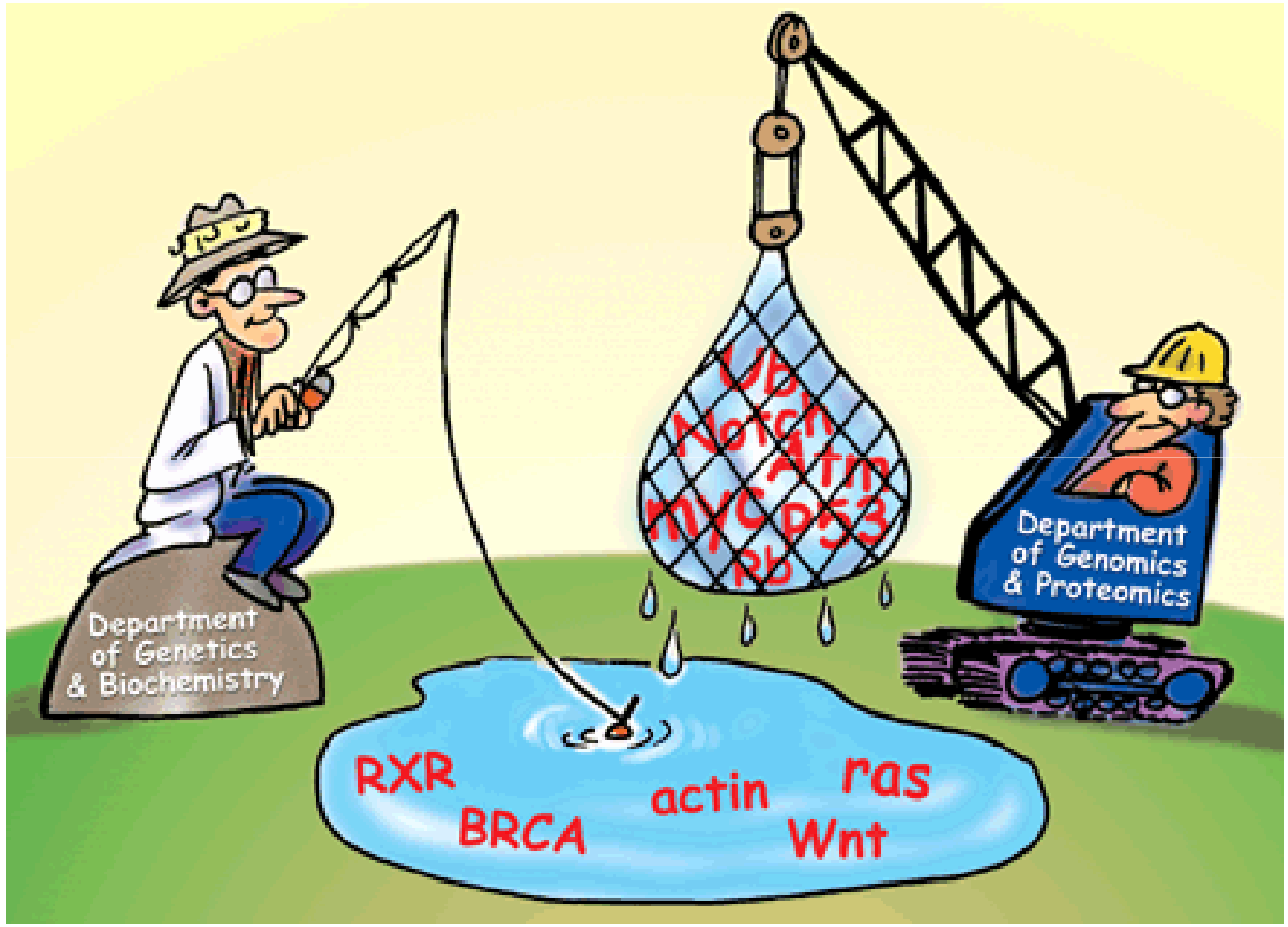
Genomica Funzionale

Genomica funzionale



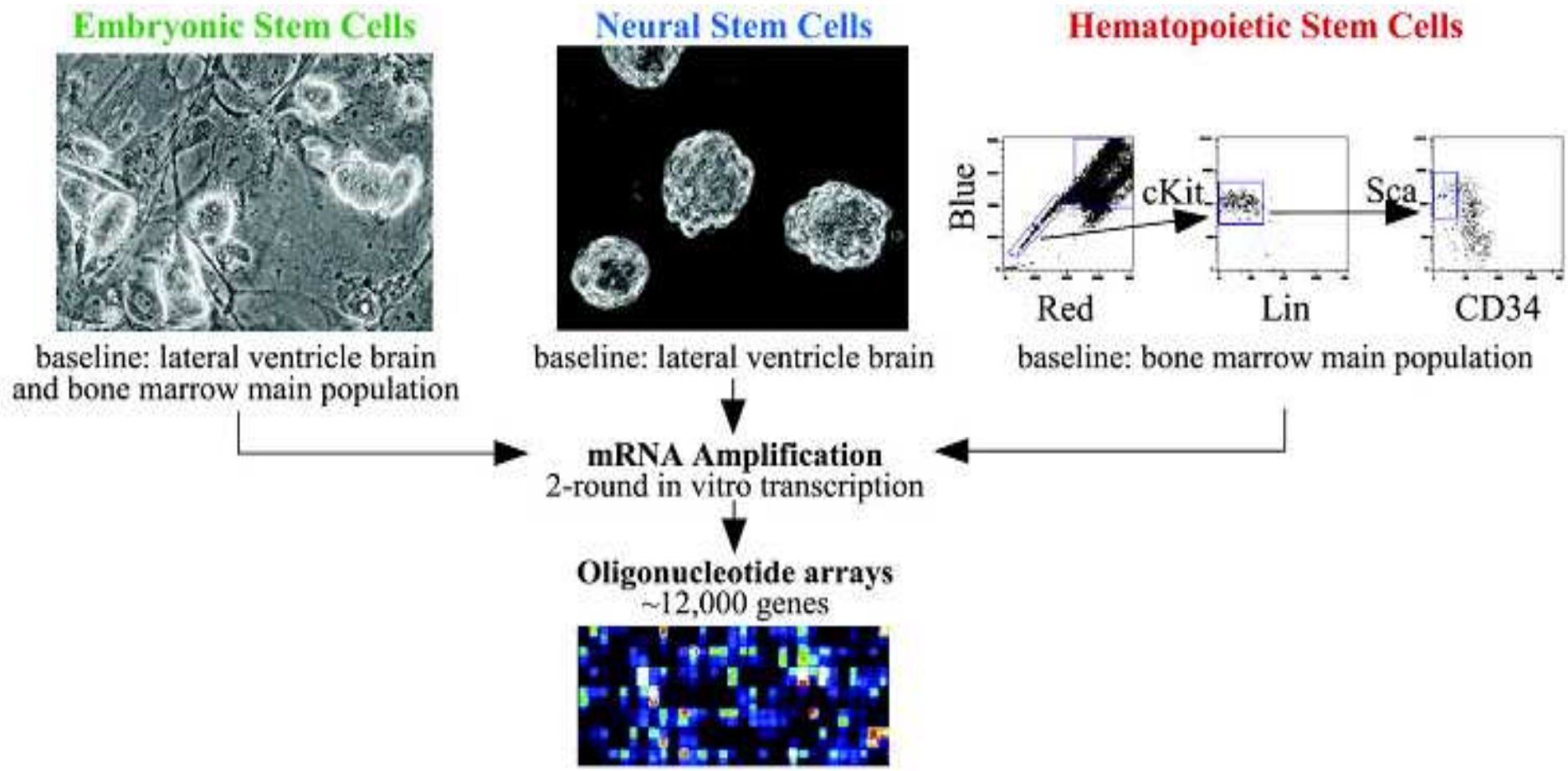
Functional Genomics

- Biochemical Genomics
- Biophysical Genomics
- Physiological Genomics
- Cell Genomics
- .....

Functional Genomics
Levels

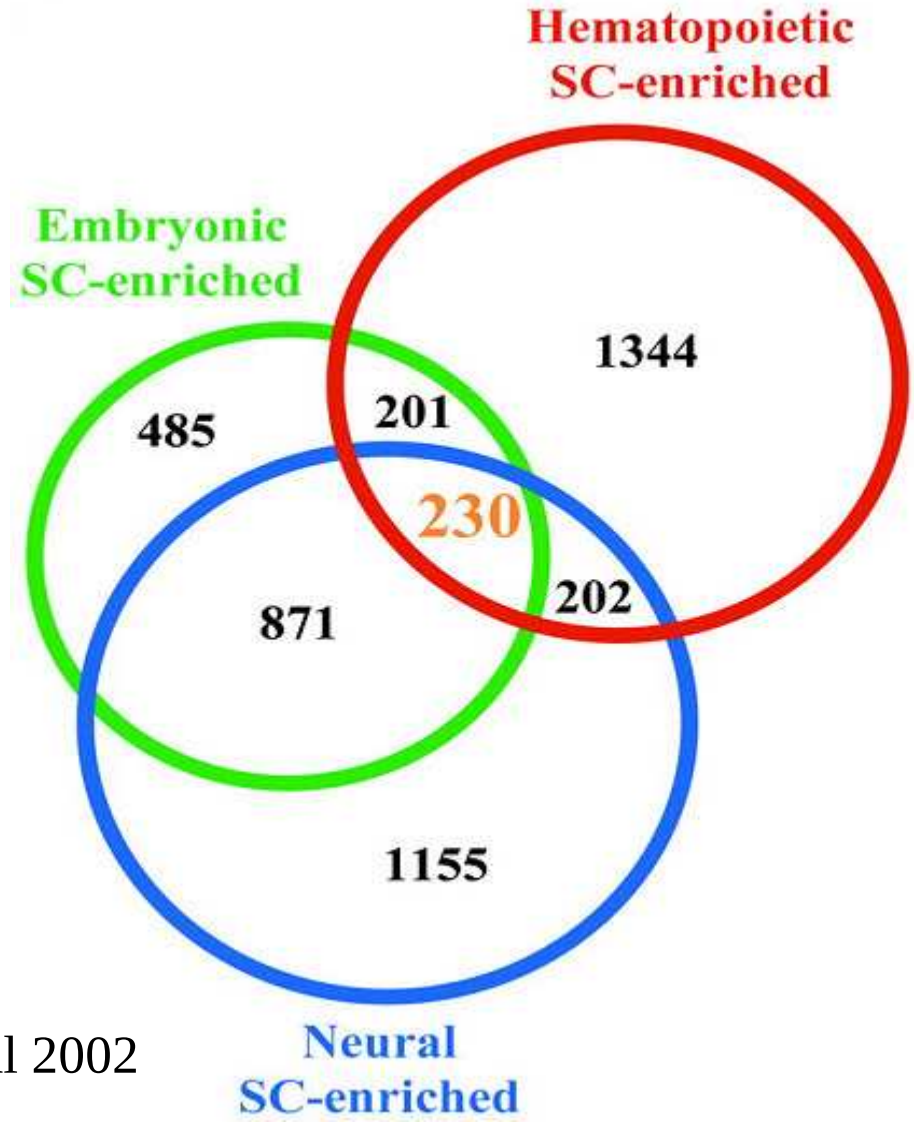
- Genome to transcriptome
- Transcriptome to proteome
- Proteome to dynamic system
- Dynamic systems to phenotype

