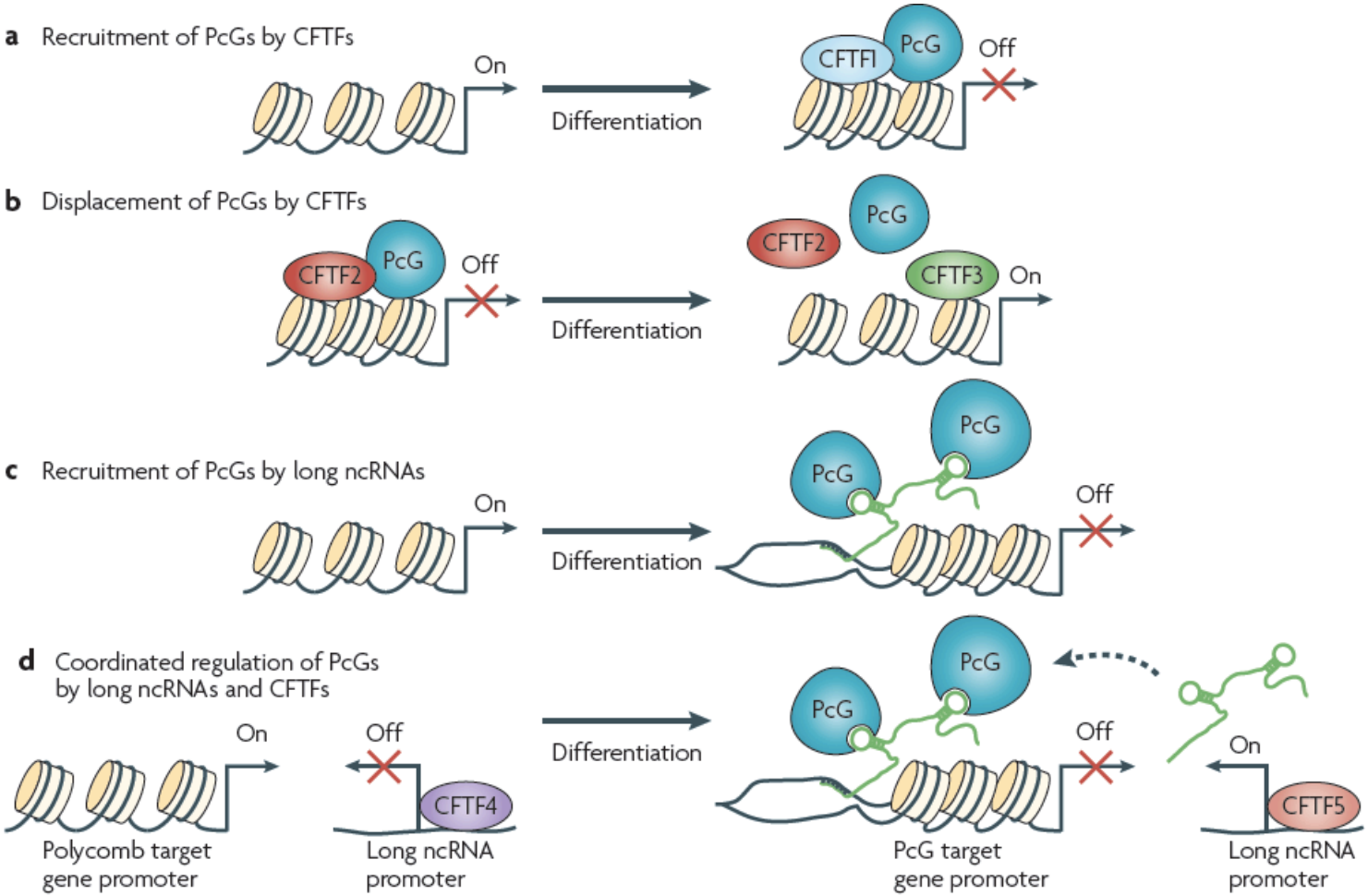
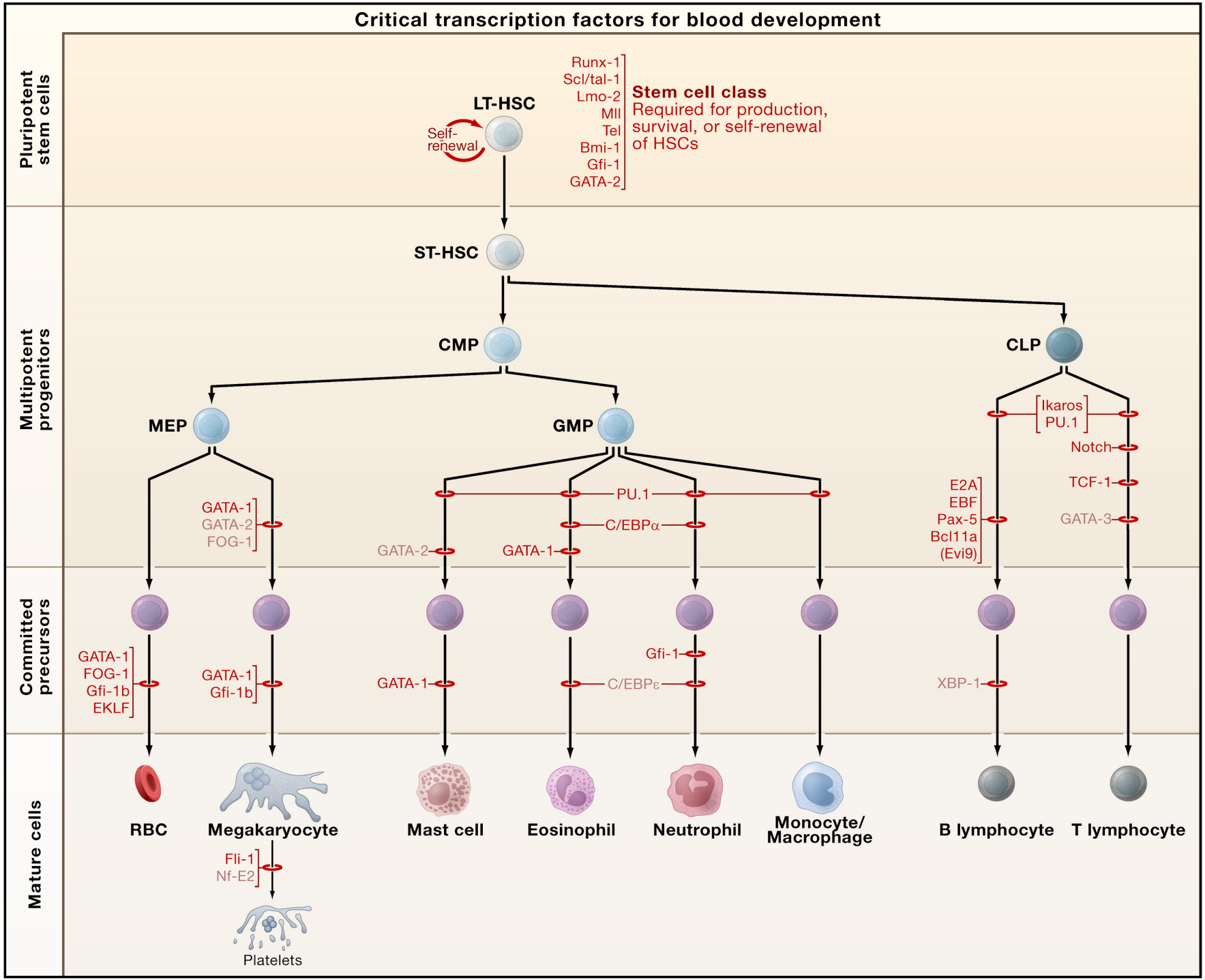


Figure 3 | **Potential mechanisms by which cell fate transcription factors and long non-coding RNAs function to regulate Polycomb group protein association with target genes during lineage choices and specification.** **a** | Cell fate transcription factors (CFTFs) recruit Polycomb group (PcG) proteins to target genes during lineage decisions. **b** | CFTFs induce the dissociation of PcG proteins from target genes during lineage decisions. **c** | Long non-coding RNAs (ncRNAs) recruit PcG proteins to target genes during lineage decisions. **d** | Coordinated action of CFTFs and long ncRNAs is necessary to recruit PcG proteins to or dissociate them from target genes during lineage determination. The long ncRNAs can function either in *cis* or in *trans*.


