CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

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Année 2012-2013 :
"Épigénétique, développement et hérédité"

11 Février, 2013

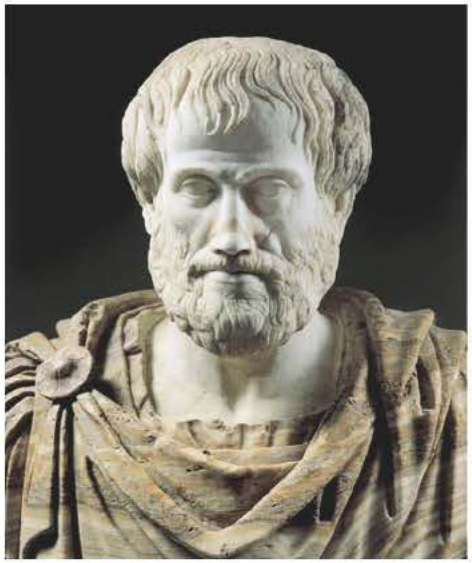
Cours I

Qu'est-ce que l'épigénétique : d'Aristote à Waddington ?

Cours II

Bases moléculaires de l'épigénétique : comment lire et mémoriser la partition du génome

Epigenesis: from simplicity to complexity



Aristotle
384–322 B.C.

Based on his own observations Aristotle rejected the theories of *spontaneous generation* and *preformation* He favored *epigenesis*, which held that the embryo started life as an undifferentiated mass, and that new parts were added during development, beginning with the heart.

He proposed that the embryo forms by coagulation in the uterus immediately after mating and the "mixing of liquids", when the *form-building* principle of the male acted on the *material substance* from the female

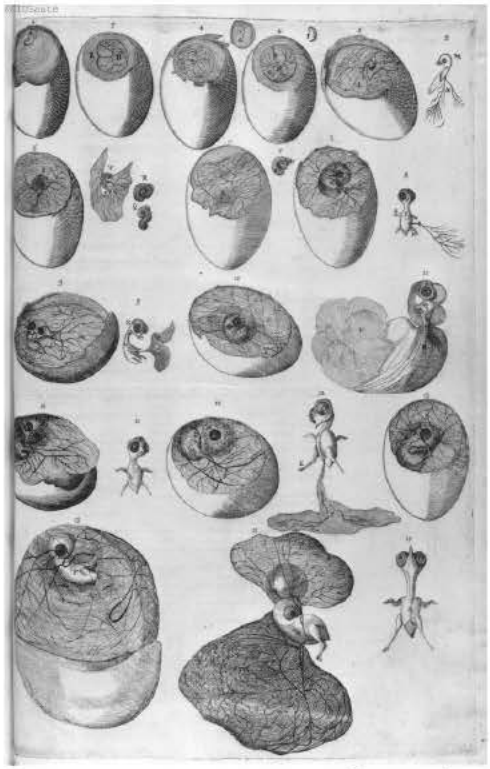
The female parent contributes only unorganized matter to the embryo – she provides the passive "support" to its growth. The semen from the male parent provided the "form," or soul, that guides development

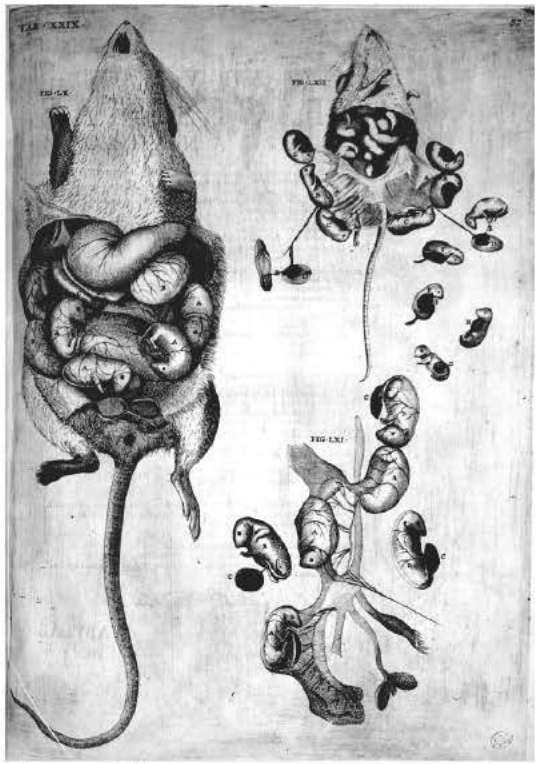
"On the Generation of Animals"
(From Ross, W. D., ed. The Oxford Translation of Aristotle. Vol. 5. Trans. Arthur Platt. Oxford: Clarendon press, 1912.)

Support for the theory of Epigenesis in the 16th – 17th Centuries

Girolamo Fabrici (ca. 1533-1619)
Italian Anatomist

William Harvey (1578–1657)
English Physician





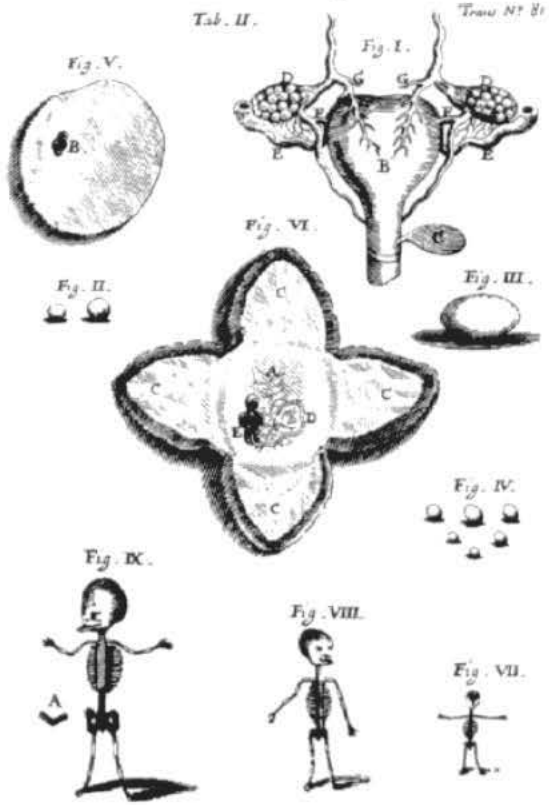
"De formatione ovi et pulli", 1621
(On the Formation of the Egg and of the Chick)

William Harvey published his work in 1651
in support of Epigenesis, but also rejected some aspects of Aristotle's theory of generation– particularly on the role of the egg:

"...an egg is a <u>true generative seed,</u> analogous to the seed of a plant; the original conception arising between the two parents, and being the mixed fruit or product of both. For as the egg is not formed without the hen, so is it not made fruitful without the concurrence of the cock."

The rise of Preformationism in the 17th and 18th Centuries

Preformation : pre-structured, pre-existing germs which were not to develop, but only to unfold



Demonstrating a clear ignorance of mammalian development, Dr. Kerkringius" illustrates "the little embryon" as a skeleton in an egg, supposedly "3 or 4 days after it was fallen into the Matrix of a woman" (1670)

"The Ovary of Eve: Egg and Sperm and Preformation" by Clara Pinto-Correia. The University of Chicago Press, 1997

The rise of Preformationism in the 17th and 18th Centuries

Was the growth, development and character of an individual pre-determined, since the beginning of time, or could it occur due to original driving forces?

Ovism

Marcello Malpighi (1628–1694) and Jan Swammerdam (1637–1680), two pioneers of microscopy, thought they could see the future parts of the adult folded up inside the eggs of frogs, chicks, insects.

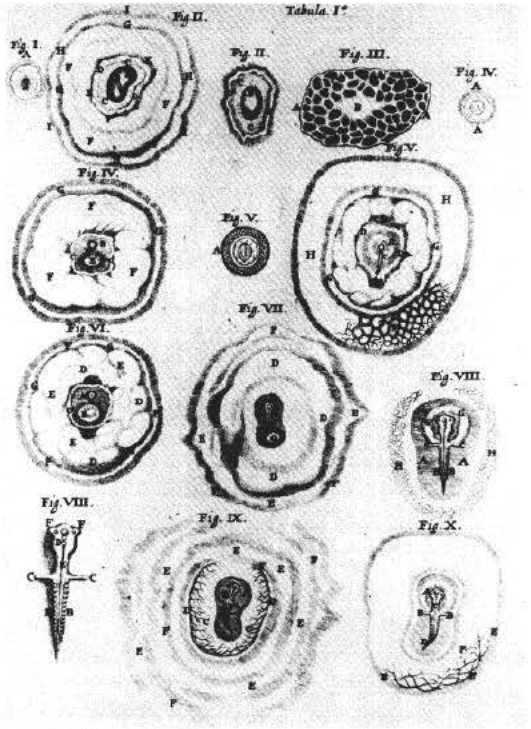
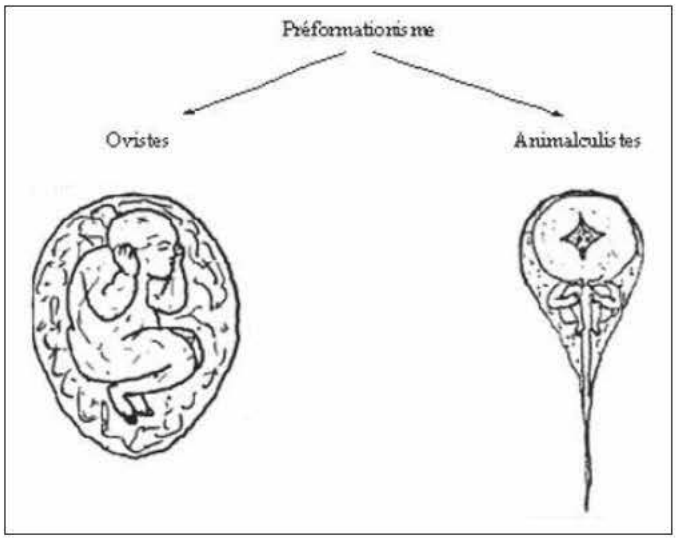


Figure 2. Illustrations from M. Malpighi (1673) of developing chicken embryos from the time of laying to 36 hours of incubation. This plate is representative of the keen observations done on early embryos during the 17th century.

E Marcello Malpighi (1628–1694)



Each new generation existed "preformed" within the egg of the preceding generation.

The whole human race must have preexisted in the ovaries of Eve!

Spermists

Early microscopes revealed the existence of "little animals" in male semen, suggesting that the preformed individuals must be present in the sperm.

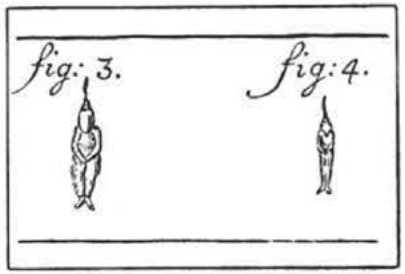


Fig. 23. Dalenpatius' drawings of human spermatozoa (from Leeuwenhoek).

Evidence for importance of sperm in reproduction came from Lazzaro Spallanzani in the late 1800's:

- filtered toad semen devoid of sperm would not fertilize eggs.

However, he concluded that the spermatic "animals" were parasites rather than the agent of fetilization…

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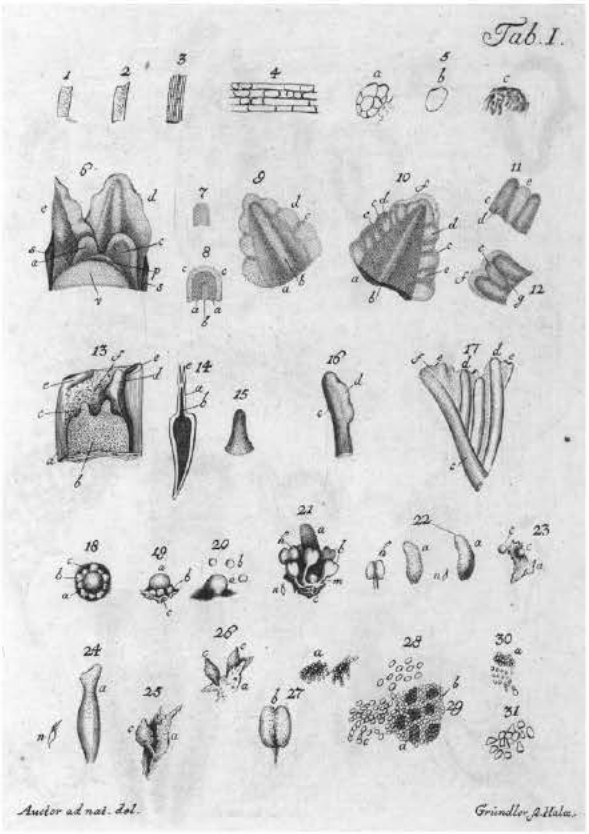
Epigenesis versus Preformation

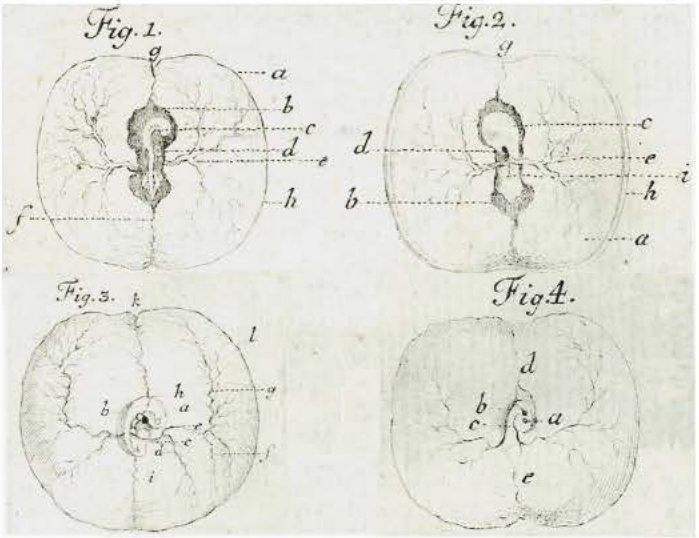
Kaspar Friedrich Wolff
(1733–1794)

Wolff examined embryos of plants & anmals meticulously.

He proposed that groups of cells, initially unspecialized, differentiated into various tissues, organs, and systems.



The First Drawing of the Shoot Apical Meristem of a Plant, from the Dissertation of Caspar Friedrich Wolff (1759).



(1): day 3.0-3.5 Chick embryo
Caspar Friedrich Wolff, 1768

The egg does not contain a formed embryo
Its structure is totally different from that of the adult.
Development is not a process of unfolding but involves continual formation, of new parts, one after the other….

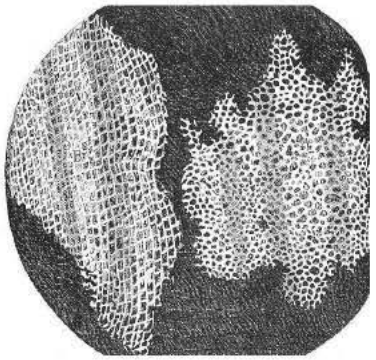
In Wolff's "Theory of Generation" (1759): bodily organs do not exist at the beginning of gestation, but develop from some originally undifferentiated material through a series of steps, termed "morphogenesis", very similar to Aristotle's original concept of epigenesis.

The Cell Theory and acceptance of Epigenesis in the 19th century

• The Cell Theory, first proposed by Matthias Schleiden, Theodor Schwann, in 1839 and completed by Rudolf Virchow in 1855, consisted of three primary points:

1. All living things are made up of cells.
2. Cells are the basic units of structure, function and physiology in living things.
3. Living cells can come only from other pre-existing cells : (*omnis cellula e cellula* – Virchow, 1855)

• With the advent of better microscopes, careful observations could now be made of a number of developing organisms: embryos were made up of cells – arising from the fertilised egg, itself a cell….



Describing the appearance of a thin layer of cork tree, in a 1665 publication, the pioneering microscopist, R. Hooke is credited with the term "cells": the cork's box-like pores looked like a Monk's living quarters, or cell (from the Latin, *cella* for "storeroom" or "small container").

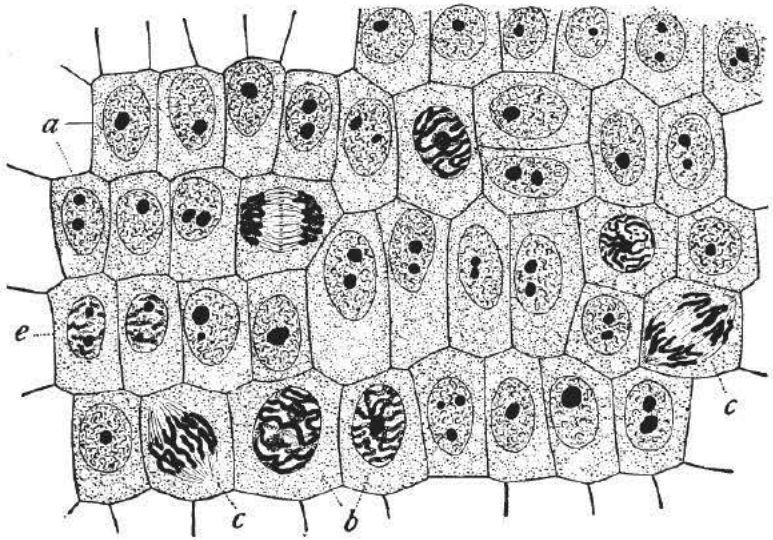


Fig. 2. — General view of cells in the growing root-tip of the onion, from a longitudinal section, enlarged 800 diameters.

a. non-dividing cells, with chromatin-network and deeply stained nucleoli ; *b*. nuclei preparing for division (spireme-stage) ; *c*. dividing cells showing mitotic figures; *e*. pair of daughter-cells shortly after division.

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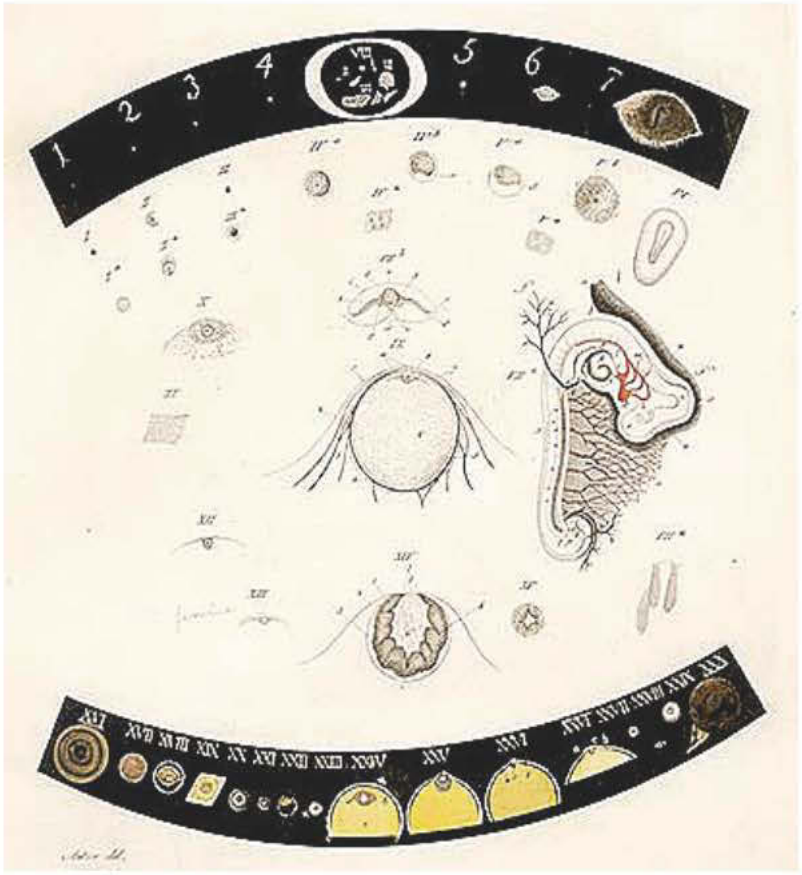
Observations on Mammalian Development : further evidence for Epigenesis and against Preformation

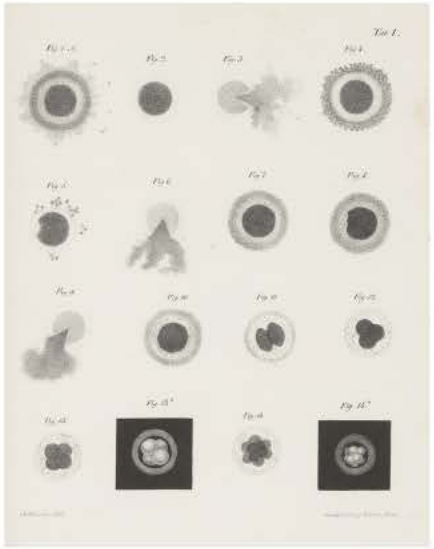
Karl Ernst von Baer
1792-1876

Discovery of the mammalian ovum:
"a body about the one hundred and twentieth of an inch in diameter, wherein lie the properties transmitting the physical and mental characteristics of the parent or grandparent, or even of more remote ancestors"
Epistola de Ovo Mammalium et Hominis Genesi (1827)

First accurate descriptions of mammalian development from the fertilised egg in History of the Evolution of Animals (1837)



Copper engravings from Karl Ernst von Baer De ovi mammalium…(1827), showing the dog embryo up to about three weeks (VII), the human egg (XIII), and for comparison, those of other vertebrates and a crayfish.



Cleaving dog embryos.
Inspired by von Baer, Lithograph by A. Schütter after Bischoff's drawings, printed by Henry & Cohen, from Th. Ludw. Wilh. Bischoff,

Inspired by von Baer, in the early 1840s, the anatomist Theodor Bischoff authored pioneering studies of early mammalian development.

E. Heard, February 11th, 2013

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The Egg and Sperm

Oscar Hertwig, Strasburger, Kölliker and others, showed in 1884-5 that eggs and sperm were single cells, the nuclei of which were found to fuse during fertilization and following their fusion, development proceeded through multiple rounds of cell division.

And, the cell nucleus was proposed to be the vehicle of inheritance

"And thus the wonderful truth became manifest that a single cell may contain within its microscopic compass the sum total of the heritage of the species".
EB Wilson, 1900

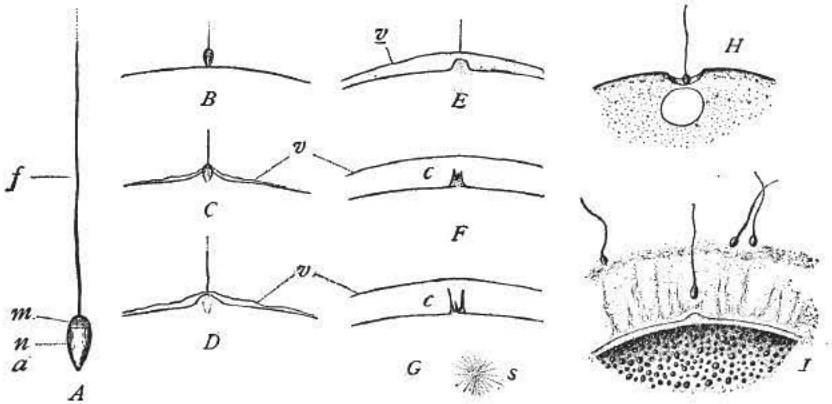


Fig. 100. — Entrance of the spermatozoön into the egg. *A–G.* In the sea-urchin, *Toxopneustes*. *H.* In the medusa, *Mitrocoma*. [METSCHNIKOFF.] *I.* In the star-fish *Asterias*. [FOL.]

A. Spermatozoön of *Toxopneustes*, × 2000; *a.* the apical body, *n.* nucleus, *m.* middle-piece, *f.* flagellum. *B.* Contact with the egg-periphery. *C. D.* Entrance of the head, formation of the entrance-cone and of the vitelline membrane (*v*), leaving the tail outside. *E. F.* Later stages. *G.* Appearance of the sperm-aster (*s*) about 3–5 minutes after first contact; entrance-cone breaking up. *H.* Entrance of the spermatozoön into a preformed depression. *I.* Approach of the spermatozoön, showing the preformed attraction-cone.