Transcriptional Profiling of Embryonic and Adult Stem Cells



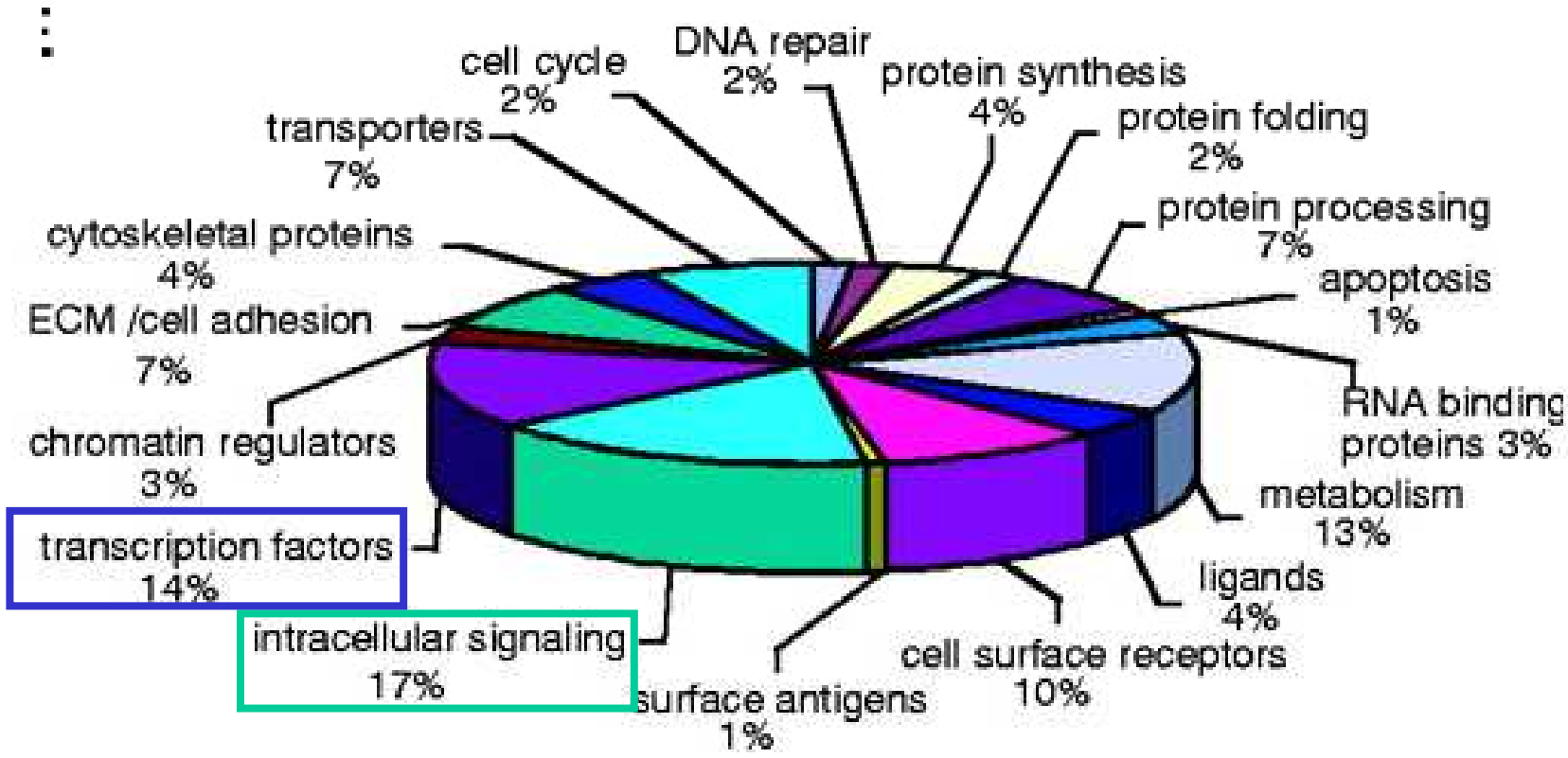
Ramalho-Santos et al 2002

Transcriptional Profiling of Embryonic and Adult Stem Cells



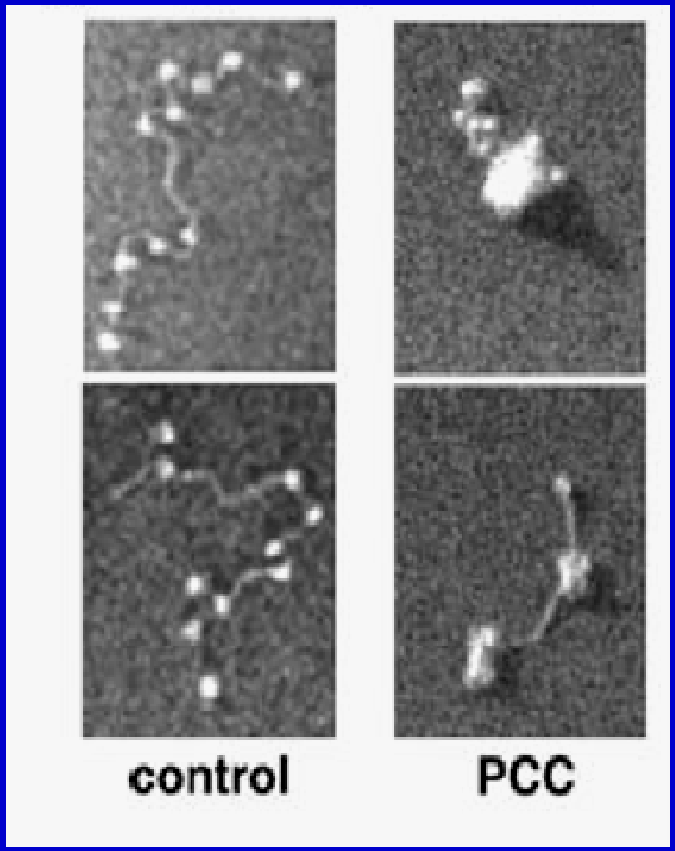
Ramalho-Santos et al 2002

A Stem Cell Molecular Signature



Ivanova et al 2002

What does Polycomb do to chromatin ?
Chromatin Condensation



Recombinant PC-containing complexes can condense nucleosomes in vitro

: Francis et al. (2004), Science 306, 1574

Polycomb complexes repress developmental regulators in murine embryonic stem cells

Laurie et al Nature 2006

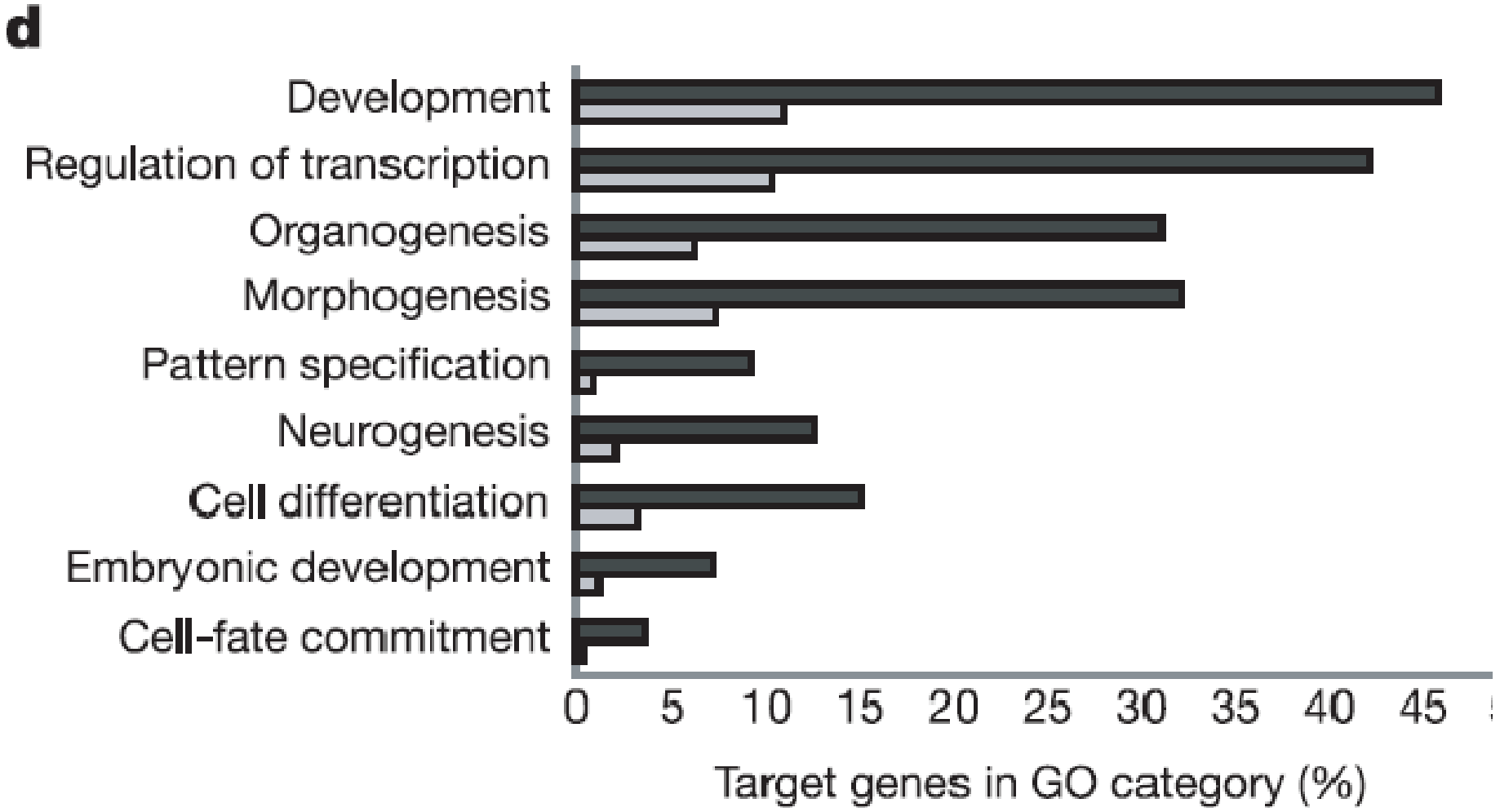
Polycomb repressive complexes PRC1 and PRC2
co-occupied 512 genes, many of which encode transcription factors with important roles in development.
All of the cooccupied genes contained modified nucleosomes (trimethylated Lys 27 on histone H3).

Polycomb

- PcG are required for the maintenance of many gene expression patterns
- These maintenance proteins form heteromultimeric complexes that bind to chromatin and alter its structure.
- PcG complexes lead to compact, transcriptionally inactive chromatin
- Several PcG complexes have been purified so far: the Polycomb Repressive Complex 1 (PRC1), the Polycomb Repressive Complex 2 (PRC2)

They are extremely large complexes that contain several proteins including chromatin modifying enzymes such as histone methyl-transferases, acetyl-transferases or deacetylases

PRC1 and PRC2 colocalize at genes encoding developmental regulators.



