Epigenetic Mechanisms and Effects

The control mechanisms used to regulate human gene expression (and more in general for advanced organisms) must be more complicated than those of inferior organisms.

- Transcriptional regulation
- Post-transcriptional regulation
- Epigenetic mechanisms and long range control of gene expression

A human cell has about 30000 genes

RNA genes

Gens coding for proteins

Each cell express only a few of all this potential in a particular time

(~ 5000 genes)

Housekeeping genes

metabolism
biosynthesis
membrane
histones
ribosomal

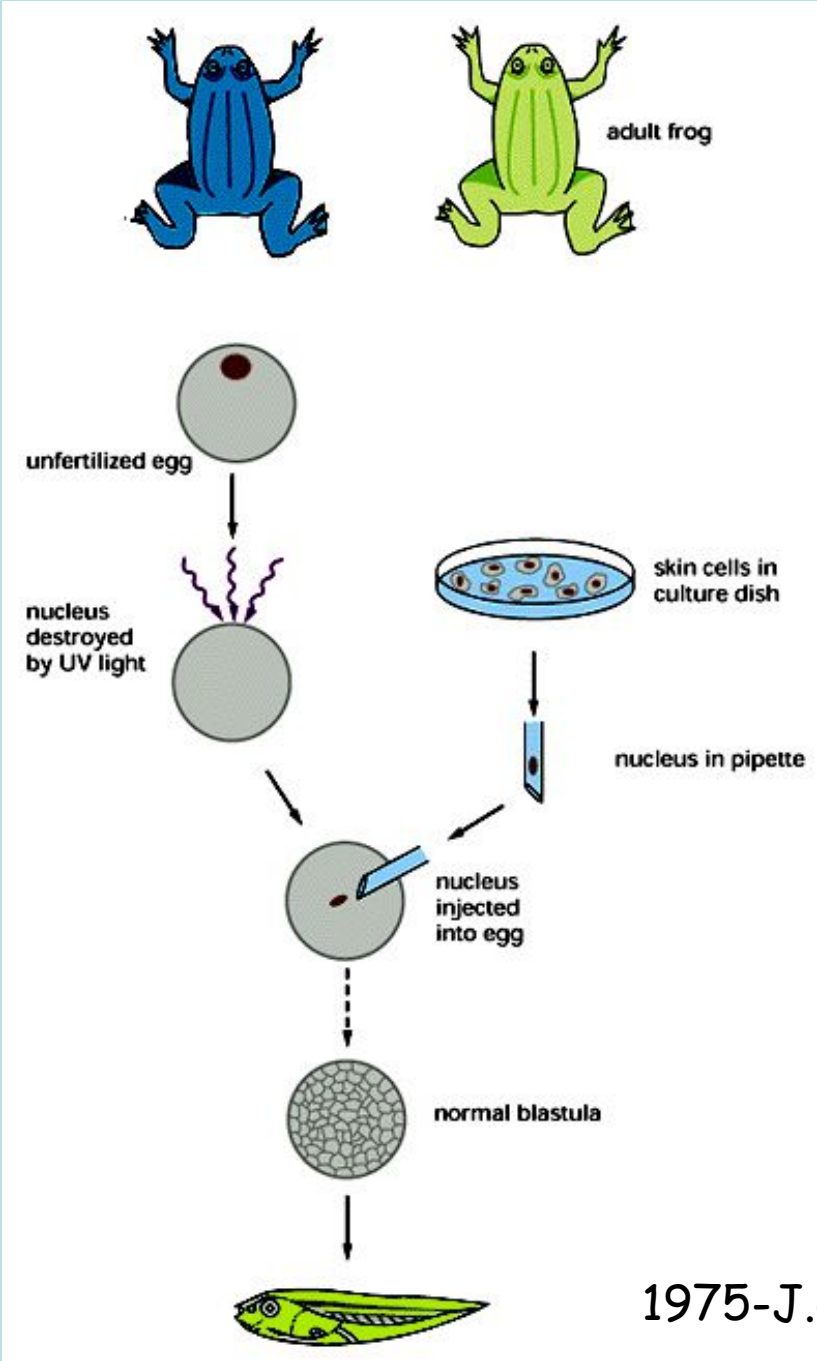
Tissue – specific genes

CELLULAR DIFFERENTIATION

THIS SELECTIVE EXPRESSION IS NOT (USUALLY) RELATED TO A DIFFERENT DNA CONTENT IN THE CELL

Each embryo nucleus is totipotent

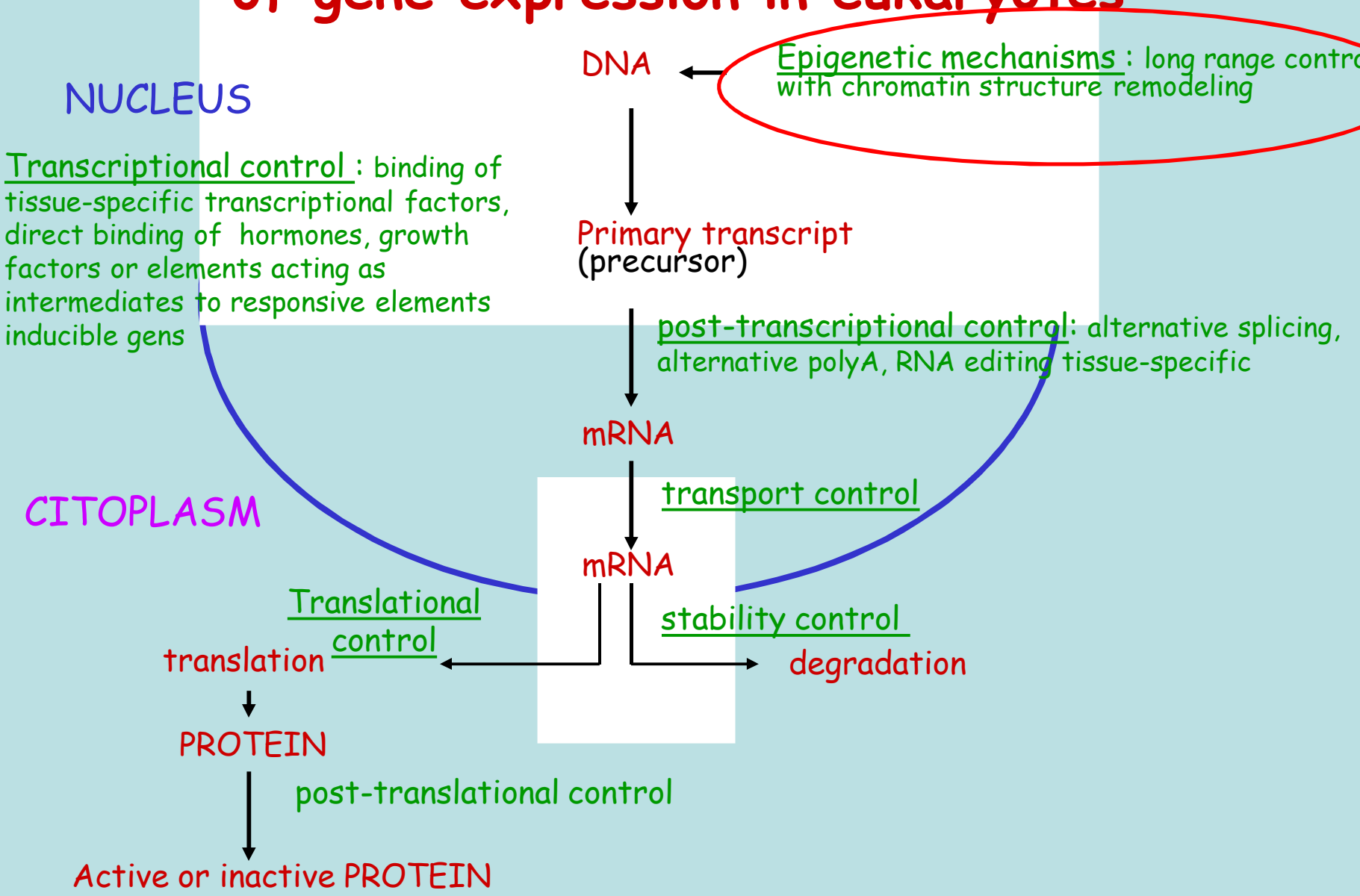
The genome is unchanged during cellular differentiation



1975-J.Gurdon


HHMI

There exist several levels of regulation of gene expression in eukaryotes



CHROMATIN

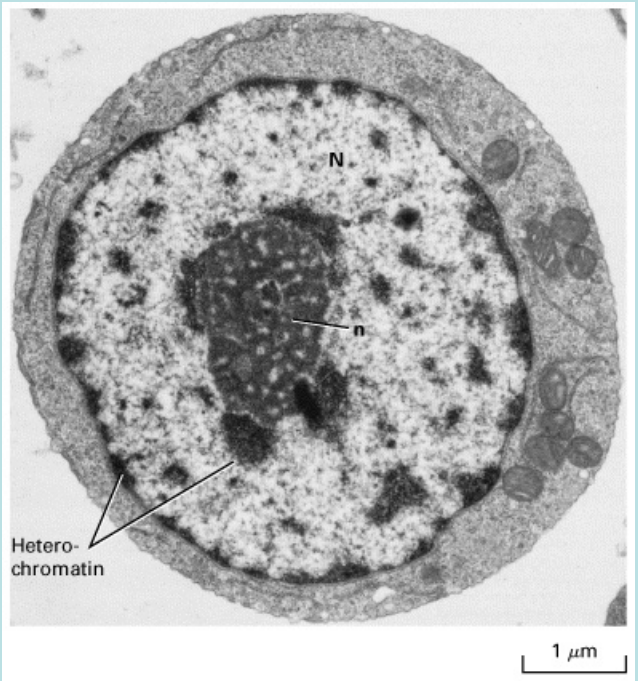
- **EUCHROMATIN** -> POTENTIALLY TRANSCRIBED

a) Housekeeping genes

b) tissue-specific genes

- **FACOLTATIVE ETHEROCROMATIN** -> Inactive if condensed

- **CONSTITUTIVE ETHEROCROMATIN** -> always inactive; localized in pericentromere and centromere regions