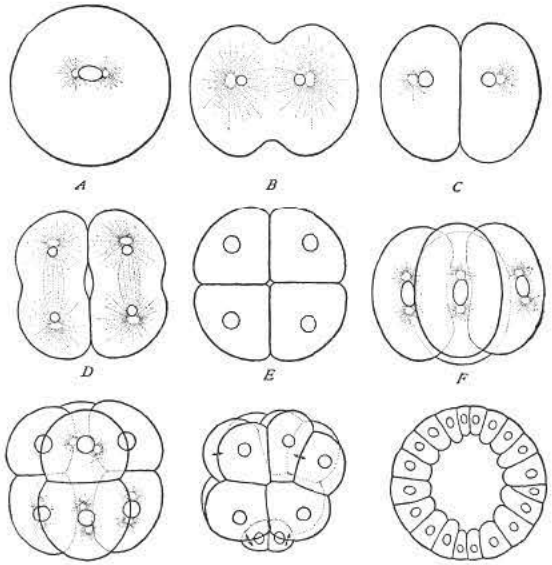
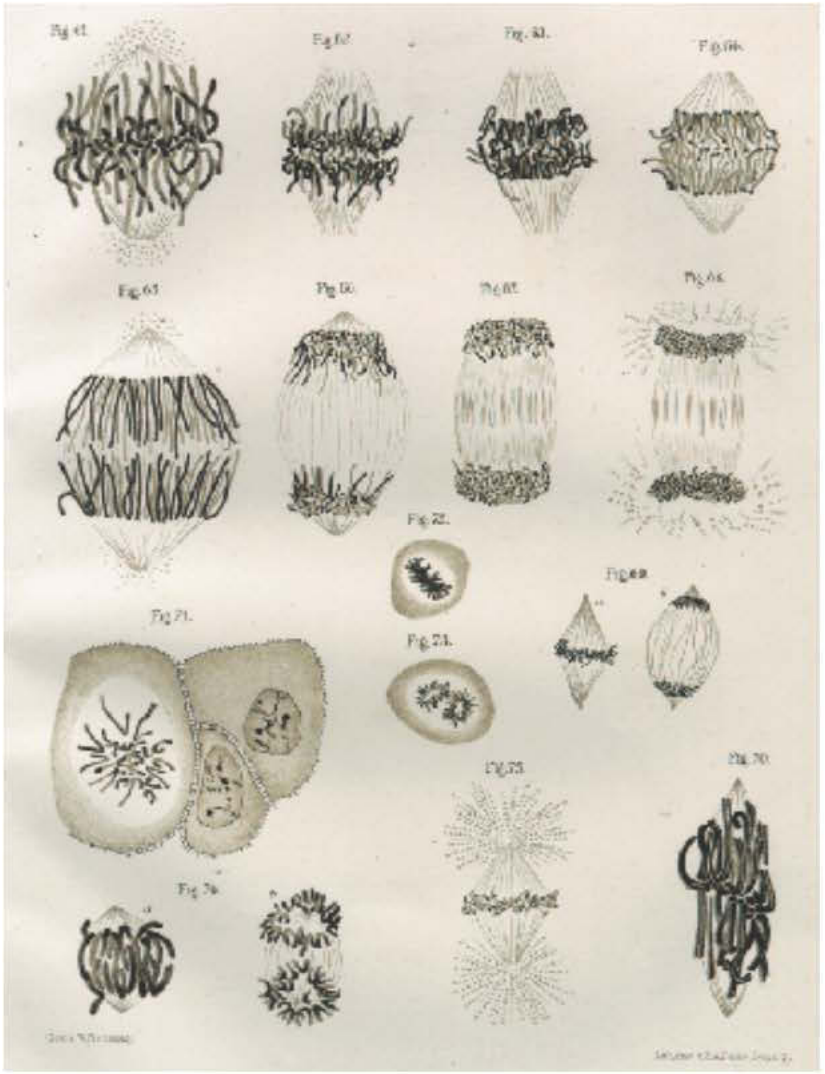


Fig. 4. — Cleavage of the ovum of the sea-urchin *Toxopneustes*, × 330, from life. The successive divisions up to the 16-cell stage (*H*) occupy about two hours. *I* is a section of the embryo (blastula) of three hours, consisting of approximately 128 cells surrounding a central cavity or blastocœl.

Cells Derive from other Cells:
Physical nature of the Hereditary Material ?

Walther Flemming (1843-1905)
German biologist



In 1882, Walther Flemming explored the fibrous network within the nucleus, which he termed chromatin, or "stainable material."

He discovered chromosomes, and mitosis: the splitting of chromosomes along their length and their partitioning into different daughter cells.

The Era of Experimental Embryology

Return to moified "Preformation" theory?

August Weismann (1834–1914)

"Germ Plasm Theory":
Inheritance only takes place via germ cells (gametes)

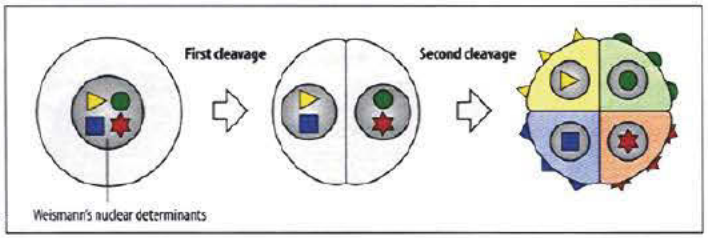
However, he proposed that genetic information *cannot* pass from soma to germ plasm and on to the next generation, "the Weismann barrier".

Weismann was concerned to rule out the inheritance of acquired characteristics as proposed by Jean Baptiste Lamarck.

From: The Developmental Mechanics of Cell Specification
Developmental Biology, Gilbert SF.

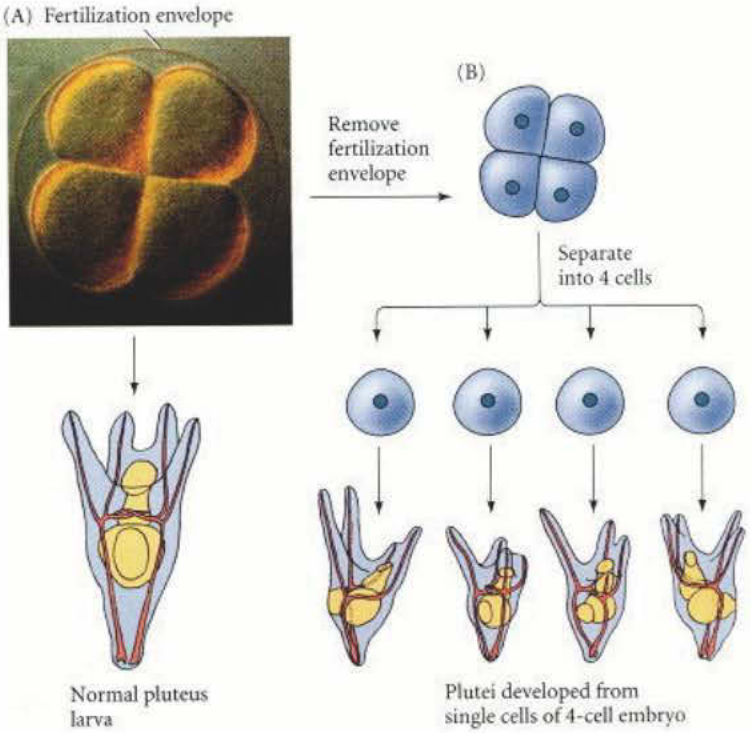
A modified view of preformation:
"determinate development"

The zygote contains all the determinants for various differentiation pathways, but as a mosaic, in which small regions of the protoplasm produce specific parts of the adult, and are unequally distributed at each cell division



Hans Driesch (1867-1941)

Any cell of an early sea urchin embryos has the ability to become an embryo.
Each cell still possesses all determinants.



Embryo grafting experiments : context and timing matter

Hans Spemann (1869–1941) and his student Hilde Mangold
discover the process of induction, the biochemical signal that lead to cellular differentiation in the nervous system and other embryonic organs.
Spemann won the Nobel Prize for Medicine in 1935

Induction: one cell or tissue directs the development of another, neighboring, cell or tissue
Organizer: control the organization of a complete embryonic body

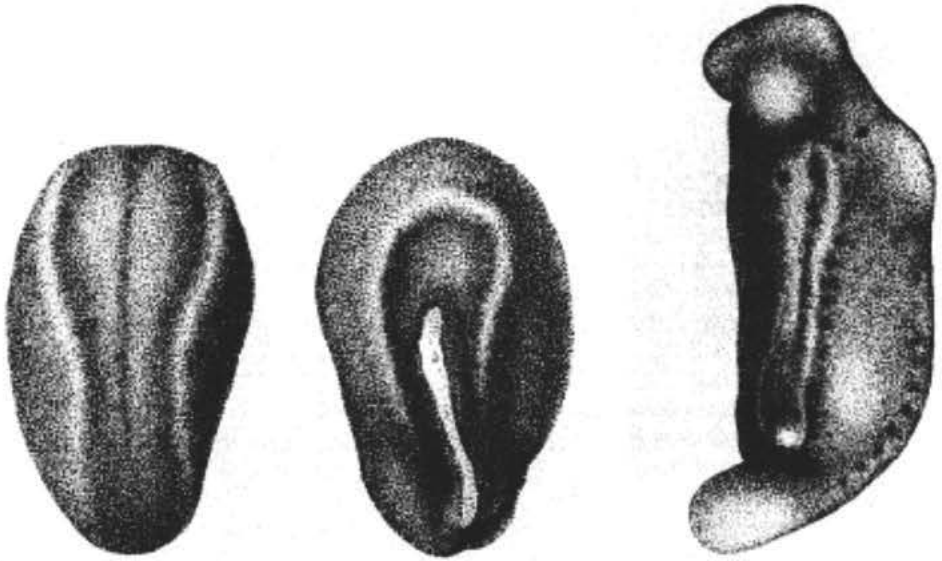


Figure 13. The classical experiments of Spemann and H. Mangold (1924) demonstrated induction in amphibian embryos. A graft of the dorsal lip of the blastopore was made from an unpigmented species to a pigmented species. The graft was placed in the future ventral region of the body. At the neurula stage, the normal neural plate is found on the dorsal surface *(left)* and a second neural plate, containing the graft on the ventral surface *(center)*. The result was the formation of a secondary embryonic axis which produced Siamese twinning *(right)*.

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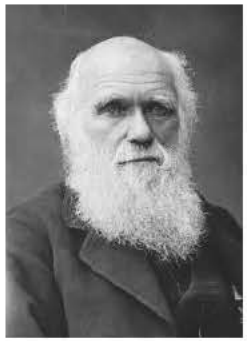
The Nature of Heredity?

The Nature of Heredity?

In 1865:
Mendel's publication of his experiments in plant hybridization &
Darwin's provisional hypothesis of pangenesis.



Charles Darwin
(1809-1882)

Darwin proposed that traits could be passed down via units he termed "gemmules," which he believed traveled from every body part to the sexual organs, where they were stored (Benson, 2001).

Gregor Mendel speculated that cells contained some type of factor that carried traits from one generation to the next

These first attempts to explain the mechanisms of heredity lacked any scientific support, their profound importance went unrecognized by the scientific community for decades. Nonetheless, Mendel and Darwin's work laid the foundation for formulating a testable, research-based theory of heredity.

The Dawn of Genetics

Gregor Mendel (1822 –1884)
Silesian biologist ,Augustinian friar

Mendel's Laws of Inheritance:
First Law of Segregation
Second Law of Independent Assortment
Third Law of Dominance

Mendel proposed the existence of hereditary 'factors' that dictate phenotypes

Only later were these called 'genes' by Johannsen in 1905 (inspired by 'pangenesis' theory of inheritance of Darwin)

Rediscovery of Mendel's work in 1900

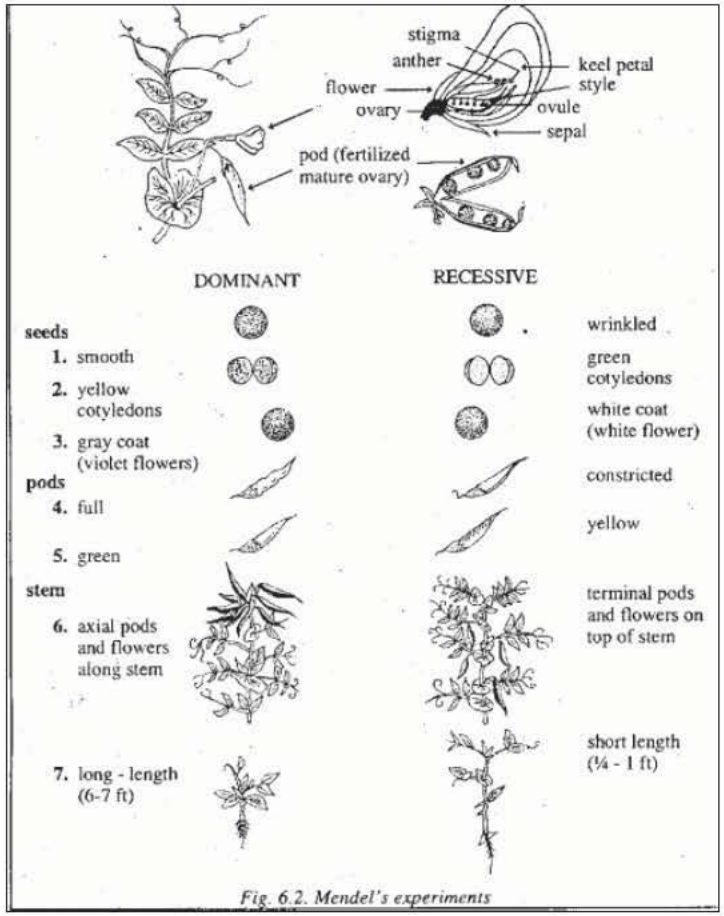


Fig. 6.2. Mendel's experiments

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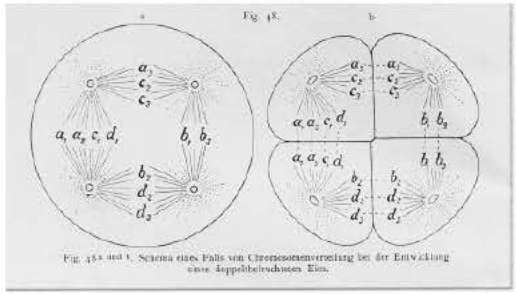
The Chromosomal basis of Mendelian Heredity

Theodor Boveri (1862-1915) and Walter Sutton (1877-1916) propose that chromosomes bear hereditary factors in accordance with Mendelian laws

In 1902, Boveri found that only sea urchin embryos possessing the full set of 36 chromosomes could develop normally. A "*specific assortment of chromosomes is responsible for normal development and this can mean only that the individual chromosomes possess different qualities*."

Boveri also realised that the Mendelian concepts of segregation and assortment could be interpreted to operate on a cellular level, with chromosomes containing Mendel's so-called hereditary "factors". In 1903 he wrote that "*the characters dealt with in Mendelian experiments are truly connected to specific chromosomes*."



Sutton worked on process of "reduction division" (later called meiosis), which gives rise to germ cells, or gametes. In meiosis, the number of chromosomes is reduced by half in sperm and egg cells, with the original number restored in the zygote, or fertilized egg, during reproduction

In 1902 Sutton suggested that "*the association of paternal and maternal chromosomes in pairs and their subsequent separation during the reduction division...may constitute the physical basis of the Mendelian law of heredity*." His "The Chromosomes in Heredity" was published in 1903.

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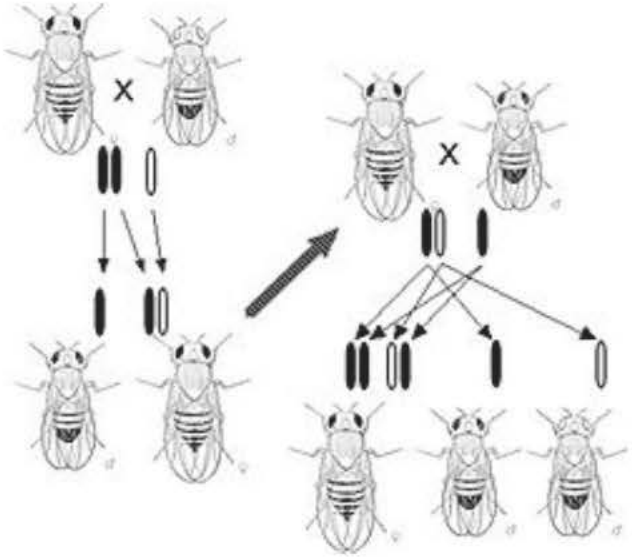
Genetic Linkage, Chromosomes and Heredity

Thomas Hunt Morgan
Embryologist, zoologist
and geneticist
1866 - 1945

Morgan discovered that eye color in *Drosophila* expressed a sex-linked trait – and must be carries on a sex chromosome. This formed the basis for the proof that the genetic factors were physically located on the individual chromosomes. Morgan went on to discover over two dozen more mutant traits between 1911 and 1914.

With evidence drawn from cytology he was able to refine Mendelian laws and combine them with the theory—first suggested by Theodor Boveri and Walter Sutton—that **the chromosomes carry the hereditary information.**

"The egg of every species of animal or plant carries a definite number of bodies called chromosomes. The sperm carries the same number. Consequently, when the sperm unites with the egg, the fertilized egg will contain the double number of chromosomes. For each chromosome contributed by the sperm there is a corresponding chromosome contributed by the egg, i.e., there are two chromosomes of each kind, which together constitute a pair."



In 1915, Morgan and his colleagues published The Mechanism of Mendelian Heredity. He received the Nobel Prize in Physiology or Medicine in 1933.

E. Heard, February 11th, 2013

Epigenesis and the Genetic Material

In summary, at the beginning of the 20th Century:

• Some experimental embryologist such as Weismann (Germ Plasm theory of heredity, 1893), believed that development proceeded by **progressive loss of genetic material**, with retention only of material relevant to **specific functions** in different cells, and preservation of the germ plasm's hereditary material to ensure the next generation.

• The new geneticists believed that the inherited nuclear material (genes and chromosomes), was the **same in every cell**, but was somehow **differently exploited**.

The formal demonstration that genetic material is in fact conserved throughout cellular differentiation – and that development depends on changes in the expression and not in the content of the genome only cam from Briggs and King, 1952 and Gurdon, 1962.

HOW is the genetic information differently exploited during cellular differentiation?

" How do the hereditary factors of genes affect development?"
from "How Animals Develop", 1936, C.H. Waddington

Birth of the discipline of Epigenetics

Conrad H. Waddington (1905-1975)
British paleontologist, zoologist
geneticist, embryologist & philosopher

- A need to establish **causal relationships between genotype** & **phenotype**, in order to understand development.
- **Epigenotype:** the processes linking genotype and phenotype
- **Epigenetics**: the study of the mechanisms of development through which genes bring about phenotypic effects
- **Epigenetics:** a discipline that would bridge the gap between genetics and experimental embryology approaches

Waddington C.H. "The Epigenotype", 1942, *Endeavour*

The fact that the word 'epigenetics' is reminiscent of 'epigenesis' is to my mind one of the points in its favour. [...] We all realize that, by the time development begins, the zygote contains certain 'preformed' characters, but that these must interact with one another, in processes of 'epigenesis,' before the adult condition is attained.

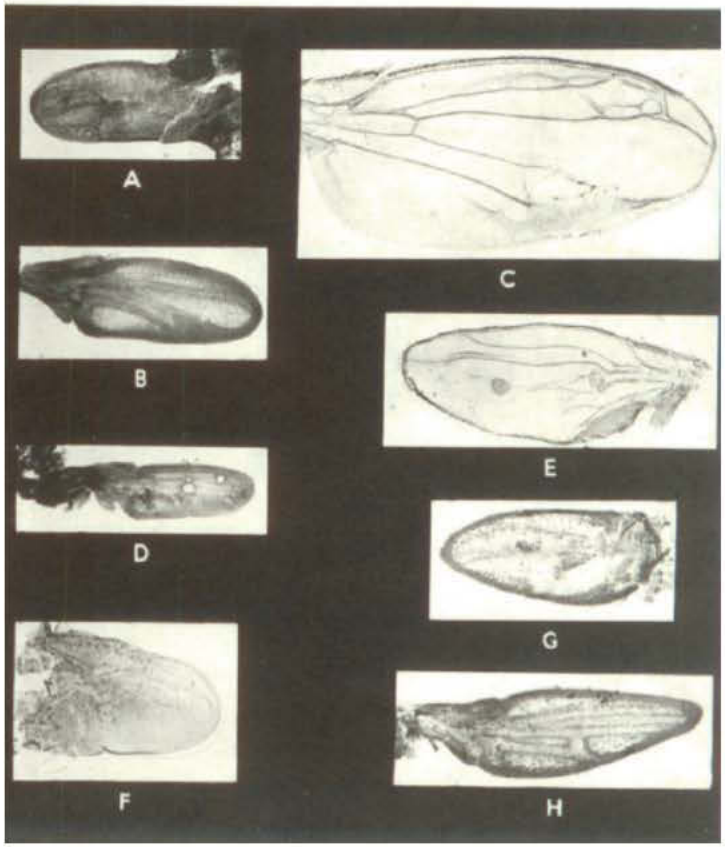
The study of the 'preformed' characters nowadays belongs to the discipline known as 'genetics;' the name 'epigenetics' is suggested as the study of those processes which constitute the epigenesis which is also involved in development.
(Waddington 1956, p. 1241)"

Waddington: the Geneticist

- Genetic perturbations in early development can cause far-reaching abnormalities in different tissues, in an analogous fashion to the mechanical manipulation of early embryos

Genetics ⇔ Experimental embryology

- A single gene can produce multiple effects on different organ and, a single organ can be affected by multiple genes



Drosophila wing development – affected by 30 loci. In first 48h after larva enters pupa, wings undergo at least 15 different processes, each of which is affected by a known gene

⇒ Genotype is in continual and unremitting control of every phase of development.

FI G UR E 3 - *Some genetically controlled abnormalities in the contraction phase of wing-development in* Drosophila.

Figures A, B, *and* C *show wings of the mutant race* net *in which there is a partial failure of contraction, which causes the formation of extra veins in some regions. D and E failure of contraction is more complete. Infigures* F, G, *and* fI *the contraction does not fail, but is abnormal, so that a wing of characteristically elongated form is produced (the mutant* blade *in* D. pseudo-obscura).

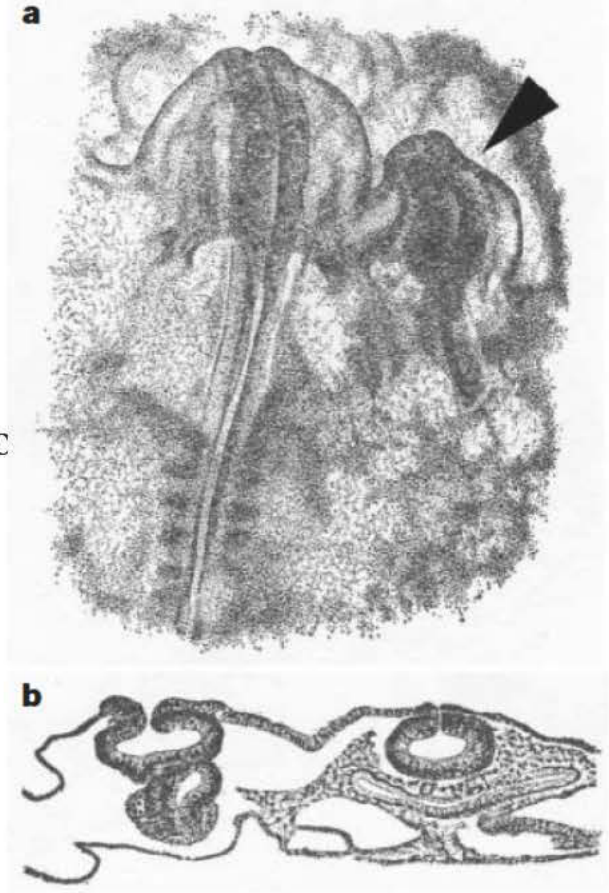
Waddington C.H. (1942) The Epigenotype, *Endeavour*.