Critical transcription factors for blood development
Pluripotent stem cells
Multipotent progenitors
Committed precursors
Mature cells
LT-HSC
Self-renewal
Runx-1
Scl/tal-1
Lmo-2
Mll
Tel
Bmi-1
Gfi-1
GATA-2
Stem cell class
Required for production, survival, or self-renewal of HSCs
ST-HSC
CMP
CLP
MEP
GMP
Ikaros
PU.1
Notch
TCF-1
GATA-3
E2A
EBF
Pax-5
Bcl11a
(Evi9)
GATA-1
GATA-2
FOG-1
PU.1
C/EBPα
GATA-2
GATA-1
GATA-1
FOG-1
Gfi-1b
EKLF
GATA-1
Gfi-1b
GATA-1
Gfi-1
C/EBPε
XBP-1
RBC
Megakaryocyte
Mast cell
Eosinophil
Neutrophil
Monocyte/ Macrophage
B lymphocyte
T lymphocyte
Fli-1
Nf-E2
Platelets

Returning to Polycombs in Hematopoiesis ….

letters to nature

Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells

In-kyung Park*, Dalong Qian*, Mark Kiel†, Michael W. Becker*, Michael Pihalja*, Irving L. Weissman‡, Sean J. Morrison† & Michael F. Clarke*

** Division of Hematology/Oncology, Internal Medicine, and † Howard Hughes Medical Institute, Department of Internal Medicine, and Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, Michigan 48109, USA*
‡ Department of Pathology, School of Medicine, Stanford University, Stanford, California 94305, USA

A central issue in stem cell biology is to understand the mechanisms that regulate the self-renewal of haematopoietic stem cells (HSCs), which are required for haematopoiesis to persist for the lifetime of the animal[1]. We found that adult and fetal mouse and adult human HSCs express the proto-oncogene *Bmi-1*. The number of HSCs in the fetal liver of *Bmi-1*$^{-/-}$ mice[2] was normal. In postnatal *Bmi-1*$^{-/-}$ mice, the number of HSCs was markedly reduced. Transplanted fetal liver and bone marrow cells obtained from *Bmi-1*$^{-/-}$ mice were able to contribute only transiently to haematopoiesis. There was no detectable self-renewal of adult HSCs, indicating a cell autonomous defect in *Bmi-1*$^{-/-}$ mice. A gene expression analysis revealed that the expression of stem cell associated genes[3], cell survival genes, transcription factors, and genes modulating proliferation including *p16*^{Ink4a} and *p19*^{Arf} was altered in bone marrow cells of the *Bmi-1*$^{-/-}$ mice. Expression of *p16*^{Ink4a} and *p19*^{Arf} in normal HSCs resulted in proliferative arrest and p53-dependent cell death, respectively. Our results indicate that *Bmi-1* is essential for the generation of self-renewing adult HSCs.

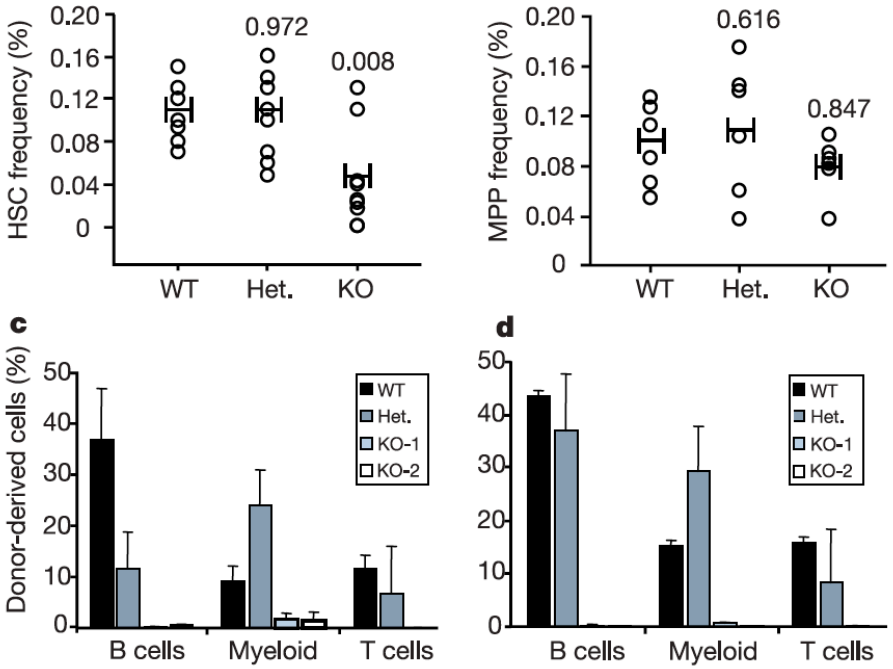
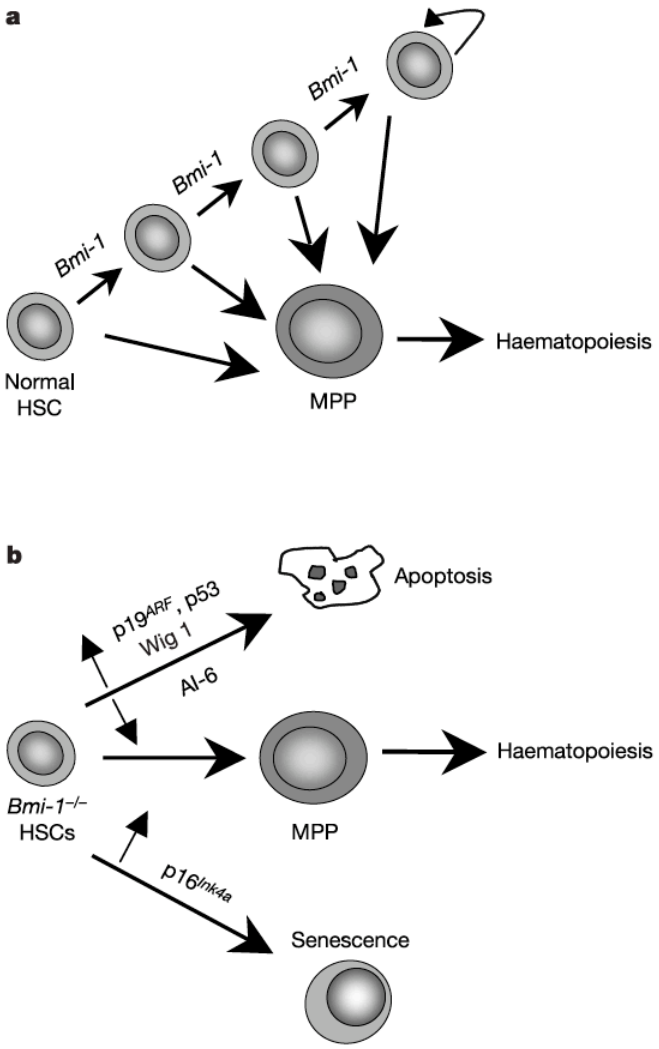
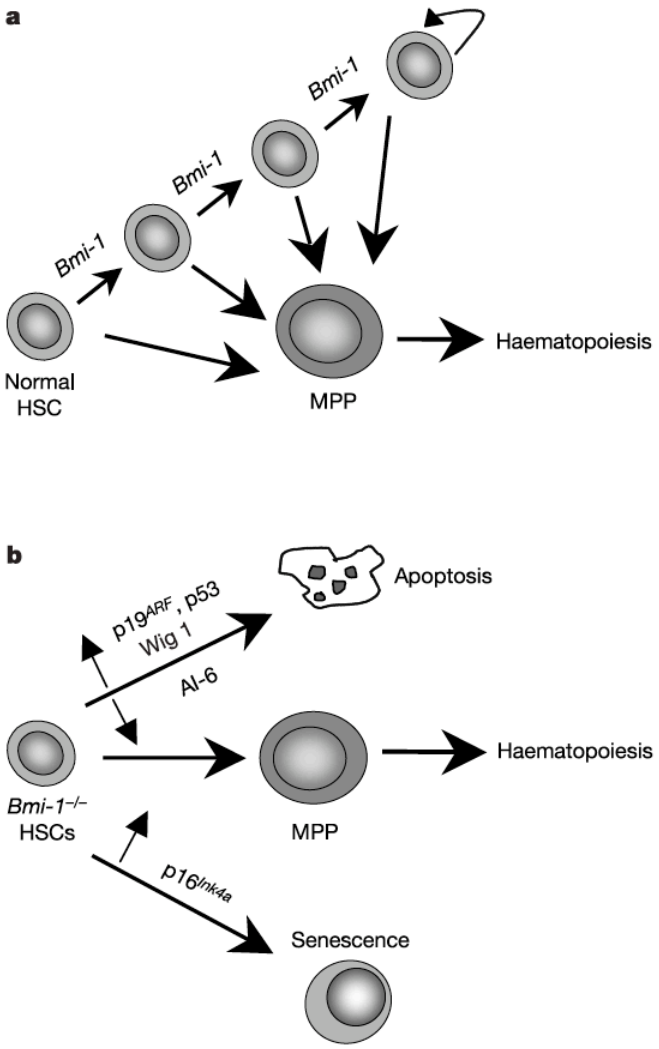


Figure 1 Analysis of adult HSCs. **a**, **b**, Effect of *Bmi-1* deletion on the frequencies of HSCs and MPPs. The bars show the average frequencies of HSCs (**a**) and MPPs (**b**) from eight individual mice in each group. *P* values (shown above the bars) were calculated with the unpaired Student's *t*-test. WT, wild type; Het., *Bmi-1*$^{+/-}$; KO, *Bmi-1*$^{-/-}$. **c**, **d**, Competitive reconstitution. Donor (Ly5.1) bone marrow cells (5×10^5) were mixed with the same number of Ly5.2 bone marrow cells and injected into lethally irradiated Ly5.2 mice ($n = 2$–4). Peripheral blood was analysed 5 weeks (**c**) and 10 weeks (**d**) after reconstitution for donor-derived myeloid, B-lymphoid and T-lymphoid cells. KO-1 and KO-2, *Bmi-1* $^{-/-}$ mice 1 and 2.