HHMI

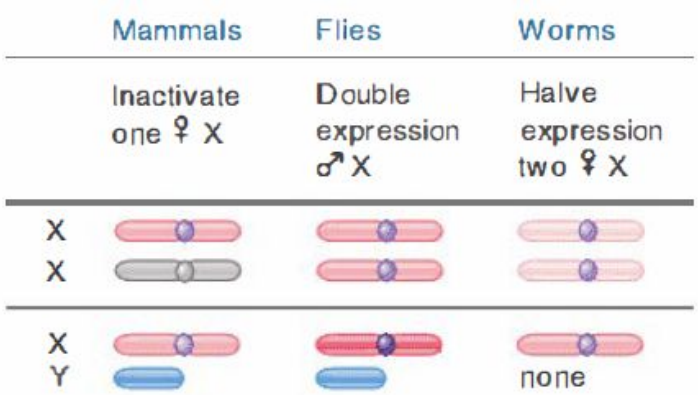


FIGURE 29.10 Different means of dosage compensation are used to equalize X chromosome expression in males and females.

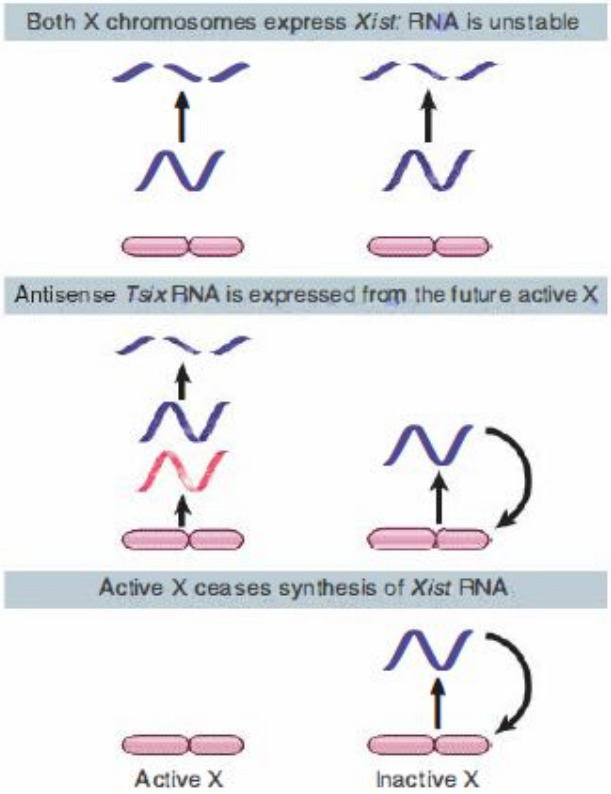


FIGURE 29.12 X-inactivation involves stabilization of *Xist* RNA, which coats the inactive chromosome. *Tsix* prevents *Xist* expression on the future active X.

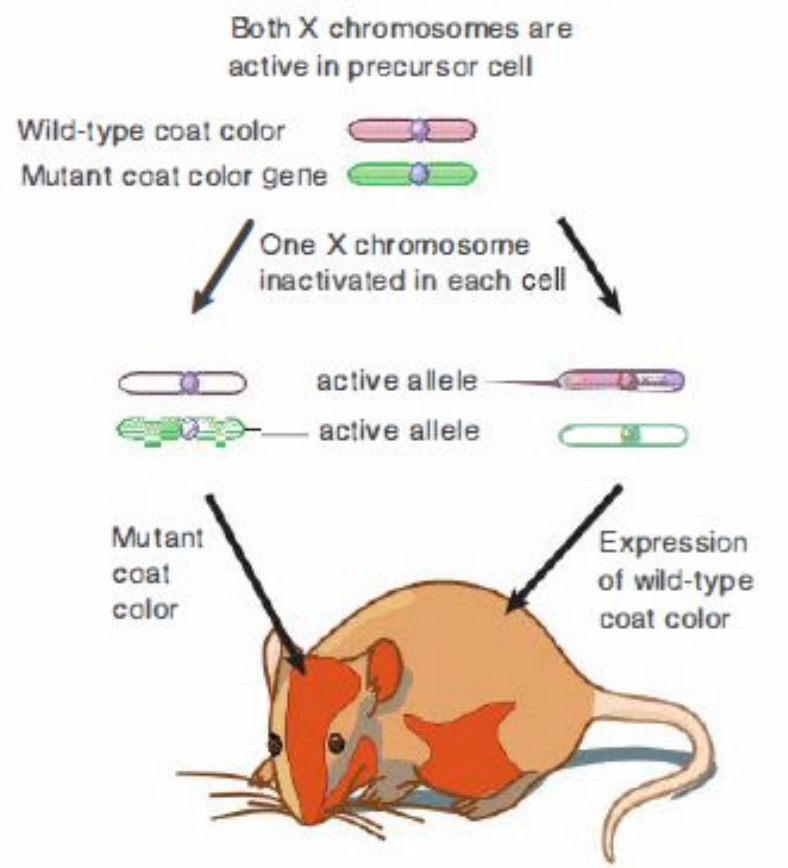
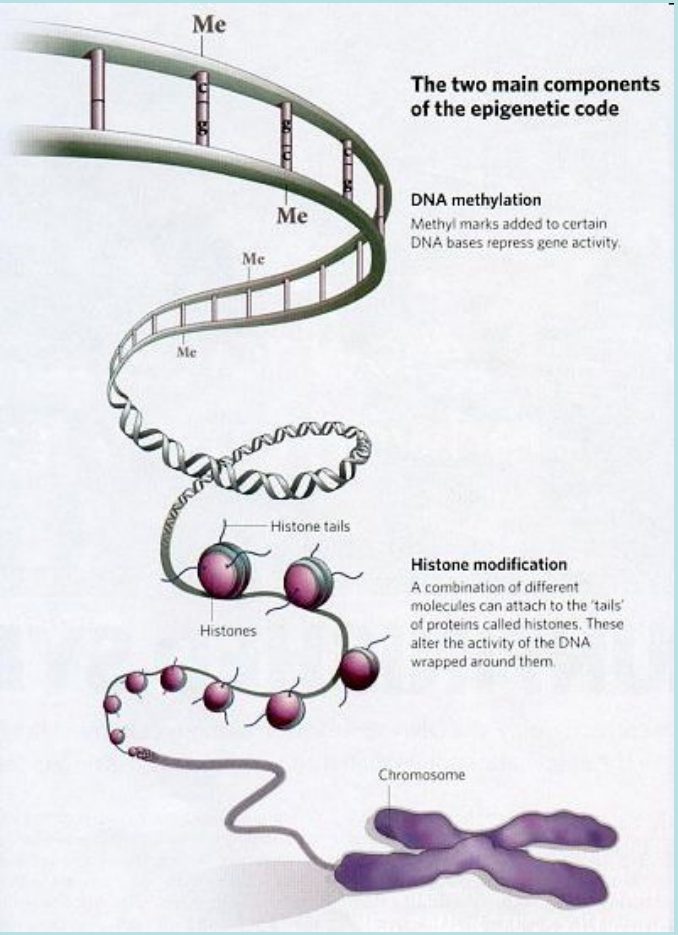


FIGURE 29.11 X-linked variegation is caused by the random inactivation of one X chromosome in each precursor cell. Cells in which the 1 allele is on the active chromosome have wild phenotype; cells in which the 2 allele is on the active chromosome have mutant phenotype.

Epigenetic effects



Factors transmitted to progeny, but can not be directly ascribed to DNS sequence.