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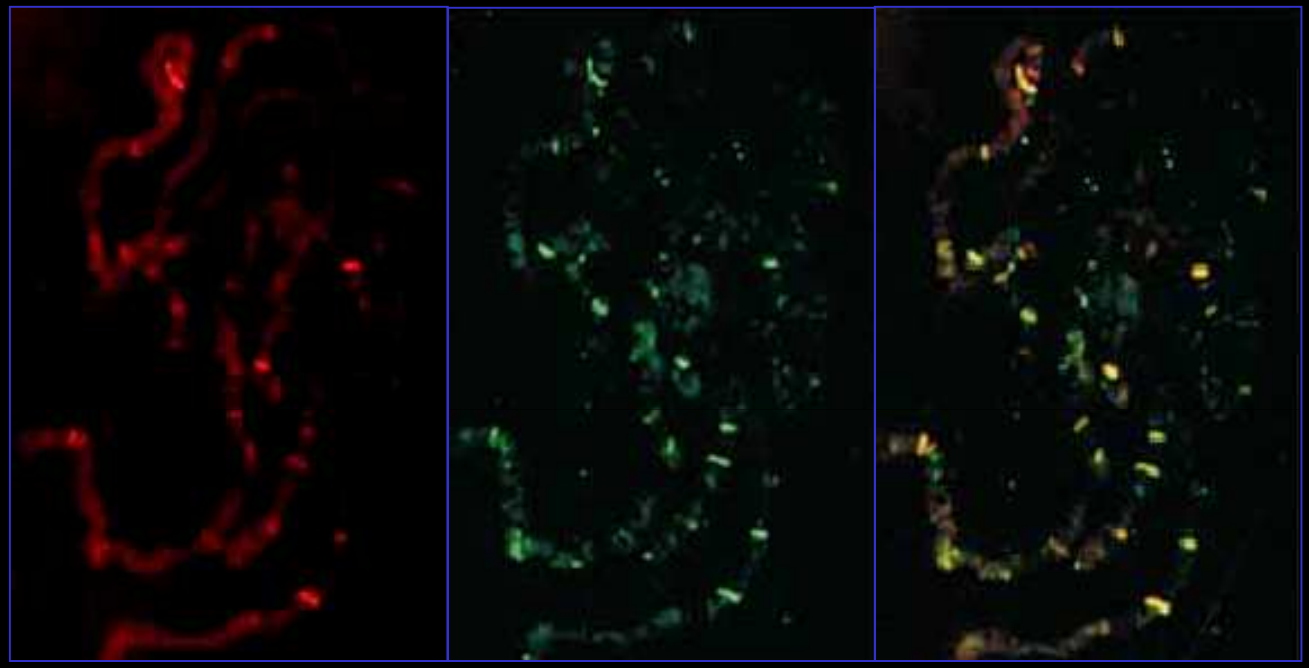
Histone H3 K27 methylation and Polycomb

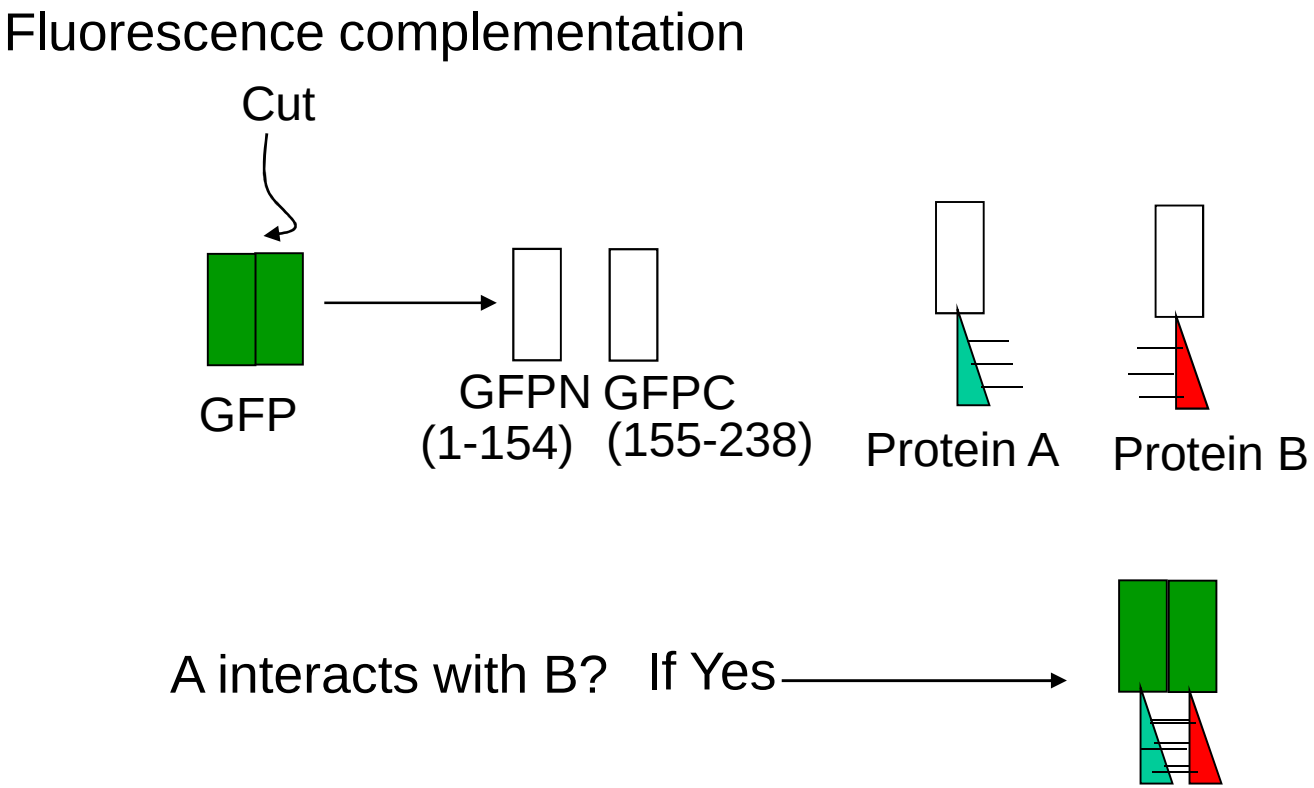


Pc H3K27me3 Merge

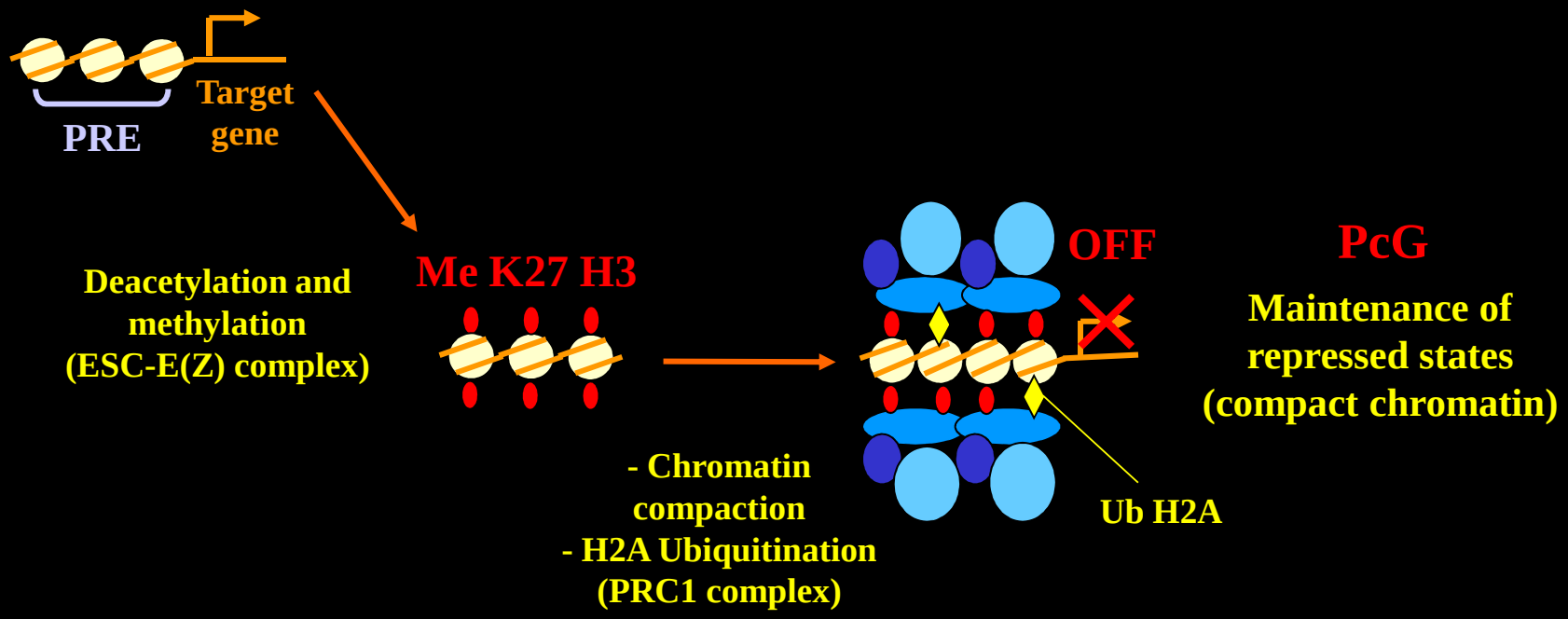
Data from:
Ringrose et al. (2004) Mol. Cell 16, 641

There is a strong correlation between trimethylation of K27 (and K9) trimethylation and Polycomb recruitment at target loci.

Biomolecular luminescence/fluorescence complementation BIC



Action of PcG and trxG complexes on chromatin



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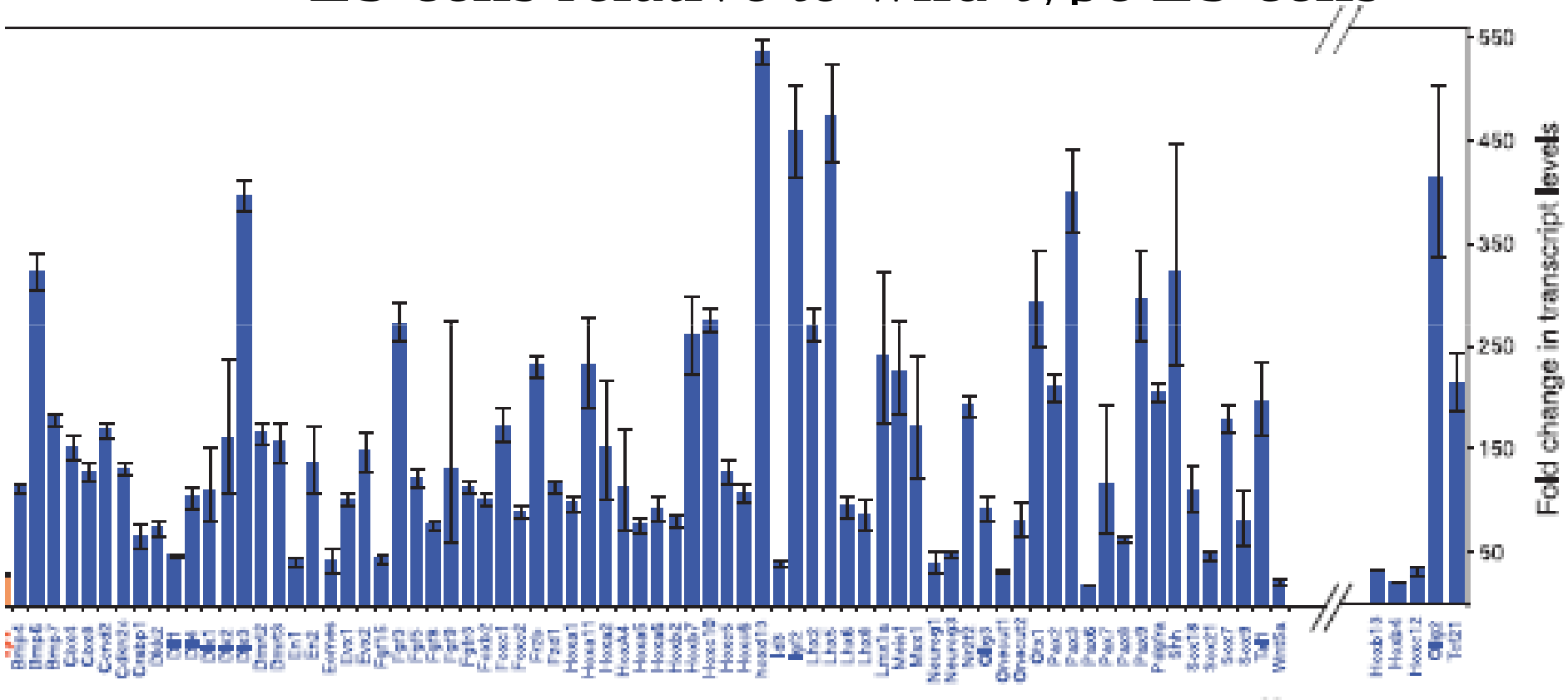
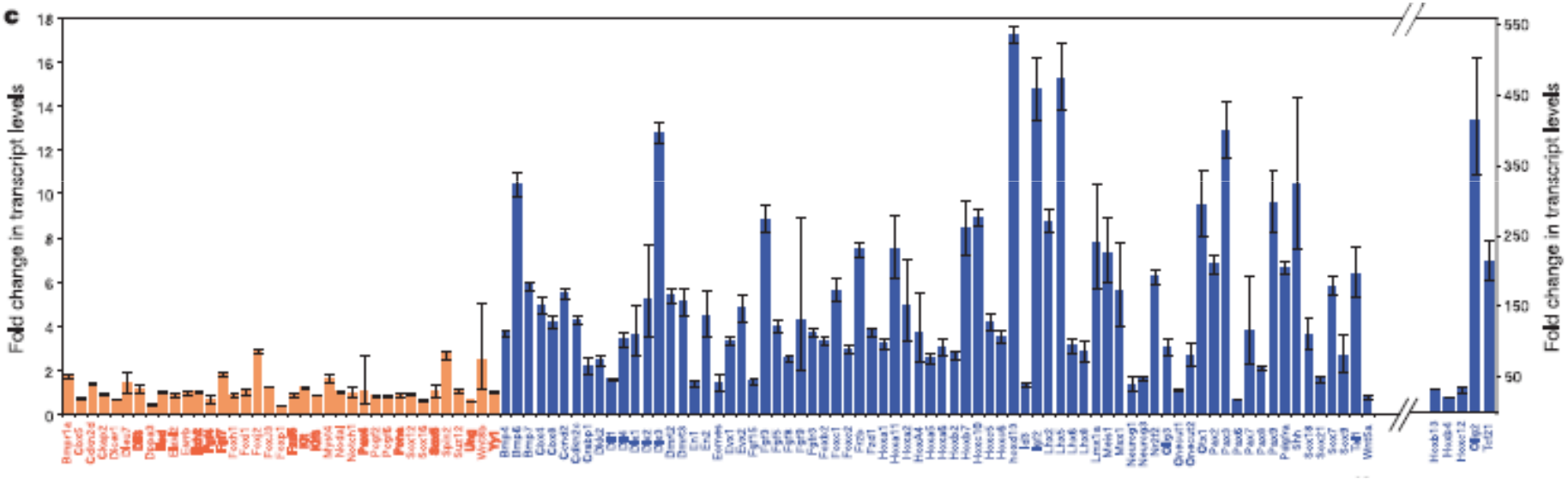
Polycomb repressive complexes PRC1 and PRC2
co-occupied 512 genes, many of which encode transcription
factors with important roles in development.
All of the cooccupied genes contained modified nucleosomes
(trimethylated Lys 27 on histone H3).
Consistent with a causal role in gene silencing in ES cells,
PcG target genes were de-repressed in cells
deficient for the PRC2 component Eed, and were preferentially
activated on induction of differentiation.
Our results indicate that dynamic repression of developmental pathways
by Polycomb
complexes may be required for maintaining ES cell pluripotency
and plasticity during embryonic development.