Waddington: the Embryologist

Waddington was very inspired by the experiments of Spemann and Mangold on amphibians demonstrating the existence of the organizing centre, that can induce a 2nd body axis in an early embryo.

In the 1930's, he himself demonstrated the existence of an organizer in both mammals and birds: Hensen's node. Grafting of a duck node onto an early chick embryo induced formation of a second body axis (primitive streak)
Furthermore a *duck* node could induce a 2nd axis in a *rabbit* embryo => organizer signal was conserved!

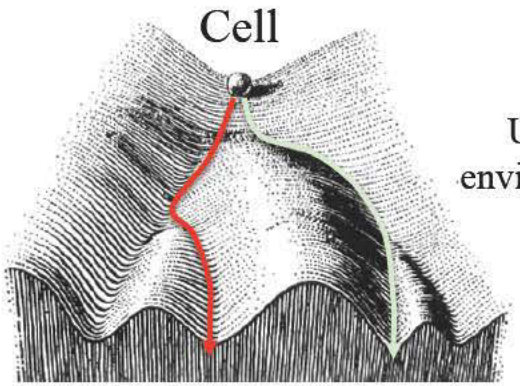
He proposed that **genes** can affect both the **organizers** (the "evocators" or inducing signals), as well as the "pattern of sensitivity" or "individuation field" and **competence** – the ability of a cell or tissue to react to an inducing signal.



Waddington, C. H. (1956) Principles of Embryology (Cambridge Univ. Press, UK)

Waddington: the Artist

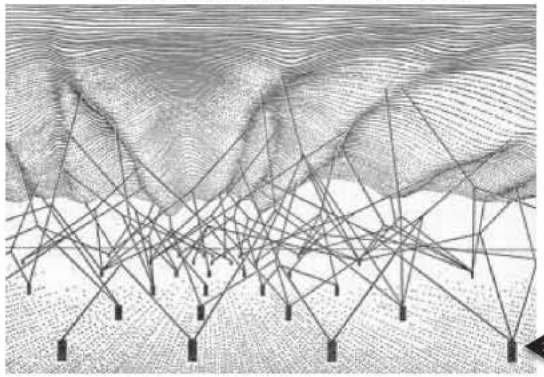
"Epigenetics is a landscape in which a cell can go down different pathways and have a different fate according to the interactions between genes and their environment"



Buffering (canalization):
Up to a certain threshold, genetic or environmental variation will not affect the pathway

Waddington proposed that **networks of genes** must be involved in defining the epigenetic landscape

A true
Systems Biologist!



Some genes can change the **topology** of the landscape. If mutated they will change the cell pathways (eg homeotic genes)

Conrad H. Waddington (1957)
The strategy of the genes (London: Allen and Unwin)

E. Heard, February 11th, 2013

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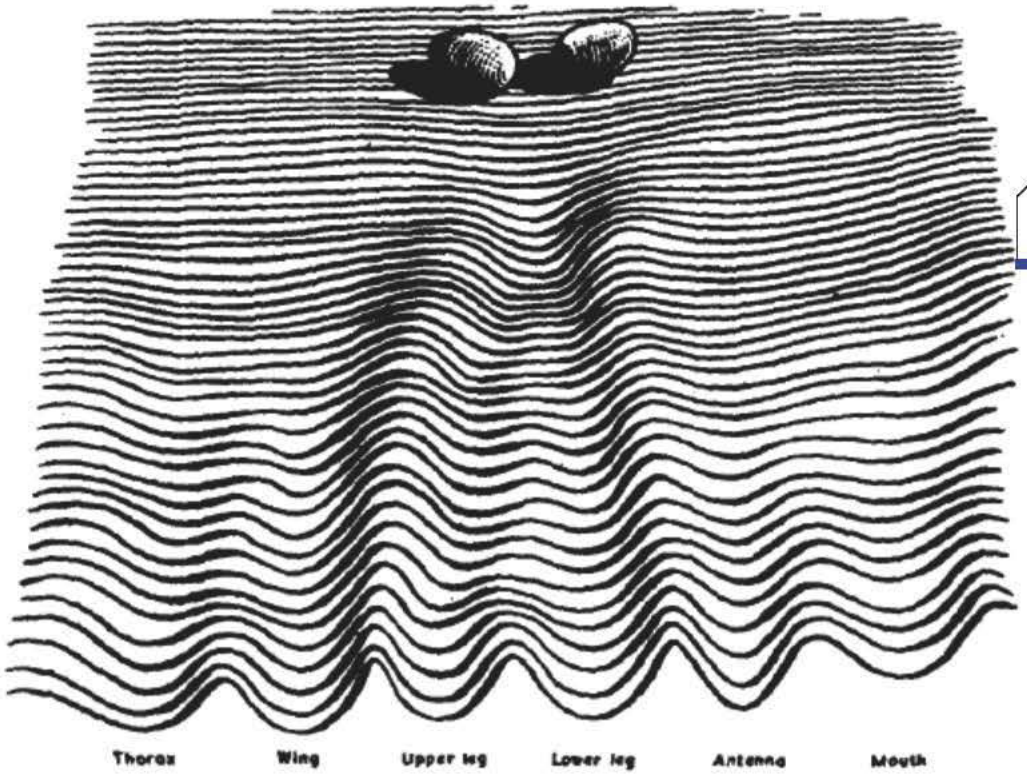
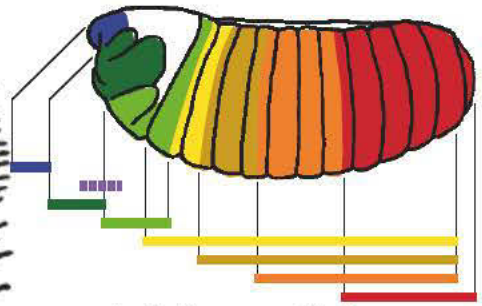
Waddington: the Artist

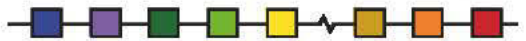
"Epigenetics is a landscape in which a cell can go down different pathways and have a different fate according to the interactions between genes and their environment"



Immature condition



Adult condition



Conrad H. Waddington (1957)
The strategy of the genes (London: Allen and Unwin)

E. Heard, February 11th, 2013

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Epigenetic Landscapes Today

Developmental potential

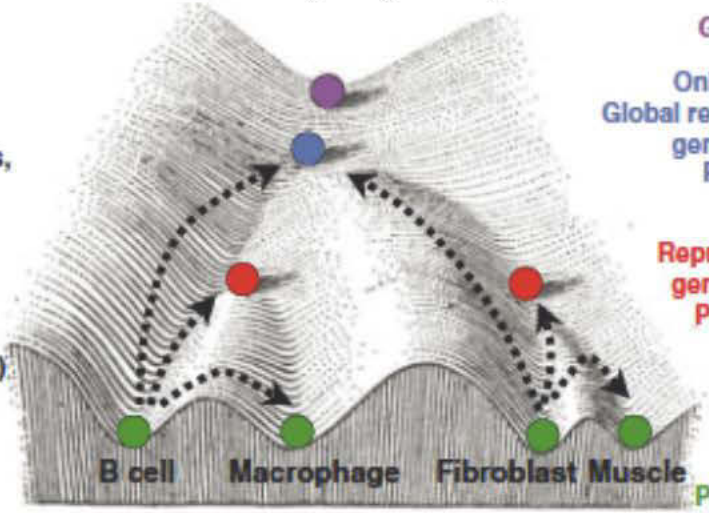
Totipotent
Zygote

Pluripotent
ICM/ES cells, EG cells, EC cells, mGS cells iPS cells

Multipotent
Adult stem cells (partially reprogrammed cells?)

Unipotent
Differentiated cell types

Epigenetic reprogramming & induced pluripotency



Epigenetic status

Global DNA demethylation

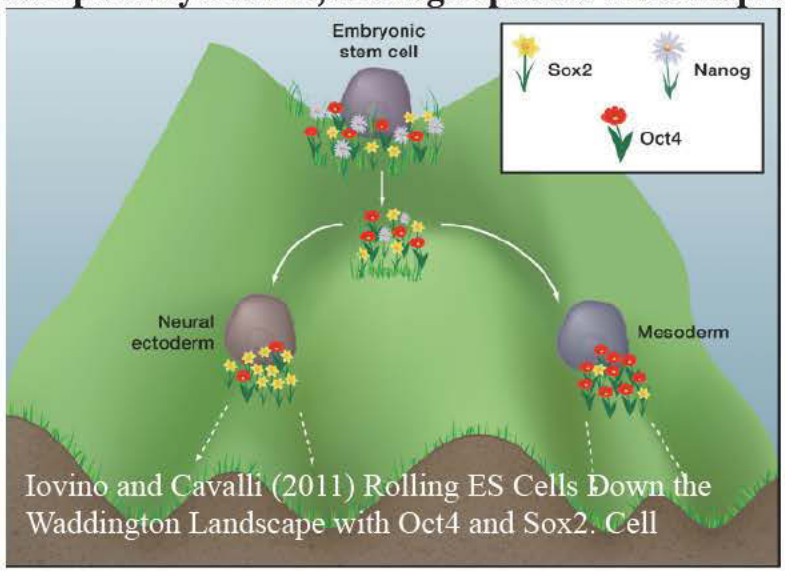
Only active X chromosomes; Global repression of differentiation genes by Polycomb proteins; Promoter hypomethylation

X inactivation; Repression of lineage-specific genes by Polycomb proteins; Promoter hypermethylation

X inactivation; Derepression of Polycomb silenced lineage genes; Promoter hypermethylation

Hochedlinger and Plath (2009) Development 136, 509-523.

Driven by Pluripotency factors, Lineage-specific Transcription Factors…



Iovino and Cavalli (2011) Rolling ES Cells Down the Waddington Landscape with Oct4 and Sox2. Cell

CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Edith Heard

Année 2012-2013 :
"Épigénétique, développement et hérédité"

Cours I

Qu'est-ce que l'épigénétique : d'Aristote à Waddington ?

Cours II

Bases moléculaires de l'épigénétique : comment lire et mémoriser la partition du génome

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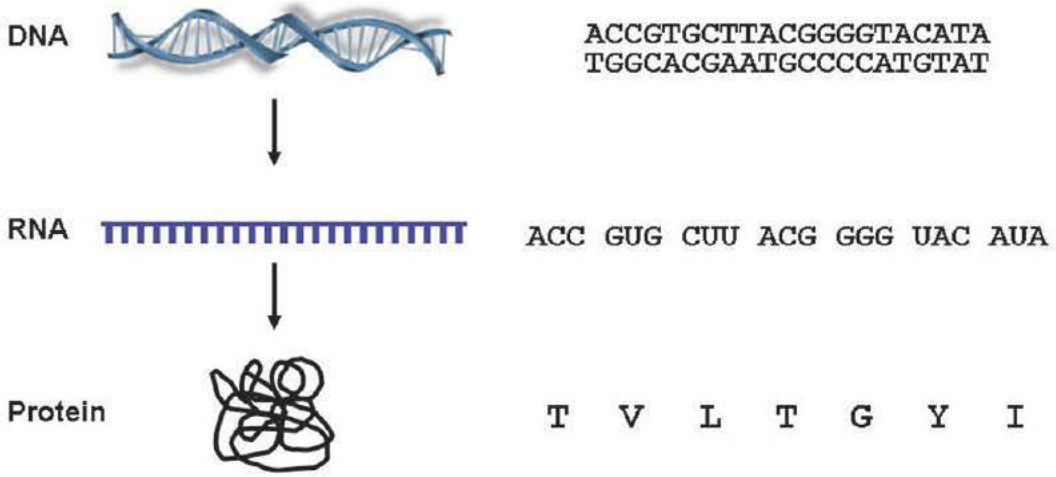
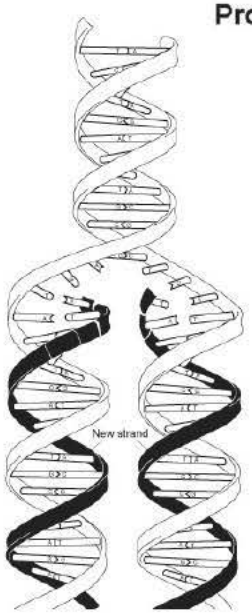
1953: Discovery of the Double Helix and all its implications



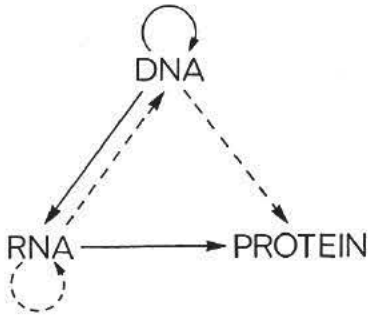


"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."
J. Watson and F. Crick, Nature, 1953

Central Dogma of Molecular Biology

by
FRANCIS CRICK
MRC Laboratory of Molecular Biology,
Hills Road,
Cambridge CB2 2QH

The central dogma of molecular biology deals with the detailed residue-by-residue transfer of sequential information. It states that such information cannot be transferred from protein to either protein or nucleic acid.



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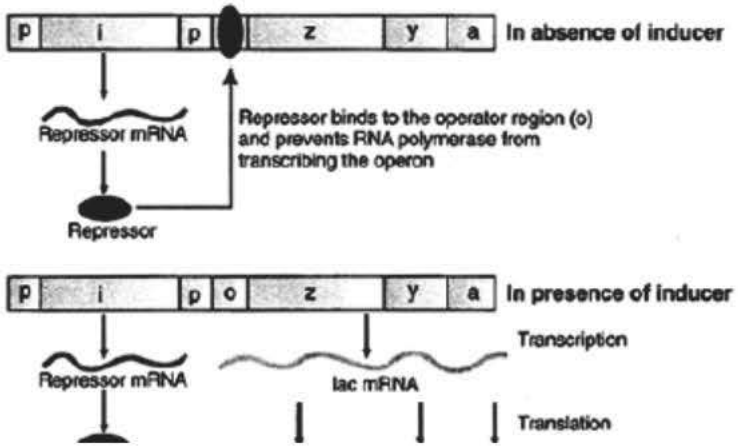
Epigenetics, Development and Genetics

How do genes and DNA control development of a complex multicellular organism from a fertilised egg ?

B. McClintock evoked "controlling elements" in the 1950's

"It is now known that controlling elements may modify gene action in a number of different ways. They may influence the time of gene action in the development of a tissue and also determine the cells in which it will occur".

The Jacob and Monod
Lac operon model

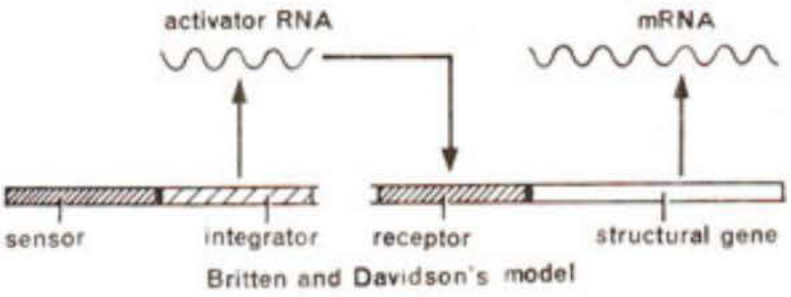


⇒ Prokaryotic and eukaryotic cells can turn genes *on* and *off*…

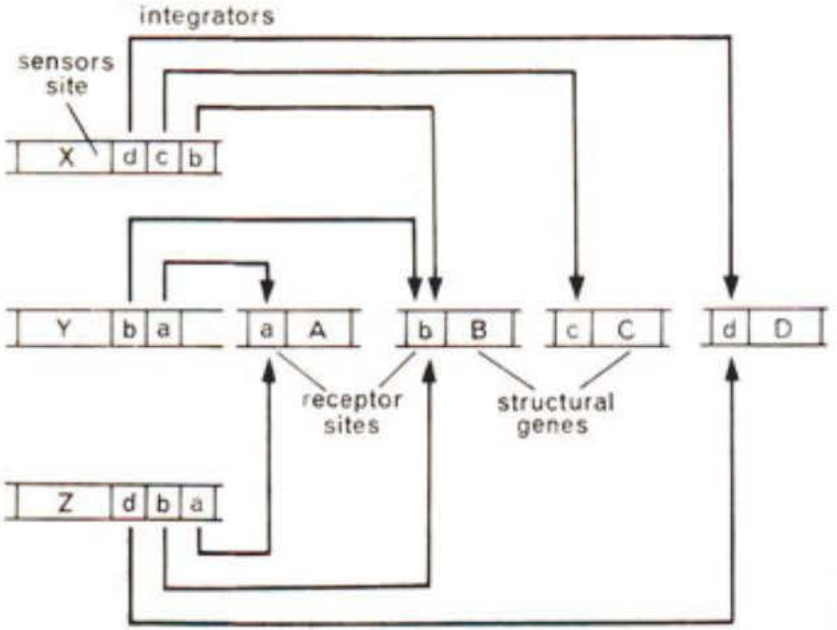
Extending the Lac Operon to Eukaryotes?

T.H. Morgan in 1934 had already evoked the idea of "gene batteries", or sets of genes that are expressed at different stages during development.

In 1969, Britten and Davidson proposed a theoretical model for how gene regulatory networks might work during differentiation, with integrated activation of large numbers of noncontiguous genes upon external signals.



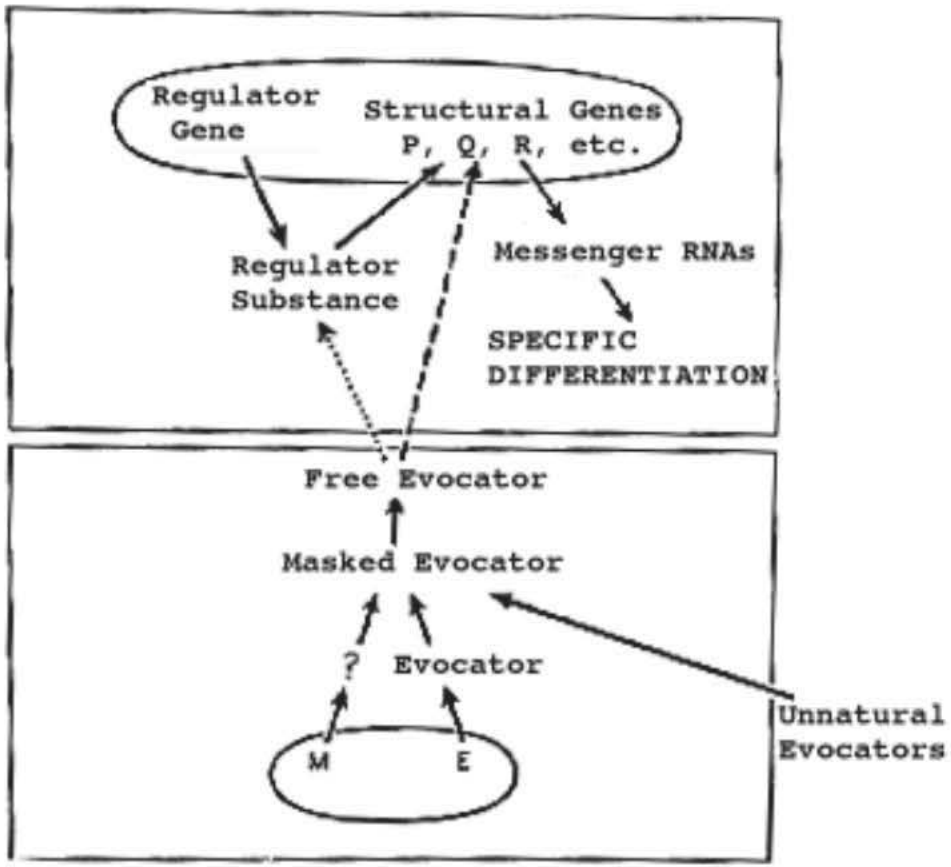
Britten and Davidson's model



Britten R.J. and Davidson E. (1969) "Gene Regulation for Higher Cells: A Theory", *Science* 165: 349-357.

Extending the Lac Operon to Eukaryotes?

Waddington also proposed a eukaryotic embryonic induction model based on the lac operon of Jacob and Monod.

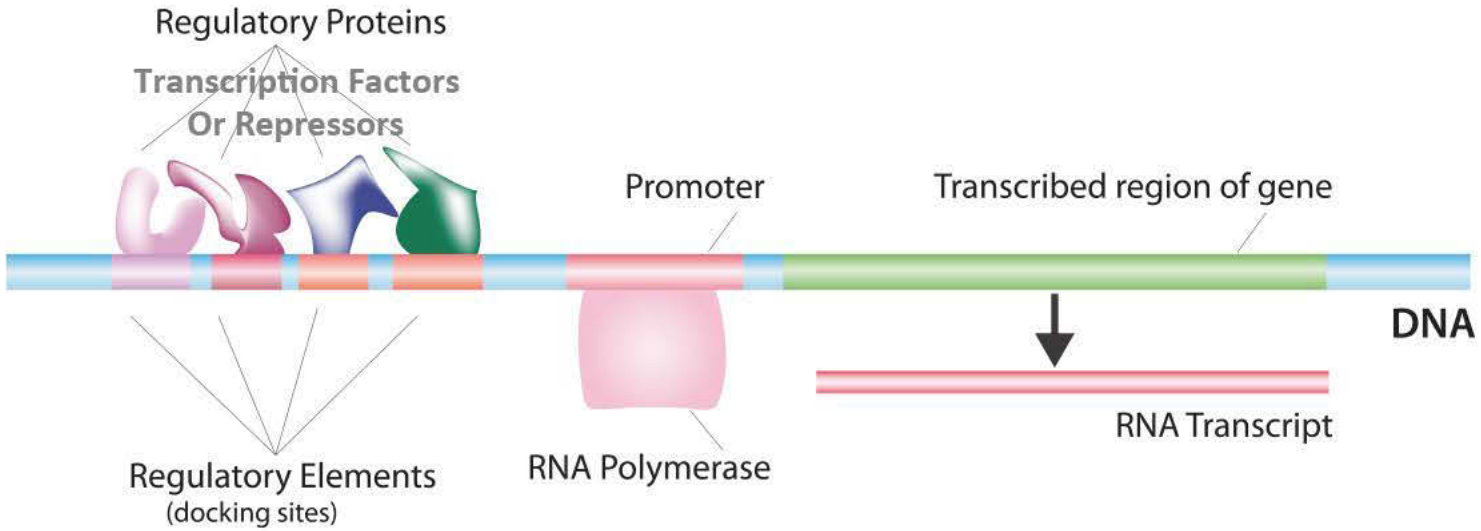


Evocator substance from one cell (below) could be freed to diffuse into neighboring cell and either directly activate a series of genes or interfere with a repressor of the transcription of those genes.

From Waddington, C. H., 1962, "New Patterns in Genetics and Development", Columbia University Press, New York.

Extending the Lac Operon to Eukaryotes?

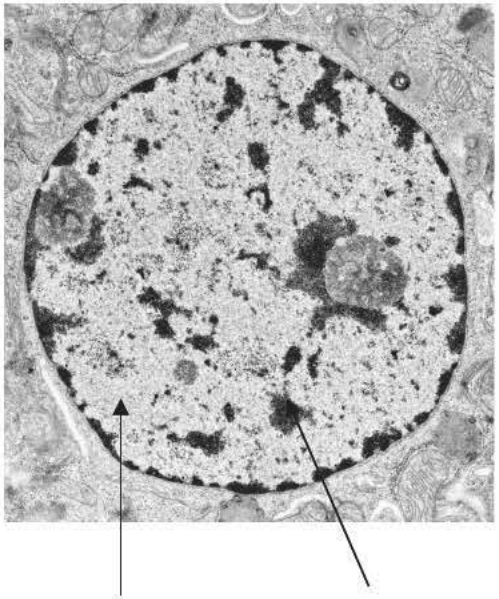
With the advent of molecular biology in 1960s and 1970s, scientists that had helped to build the basic concepts, tools and procedures of molecular genetics, including the genetic code and the operon model turned to address the facts in eukaryotic organisms.



Although basic principles of gene regulation could surely apply, eukaryotes clearly differed from prokaryotes, for example in the complexity of their genomes and of their chromatin.