- DNA methylation;

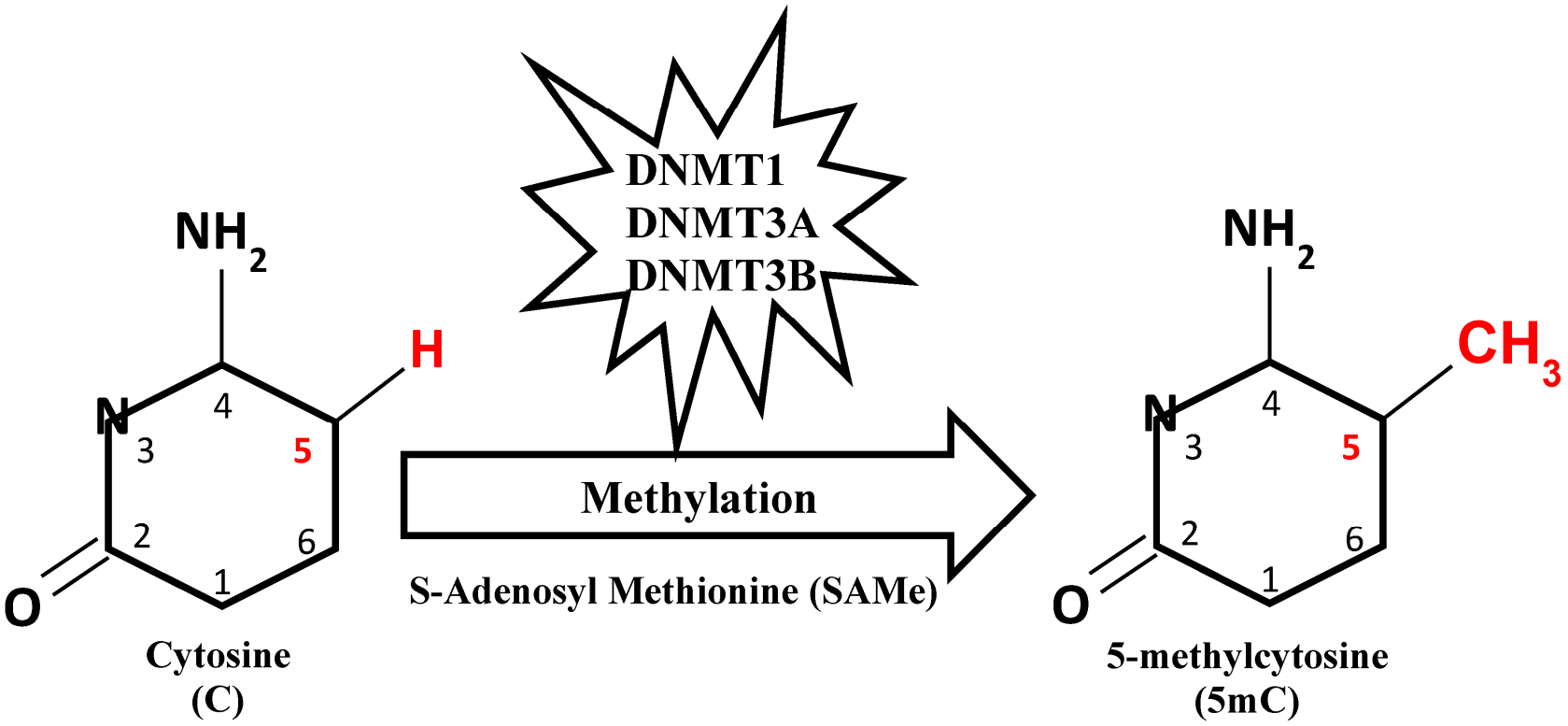
In eukaryotic cells methylation is only on C. Inly the 3% of all C are methylated and usually the C forming CpG duplets is target for methylation.

- Histones modification;

Acetylation, phosphorylation and methylation, responsible for conformational changes of chromatin.

Epigenetic mechanisms: DNA methylation

DNA methylation is a post-replicative process. The extension of modifications concerning DNA methylation is essentially determined during development. DNA methylation is indeed one of those mechanisms correlated to cellular differentiation, trough the inhibition of gene expression at the transcriptional level.



Which are the regions that are target of methylation?

- Vertebrate genes that are actively transcribed are "branded" by " CpG islands" at the 5' end. In these regions CpG frequency is comparable to the one is expected for all DNA (40% GC), into the remaining part of the genome is instead lower than the expected of 20%.

- In the human genome the 56% of all genes are associated to CpG islands: all the housekeeping genes and the 40% of all that genes with tissue-specific expression.

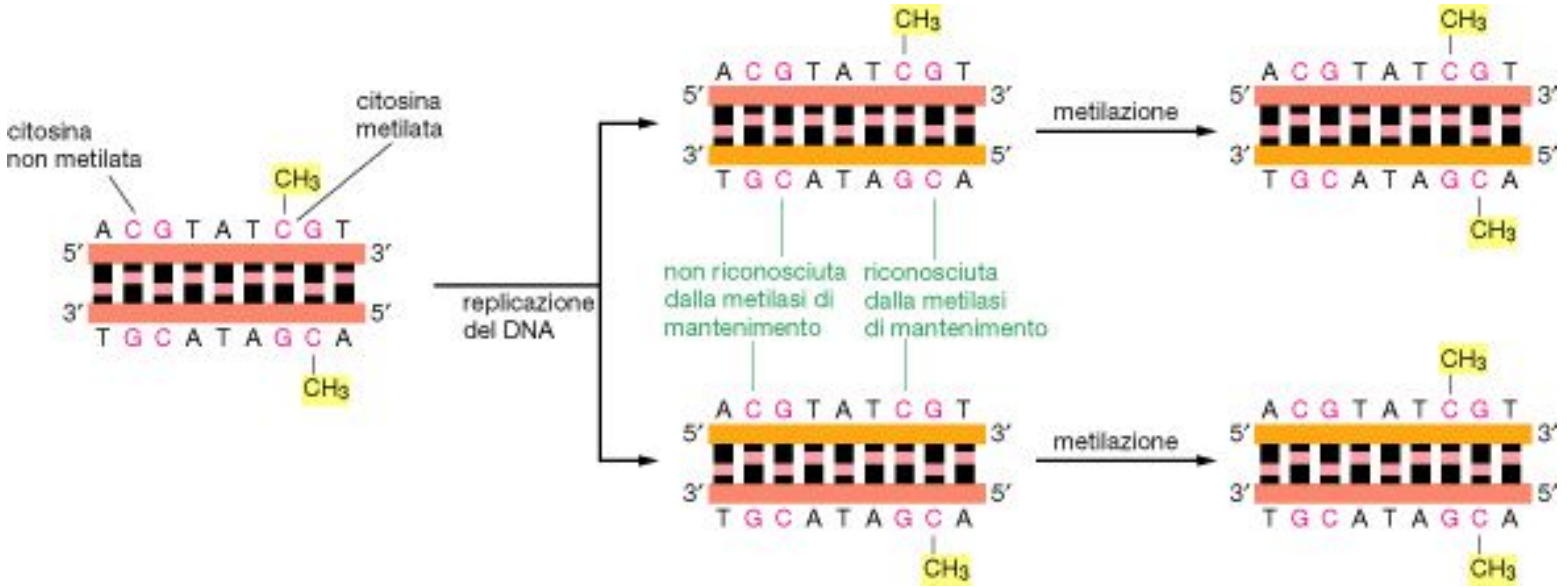
- Tissue-specific genes are methylated in CpG in those tissues where they are silenced.

Main functions of methylation are related to repression of gene transcription:

- **Difens against transposons**: methylation is essential to maintain silenced transposon and retrotransposon genomes
- **Gene regulation**: methylation helps to establish and maintain an transcriptional inactive state (heterochromatin)
- **Chromosomial stability**

In non embryonal cells, 80% of CpG are methylated (LINE, SINE, LTRs), with exception of CpG islands located into the promoters that usually are maintained hypomethylated.
About 40,000 CpG island in the mammalian genome, into the promoters of ~50% of the known genes.

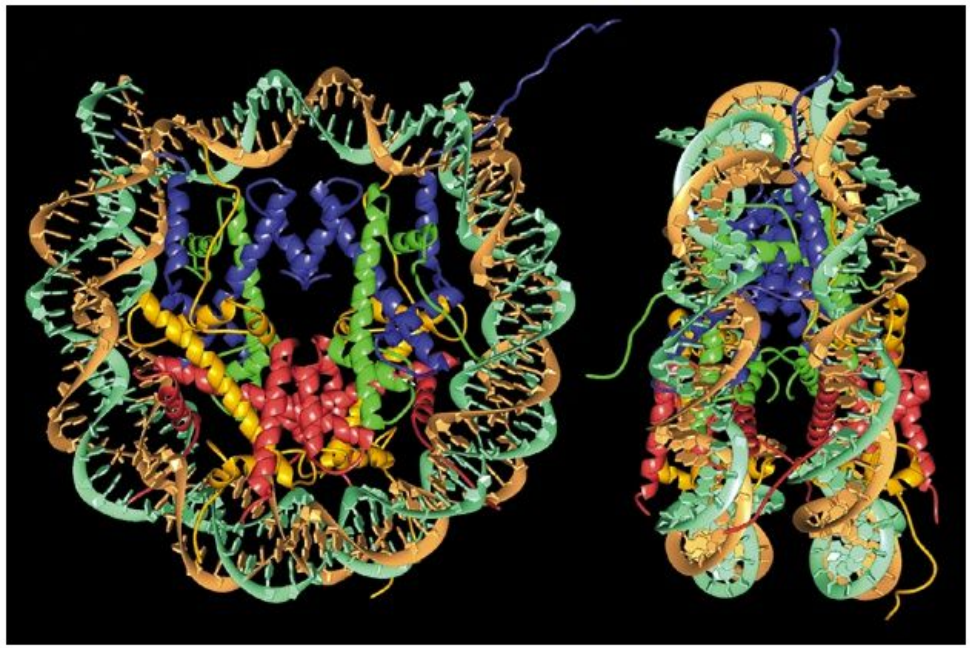
Once that it is established, the methylation scheme is maintained by a <u>MAINTENANCE</u> DNA-methyl transferase, that shows a particular affinity to emi-methylated sequences: then they tend to remethylate the new strand that have been formed on a methylated template.