Quantification of transcript levels in Eed mutant ES cells relative to wild-type ES cells



Quantification of transcript levels in Eed mutant ES cells relative to wild-type ES cells

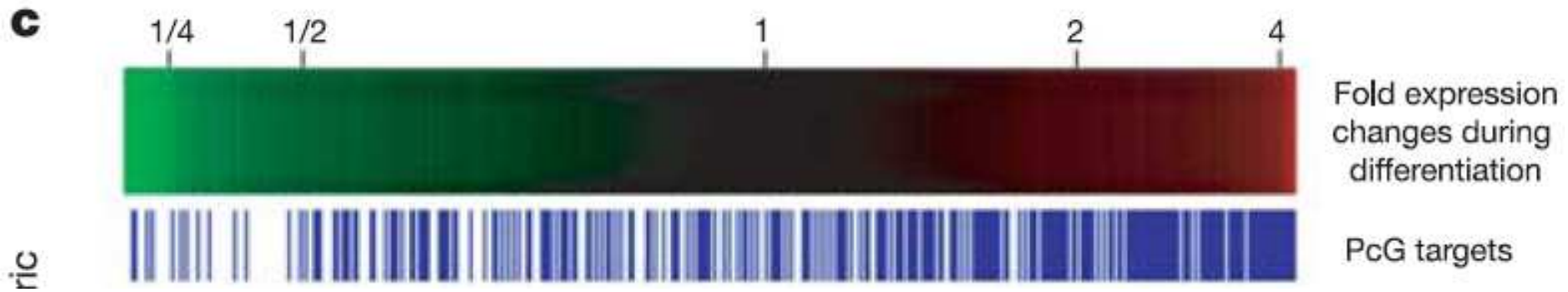


PcG and TrxG

- PcG and TrxG proteins are required for the maintenance of many gene expression patterns [5]. These maintenance proteins form heteromultimeric complexes that bind to chromatin and alter its structure.
- Current models propose that PcG complexes lead to compact, transcriptionally inactive chromatin, whereas TrxG complexes maintain chromatin in an open conformation that facilitates transcription.
- In *Drosophila*, several PcG and TrxG complexes have been purified so far: the Polycomb Repressive Complex 1 (PRC1), the Polycomb Repressive Complex 2 (PRC2), the PhoRC complex, the Pcl-PRC2 complex, the Trithorax Activating Complex 1 (TAC1) and the Brahma Complex (BRM) also called SWI/SNF complex.
- They are extremely large complexes that contain several proteins including chromatin modifying enzymes such as histone methyl-transferases, acetyl-transferases or deacetylases

Polycomb

- Polycomb group (PcG) transcription regulatory proteins maintain cell identity by sustained repression of numerous genes. Differentiation of embryonic stem (ES) cells induces a genome-wide shift in PcG target gene expression. We investigated the effects of differentiation and protein interactions on CBX family PcG protein localization and dynamics using fluorescence imaging. In mouse ES cells, different CBX proteins exhibited distinct distributions and mobilities. Most CBX proteins were enriched in foci known as polycomb bodies. Focus formation did not affect CBX protein mobilities, and the foci dispersed during ES cell differentiation. The mobilities of CBX proteins increased upon induction of differentiation, and decreased as differentiation progressed. Deletion of the chromobox, which mediates interactions with RING1B, prevented the immobilization of CBX proteins.
- In contrast, deletion of the chromodomain, which can bind trimethylated lysine 27 of histone H3, had little effect on CBX protein dynamics. The distributions and mobilities of most CBX proteins corresponded to those of CBX-RING1B complexes detected using bimolecular fluorescence complementation (BiFC) analysis.
- Epigenetic reprogramming during ES cell differentiation is therefore associated with global changes in the subnuclear distributions and dynamics of CBX protein complexes.



expression profiles were compared between
undifferentiated ES cells and cells after 14 days of differentiation.

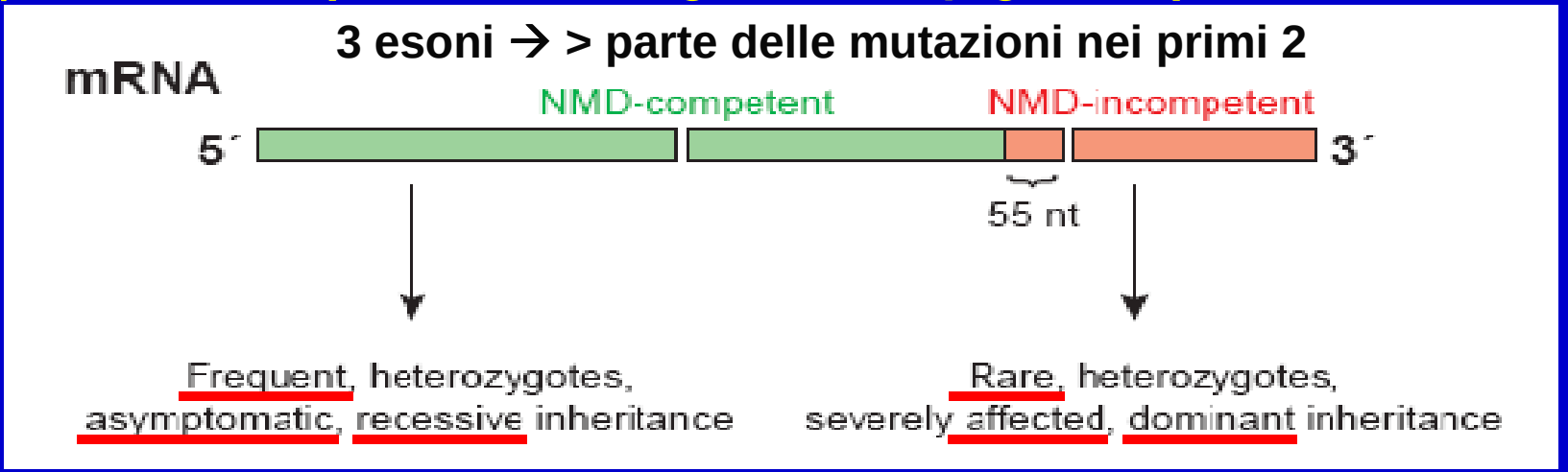
NMD e patologie

• Circa il 30% delle malattie ereditarie è causato da mutazioni nonsenso o frameshift che generano codoni nonsenso

• Il NMD PROTEGGE i portatori eterozigoti di un allele contenente un PTC → forma recessiva dovuta alla proteina Wt prodotta dall'allele normale

• Nel caso di oncosoppressori (es. BRCA1) → PROTEZIONE degli eterozigoti finché l'allele Wt rimane intatto → NO NMD = produzione di oncoproteine a carattere dominante negativo → sviluppo tumore

Es. **β-talassemia (mutazioni nel gene della β-globina)**:

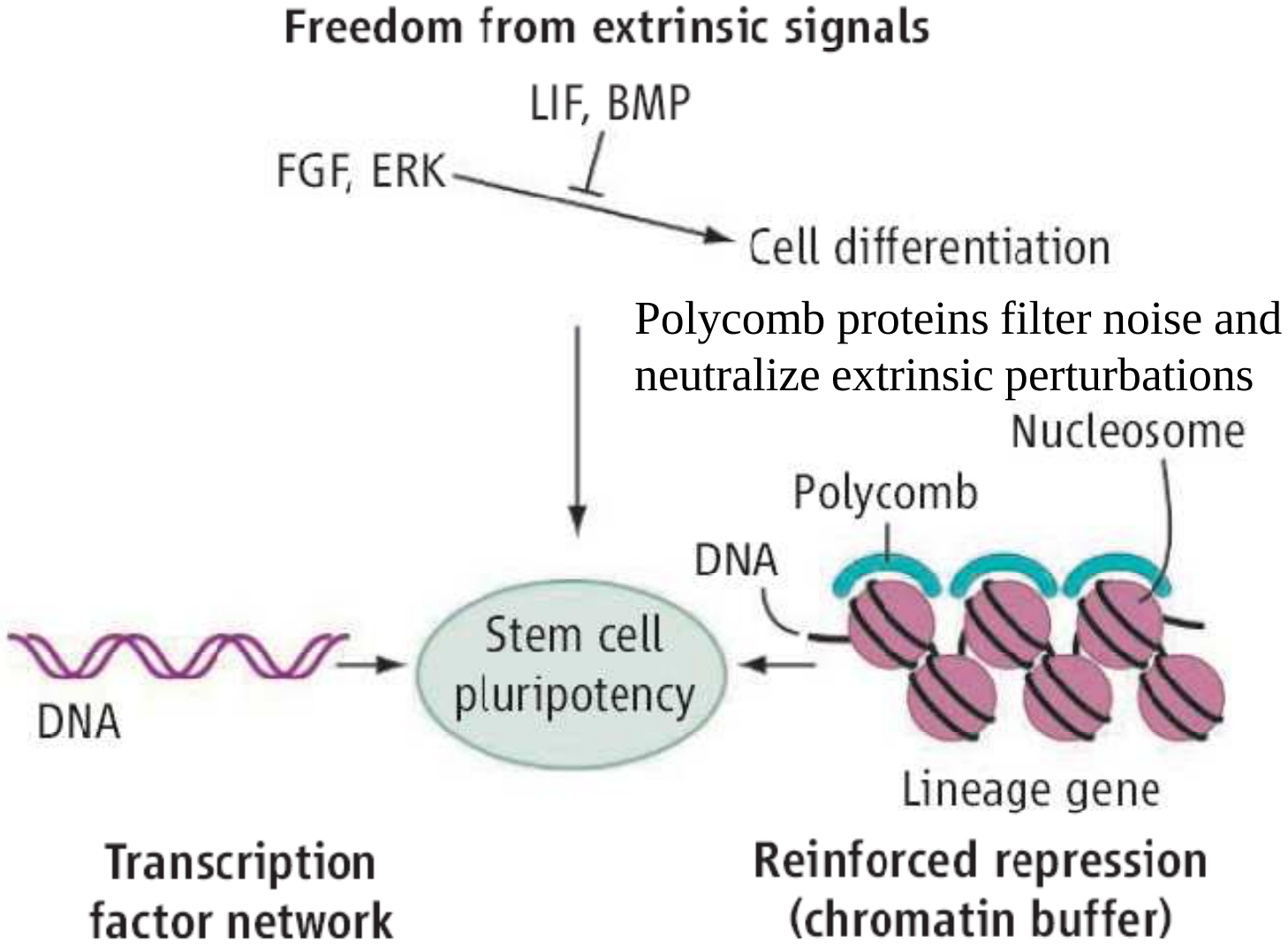


Mantenuti bassi i livelli del trascritto mutato

Livelli circa normali del trascritto
β-globina tronca → catene insolubili
Precipitazione e fenotipo clinico

