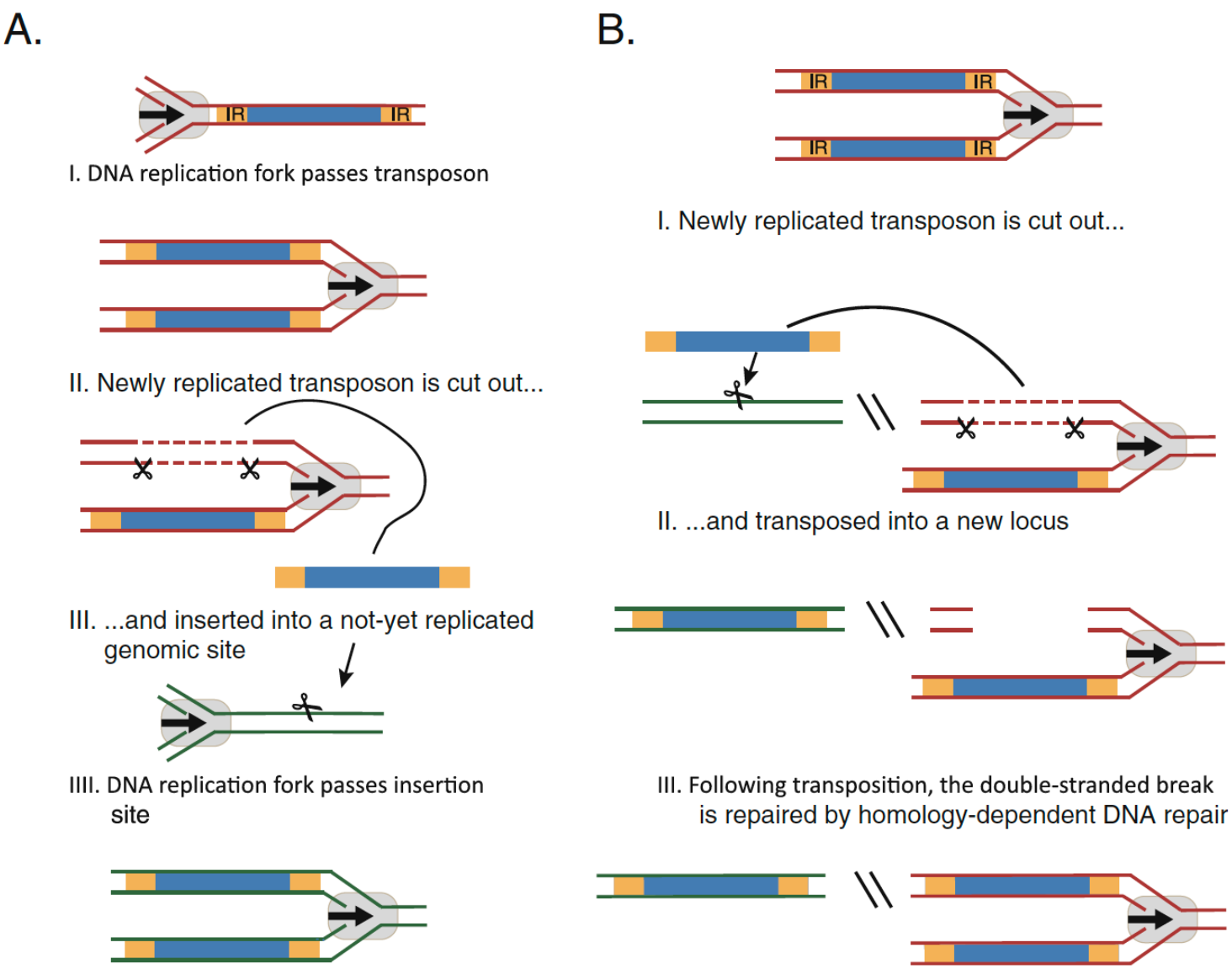


Figure 3 Models of replicative transposition. (A) After replication, the transposon is excised and integrated into a yet unreplicated genomic site thus duplicating the newly inserted transposon. **(B)** The double-stranded break created by transposition at newly replicated DNA is repaired using the sister chromatid as a template for homology-directed DNA repair, leading to reconstitution of the excised transposon. IR, terminal inverted repeat.

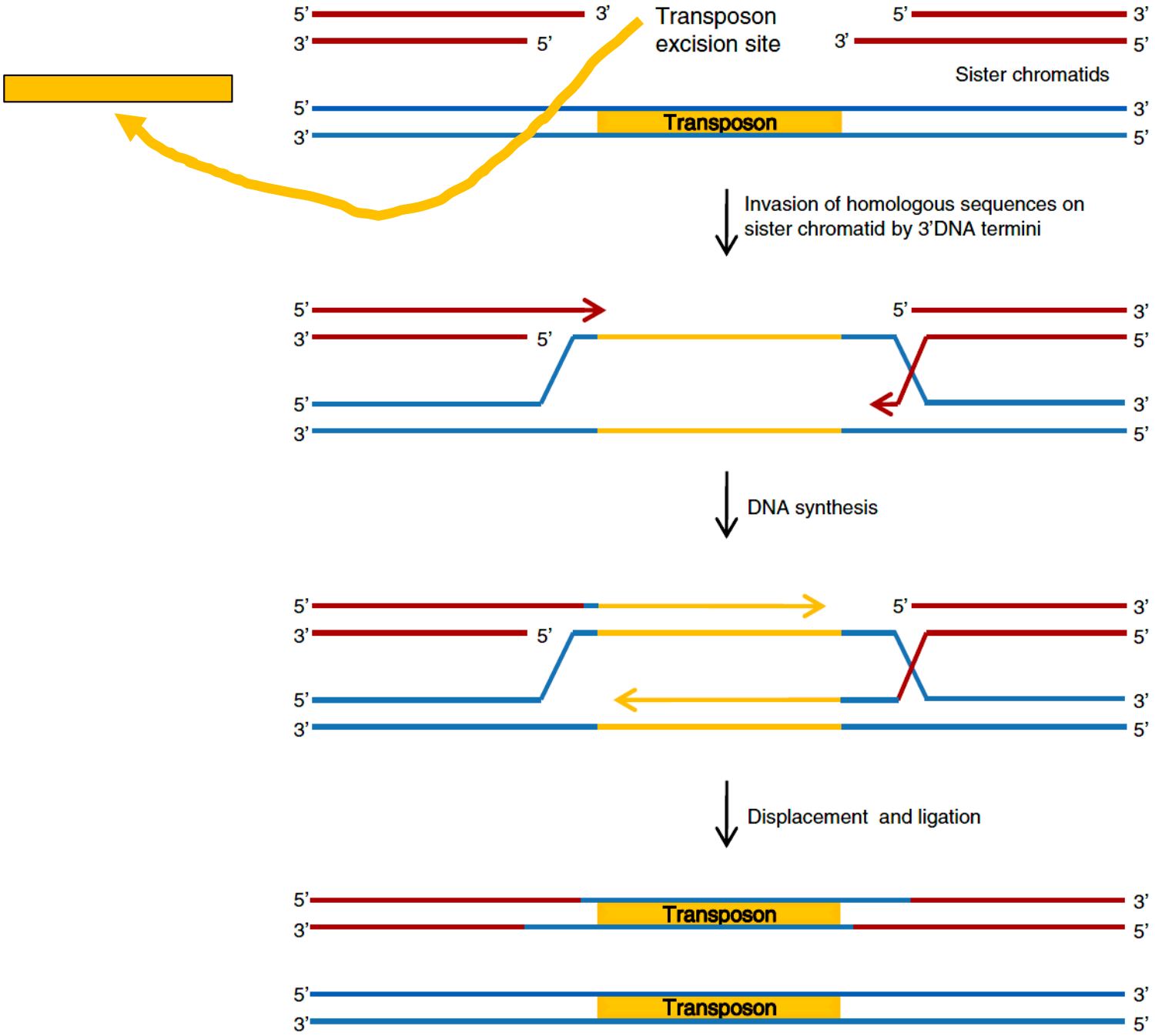
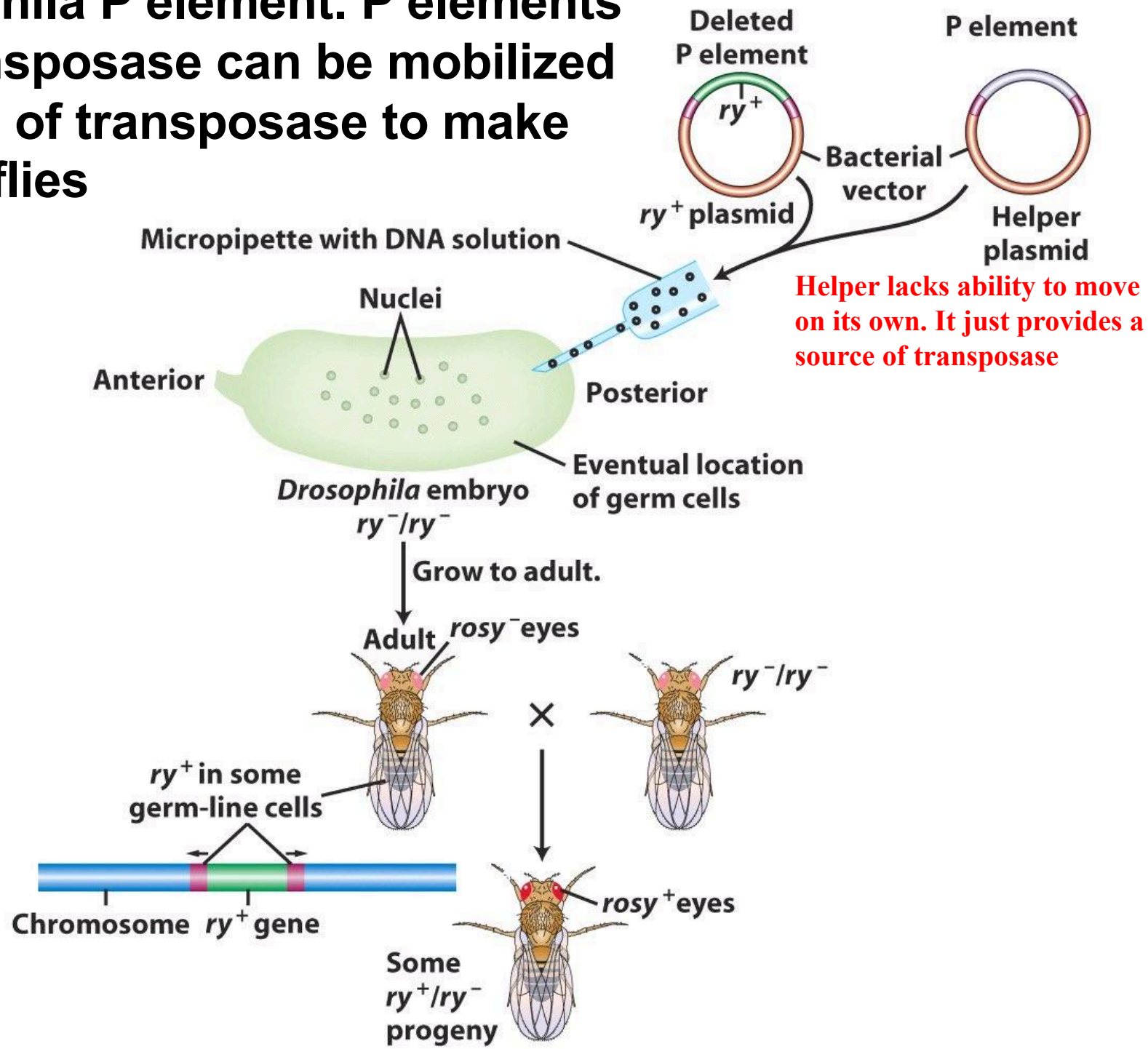


Figure 4 Homology-dependent DNA repair by synthesis-dependent strand annealing (SDSA). Following excision, the 3′-DNA termini from the double-stranded breaks invade the homologous sequence on the sister chromatid. The homologous sequence is then used as template for DNA synthesis and the final, elongated 3′-DNA termini anneal to each other and are joined with the 5′ ends by ligation.

The Drosophila P element. P elements lacking transposase can be mobilized by a source of transposase to make transgenic flies

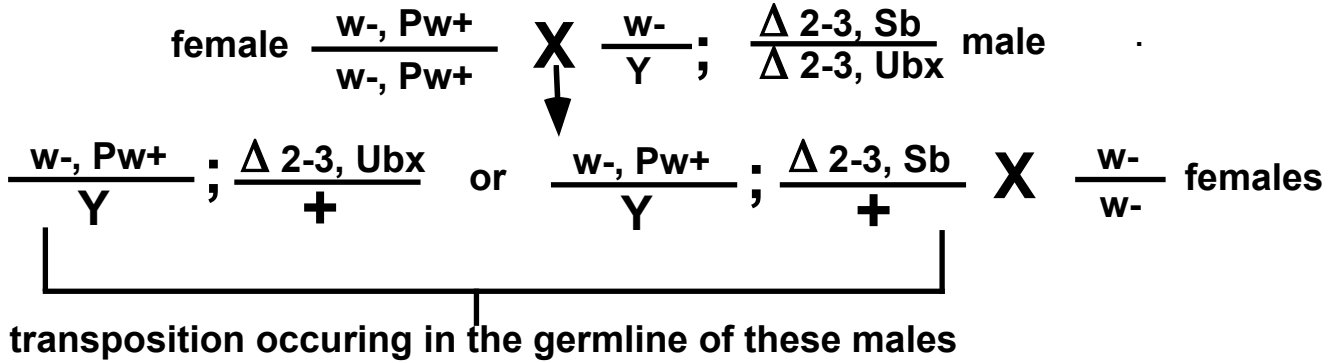


Helper lacks ability to move on its own. It just provides a source of transposase

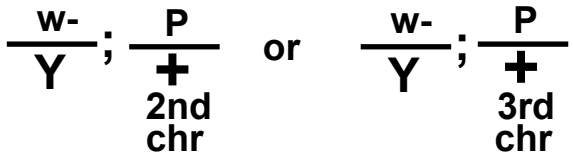
Hopping a transposon in flies off the X chromosome, and onto an autosome.

Pw+ = a transposon that carries a wildtype copy of the w gene on the X chromosome.

Δ 2-3, Ubx and Δ 2-3, Sb = these are third chromosomes that carry an immobilized source of transposase (Δ 2-3), as well as a dominant visible marker (Ubx or Sb). Transposase is normally only active in the germline. However, Δ 2-3 is active in somatic cells as well. This has the consequence that transposons are continually being mobilized in somatic tissues. If the P element contains a cell autonomous marker gene such as w+ (red pigment) this mobilization can be observed as eye color mosiacism.



The P element can hop into the + version of the second or third chromosome. If this occurs, and the meiotic product (the sperm) also carries a Y chromosome, then the progeny will be a red-eyed male. If no transposition occurs or the P hops into outer space then male progeny will have white eyes.



There are a number of other male progeny types that will also be genrated. Some of these will still have the Δ 2-3 source of transposase. Others will simply have no P element transposition onto one of the chromosomes present in the gamete. These will all lead to progeny that are w-

1. Any red eyed male resulting from the above crossmust have had a transposition event off the X chromosome and onto an autosome or onto the Y chromosome (happens rarely), since males get their X (which in this case is w-) from the female.

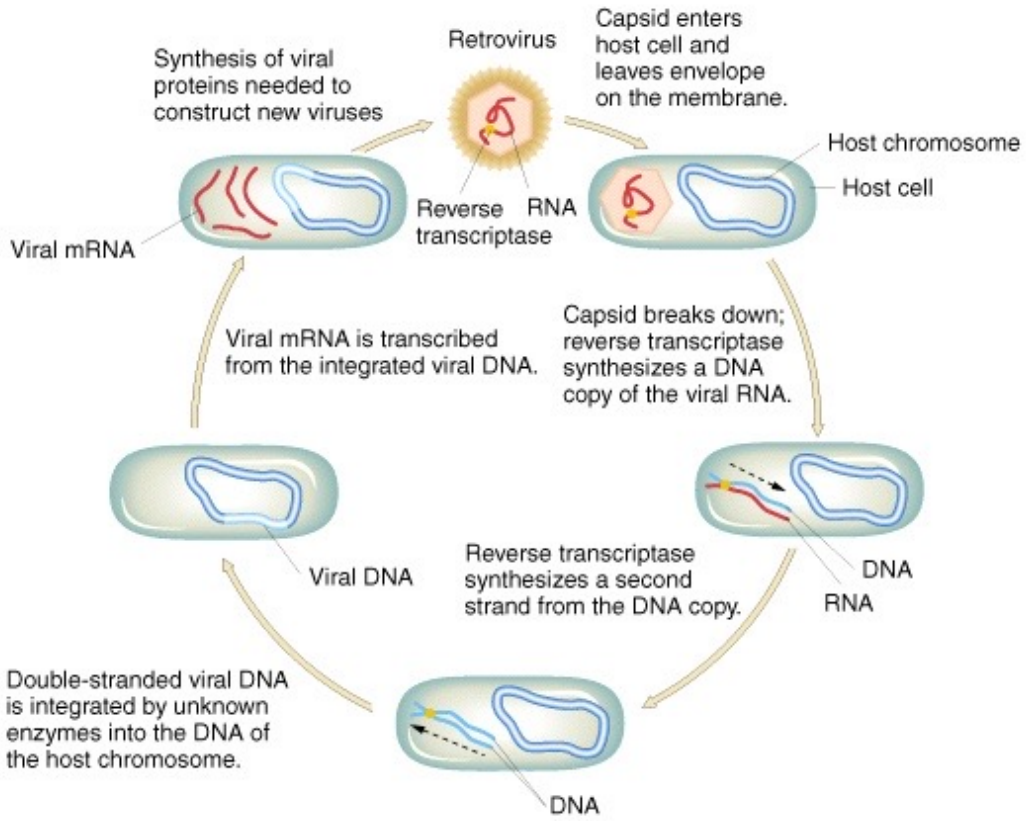
2. Males that have both a P element and Δ 2-3 will have red and white variegated eyes as a result of somatic transposition of the Pw+ in somatic eye tissue.

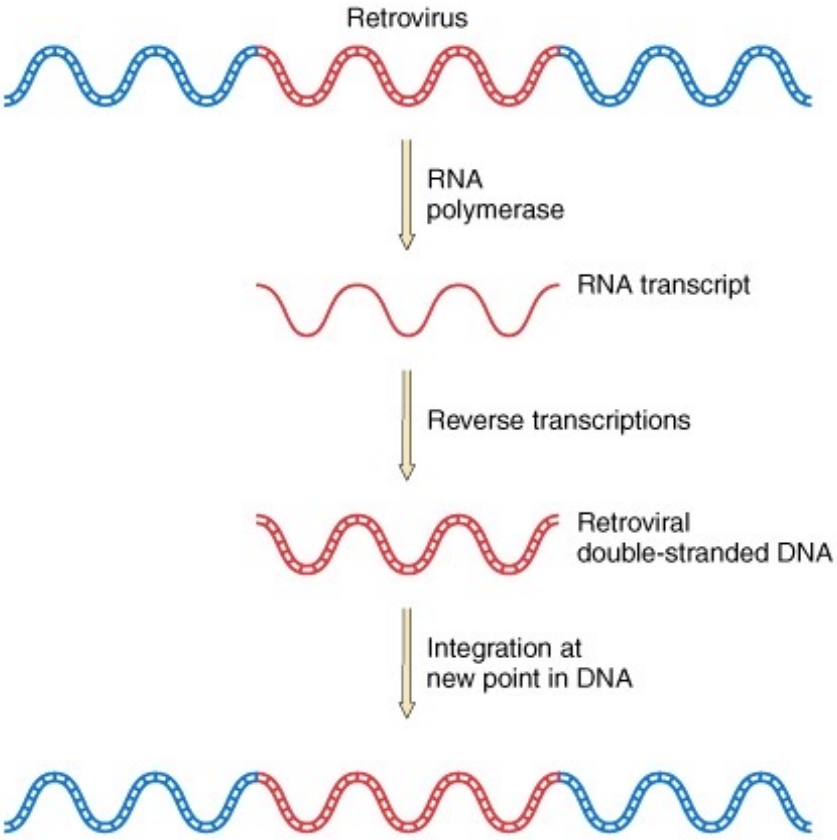
The power of the P element

In the early 20th century wild Drosophila melanogaster populations lacked the P element. We know this because people collected animals from throughout the world and kept them as stocks up through the present. They lack any P elements.