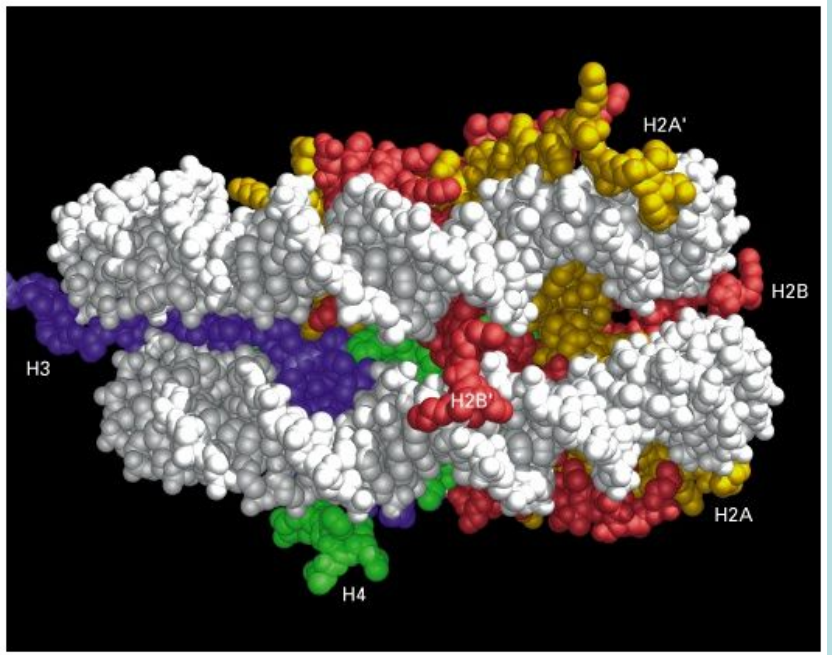


=> methylation pattern maintenance
(epigenetic modification - cellular memory)

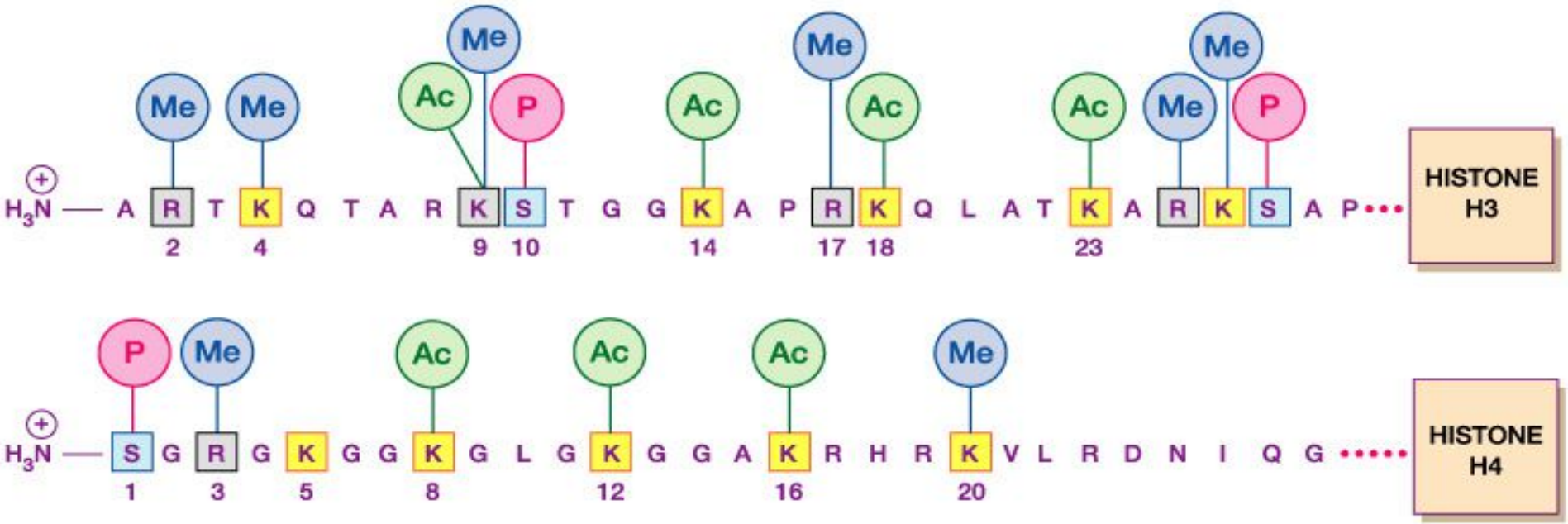
Epigenetic mechanism: Histone Modifications

The aminoacidic residues at the N-terminal of each histone (20-60 residues) extend over the surface of the nucleosome. These regions are particularly rich in lysine (K) that can be reversibly modified by acetylation, phosphorylatione and methylation.





Modifications for H3 and H4 histones



Lysine 9 in H3 can be either acetylated or methylated. Acetylation is associated to transcriptionally active chromatin, but if the chromatin region is methylated on DNA (CpG), proteins that binds to methylated DNA recall histone deacetylases, that remove acetyl groups and histone methyltransferase, bound to CpG binding proteins, that methylate histones.

The final result is chromatin condensation.

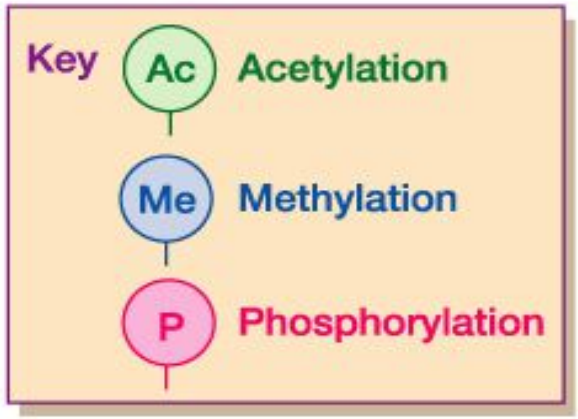
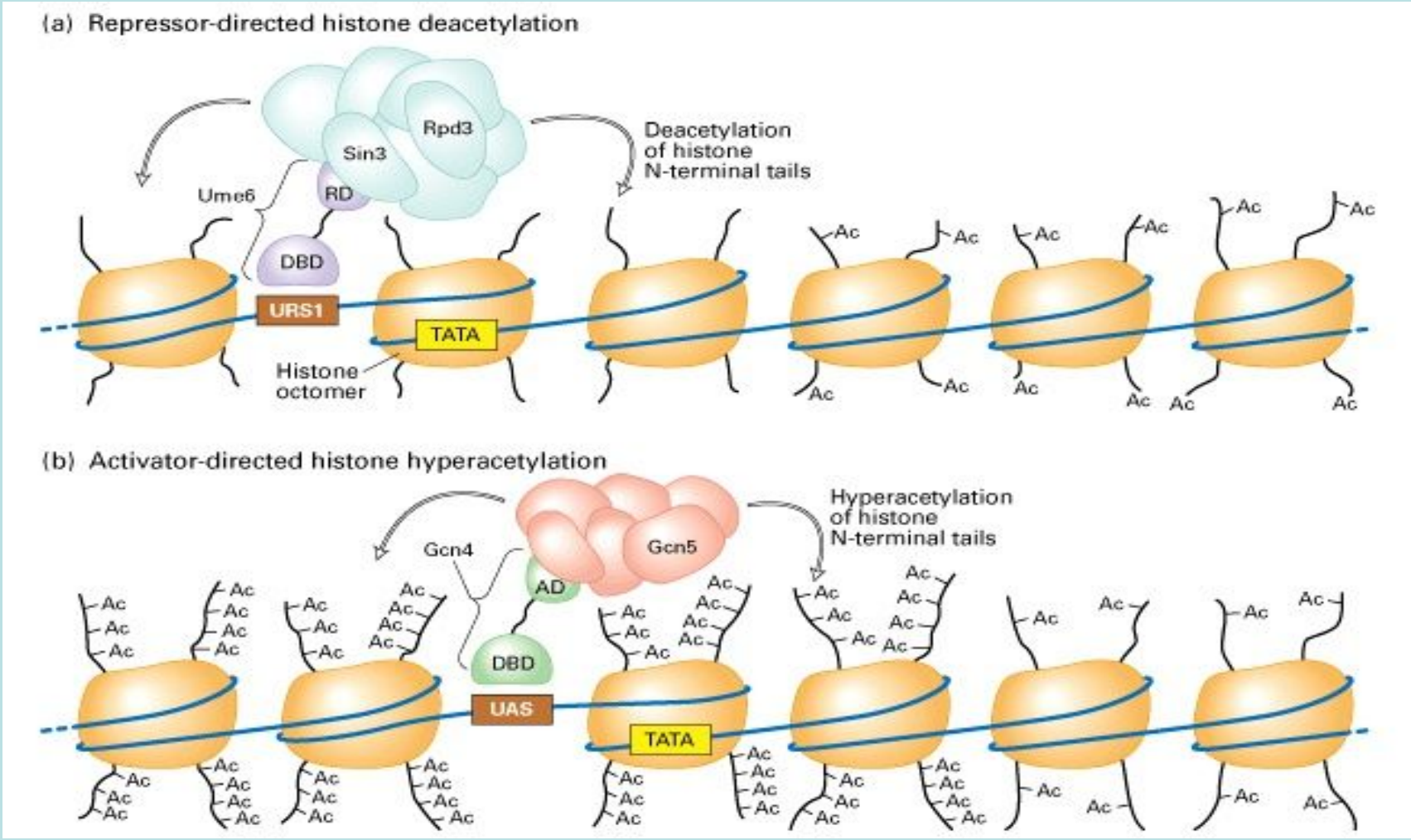


Figure 10-1 Human Molecular Genetics, 3/e. (© Garland Science 2004)

Repressors and activators can direct histon deacetylation/acetylation at the level of specific genes



CHROMATIN CHARACTERISTICS

Characteristic	Active chromatin	Inactive chromatin
Chromatin conformation	Extended, opened	Condensed
Methylation of DNA	Poorly methylated especially in the promoter regions	Methylated
Acetylation of histones	Acetylated histones	Non acetylated histones

Transcriptional Control

The principal method used for the gene expression control in eukaryotes is a selective transcription, that can be obtained with specific DNA binding proteins.