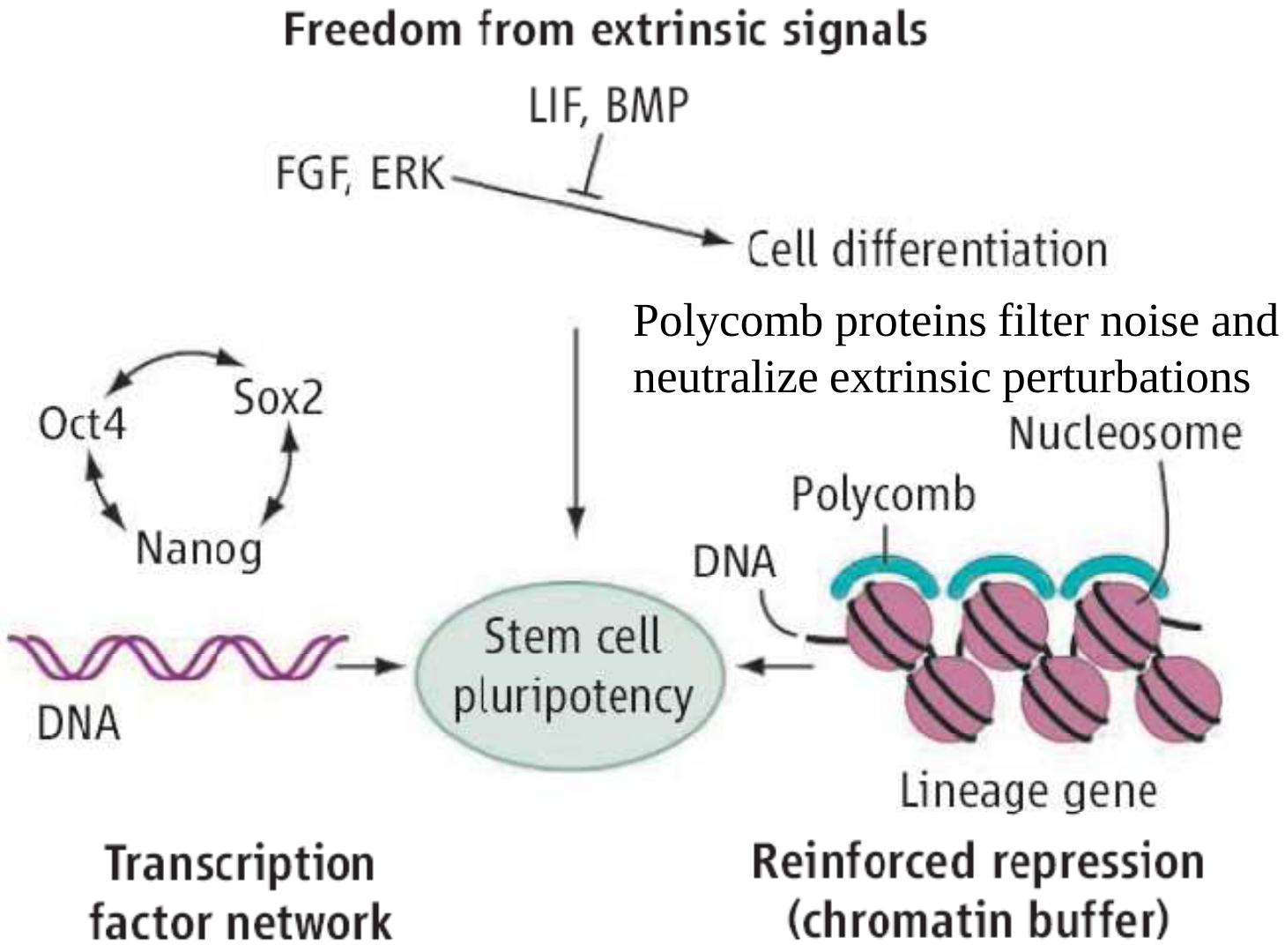
Recipe for pluripotency

Science 2009



Recipe for pluripotency

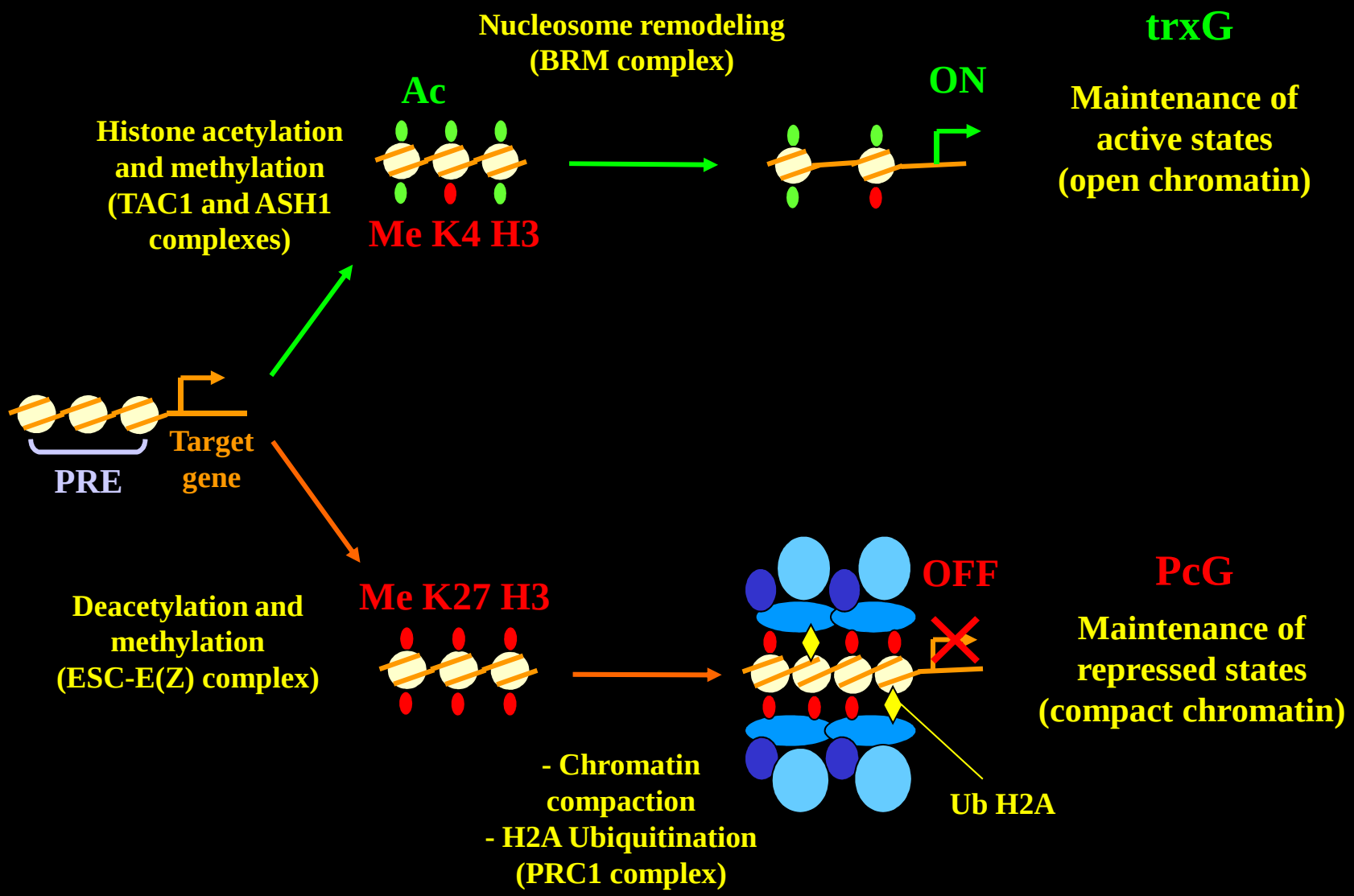
Science 2009

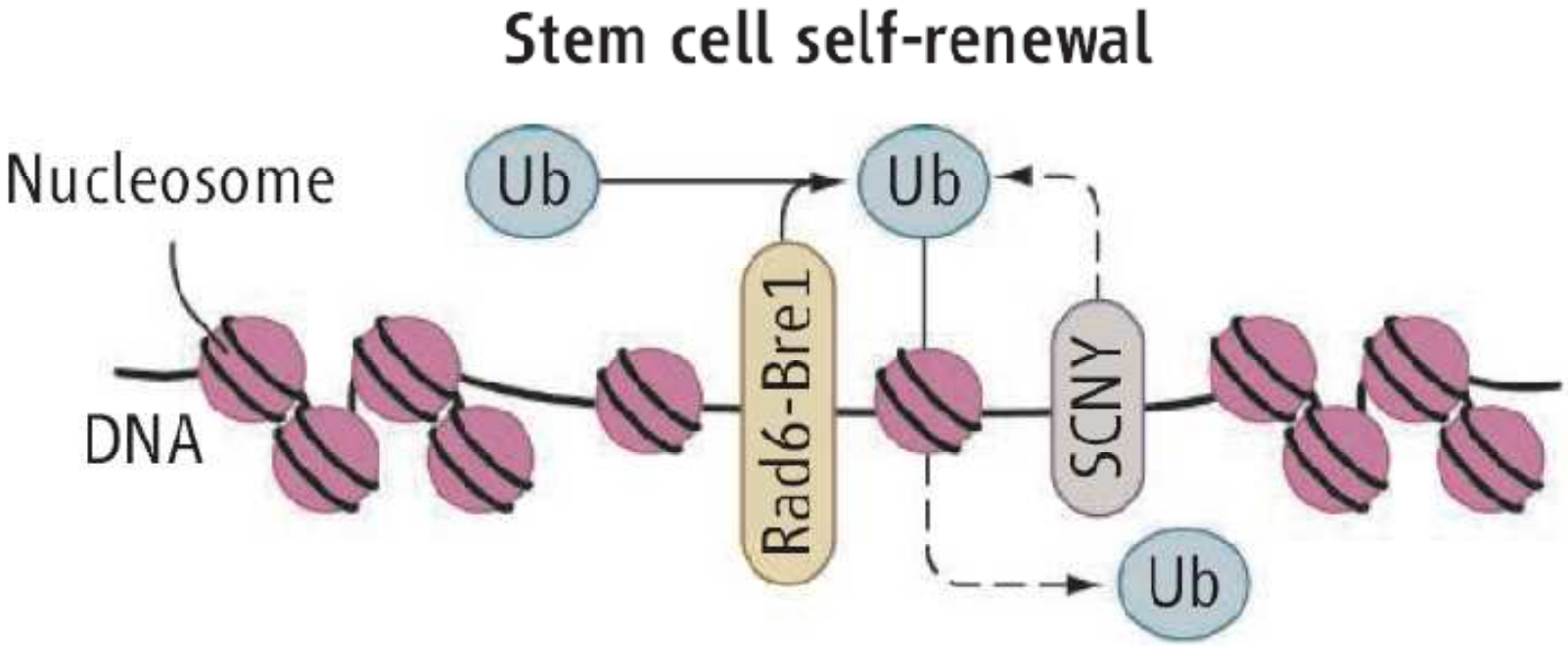


Polycomb

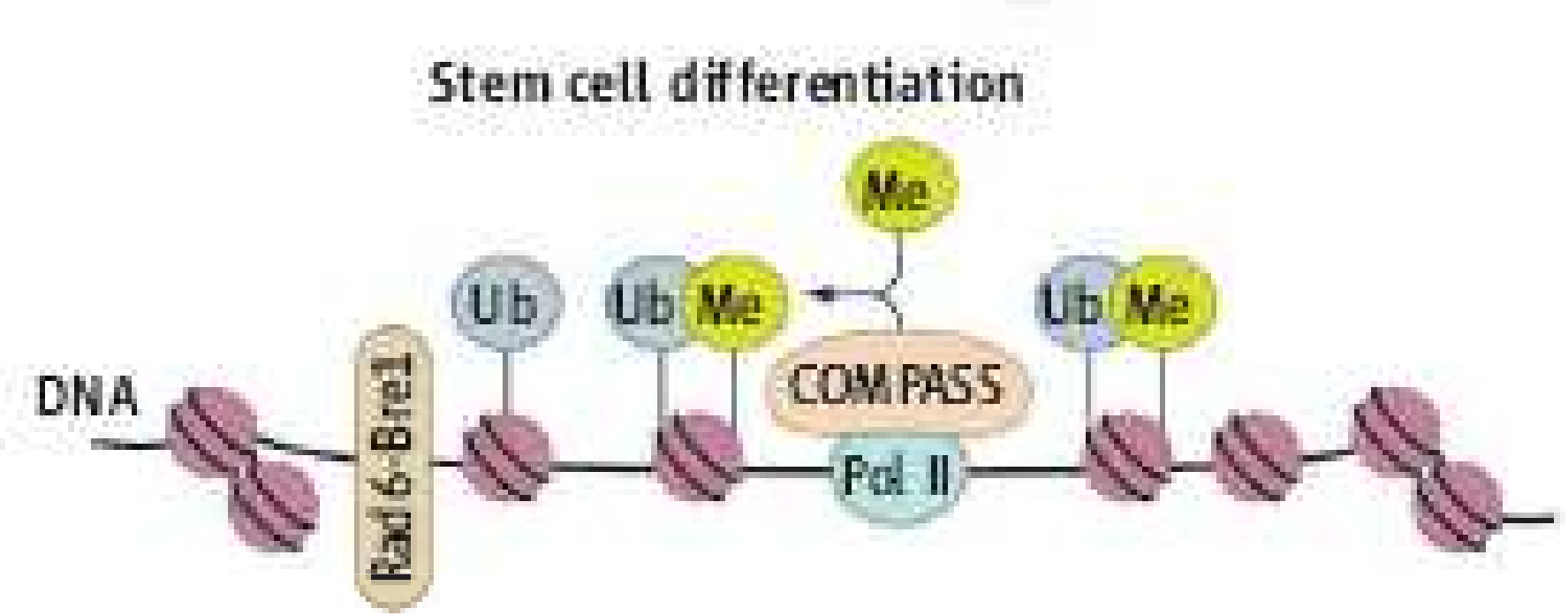
- The roles of PcG proteins in the maintenance of pluripotencysuggest that they constitute a cellular memory.

Action of PcG and trxG complexes on chromatin





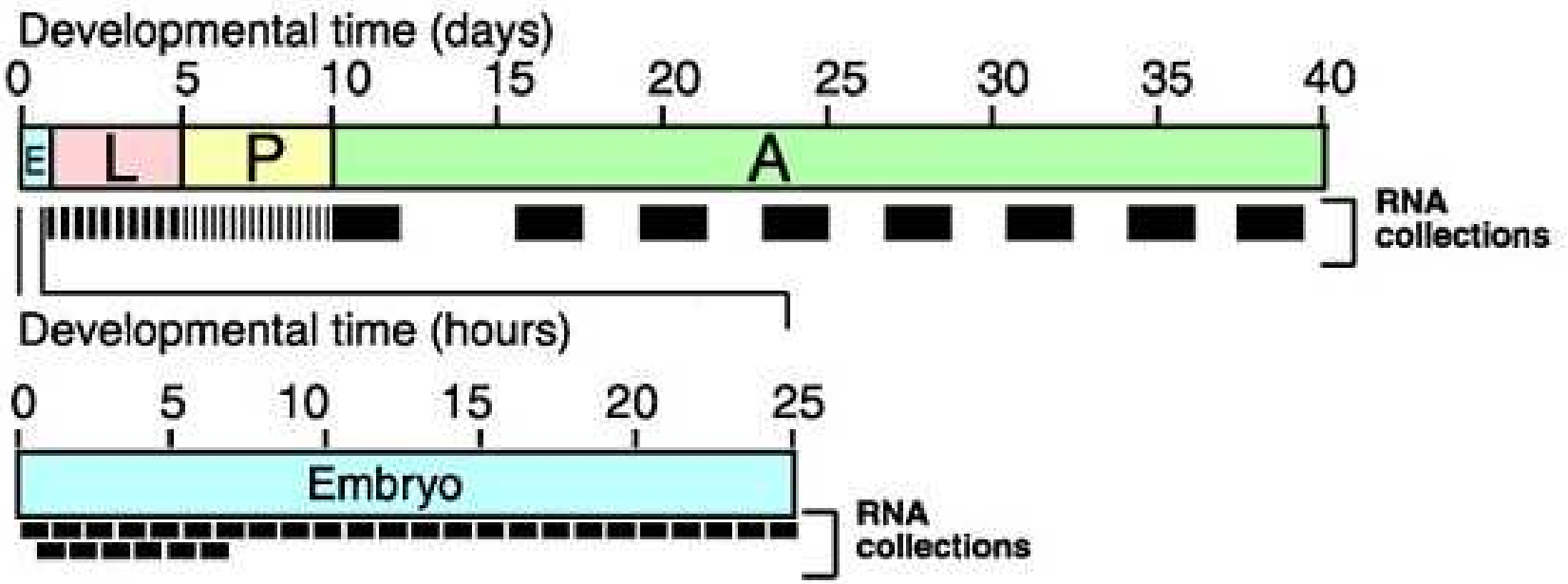
- In adult *Drosophila* stem cells, SCNY removes ubiquitin (Ub) from histone H2B at promoters of genes that need to stay silent to maintain stem cell identity.



During stem cell differentiation, SCNY is inactivated to allow Rad6-Bre1 to monoubiquitinate histone H2B.
This modification is required for recruitment and activation of the COMPASS histone methylase complex, which methylates (Me) histone H3 (H3K4).

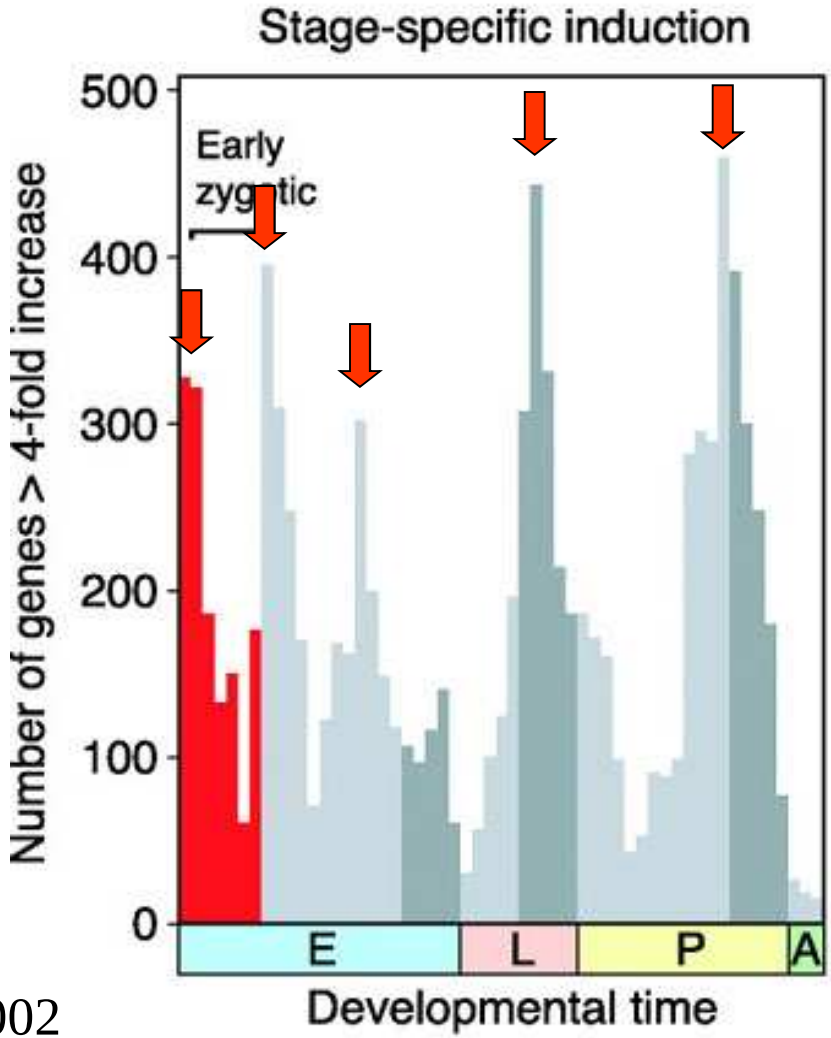
Gene Expression During the Life Cycle of *Drosophila melanogaster*