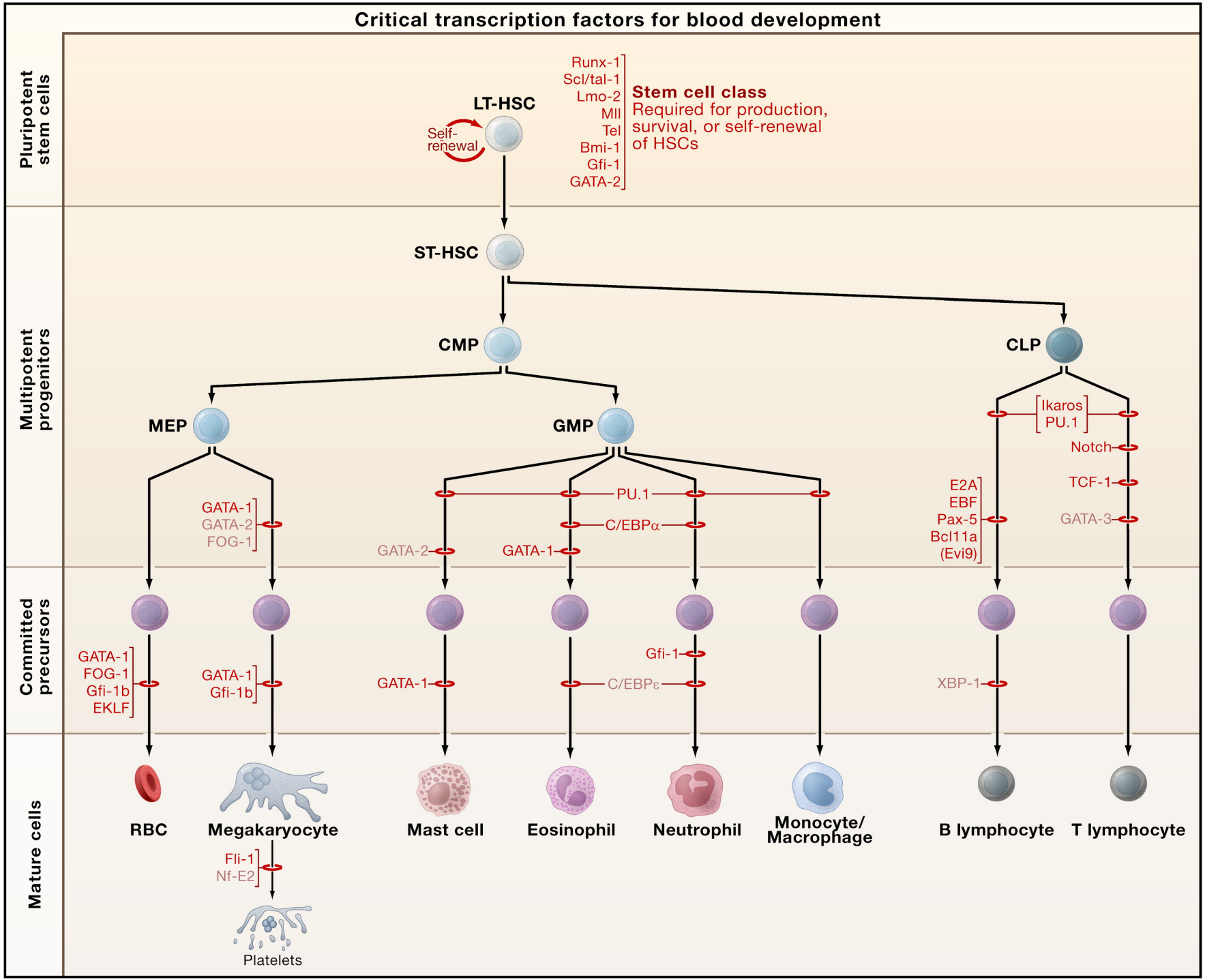
Returning to Polycombs in Hematopoiesis ….



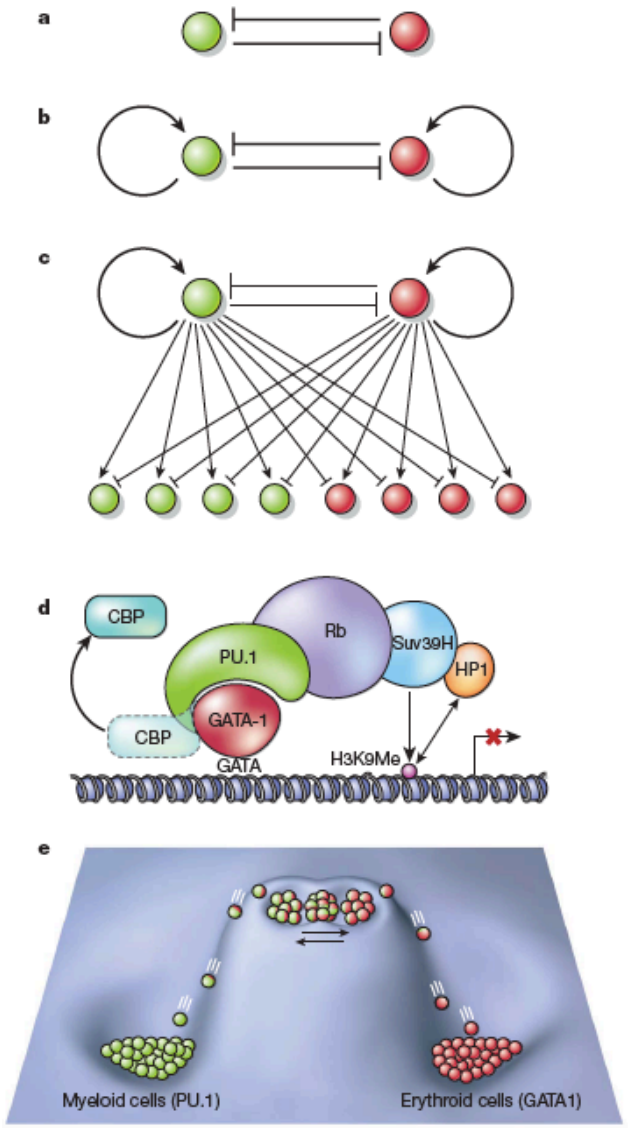
Returning to Polycombs in Hematopoiesis ….



Do Polycombs also have a role in regulating ADULT stem cell lineage choices??


Critical transcription factors for blood development
Pluripotent stem cells
Multipotent progenitors
Committed precursors
Mature cells
LT-HSC
Self-renewal
Runx-1
Scl/tal-1
Lmo-2
Mll
Tel
Bmi-1
Gfi-1
GATA-2
Stem cell class
Required for production, survival, or self-renewal of HSCs
ST-HSC
CMP
CLP
MEP
GMP
Ikaros
PU.1
Notch
TCF-1
GATA-3
E2A
EBF
Pax-5
Bcl11a
(Evi9)
GATA-1
GATA-2
FOG-1
PU.1
C/EBPα
GATA-2
GATA-1
GATA-1
FOG-1
Gfi-1b
EKLF
GATA-1
Gfi-1b
GATA-1
Gfi-1
C/EBPε
XBP-1
RBC
Megakaryocyte
Mast cell
Eosinophil
Neutrophil
Monocyte/ Macrophage
B lymphocyte
T lymphocyte
Fli-1
Nf-E2
Platelets