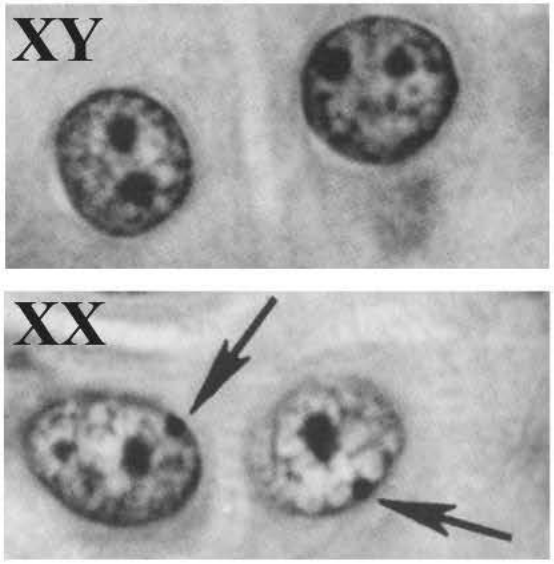
Packaging and organisation of the genetic material in the nucleus

Heteorchromatin and Euchromatin
Emile Heintz, 1929

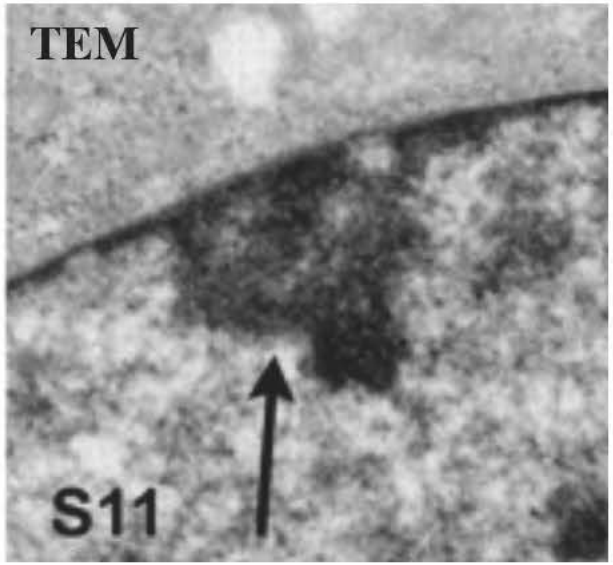


http://medcell.med.yale.edu/histology/

The Barr Body



Bertram et Barr, 1949



Rego et al, 2008

Female Mammals have one active (euchromatic) X chromosome and one inactive (heterochromatic) X
M. Lyon, 1961

Barbara McClintock proposed that "*changes in quantity, quality or structural organization of heterochromatic elements may well alter the kind and/or degree of particular exchanges that occur, and in this way control the chromosome organization and the kind and the relative effectiveness of genic action*" (McClintock, 1950).

Chromatin states are proposed to influence transcription

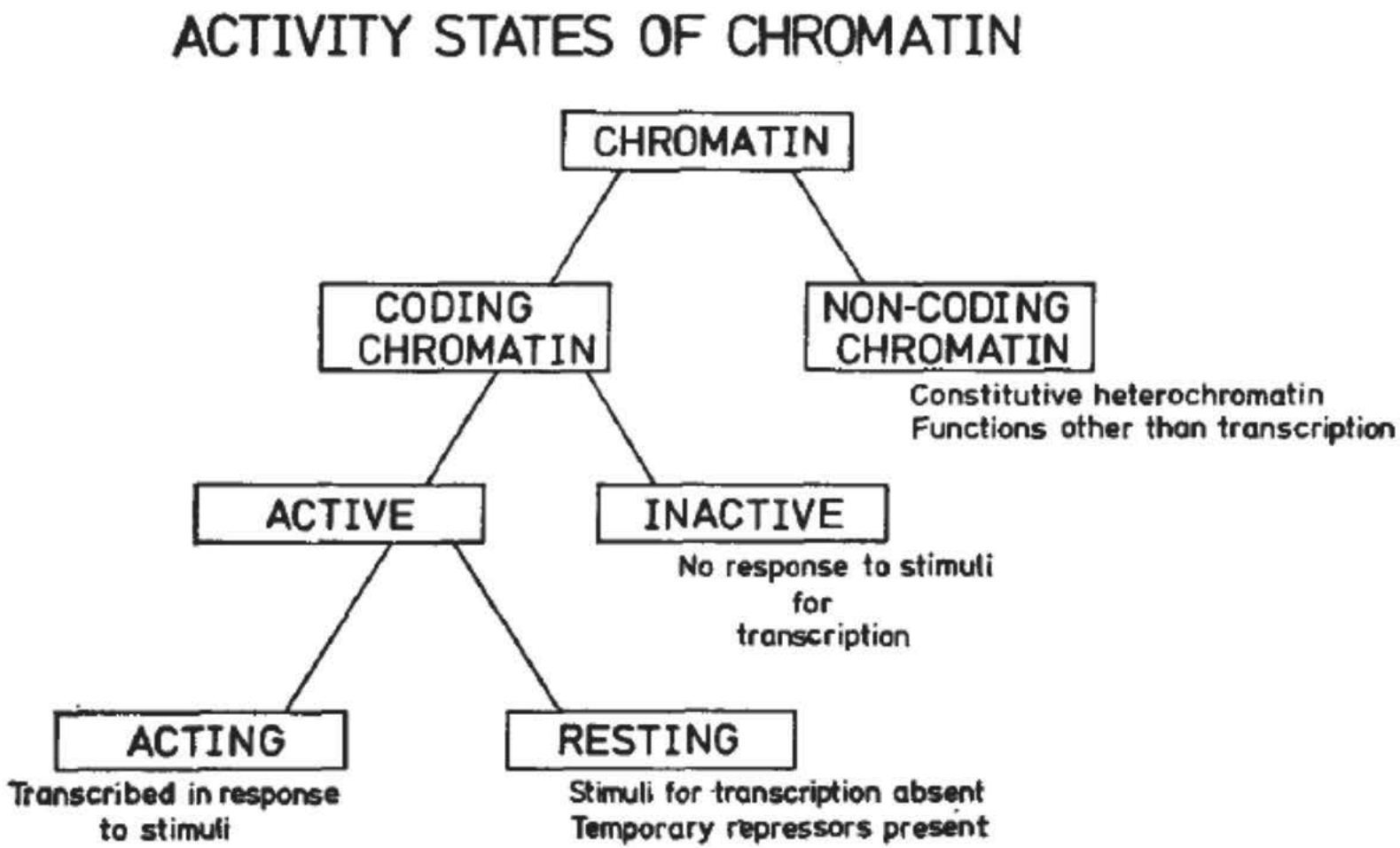


FIGURE 1.—Classification of eukaryote chromatin according to its functional state.

Genetics 78: 305–309 September, 1974.

Mary Lyon, 1974

1970's & 80's: Epigenetics and the notion of Inheritance

In Waddington's definition of Epigenetics, changes in gene regulation and activity during development were implicit; the notion of heritability less so.

In the 1970's-80's a major shift took place in the use of the word, to include the notion of *transmission* or *heritability* of gene expression states.

WHY?

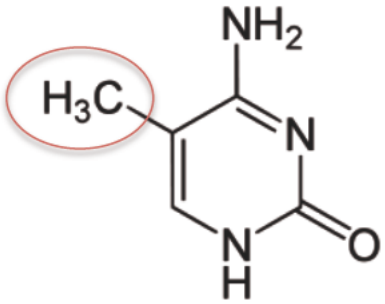
1. **Observations from cultured cells** raised the question of **somatic inheritance** : how could replicating cells "remember" their differentiation state with such high fidelity?

2. **Stem cell differentiation**: what caused **switches in gene activity**? The realization that some specialized genes, which determine the phenotype of differentiated cells are permanently turned on, and other genes—active in some other cell type—are permanently turned off. Some of these controls must be **mitotically heritable** – how?

3. **X-chromosome inactivation (XCI)**: how is one of the 2 X chromosomes stably shut down during development – what triggers the **switch in gene activity** and how is it subsequently made **somatically heritable**?

4. Phenomena with **unusual (non-Mendelian) inheritance** eg XCI, Paramutation in maize, imprinting….

Proposal of DNA Methylation as an Epigenetic modification



5-Methylcytosine

In 1975, Robin Holliday and Art Riggs independently postulated that:

1. DNA methylation might affect gene expression

2. Changes in DNA methylation could explain switching on & off of genes in development.

3. Predicted existence of enzyme(s) methylating a particular region of DNA – either by sequence specific binding, or via interaction with other proteins that were sequence specific

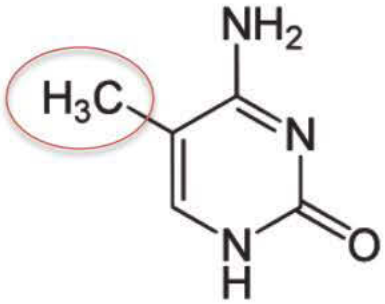
4. DNA methylation pattern could be heritable, if maintenance methylases existed that recognize hemi-methylated DNA soon after replication, but do not act on unmethylated DNA

⇒ mechanism for heritability of the methylated and non-methylated DNA

⇒ heritability of a given pattern of gene activities

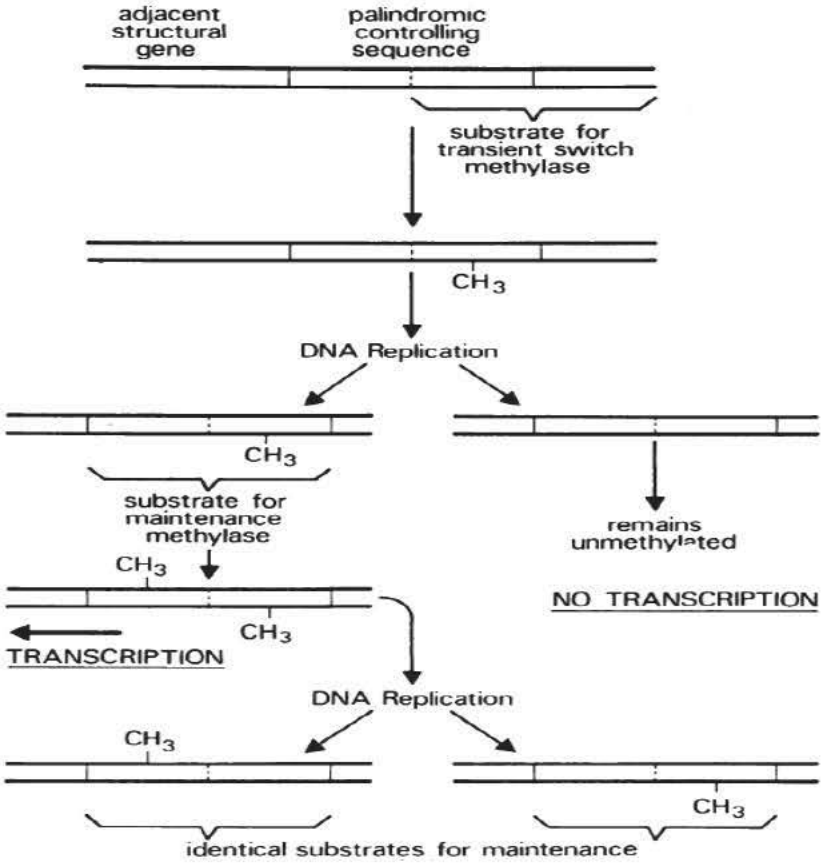
Proposal of DNA Methylation as an Epigenetic modification

Robin Holliday
Geneticist



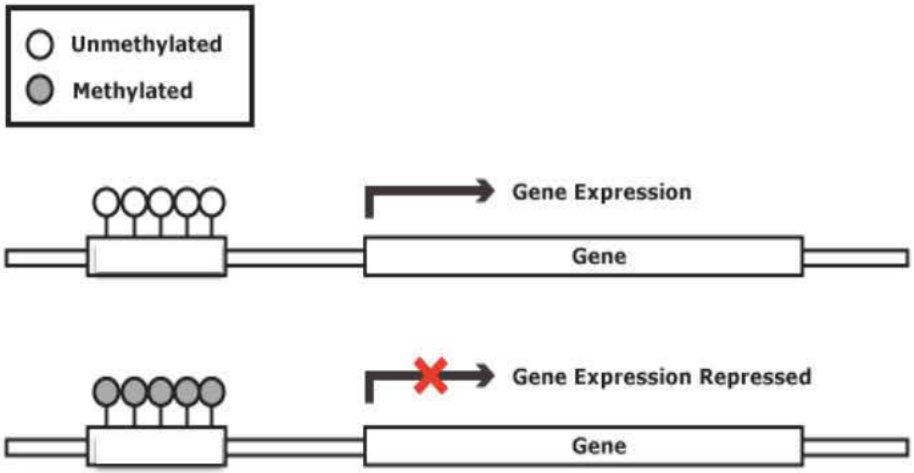
5-Methylcytosine

Art Riggs
Biochemist



X inactivation, differentiation, and DNA methylation

A.D. RIGGS



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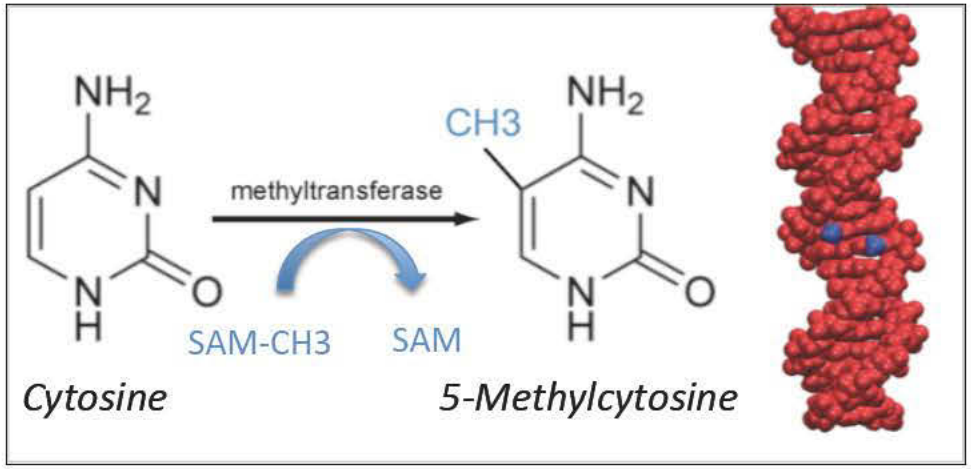
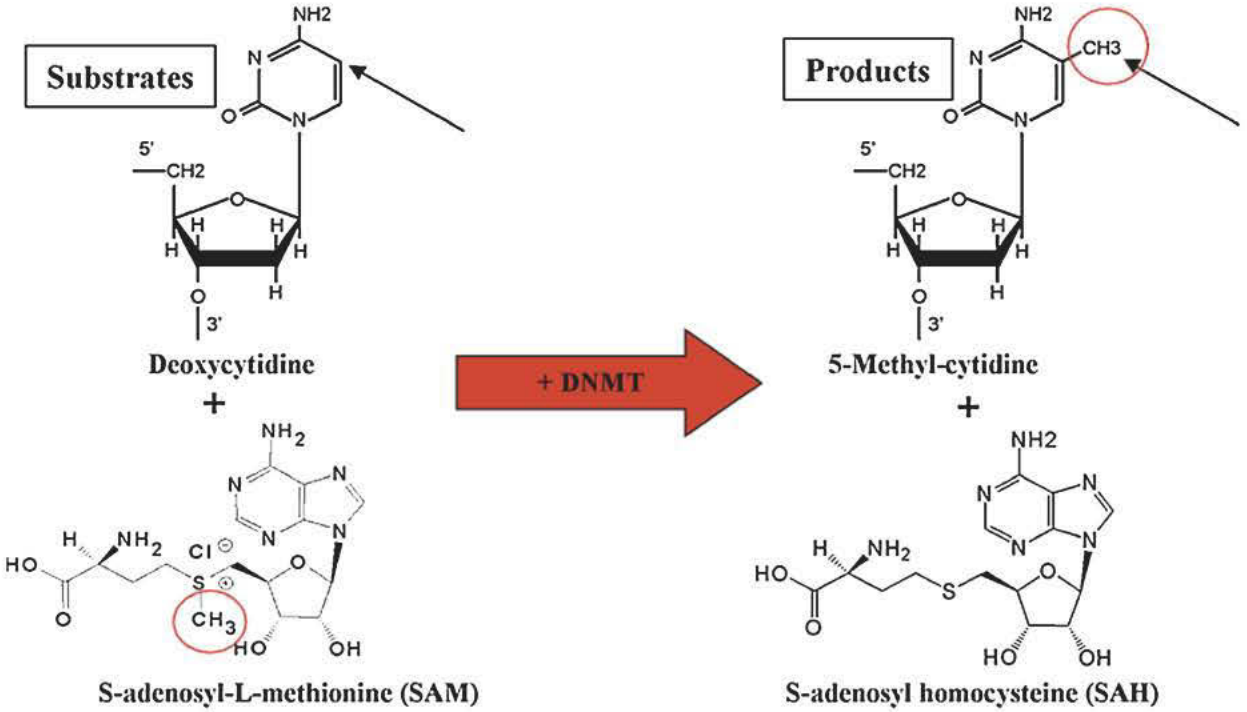
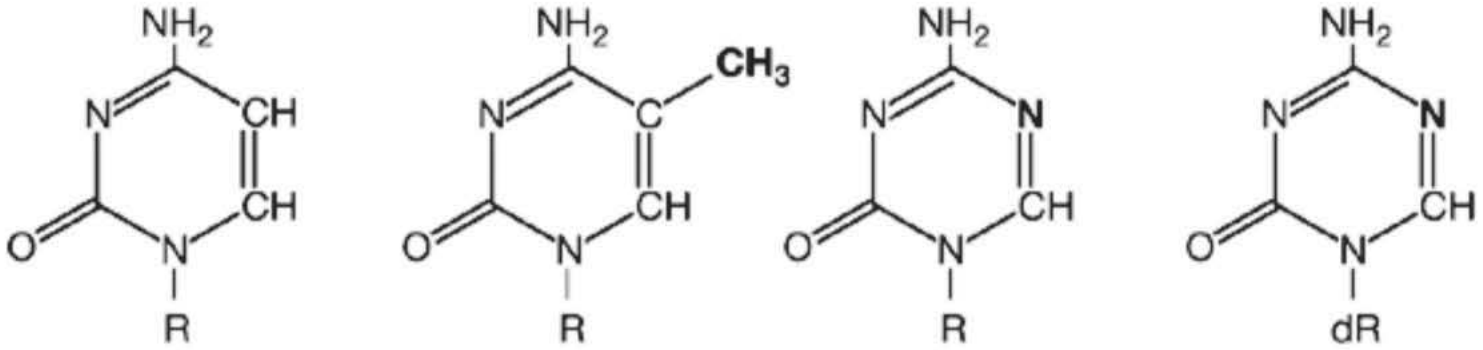
Proposal of DNA Methylation as an Epigenetic modification





http://www.ks.uiuc.edu/Research/methylation/

Inhibition of DNA Methylation could affect gene expression

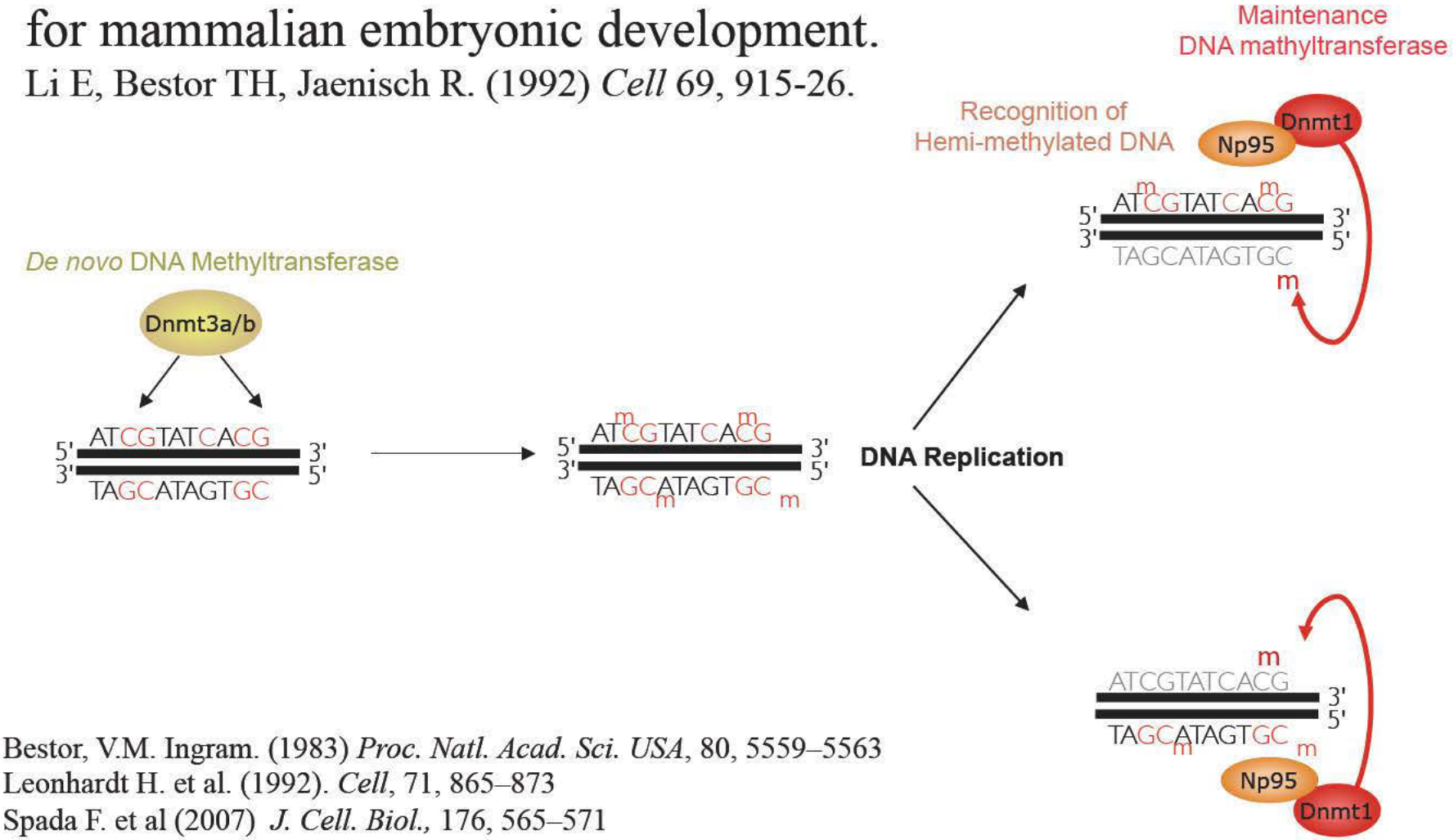


With the advent of the DNA methylation inhibitor 5-azacytidine (Jones, 1984), data on cultured mammalian cells showed that gene expression could be affected by methylation, and it was proposed that the inactive expression state may be "locked in" by DNA methylation (Razin and Riggs,1980; Lock et al., 1987).

DNA Methylation of Cytosine in CpG dinucleotides

Dnmt1 and DNA methylation are essential for mammalian embryonic development.

Li E, Bestor TH, Jaenisch R. (1992) *Cell* 69, 915-26.



Bestor, V.M. Ingram. (1983) *Proc. Natl. Acad. Sci. USA*, 80, 5559–5563
Leonhardt H. et al. (1992). *Cell*, 71, 865–873
Spada F. et al (2007) *J. Cell. Biol.,* 176, 565–571
Bostick, M. et al (2007) *Science* ;317, 1760-4.
Sharif, J. et al (2007) *Nature* 450, 908-12.

Epigenetics and Heritable States

A new definition of Epigenetics:

The study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence.

