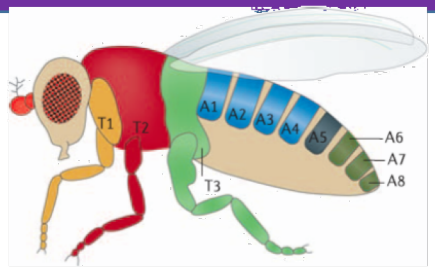
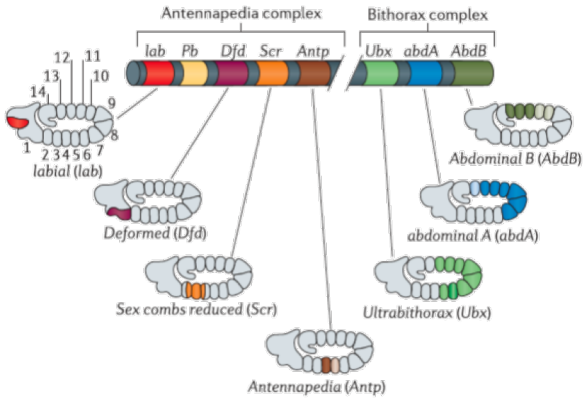
Post-transcriptional gene regulation by Polycomb and Trithorax group genes

Homeotic genes and Drosophila melanogaster development



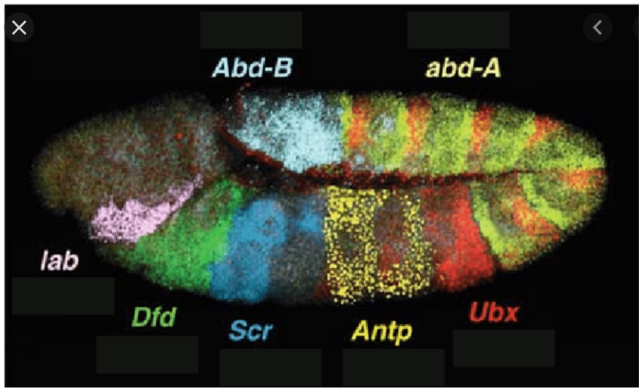


Colinearity – poterior/anterior gene arrangement corresponds with posterior/anterior gene expression (conserved in vertebrates – Hox gene cluster)

Polycomb silencers control cell fate, development and cancer

Anke Sparmann and Maarten van Lohuizen

Body patterning in D. melanogaster is controlled by Hox genes



Antennapedia and Bithorax complexes are gene clusters that encode a series of homeotic genes (Hox genes). Hox genes are powerful transcription factors.

Hox genes show body segment specific gene expression and are essential to define the gene expression patterns of each body segment
- Expression is specific in the larvae but also
- in the body segment that has been developed from the larval segement

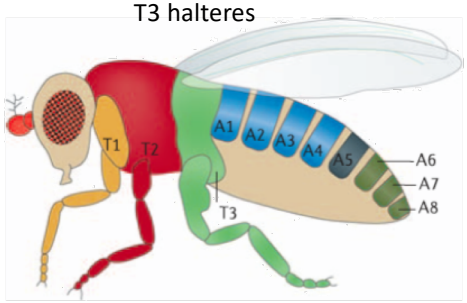
Mutation of Hox genes cause characteristic developmental defects in the body segment that lack hox gene expression

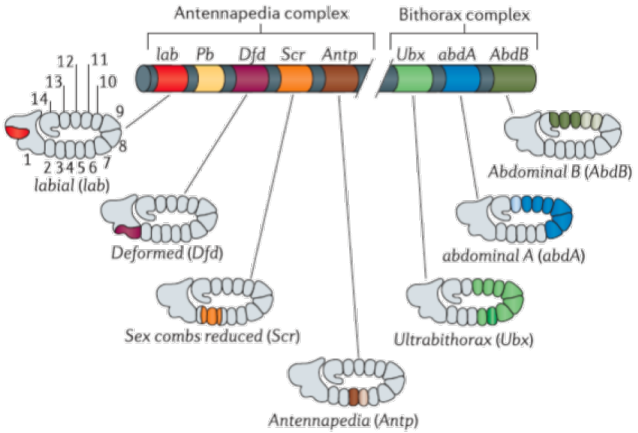
Homeotic transformation: a normal body part is replaced by a body part which is regularly found in other regions.

Homeotic genes and Drosophila melanogaster development

a

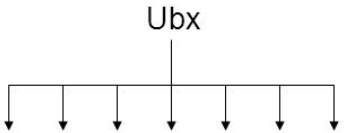
T2 wings
T3 halteres



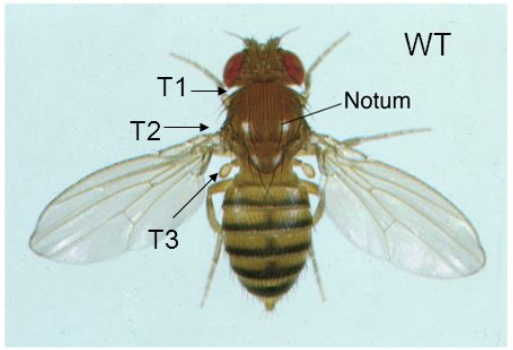


Homeotic Phenotypes

Development of an inappropriate body part in place of the correct body part.



Ubx ⊣ Antp off in T3 cells
Ubx⁻ → Antp on in T3 cells

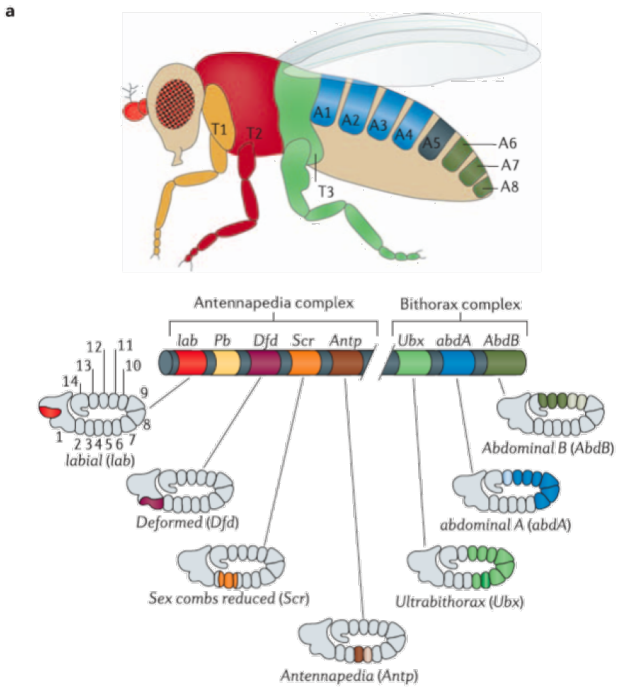


(b)

(c)

Ubx controls formation of halteres
Loss of Ubx expression → Antp ectopically expressed in T3 → wing formation induced

Homeotic genes and Drosophila melanogaster development

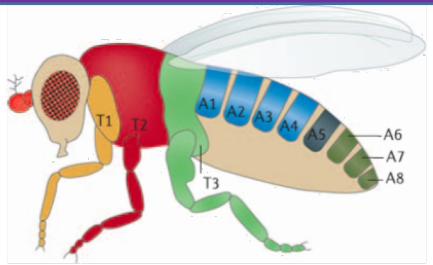


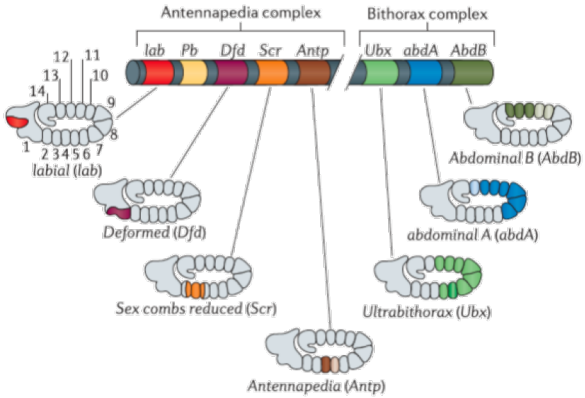
Wild-type fly

Ectopic expression of antennapedia (using another promoter) in the larval segment that gives rise to antenne

Homeotic transformation: a normal body part is replaced by a body part which is regularly found in other regions.

Polycomb group genes were first defined in Drosophila melanogaster





Colinearity – poterior/anterior gene arrangement corresponds with posterior/anterior gene expression (conserved in vertebrates – Hox gene cluster)

Antennapedia activates the "leg" gene expression program in the antenne

HOWEVER:

Other fly mutations were isolated that did not impact on Antennapedia or Bithorax complexes but caused homeotic transformations!!!

Wild-type fly

Ectopic expression of antennapedia in the larval segment that gives rise to antenne

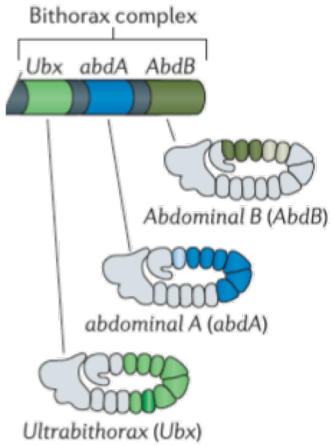
Homeotic transformation: a normal body part is replaced by a body part which is regularly found in other regions.

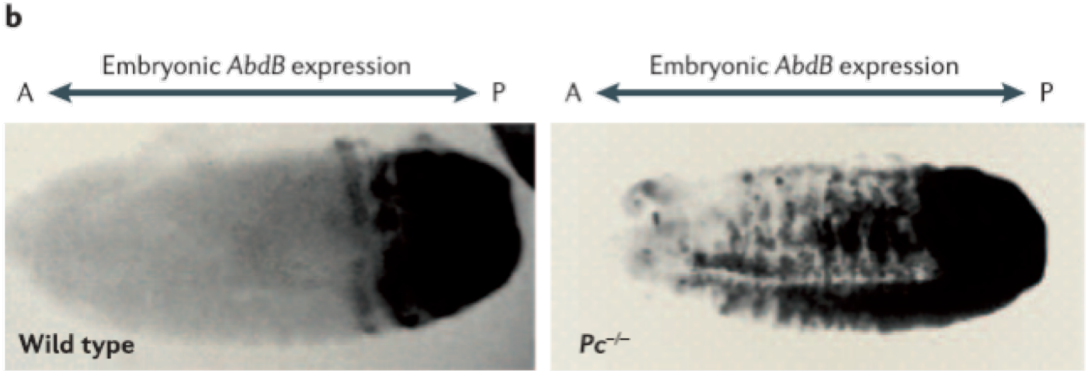
Polycomb silencers control cell fate, development and cancer

Anke Sparmann and Maarten van Lohuizen

Discovery of mutations that are not located in Hox genes, but cause homeotic transformations

Mutations in Pc (Polycomb) and other related genes results in aberrant expression of abdominal B in larvae





Polycomb (Pc) is required to limit the gene expression of Hox genes to the relevant larval segment

- → **Pc does represses Hox genes in segements where respective Hox gene shoulds not activate its respective gene expression program**
- → **Pc is expressed in many segments**
- → **Pc is not the only gene that has this function: several gene that similar phenotypes were grouped together: POLYCOMB GROUP GENES (PcG)**

→ **IMPORTANT: Pc mutations can recapitulate a gain of function phenotype of a Hox gene**

Polycomb silencers control cell fate, development and cancer

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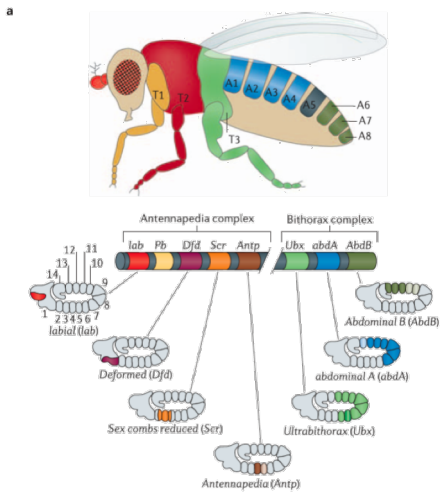
PcG mutations can recapitulate a gain of function phenotype of a Hox gene



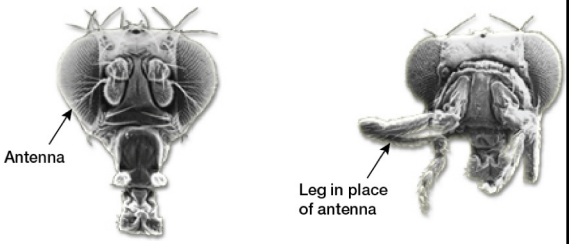
Wild-type fly

Ectopic expression of antennapedia in the larval segment that gives rise to antenne

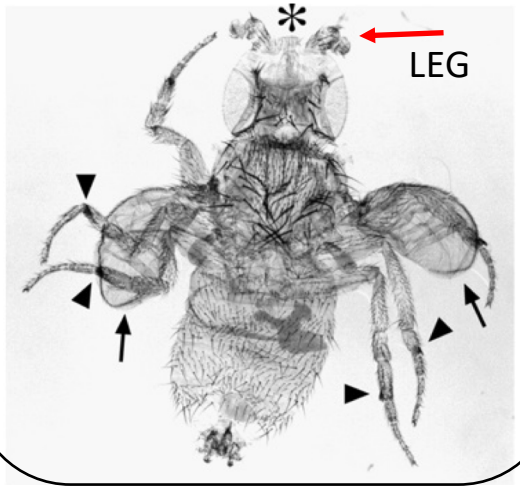
Polycomb mutants recapitulate Hox gene mutations

Wild type

Mutation in Antennapedia gene



Su(z)12 mutant (polycomb group gene) with antennapedia phenotype



Important:

PcG phenotypes are complex, several body segements can be affected.

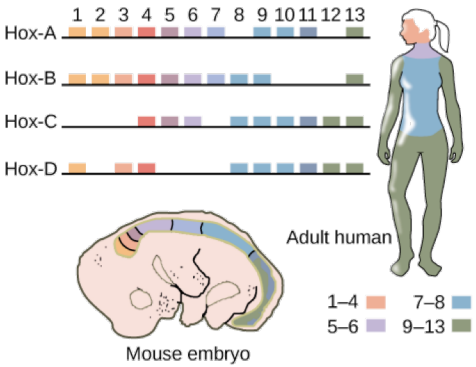
→ PcG group proteins regulate many genes and maintain gene expression programs in different segments

PcG group genes are key regulators of gene expression that repress silence genes on the epigenetic level

Polycomb group genes are highly conserved!

Polycomb group genes are conserved regulators of development in vertebrated

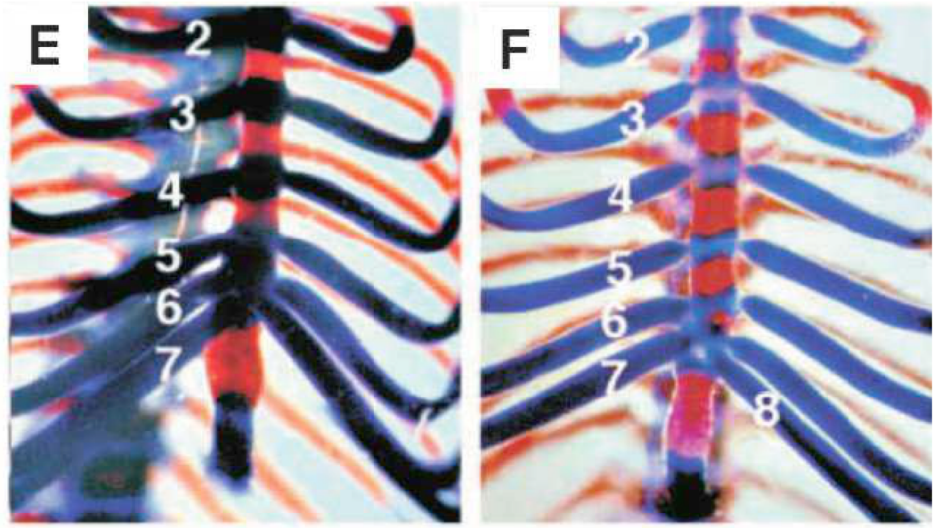
Family	Gene symbol	Gene name
Cdx	*CDX1*	caudal type homeobox 1
	CDX2	caudal type homeobox 2
	CDX4	caudal type homeobox 4
Evx	*EVX1*	even-skipped homeobox 1
	EVX2	even-skipped homeobox 2
Gbx	*GBX1*	gastrulation brain homeobox 1
	GBX2	gastrulation brain homeobox 2
Gsx	*GSX1*	GS homeobox 1
	GSX2	GS homeobox 2
Hox1	*HOXA1*	homeobox A1
	HOXB1	homeobox B1
	HOXD1	homeobox D1
Hox2	*HOXA2*	homeobox A2
	HOXB2	homeobox B2
Hox3	*HOXA3*	homeobox A3
	HOXB3	homeobox B3
	HOXD3	homeobox D3
Hox4	*HOXA4*	homeobox A4
	HOXB4	homeobox B4
	HOXC4	homeobox C4
	HOXD4	homeobox D4
Hox5	*HOXA5*	homeobox A5
	HOXB5	homeobox B5
	HOXC5	homeobox C5
Hox6-8	*HOXA6*	homeobox A6
	HOXB6	homeobox B6
	HOXC6	homeobox C6
	HOXA7	homeobox A7
	HOXB7	homeobox B7
	HOXB8	homeobox B8
	HOXC8	homeobox C8
	HOXD8	homeobox D8
Hox9-13	*HOXA9*	homeobox A9
	HOXB9	homeobox B9
	HOXC9	homeobox C9
	HOXD9	homeobox D9
	HOXA10	homeobox A10
	HOXC10	homeobox C10
	HOXD10	homeobox D10
	HOXA11	homeobox A11
	HOXC11	homeobox C11
	HOXD11	homeobox D11
	HOXC12	homeobox C12
	HOXA13	homeobox A13
	HOXB13	homeobox B13
	HOXC13	homeobox C13
	HOXD13	homeobox D13
Mnx	*MNX1*	motor neuron and pancreas homeobox 1
Meox	*MEOX1*	mesenchyme homeobox 1
	MEOX2	mesenchyme homeobox 2
Pdx	*PDX1*	pancreatic and duodenal homeobox 1