The PU.1 : GATA1 Paradigm

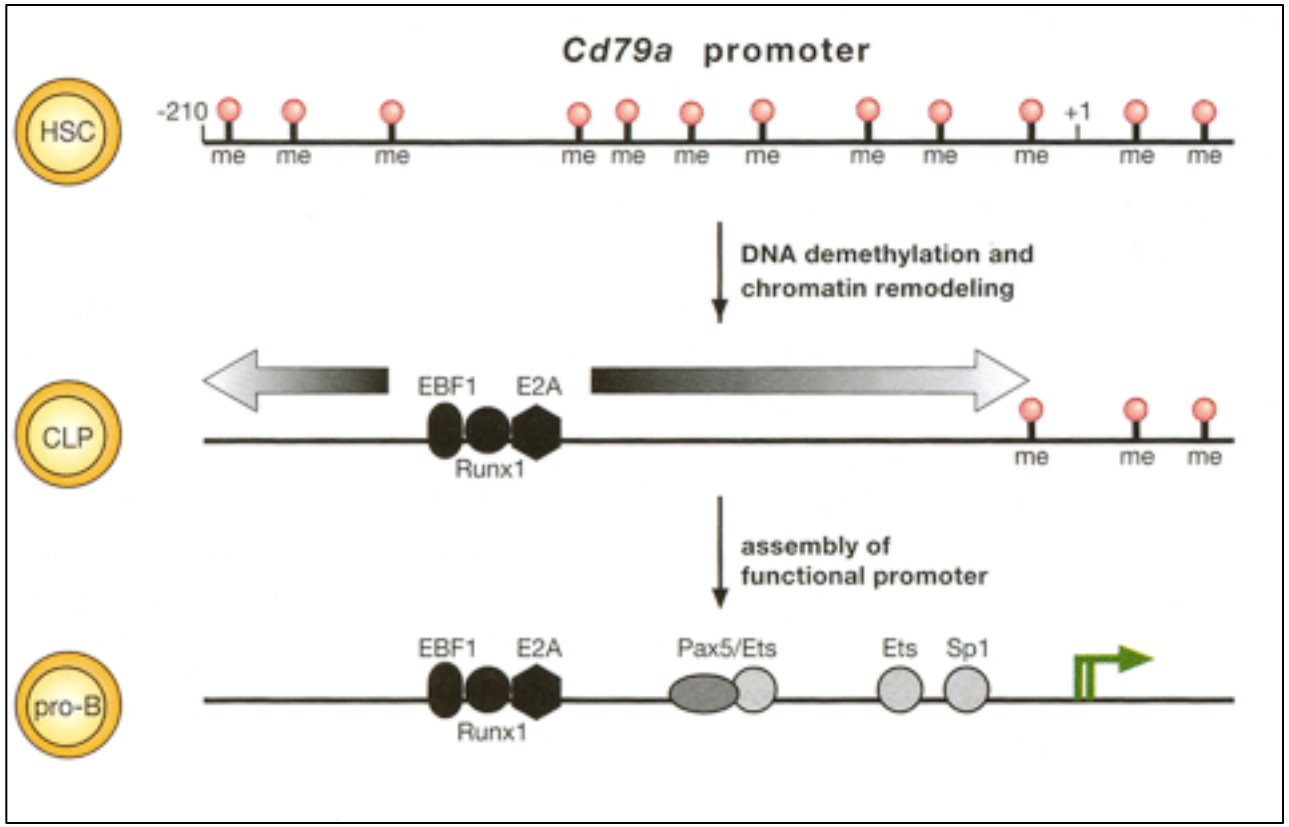


- Lineage specific TFs accelerate (activate genes of) one lineage while at the same time put on the breaks (repress genes) of another lineage.
- Once one TF becomes dominant the conflict is resolved and cellular commitment ensues

Figure 3 | Transcription factor cross-antagonism: the PU.1:GATA1 paradigm. **a**, In the simplest formulation of cross-antagonism, the two regulators (represented as green and red spheres, respectively) negatively influence each other. **b**, Representation of a cross-antagonistic motif in which the transcription factors also autoregulate. **c**, Here the two factors are shown to positively or negatively regulate the repertoire of their own and each other's target genes. **d**, Scheme of the biochemical mechanisms that underlie the GATA1 arm of the PU.1:GATA1 antagonism. To activate a target gene in erythroid cells GATA1 recruits the histone acetylase CREB-binding protein. Overexpressed PU.1 displaces CREB-binding protein (CBP) by binding to GATA1 and recruits Rb as well as Suv39H protein. This results in methylation of lysine 9 in histone H3 and recruitment of HP1a, causing repression of the target gene[34]. **e**, Representation of the PU.1:GATA1 antagonism as a binary attractor model in a modified Waddingtonian epigenetic landscape. Bicoloured marbles in the upper, shallow basin represent monocytic/erythroid progenitors that express different ratios of PU.1 and GATA1. These progenitors fluctuate between different states determined by the relative amount of PU.1 and GATA1. Cells at both ends of the spectrum are biased towards either monocytic or erythroid differentiation. During spontaneous or induced commitment they move out of the basin and roll into the attractor basins below. Green marbles represent monocytic cells expressing high levels of PU.1; red marbles erythroid cells expressing high levels of GATA1.

An example of Transcription Factors affecting DNA methylation during differentiation

The CD79a gene encodes an important protein for B cells



Off in HSC

On in pro-B cells

methylated in HSC

unmethylated in pro-B cells

Somehow, TFs recruit DNA demethylation enzymes?

Maybe affect histone modifications also?

X-chromosome Inactivation
or
Dosage Compensation

Chromosomal dosage and compensation

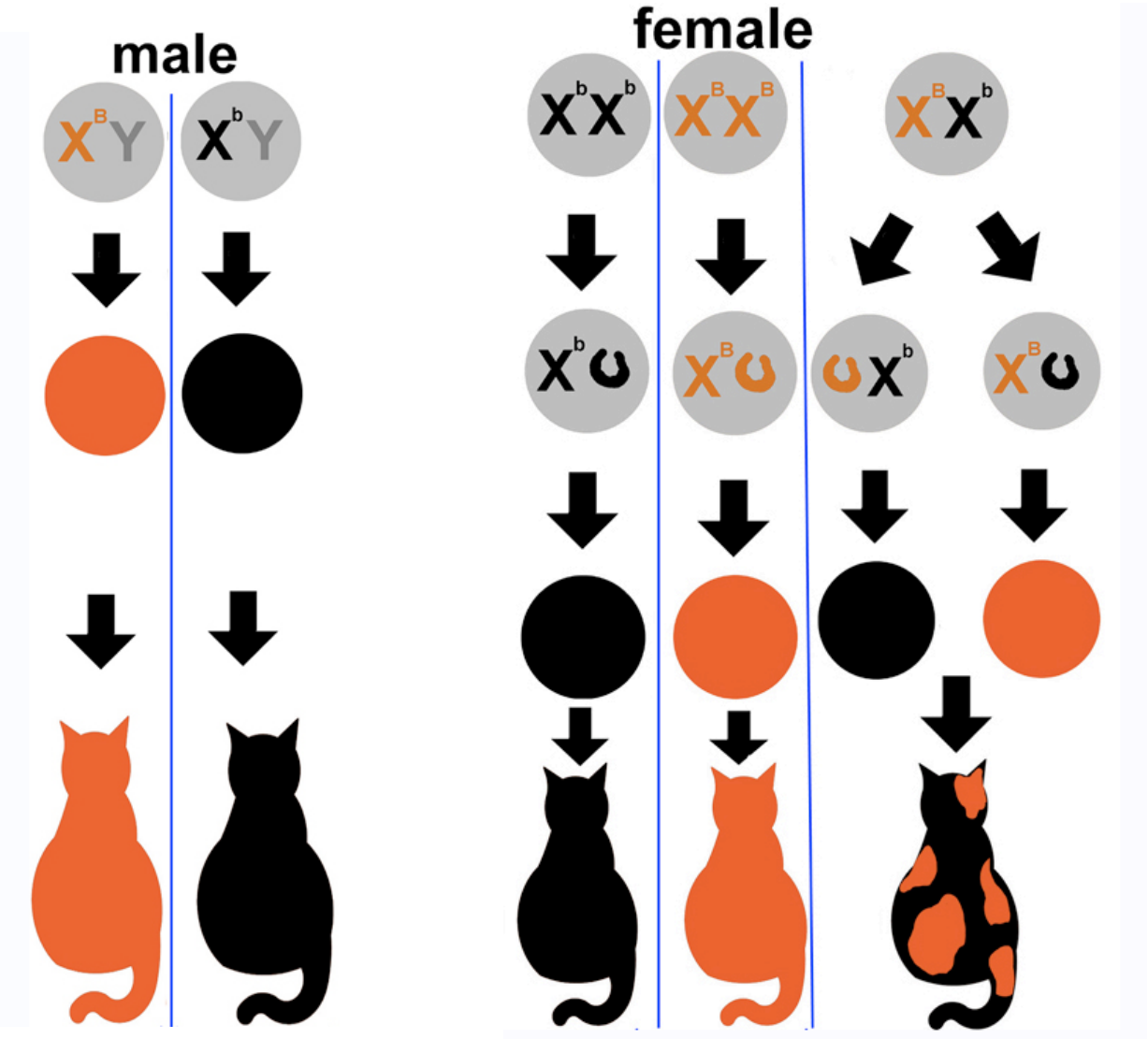
- Women are XX, men are XY
- Y – encodes genes needed to develop as a male
- Since women have double the "**dose**" of X they need to **compensate** somehow…

How are levels of all essential X-encoded gene products similar between men and women if women have twice the number of alleles?