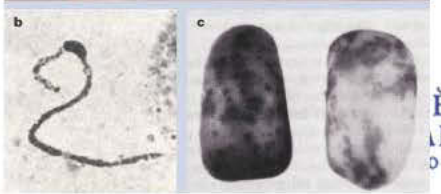
Holliday, R. (1987) "The inheritance of epigenetic defects." *Science* 238:163-70.
Holliday, R. (1994) Holliday R. "Epigenetics: an overview". *Dev Genet* 15:453-7.
Russo, V.E.A., R.A. Martienssen & A.D. Riggs Eds. (1996) "Epigenetic mechanisms of gene regulation." *Cold Spring Harbor Laboratory Press*.. p. 1.

Epimutation:

Heritable changes in genes that are not due to changes in DNA sequence.
(Holliday, 1987)

Processes that could now be classified under this new definition

X-chromosome Inactivation
Genomic Imprinting
Paramutation
Transposon silencing
Changes in Phase
Position Effect Variegation



E. Hea

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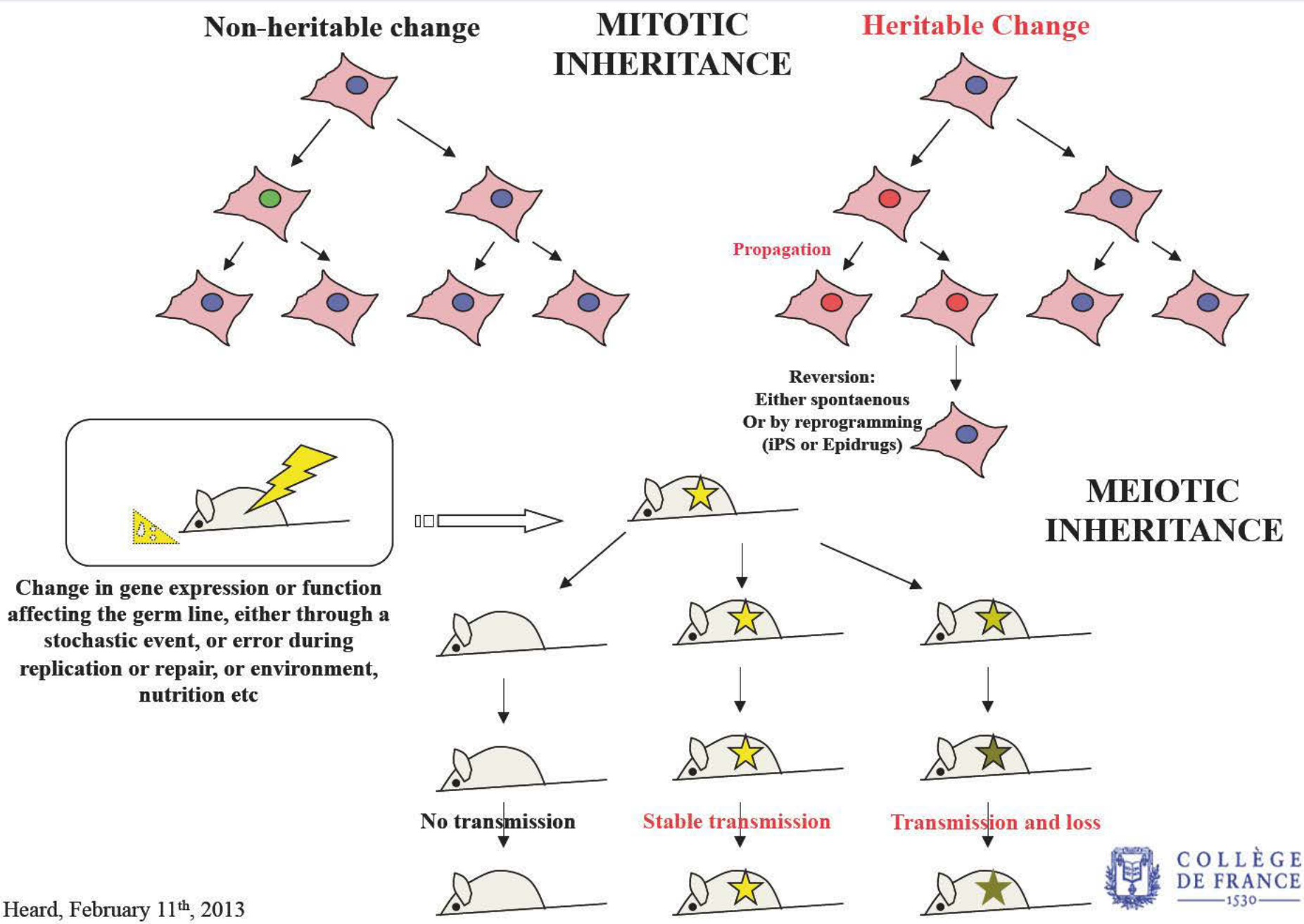
Epigenetics and Heritable States



E. Heard, February 11th, 2013

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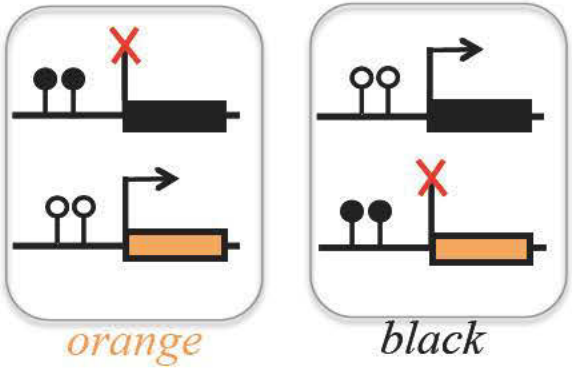
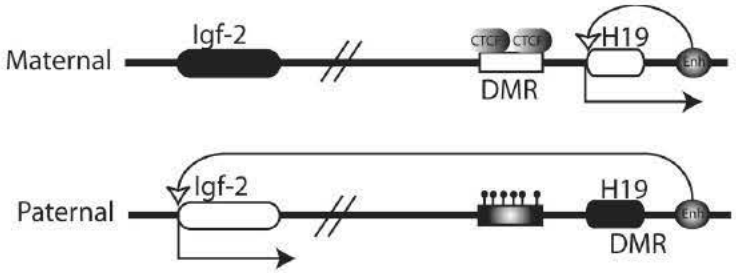
Many Epigenetic Processes are dependent on DNA Methylation

X inactivation

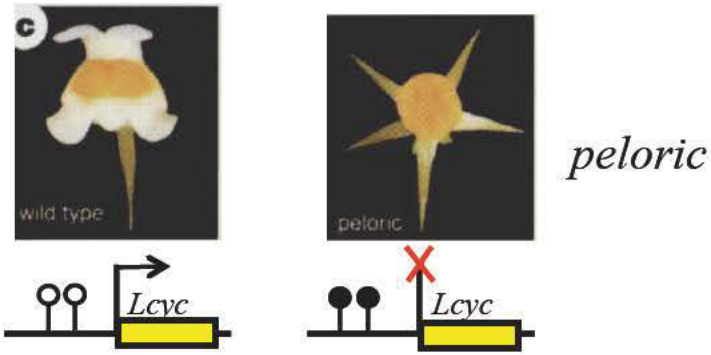


Imprinting



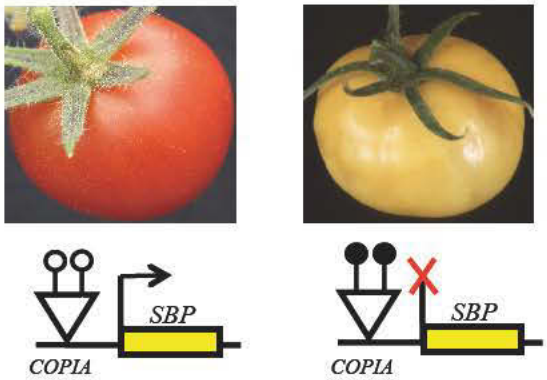
methylation

Morgan et al., 1999



peloric

(Cubas et al, Nature, 1999)



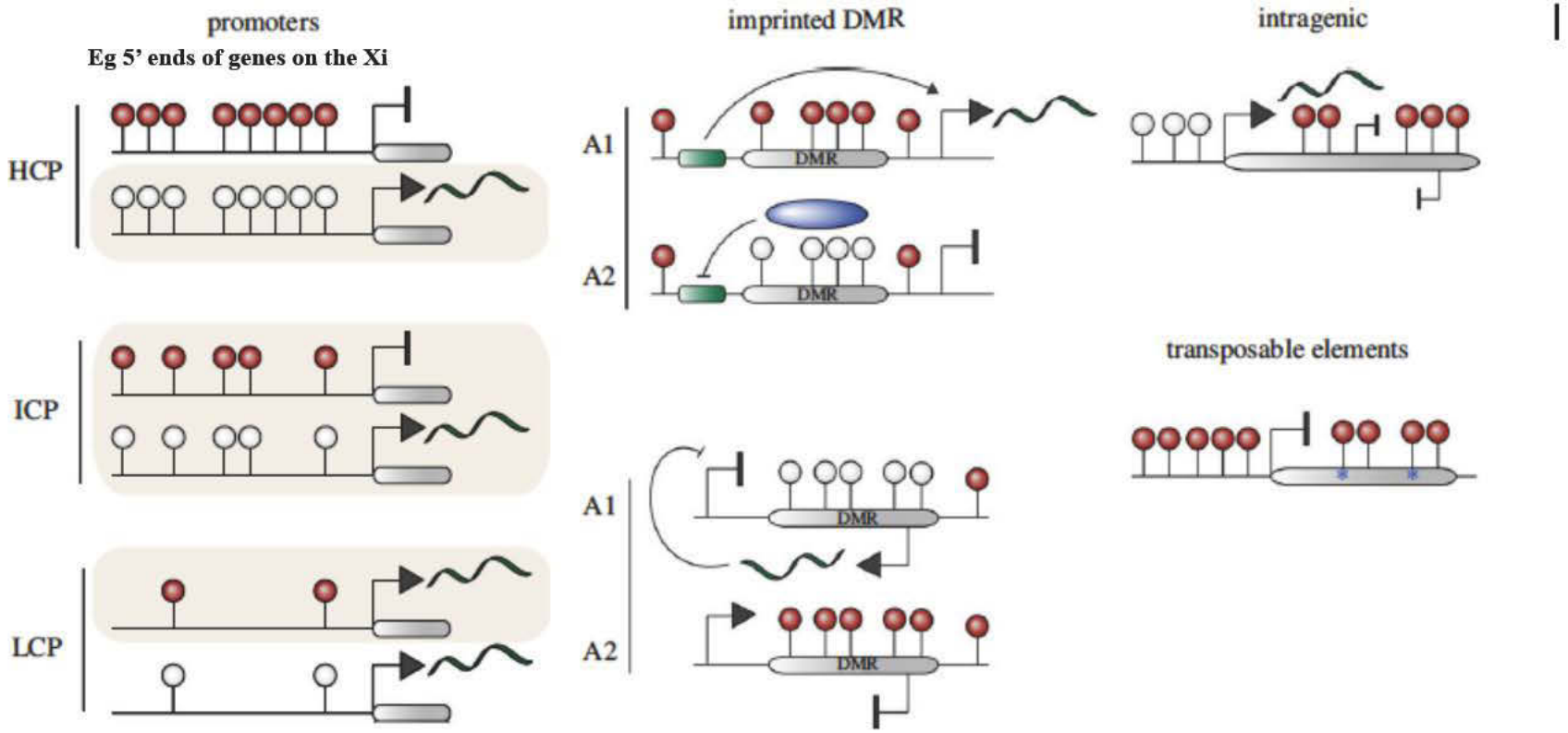
cnr

(Manning et al, Nat Genet, 2006)

Where is DNA Methylation in the genome?

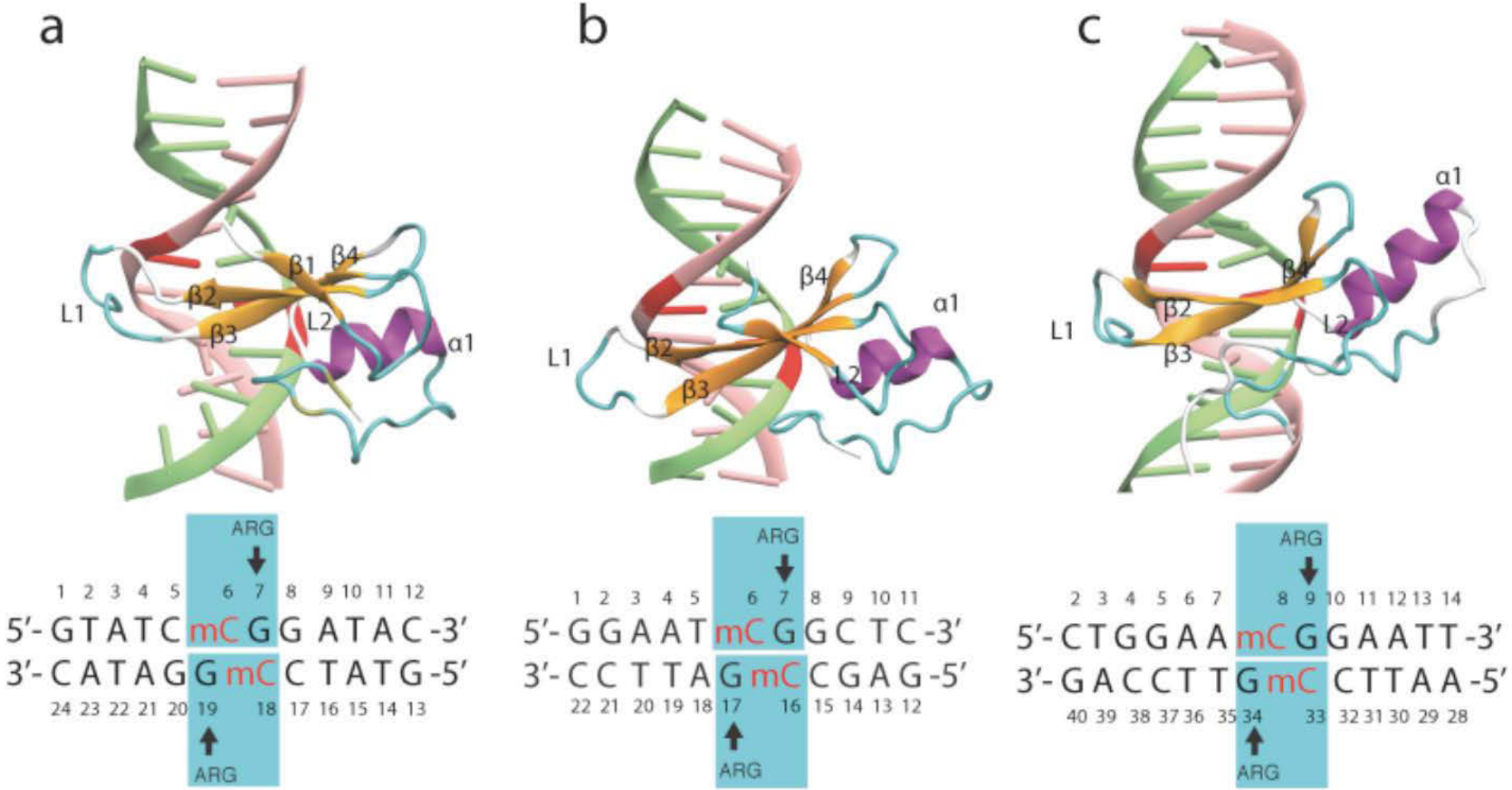
What does it do there?

- Prevent binding of factors (CTCF, transcription machinery…?)
- Facilitate recruitment of factors (MBD proteins, co-repressor complexes…?)



Reviewed by Hackett J. and Surani, A. "DNA Methylation dynamics during the mammalian life cycle" *Phil.Trans. Of the Royal Soc.* (2013)

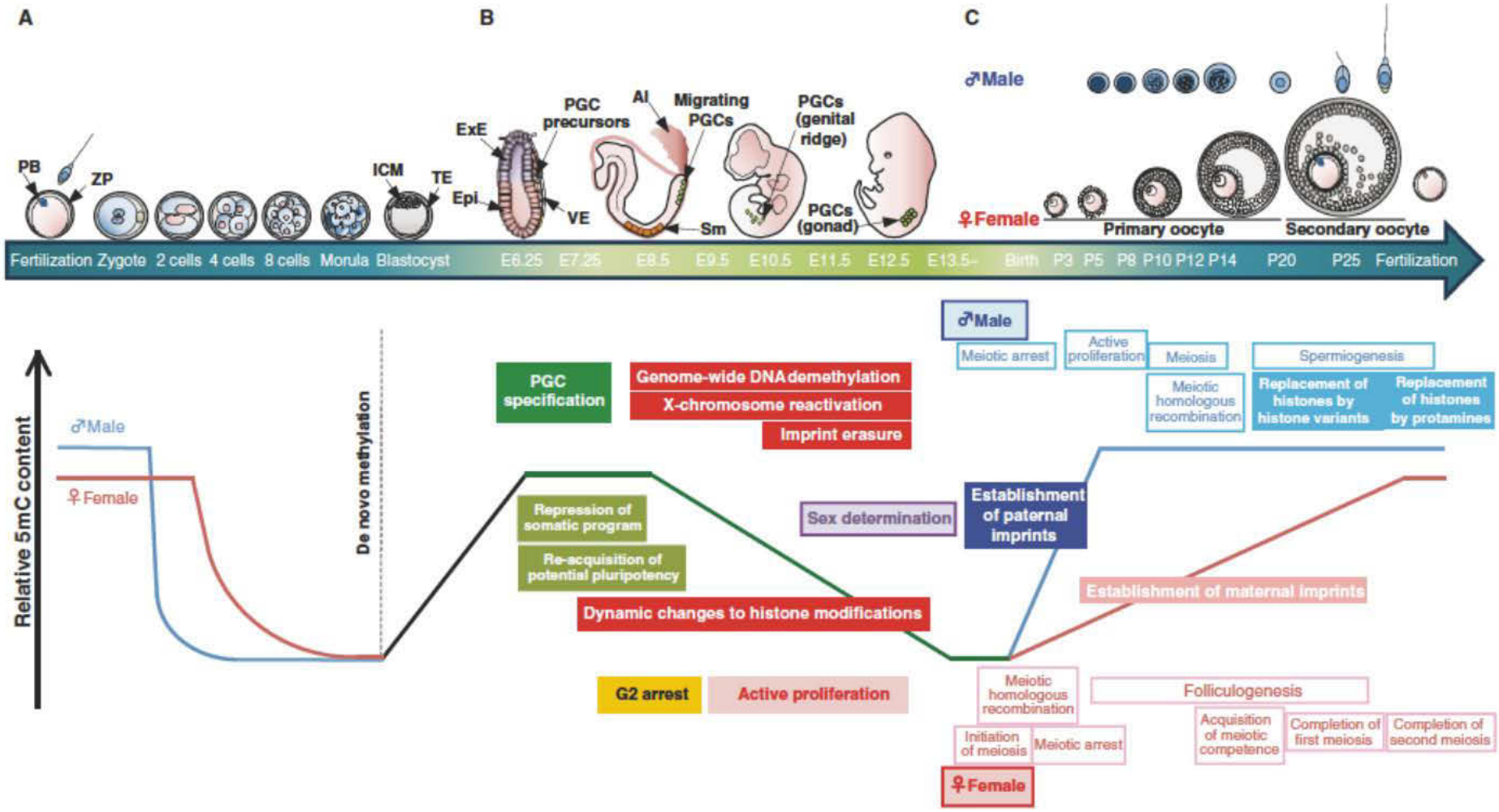
DNA Methylation Binding Proteins



MBD proteins binding to mDNA. Shown are the structures of
(a) MBD1-mDNA, (b) MBD2-mDNA and (c) MeCP2-mDNA complexes.

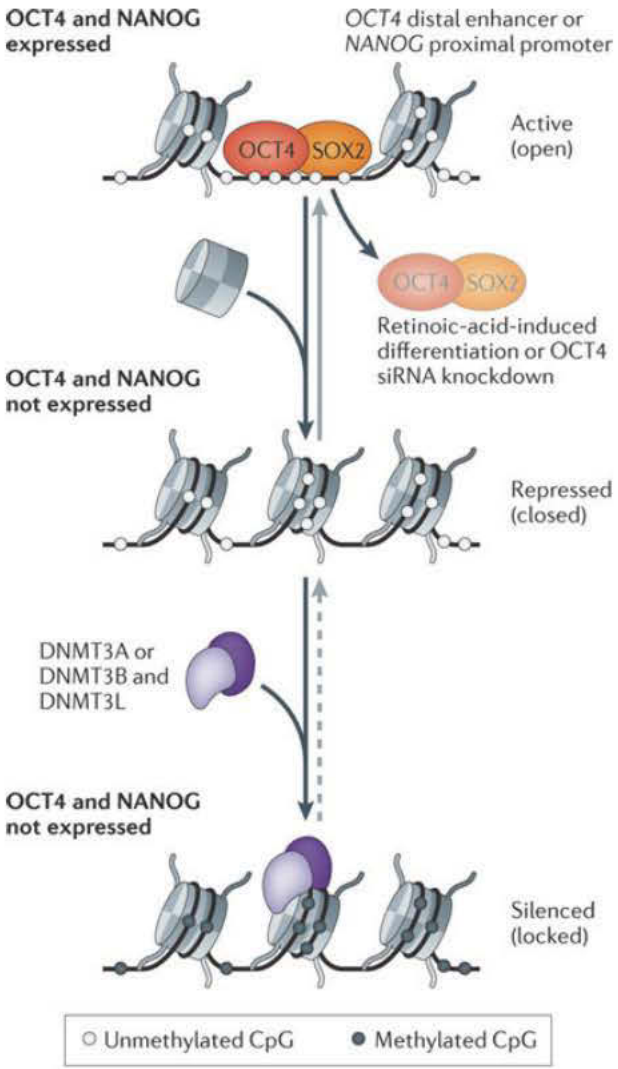
http://www.ks.uiuc.edu/Research/methylation/

How do DNA Methylation patterns change during development?



Saitou, M. (2012) Development

How do DNA Methylation patterns change during development?



Silencing usually precedes DNA methylation:

Active promoters and enhancers have nucleosome-depleted regions (NDRs) that are often occupied by transcription factors and chromatin remodellers.

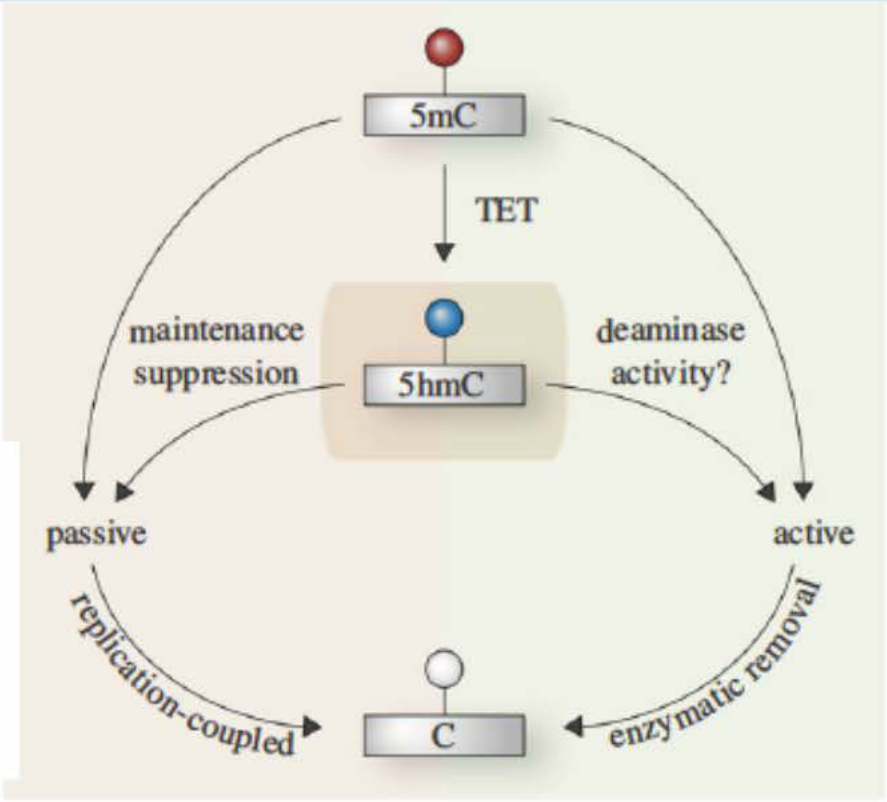
Loss of factor binding — for example, during differentiation — leads to increased nucleosome occupancy of the regulatory region, providing a substrate for *de novo* DNA methylation.