The P element entered D. melanogaster sometime in the 20th century, from another species. It is now ubiquitous (30+ copies) in ALL wild populations.

Retroviruses are RNA viruses that generate a double-stranded DNA intermediate using the enzyme reverse transcriptase. The double-stranded DNA intermediate can integrate into the genome, generating a provirus.





Retrovirus-like Retrotransposon

Yeast Ty1

LTR: Long Terminal Repeat contains both promoter and poly-A sites

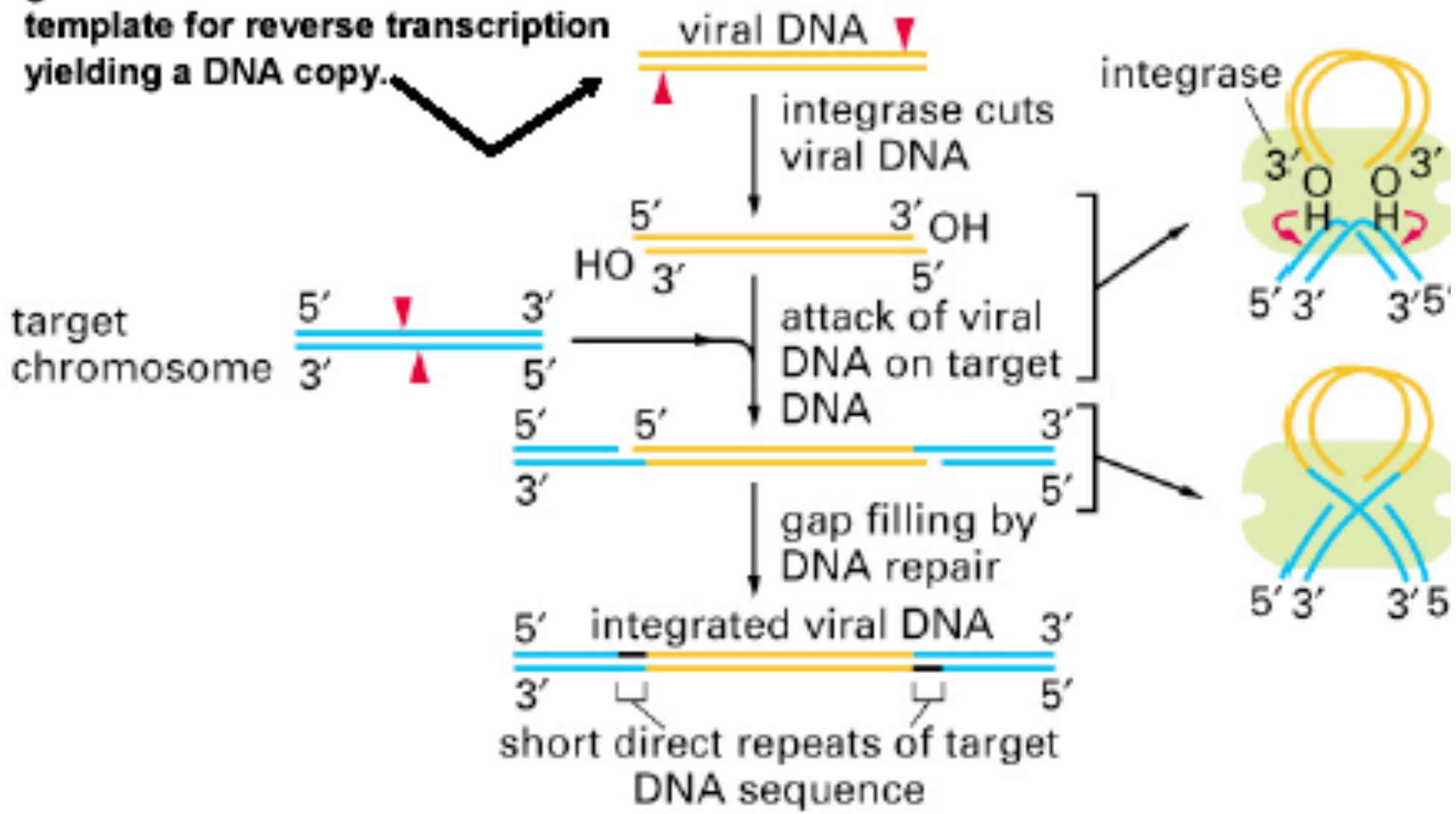
TYA: encoding a polyprotein for the capsid (*gag*-like proteins)

TYB: encoding a polyprotein for enzymes used in transposition

- ***pro*** **proteinase**
- ***int*** **integrase**
- ***rt*** **reverse transcriptase**
- ***rnh*** **RNaseH**

Integrase Catalyzes Insertion of Retrotransposons

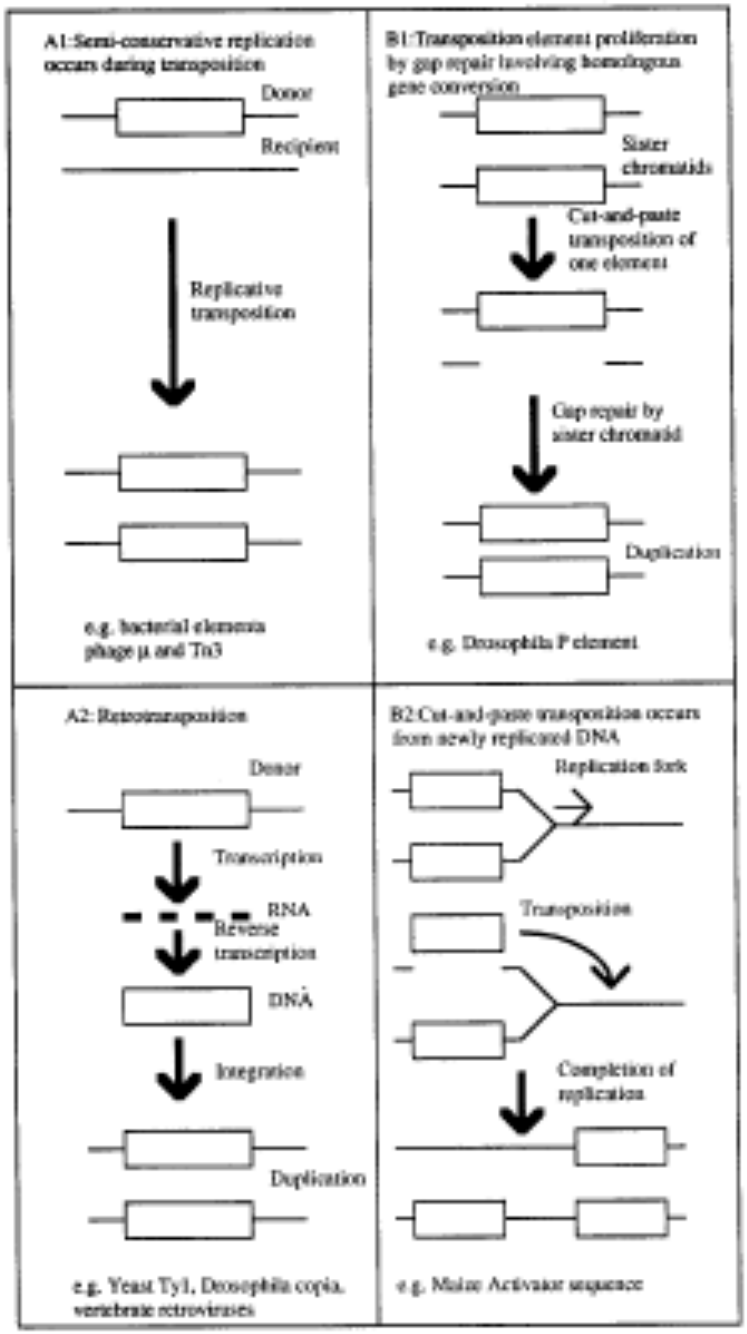
Retrotranposon transcription generates RNA that serves as a template for reverse transcription yielding a DNA copy.



Mechanisms by which transposons and retrotransposons increase in copy number

Replicative transposition

RNA synthesis followed by reverse transcription



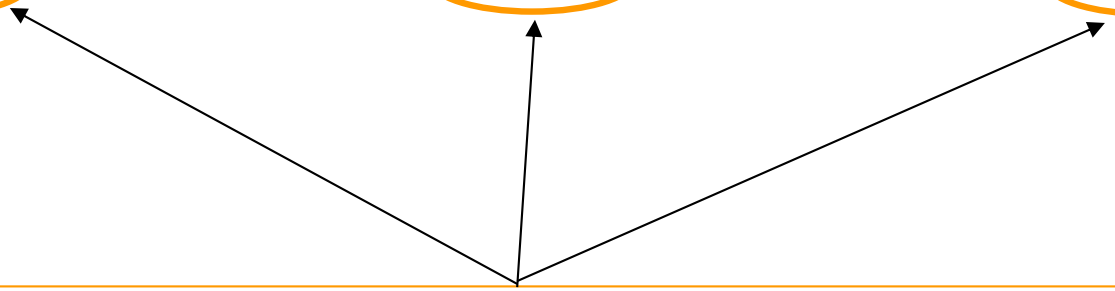
Repair of a DNA Double-stranded break

Movement in front of a replication fork

Fig. 1 Four ways in which transposition can lead to an increase in transposable element copy number. For

Number and nature of interspersed repeats in human, fly and worm

	Human		Fly		Worm	
	Percentage of bases	Approximate number of families	Percentage of bases	Approximate number of families	Percentage of bases	Approximate number of families
LINE/SINE	33.40%	6	0.70%	20	0.40%	10
LTR	8.10%	100	1.50%	50	0.00%	4
DNA	2.80%	60	0.70%	20	5.30%	80
Total	44.40%	170	3.10%	90	6.50%	90

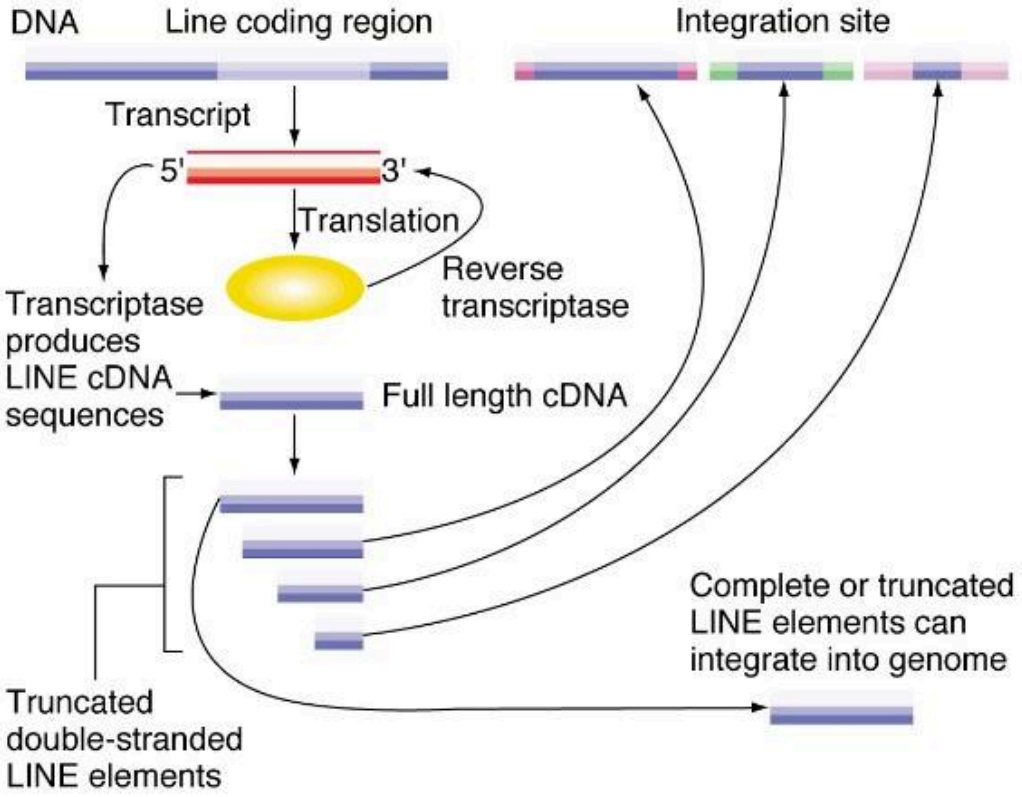


The euchromatic portion of the human genome has a much higher density of transposable element copies than fly and worm

Transposons and genome structure

Drosophila melanogaster: 90 different elements = 3% of genome
Mammals: LINES and SINES = 34% of genome

LINES are retroposons that derive from a "selfish" DNA sequence that encodes reverse transcriptase. A complete LINE sequence can be copied into RNA. Reverse transcriptase makes cDNA copies from the RNA. These copies may be complete or truncated. Both may then integrate into other sites in the Genome.

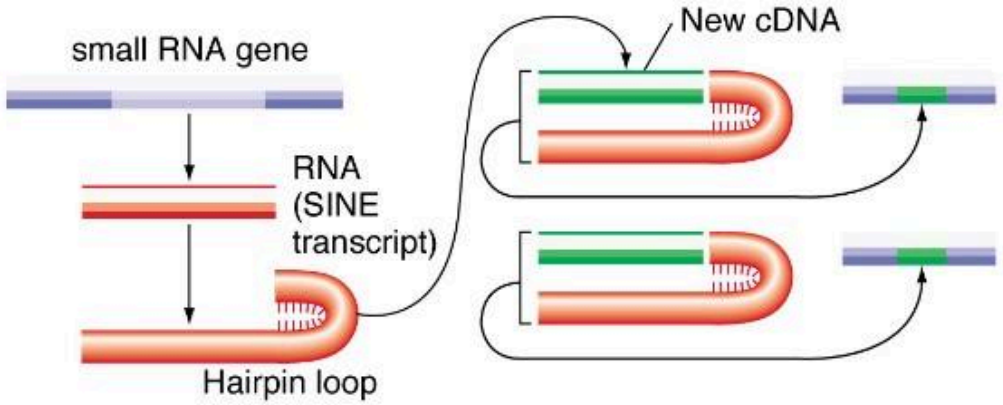


SINES replicate through an RNA intermediate, but do not encode reverse transcriptase themselves. Instead They utilize reverse transcriptase present in the cell.

The Alu family of repetitive elements in the human Genome is an example of a SINE family. There are Than 500,000 Alu elements/ genome at 300bp/element.

The SINE element has evolved from normal cellular RNAs, most often tRNAs, but also an RNA that forms A component of the signal recognition particle, which Is required for protein translocation across the Endoplasmic reticulum membrane.

The important modification of these cellular RNAs that Has occurred are modifications in the 3' end of the Transcript that lead to self complementarity. This Provides a binding structure for reverse transcriptase.



How a Quarter of Cow DNA Came From Reptiles

By hopping between species, jumping genes have radically altered the course of animal evolution.

By Ed Yong

Transposon mobilization: Consequences for genome evolution over time

1. Inversions as a result of recombination between inverted repeats.

2. Deletions as a result of recombination between direct repeats.

3. Unequal crossing over between repeats (regions of micro-homology) leads to translocations, deletions, and gene duplications.

4. Gene inactivation as a result of transposon insertion.

5. Gene activation as a result of a transposon (or retrovirus) inserting near a gene, and driving the transcription of that gene from a transposon promoter.

6. Once transposons enter the germline of a species they can multiply and become ubiquituous very rapidly.

   Drosophila P elements. World-wide spread within 30 years

   Alu sequences in primates and humans.

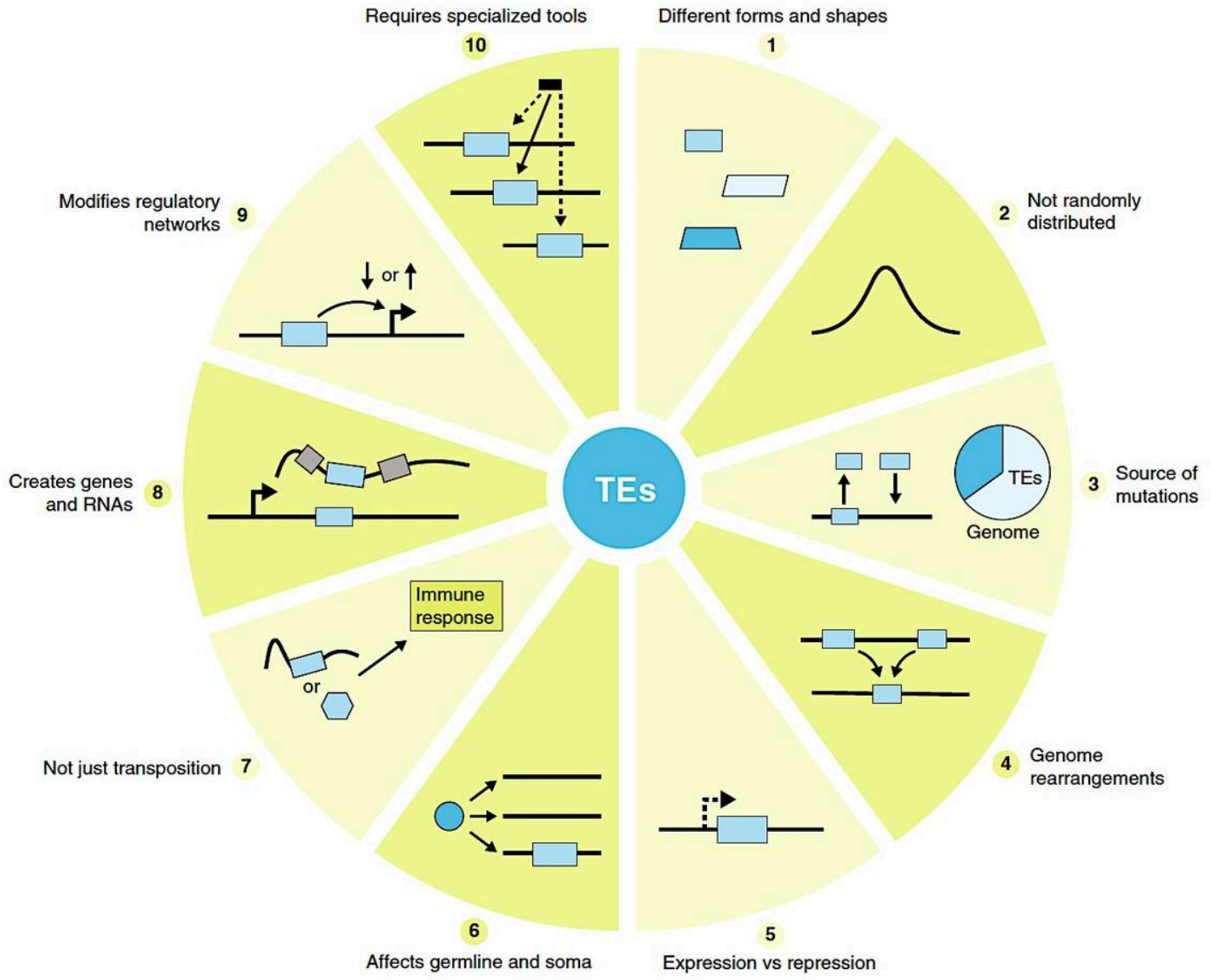


Fig. 2 Ten things you should know about transposable elements (TEs). Examples of how TEs can impact genomes in direct and indirect ways. Blue boxes represent TEs, gray boxes represent canonical exons, and the black box represents a sequencing read. Right-angled arrows represent gene or TE promoters

Epigenetics

The histone code

Imprinting

and cloning

Fertilization is a very efficient way of producing a healthy embryo (otherwise we would not be here)

Cloning animals from somatic cells is very inefficient: only 1-5% of embryos survive

WHY??!!!

Broadly speaking

An “epigenetic” effect on the genome changes the phenotype without changing the genotype.