How is this achieved???

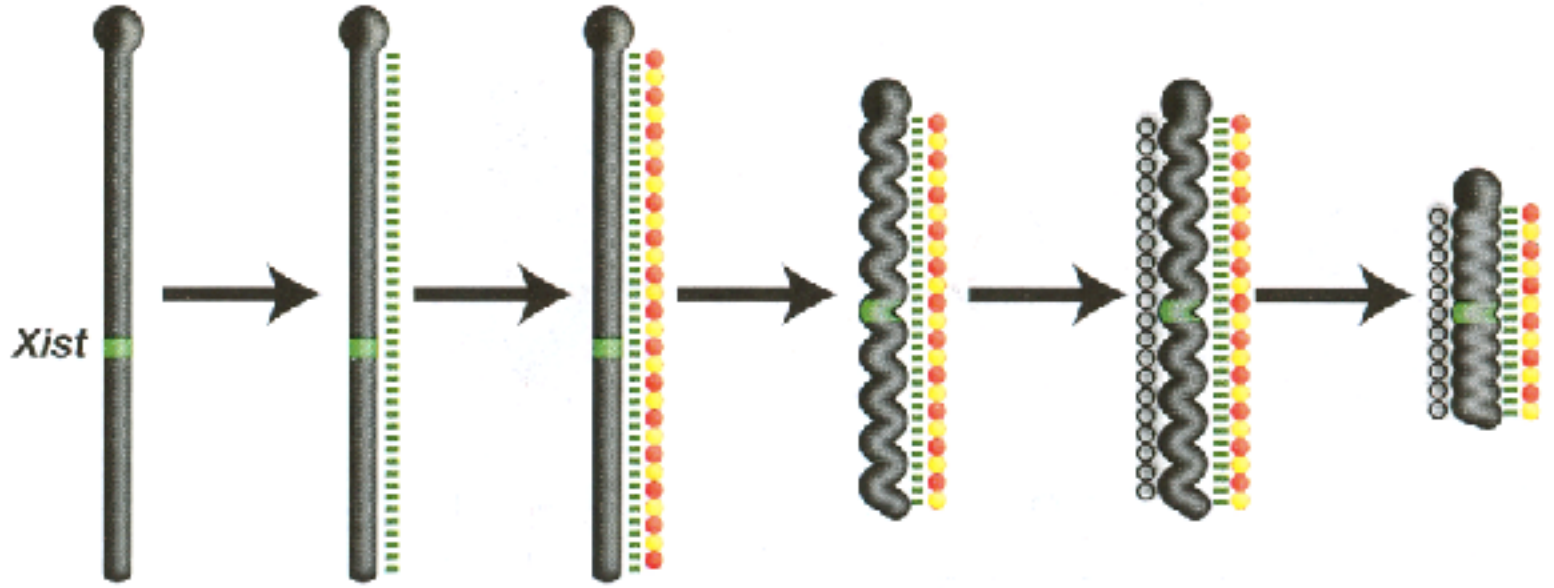
Flies – double the rate of transcription on the single male X chromosome
Worms – half rate of transcription on both female X's
Mammals – Switch off one female X chromosome in a **random** fashion

Previously we discussed the calico cat…
Now let's investigate the molecular mechanisms involved…



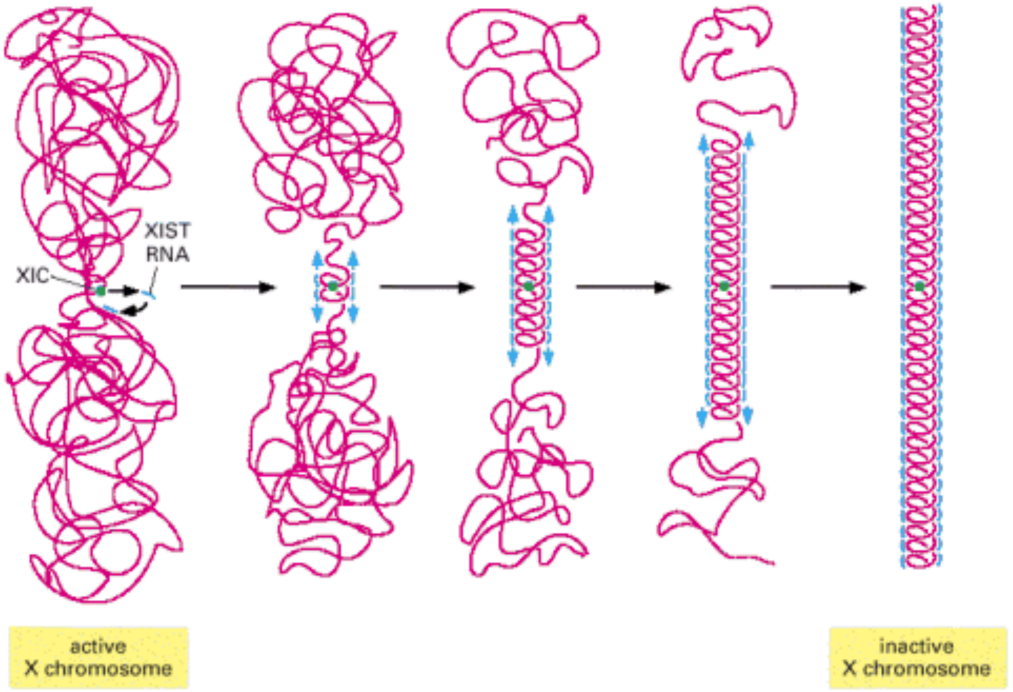
The X-inactivation center (Xic) and the X inactive specific transcript (Xist)

- Both are required for silencing of the X-chromosome
- This locus produces a large ncRNA called Xist
- Xist binds and coats the X-chromosome in cis and triggers silencing
- This leads to progressive DNA and Chromatin modifications



Mechanistically – what happens?

- Xist levels increase in pre-implantation embryos prior to X-chromosome inactivation
- It binds to and coats ~85% of the X-chromosome it is transcribed from and leads to chromosome condensation and silencing