E: Embryo L: Larva P: Pupa A: Adult *Drosophila*



Arbeitman et al 2002

Gene Expression During the Life Cycle of *Drosophila melanogaster*



Arbeitman et al 2002

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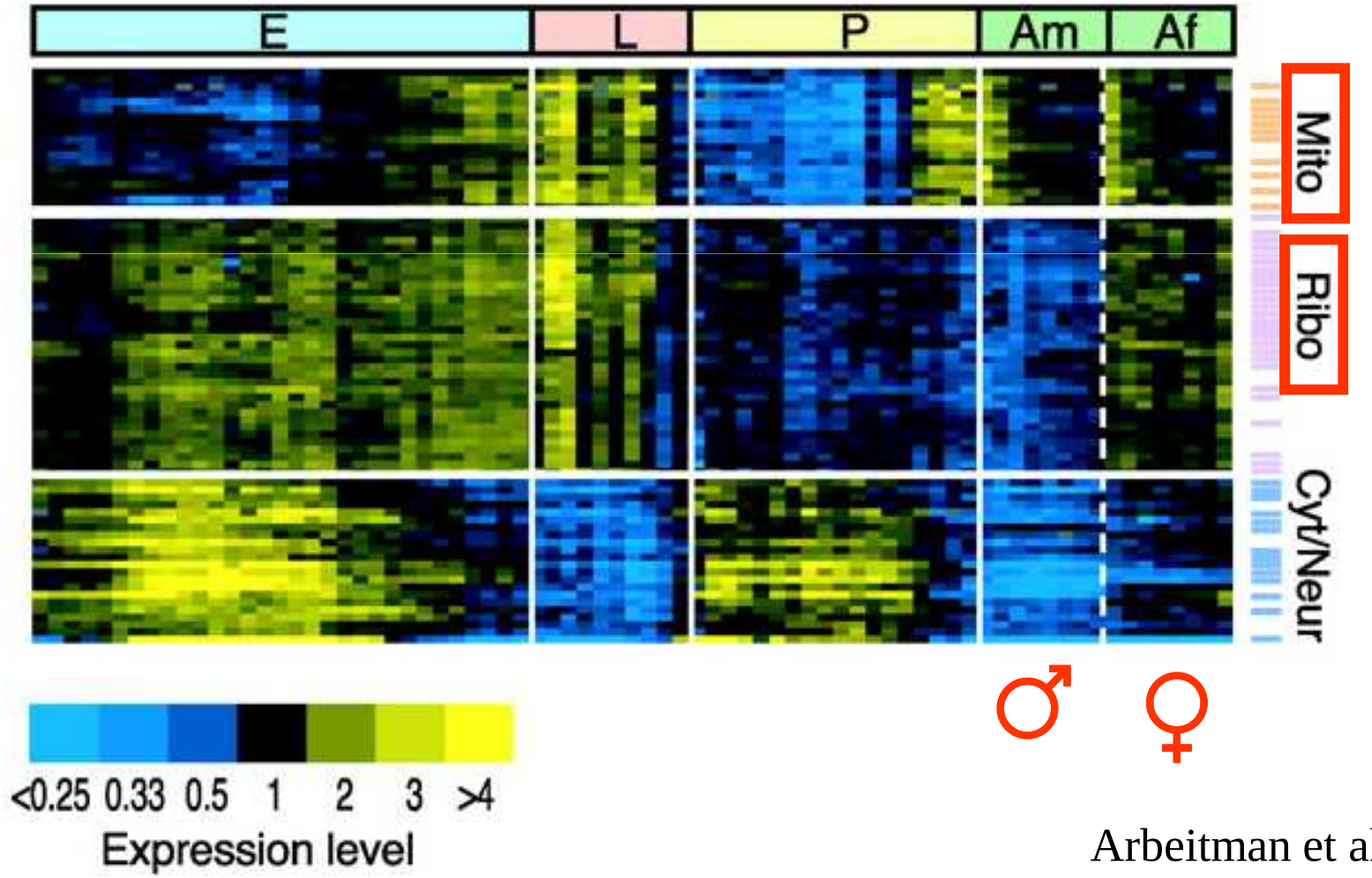
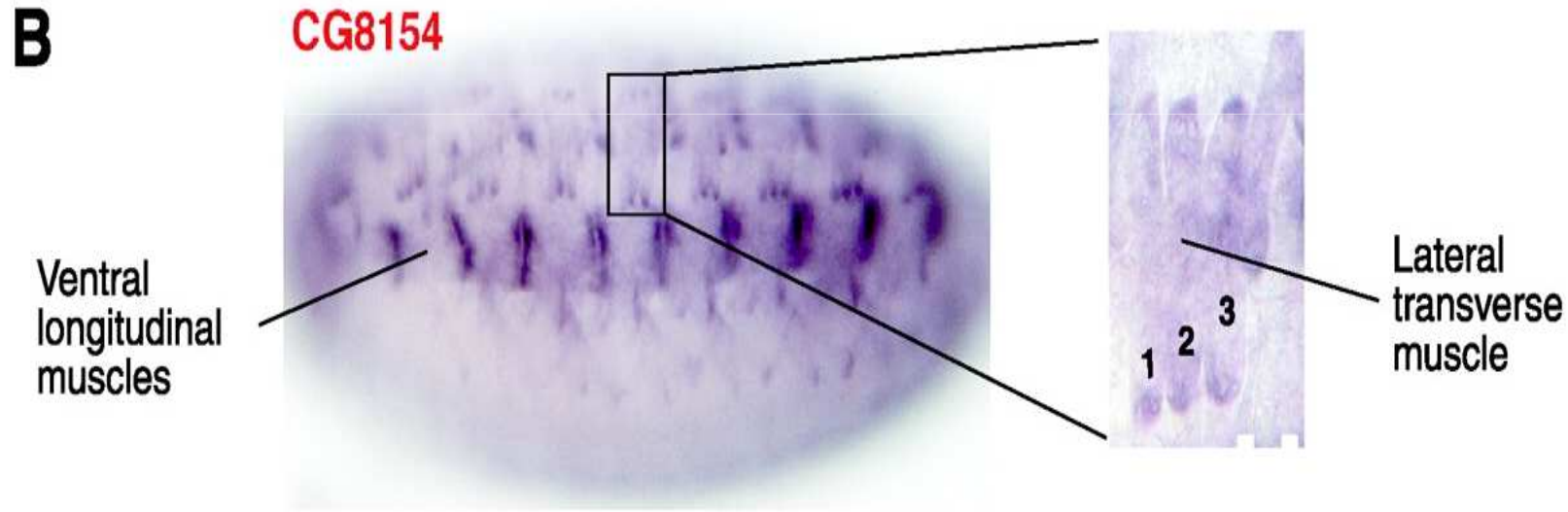


Arbeitman et al 2002

- Three selected clusters of genes with similar expression profiles and related biological functions: components of mitochondria (Mito), ribosome (Ribo), and cytoskeletal/neural genes (Cyt/Neur). Genes within each cluster that are known to share a common biological function are indicated by a colored bar.

Gene Expression During the Life Cycle of *Drosophila melanogaster*



Arbeitman et al 2002

Functional Genomics
Levels

- Genome to transcriptome
- Transcriptome to proteome
- Proteome to dynamic system
- Dynamic systems to phenotype

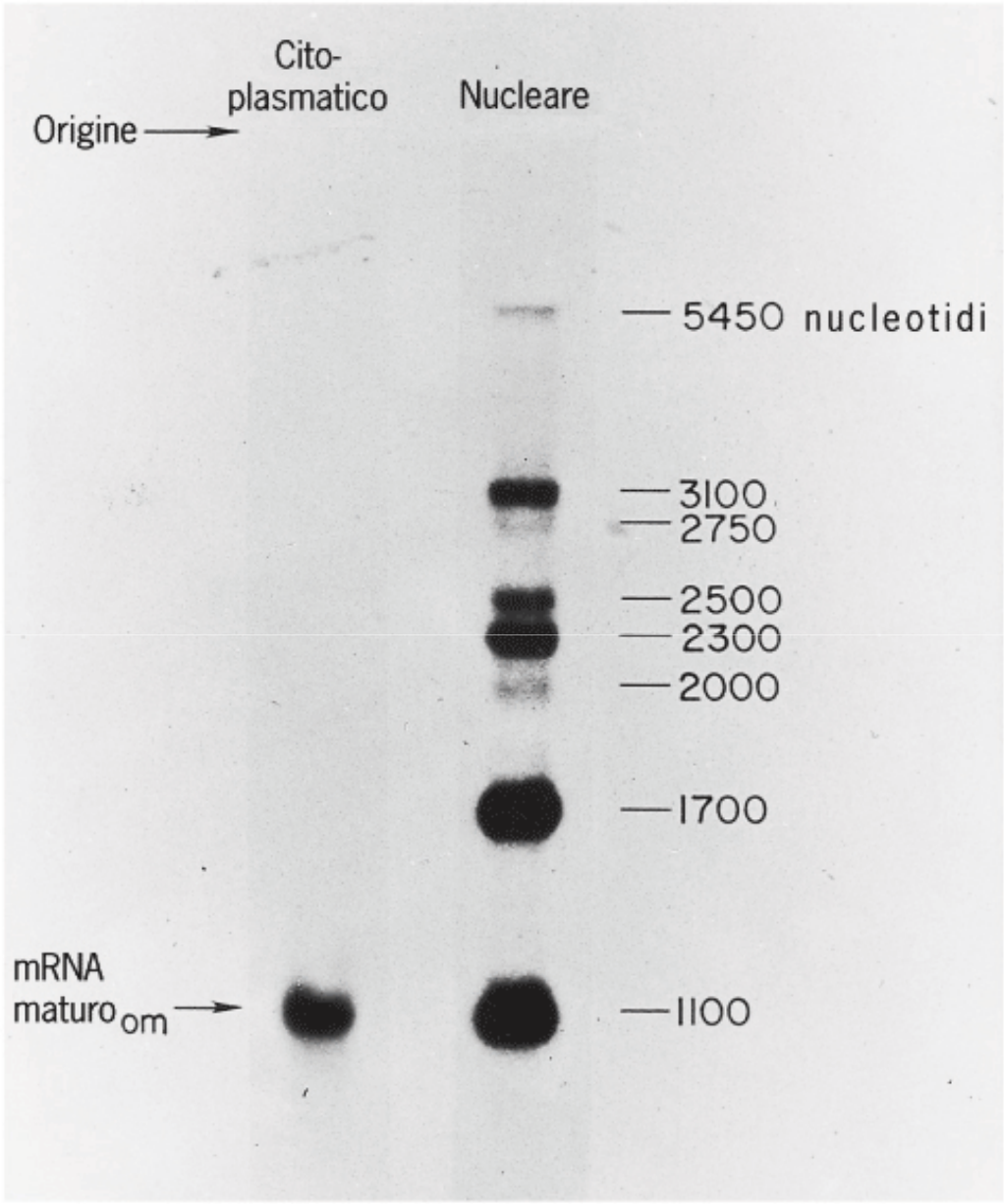
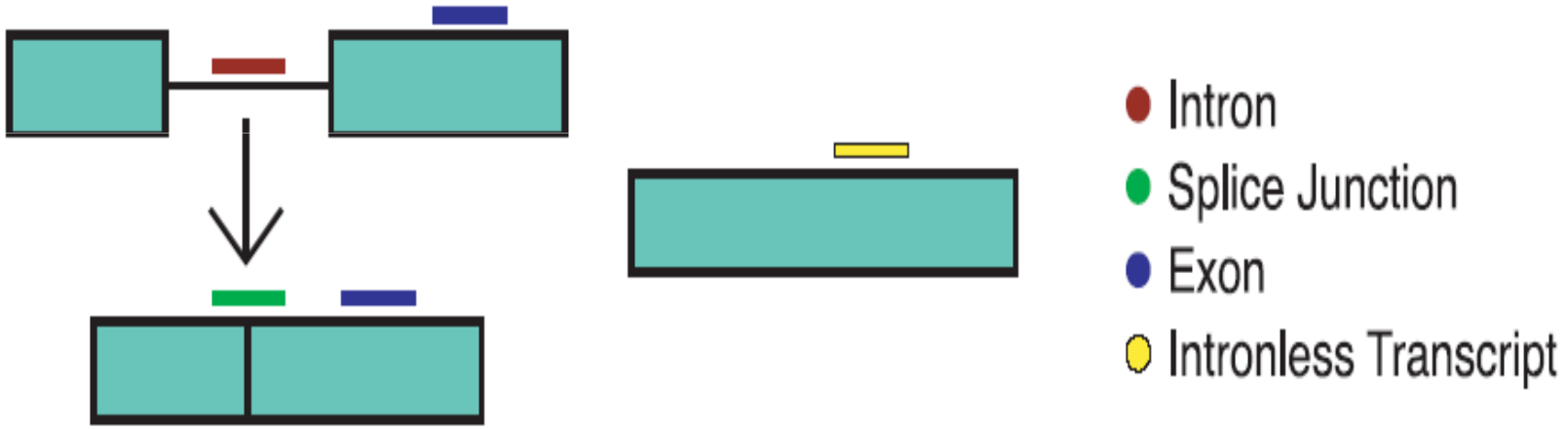
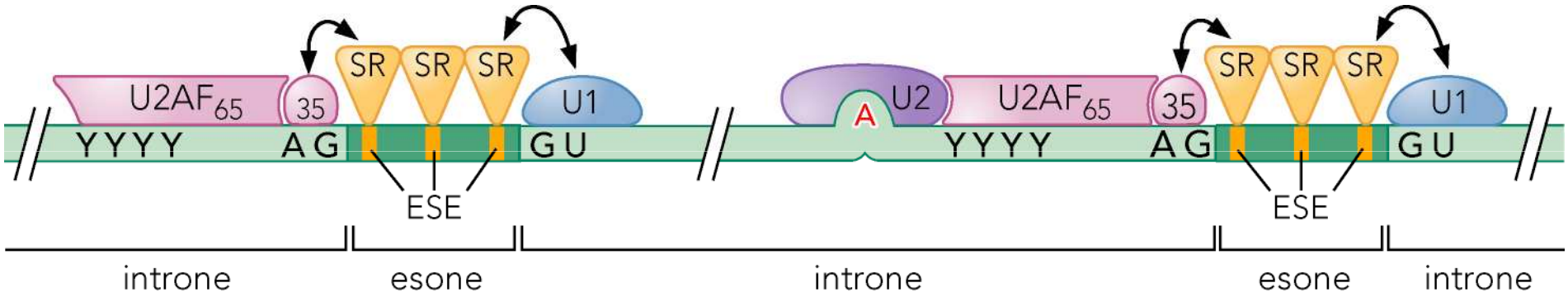