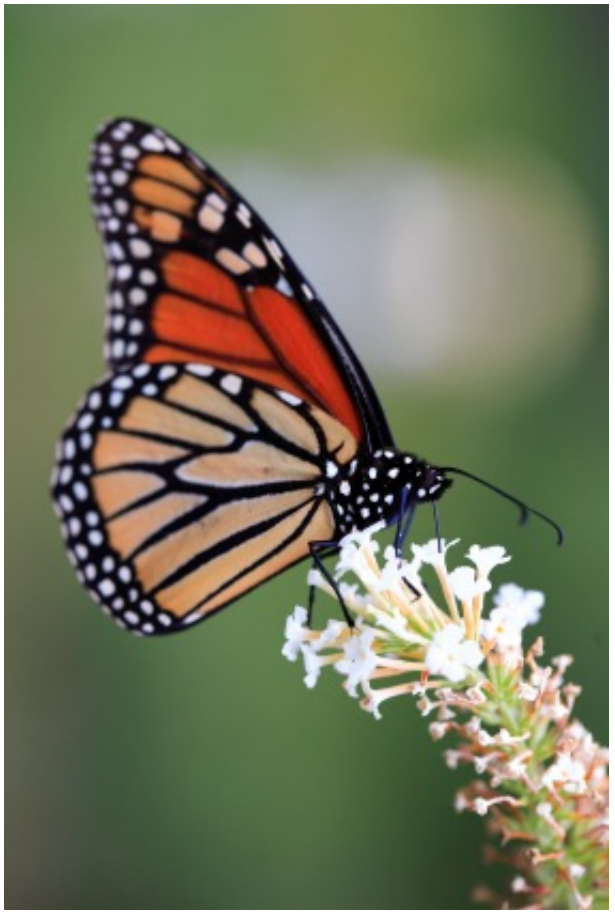
→ The power of the environment and of life history

Technically

"A mitotically or meiotically heritable change in gene expression state (or genome functional state) that is not associated with a change in the primary sequence of DNA."

The Power of Epigenetics

Epigenetic processes generate
multiple phenotypes from the same genotype



In other words

<u>Genetics</u>

Organism (or a cell) with a phenotype

↓

Mutation (change in DNA)

↓

Different phenotype

<u>Epigenetics</u>

Organism (or a cell) with a phenotype

↓

Something happens, but **not** a change in the DNA

↓

Different phenotype

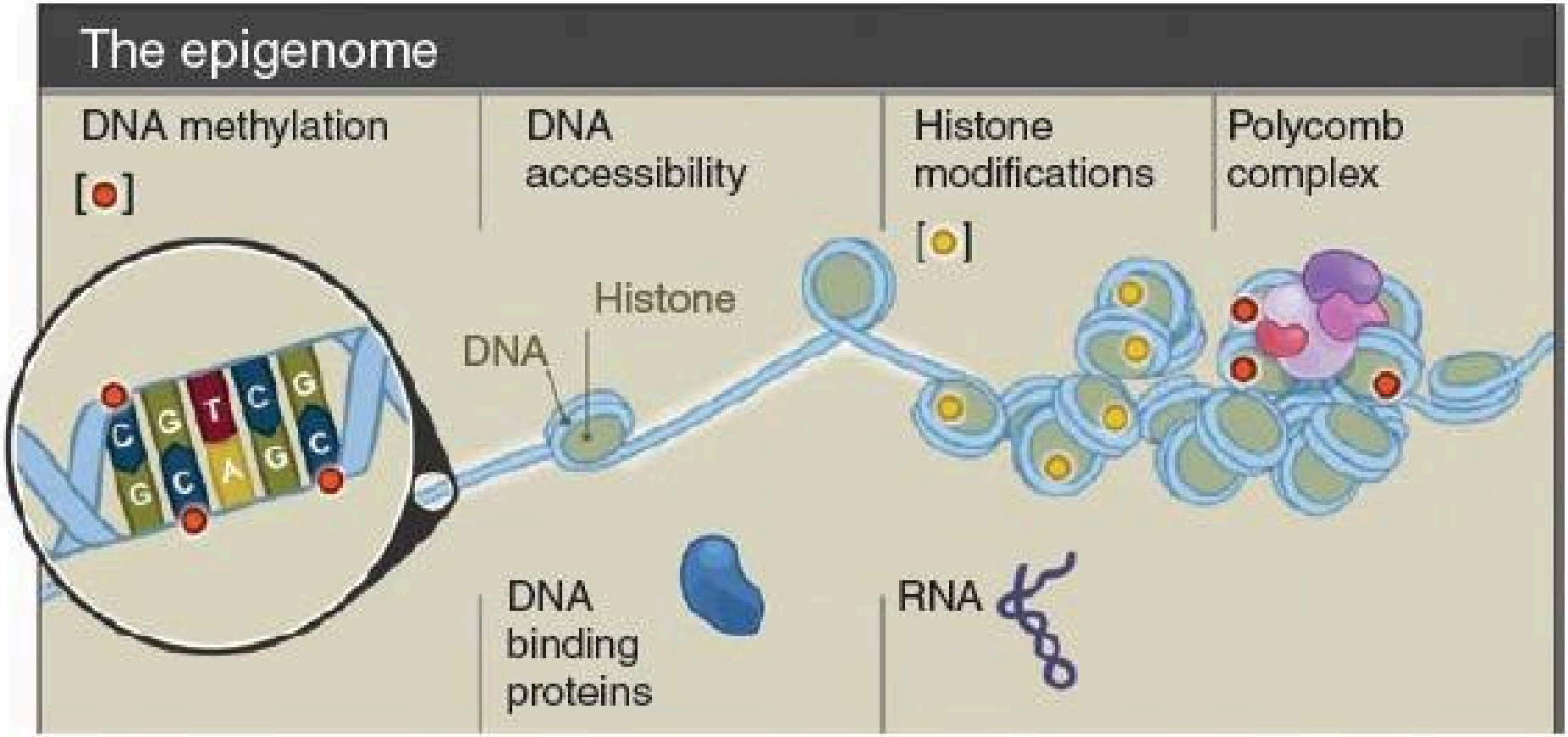


Figure 1 Layers of genome organization. Genome function and cellular phenotypes are influenced by DNA methylation and the protein-DNA complex known as chromatin. In mammals, DNA methylation occurs on cytosine bases, primarily in the context of CpG dinucleotides. Accessible chromatin that is hypersensitive to DNase I digestion marks promoters and functional elements bound by transcription factors or other regulatory proteins. Histone modifications, associated proteins such as Polycomb repressors and noncoding RNAs constitute an additional layer of chromatin structure that affects genome function in a context-dependent manner.

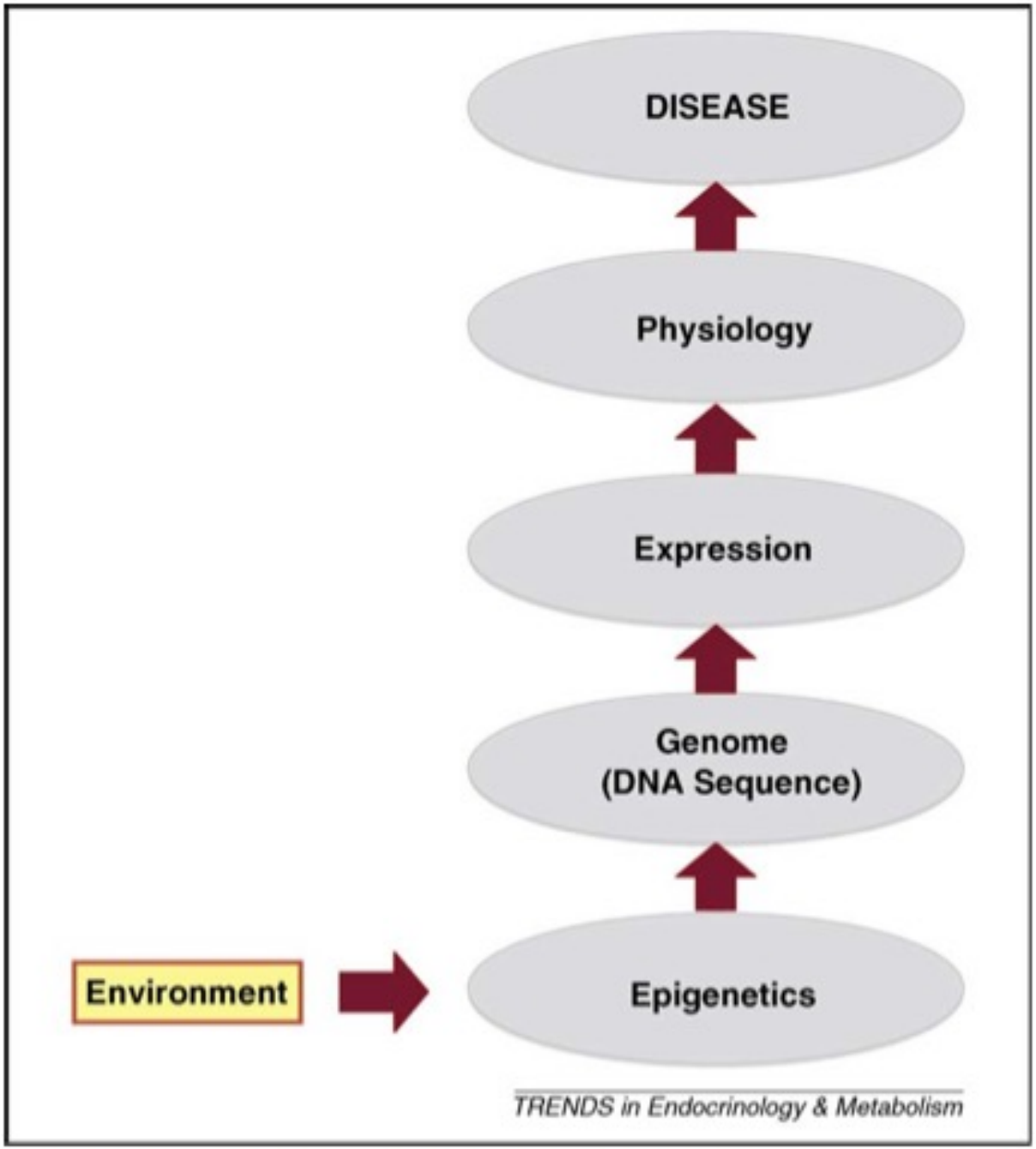
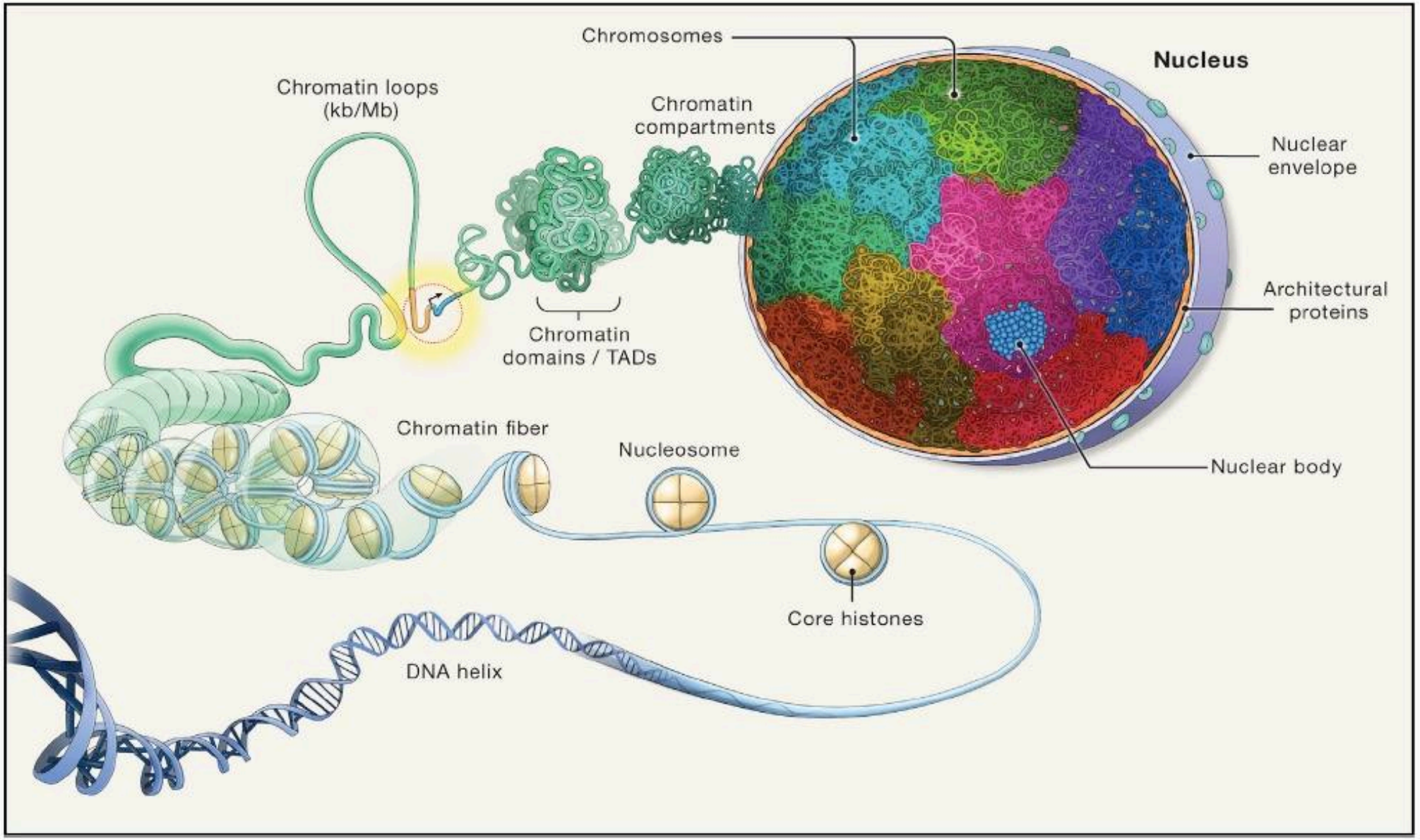
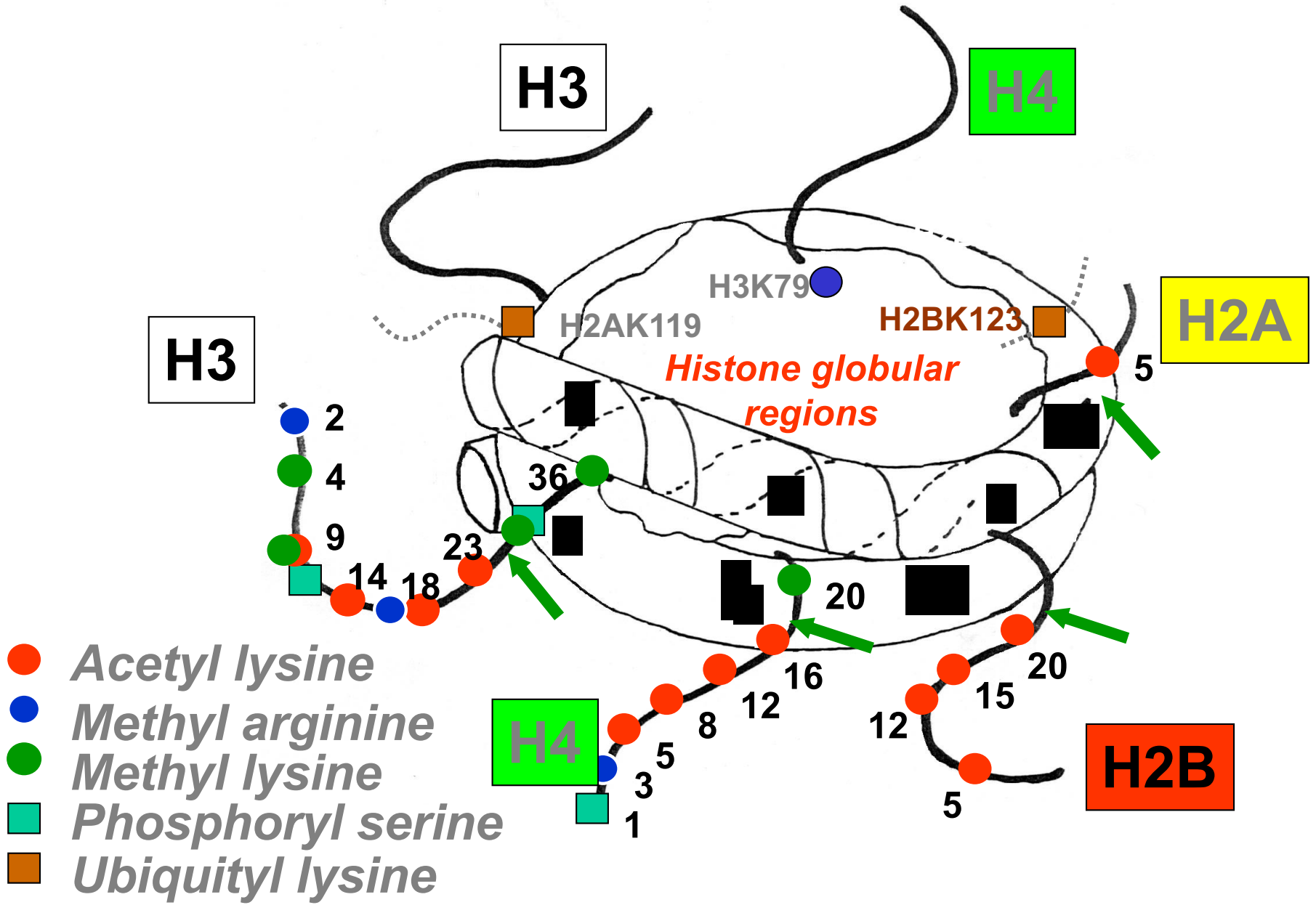
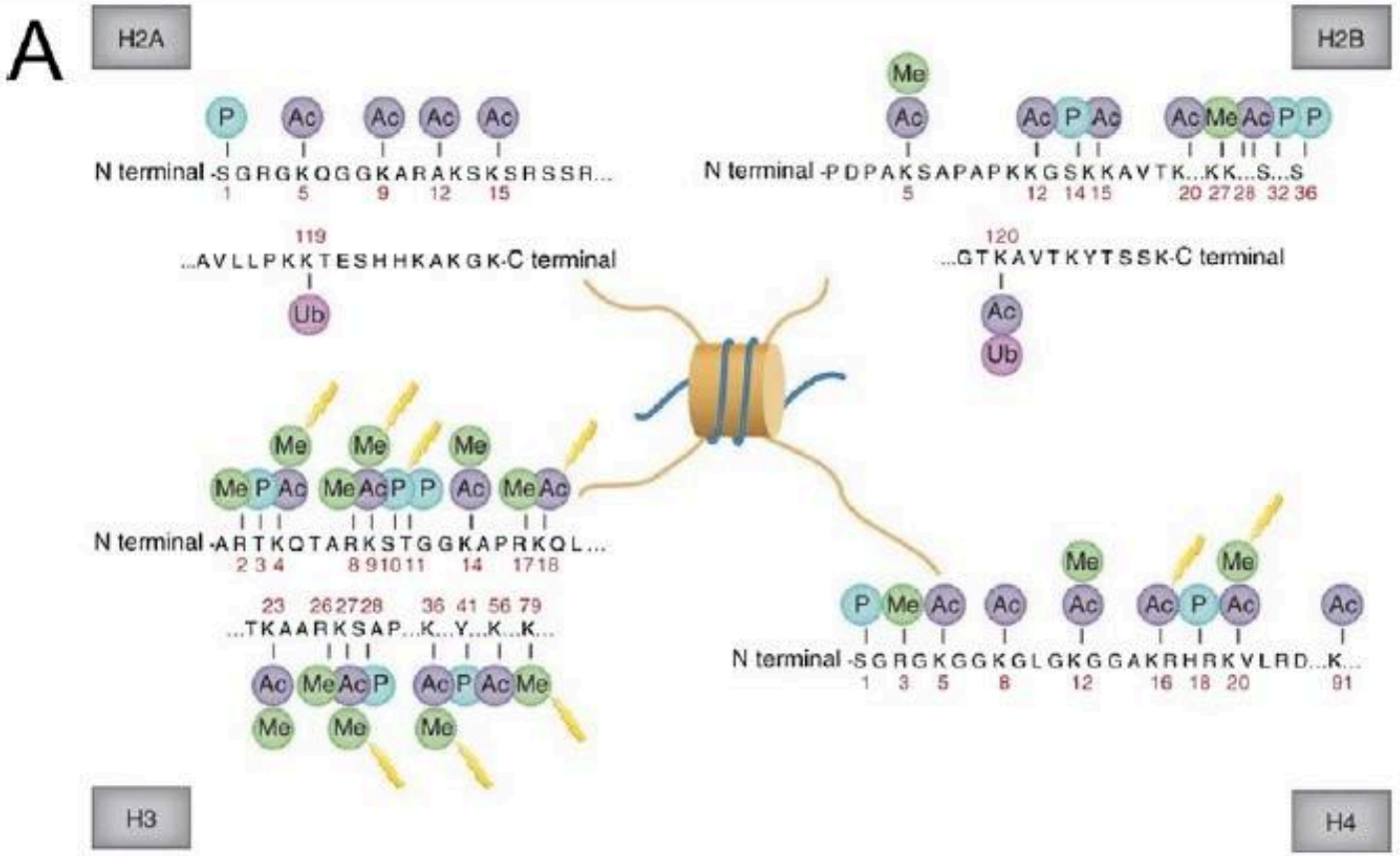


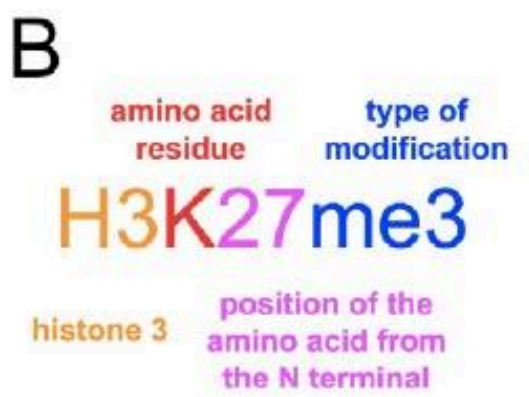
Figure 1. Proposed etiology of how the environment effects disease. The cascade of molecular and physiological processes following an environmental exposure to promote disease is shown.