Homeotic transformation of vertebrae:
Ring 1A-/- mice (PcG gene)

8 instead of 7 vertebrae

Polycomb group genes are key regulators of
- Development
- Disease



Polycomb group genes are conserved in evolution

DNA binding

Polycomb repressive complex 2 (PRC2)

Polycomb repressive complex 1 (PRC1)

Drosophila melanogaster			*Mus musculus*	*Arabidopsis thaliana*	*Caenorhabditis elegans*
PcG DNA binding proteins					
PHO	Pleiohomeotic	Zinc finger	YY1		
PHOL	Pleiohomeotic-like	Zinc finger			
PSQ	Pipsqueak	BTB-POZ domain			
DSP1	Dorsal switch protein 1	HMG domain protein	HMGB2		
PRC2 core proteins					
ESC	Extra sex combs	WD 40 repeats	EED	FIE	MES-6
E(Z)	Enhancer of zeste	SET domain	EZH1/ENX2 EZH2/ENX1	CLF MEA SWN	MES-2
SU(Z)12	Suppressor of zeste 12	Zinc finger VEFS box	SU(Z)12	FIS2 VRN2 EMF2	
p55	p55	Histone-binding domain	RBAP48 RBAP46	MSI1 (MSI2/3/4/5)	
PRC1 core proteins					
PC	Polycomb	Chromodomain	CBX2/M33 CBX4/MPC2 CBX6 CBX7 CBX8/MPC3		
PH	Polyhomeotic	Zinc finger SAM/SPM domain	EDR1/MPH1/RAE28 EDR2/MPH2 (EDR3)		SOP-2
PSC	Posterior sex combs	Zinc finger HTH domain	BMI1 MEL18/RNF110/ZFP144	AtBMI1A AtBMI1B AtBMI1C	MIG-32
SCE/dRING	Sex combs extra/dRing	RING zinc finger	RING1/RING1A RNF2/RING1B	AtRING1A AtRING1B	SPAT-3

Epigenetic writers

PcG mutations can recapitulate a gain of function phenotype of a Hox gene

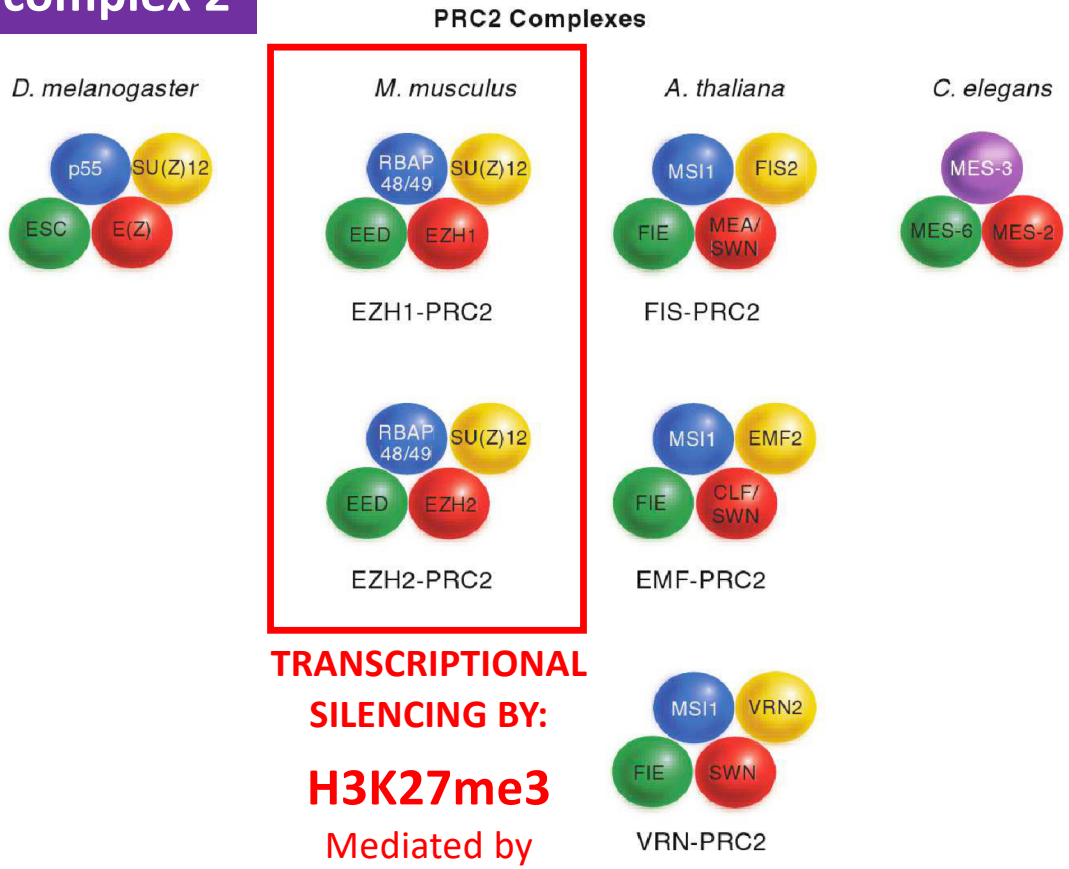
PcG loss of function gives complex phenotypes, many PcG genes have been discovered in mutational screens

→ might function as complex?

HOW CAN I PURIFY THE POLYCOMB PROTEIN IN A COMPLEX??

Polycomb complexes induce histone modifications

PRC2 – Polycomb repressive complex 2

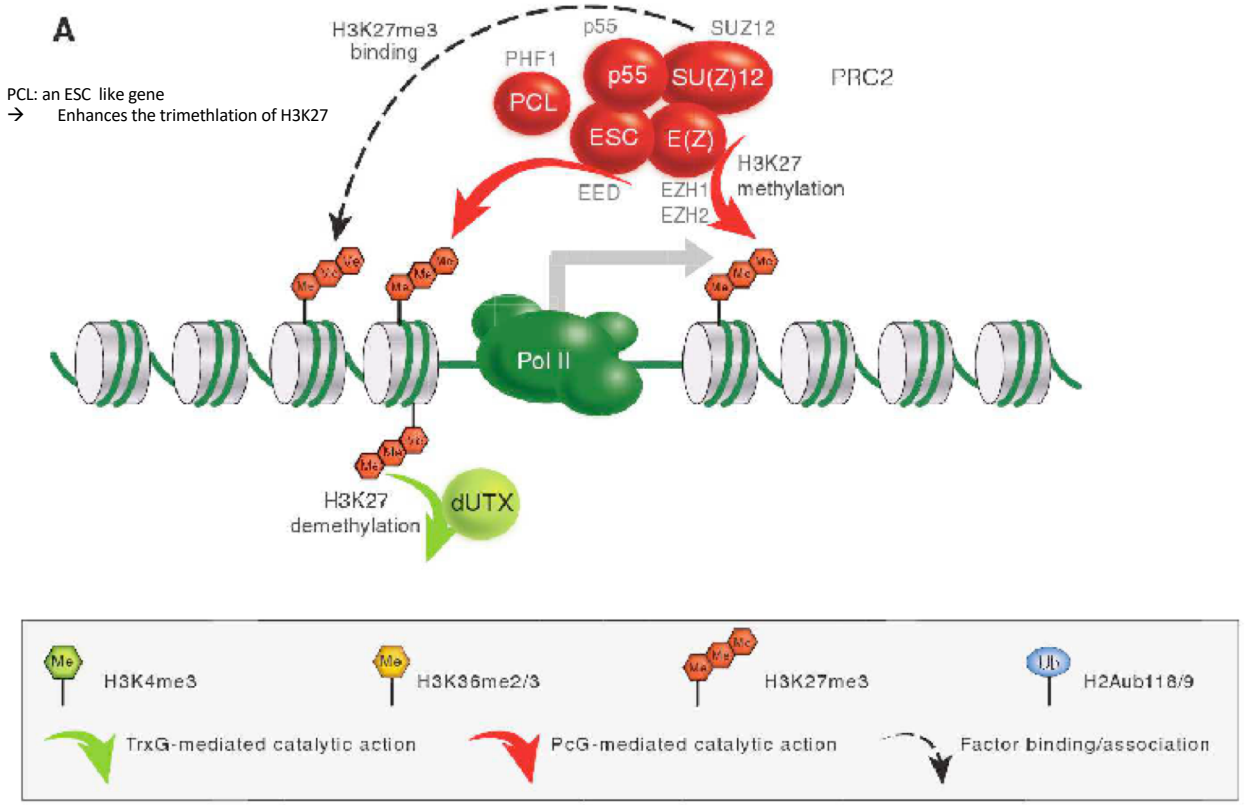


TRANSCRIPTIONAL SILENCING BY:

H3K27me3

Mediated by **EZH1** or EZH2 (EZH2 is more important; EZH1 has rather low expression)

PRC2 – Polycomb repressive complex 2



PCL: an ESC like gene
→ Enhances the trimethlation of H3K27

UTX is a de-methylase of the TRITHORAX group
→ Antagonism Polycomb - Trithorax

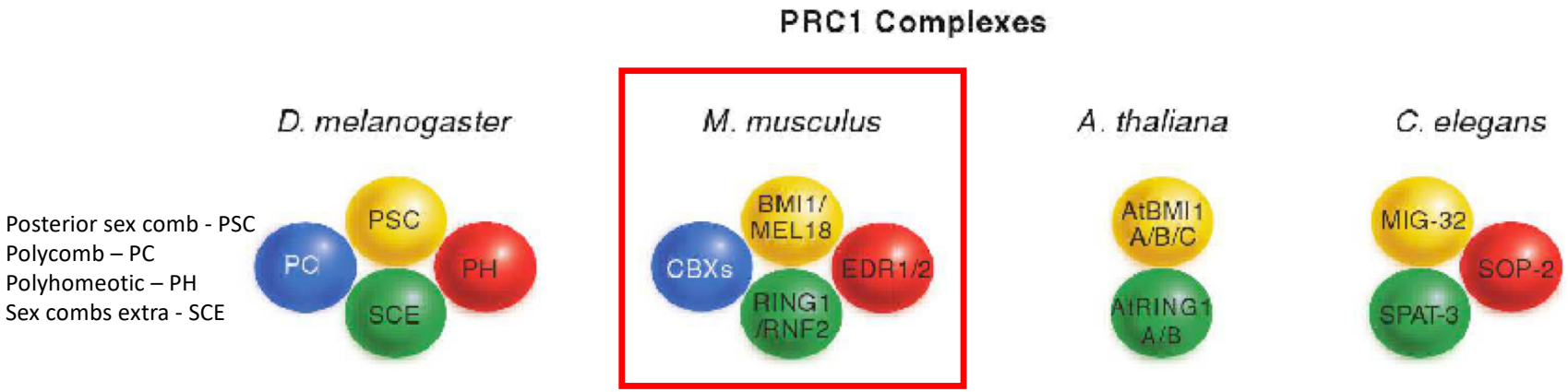
PRC2 imposes H3K27me3, however the direct structural impact of H3K27me3 on chromatin structure is not known.

H3K27me3 form a platform for binding of EED → entire PRC2 complex is stabilized
→EZH induces more H3K27me3 → **self re-inforcing loop**

→ Heredity of gene silencing

→ H3K27me3 is also a binding platform for PRC1 complex

Polycomb complexes induce histone modifications



Uniform complex

Multiple exchangeable subunits

Heterogeneity of PRC1 complex components
TRANSCRIPTIONAL
SILENCING "MEASURABLE" BY:

ubH2AK119 and ubH2AK118

Mediated by
Ring1a/Ring1b Ubiquitin ligases

PRC1 – Polycomb repressive complex 1

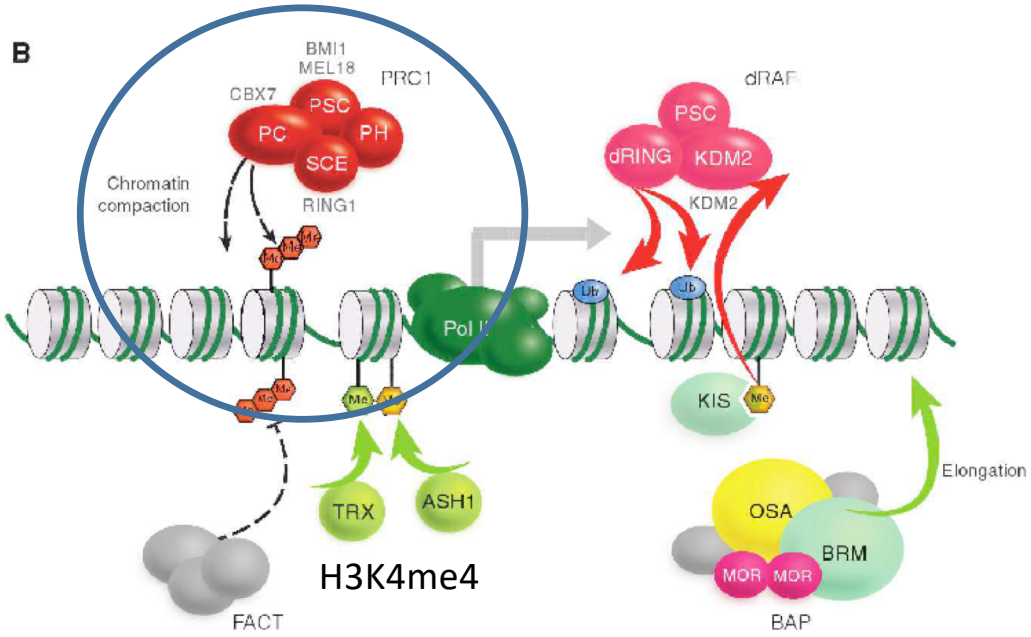


Figure 5. Schematic representation of the core PcG and TrxG protein complexes and their functions at promoters. *Drosophila* PcG proteins are depicted as red ovals with selected mammalian orthologs indicated in gray text. (*A*) Components and function of the PRC2 and counteracting activities of TrxG proteins (light green). (*B*) Components and functions of PRC1 and dRING-associated factor (dRAF) and the counteracting activities of the BAP SWI/SNF, facilitates chromatin transcription (FACT) remodeling complexes, and SET-domain histone KMTs TRX and ASH1. The TrxG protein Kismet-L is a member of the chromatin-helicase-DNA-binding (CHD) subfamily of chromatin-remodeling factors, stimulating elongation of Pol II. (Adapted from Enderle 2011.)

The FACT complex has been shown to destablilize the interaction between the H2A/H2B dimer and the H3/H4 tetramer of the nucleosome, thus reorganizing the structure of the nucleosome. In this way, the FACT complex may play a role in DNA replication and other processes that traverse the chromatin, as well as in transcription elongation.

PRC1 is recruited byH3K27me3

→ PRC1 restricts access of RNA Pol II and SNI/SNF chromatin remodeling complexes → impede gene activation.

Role of ubH2A118/119:

- Inhibit the recruitment of the FACT chromatin remodeling complex

The PSC/dRING containing dRAF complex

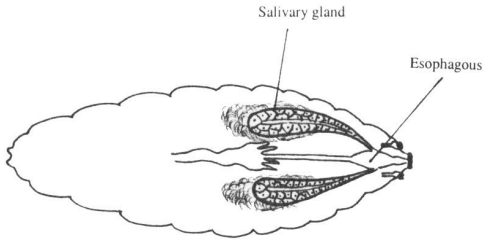
dRAF complex contains PRC1 proteins and the KDM2 jumonji domain protein

- dRING makes ubH2AK118/119
- KDM2 demethylates H3K36me; Kis7 looses access

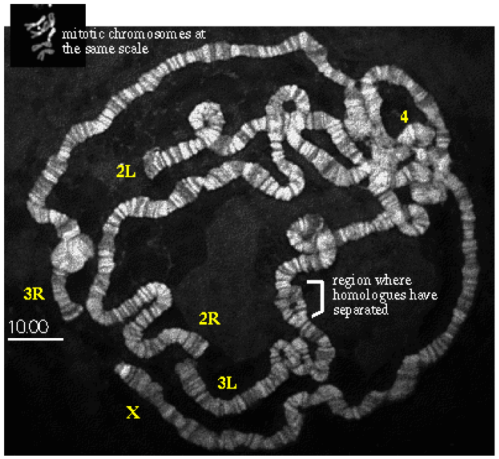
→ **Antagonism Polycomb – Trithorax**

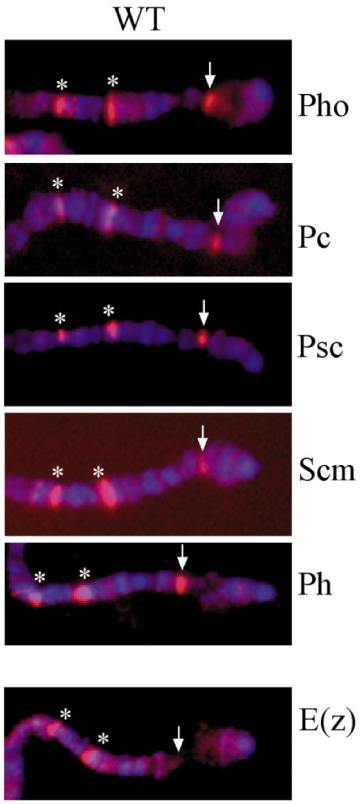
TRX and ASH1 place active methylation marks; Kismet-L binds active methylation mark and stimulates transciptional elongation

PRC2 and PRC1 gene silencing is highly relevant for gene expression on the entire genome level