The allelic exclusion: even if two copies of each gene are present, in some cases only one of them is expressed

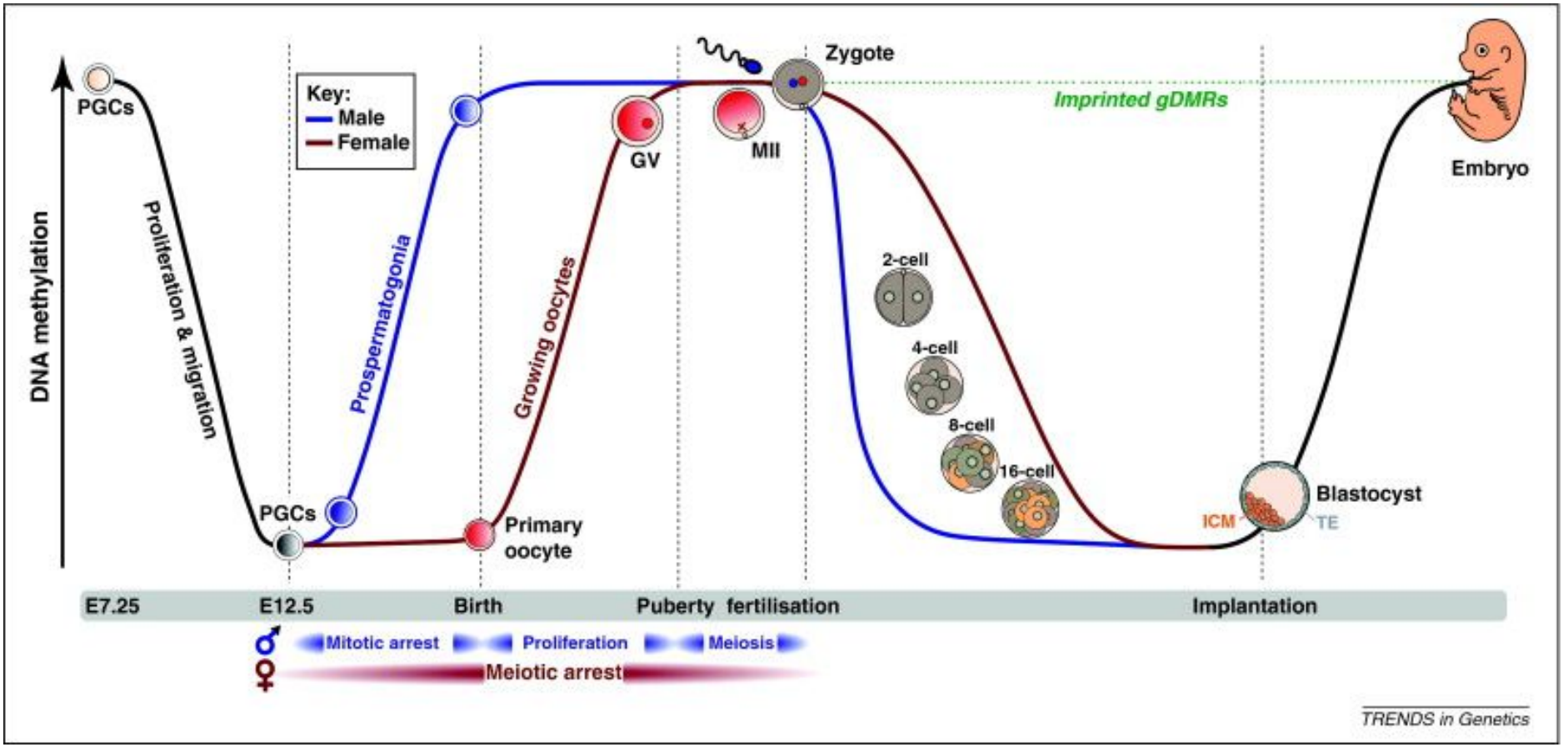
- It can be depending on the parental origin of the allele, and in this case it is called imprinting.

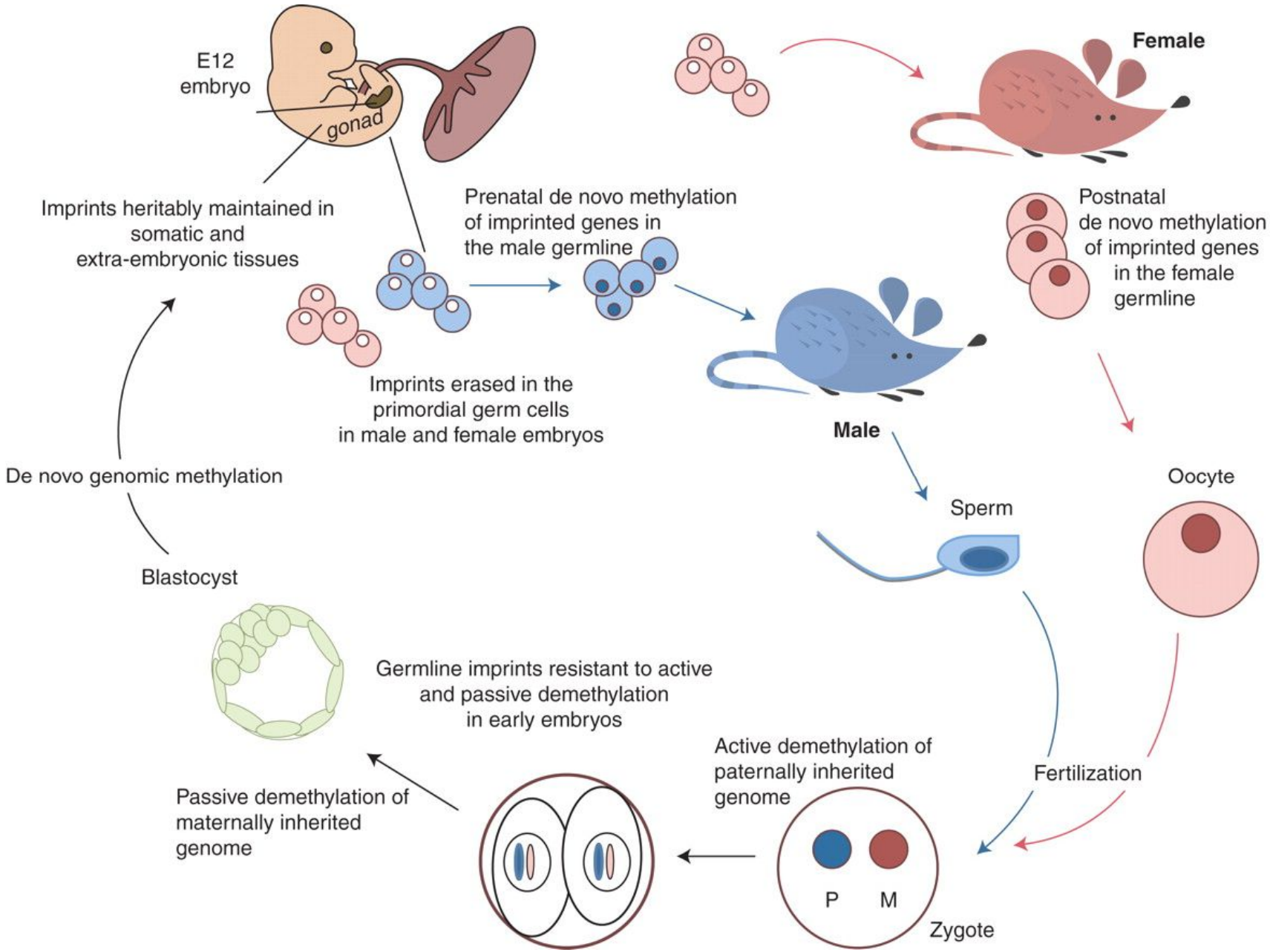
- It can be independent from the parental origin (e.g. X chromosome inactivation in the female somatic cells).

- In both cases it can be tissue-specific.

Changes in DNA methylation state during mammalian development

During segmentation there is immediately a demethylation phase, followed by a "de novo" methylation dispersed on the entire genome, after the blastocyst implant.




E12
embryo
gonad
Imprints heritably maintained in
somatic and
extra-embryonic tissues
Female
Prenatal de novo methylation
of imprinted genes in
the male germline
Postnatal
de novo methylation
of imprinted genes
in the female
germline
Imprints erased in the
primordial germ cells
in male and female embryos
Male
De novo genomic methylation
Sperm
Oocyte
Blastocyst
Germline imprints resistant to active
and passive demethylation
in early embryos
Active demethylation of
paternally inherited
genome
Fertilization
Passive demethylation of
maternally inherited
genome
P
M
Zygote

Imprinting: A change in a gene that occurs during passage through the sperm or egg with the result that the paternal and maternal alleles have different properties in the very early embryo. This is caused by methylation of DNA.