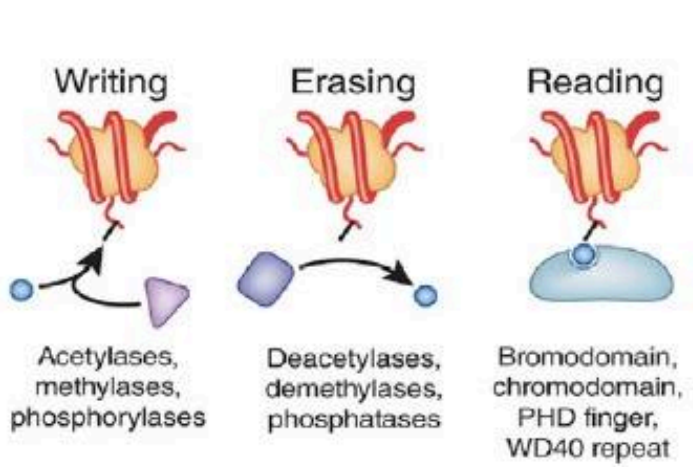

Chromosomes
Nucleus
Chromatin loops
(kb/Mb)
Chromatin
compartments
Nuclear
envelope
Architectural
proteins
Chromatin
domains / TADs
Chromatin fiber
Nucleosome
Nuclear body
Core histones
DNA helix

Histone tails are subject to multiple, enzyme-catalyzed post-translational modifications (PTM)

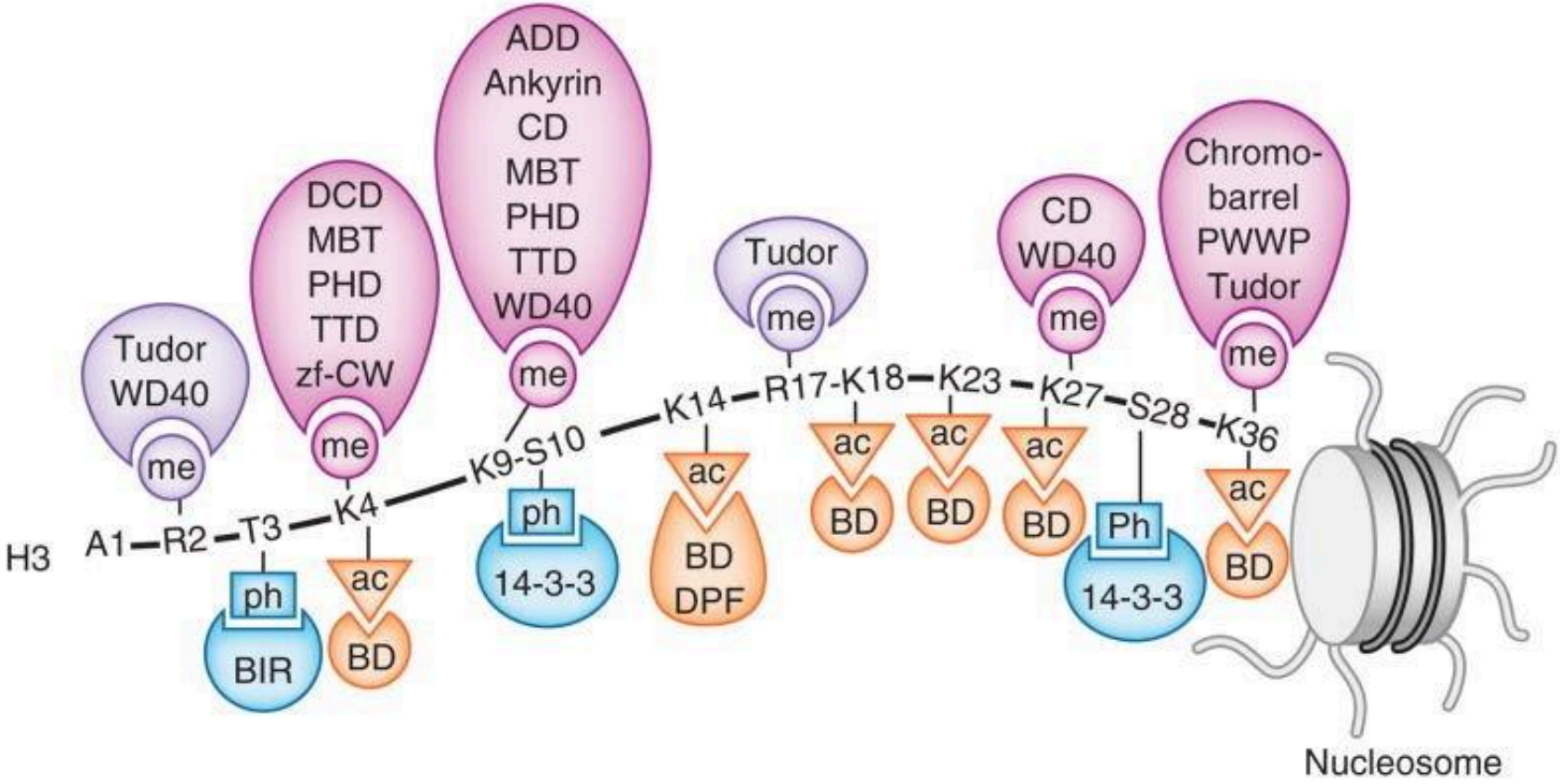



A
H2A
H2B
H3
H4
N terminal -SGRGKQGGKARAKSKSRSSR...
N terminal -PDPAKSAPAPKKGSKKAVTK...KK...S...S
...AVLLPKKTESHHKAKGK-C terminal
...GTKAVTKYTSSK-C terminal
N terminal -ARTKQTARKSTGGKAPRKQL...
...TKAARKSAP...K...Y...K...K...
N terminal -SGRGKGGKGLGKGGAKRHRKVLRD...K...


B
amino acid residue
type of modification
H3K27me3
histone 3
position of the amino acid from the N terminal


C
Writing
Erasing
Reading
Acetylases, methylases, phosphorylases
Deacetylases, demethylases, phosphatases
Bromodomain, chromodomain, PHD finger, WD40 repeat

Multiple protein binding domains recognise specific modified amino acids…a code?



Musselman et al (2012) Nature Struct.Mol.Biol. 19 (12) 1718-27

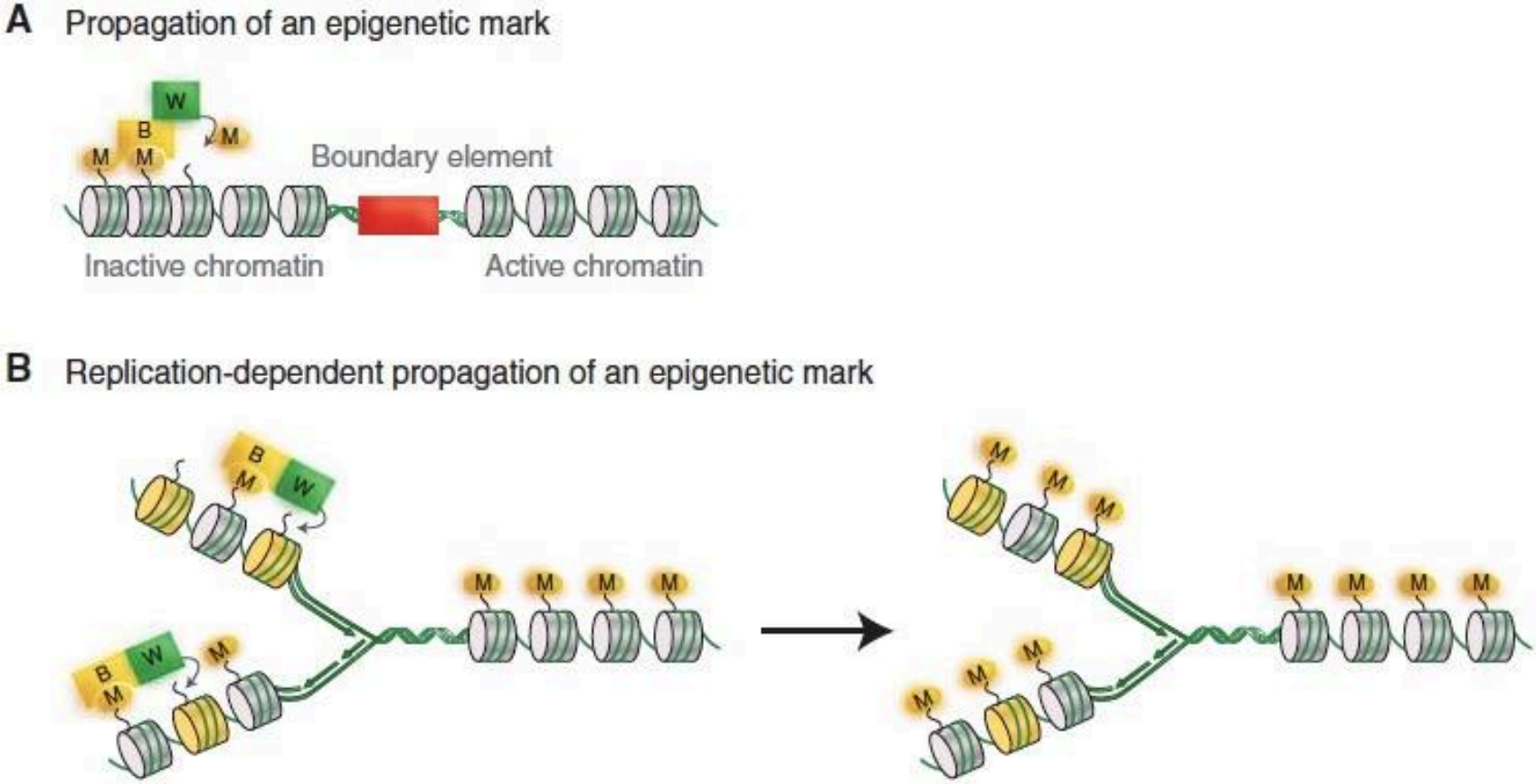
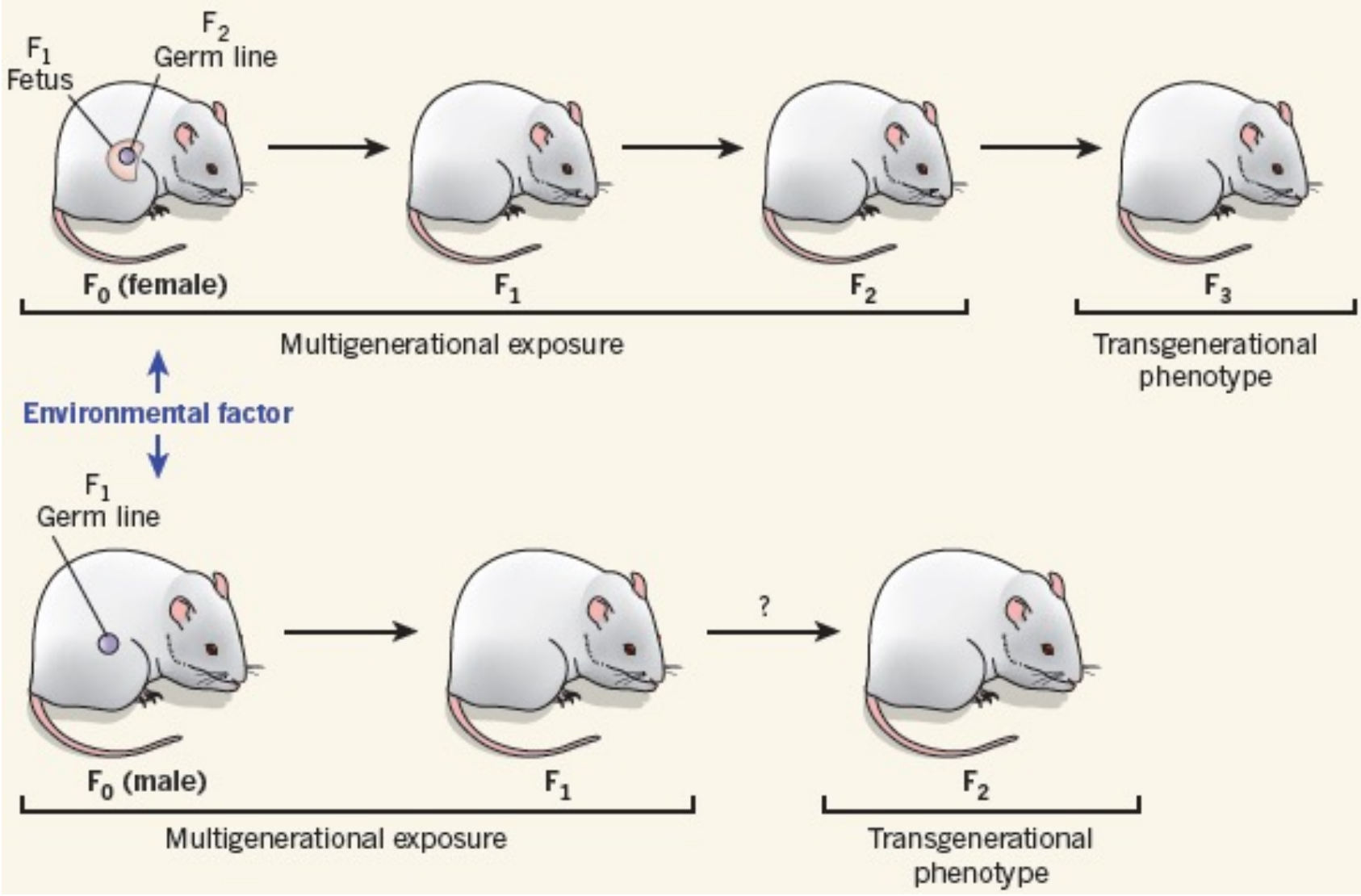


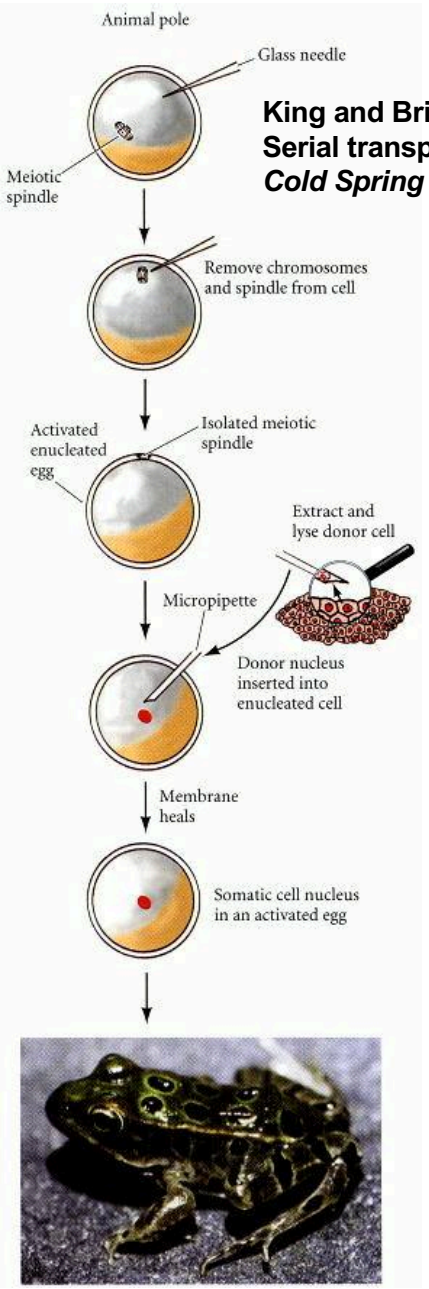
Figure 1. Propagation of epigenetic marks. (*A*) A general mechanism for propagating a histone modification such as H3K9 methylation typically found in heterochromatic regions. The modified histone tail (M) interacts with a protein binder (B) that has a binding site specific for that modification. B also has a specific interaction site with an enzyme "writer" (W) that carries out the same histone modification on an adjacent nucleosome (gray cylinder). Spreading of the histone mark will continue until the modifying machinery reaches a boundary element, delineating the boundary between heterochromatin and euchromatin. (*B*) A general mechanism for maintaining a histone modification during replication. Newly deposited nucleosomes (yellow), which may incorporate histone variants, are interspersed with parental nucleosomes (shaded in gray) following DNA replication. The modified histone tail (M) on the parental nucleosome interacts with a protein binder (B). As in *A*, B interacts with a "writer" (W), which catalyzes a histone modification on the histone tail from an adjacent daughter nucleosome.

Fathers' nutritional legacy

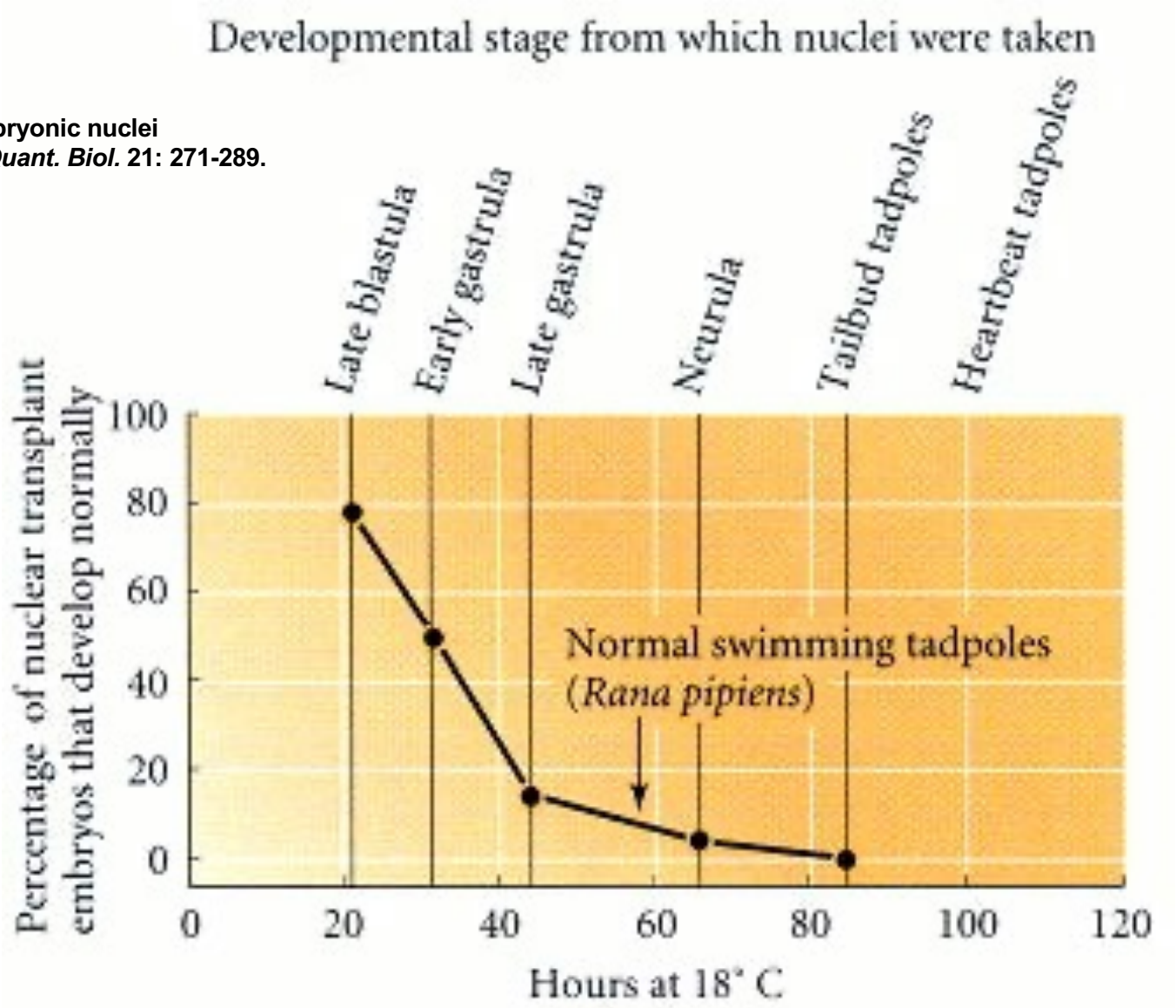
A female can develop a diabetes-like disease due to a high fat content in her father's diet before she was conceived. Epigenetic modifications of the father's sperm DNA might underlie this peculiar observation. SEE LETTER P.963



Nuclei taken from older developmental stages are less able to promote normal development. Their potential becomes restricted



King and Briggs (1956).
Serial transplantation of embryonic nuclei
Cold Spring Harbor Symp. Quant. Biol. 21: 271-289.