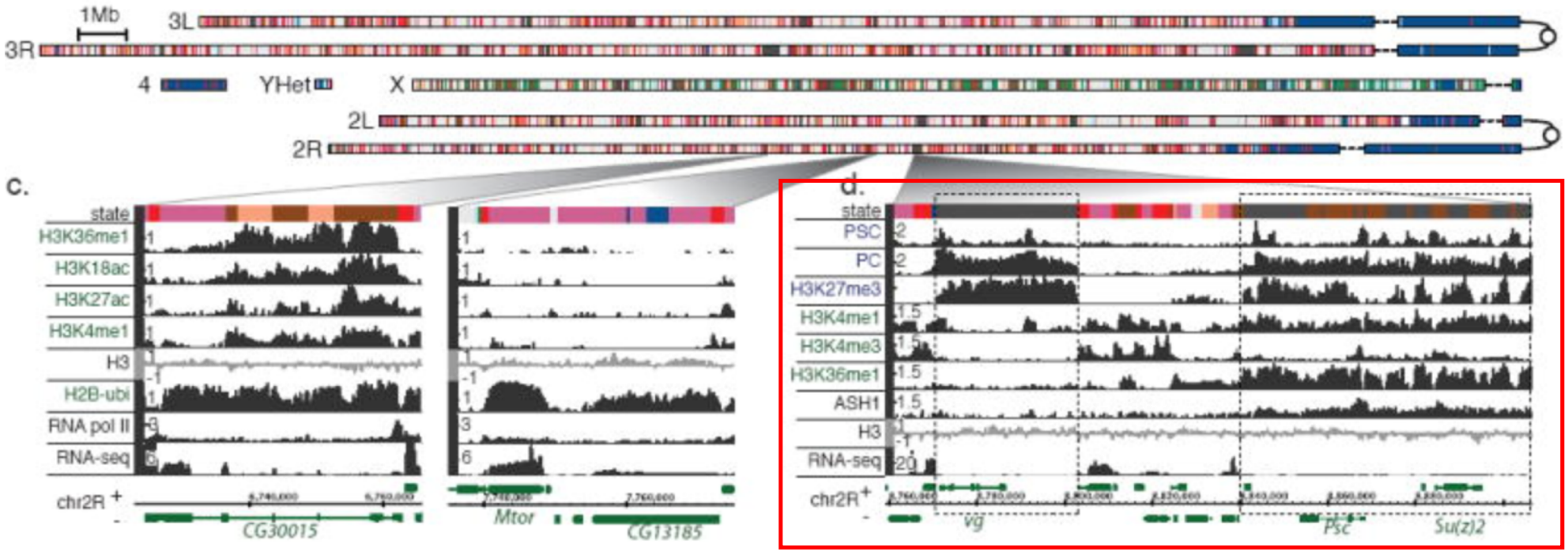
Un cromosoma politenico è un cromosoma gigante. I cromosomi politenici si formano in seguito a vari cicli di replicazione che producono molte copie (anche centinaia) di cromatidi fratelli che rimangono uniti.La formazione dei cromosomi politenici ha la funzione di aumentare il volume cellulare ma può anche comportare un vantaggio metabolico dato che l'elevato numero di copie di geni permette un alto livello di espressione genica. In Drosophila melanogaster, per esempio, i cromosomi delle ghiandole salivari delle larve subiscono numerosi cicli di endoreplicazione, e questo consente di produrre grandi quantità di secreto prima dell'impupamento.





D. Melanogaster polythene chromosomes

PRC2 and PRC1 gene silencing is highly relevant for gene expression on the entire genome level



D. Melanogaster chromatin map

PSC and PC locate in regions with no H3K4me3: inactive monovalent chromatin → no RNA expression

PSC and PC locate in regions with H3K4me3: inactive bivalent chromatin → no RNA expression

PSC and PC locate excluded from regions with H3K4me3: active monovslent chromatin → RNA expression

MECHANISMS OF POLYCOMB GROUP GENE- DEPENDENT SILENCING

1. PRC1 induces a stalling of RNA Polymerase at promoters of Polycomb target genes (in drosophila)

What is a stalled RNA Polymerase II?

- Replication, transcription, and translation stress lead to stalling of respective polymerases
- When stalling at promoters, RNA Polymerase produces a significant amount of short ncRNAs –> detectable by RNA-Seq
- Important: Overlapping RNA-Seq data and Polycomb ChIP seq revealed that many PRC1 target genes produce short ncRNAs from promoters → PRC1 stalls RNA Pol II

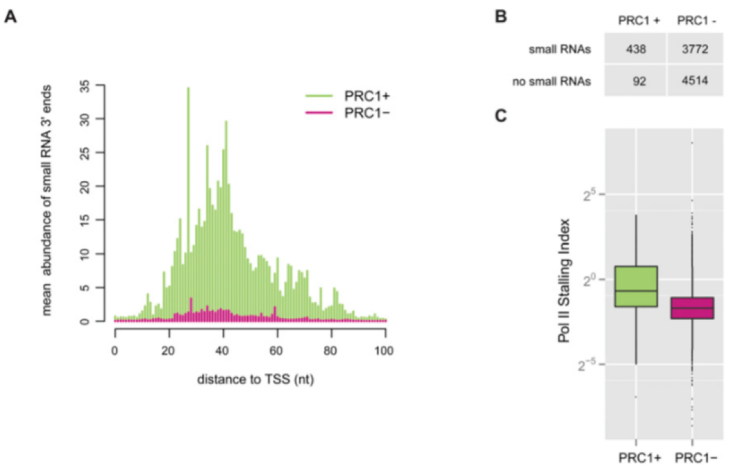
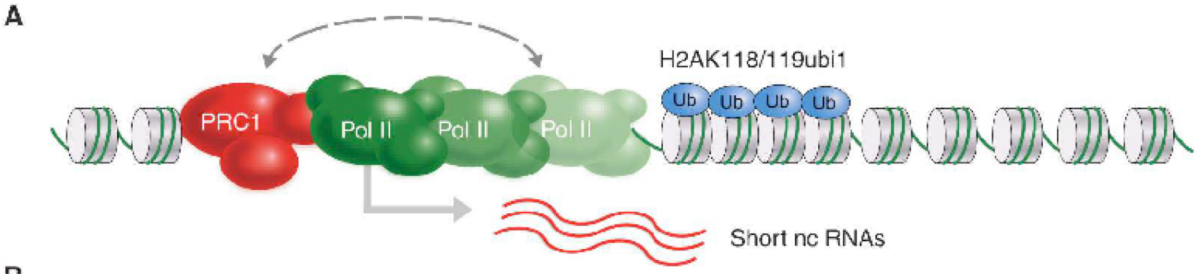


Figure 3. PRC1 preferentially binds stalled promoters in S2 cells. (*A*) PRC1-bound promoter exhibits a higher abundance of promoter-proximal short RNA 3′-ends indicative of increased Pol II stalling (Nechaev et al. 2010). (*B*) PRC1 largely binds promoter producing small RNAs (≥1 read from 5′- and 3′-end libraries). (*C*) Pol II preferentially remains stalled at PRC1-bound promoter as calculated by the ratio of promoter-proximal occupancy versus gene body. The two populations are significantly different (*P*-value < 2.2×10^{-16}, two-sample Kolmogorov-Smirnov test).

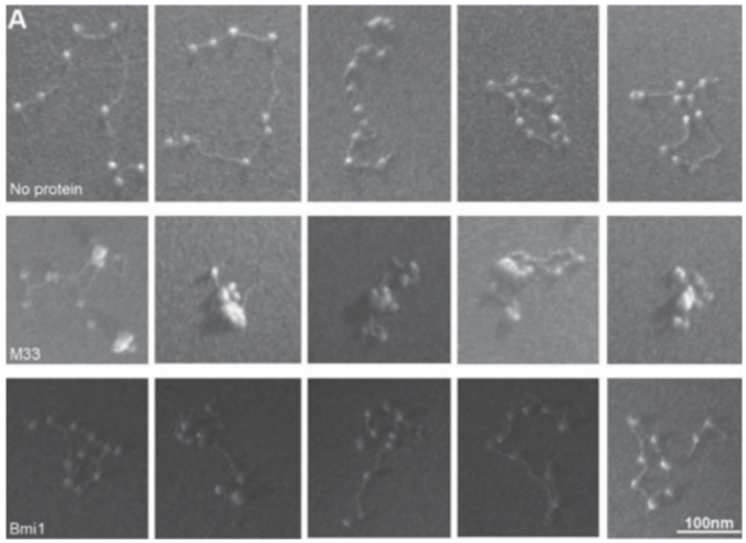


RNA Pol II stalling: production of ncRNAs from PRC1 target gene promoters

Transcription elongation is not a smooth ride along the DNA railway. For proofreading, the polymerase is made to back-up, erase some of the RNA it has already made and have another go at transcription. In general, RNA polymerase does not transcribe through a gene at a constant pace. Rather it pauses periodically at certain sequences, sometimes for long periods of time before resuming transcription. In extreme cases, for example, when the polymerase encounters a damaged nucleotide, it comes to a complete halt. More often, an elongating polymerase is stalled near the promoter. Promoter-proximal pausing during early elongation is a commonly used mechanism for regulating genes poised to be expressed rapidly or in a coordinated fashion.

MECHANISMS OF POLYCOMB GROUP GENE- DEPENDENT SILENCING

2. PRC1 induces chromatin compaction (aggregation)



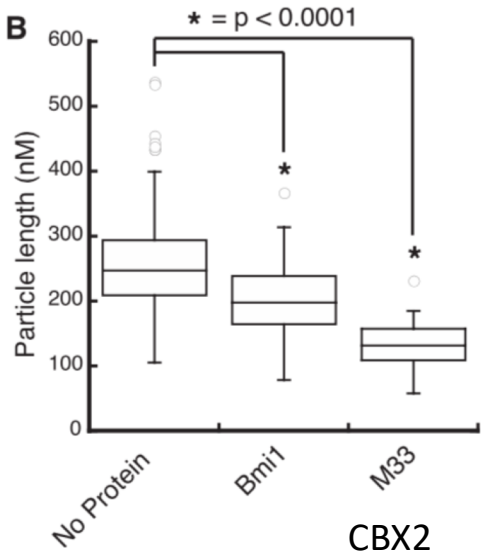


Figure 5. Compaction of nucleosomal arrays by mouse PcG proteins. (*A*) Representative EM images of nucleosomal arrays incubated with the indicated PcG protein. (*B*) Box plot representation of the measured maximal diameter of nucleosomal array particles. Particle length is the diameter of the smallest circle that can entirely surround one nucleosomal array. The box represents the upper and lower quartile, and the line splitting the box represents the mode. The open circles represent outliers, and the asterisks indicate a *P*-value of <0.0001 using Student's *t*-test. No protein, $n = 72$; Bmi1, $n = 50$; M33, $n = 30$.

Purified nucleosomal arrays incubate with recombinant polycomb proteins
→ Arrays are compacted

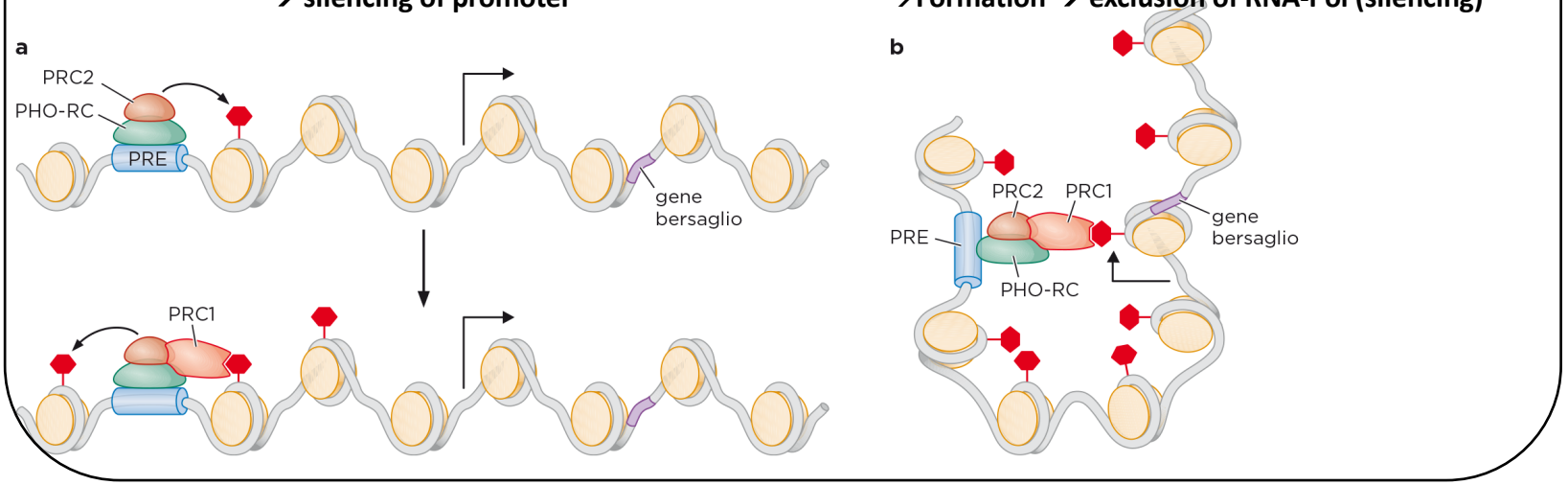
MECHANISMS OF POLYCOMB GROUP GENE- DEPENDENT SILENCING

3. PRC2 - PRC1 induces chromatin looping – RNA polymerase exclusion

PRC2 is recruited by DNA elements (polycomb responsive elements) that are localized in vicinity of promoters

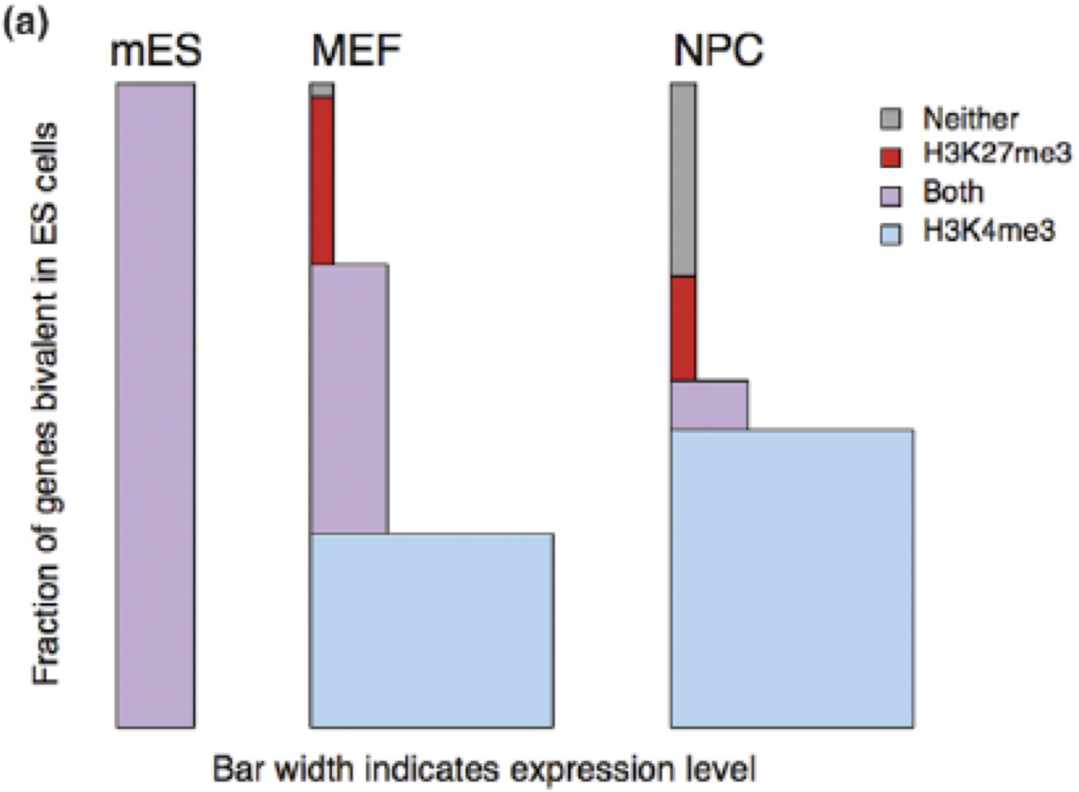
PRC2 recruited by PRE (polycomb responsive elements) →mediates H3K27me3→ PRC1 recruitment → silencing of promoter

PRC2 recruited by PRE (polycomb responsive elements) →mediates H3K27me3→ PRC1 recruitment → loop →Formation → exclusion of RNA-Pol (silencing)



PRC2 is essential for gene expression control in development/differentiation/disease

(a)



(b)

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

mES: mouse embryonic stem cells
MEFs:Mouse embryonic fibroblasts
NPC: neuronal progenitor cells

Stem cell regulation by polycomb repressors: postponing commitment
Alexandra M Pietersen and Maarten van Lohuizen

Current Opinion in Cell Biology 2008, **20**:201–207

HOW ARE PcG PROTEINS RECRUITED TO TARGET GENES? – POLYCOMB RESPONSE ELEMENTS

Polycomb repressive complex 1/2 interacts with defined DNA sequences = PRE Polycomb responsive elements

PRE elements were discovered in Drospophila → enrichment of PRC1/2 components at PRE sites

PRC2 and PRC1 components do not have DNA binding domains

PRC2 and PRC1 interact with specialized PcG group proteins that bind PREs
D.melanogaster: PHO Pleiohomeotic; PHO-L Pleiohomeotic like → form PhoRC complex

PhoRC complex at app 45% of all PRC1/2 target genes; other sites: PRC1/PRC2 interacts with other DNA binding proteins

Polycomb group genes are conserved in evolution

DNA binding

Polycomb repressive complex 2 (PRC2)

Polycomb repressive complex 1 (PRC1)