Changes in DNA methylation state during mammalian development.

- Methylation is usually associated with inactivation of the gene.
- When genes are differentially imprinted, survival of the embryo may require that the functional allele is provided by the parent with the unmethylated allele
- Survival of heterozygotes for imprinted genes is different, depending on the direction of the cross.

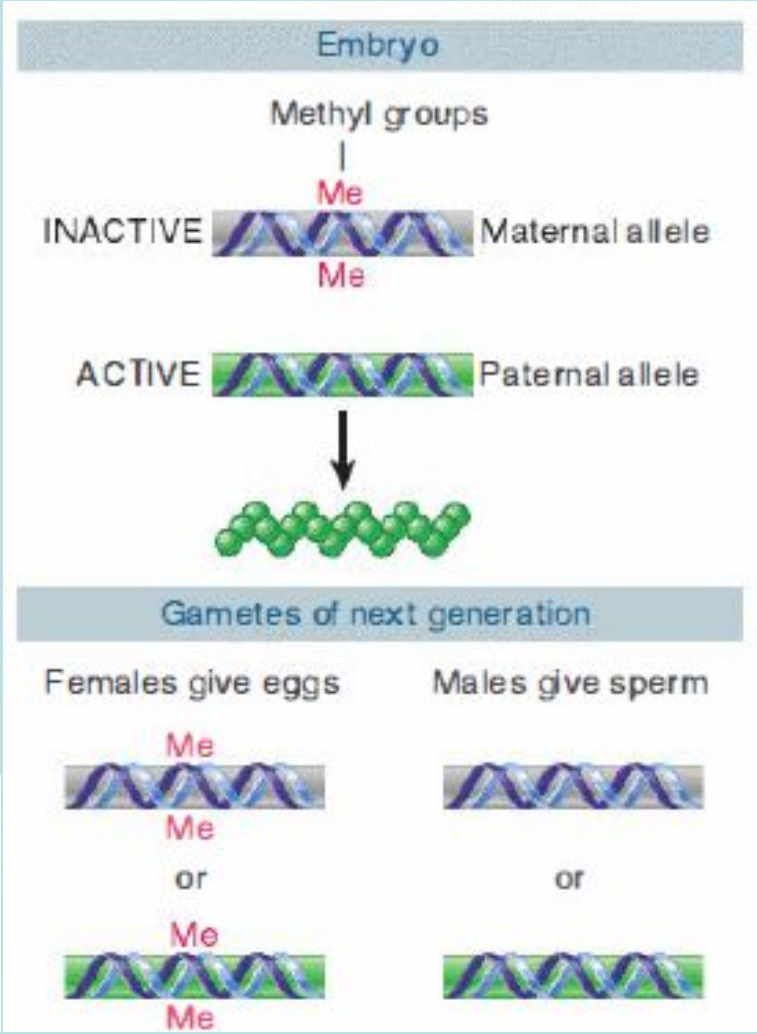


FIGURE 29.23 The typical pattern for imprinting is that a methylated locus is inactive. If this is the maternal allele, only the paternal allele is active, and it will be essential for viability. The methylation pattern is reset when gametes are formed, so that all sperm have the paternal type and all oocytes have the maternal type.

Genomic imprinting implies the germ line footprint deletion.

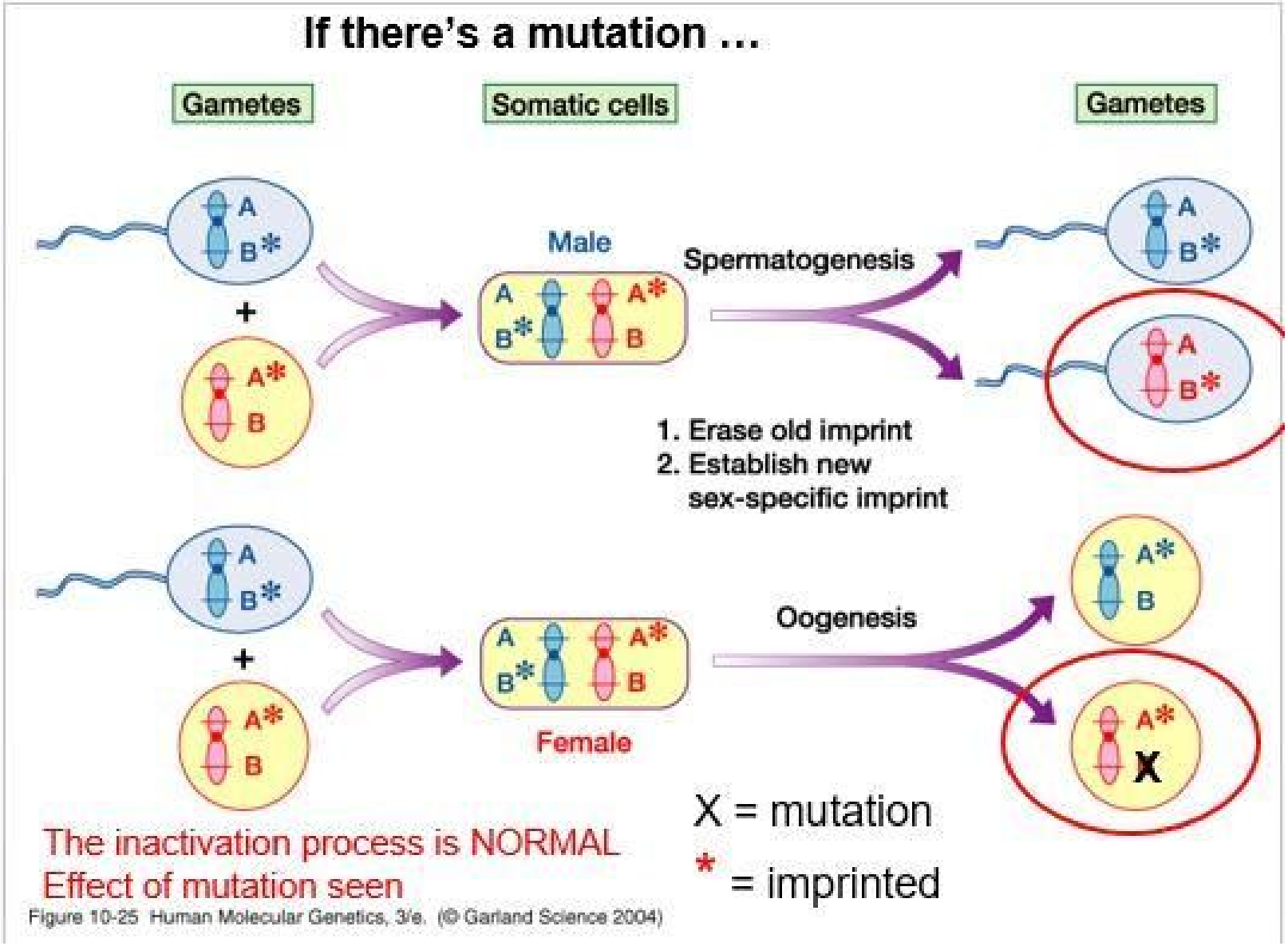


Figure 10-25 Human Molecular Genetics, 3/e. (© Garland Science 2004)

- **Imprinted genes are controlled by methylation of cis-acting sites.**
- **Methylation may be responsible for either inactivating or activating a gene.**

These two genes react oppositely to the state of methylation at the ICR located between them. The ICR is methylated on the paternal allele. H19 shows the typical response of inactivation. Note, however, that Jgf2 is expressed. The reverse situation is found on a maternal allele, where the ICR is not methylated. H19 now becomes expressed, but lgj2 is inactivated.

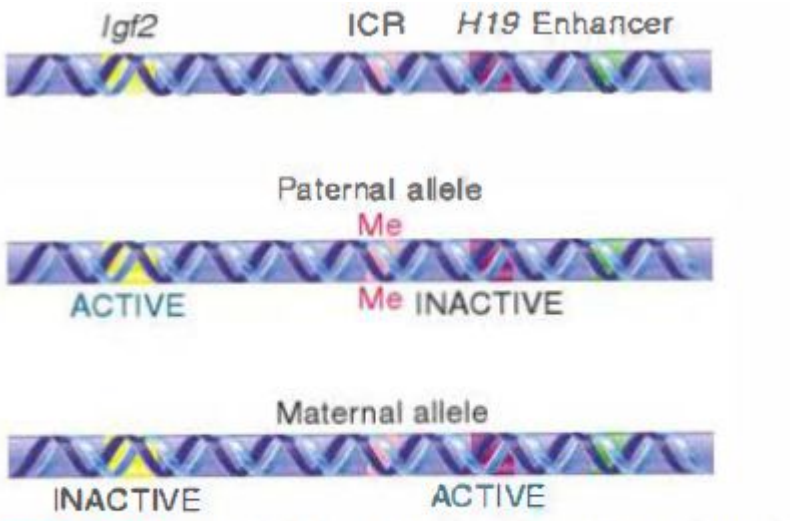


FIGURE 29.24 The ICR is methylated on the paternal allele, where *Igf2* is active and *H19* is inactive. ICR is unmethylated on the maternal allele, where *Igf2* is inactive and *H19* is active.