McKinnell, R. G. 1978. *Cloning: Nuclear Transplantation in Amphibia.* University of Minnesota Press, Minneapolis.

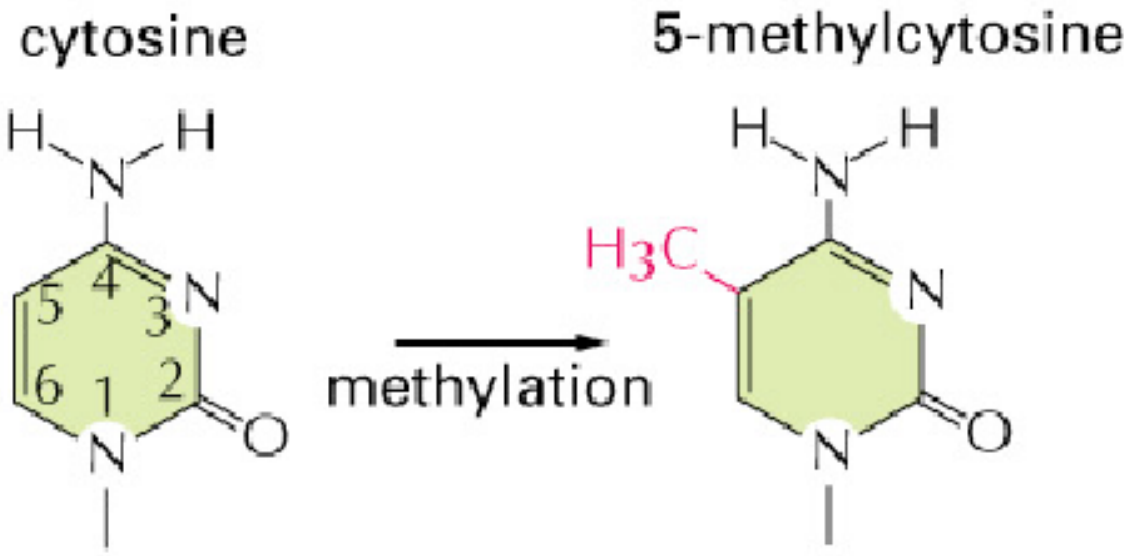
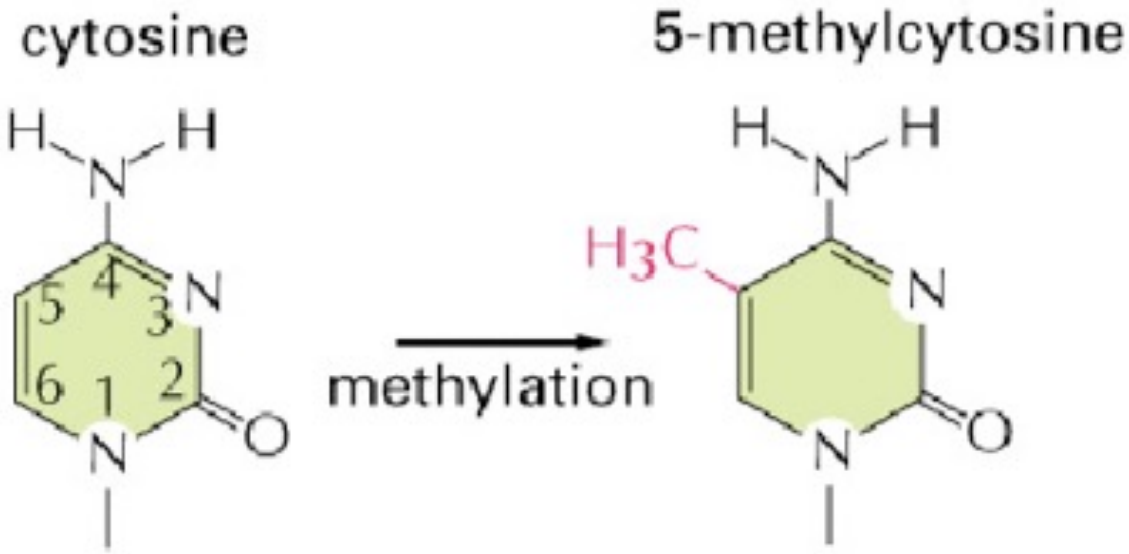


Figure 7–80. Molecular Biology of the Cell, 4th Edition.

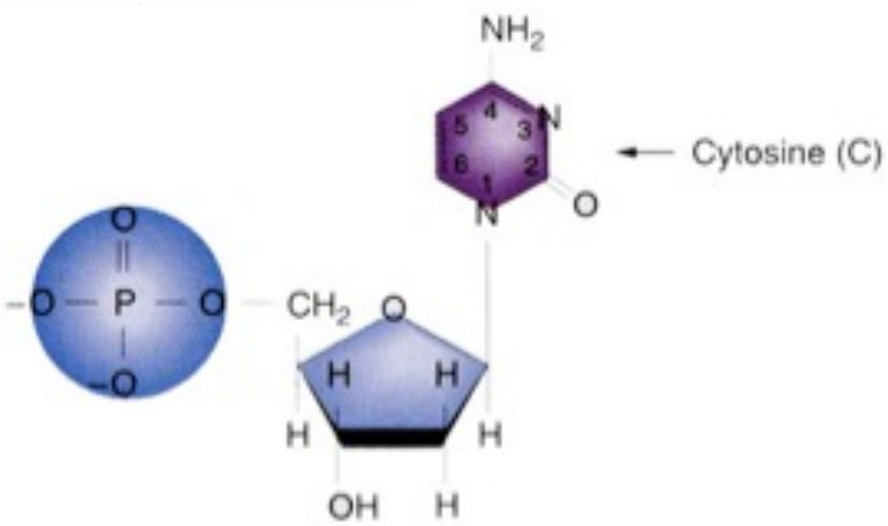
In vertebrates, cytosines in the sequence 5'-C-PO_4-G-3' <u>can</u> be methylated at the 5 position. This does not affect base pairing. These sequences are rare in DNA, probably because if the 5-CH_3-C deaminates, it is converted to a T, which is not easily distinguished from "normal" T for repair purposes. (Remember that a deaminated C is U, which, since it does not belong in DNA, is easily distinguished for repair.)

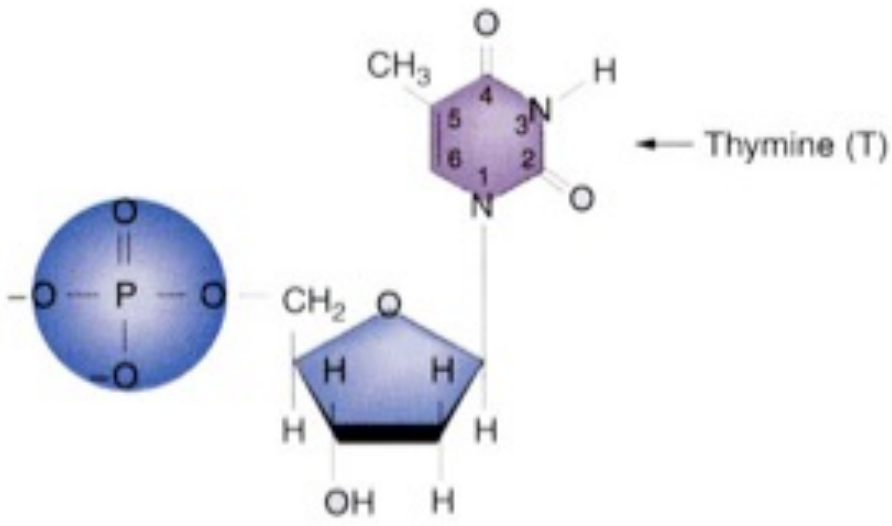
What is the role of DNA methylation? At least five separate functions in vertebrates.

1. **Gene inactivation, both of specific genes and perhaps of endogenous viruses, transposable elements, and retroviruses.**
2. **X chromosome inactivation – Dosage compensation**
3. **Genomic imprinting**
4. **Cancer**
5. **Centrometric heterochromatin**



Pyrimidine nucleotides





Gene inactivation: DNA methylation has the important advantage that it can be preserved easily during DNA replication, maintaining the regulated state. *DNMT1* can pass this "epigenetic" marking down during cell division. This type of methylation in vertebrates is usually associated with gene inactivation. Specific methyl binding proteins are also associated with gene inactivation and CpG methylation

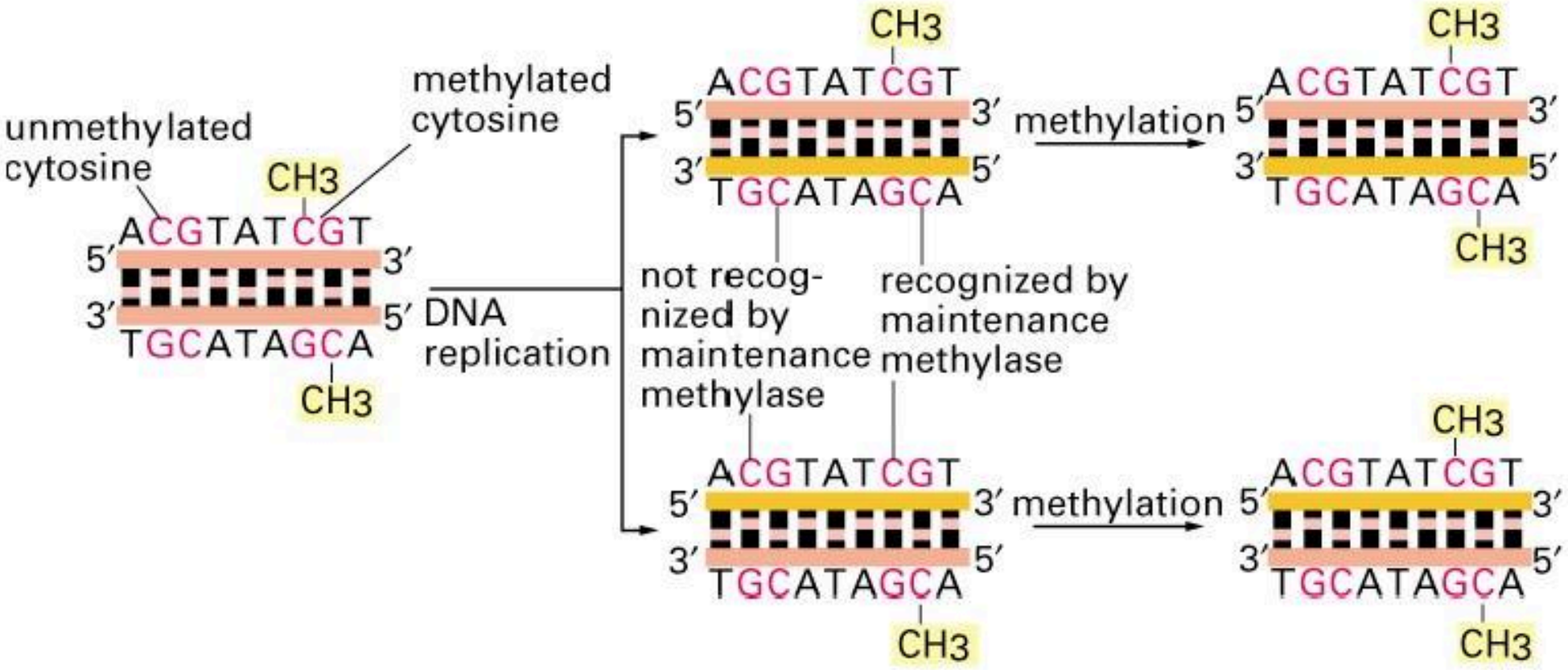
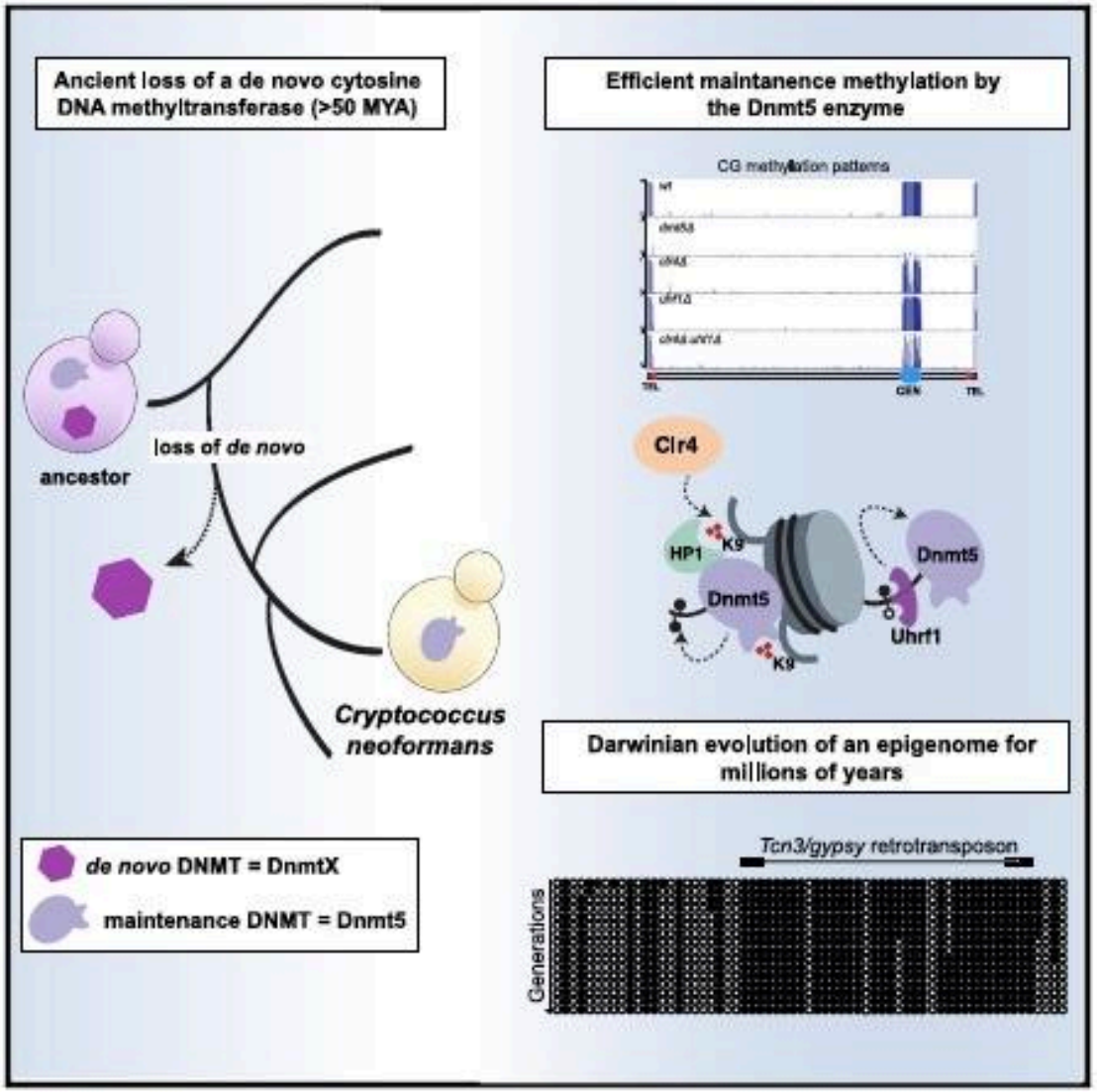


Figure 7–81. Molecular Biology of the Cell, 4th Edition.

Evolutionary Persistence of DNA Methylation for Millions of Years after Ancient Loss of a *De Novo* Methyltransferase

Graphical Abstract



Authors

Sandra Catania, Phillip A. Dumesic, Harold Pimentel, ..., Christina A. Cuomo, Jonathan K. Pritchard, Hiten D. Madhani

Correspondence

hitenmadhani@gmail.com

In Brief

The pathogenic fungus *Cryptococcus neoformans* encodes a functional maintenance methyltransferase for 5mC but no *de novo* enzyme; 5mC has been maintained for millions of years by a combintation of random epimutation and natural selection.

Monoallelic expression in mammals

There are three known situations that lead to monoallelic expression in mammalian genomes during normal organism development and function.

1. Genomic imprinting

2. X-chromosome inactivation in females

X inactivation occurs in female mammals at an early stage in development. X inactivation is random.

--Distinct mechanisms are involved in the initiation and the maintenance of inactivation.
--XIST is expressed from the inactivated X chromosome. It paints the inactive X, initiating the process of inactivation.
--Deletion of the XIST locus on one X chromosome leads to that chromosome never being inactivated. Always the other one. The gene acts in CIS, not TRANS.

3. Allelic exclusion
Ig gene expression in B lymphocytes
T cell receptor gene expression in T lymphocytes

+ one more - monoallelic expression in many genes in many tissues significance unknown

Monoallelic gene expression is shockingly common. The reason why it occurs is unknown

Widespread Monoallelic Expression

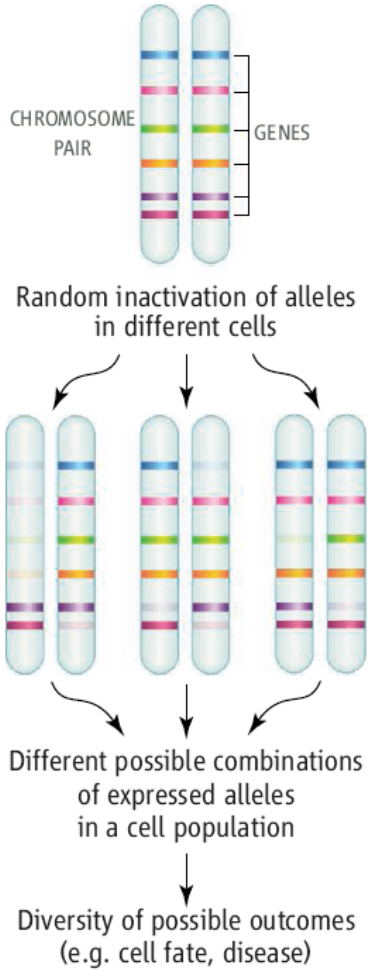
The surprisingly high prevalence of random allele inactivation in human cells can generate diversity in gene expression that affects cell fate and physiology.

Rolf Ohlsson

Most eukaryotic cells have two copies of autosomal (non–sex-determining) chromosomes. Although both copies (alleles) of individual genes are usually expressed in each diploid cell, one of the alleles is inactivated in a subset of genes (*1*). It is of profound interest that monoallelic expression in somatic cells does not simply represent a rheostat control for gene expression. Rather, it often operates in some selective function, such as determining the repertoire of odorant receptors or T cell receptors that are expressed (*2*, *3*). Moreover, the parental alleles of some mouse genes, such as those that encode cytokines, are expressed in random patterns, in which either, neither, or both alleles are inactivated, potentially influencing the selection and expansion of particular on T cells (*4*).