Drosophila melanogaster			*Mus musculus*	*Arabidopsis thaliana*	*Caenorhabditis elegans*
PcG DNA binding proteins					
PHO	Pleiohomeotic	Zinc finger	YY1		
PHOL	Pleiohomeotic-like	Zinc finger			
PSQ	Pipsqueak	BTB-POZ domain			
DSP1	Dorsal switch protein 1	HMG domain protein	HMGB2		
PRC2 core proteins					
ESC	Extra sex combs	WD 40 repeats	EED	FIE	MES-6
E(Z)	Enhancer of zeste	SET domain	EZH1/ENX2 EZH2/ENX1	CLF MEA SWN	MES-2
SU(Z)12	Suppressor of zeste 12	Zinc finger VEFS box	SU(Z)12	FIS2 VRN2 EMF2	
p55	p55	Histone-binding domain	RBAP48 RBAP46	MSI1 (MSI2/3/4/5)	
PRC1 core proteins					
PC	Polycomb	Chromodomain	CBX2/M33 CBX4/MPC2 CBX6 CBX7 CBX8/MPC3		
PH	Polyhomeotic	Zinc finger SAM/SPM domain	EDR1/MPH1/RAE28 EDR2/MPH2 (EDR3)		SOP-2
PSC	Posterior sex combs	Zinc finger HTH domain	BMI1 MEL18/RNF110/ZFP144	AtBMI1A AtBMI1B AtBMI1C	MIG-32
SCE/dRING	Sex combs extra/dRing	RING zinc finger	RING1/RING1A RNF2/RING1B	AtRING1A AtRING1B	SPAT-3

Mammals: Function in PRC recruitment debated

PRE BINDING PROTEINS

Epigenetic writers

PREs have many binding sites for certain TFs – not only for Pho/Phol

Development 134, 223-232 (2007) doi:10.1242/dev.02723

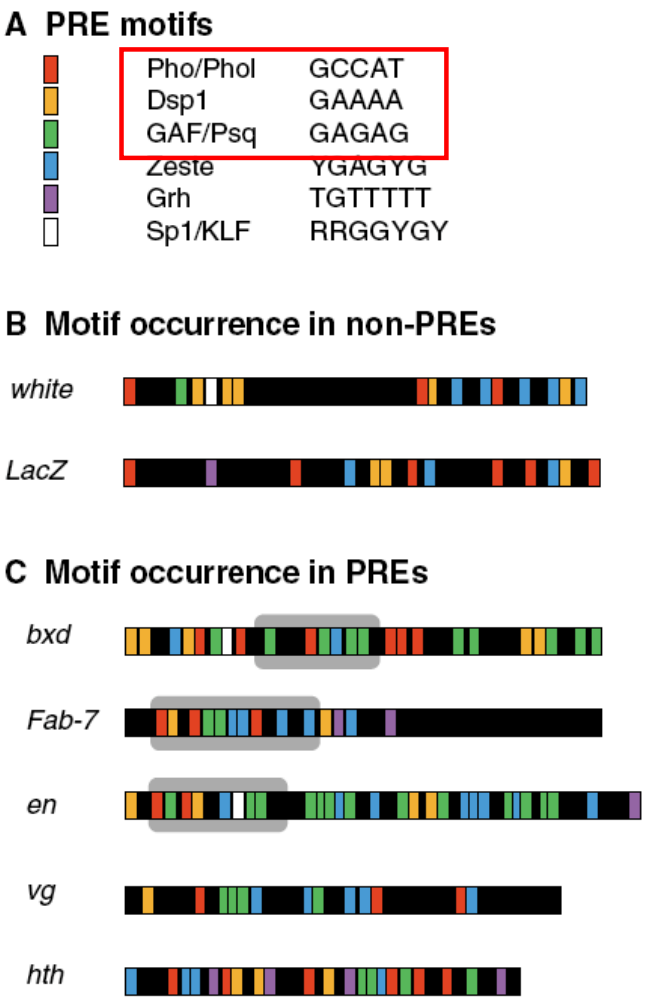
Polycomb/Trithorax response elements and epigenetic memory of cell identity

Leonie Ringrose[1] and Renato Paro[2]

PREs consist of modules of sequences that can recruit multiple transcriptional regulators that interact
Polycomb group proteins.

The set of transcription factors available for binding to
PRE elements is defined by the cell-identity (gene expression
Profile of the respective cell)

(NOTE: also TrxG proteins can be found at PREs)



A. DNA motifs shown to be important for PRE/TRE function, bound by specific proteins

B. Motifs are short an occur frequently in regulatory regions of random genes. Many of these motifs are important for regulating genes that do not have PRE/TREs, for example the Drosophila white gene which is regulated by the Zeste protein (600 bp of upstream regulatory region are shown). These motifs are also short and occur randomly in DNA, such as in the bacterial LacZ gene (the first 600 bp of the coding sequence are shown).

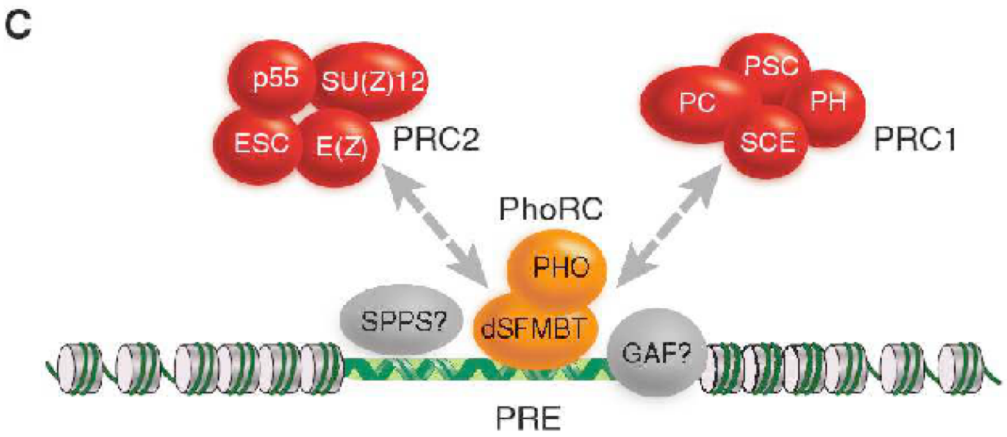
C. PRE/TREs have different combinations of motifs, with no preferred order or number. Shown here are ~600 bp of the bxd and Fab-7 PREs from the Drosophila Bithorax complex, and of PRE/TREs from the Drosophila engrailed (en), vestigial (vg) and homothorax (hth) loci. Grey boxes show minimal PRE/TREs where these have been defined (Dejardin et al., 2005; Brown et al., 2005). Flanking sequences contain additional motif clusters which may contribute to the function of these PRE/TREs in their endogenous context.

HOW ARE PcG PROTEINS RECRUITED TO TARGET GENES? – POLYCOMB RESPONSE ELEMENTS

Drosphila

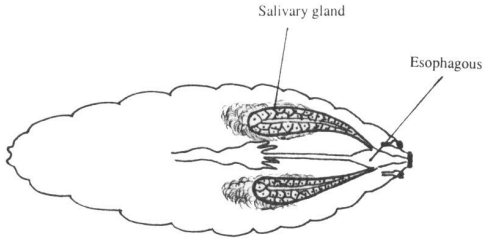
Classic PRE elements are composed of several motifs, identified by matematical prediction and need experimental validation

MINIMAL CRITERIA FUNCTIONAL RELEVANT PRE ELEMENTS:

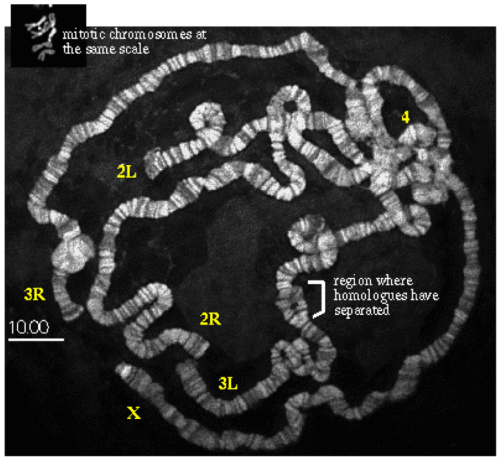


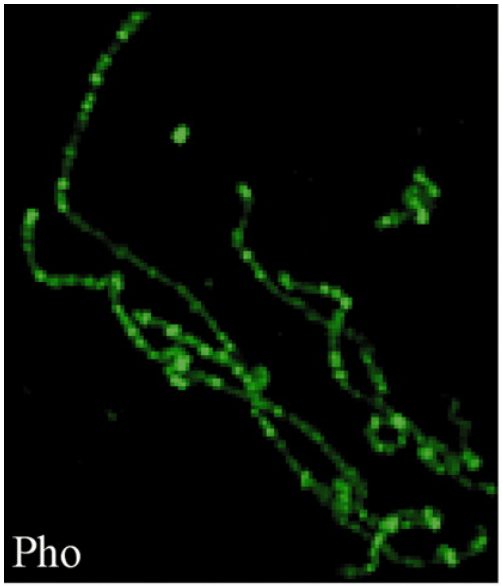
(1) PREs attract H3K27me3,
(2) A PRE forms a new binding site for PcG proteins when inserted at a new location within the genome, and
(3) they confer PcG-based repression to a reporter gene.

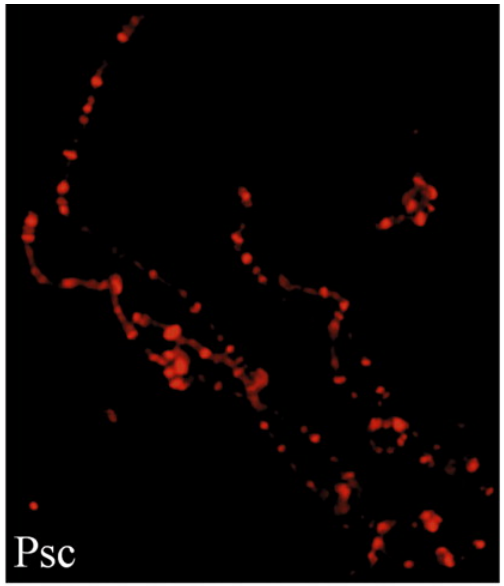
Pho RECRUITS POLYCOMB PROTEINS TO DEFINED GENES

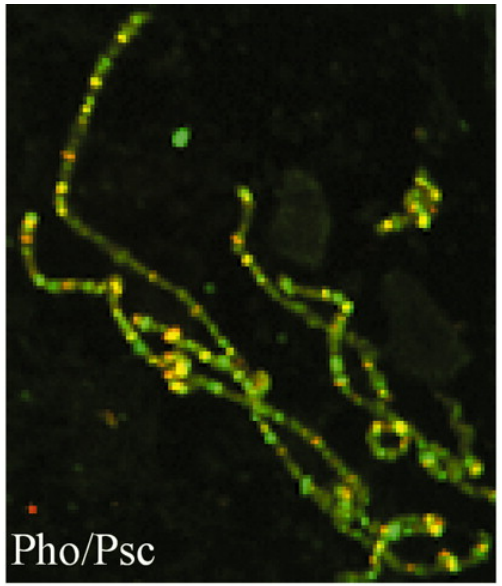


Un cromosoma politenico è un cromosoma gigante. I cromosomi politenici si formano in seguito a vari cicli di replicazione che producono molte copie (anche centinaia) di cromatidi fratelli che rimangono uniti.La formazione dei cromosomi politenici ha la funzione di aumentare il volume cellulare ma può anche comportare un vantaggio metabolico dato che l'elevato numero di copie di geni permette un alto livello di espressione genica. In Drosophila melanogaster, per esempio, i cromosomi delle ghiandole salivari delle larve subiscono numerosi cicli di endoreplicazione, e questo consente di produrre grandi quantità di secreto prima dell'impupamento.









HOW ARE PcG PROTEINS RECRUITED TO TARGET GENES? – POLYCOMB RESPONSE ELEMENTS

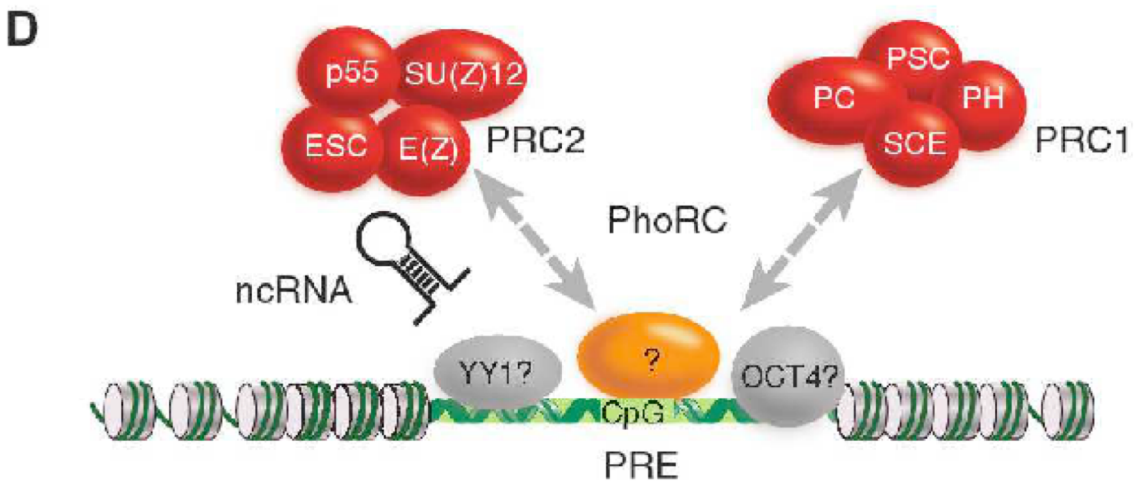
Vertebrates

PREs are ill defined in vertebrates: a ”consensus PRE site” does not exist

Polycomb proteins accumulate around gene promoters

PRC2/PRC1 target sites can be defined by ChIP Seq

Often YY1 co-localizes with PRC1/2; however there is no defined mechanism that explains PRC1/2 complex recruitment to PRC1/PRC2 target genes → multiple transcription factors can interact with PRC1/2 (i.e. OCT4)



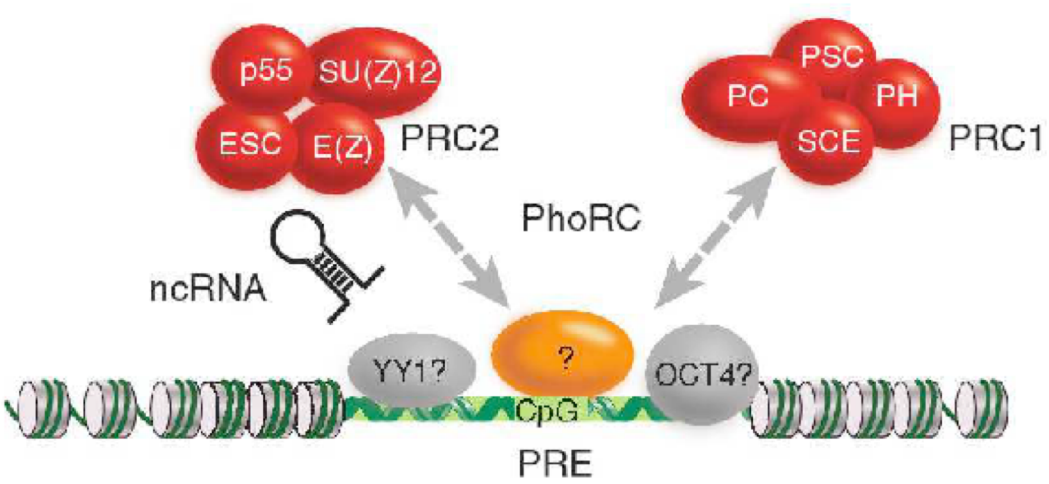
PRC1/2 Recruitment:

- TRANSCRIPTION FACTORS (Repressors)
- ncRNA COMPONENTS
- YY1?

HOW ARE PcG PROTEINS RECRUITED TO TARGET GENES? – POLYCOMB RESPONSE ELEMENTS

A LINK BETWEEN H3K27me3 – PRC1 – PRC2 – CpG DNA METHYLATION

Vertebrates



In **self-renewing embryonic stem cells**
CpG islands show low DNA methylation but are enriched for H3K27me3

During differentiation most H3K27me3 CpGs gain DNA methylation

PRC2 interact with DNMTs (IP)

MODEL: H3K27me3 (PRC1/2) at CpG islands of bivalent genes mediate
the inactivation of the gene by DNA methylation during differentiation

Recruitment of PcG proteins by ncRNAs

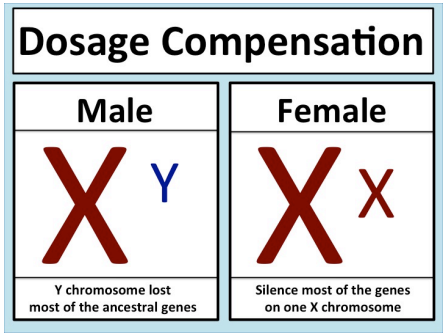
- Xist RNA: Vertebrate X chromosome inactivation
- HOTAIR lncRNA in human cancer
- lncRNAs in genomic imprinting

DOSAGE COMPENSATION IN VERTEBRATES: X chromosome inactivation (XCI)

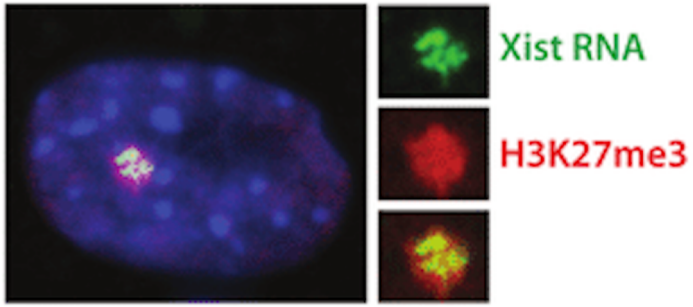
	Male		Female		
Drosophila melanogaster	X Y	AA	XX	AA	**rox lncRNA**
Homo sapiens	X Y	AA	X x	AA	**Xist lncRNA - POLYCOMB**
Caenorhabditis elegans	X	AA	XX	AA	

PRC1/2 CAN INTERACT WITH ncRNA

X chromosome inactivation = process of dosage compensation of X-linked gene expression in vertebrate species

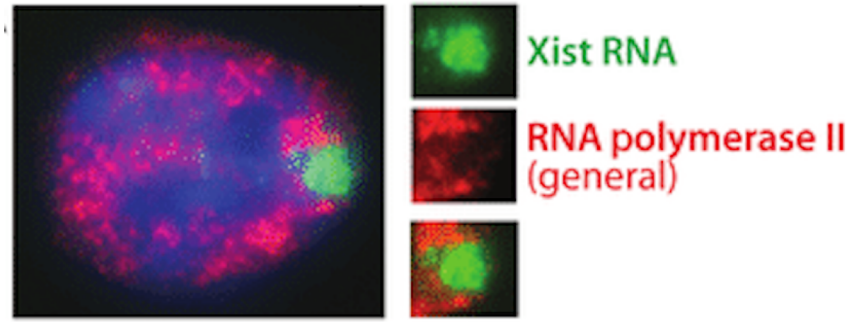


Female Cell



- X-linked Xist gene transcribes Xist ncRNA that localizes along X-chromosome
- Xist marks the territory of the inactive X chromosome
- Xist recruits as first step during XCI PRC2 and PRC1

Female Cell



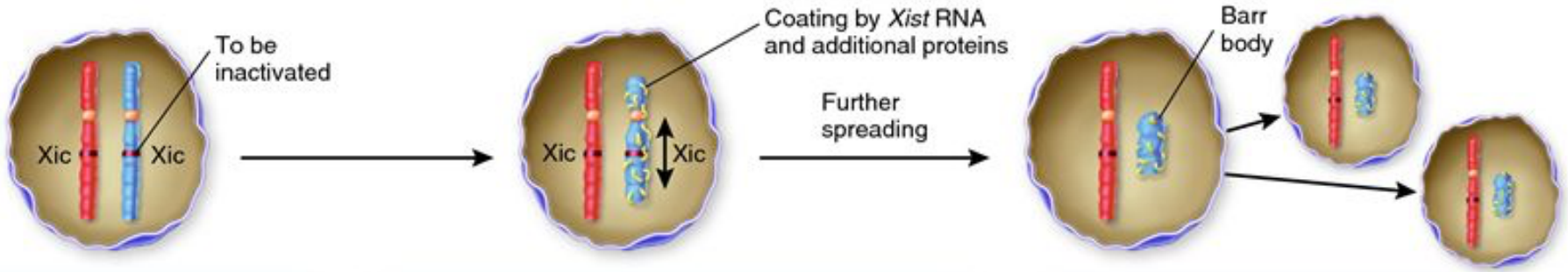
- Silencing of most genes on inactive X chromosome
- RNA PolII excluded from Xist territories

PRC1/2 CAN INTERACT WITH ncRNA

Xic: (X inactivation center)

Encodes a series of ncRNAs that are involved in **choice** and **silencing** of one specific X chromosome.