X inactivation is developmentally regulated

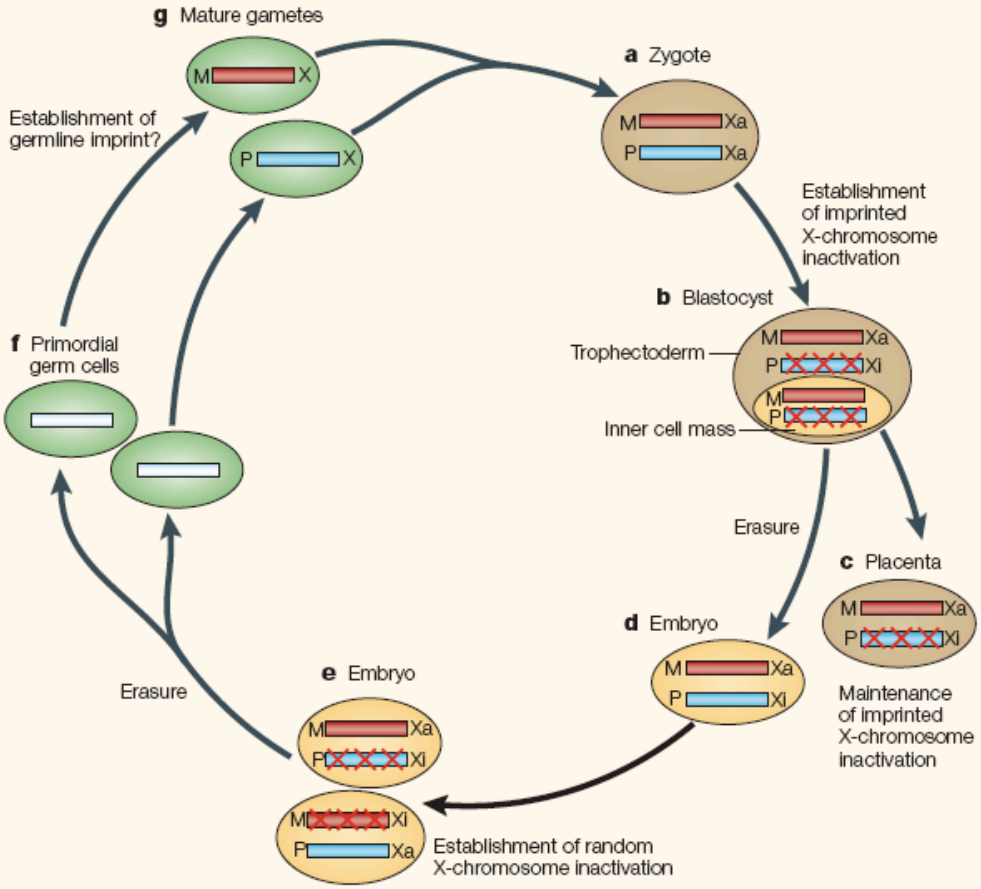
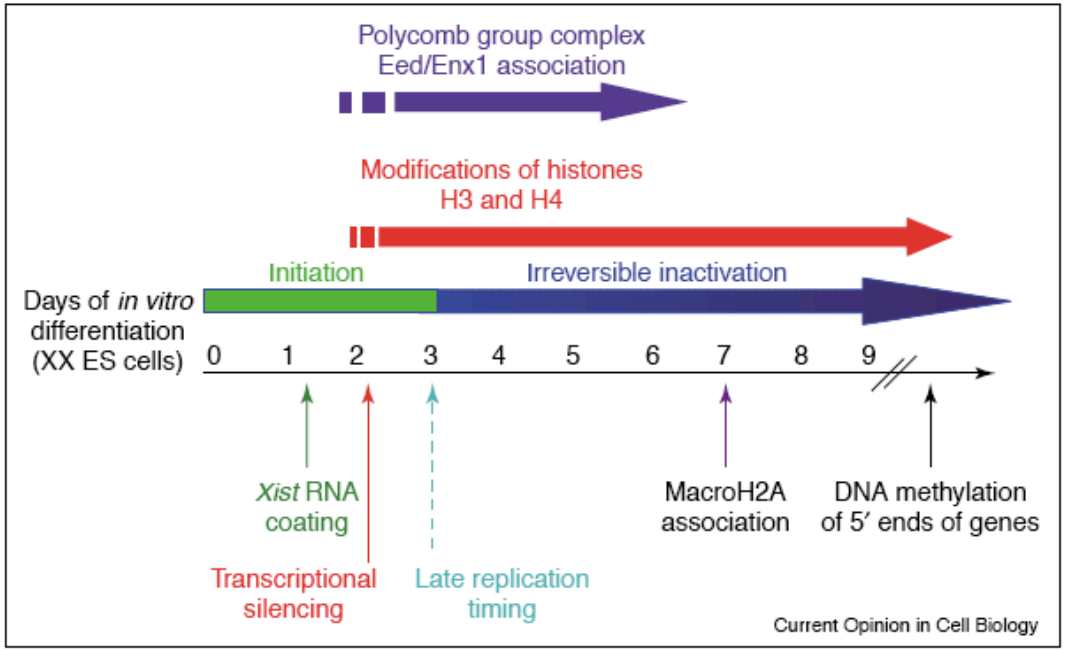


Figure 2 | **Life cycle of X-chromosome inactivation in the mouse.** Red rectangles indicate the X chromosome of maternal origin (M), blue rectangles indicate that of paternal origin (P) and white rectangles indicate X chromosomes in primordial germ cells, from which all parental epigenetic marks have been fully erased. The active and inactive X chromosomes are indicated by Xa and Xi, respectively. The X chromosomes in the zygote (**a**) are both potentially active. Imprinted X-chromosome inactivation of the paternal X chromosome is established in all cells during the pre-implantation stages (represented by crosses in the blastocyst stage (**b**)). This is maintained in the placenta and other extra-embryonic tissues (**c**), but is erased in the embryonic tissues (**d**). Random X-chromosome inactivation is then established in the embryonic cells (**e**) and maintained throughout adult life, except in the developing germline (**f**) where the X chromosomes are reprogrammed. It is not yet known if and when a germline imprint is established to differentially mark the parental X chromosomes (**g**).

Mechanistically – what happens?



Kinetics of X inactivation in differentiating female ES cells. The timing of the various events that characterise random X inactivation in differentiating female ES cells are shown. This summary is compiled based on findings reviewed here and elsewhere. The earliest time point at which each of these characteristics is first detected is indicated. The time periods corresponding to a transition from a Xist RNA dependent 'initiation' phase (green) and a Xist RNA independent 'irreversible' phase (blue) are indicated and are based on the study of Wutz *et al.* [20].

- DNA methylation appears to be a very late event
- Polycombs do not seem to be retained on silent somatic X-chromosomes…

Genomic Imprinting

Genomic Imprinting

An epigenetic phenomenon which restricts the expression of a gene from either the maternal (m) or paternal (p) locus

- Only about 80 genes identified so far are "imprinted"

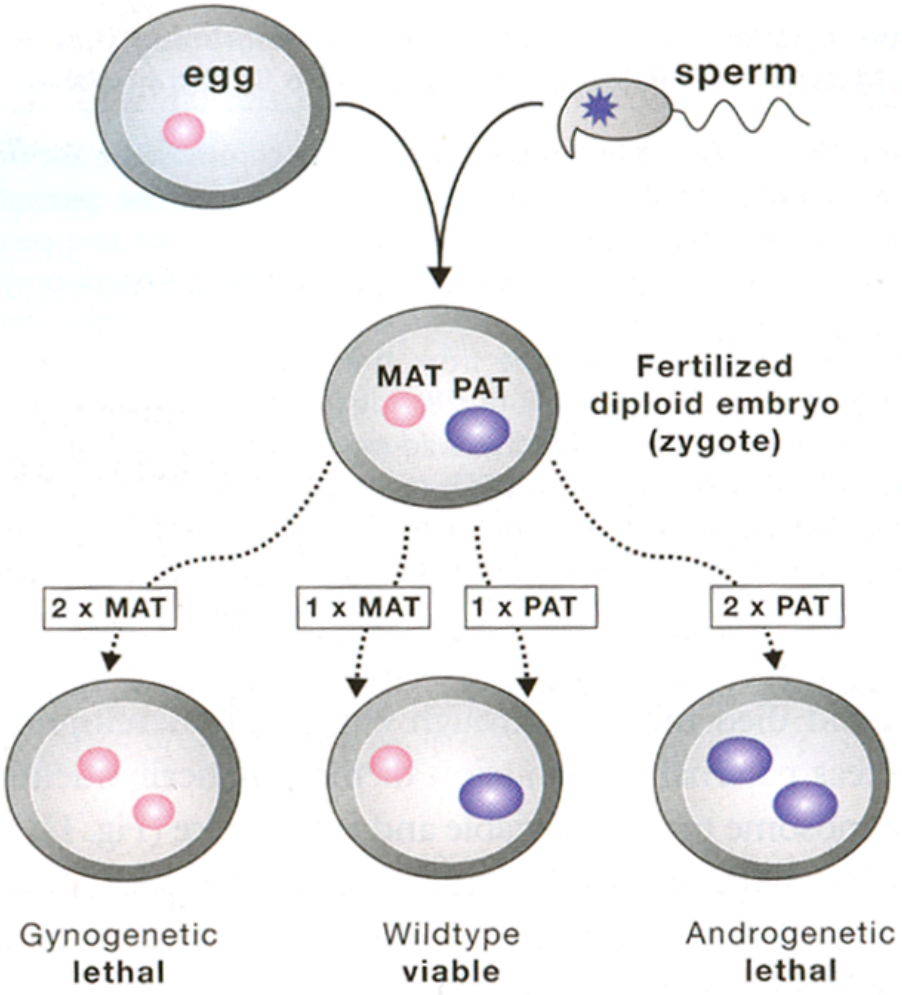
- Some examples include H19, CDKN1C and IGF2

Maternally expressed	**Paternally expressed**
H19	IGF2
CDKN1C	

- Not sex specific. Therefore, IGF2 is silenced on the paternal copy in both boys and girls

- <u>Why</u> does it happen????
  - "Paternal Conflict hypothesis" a male-female tug-of-war
  - The father is more 'interested' in the growth of his offspring, at the expense of the mother…p57…

Key experiments using nuclear transfer in mice



Conclusion:

- BOTH maternal and paternal genomes express different sets of genes
- These genes, albeit the minority (so far 80 out of 25k) are necessary for embryonic development