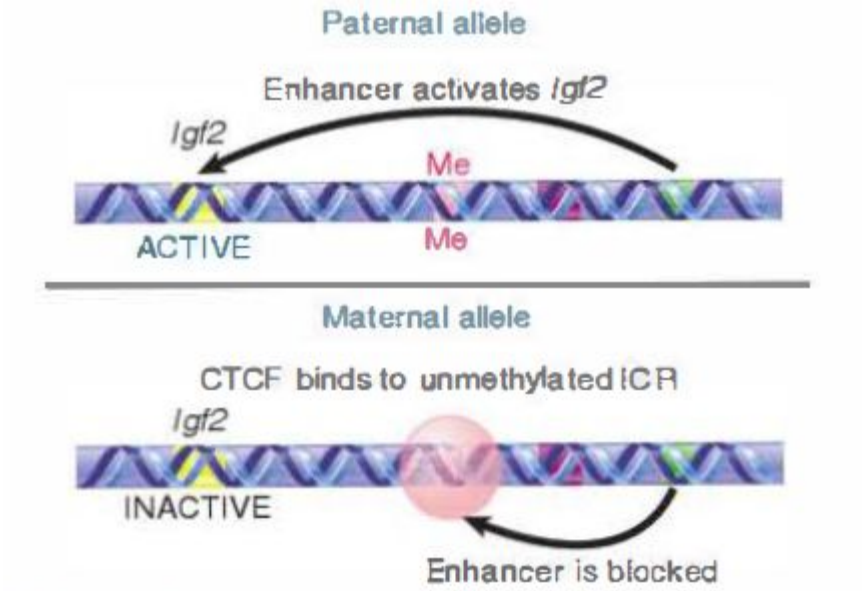
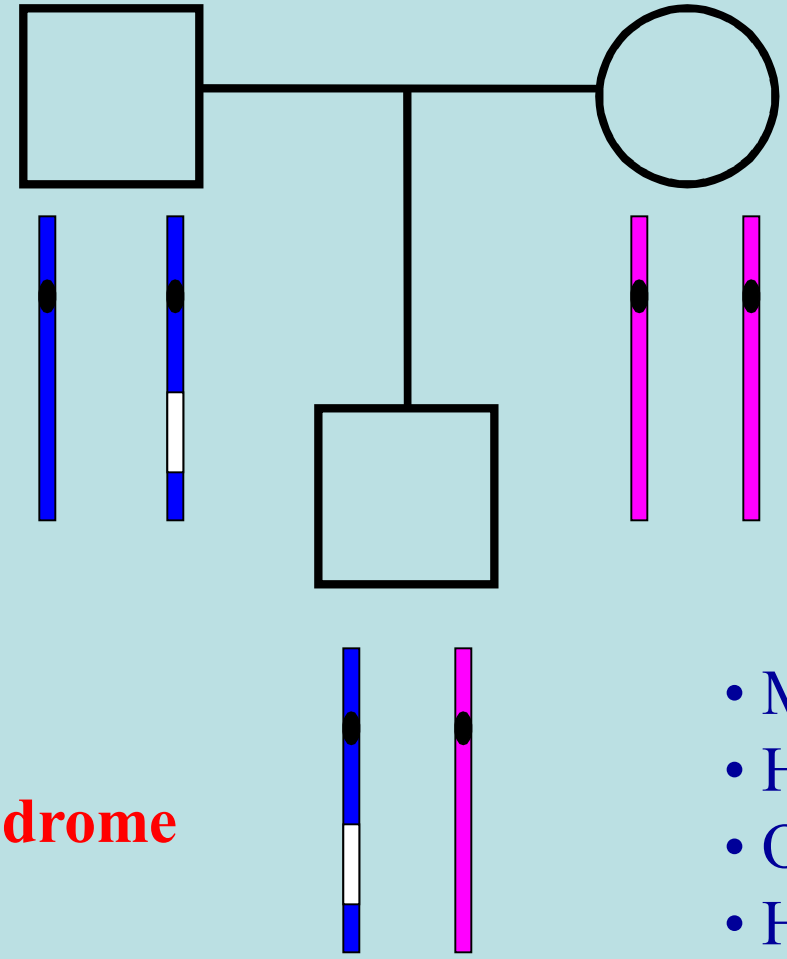
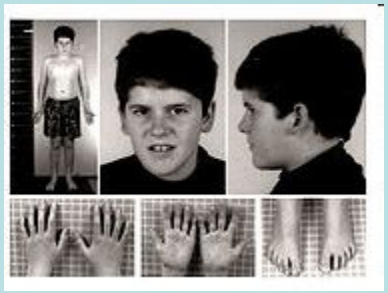


FIGURE 29.25 The ICR contains an insulator that prevents an enhancer from activating *Igf2*. The insulator functions only when CTCF binds to unmethylated DNA.

Imprinting examples:
Deletion of the regions 15q11-q13 in heterozygosis

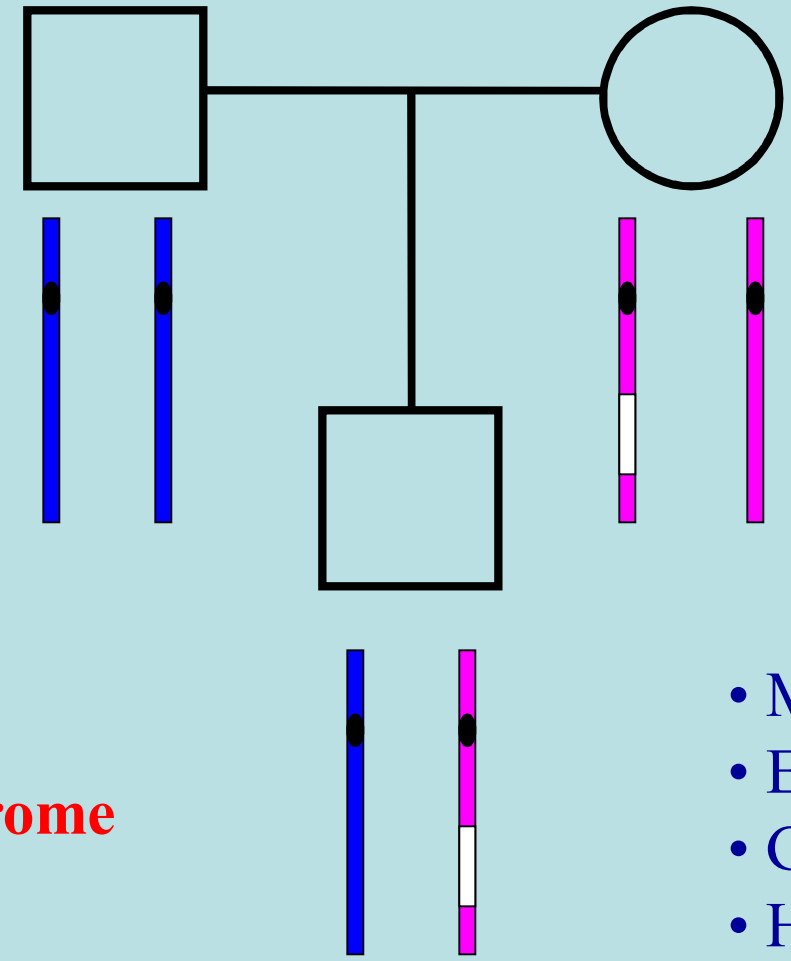




Prader-Willi syndrome

- Mental retard
- Hypotony
- Obesity
- Hypogonadism

Imprinting examples:
Deletion of the regions 15q11-q13 in heterozygosis



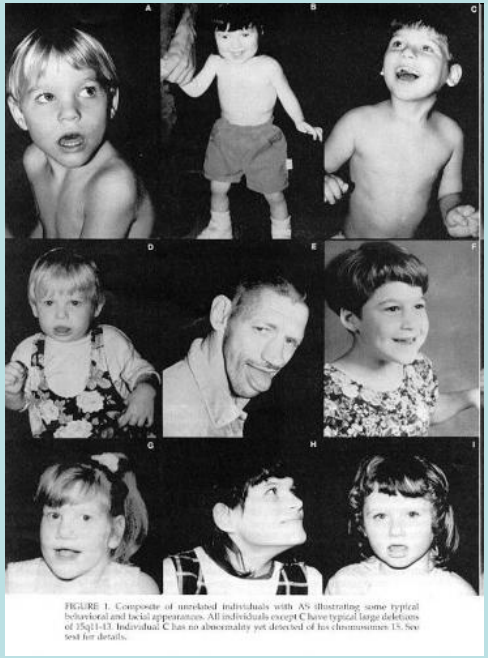


FIGURE 1. Composite of unrelated individuals with AS illustrating some typical behavioral and facial appearances. All individuals except C have typical large deletions of 15q11-13. Individual C has no abnormality yet detected of his chromosomes 15. See text for details.