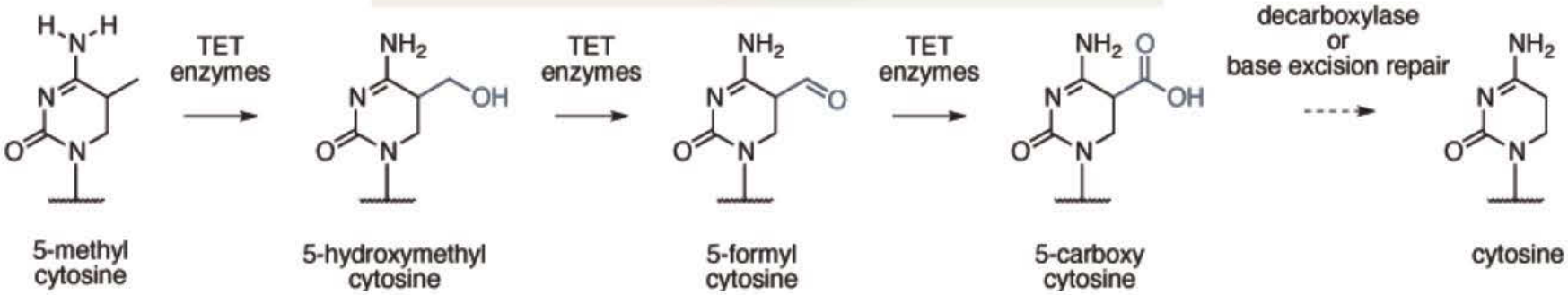
DNA methylation subsequently provides added stability to the silent state and is likely to be a mechanism for more accurate epigenetic inheritance during cell division.

Jones, P. (2012) Functions of DNA methylation: islands, start sites, gene bodies and beyond
Nature Rev. Genetics 13, 484-493

DNA Methylation can be lost passively, or actively eg via the TET enzymes, creating new modifications

NB similarity between 5mC and its oxidation derivatives means that conventional techniques used for 5mC analysis cannot distinguish between 5mC and 5hmC/ 5fC/5caC.





Active demethylation of 5-methylcytosine by iterative oxidation to 5-hydroxycytosine, 5-formylcytosine and 5-carboxycytosine, followed by decarboxylation/base excision repair.
Adapted from Hackett and Surani (2013), and Wu and Zhang (2010)

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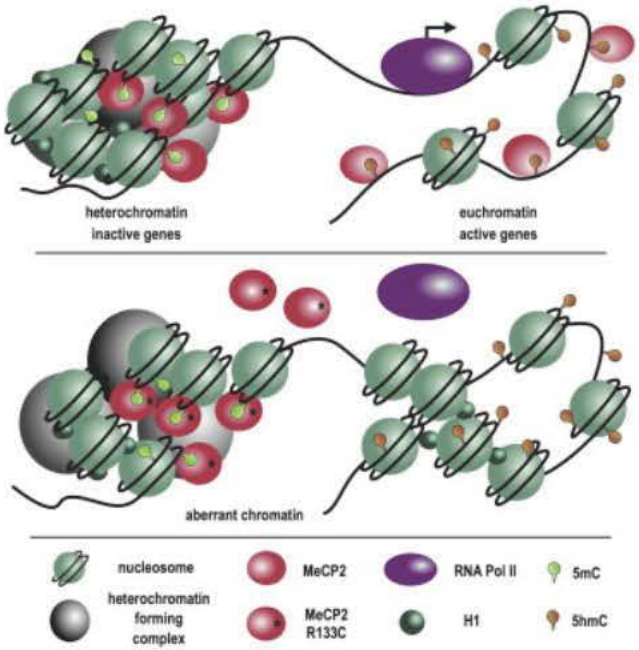
TET enzymes and DNA methylation derivatives: the expanding horizon of epigenetics

MeCP2 Binds to 5hmC Enriched within Active Genes and Accessible Chromatin in the Nervous System

Mellén et al (2012), Cell 151:1417-1430.

MeCP2 binds not only 5mC, but also 5hmC, a cytosine derivative enriched in active genes in neurons. A Rett syndrome mutation in MeCP2 preferentially inhibits 5hmC binding, suggesting a possible involvement of MeCP2's ability to "read" 5hmC in normal brain development.



Tet3 CXXC Domain and Dioxygenase Activity Cooperatively Regulate Key Genes for Xenopus Eye and Neural Development. Xu et al (2012), Cell 151:1200-1213.

Xenopus Tet3 plays an essential role in early eye and neural development by directly regulating a set of key developmental genes. Tet3 is an active 5mC hydroxylase regulating the 5mC/5hmC status at target gene promoters.

Strong links between TET mutations and cancer:

Mutations of TET2 are associated with decreased 5hmC levels in various myeloid leukemias (Delhommeau et al., 2009; Langemeijer et al., 2009), and Tet2 deficiency leads to increased hematopoietic stem cell self-renewal and myeloid transformation in mouse (Moran-Crusio et al., 2011; Quivoron et al., 2011). TET1 and TET2 play critical roles in melanoma and breast cancer (Hsu et al., 2012; Lian et al., 2012).

DNA methylation is not a universal Epigenetic modification

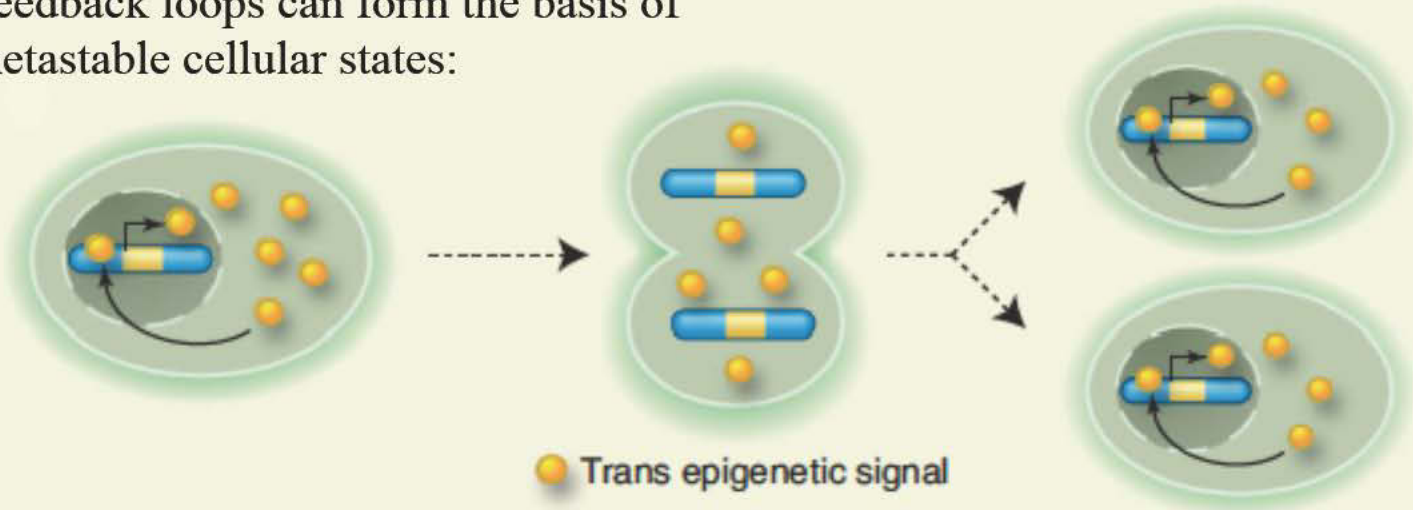
- Important maintenance mark in mammals and plants
  – enabling somatic memory and trans-generational memory

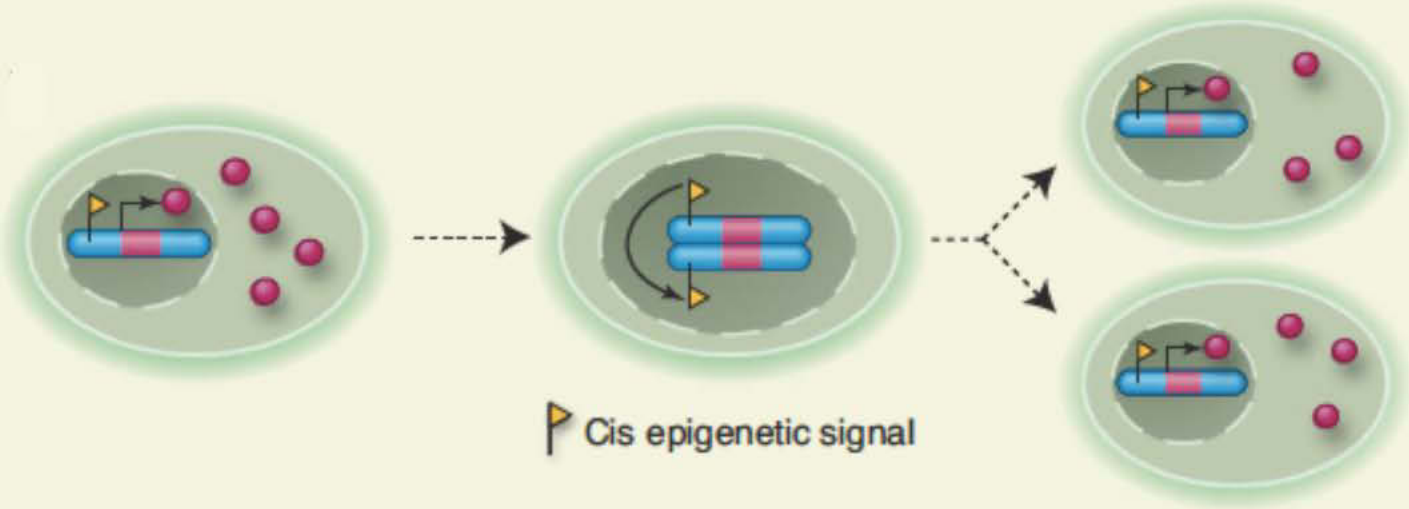
- Essential roles in mammals and other vertebrates, as well as plants and some fungi

- But lacking in several organisms, eg *Drosophila*, *C. elegans*

Heritable changes in gene expression: Other mechanisms?

Feedback loops can form the basis of metastable cellular states:



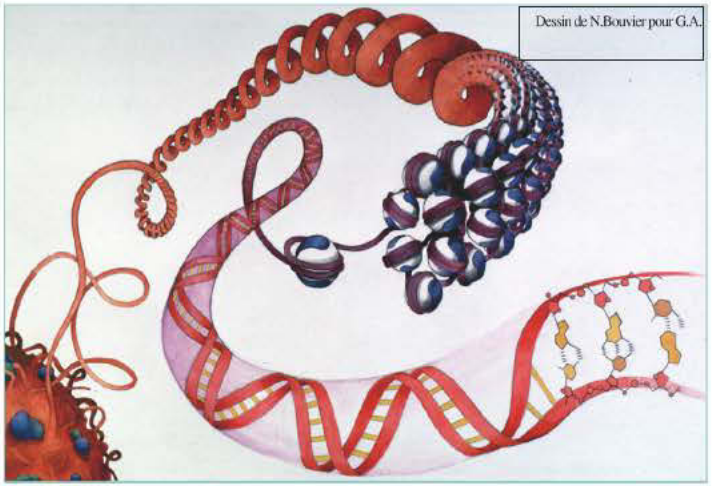


Roberto Bonasio, *et al.*
Molecular Signals of Epigenetic States, Science, 2010.

EPIGENETIC MECHANISMS

Actors involved in cellular memory

Chromatin



Histone modifications and variants

Chromatin associated proteins eg PcG, TrX

Chromatin remodelling

DNA methylation

Bookmarking factors (eg FoxA)

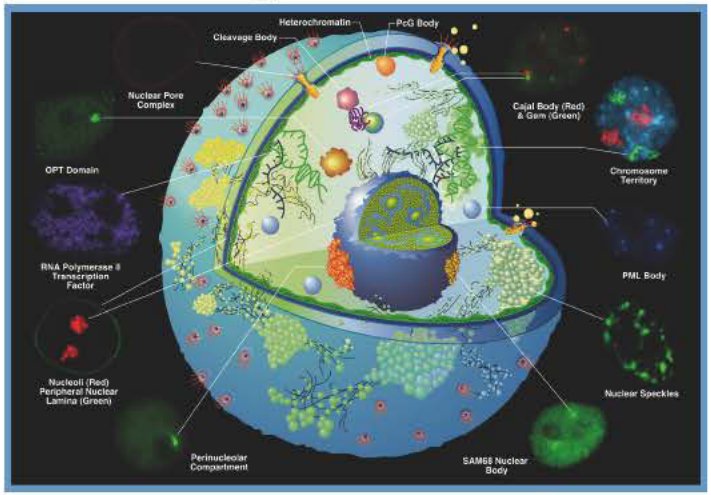
Non-coding RNAs

Long non-coding RNAs (eg XIST, Airn…)

Intergenic trancripts

Small RNAs (siRNAs, miRNAs…)

Nuclear Organisation



Nuclear compartments and bodies

3D domain topology

Cis and *trans* interactions

Cytoplasmic components

Prions…

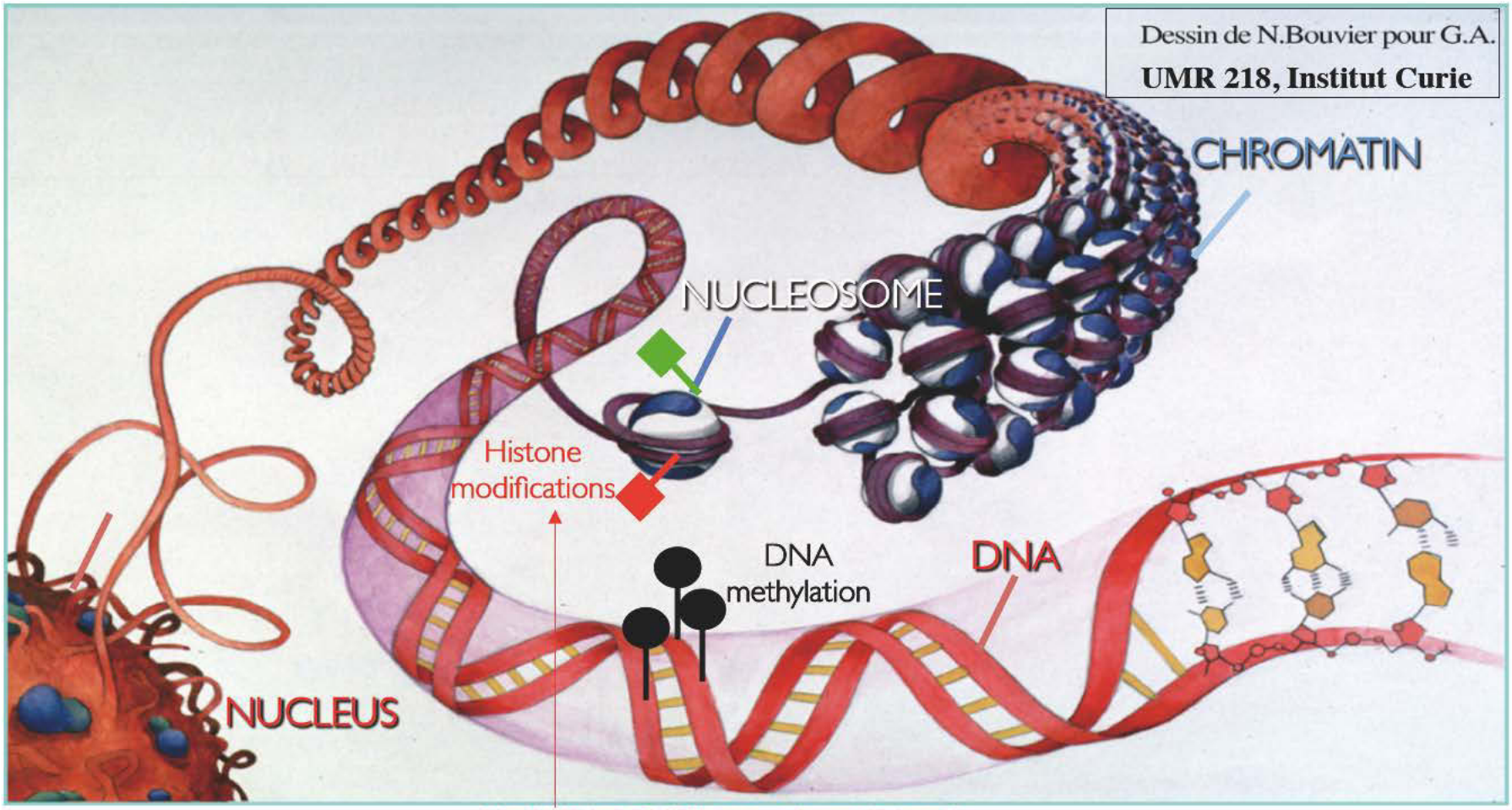
Cell Structures

Cell surface structures

(eg cilia)

Chromatin-based Epigenetic Mechanisms

Packaging of the genome; Regulation of gene activity,
Perpetuation of activity states through the cell cycle, DNA replication and repair

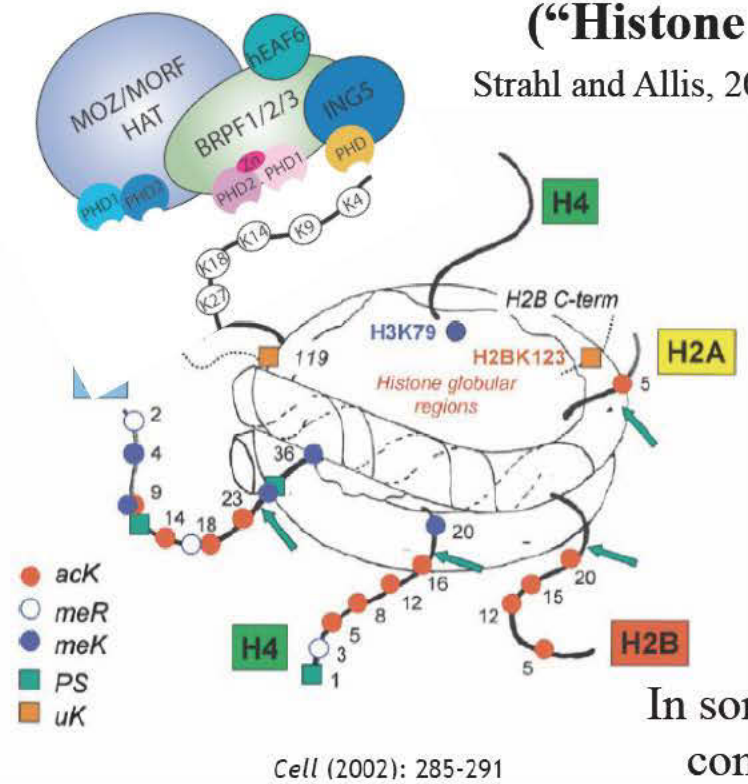


Methylation (HMTases - demethylases);
Acetylation (HATs - HDACs)
Phosphorylation (kinases - phosphatses)etc

Histone modifications

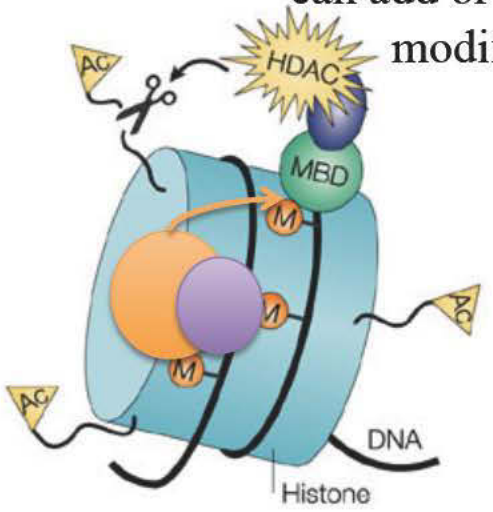
Mediators of chromatin accessibility
Platforms for binding proteins
("Histone Code"?)

Strahl and Allis, 2001; Turner 2001



Cell (2002): 285-291

Histone modifying enzymes can add or remove these modifications



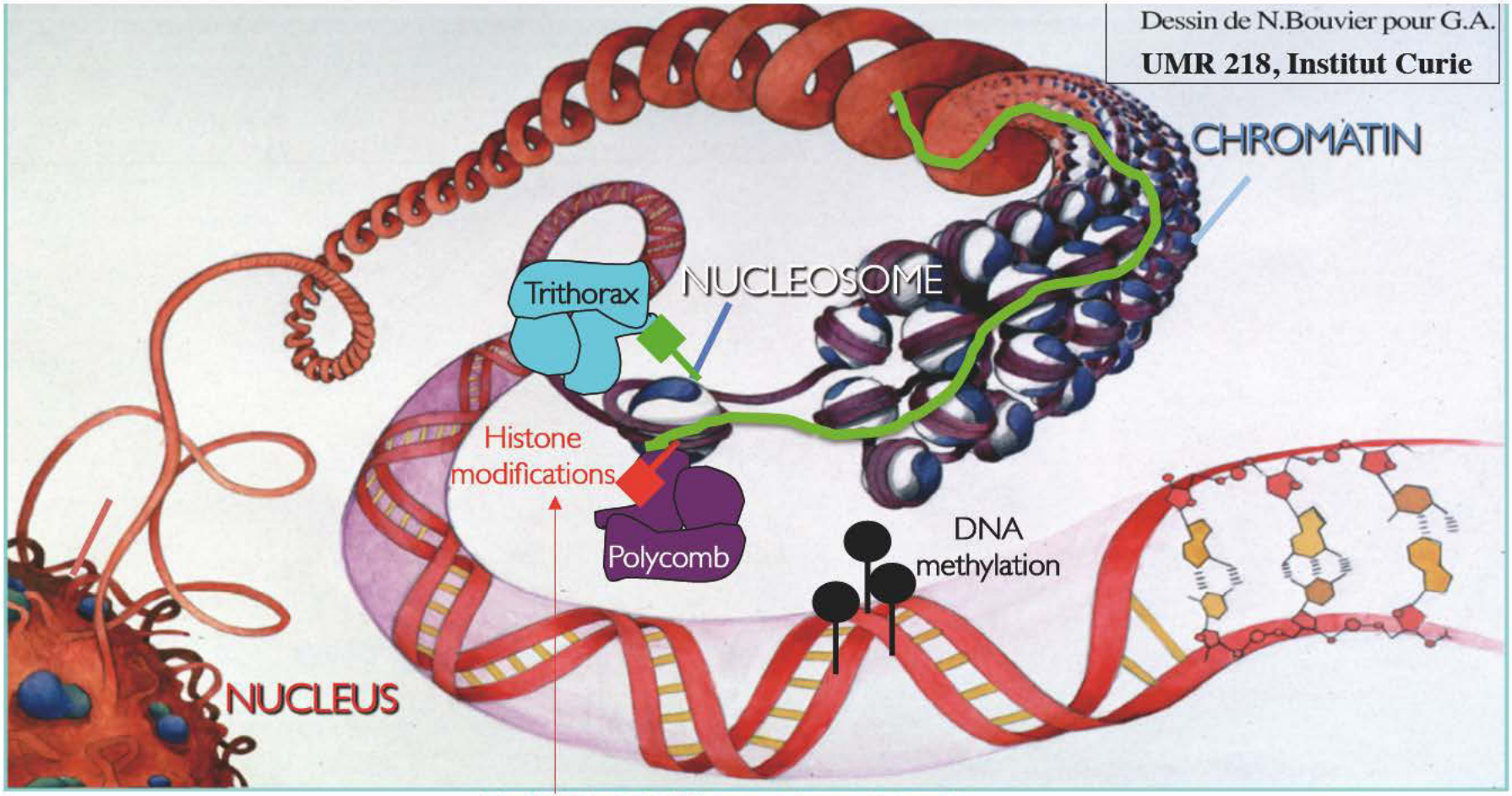
In some cases, histone 'readers' and 'writers' are in complexes, possibly enabling self-perpetuation and/or spreading in *cis*
eg HP1/H3K9me3; PRC2/H3K27me3 etc

Kornberg RD (1974) "Chromatin structure: a repeating unit of histones and DNA". Science 184, 868–871.
Lorch Y et al. (1987) "Nucleosomes inhibit the initiation of transcription but allow chain elongation". Cell 49, 203-210.
Han M, Grunstein M (1988). "Nucleosome loss activates yeast downstream promoters in vivo". Cell 55, 1137-1145.