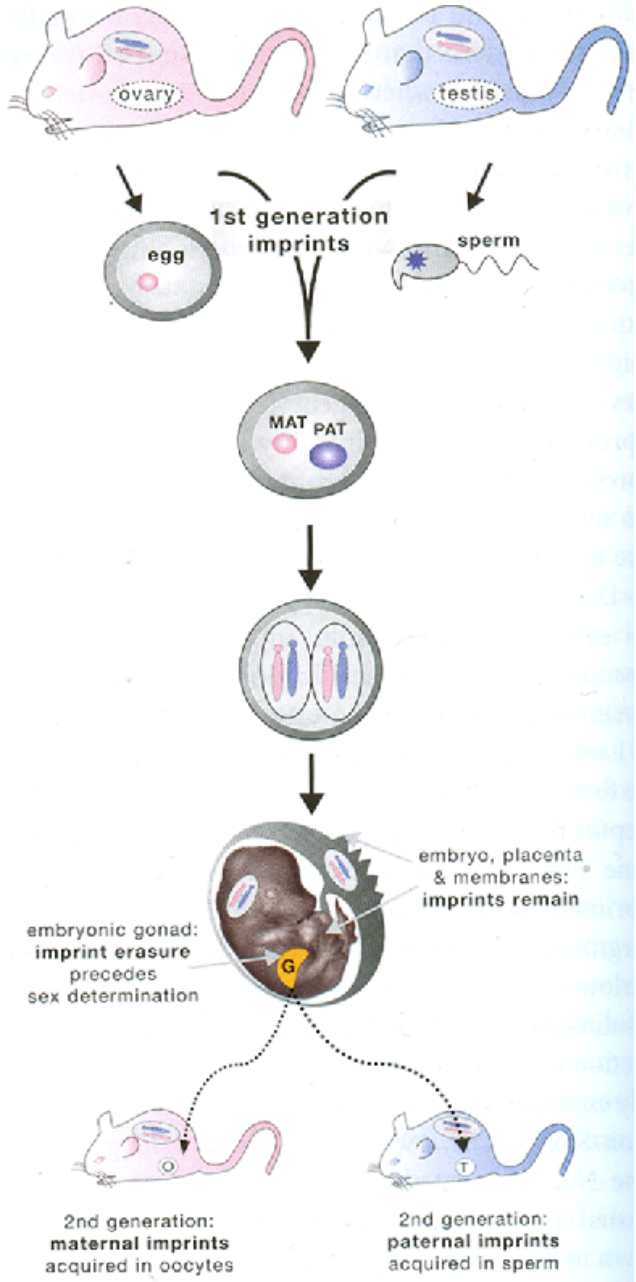
Examples so far….

Table 1. The function of imprinted genes as determined by gene inactivation

Maternal	Gene function	Paternal
–Igf2r	growth defects in	*+Igf2*
–Gnas	embryo, placenta,	*+Gnasxl*
–Tssc3/Ipl	or postnatal stage	*+Peg1/Mest*
–Mash2		*+Peg3/Pw1*
–Grb10/Meg1		*+Rasgrf1*
–/+ Cdkn1c		*+Dlk1*
Nesp	behavioral or	*+Peg1/Mest*
Ube3a	neurological	*+Peg3/Pw1*
*Kcnq1**	defects	*+Rasgrf1*
Asb1[spermatogenesis]	other defects	*Ndn*[strain-specific lethality]
Dcn[Tumor suppressor]		
H19 ncRNA	no obvious defects	*Snrpn/Snurf*
Slc22a2	in embryo or	*Frat3*
Slc22a3	neonate	*Ins2*

(Maternal) Maternally expressed imprinted gene, (Paternal) paternally expressed imprinted genes, (+) growth promoting effect, (–) growth suppressing effect, (–/+) defect in differentiation but growth regulatory status unclear, (*) additional differentiation defect. (Reference to the primary data can be found at: http://www.mgu.har.mrc.ac.uk/research/imprinting/function.html).

When does it happen???



- Most likely the gametic imprints are placed on paternally imprinted genes during sperm production and on maternally imprinted genes during egg production
- Women making eggs need to remove imprinting marks on paternally imprinted genes
- Men making sperm need to remove imprinting marks on maternally imprinted genes

Imprinted genes are generally found in clusters and contain both coding and non-coding RNAs

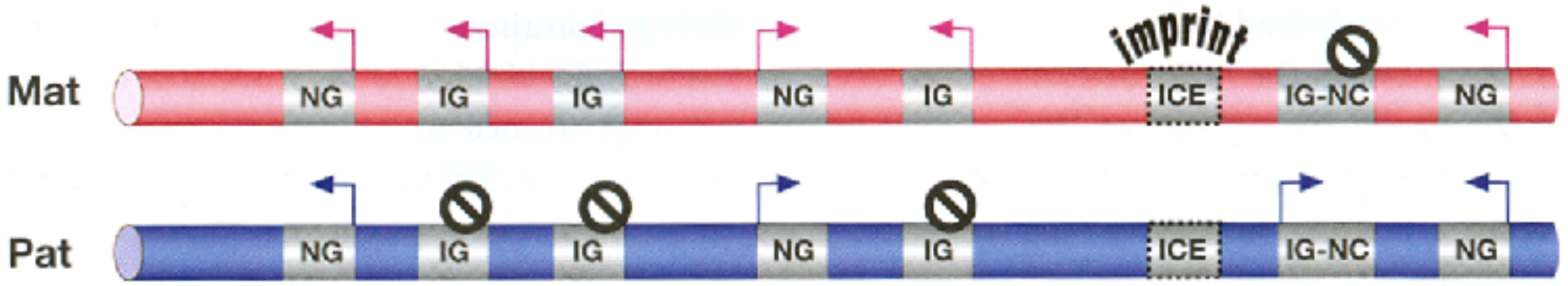
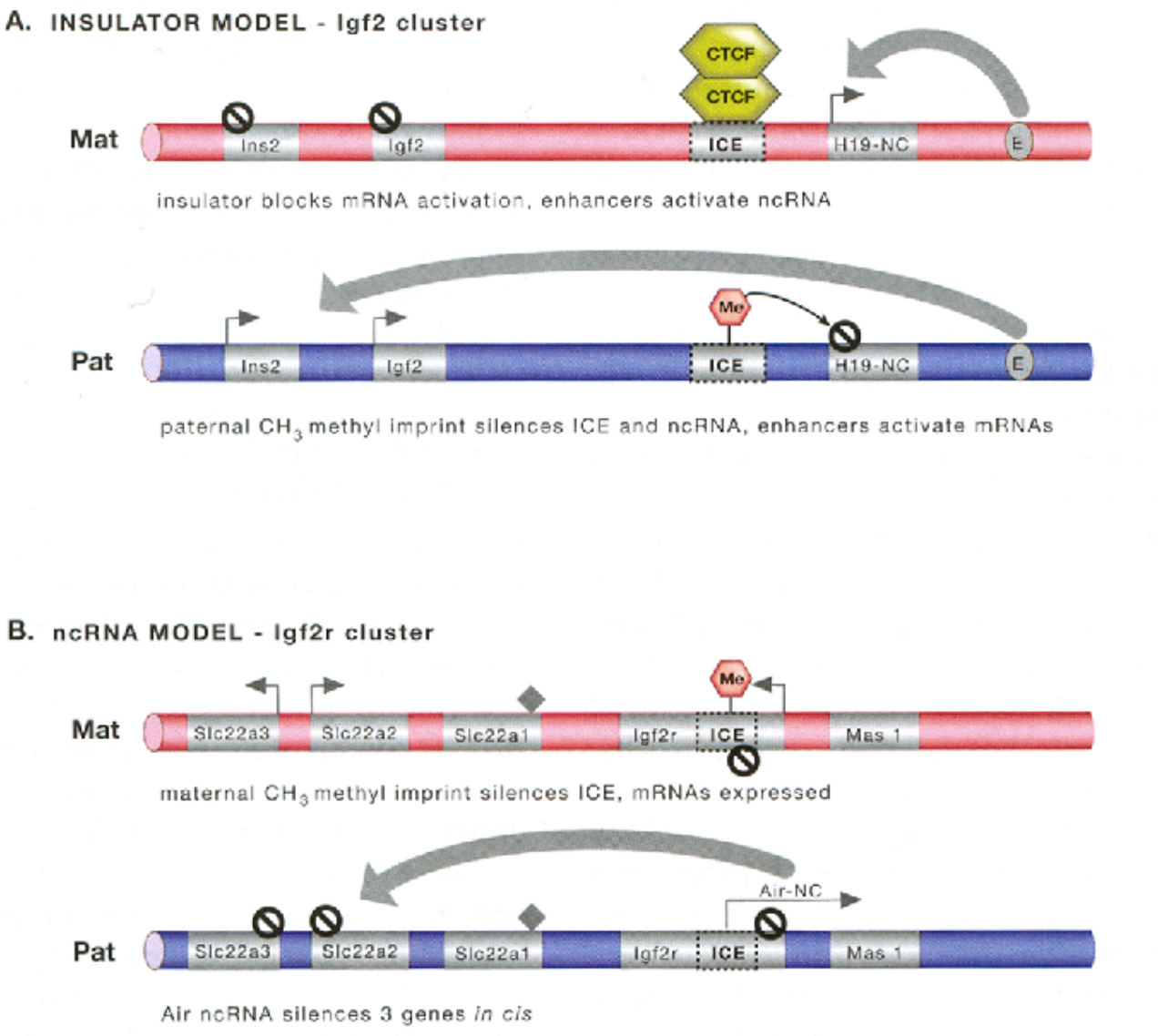


Figure 5. Imprinted Genes Are Expressed from One Parental Allele and Often Clustered

Most imprinted genes are found in clusters that include multiple protein-coding mRNAs and at least one noncoding RNA (ncRNA). Non-imprinted genes can also be present. The imprinting mechanism is *cis*-acting, and imprinted expression is controlled by an imprint control element that carries an epigenetic imprint inherited from one parental gamete. One pair of diploid chromosomes is shown pink (maternally expressed imprinted gene) and blue (paternally expressed imprinted gene). (IG) Imprinted mRNA gene, (IG-nc) imprinted ncRNA gene, (NG) non-imprinted gene, (ICE) imprint control element, (*arrow*) expressed gene, (*filled circle*) repressed gene.