Xist is upregulate from one of the 2 X chromosomes and drives the silencing of an entire (most) X chromosome



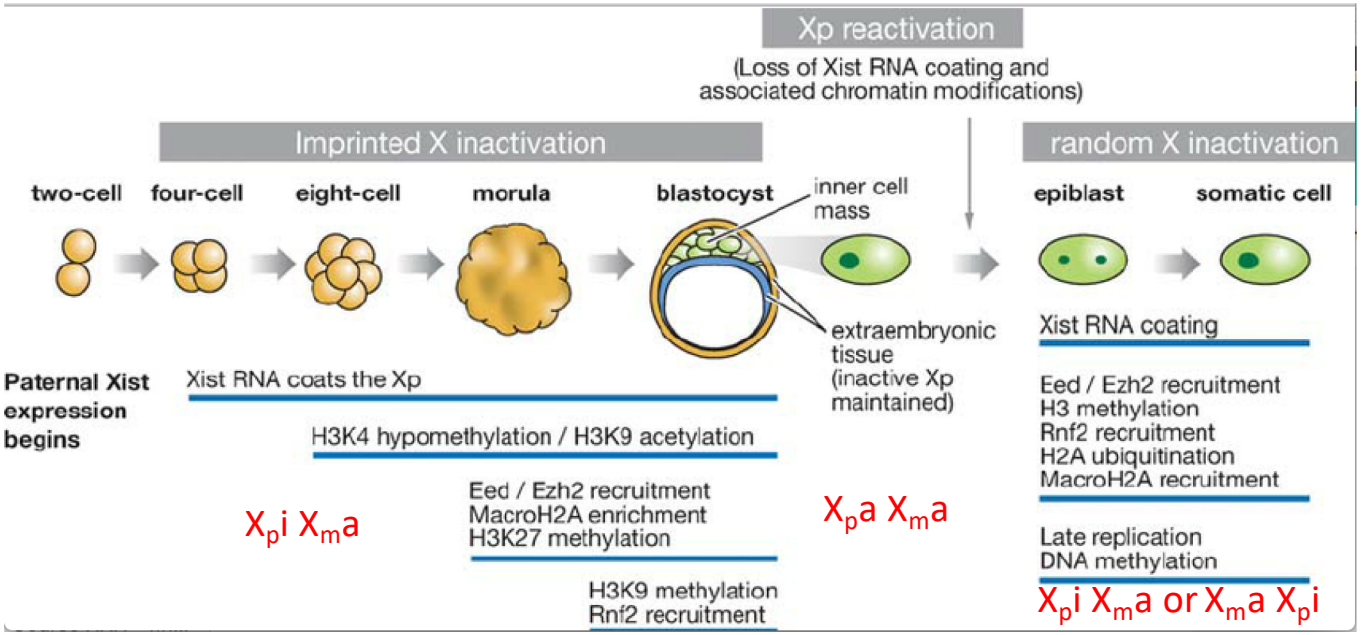
In vivo: Blastocyste stage

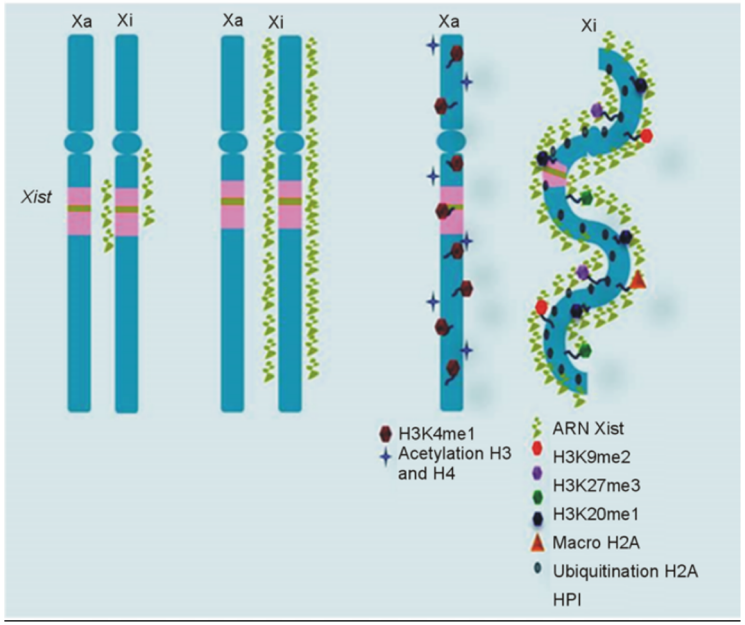
In vitro: Studied in cells that correspond to the blastocyst stage = embryonic stem cells:

- Differentiating female embryonic stem cells (endogenous Xic) or
- Male embryonic stem cells that carry an inducible Xist transgene on autosome (inactivation by Xist occurs on autosome)

PRC1/2 CAN INTERACT WITH ncRNA

Xist and PRC2





Inner cell mass = epiblast cells: BOTH X chromosomes are active in female cells

Embryonic stem cells are cultivated from the inner cell mass and contain active X chromosome chromatin

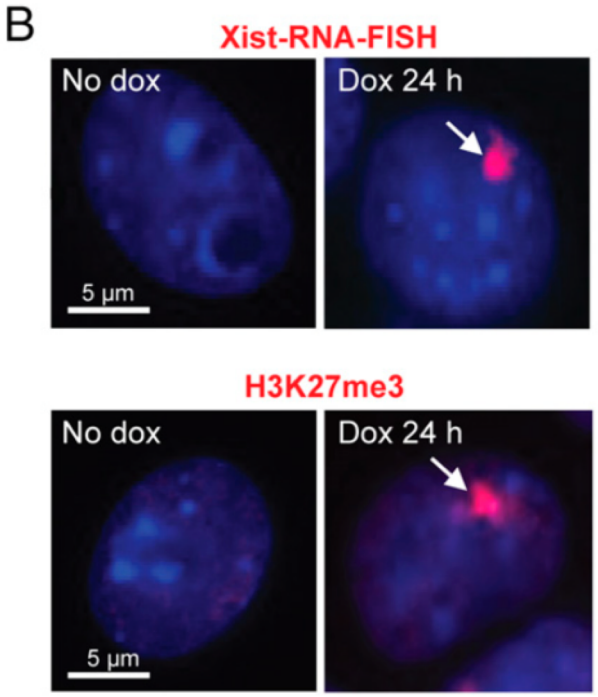
Model systems to study the epigenetics of XCI:

In vivo: Blastocyste stage

In vitro: Studied in cells that correspond to the blastocyst stage = embryonic stem cells:

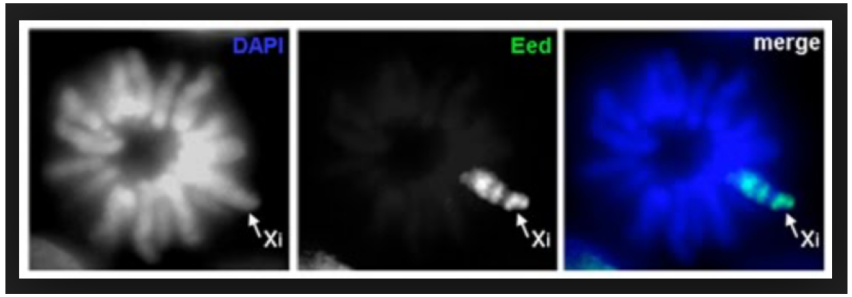
- Differentiating female embryonic stem cells (endogenous Xic) or
- Male embryonic stem cells that carry an inducible Xist transgene on autosome (inactivation by Xist occurs on autosome)

Embryonic stem cells revealed Xist dependet XCI



C

Male embryonic stem cell with a doxycyclin inducible Xist Transgene
Addition of Doxycyclin to the medium mediates Xist expression



A stem-loop repeat motif of the Xist lncRNA recruits PRC2 to the future Xi

Xist RNA spreads along the entire X chromosome (model: Xist expression from inducible Promoter)

Also PRC2 components (i.e. Eed) spread across The future Xi

PRC2 (Ezh2) mediates chromosome wide H3K27me3 methylation

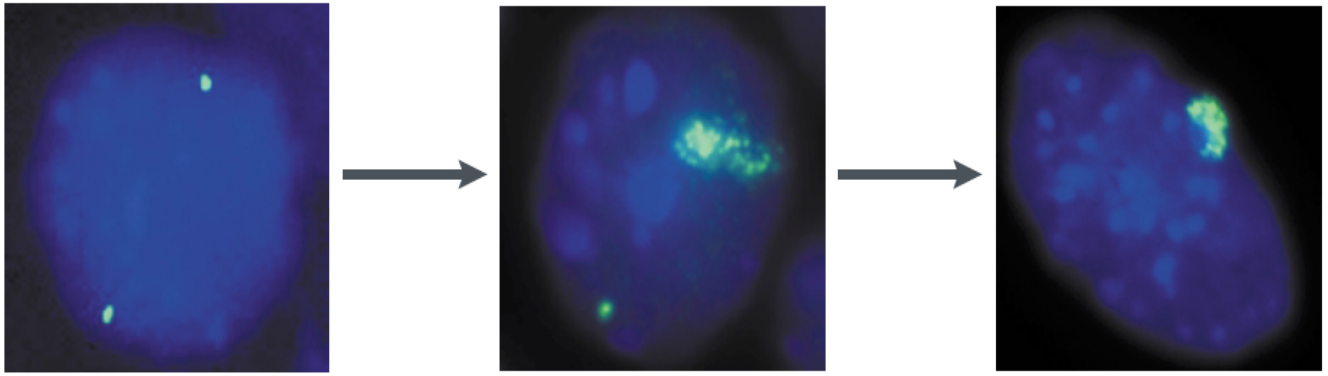
PRC1 is also recruited to the Xi (PRC2 independent) H2AK119 ubiquitination

Deacetylation
Imposition of DNA methylation
Incorporation of histone variants

CHROMOSOME WIDE GENE SILENCING (redundant pathways)

Embryonic stem cells revealed Xist dependet XCI

Xist RNA-FISH on female embryonic stem cells that initiate X inactivation



2 X chromosomes
Express Xist at low leves
(both X active)

1 X chromosome
Express Xist at high levels
(choice,
silencing of 1 X chromosome)

1 X chromosome
Express Xist at high levels
(silencing of 1 X chromosome)
Other X chromosome has silenced
comoletely its Xist gene

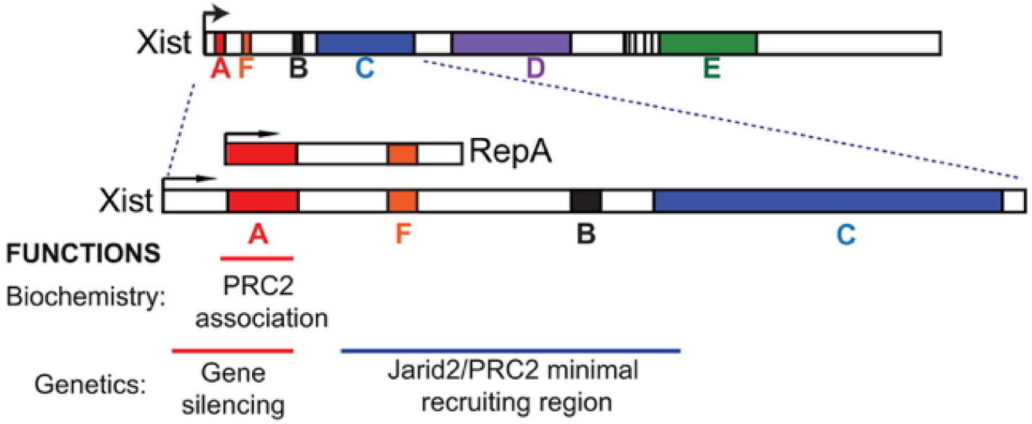
48 hours indiction of mESC differentiation

Developmental program activates counting – choice - silencing

PRC1/2 CAN INTERACT WITH ncRNA

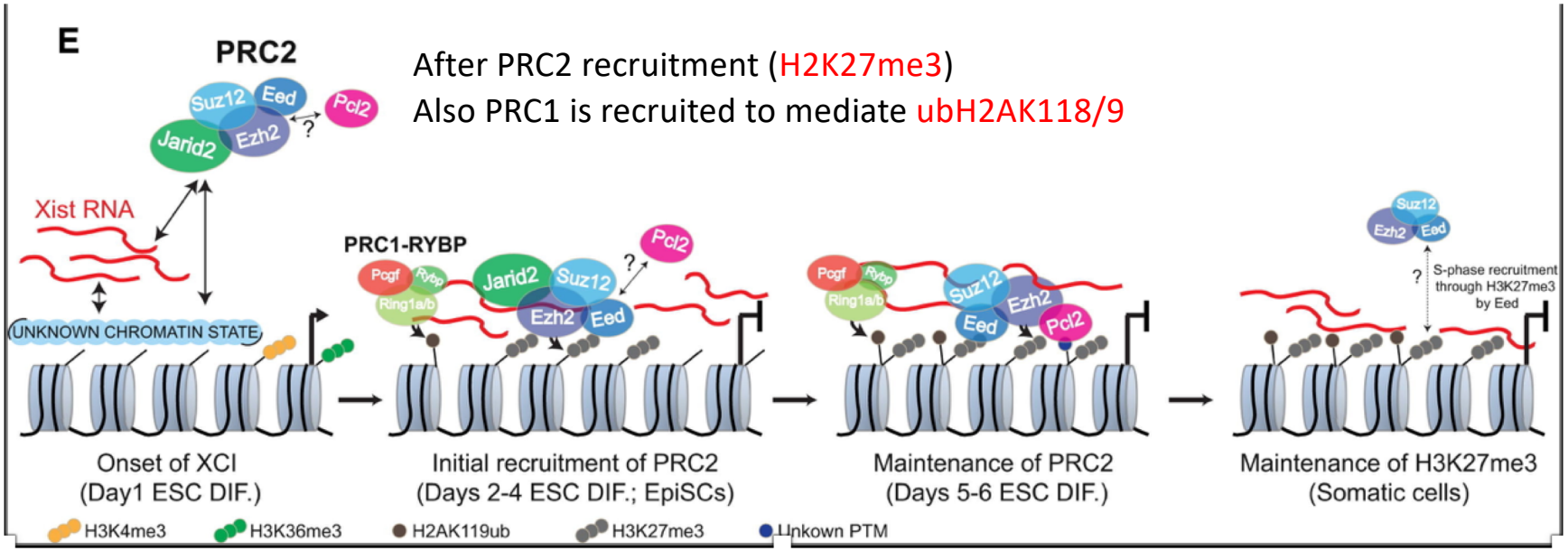
Gene

Function of relevant Xist RNA region



Xist contains RNA regions that:
- **Recruit PRC2**
- **interact with the PRC2 co-factor Jarid2**

(important: Jarid helps RPC2 to interact with chromatin → general function of Jarid2 in PRC2 complex

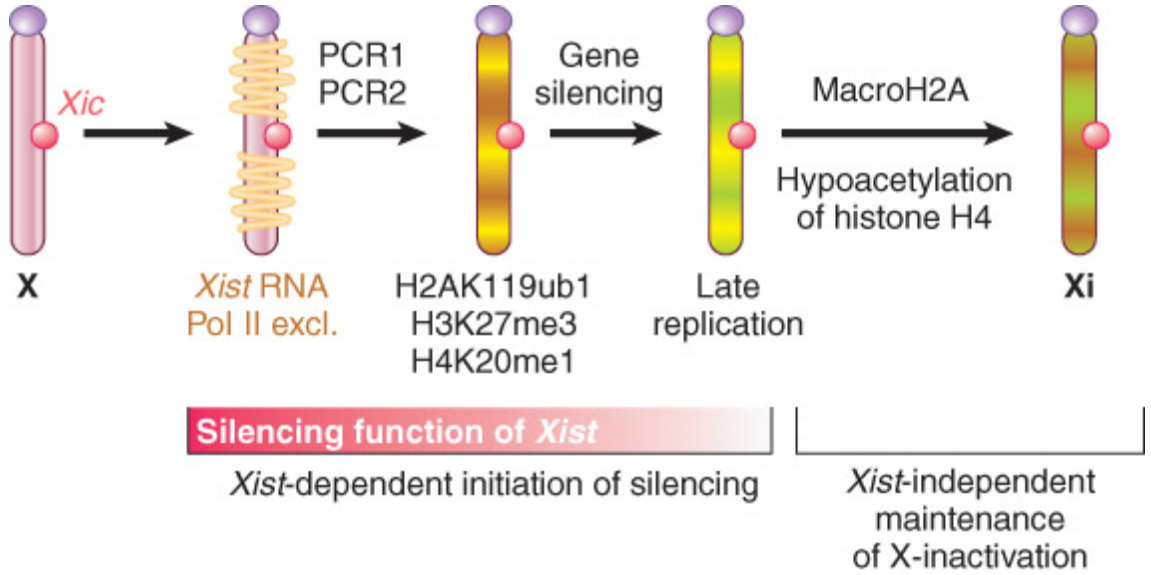


After PRC2 recruitment (H2K27me3)

Also PRC1 is recruited to mediate ubH2AK118/9

PRC1/2 CAN INTERACT WITH ncRNA

- *Xist* recruits Polycomb complexes, which modify histones on the inactive X



Xist RNA produced from the Xic locus accumulates on the future inactive X (Xi).