On page 1136 in this issue, Gimelbrant et al. (*5*) report that the mammalian genome employs random, monoallelic expression more extensively than thought. This may be to generate diversity in expression patterns on an unprecedented scale, which has important impli-

polymorphisms (SNPs). By modifying a method for detecting SNPs in DNA sequences, Gimelbrant *et al*. were able to track expressed messenger RNA (mRNA) sequences. Because most of the SNPs for the 3939 genes that were assessed are located in noncoding introns that are not present in mature mRNA, the authors included precursor forms of mRNAs (which contain introns) in the samples that were analyzed. They identified 371 genes (nearly 10%) as monoallelically expressed in epigenetically stable patterns in at least one population of cells derived from a single B cell clone. Although most of these genes



Generating diversity. Alleles are randomly inactivated on a pair of chromosomes in a human somatic cell. The various patterns of inactivation in progeny cells are then stabilized (epigenetically). This can generate diverse cellular and physiological outcomes.

than 1000 genes in the human genome can potentially be monoallelically expressed at any given time, indicating an amazing degree of diversity in possible combinations of expressed alleles. Interestingly, genes encoding cell surface receptors are overrepresented in this subset, suggesting the enormous potential for epigenetic regulation of receptor-mediated cell-cell communications, and hence, the regulation of cell diversity and cell fate (*8*). Second, Gimelbrant *et al*. hint at the fascinating possibility that the

Genomic imprinting

Maternal alleles are unequal to paternal alleles

1. mouse embryos with two maternal or paternal genomes fail to develop.

2. human triploid abortuses are phenotypically different depending on whether the extragenome is maternal or paternal.

3. Certain human characters are autosomal dominant, but manifest themselves only when inherited from one parent. For example, in some families, glomus tumors are inherited as an autosomal dominant character, but expressed only in people who inherit the gene from their father.

4. Deletion of certain chromosomal regions produces a different phenotype when the deletion is present on the maternal or paternal chromosome.

EXAMPLE:

deletion of 15q12 (paternal): Prader-Willi syndrome (mental retardation, gross obesity)

deletion of 15q12 (maternal): Angelman syndrome (mental retardation, growth retardation, hyperactivity, inappropriate laughter).

5. Allele loss in many cancers preferentially involves the paternal allele.

Genomic imprinting involves the selective silencing of alleles of specific genes of either paternal or maternal origin. Imprinting is described as being epigenetic, meaning "out side the genes". Imprinting does not involve a change in DNA sequence, but yet an allele expression state is transmitted from one somatic cell generation during the development and growth of an organism. Paternal imprinting means that the allele of a particular gene inherited from the father is silenced. Maternal imprinting is the opposite.

Imprinting correlates with DNA methylation. When DNA is heavily methylated, it tends to be transcriptionally inactive. Mice that are disrupted for the activity of a DNA methyltransferase some aspects of imprinting are disrupted. However, really our understanding of how imprinting occurs, how loci are chosen to be imprinted, how the imprint is erased, and last of all, why imprinting occurs, is still unclear.

Mono-parental embryos do not develop normally

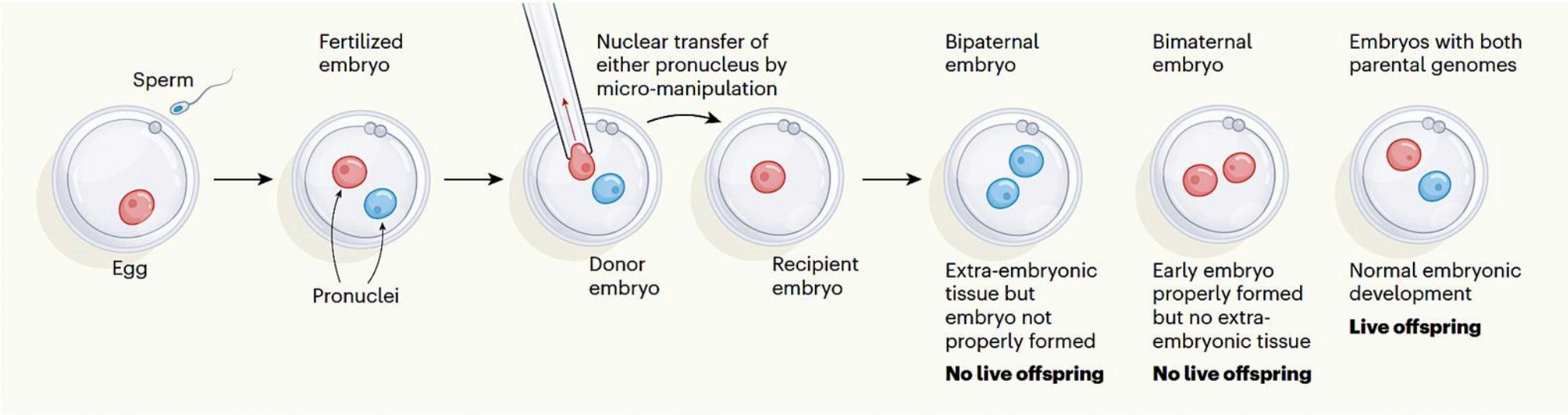
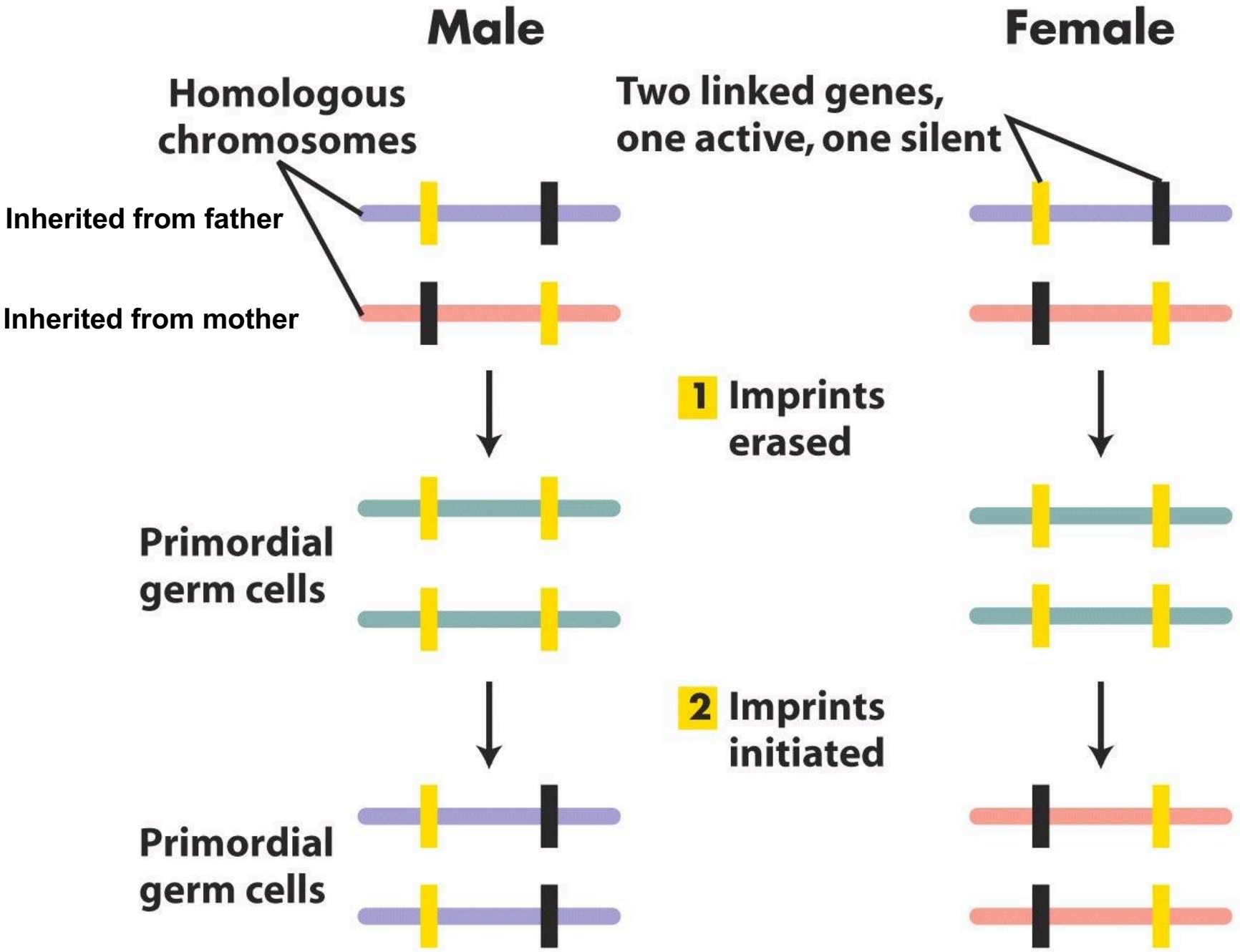
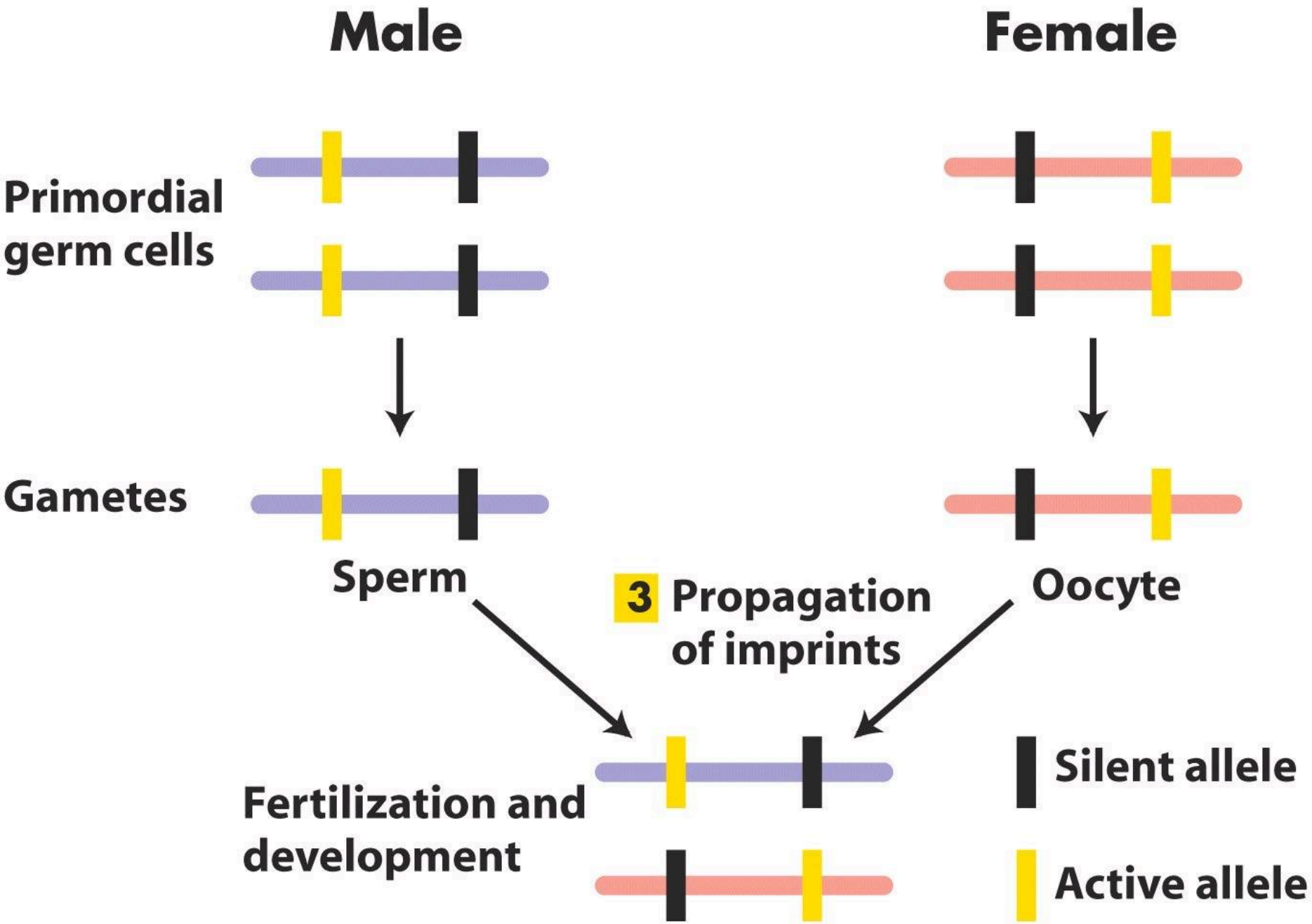
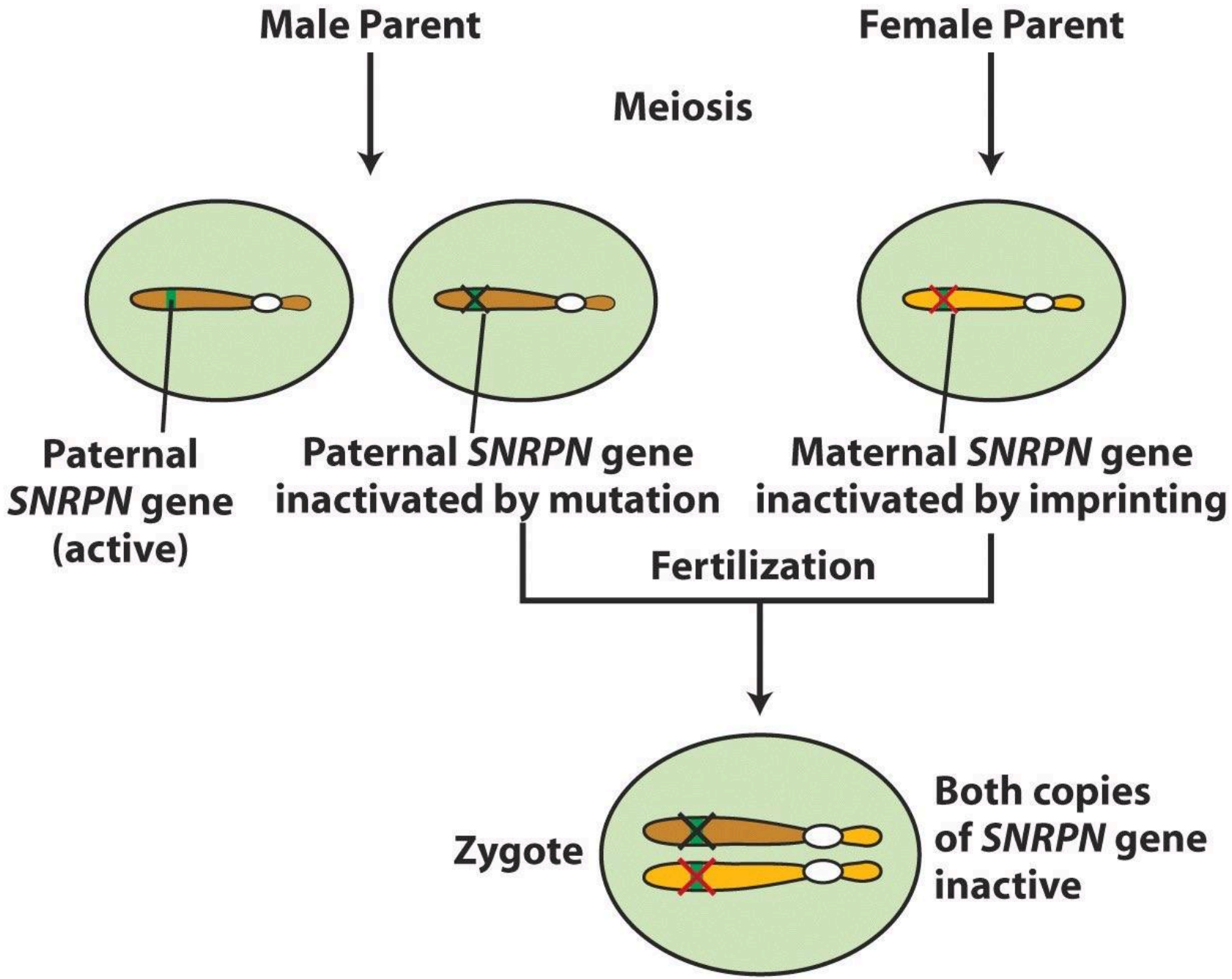


Figure 1 | Embryo manipulation experiments. Forty years ago, Surani, Barton and Norris[1,3], and McGrath and Solter[2] showed that both parental genomes are required for mammalian offspring to be viable. A newly fertilized mouse embryo contains two pronuclei: one from the sperm and one from the egg, each containing a copy of the genome. Micromanipulation was used to transfer pronuclei between donor and recipient embryos to generate reconstituted embryos with two copies of the paternal genome (bipaternal) or maternal genome (bimaternal), or one of each. Bipaternal embryos failed to form properly, whereas bimaternal early embryos formed correctly but had poor development of the extra-embryonic tissues needed to support development. Only manipulated embryos that contained one genome copy from each parent developed normally and gave rise to viable and fertile offspring. These experiments proved that the two parental genomes are not functionally equivalent, and that each copy carries distinguishing 'imprints' – now known to be epigenetic modifications to DNA that affect the expression or repression of certain genes throughout life.