- If the Imprint control element (ICE) is removed on the silent (but not the active) X-chromosome then silencing does not occur…therefore the effect is in cis

Two representative examples of cis-acting silencing mechanisms at two different imprinted gene clusters



The *Air* Noncoding RNA Epigenetically Silences Transcription by Targeting G9a to Chromatin

Takashi Nagano,[1,2]* Jennifer A. Mitchell,[1] Lionel A. Sanz,[3] Florian M. Pauler,[4] Anne C. Ferguson-Smith,[5] Robert Feil,[3] Peter Fraser[1]*

A number of large noncoding RNAs (ncRNAs) epigenetically silence genes through unknown mechanisms. The *Air* ncRNA is imprinted—monoallelically expressed from the paternal allele. *Air* is required for allele-specific silencing of the cis-linked *Slc22a3*, *Slc22a2*, and *Igf2r* genes in mouse placenta. We show that *Air* interacts with the *Slc22a3* promoter chromatin and the H3K9 histone methyltransferase G9a in placenta. *Air* accumulates at the *Slc22a3* promoter in correlation with localized H3K9 methylation and transcriptional repression. Genetic ablation of G9a results in nonimprinted, biallelic transcription of *Slc22a3*. Truncated *Air* fails to accumulate at the *Slc22a3* promoter, which results in reduced G9a recruitment and biallelic transcription. Our results suggest that *Air*, and potentially other large ncRNAs, target repressive histone-modifying activities through molecular interaction with specific chromatin domains to epigenetically silence transcription.

Kcnq1ot1 ncRNA recruits BOTH G9a and PRC2

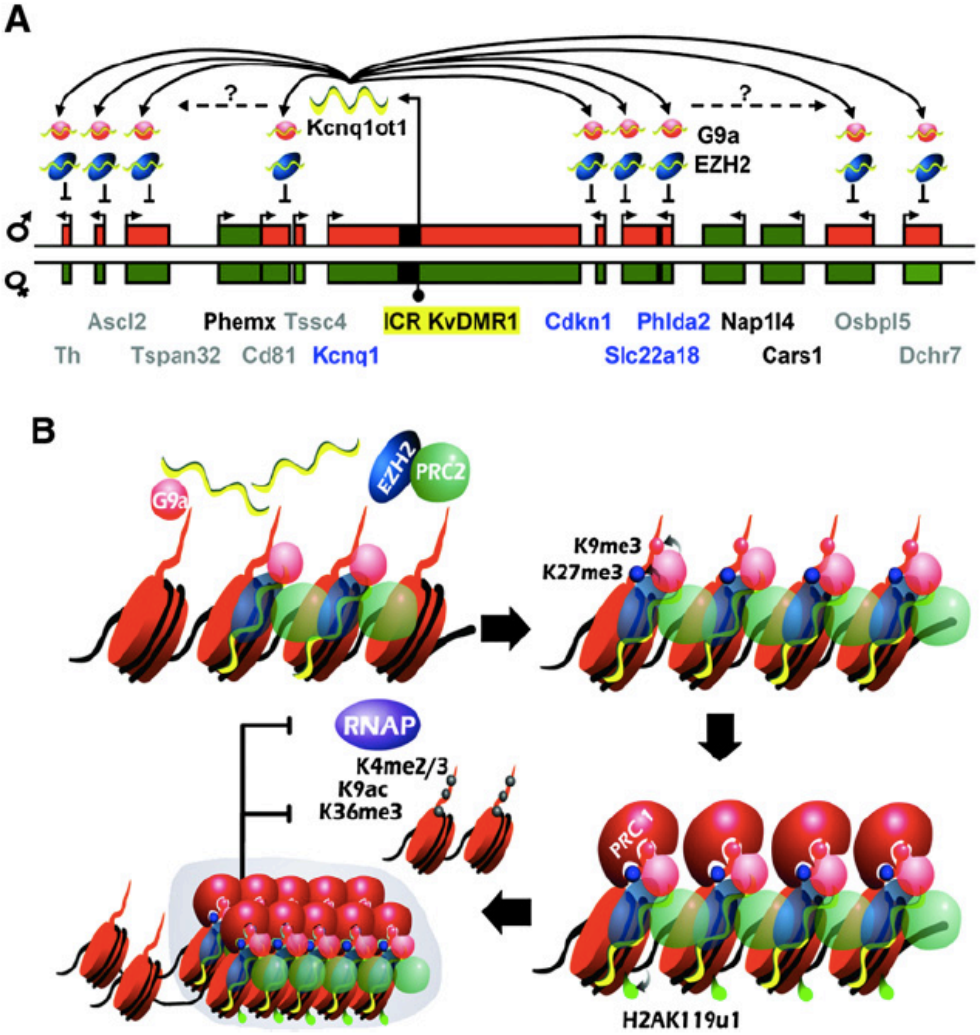


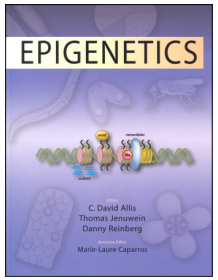
Figure 1. ncRNA and PcG-Mediated Chromatin Compaction of the Kcnq1 Cluster

(A) Kcnq1ot1 transcript regulates Kcnq1 imprinting cluster. Kcnq1ot1-dependent imprinting requires HMTs G9a and Ezh2 and their interaction with Kcnq1ot1 RNA appears critical for paternal imprinting in extraembryonic tissue. The dashed line represents the possible mechanism of spreading repression by HMT complexes containing Kcnq1ot1. Genes in blue text indicate "inner" imprinted genes, in gray text indicate "outer" imprinted genes, and in black text indicate nonimprinted genes. Paternal and maternal alleles are indicated. Red boxes represent paternally imprinted genes, and green boxes represent paternally and maternally expressed genes. The black circle represents DNA methylation of Kcnq1ot1 promoter (KvDMR1) on maternal allele.

(B) Polycomb repressive complex 2 (PRC2) containing Ezh2 and G9a associate with Kcnq1ot1 and are recruited to imprinted genes in Kcnq1 cluster. Repressive histone PTMs H3K9me3 and H3K27me3 are directed by G9a and Ezh2, respectively. Small pink circles on histone tails indicate H3K9me3; small blue circles represent H3K27me3. PRC1 complex recognizes repressive histone PTMs via PcG protein interaction, and PRC1 member Rnf2 catalyzes the repressive PTM H2AK119u1. However, PRC1 targeting may be independent of PRC2 as shown by Terranova et al. (2008). Ubiquitin is depicted with green circles. PRC1 and PRC2 complexes direct genomic contraction and higher-order chromatin condensation. Shaded area represents a distinct nuclear repressive compartment, devoid of RNAP and active histone PTMs, H3K4me3, H3K9ac and H3K36me3, indicated by small gray circles.

Recommended reading material

- Epigenetics
  by C. David Allis, Thomas Jenuwein, Danny Reinberg
  CHAPTER 17: Dosage Compensation in Mammals
  CHAPTER 19: Genomic Imprinting in Mammals



- Epigenetic signatures of stem-cell identity

  Mikhail Spivakov and Amanda G. Fisher

  NATURE REVIEWS | GENETICS VOLUME 8 | APRIL 2007 | 263

Some Extra reading:

Review TRENDS in Genetics Vol.23 No.6

Silencing by imprinted noncoding RNAs: is transcription the answer?

Florian M. Pauler*, Martha V. Koerner* and Denise P. Barlow

New twists in X-chromosome inactivation
Jennifer A Erwin[1,2] and Jeannie T Lee[1,2]

Current Opinion in Cell Biology 2008, **20**:349–355

Review

Genetics and epigenetics: stability and plasticity during cellular differentiation

Fabio Mohn and Dirk Schübeler

Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, 4058 Basel, Switzerland