Adapted from A. Wutz and J. Gribnau, Curr. Opin. Genet. Dev. 17 (2007): 387-393.

Recruitment of PcG proteins by ncRNAs

- Xist RNA: Vertebrate X chromosome inactivation
- HOTAIR lncRNA in human cancer
- lncRNAs in genomic imprinting

PRC1/2 CAN INTERACT WITH lncRNA in cancer

HOTAIR lncRNA interacts with PRC2 → DIRECTS PRC2 TO TARGET GENES (i.e Hox4)

HOTAIR lncRNA is overexpressed in tumors → → altered gene expression →→ tumorformation and progression

HOTAIR lncRNA expression is a prognostic marker (poor survival)

HOTAIR IS OVEREXPRESSED IN HUMAN CANCER

Table 1

Overexpression of *HOTAIR* in different cancers

Type	Overexpression of *HOTAIR*	References
Breast cancer	Poor prognosis, metastasis, invasion, and short overall survival	21,35
Esophageal squamous cell carcinoma (ESCC)	Poor prognosis, high TNM stage, invasion, metastasis, and short overall survival	36,37
Gastric cancer	Tumor staging, venous infiltration, and lymph node metastasis	38,39
Hepatocellular carcinoma	Invasion of HCC cells, possibility of recurrence	40-44
Colorectal cancer	Poor prognosis, low survival, and metastasis promotion	45-47
Gallbladder cancer (GBC)	Promoting carcinogenesis	29
Bladder cancer (BC)	Poor prognosis and high recurrence rate	48
Renal carcinoma	Proliferation, invasion, and promotion of tumor growth	49
Cervical cancer	FIGO stage, aggression, and lymph node metastasis	30
Epithelial ovarian cancer	Poor prognosis, FIGO stage, lymph node metastasis, overall survival, and metastatic stage of EOC	50
Endometrial carcinoma	Poor prognosis, lymph node metastasis, EC grade, and overall survival	51,52
Lung cancer	Invasion and metastasis	53
Non-small cell lung cancer	Promotion of lymph node metastasis	54,55
Small-cell lung cancer	Poor prognosis, proliferation and invasion	56
Nasopharyngeal carcinoma	Poor prognosis, overall survival, proliferation, invasion, and promotion of tumor stage	31
Melanoma	Invasion and metastasis	57
Glioma	Poor prognosis, cell cycle progression, and glioma grade	58
Pancreatic cancer	Proliferation and aggression of tumors	59

PRC1/2 CAN INTERACT WITH lncRNA in cancer

HOTAIR lncRNA interacts with PRC2 → DIRECTS PRC2 TO TARGET GENES (i.e Hox4)

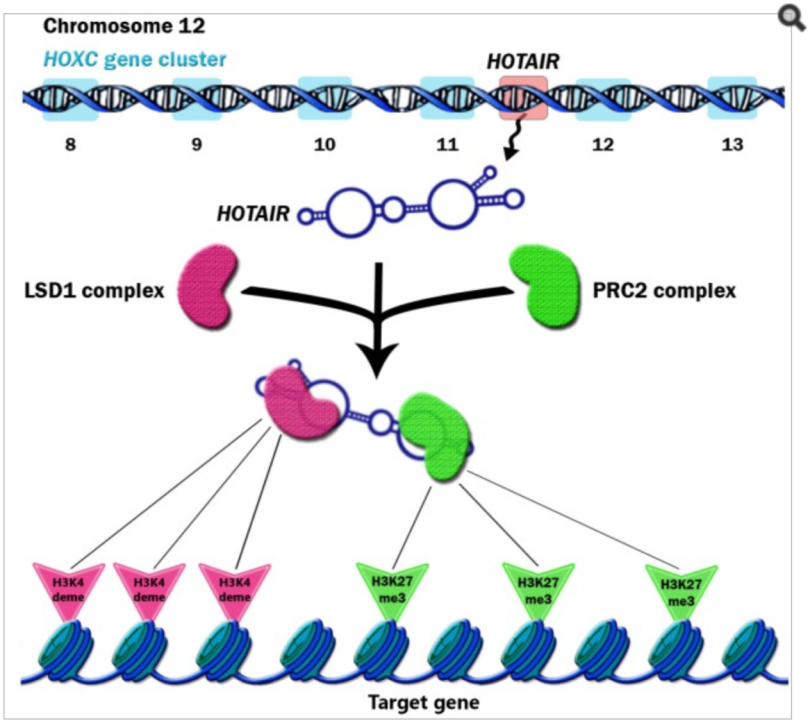
HOTAIR lncRNA is overexpressed in tumors → → altered gene expression →→ tumorformation and progression

HOTAIR lncRNA expression is a prognostic marker (poor survival)

HOTAIR Complexes with LSD1 and PRC2

LSD1: DEMTHYLATION OF H3K4
PRC2: METHYLATION OF H3K27

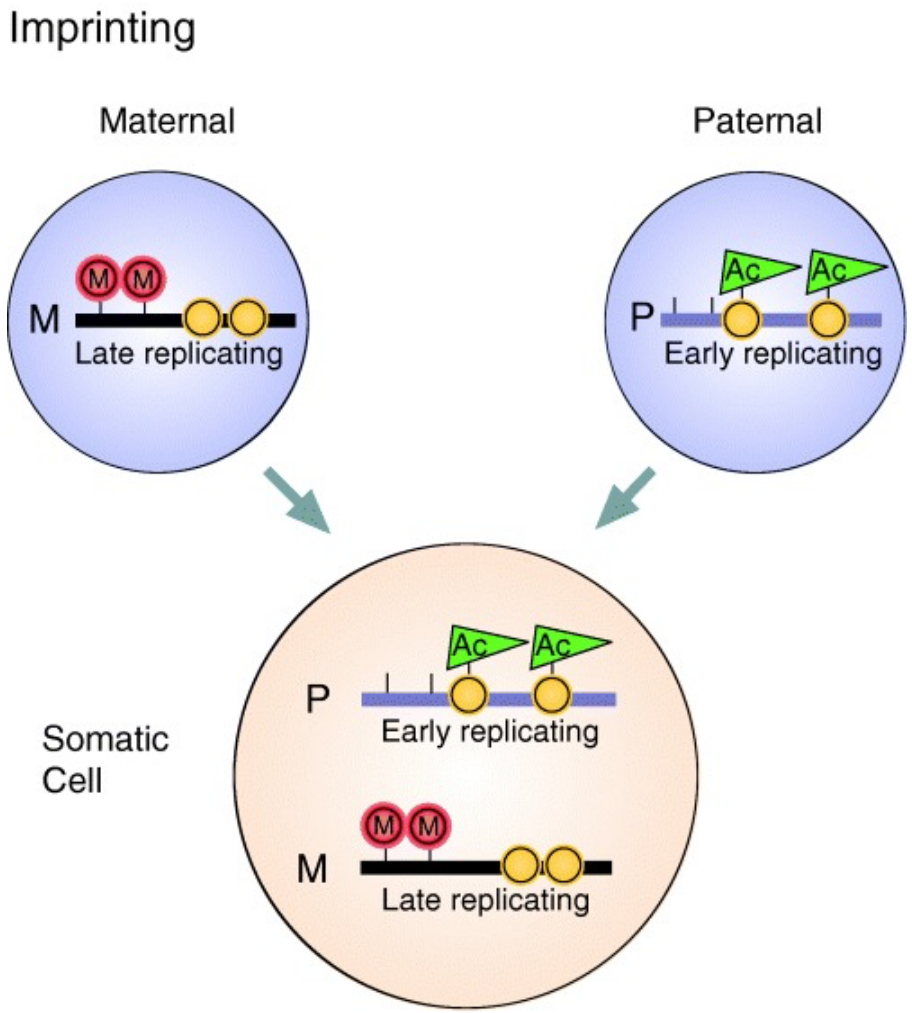
Aberrantly expressed HOTAIR targets gene silencing to genes promoters of tumor suppressor genes



Recruitment of PcG proteins by ncRNAs

- Xist RNA: Vertebrate X chromosome inactivation
- HOTAIR lncRNA in human cancer
- lncRNAs in genomic imprinting

PRC1/2 CAN INTERACT WITH ncRNA – genomic imprinting



Epigenetic information is not erased from all genes after fertilization.
Some genes maintain the epigentic information from the paternal/maternal chromosomes

= IMPRINTED GENES

→ Exclusive expression of the maternal or paternal gene

→ For example imprinted XCI

PRC1/2 CAN INTERACT WITH lncRNA to mediate genetic imprinting

Genomic Imprinting: Kcnq1 LOCUS IN MOUSE

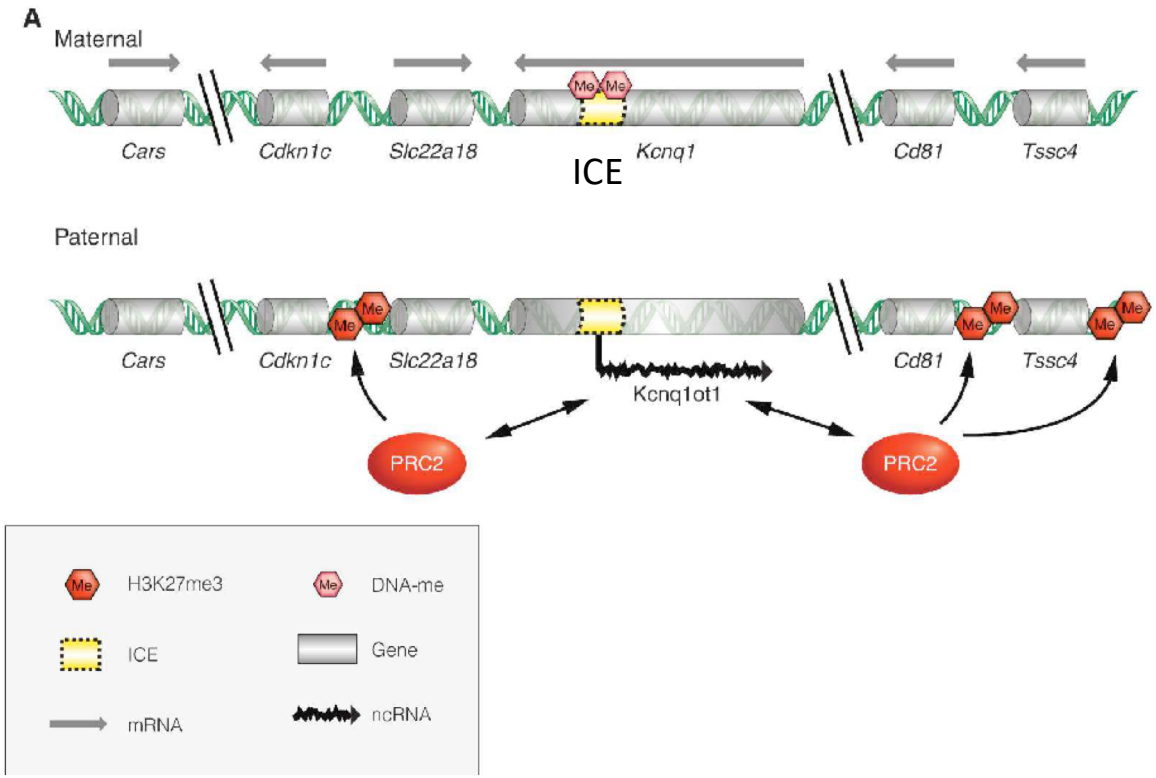


Figure 8. Interplay of PcG-mediated repression and DNA methylation regulates genomic imprinting in plants and mammals. (*A*) Regulation of genomic imprinting at the *Kcnq1* domain on distal chromosome 7. The imprinting control element (ICE) is maternally methylated and prevents the transcription of the lncRNA *Kcnq1ot1* from the maternal chromosome. The paternally expressed *Kcnq1ot1* associates with chromatin and recruits chromatin modifying complexes, such as PRC2, to mediate and maintain transcriptional silencing of several paternal, protein-coding alleles. (*B*) In *Arabidopsis* seeds, the paternally

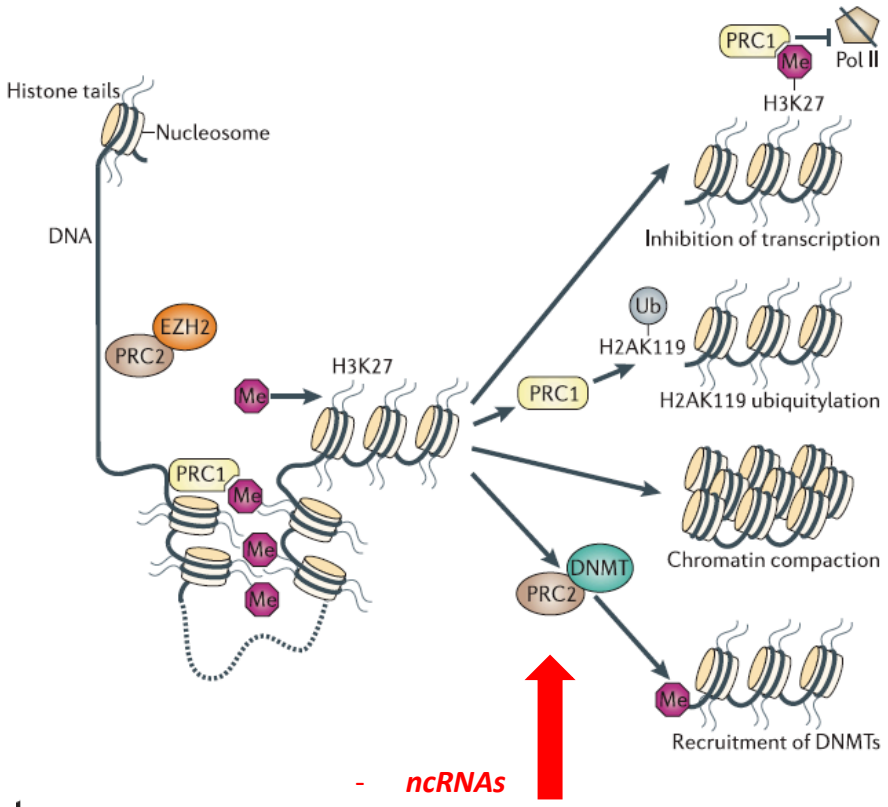
Maternal allele: ACTIVE – expression of protein coding genes around Kcnq1.
WHY: Kcnq1 lncRNA has ICE (CpG island) methylation at promoter (ICE: Imprinting control element) → Kcnq1 is silenced

Paternal allele: INACTIVE – genes around Kcnq1 are silenced.
WHY: Kcnq1 CpG island is unmethylated. Kcnq1 lncRNA is expressed.
lncRNA recruits PRC2 that silences nearby genes

Insertion of premature poly-A signal into Kcnq1 gene:

- On maternal allele: no effect
- On paternal allele: loss of silencing of imprinted genes

OVERVIEW: IMPACT OF POLYCOMB ON GENE EXPRESSION



- ***ncRNAs***
- ***trancription factors***
- ***Polycomb DNA bindig proteins***

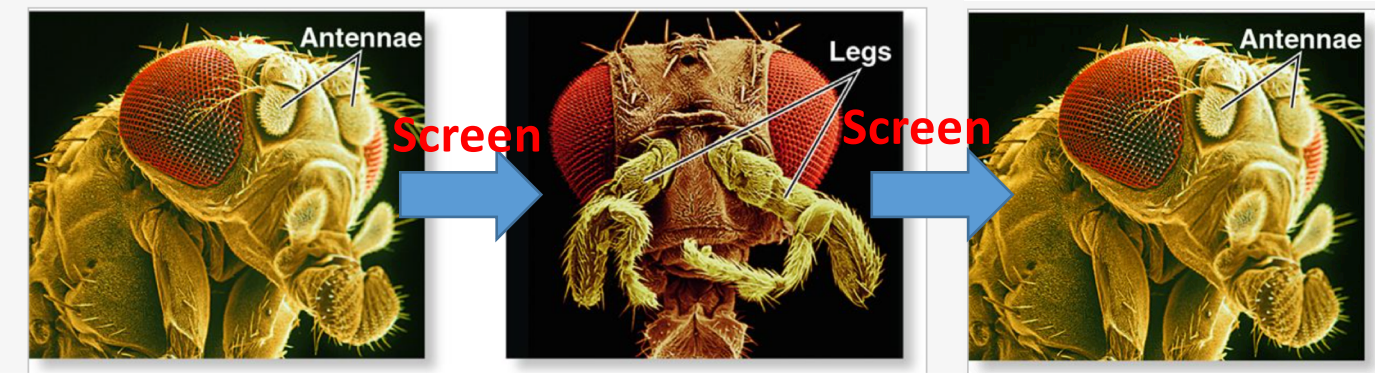
- **Chromatin remodeling (i.e. FACT)**
- **H3K36me3 (i.e. KDM2)**