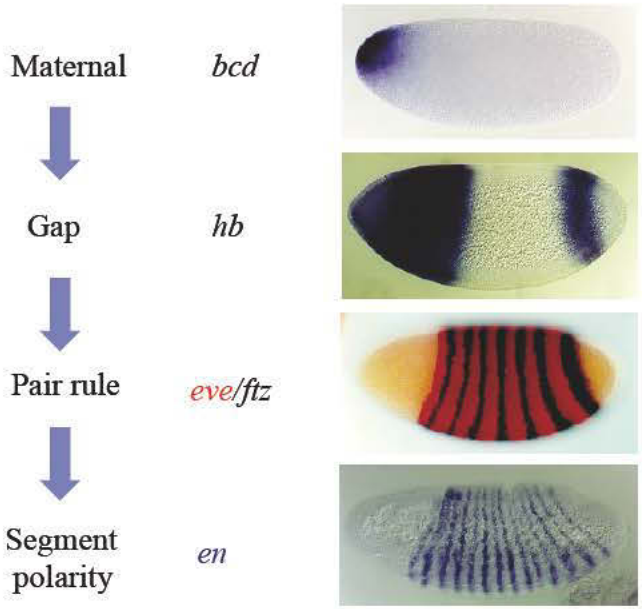
Chromatin-based Epigenetic Mechanisms

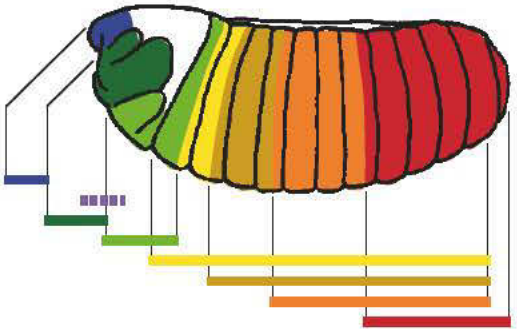
- Some ncRNAs can participate in recruiting and/or maintaining chromatin factors

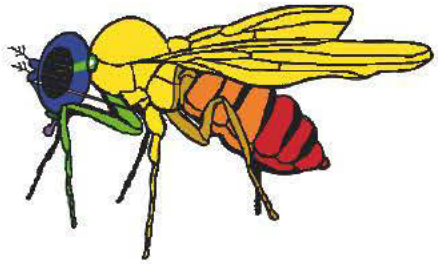
- Polycomb and Trithorax Group complexes participate in changing chromatin states and in establishment and maintenance of inactive and active states respectively

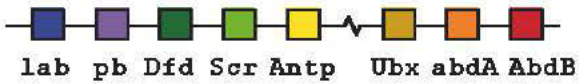


Polycomb and Trithorax Group Proteins





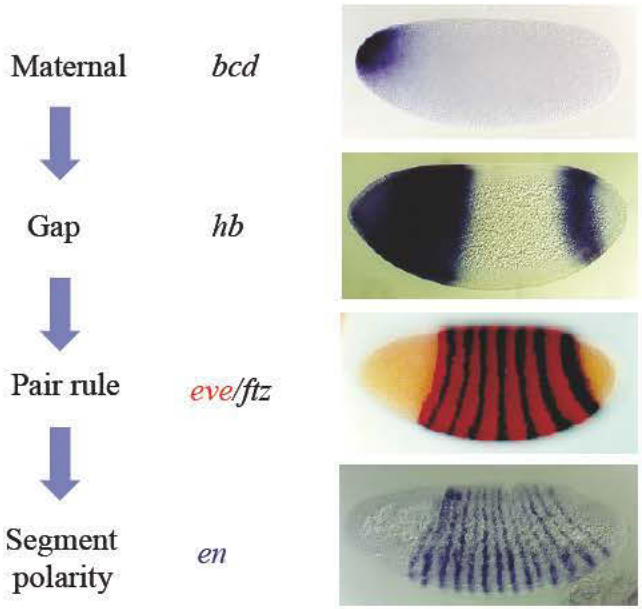




In *Drosophila*, the Gap and Pair-Rule transcription factors first established the homeotic genes pattern of expression.

The memory of this positional information has to be conserved up to the adult stage.

Polycomb and Trithorax Group Proteins

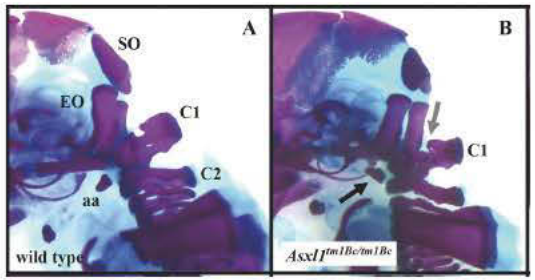


Polycomb and Trithorax group proteins are involved in **maintaining** patterns of gene expression

Mutations lead to ectopic expression of homeotic genes resulting in transformations of segments and body structures in flies and mammals.

In *Drosophila*, the Gap and Pair-Rule transcription factors first established the homeotic genes pattern of expression.


Birve et al., Dev. 2001



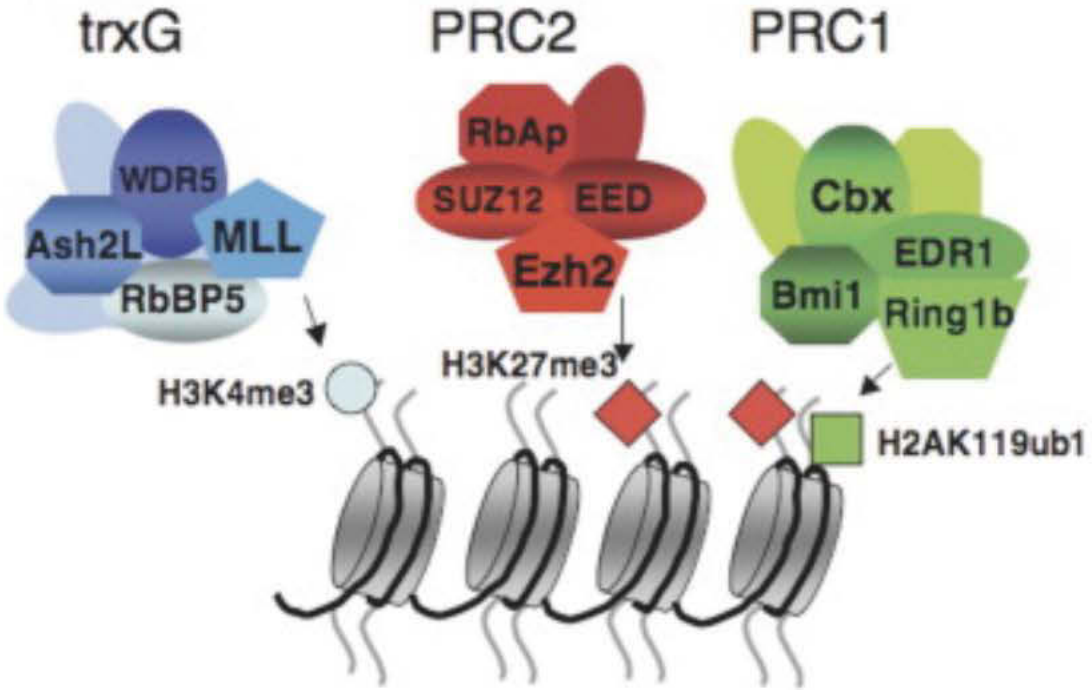
Fisher et al., Dev Biol. 2010

The memory of this positional information has to be conserved up to the adult stage.

Ingham, P. W., and Whittle, R. (1980). Trithorax: A new homeotic mutation of Drosophila melanogaster causing transformations of abdominal and thoracic imaginal segments. Mol. Gen. Genet. 179, 607–614.

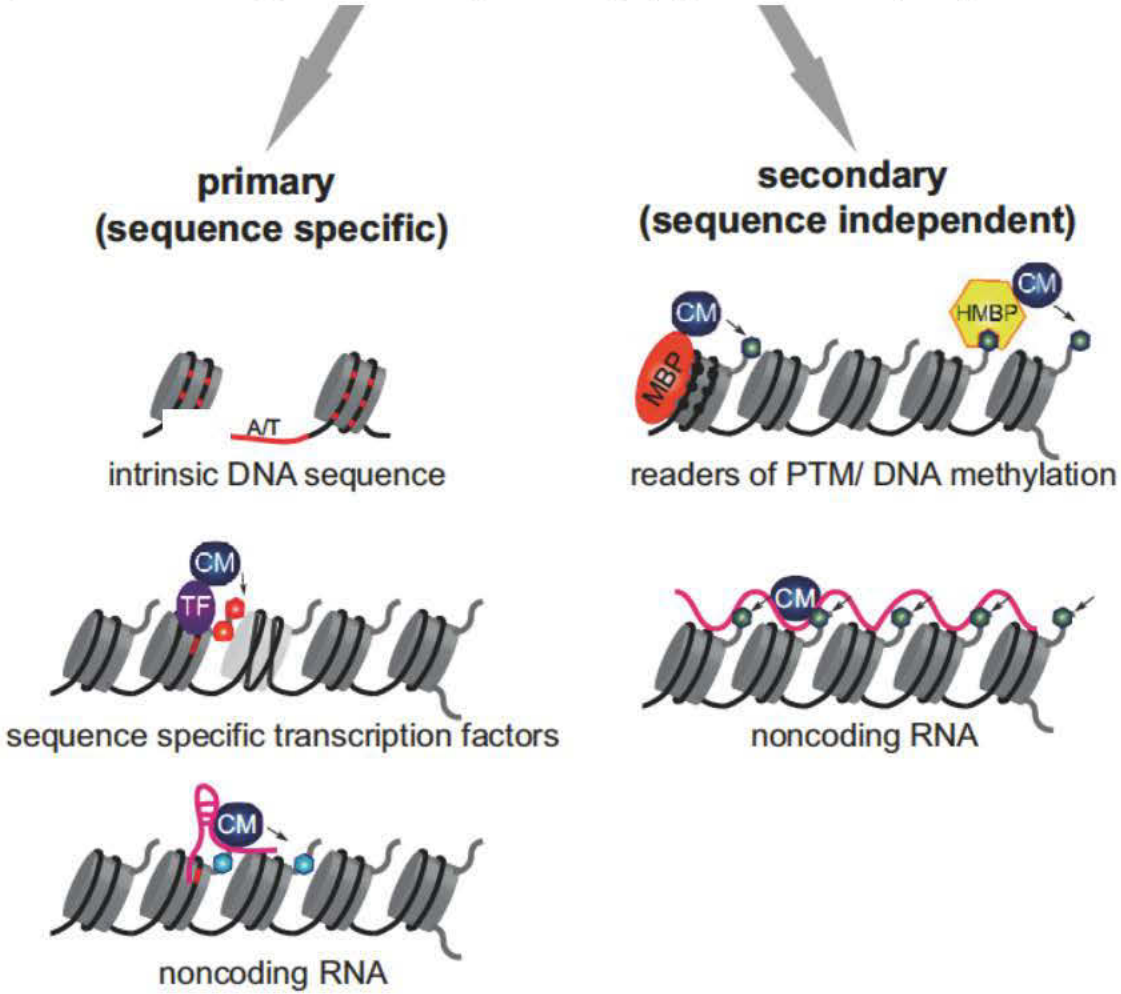
Polycomb and Trithorax Group Proteins

Multi-protein complexes
Associate with and modify chromatin (states +compaction)
Mainly developmental roles
Highly conserved across multicellular organisms
Cross-talk with DNA methylation and non-coding RNAs
(eg mammalian XCI, imprinting, vernalization in plants)



Setting Chromatin Marks

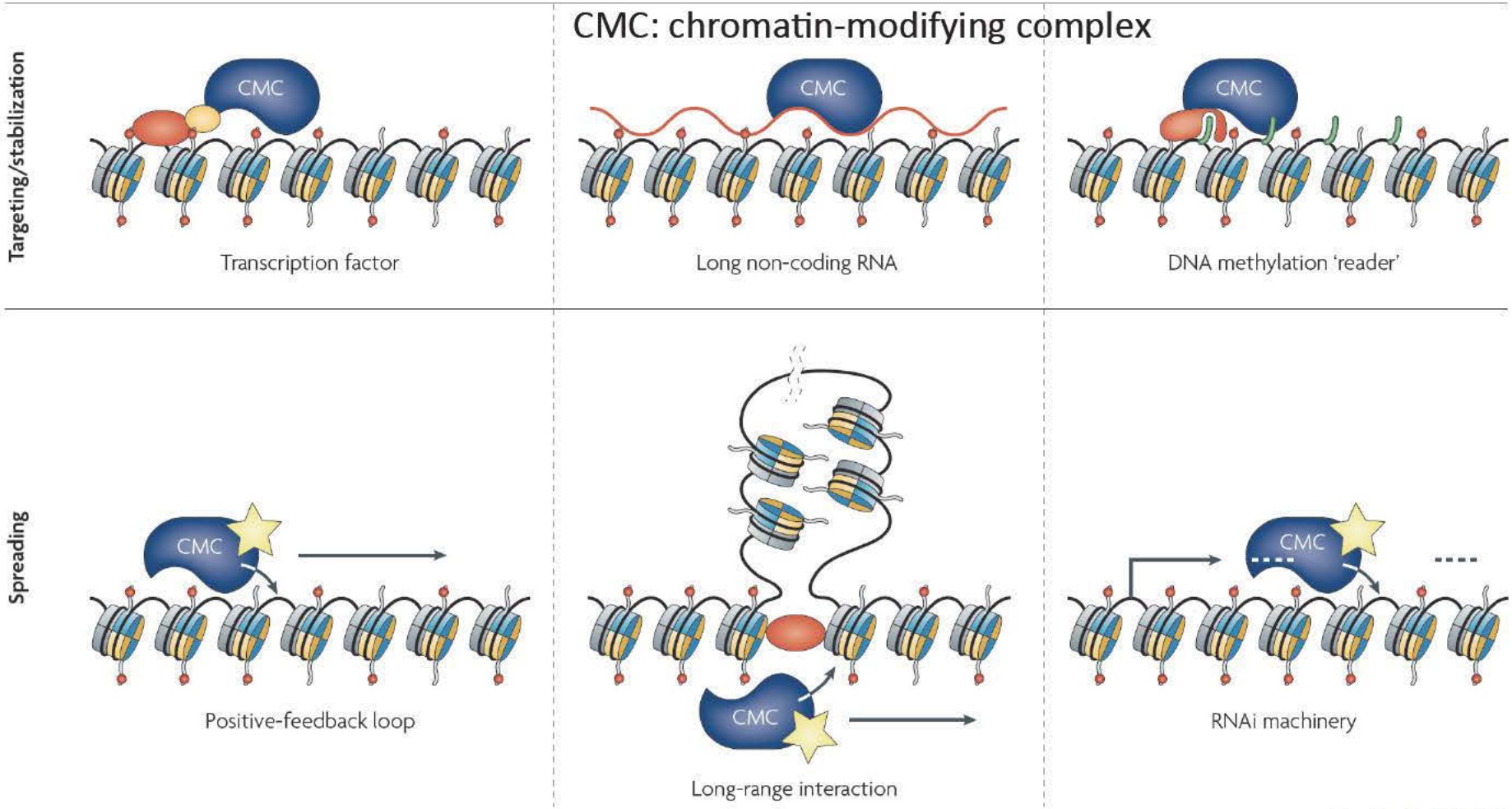
The targeting of chromatin modifying activities and DNA methyl binding proteins is still quite poorly understood in most cases:
during development, in response to signalling (eg hormonal), upon DNA damage….



From: Hassler and Egger (2012), *Biochimie* 94, 2219-2230

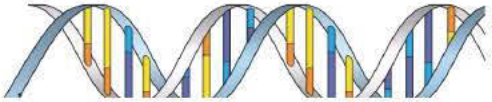
Perpetuating Chromatin Marks

One of more of these processes can participate in maintaining a chromatin mark over time.
To what extent chromatin marks are truly 'epigenetic' in the heritable sense remains very much an open question.



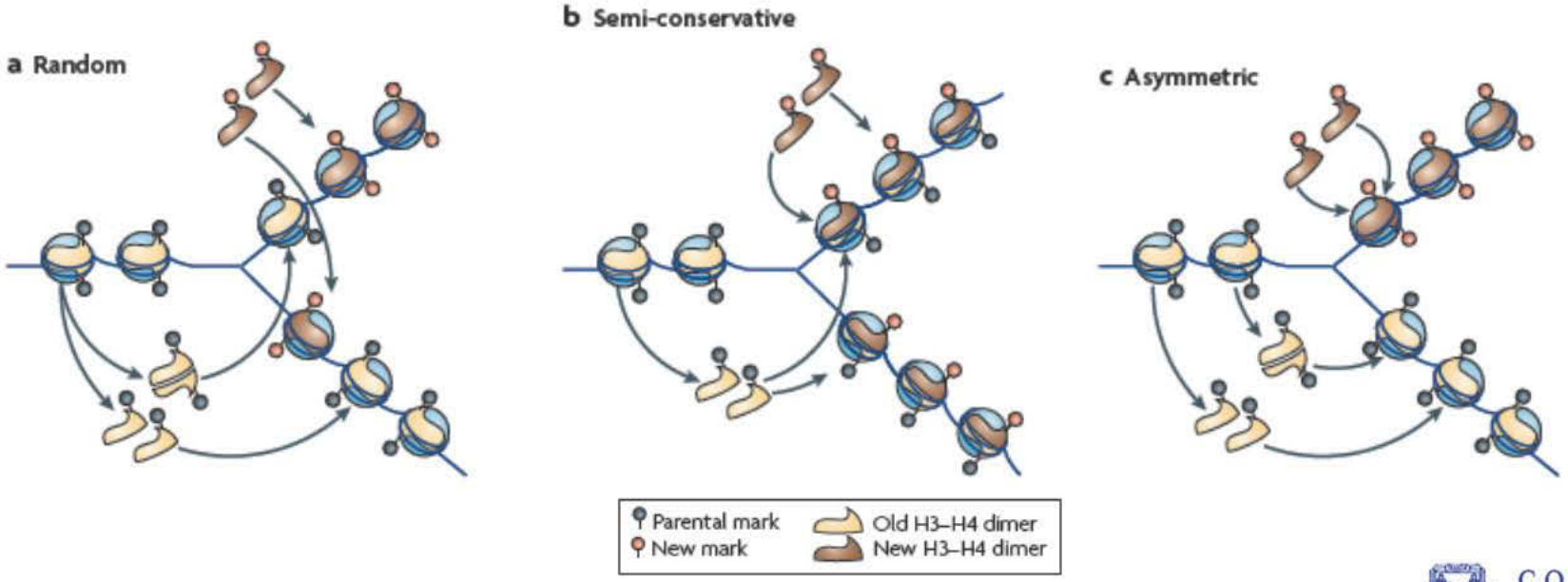
Perpetuating Chromatin Marks through DNA Replication

DNA: Semi-conservative replication



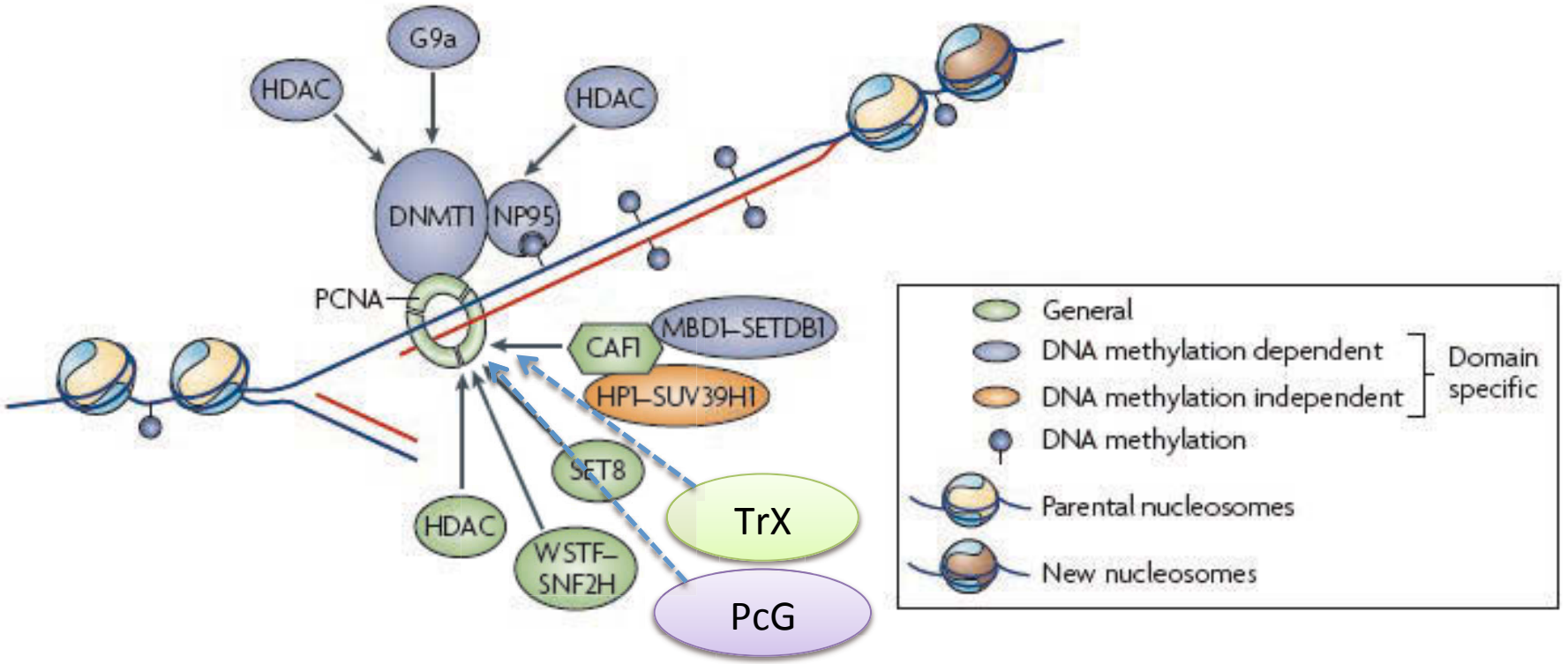
"Hemi-modified" nucleosomes (as in DNA methylation)?
Recycling of old (modified) and new (unmodified or modified) histones?
Persistence of the histone modifiers at the replication fork?