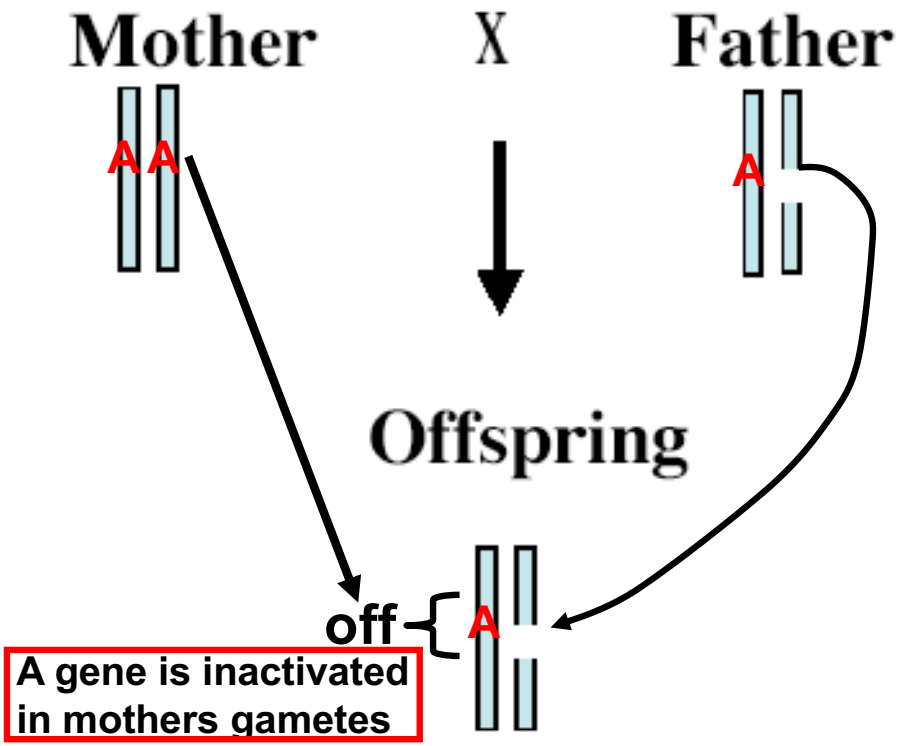

Male
Female
Homologous chromosomes
Two linked genes, one active, one silent
Inherited from father
Inherited from mother
1 Imprints erased
Primordial germ cells
2 Imprints initiated
Primordial germ cells

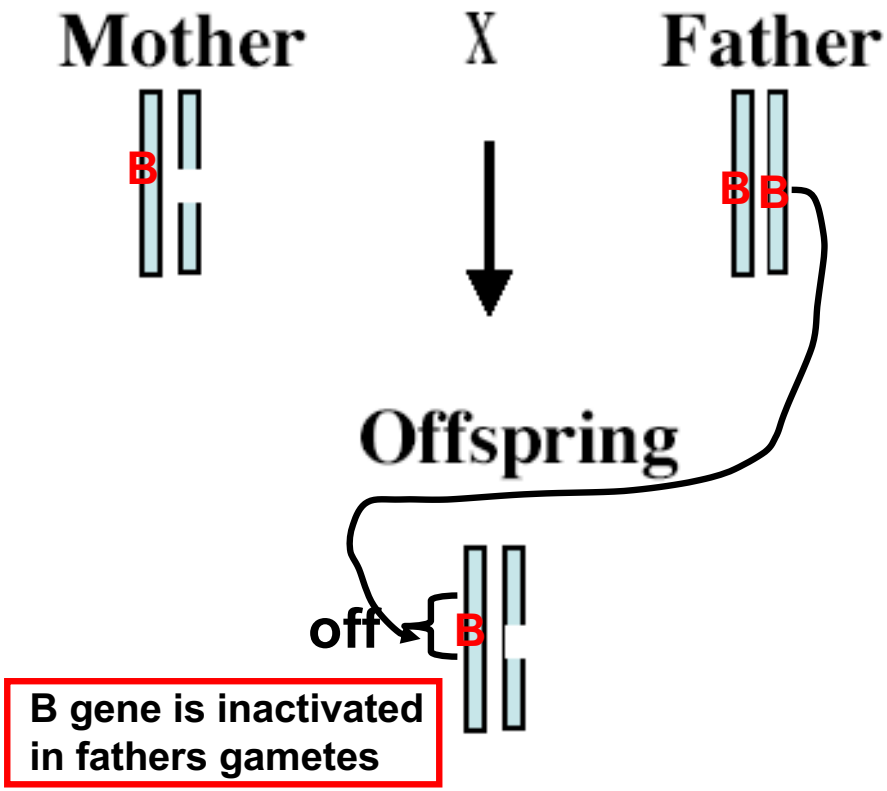

Male
Female
Primordial germ cells
Gametes
Sperm
Oocyte
3 Propagation of imprints
Fertilization and development
Silent allele
Active allele


Male Parent
Female Parent
Meiosis
Paternal SNRPN gene (active)
Paternal SNRPN gene inactivated by mutation
Maternal SNRPN gene inactivated by imprinting
Fertilization
Zygote
Both copies of SNRPN gene inactive

Imprinting

Ex: Prader-Willi syndrome/ Angelman syndrome





Prader-Willi syndrome

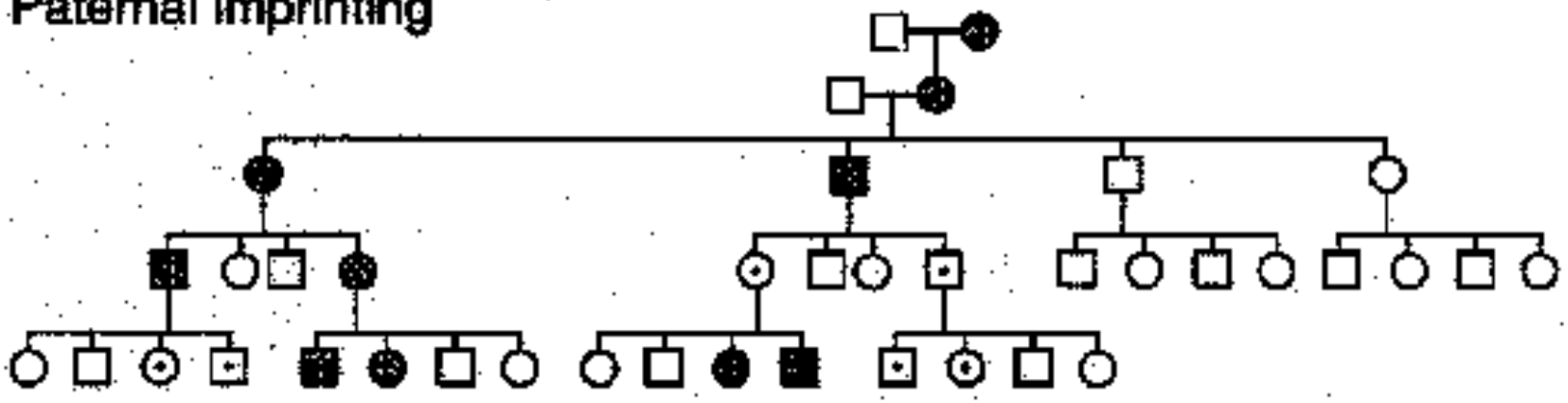
The maternal alleles of certain genes in the deletion region are shut off

Angelman syndrome

The paternal alleles of a different set of genes in the same region are shut off

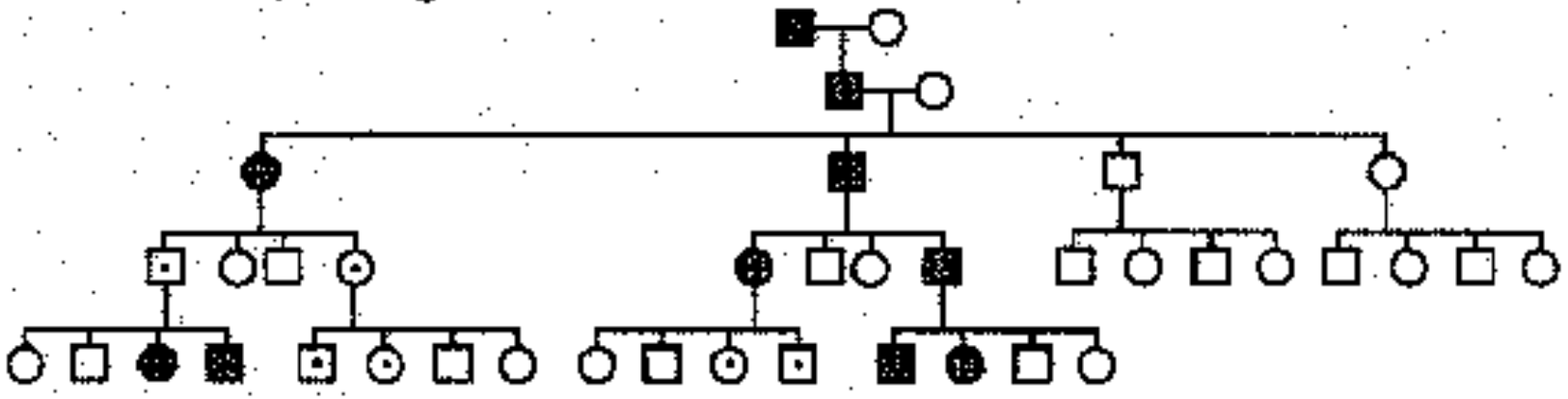
The paternal copy of the gene is inactivated

Paternal imprinting



The maternal copy of the gene is inactivated

Maternal imprinting



Pedigrees reflecting maternal and paternal imprinting. In both pedigrees, affected individuals (represented by the filled-in circles and squares) are heterozygous for a deletion removing a gene that has either a paternal or a maternal imprint. With paternal imprinting, affected individuals receive a deleted chromosome from their mother and an inactivated copy of the gene from their father. Therefore, they have no functional copy of the gene in question. The opposite is true with maternal imprinting. The deleted chromosome comes from the father, while the inactivated copy of the gene comes from the mother. In these pedigrees, a dotted symbol indicates individuals carrying a deleted chromosome but not displaying the mutant phenotype.

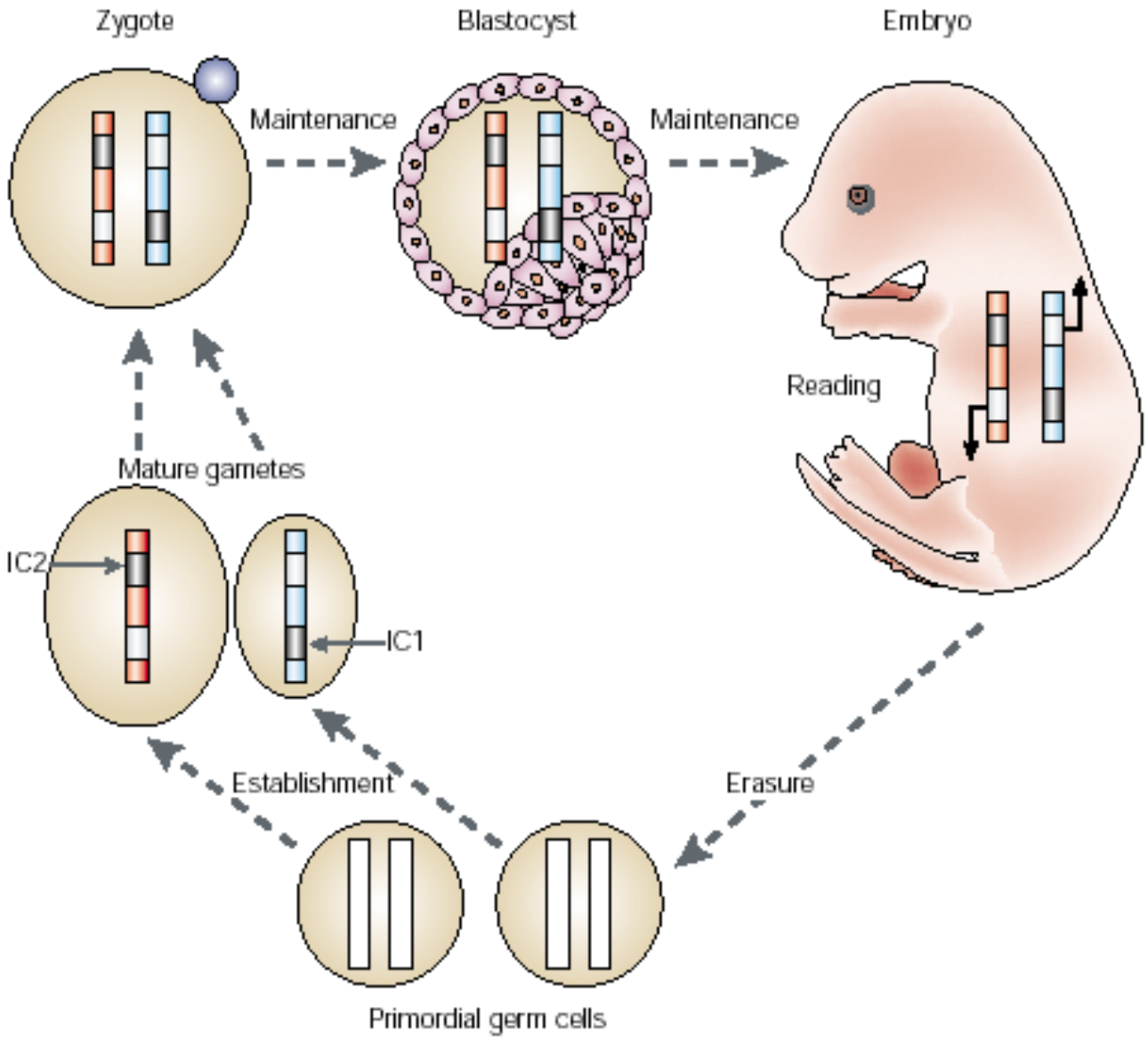


Figure 3 | **Life cycle of methylation imprints.** Erasure, establishment and maintenance of methylation imprints at imprinting centres during germ cell and embryonic development. Imprinting control elements 1 (IC1) and IC2 are shown as examples (see chromosome 11p15.5 in FIG. 1). Grey indicates modification and white indicates no modification at the corresponding alleles. Parental chromosomes are marked according to their sex in blue (male) or red (female). The reading (transcriptional interpretation of the primary imprints) in the developing embryo is indicated by arrows.

Age-Associated Activation of Epigenetically Repressed Genes in the Mouse

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ABSTRACT

Epigenetic control of gene expression is a consistent feature of differentiated mammalian cell types. Epigenetic expression patterns are mitotically heritable and are stably maintained in adult cells. However, unlike somatic DNA mutation, little is known about the occurrence of epigenetic change, or epimutation, during normal adult life. We have monitored the age-associated maintenance of two epigenetic systems—X inactivation and genomic imprinting—using the genes *Atp7a* and *Igf2*, respectively. Quantitative measurements of RNA transcripts from the inactive and active alleles were performed in mice from 2 to 24 months of age. For both genes, older animal cohorts showed reproducible increases in transcripts expressed from the silenced alleles. Loss of X chromosome silencing showed cohort mean increases of up to 2.2%, while imprinted-gene activation increased up to 6.7%. The results support the hypothesis that epigenetic loss of gene repression occurs in normal tissues and may be a contributing factor in progressive physiological dysfunction seen during mammalian aging. Quantitatively, the loss of epigenetic control may be one to two orders of magnitude greater than previously determined somatic DNA mutation.

Global loss of imprinting leads to widespread tumorigenesis in adult mice

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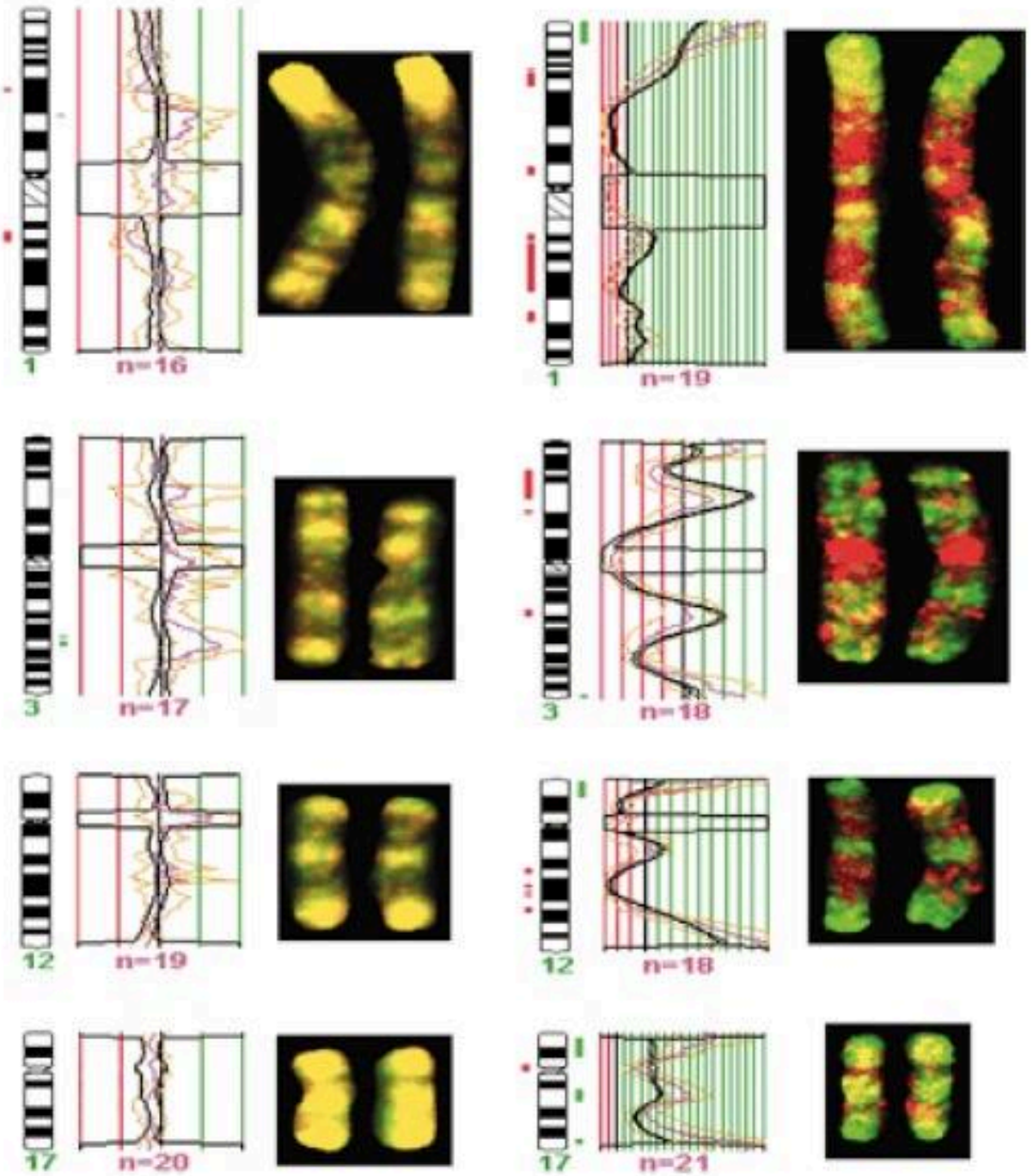
Summary

Loss of imprinting (LOI), commonly observed in human tumors, refers to loss of monoallelic gene regulation normally conferred by parent-of-origin-specific DNA methylation. To test the function of LOI in tumorigenesis, we developed a model by using transient demethylation to generate imprint-free mouse embryonic stem cells (IF-ES cells). Embryonic fibroblasts derived from IF-ES cells (IF-MEFs) display TGFβ resistance and reduced p19 and p53 expression and form tumors in SCID mice. IF-MEFs exhibit spontaneous immortalization and cooperate with H-Ras in cellular transformation. Chimeric animals derived from IF-ES cells develop multiple tumors arising from the injected IF-ES cells within 12 months. These data demonstrate that LOI alone can predispose cells to tumorigenesis and identify a pathway through which immortality conferred by LOI lowers the threshold for transformation.

(Fraga et al., 2005 PNAS)

Mapping chromosomal regions with differential DNA methylation in MZ twins by using comparative genomic hybridization for methylated DNA. Competitive hybridization onto normal metaphase chromosomes of the AIMS products generated from 3- and 50-year-old twin pairs. Examples of the hybridization of chromosomes 1, 3, 12, and 17 are displayed. The 50-year-old twin pair shows abundant changes in the pattern of DNA methylation observed by the presence of green and red signals that indicate hypermethylation and hypomethylation events, whereas the 3-year-old twins have a very similar distribution of DNA methylation indicated by the presence of the yellow color obtained by equal amounts of the green and red dyes.



Among vertebrates, only mammals imprint their genomes. Why?

Seems like a bad idea because it makes an offspring potentially more sensitive to genomic damage at the imprinted loci: If the embryo is heterozygous at an imprinted locus it may be dead, whereas if the locus was not imprinted the animal would be viable.

A hypothesis: The struggle between the sexes?

--Females can mate with multiple males, generating embryos fathered by different males.

--If a male could underexpress genes that retard growth, his embryos would grow faster, outcompeting those of the other males.

--This would be draining to the mother. In response (in the evolutionary sense) she would benefit by downregulating genes that enhance growth. This gives each embryo an equal chance and does not drain all her energy.