Angelman syndrome

- Mental retard
- Elocution difficulties
- Growth delay
- Hyperactivity
- Inappropriate laughter

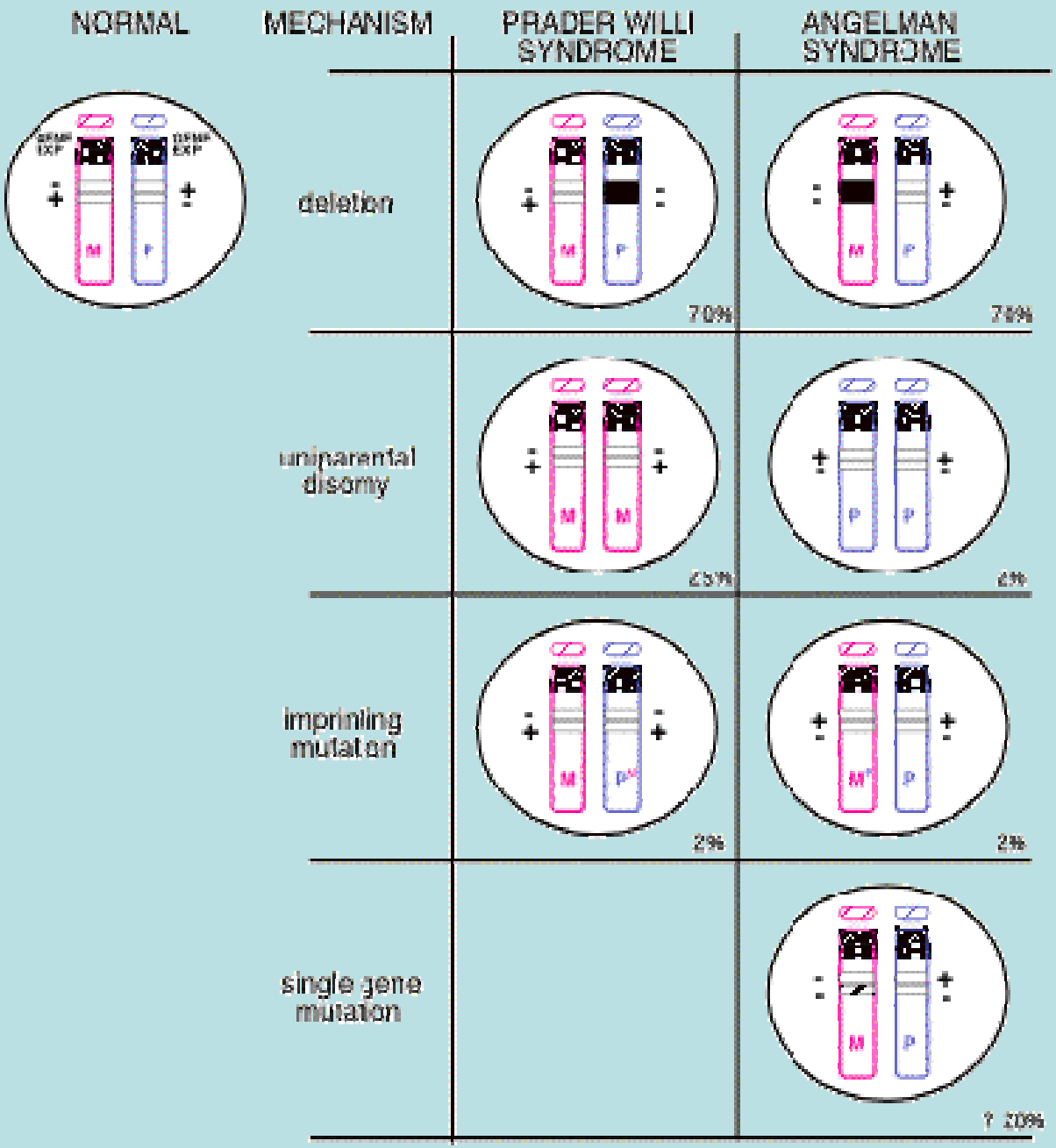
NON TRADITIONAL INHERITANCE

Mendel: genes transmitted by one on the other parent have the same phenotypic effect: the expression of a gene is the same independently from the parent that transmitted it.

GENOMIC O PARENTAL IMPRINTING: differential expression of one gene or of part of chromosomes depending on the parent that transmitted it (allelic exclusion depending on the parental origin).

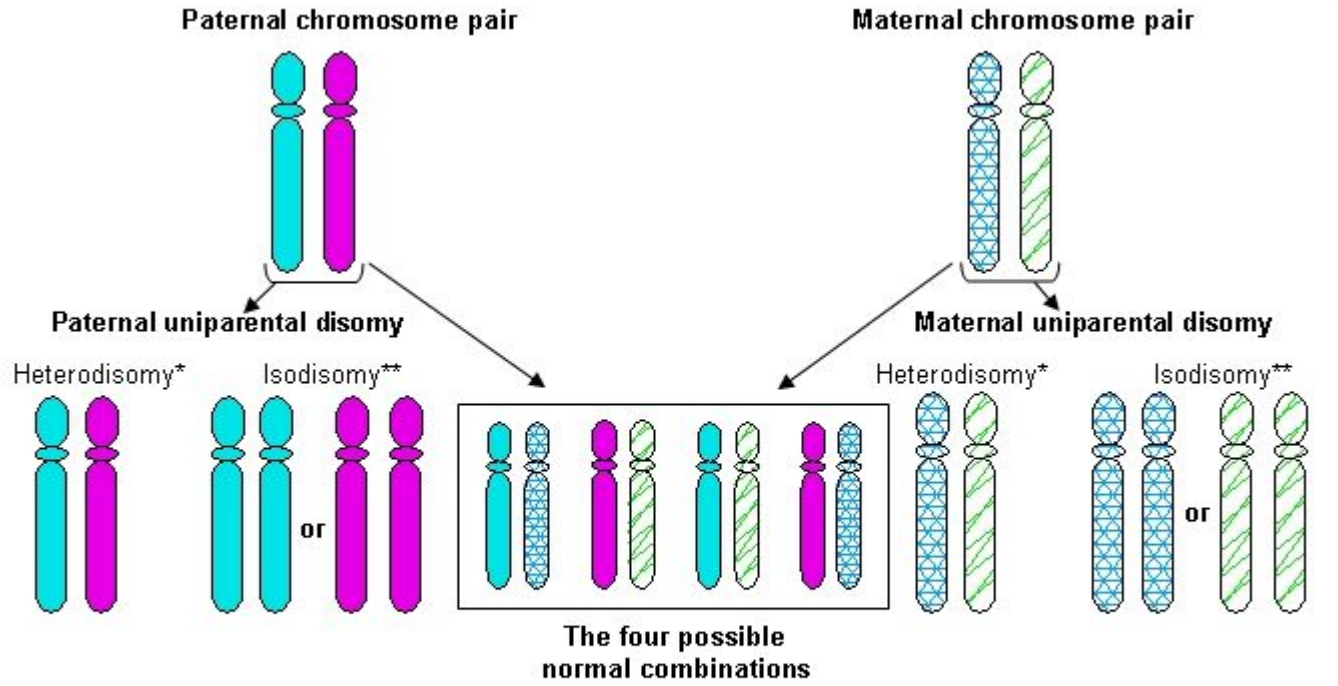
MATERNAL or PATERNAL IMPRINTING: gametogenesis in one sex "mark" one gene to make it different from the counterpart given from the opposite sex parent. During gametogenesis some genes are inactivated in differential way in both sexes; whereby their expression depends on the parent who transmitted it.

MECHANISMS OF IMPRINTED GENE DISEASE



UNIPARENTAL DISOMY-UPD

DIPLOID ORGANISM WITH TWO COPIES OF A CHROMOSOME (OR OF A PART OF A CHROMOSOME) COMING FROM THE SAME PARENT



* Heterodiomy = both homologs from a single parent are present
** Isodisomy = identical chromosome is present in duplicate

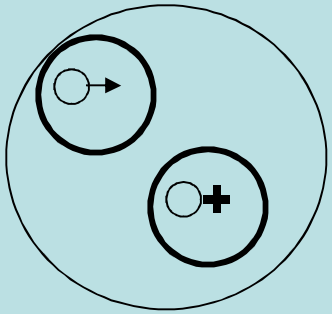
Experiments made in mice show that many UPD have no phenotypic effect.

UPDs can be isodisomy when both the homologs are identical, or heterodisomy when comes from both the homologs of one parent.

The most common cause of UPD is thought to be the recovery of a trisomy: one trisomyc product of conception that would be destined to die, occasionally loses from a single cell that is still totipotent one of the chromosomes, for example due to a wrong mitotic disjunction or an anaphase delay.
The euploid progeny from this cell will give the embryo, while all other cells die.

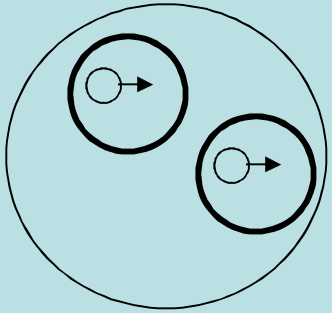
Many human UPDs are not usually diagnosed,
because are not cause of a pathology.

Uniparental diploidy are lethal

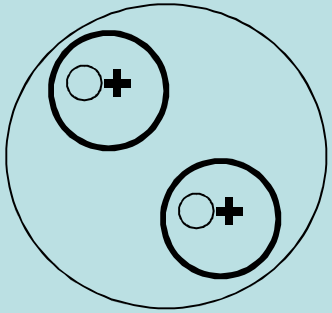


NORMAL DEVELOPMENT

In humans, 46XX from an exclusive paternal origin gives hidatiform moles, often developing in a choriocarcinoma.
The ovarian teratomas instead result from a uniparental maternal diploidy.



LETHAL



LETHAL

NORMAL DEVELOPMENT REQUIRES THE PRESENCE OF BOTH A MATERNAL AND A PATERNAL GENOME RUNNING IN A DIFFERENT WAY.

THE ACTIVITY OF SOME GENES IN EMBRYO DEPENDS ON THE PARENTAL ORIGIN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POTENT BIOLOGY
Stem Cells, Cloning,
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