Chromatin: Still an open question…



Probst et al. (2009) *Nat. Rev. Mol. Cell Biol.*

Perpetuating Chromatin Marks through DNA Replication



Probst et al. (2009) *Nat. Rev. Mol. Cell Biol.*

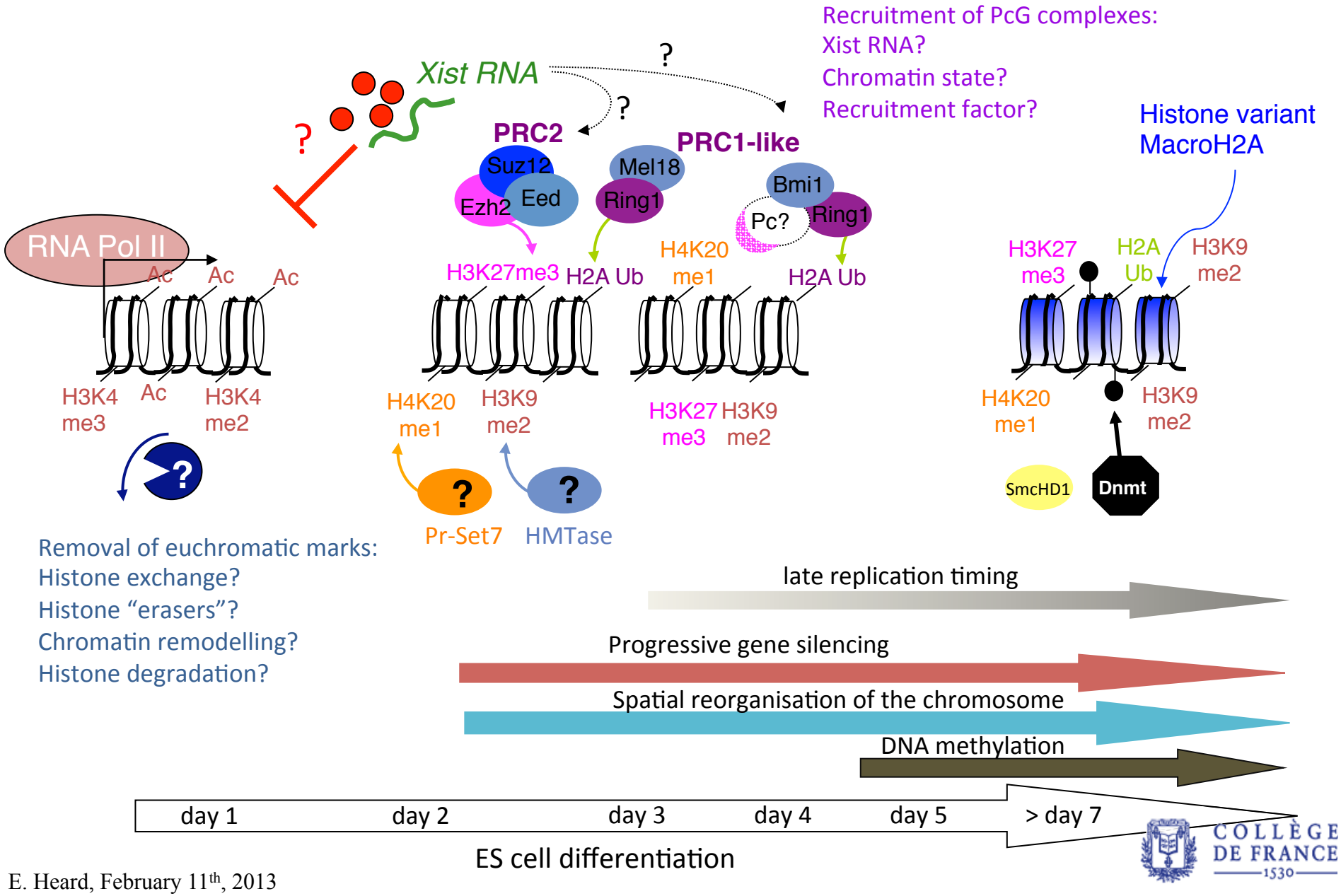
Removing Chromatin Marks

1. Passive loss (absence of maintenance mechanisms)
2. Active loss (enzymatic removal of histone modifications, histone exchange, nucleosome eviction, chromatin remodelling, etc)

Chromatin is highly dynamic
Yet states of gene activity can be stably propagated over hundreds of cell divisions in many cases
⇒ Synergy between chromatin, RNA-based, DNA methylation-based, nuclear organization and other mechanisms?

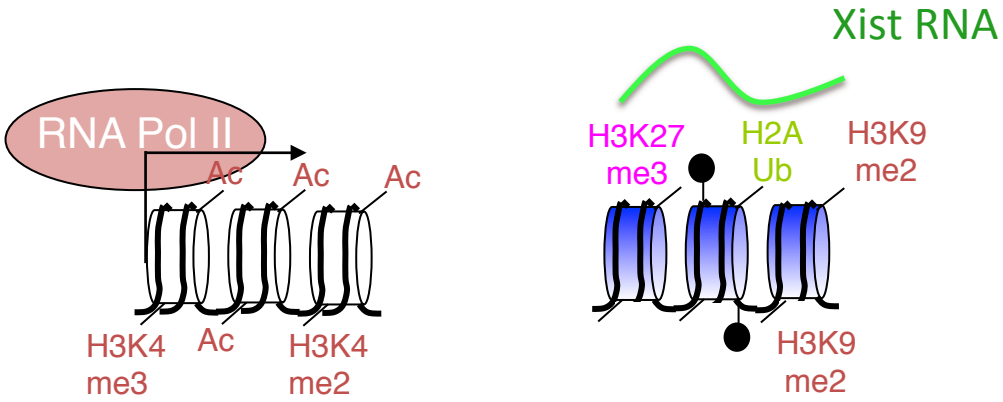
Reprogramming may involve both active and passive removal.
"Accidental" loss may occur sporadically, or after DNA damage, or with ageing– and may lead to epimutation and disease.

Setting up and Propagating Heterochromatin during XCI



Setting up and Propagating Heterochromatin during XCI

Identical DNA sequences
Opposite gene activity states



Active X chromosome

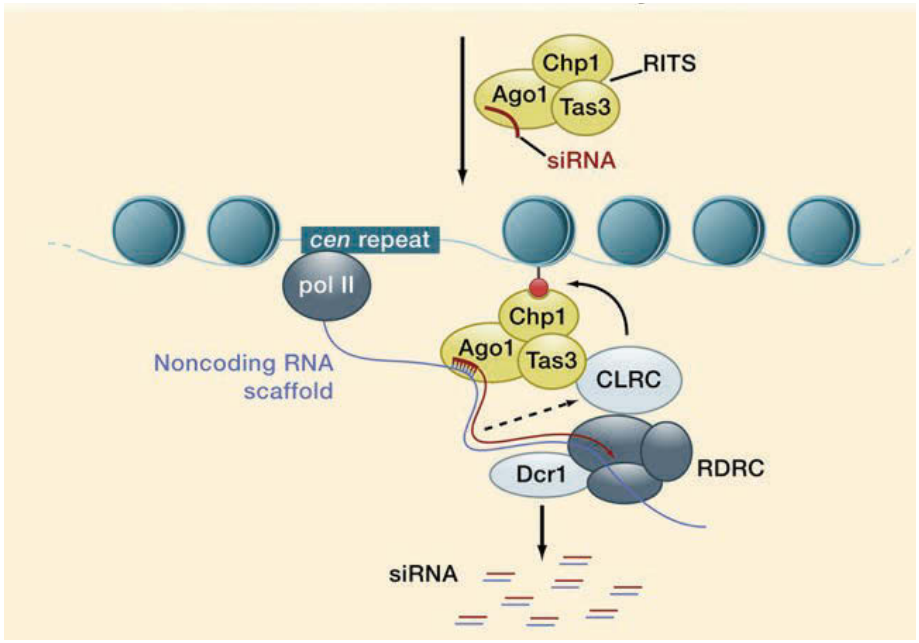
Inactive X chromosome

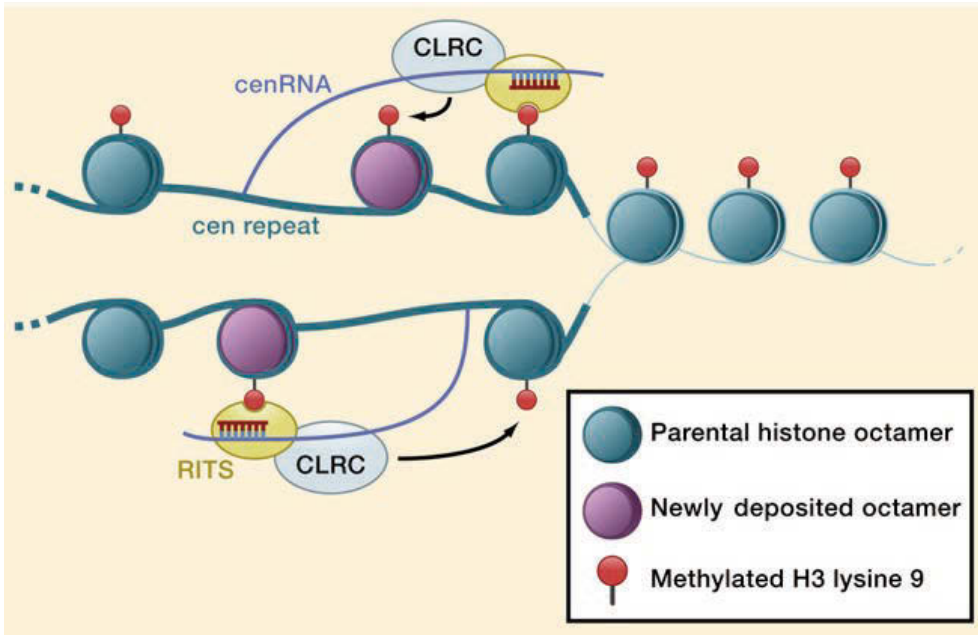
Synergy of epigenetic marks provides extremely stable heritable silencingover hundreds of cell divisions.

Fully reversible in the germ line and in iPS

Setting up and Propagating Heterochromatin via RNAi

Heterochromatin Assembly and Replication at Pericentromeric DNA Repeats in Fission Yeast





During replication of heterochromatin, the silent state is efficiently re-established as RITS complex can bind cooperatively via siRNA-mediated base pairing and association with H3K9 methylation. RITS-mediated recruitment of CLRC then results in methylation of newly deposited histones and re-establishment of silencing.

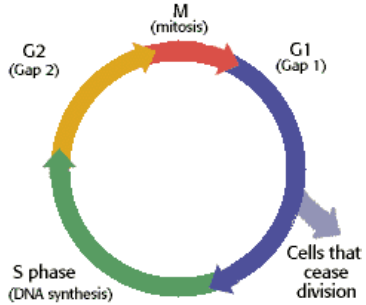
Moazed D. (2011) Cell 146.

OPEN QUESTIONS

When and how are epigenetic mechanisms important in development ?

How are epigenetic states perpetuated across the cell cycle?
(RNAi /HP1; PcG/TrX; DNA methylation; TFs…)
And in non-dividing cells?

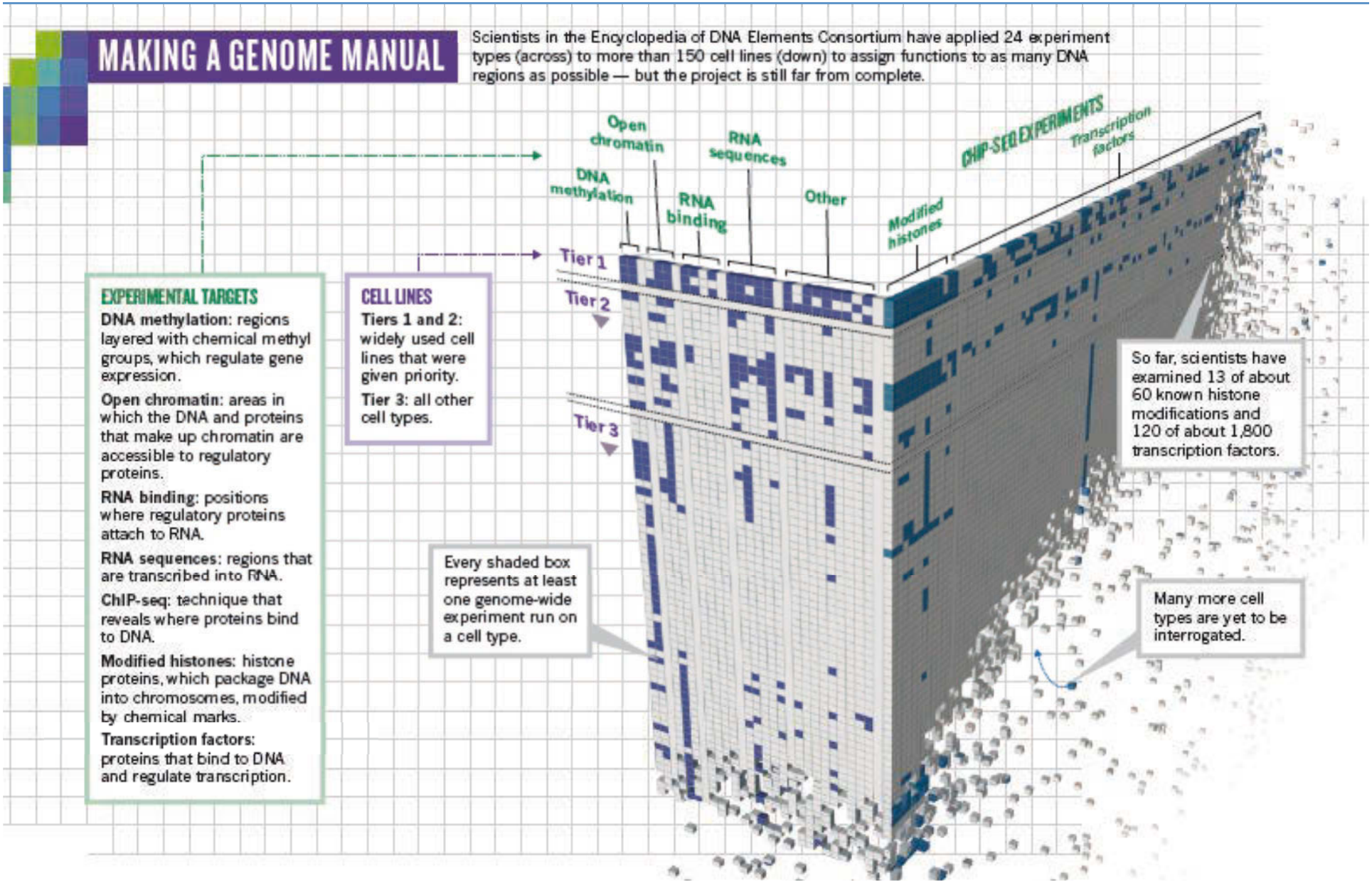
How is reprogramming achieved?
(whether *in vivo* or experimentally)



How are epigenetic processes affected by:
Ageing
Disease (eg cancer)
Environmentally induced changes
Nutritionally induced changes

Which epigenetic marks can be perpetuated across generations and how?

The Epigenomics Era



The Epigenomics Era

Genome-wide analysis of chromatin marks, binding proteins, RNAs…

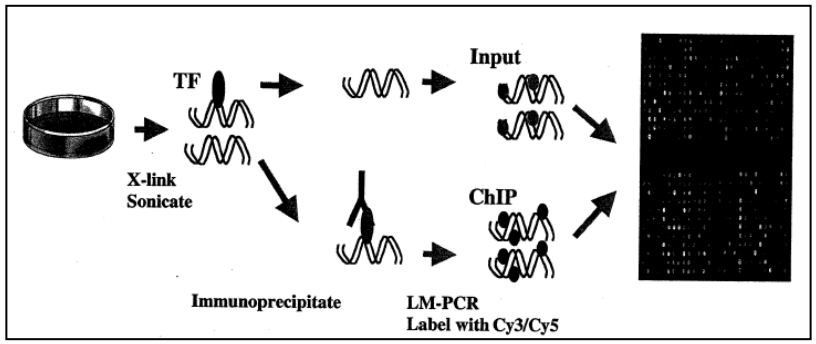
1. Chromatin, protein and DNA methylatuin mapping:

   ChIP-Seq, MeDIP Seq etc (Chromatin immunoprecipitation on micorarrays or sequencing

2. Mapping of long range *cis* and *trans* interactions in the nucleus :

   Chromosome conformation capture

3. Identification of small RNAs (miRNAs, siRNAs, piRNAs…) by deep sequencing



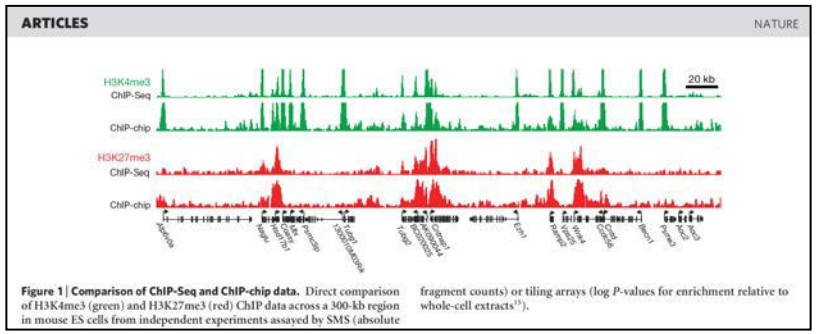
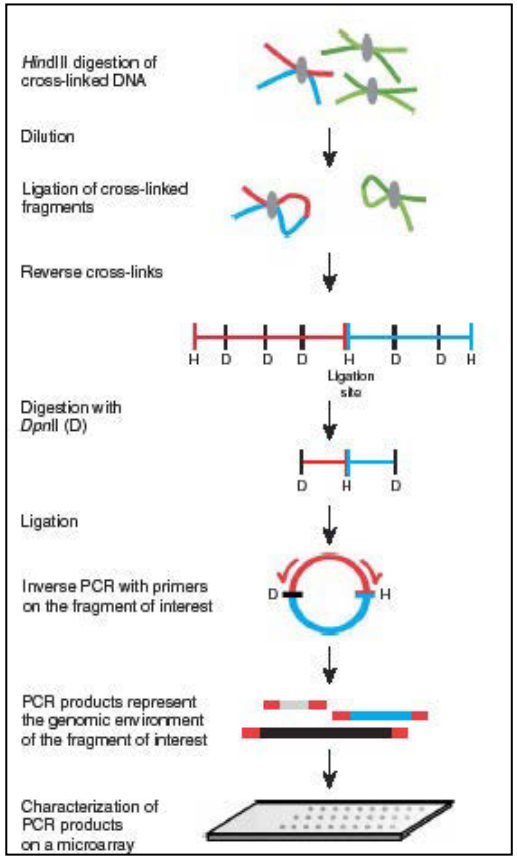


Figure 1 | Comparison of ChIP-Seq and ChIP-chip data. Direct comparison of H3K4me3 (green) and H3K27me3 (red) ChIP data across a 300-kb region in mouse ES cells from independent experiments assayed by SMS (absolute fragment counts) or tiling arrays (log *P*-values for enrichment relative to whole-cell extracts[15]).



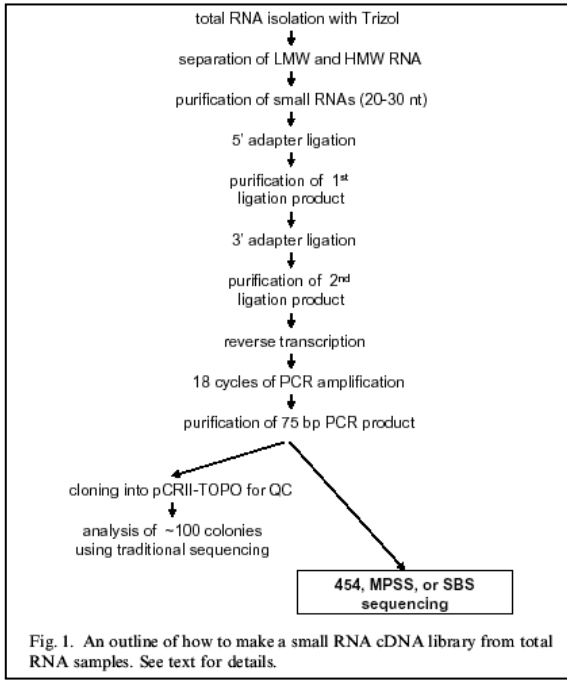


Fig. 1. An outline of how to make a small RNA cDNA library from total RNA samples. See text for details.

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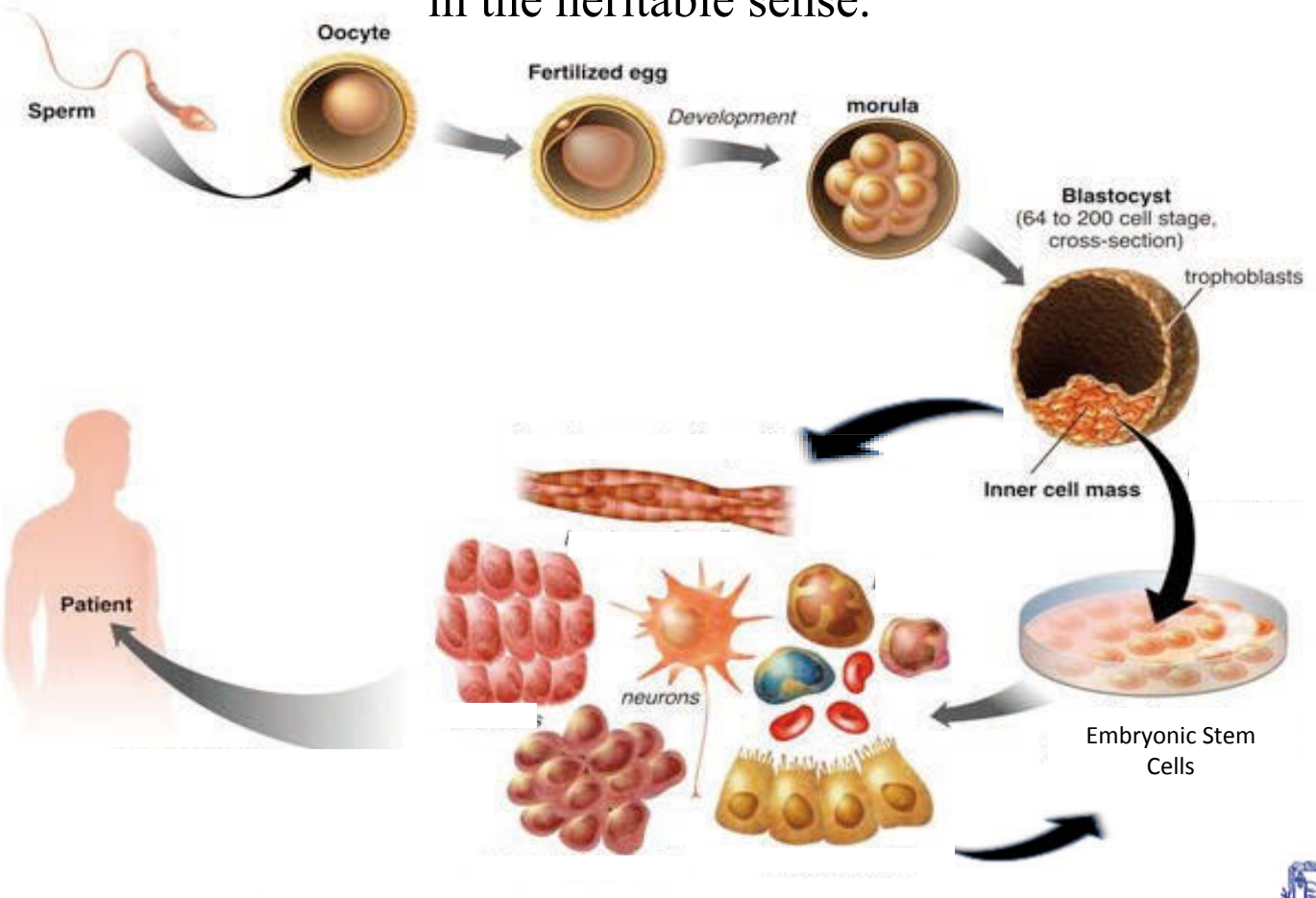
The Epigenomics Era

Combined with the power of genetics and biochemistry, the identification of the epigenomic changes during development, reprogramming and disease, as well as across generations, should help us understand the underlying epigenetic mechanisms; And to what extent, if at all, agiven epigenomic change can be considered epigenetic in the heritable sense.



Evolution of ‘Epigenetics’ since 1942

The study of the mechanisms of development through which genes bring about phenotypic effects. *Conrad H. Waddington 1942*

The study of mitotically or meiotically heritable changes in gene expression that are not accompanied by changes in the DNA sequence. *Robin Holliday, 1994, Art Riggs, 1996*

All the weird and wonderful things that cannot be explained by genetics” *Denise Barlow, Epigenome Network of Excellence (website)*

Everything we do—everything we eat or smoke—can affect our gene expression and that of future generations. Epigenetics introduces the concept of free will into our idea of genetics. *Randy Jirtle*, 2007

Epigenetic events correspond to the structural adaptation of chromosomal regions so as to register, signal, or perpetuate altered activity states. *Adrian Bird, 2007*