Polycomb silencers control cell fate, development and cancer

Anke Sparmann and Maarten van Lohuizen

EXPERIMENTAL APPROACH IN DROSOPHILA:

A SUPPRESSOR MUTATION SCREEN TO IDENTIFY EPIGENTIC REGULATORS THAT ACTIVATE GENE EXPRESSION --- TRITHORAX GROUP GENES ---



WILD-TYPE

PRC2 mutation
In Su(z)12

(partial) rescue
of phenotype

Mutation screen

Supressor - Mutation screen

POLYCOMB GROUP GENES (PcG)

TRITHORAX GENES (TrxG)

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--- TRITHORAX GROUP GENES ---

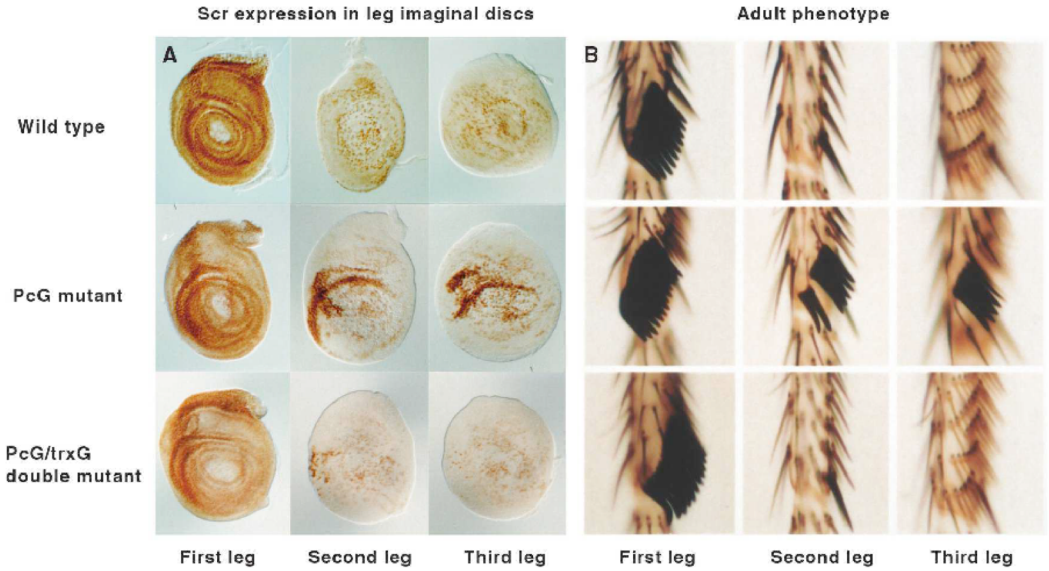

Figure 4. trxG mutations block the derepression of Hox genes in PcG mutants. (*A*) Leg imaginal discs stained with antibodies against the protein encoded by the Hox gene, *Scr*, which specifies the identity of the labial and first thoracic segments, including the first leg. (*B*) Basitarsal segments of the legs of wild-type and mutant adults. Note the presence of sex comb teeth on the first leg, but not the second and third legs of wild-type adults. The *Scr* gene is partially derepressed in the second and third leg discs, in which it is normally silent, in individuals heterozygous for mutations in PcG genes leading to the appearance of ectopic sex comb teeth on the second and third legs. These phenotypes are suppressed by mutations in *brm* and many other trxG genes. (*A*, Reprinted, with permission, from Tamkun et al. 1992, © Elsevier; *B*, portion modified, with permission, from Kennison 2003, © Elsevier.)

Drosophila males use their sex combs to grasp the females' abdomen and genitalia and to spread their wings prior to copulation.

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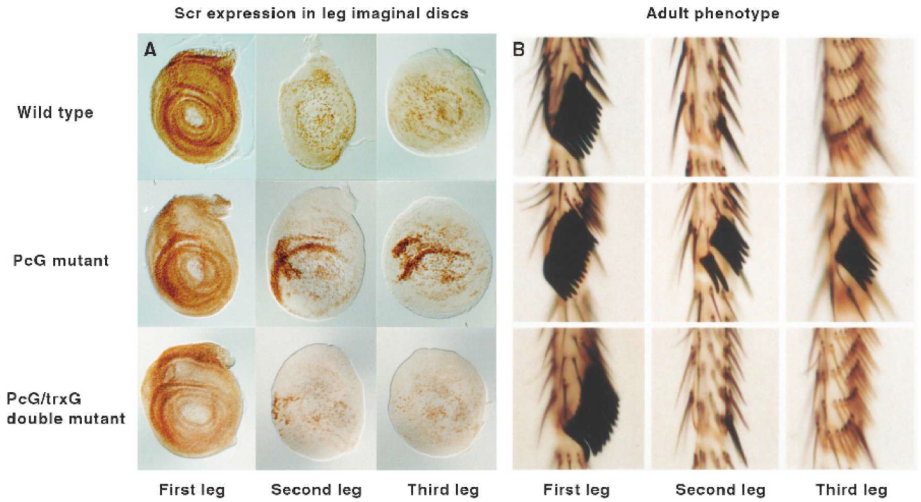

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	SNR1	hSNF5/INI1	Snf5, Sfh1	Yes (5–10)
	Kismet (KIS)	CHD7	–	NK
Histone methyltransferases	Trithorax (TRX)	MLL1, MLL2, MLL3	Set1	Yes (5–20)
	Absent, small or homeotic 1 (ASH1)	MlLL4, hASH1	hSET1 –	NK
Mediator subunits	Kohtalo (KTO)	TRAP230	Srb8	Yes (13–24)
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Cohesin subunit	Verthandi (VTD)	Rad21	Scc1/Rad21	Yes (>3)
Transcription factor	Trithorax-like (TRL)	BTBD14B	–	No
Growth factor receptor	Breathless (BTL)	FGFR3	–	NK
Other	Sallimus (SLS)	Titin		NK

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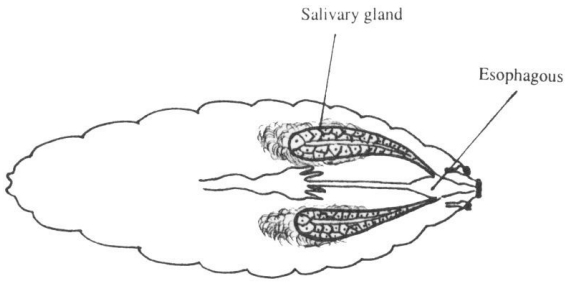
TRITHORAX GROUP GENES TrxG

Selected *D. melanogaster* TrxG proteins	Mammalian homologues	Biochemical role(s)	
Trithorax	Myeloid/lymphoid or mixed-lineage leukaemia proteins 1–3	SET domain subunit of a methyltransferase for H3K4	**CHROMATIN MODIFCATION H3K4 METHYLATION**
Absent small and homeotic disks protein 1 (ASH1)	ASH1-like	SET domain subunit of a methyltransferase for H3K4 and/or H3K36	**CHROMATIN MODIFCATION H3K4 – H3K36 METHYLATION**
Brahma (BRM)	BRM (also known as SMARCA2) and BRG1 (also known as SMARCA4)	ATPase subunit of a SWI/SNF-type nucleosome remodelling complex	**CHROMATIN REMODELLING**
Kismet	Chromodomain helicase DNA-binding protein 7	ATPase subunit of a presumed chromodomain-type nucleosome remodelling complex, which functions in transcription elongation	**CHROMATIN REMODELLING**
Nejire (also known as CBP)	?	Subunit of the TAC1 complex, which mediates histone acetylation	**CHROMATIN MODIFCATION**

Simon and Kingston, 2009

TRITHORAX GROUP GENES ARE LOCALIZED TO DEFINED POSTIONS IN THE GENOME



Un cromosoma politenico è un cromosoma gigante. I cromosomi politenici si formano in seguito a vari cicli di replicazione che producono molte copie (anche centinaia) di cromatidi fratelli che rimangono uniti.La formazione dei cromosomi politenici ha la funzione di aumentare il volume cellulare ma può anche comportare un vantaggio metabolico dato che l'elevato numero di copie di geni permette un alto livello di espressione genica. In Drosophila melanogaster, per esempio, i cromosomi delle ghiandole salivari delle larve subiscono numerosi cicli di endoreplicazione, e questo consente di produrre grandi quantità di secreto prima dell'impupamento.

BRM TRX

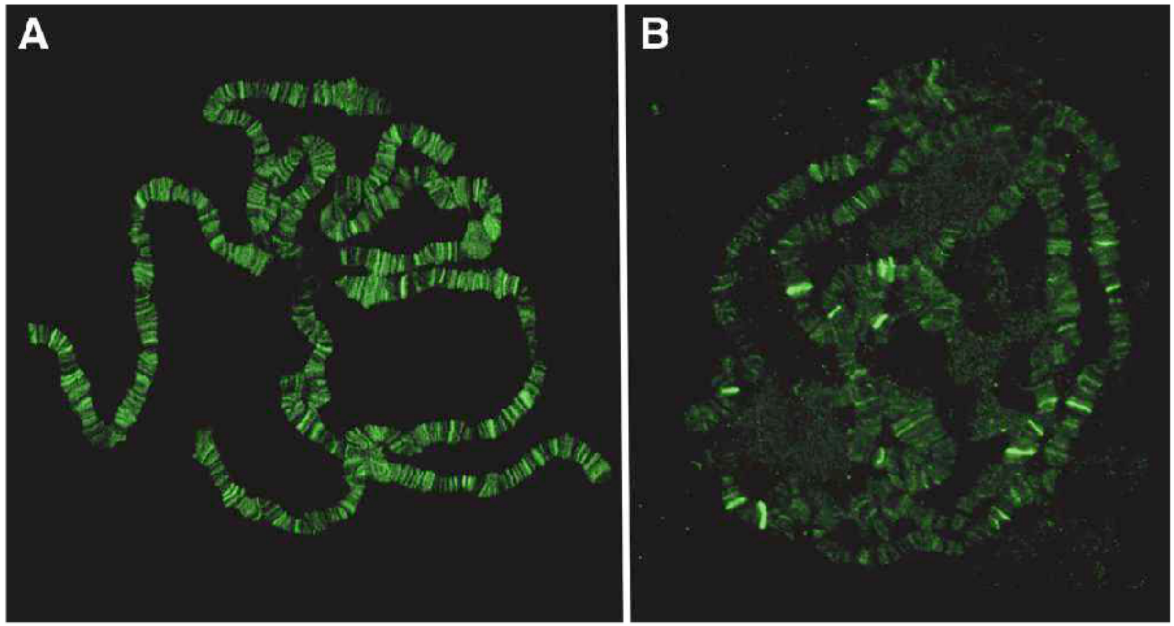


Figure 6. Chromosomal distribution of trxG proteins. The genome-wide distribution of trxG proteins was examined by staining *Drosophila* salivary gland polytene chromosomes with antibodies against BRM (*A*) or TRX (*B*). Consistent with a relatively global role in transcriptional activation, BRM is associated with hundreds of sites in a pattern that overlaps extensively with RNA Pol II. In contrast, strong TRX signals are detected at a much smaller number of sites on polytene chromosomes.

1. TRITHORAX GROUP GENES COVALENTLY MODIFY HISTONES

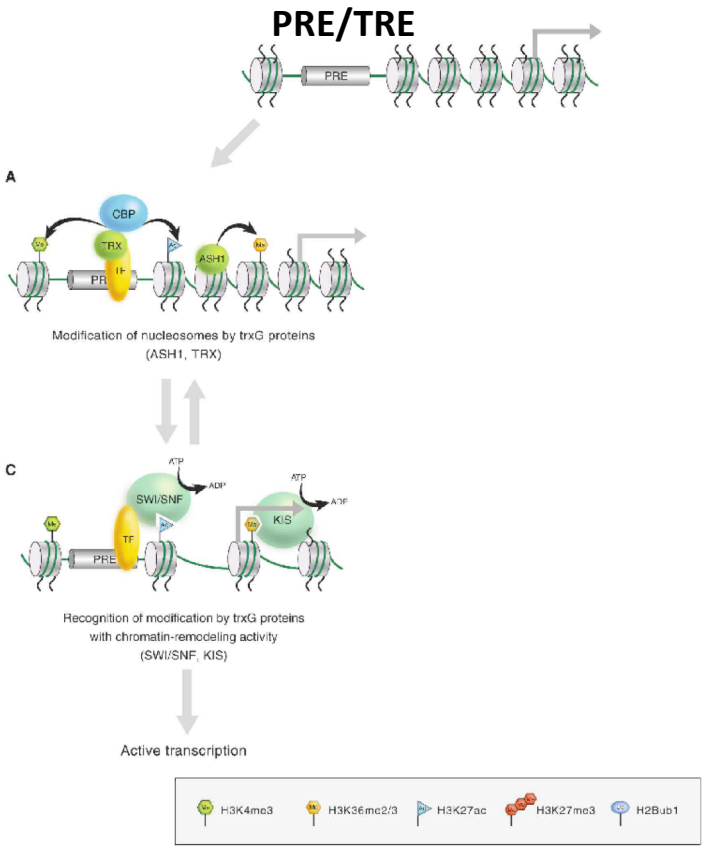


Figure 7. trxG and PcG functions and interactions. Both trxG and PcG families include proteins that covalently modify histones and those that noncovalently modify chromatin. Covalent modifications on histones can promote or block the binding or activity of trxG complexes (e.g., SWI/SNF and KIS), PcG complexes (e.g., PRC1 and PRC2), or other factors involved in the maintenance of active or repressed states. Binding by these latter complexes has the potential to lead to further covalent modification, thus leading to iterative cycles of covalent modification and recognition of the covalent marks.

Fly **TRX** has 6 HKMTs homologs in humans:

- hSET1A
- hSET1B

COMPASS complex components: H3K4 methylation and promotion of shift from transcriptional initiation to elongation; in general H3K4me associates with active transcription

- MLL1
- MLL2
- MLL3
- MLL4

H3K4me associates with active transcription

Fly **ASH1** has 1 HKMT homolog in humans:

- hASH1

H3K4me and H3K36 methylation → activation of transcription

REMEMBER THE LINK BETWEEN H3K4me and DNA methylation

2. A LINK BETWEEN TrxG PROTEINS AND HISTONE ACETYLATION

Fly:

-

TRX is associated with dCBP: a histone lysine acetyl transferase (dCBP is not a TrxG gene) (humans: CBP/p300 HAT complex!!!). Recruitment of TrxG proteins by transcrition factor brings HAT and leads to histone acetylation

- TrxG protein kismet (kis) contains a helicase domain is recruited after ASH1 activity → chromatin remodeling

→ *H3K4 methylation, H3K36 methylation*
→ *H3 and H4 acetylation*
→ *Reduced H3K9 methylation*
→ *Reduced H3K27 methylation*
→ *Reduced DNA methyaltion (humans)*

EPIGENETIC ACTIVATION OF GENE EXPRESSION

3. TRITHORAX GROUP PROTEINS ARE CENTRAL CHROMATIN REMODELERS

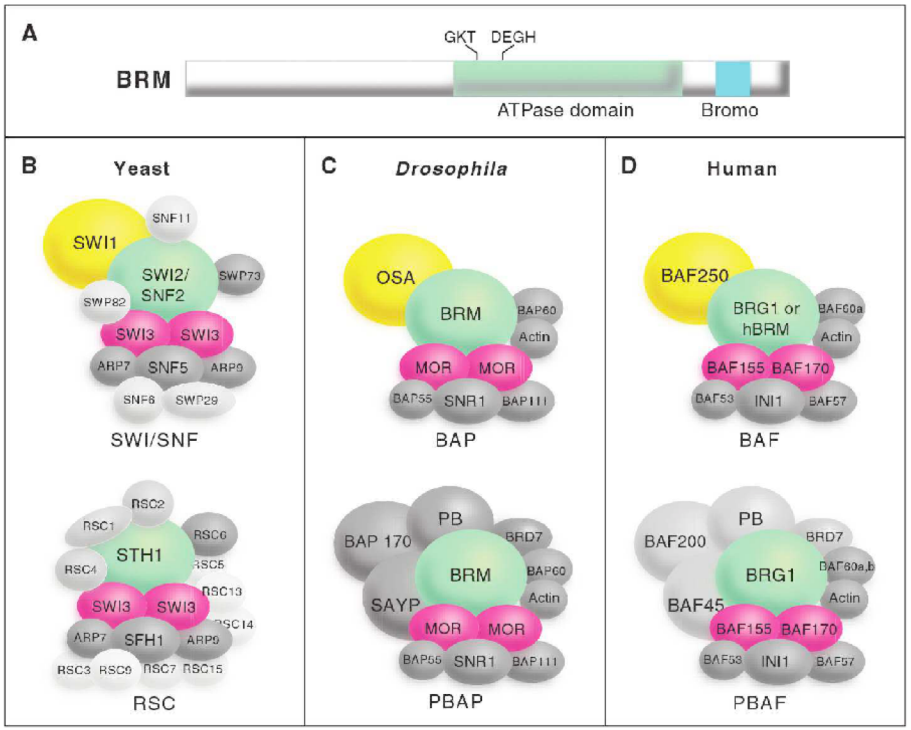


Figure 5. The SWI/SNF family of remodeling complexes. Each complex contains a member of SNF2/SWI2 family of ATPases and at least eight other subunits. (*A*) Schematic diagram of the BRM protein showing the location of the ATPase domain and carboxy-terminal bromodomain (which shows affinity to acetylated lysine residues in histone tails), which are conserved in all SNF2/SWI2 family members. SWI/SNF complexes in yeast (*B*), *Drosophila* (*C*), and humans (*D*) are shown. *Drosophila* trxG proteins (BRM, MOR, and OSA) and their counterparts in other organisms are shown in color. Further information about these complexes and their subunits may be found in Mohrmann and Verrijzer (2005).