FIGURA 11.36 **La maturazione del pre-mRNA per l'ovomucoide.**

Genomewide Analysis of mRNA Processing in Yeast Using Splicing-Specific Microarrays



Clark et al 2002



PRP4 – member of the SR protein family of splicing factors.

pre-mRNA from intron-containing genes was found to accumulate in a temperature-sensitive mutant

- PRP4 –

  member of the cellular SR protein family of splicing factors.

  ubiquitously expressed

  belongs to the serine-arginine-rich protein-specific kinases, recognizing serine-arginine-rich substrates.

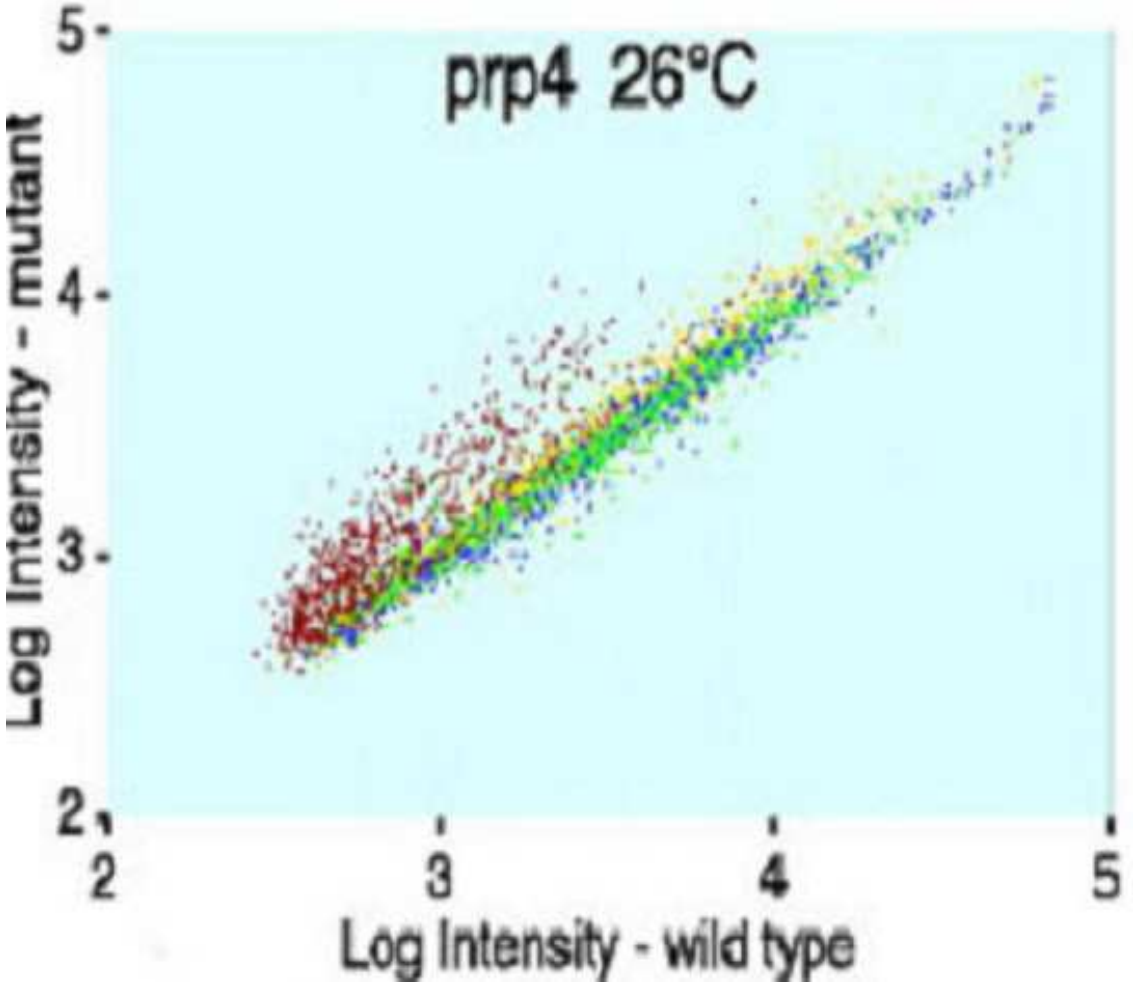
  identified in yeast, through its role in pre-mRNA splicing

  pre-mRNA from intron-containing genes was found to accumulate when a temperature-sensitive mutant was maintained at the restrictive temperature.

Genomewide Analysis of mRNA Processing in Yeast Using Splicing-Specific Microarrays

- Intron
- Splice Junction
- Exon
- Intronless Transcript

Wild Type



Clark et al 2002

Genomewide Analysis of mRNA Processing in Yeast Using Splicing-Specific Microarrays

- Intron
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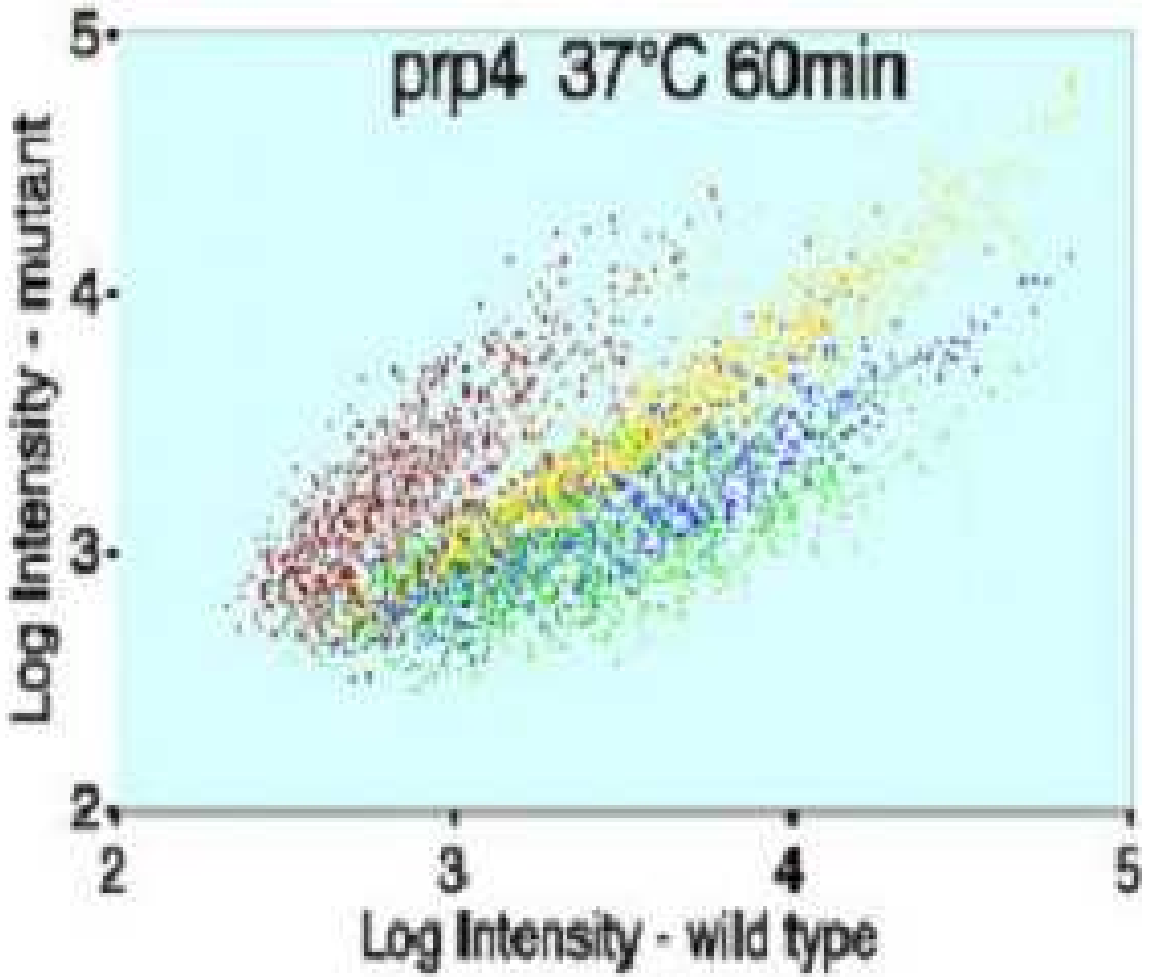
Mutant



Clark et al 2002

Identification of a functional network of human epigenetic silencing factors. JBC 2010

- Epigenetic silencing is mediated by families of factors that place, remove, read, and transmit repressive histone and DNA methylation marks on chromatin.
- How the roles for these functionally diverse factors are specified and integrated is the subject of intense study.
- To address these questions, HeLa cells harboring epigenetically silent green fluorescent protein reporter genes were interrogated with a small interference RNA library targeting 200 predicted epigenetic regulators, including potential activators, silencers, chromatin remodelers.
- Specific epigenetic silencing factors could be detected by measuring green fluorescent protein reactivation after small interference RNA-based factor knockdown.

Identification of a functional network of human epigenetic silencing factors. JBC 2010

In our analyses, we identified a specific subset of 15 epigenetic factors that are candidates for participation in a functional epigenetic silencing network in human cells. These factors include

- histone deacetylase 1,
- de novo DNA methyltransferase 3A,
- components of the polycomb PRC1 complex (RING1 and HPH2),
- and the histone lysine methyltransferases KMT1E and KMT5C.
- Consistent with this interpretation, knockdown of either KMT1E or CHAF1A resulted in a loss of multiple histone-repressive marks and concomitant gain of activation marks on the promoter during reactivation.
- These results reveal how functionally diverse factors may cooperate to maintain gene silencing during normal development or in disease.
- Furthermore, the findings suggest an avenue for discovery of new targets for epigenetic therapies.