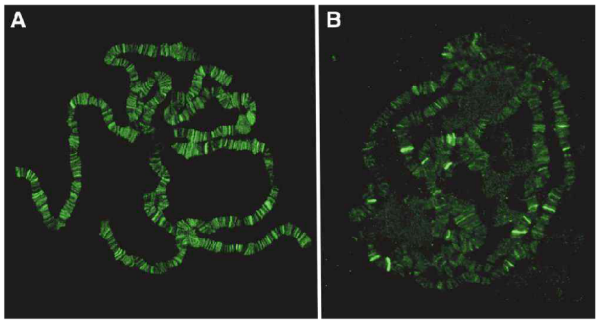
Trithorax group proteins BRM (fly) BRG1 (human) belong to the SWI/SNF family of chromatin remodelers.

SWI/SNF family chromatin remodeling complexes contain up to 18 subunits and can shift nucleosomes to increase the accessibility of regulatory sequences

Chromatin remodelers are recruited by transcription factors but are also recruited during transcriptional elongation.

→ Chromatin remodeling is essential during all phases of transcription

→ #25000 complexes in a human cell: highly important for gene expression control



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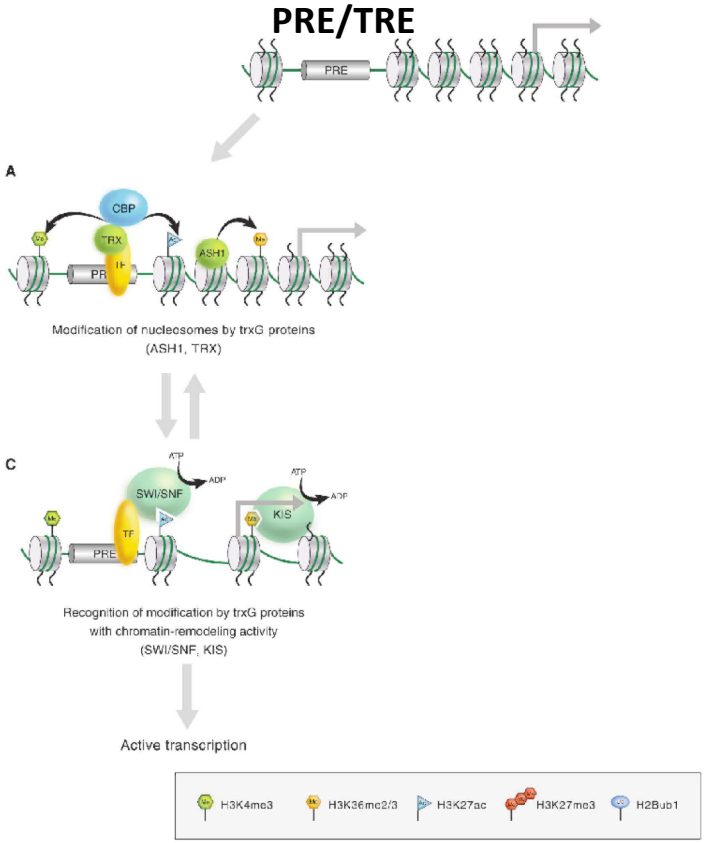

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Kismet (fly) CHD7 (human) has affinity for H3K36methylated chromatin

→ Chromatin remodelling complex (multiprotein)

→ Associated with RNA polymerase

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CHARGE SYNDROME IS CAUSED BY MUTATION IN HUMAN kis (CHD7)

CHARGE syndrome (formerly known as CHARGE association), is a syndrome caused by a genetic disorder. It was first described in 1979.In 1981, the term "CHARGE" came into use as an acronym for the set of unusual congenital features seen in a number of newborn children.
The letters stand for: coloboma of the eye, heart defects, atresia of the nasal choanae, retardation of growth and/or development, genital and/or urinary abnormalities, and ear abnormalities and deafness.
These features are no longer used in making a diagnosis of CHARGE syndrome, but the name remains. CHARGE syndrome is the leading cause of congenital deafblindness.

CHARGE syndrome was formerly referred to as CHARGE association, which indicates a non-random pattern of congenital anomalies that occurs together more frequently than one would expect on the basis of chance. Very few people with CHARGE will have 100% of its known features. In 2004, mutations on the CHD7 gene (located on Chromosome 8) were found in 10 of 17 patients in a study conducted in the Netherlands, making CHARGE an official syndrome. A further study in the US of 110 individuals with CHARGE syndrome showed that 60% of those tested had a mutation of the CHD7 gene. The trithorax group member CHD7 is parto of the chromodomain helicase DNA-binding (CHD) protein family that plays a role in transcription regulation by chromatin remodeling.

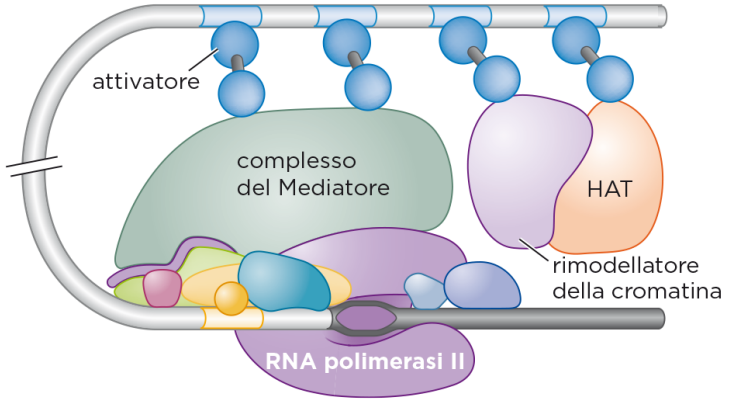
1/10000 births
500 different mutations in CDH7 identified

Major Features of CHARGE Syndrome (very common in CHARGE and relatively rare in other conditions)

FEATURE	INCLUDES	FREQUENCY
Coloboma of the eye	Coloboma (sort of like a cleft) of the iris, retina, choroid, macula or disc (not the eyelid); microphthalmos (small eye) or anophthalmos (missing eye): CAUSES VISION LOSS Pictures	80%-90%
Choanal atresia or stenosis	The choanae are the passages that go from the back of the nose to the throat. They can be narrow (stenosis) or blocked (atresia). It can be unilateral (one-sided) or bilateral (both sides), bony or membranous. **Unilateral atresia or stenosis can be difficult to diagnose** Pictures	50%-60%
Cranial nerve abnormality	I - Missing or decreased sense of smell	90-100%
	IX/X - Swallowing difficulties, aspiration - Pictures	70%-90%
	VII - Facial palsy (one side or both) - Pictures	40%
CHARGE outer ear	Short, wide ear with little or no lobe, "snipped off" helix (outer fold), prominent antihelix (inner fold) which is discontinuous with tragus, triangular concha, decreased cartilage (floppy), often stick out, usually asymmetric - Pictures	>50%
CHARGE middle ear	Malformed bones of the middle ear (ossicles): CAUSES CONDUCTIVE HEARING LOSS	Common
CHARGE inner ear	Malformed cochlea (Mondini defect); small or absent semicircular canals: CAUSE HEARING LOSS AND BALANCE PROBLEMS - Pictures	90%

4. TRITHORAX GROUP PROTEINS ARE ASSOCIATED WITH THE TRANSCRIPTIONAL COMPLEX

- The mediator complex is a large protein complex (<20 proteins) that communicates between the basal transcription factors and activating regulatoy elements. TRP230, TRAP240
- Essential for the initiation of transcription!!



5. TRITHORAX GROUP PROTEINS ARE LINKED WITH LONG-RANGE CHROMATIN INTERACTION

Rad21 is member of trithorax group genes!
Has a role in long range chromatin interaction
Links enhancers with promoters via loop formation

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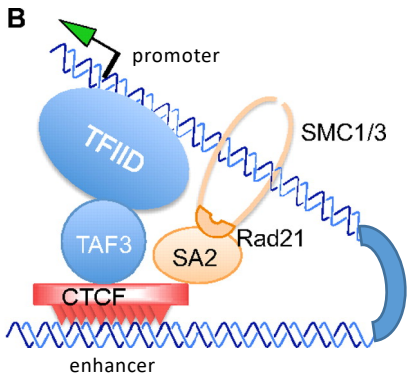

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Trithorax group proteins are recruited by TRE – Trithorax reponse elements

DROSOPHILA

TRE elements are concentrated DNA binding sites for Trithorax group proteins
→Recruitment function

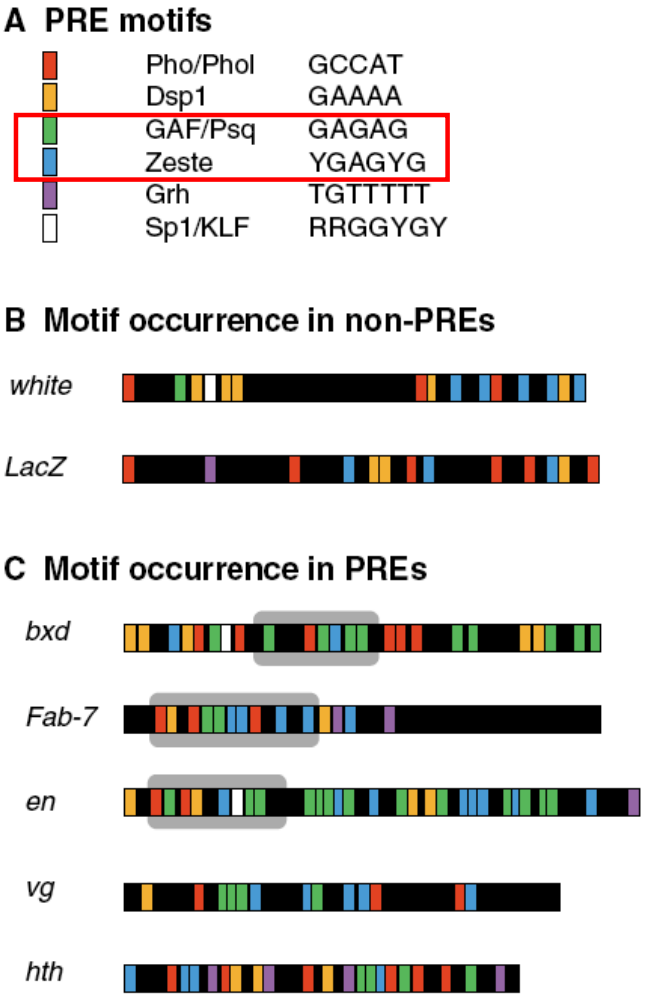
NOTE: MOST TRE elements overlap with PRE elements

For example: GAF/Psq and Zeste have an important role in Trithorax recruitment
And gene activation.
However, sites are located in PREs
→ Cell type specific transcription program (type and dosage of expressed transcription factors co-ordinate balance of Polycomb/Trithorax function

Development 134, 223-232 (2007) doi:10.1242/dev.02723

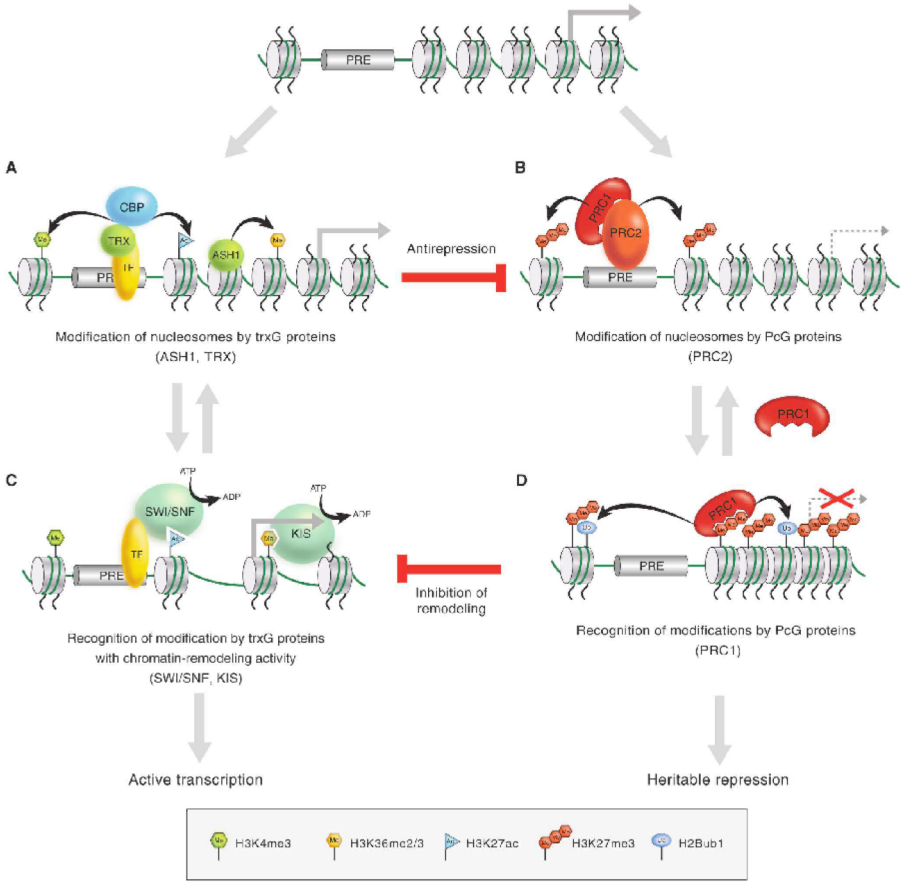
Polycomb/Trithorax response elements and epigenetic memory of cell identity

Leonie Ringrose[1] and Renato Paro[2]



PRE/TRE motifs and flexibility of PRE/TRE design. (A) DNA motifs shown to be important for PRE/TRE function. The Grh (Grainy head) protein binds to several different PRE/TRE sites. The motif shown is that found in PRE/TREs by Blastyak et al. (Blastyak et al., 2006). The Dsp1 protein also has broad DNA-binding specificity (Brickman et al., 1999). The motif shown is that used by Dejardin et al. (Dejardin et al., 2005). Gaf binds the same target sequence as Pipsqueak (Psq), suggesting that the two proteins may compete or cooperate at closely spaced sites. (B) Many of these motifs are important for regulating genes that do not have PRE/TREs, for example the Drosophila white gene which is regulated by the Zeste protein (600 bp of upstream regulatory region are shown). These motifs are also short and occur randomly in DNA, such as in the bacterial LacZ gene (the first 600 bp of the coding sequence are shown). (C) PRE/TREs have different combinations of motifs, with no preferred order or number. Shown here are ~600 bp of the bxd and Fab-7 PREs from the Drosophila Bithorax complex, and of PRE/TREs from the Drosophila engrailed (en), vestigial (vg) and homothorax (hth) loci. **Grey boxes show minimal PRE/TREs where these have been defined (Dejardin et al., 2005; Brown et al., 2005).** Flanking sequences contain additional motif clusters which may contribute to the function of these PRE/TREs in their endogenous context.

COMPETEING FUNCTION OF POLYCOMB AND TRITHORAX GROUP PROTEINS AT PRE/TREs



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→ **ASH1 mediates H3K4me3 and H3K36me3**

→ **H3K36 methylation enhances transcriptional elongation**

→ **TRX/CBP complex mediates H3K4methyaltion and H3K27acetylation**

→ **H3K27acetylation prevents H3K27methyaltion by E(z)**

→ **H3K4me3 inhibits the recruitment of PRC2**

→ **H3K36methylation inhibits the activity of PRC2**

FUNCTIONAL ANTAGONISM + PREVENTION OF SPREADING OF PcG and TrxG TYPE OF CHROMATIN

REMEMBER: TRANSCRIPTION FACTORS CONTROL THE EQUILIBRIUM BETWEEN PcG and TxG

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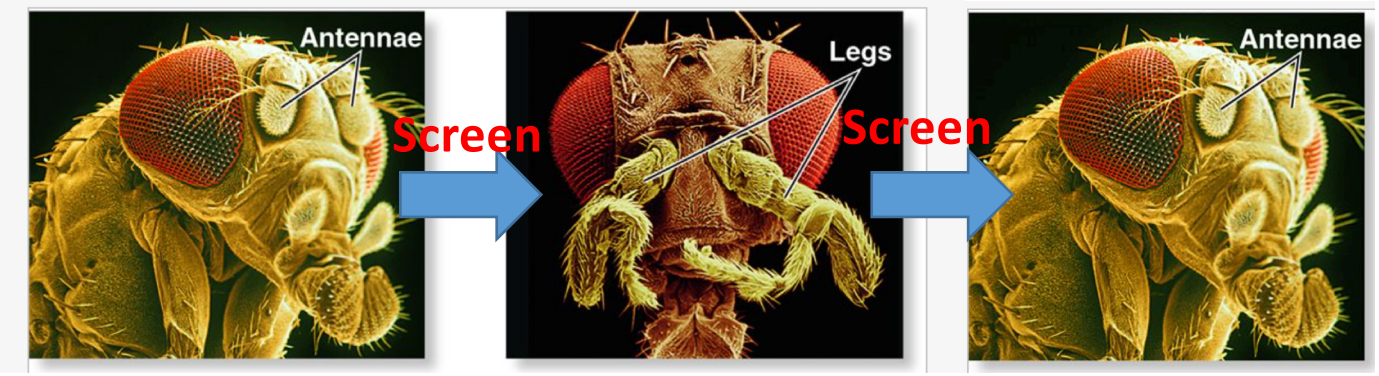

WILD-TYPE

PRC2 mutation
In Su(z)12

(partial) rescue
of phenotype

Mutation screen

NO REPRESSIVE EFFECT FROM PRE → TrX group genes take their chance to activate gene expression → homeotic transformation

POLYCOMB GROUP GENES(PcG)

Supressor - Mutation screen

PcG mutated: no repression; TrX group gene mutation → no activation
rescue of homeotic transformation caused by PcG mutation

TRITHORAX GROUP GENES(TrxG)