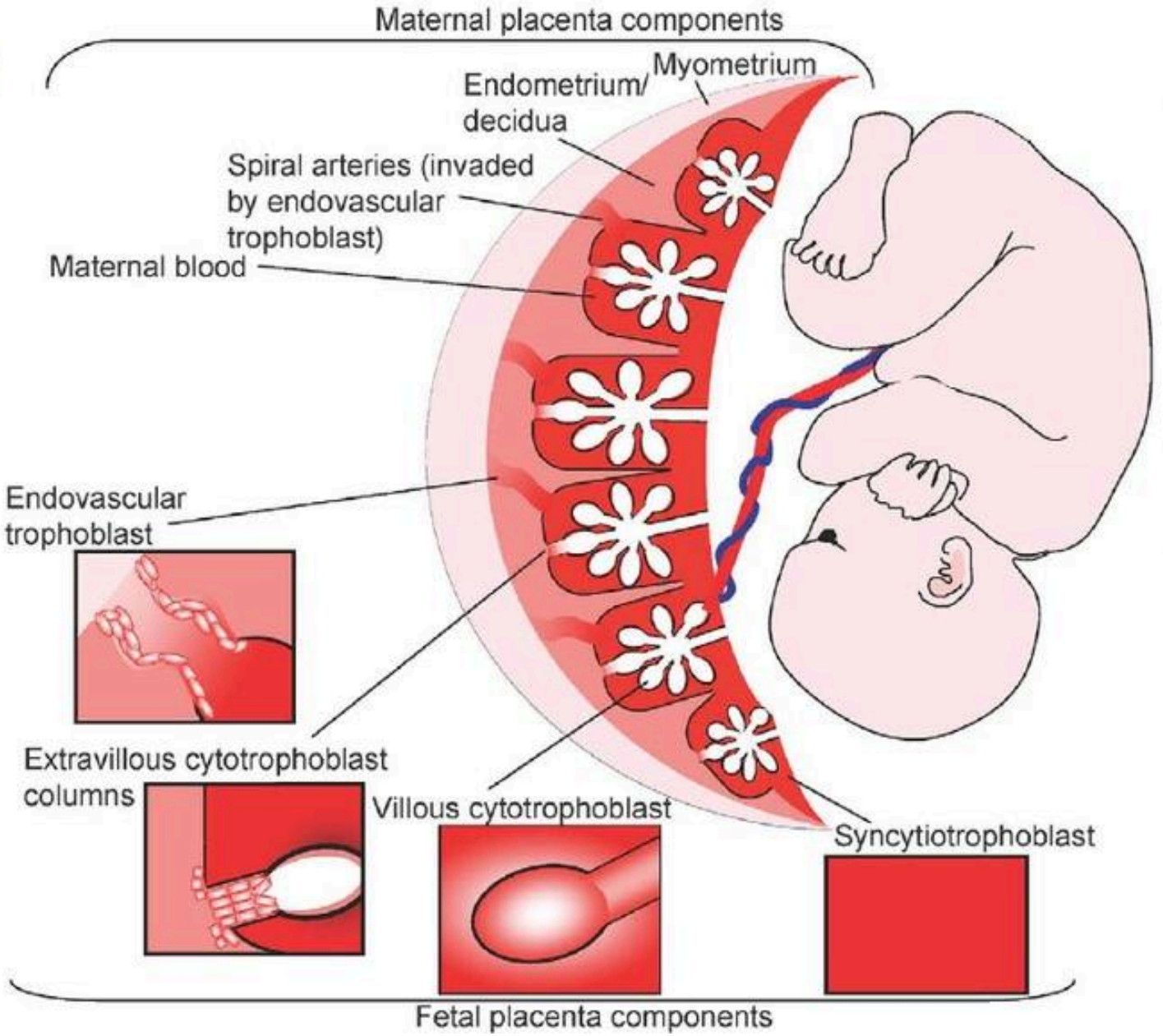
--So, the argument goes, imprinting will silence growth enhancing genes in the female and silence growth suppressing mutations in the male.

Some supporting evidence

--Only in mammals do embryos compete for maternal resources in utero.

--Many of the genes identified as being imprinted are growth regulators.

Potential imprinted gene action in the placenta



Imprinted gene action in the fetus

Thyroid
GNAS ♀

Brain
UBE3A ♀

Pituitary gland/ Pancreas
PLAGL1 ♂

Imprinted genes often regulate fetal growth

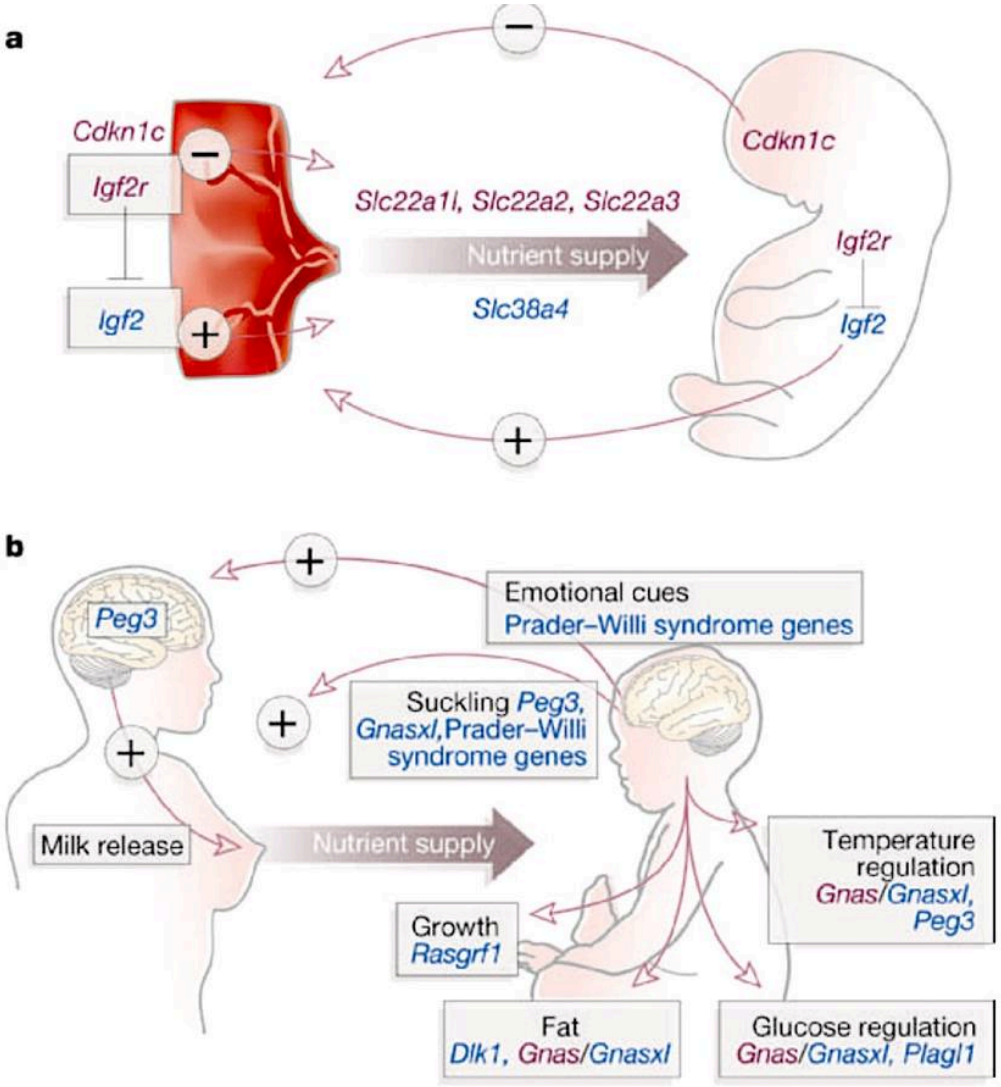


Figure 2 **Effects of imprinted genes on resource acquisition by offspring[6]. Imprinted genes that are expressed from the maternally derived copy are in purple; those expressed from the paternally derived copy are in blue.** a, **Growth of the fetus — promoted by, for example, *Igf2*, a paternally expressed imprinted gene — may signal increased demand to the placenta. *Igf2* also increases the placenta's nutrient-transport capacity[9,10]. Several nutrient-transporter-encoding genes (*Slc* genes) are also imprinted. Maternally expressed genes (such as *Igf2r* and *Cdkn1c*) may reduce nutrient supply or demand[6].** b, **Imprinted genes might also control resource provision after birth, acting in the mother's brain to regulate milk release, or in the infant to regulate nipple attachment, suckling and feeding behaviours, including emotional interactions with the mother. Within the infant, imprinted genes may also affect the allocation of acquired resources into growth, fat reserves and homeostatic mechanisms, such as glucose and temperature regulation. Most genes shown were discovered through experiments in mice, but might be common to humans.**

Annu. Rev. Ecol. Syst. 2000. 31:9–32

THE KINSHIP THEORY OF GENOMIC IMPRINTING

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Key Words genetic conflict, inclusive fitness, parent-offspring conflict, kin selection, sex chromosomes

■ **Abstract** The inclusive fitness effect attributable to an allele can be divided into an effect on matrilineal kin when the allele is maternally derived and an effect on patrilineal kin when paternally derived. However, the allele is not subject to selection on its effects on patrilineal kin when maternally derived nor on its effects on matrilineal kin when paternally derived. As a result, natural selection may favor alleles with effects that differ, depending on the allele's parental origin. At autosomal loci, this process is predicted to lead to the silencing of alleles when inherited from one or the other parent. At X-linked loci subject to random X inactivation, the process is predicted to lead to quantitative differences of expression between maternal and paternal alleles but not to complete silencing of one allele. The implications of this theory and some challenges to the theory are reviewed.

A Custody Battle for the Mind: Evidence for Extensive Imprinting in the Brain

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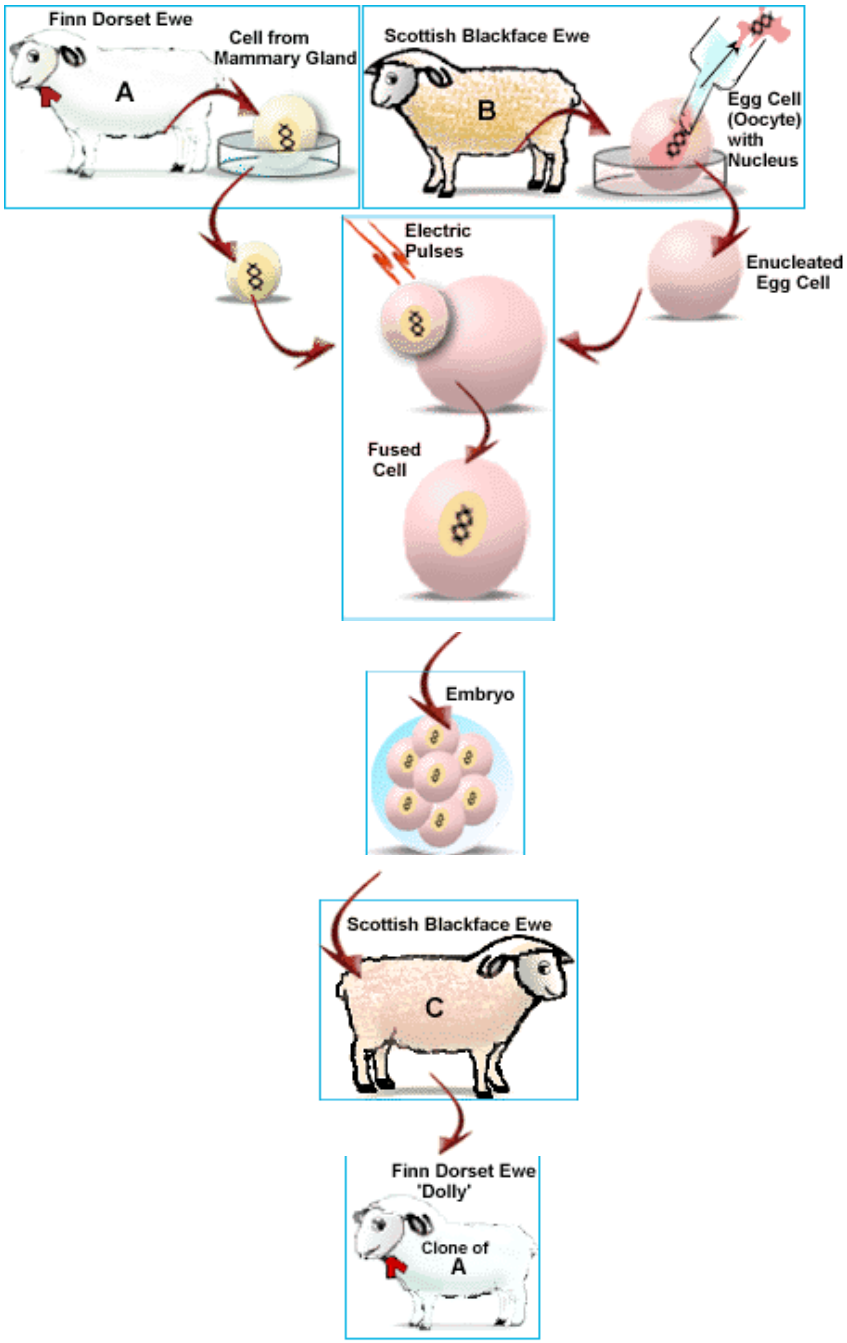
DOI 10.1016/j.neuron.2010.07.026

Relatively few genes (~100) have previously been shown to be imprinted such that their expression in progeny derives from either the maternal or paternal copy. Two recent studies by Gregg et al. (2010a, 2010b) in *Science* expand this list by an order of magnitude, revealing complex patterns of parent-of-origin bias in gene expression in the brain that are developmentally and regionally restricted, and in many cases, sexually dimorphic.



Nucleus removed From egg cell

Cell with DNA fused Into enucleated egg cell

Implantation into The surrogate mother

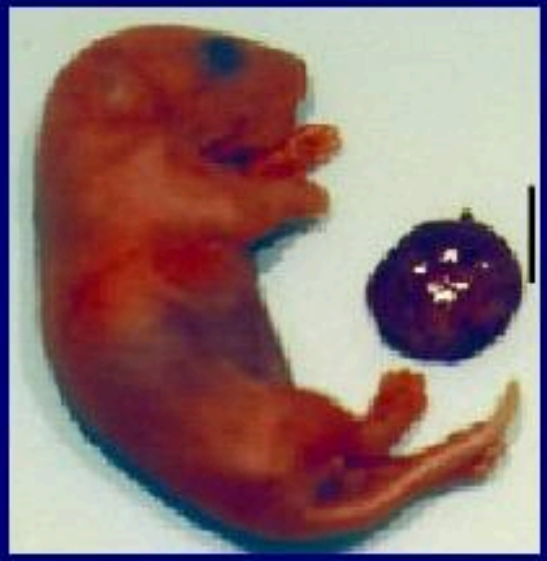
Birth of clone of Sheep A (Dolly)

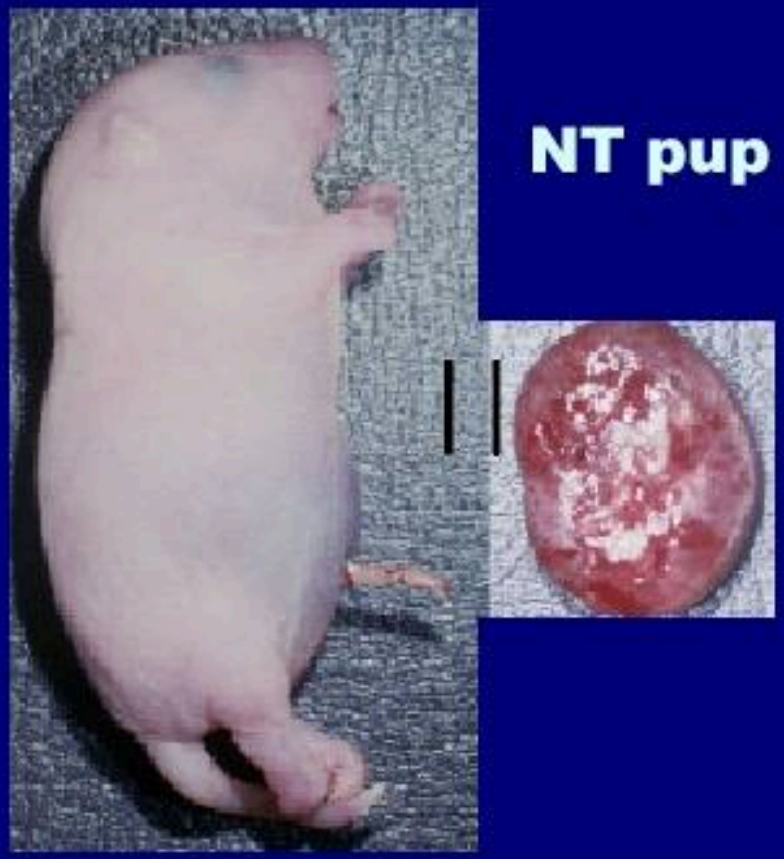
Although the clone of sheep A (Dolly) could have been created using only sheep A, sheep B and C were used in the cloning process in order to ensure that Dolly was truly a clone and the not the result of unexpected mating.

Cloned Mice often Display Severe Overgrowth

Control

NT pup





Similar Problems seen in Cloned Animals of all Species: *"Large Offspring Syndrome"*

A. Most common problems

- **Fetal overgrowth**
- **Defective placenta**
- **Respiratory distress**
- **Abnormal heart, circulatory system**

→ ***May be immediate cause of perinatal death***

B. Problems seen in some clones

- **Abnormal immune system**
- **Skeletal abnormalities**
- **Abnormalities in brain, kidney, ...**

Abnormal Development of Nuclear Clones: Two Possibilities

- ***Due to genetic changes?***

  → **Offspring of cloned animals are normal: argues against genetic defects**

Defects likely caused by stochastic errors in the correct activation of imprinted or non-imprinted genes

Proof that these abnormalities are entirely epigenetic

Dolly's lambs, and the offspring of all cloned animals, are normal.

"Reprogramming" in *Normal* Development

Epigenetic state is "reset" at two Stages:

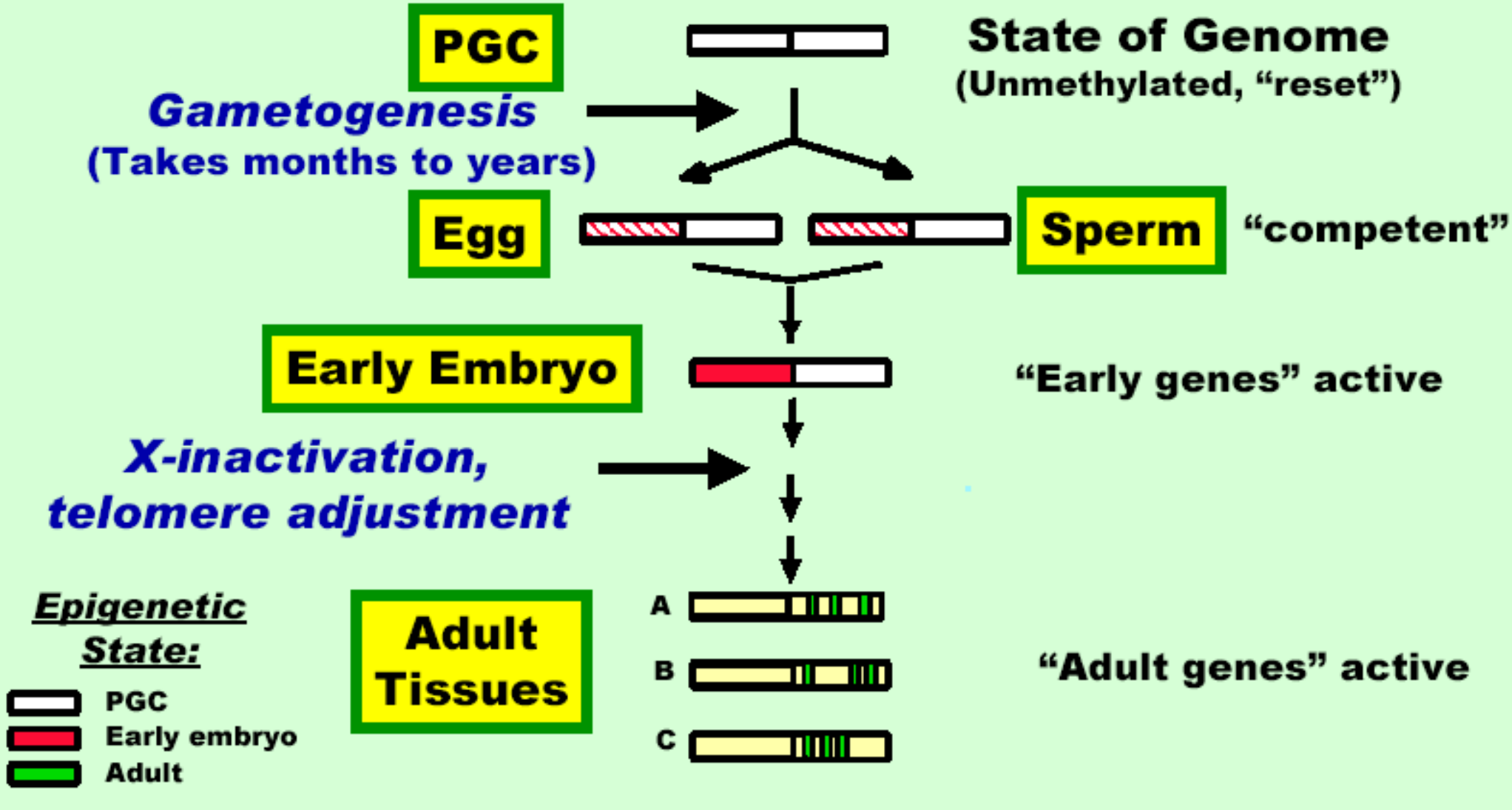
A. During gametogenesis

-- imprinted, non-imprinted genes

B. Postzygotically

-- X inactivation, telomere length

Reprogramming in *Normal* Development
(Fertilization)



The Problem of *Reprogramming* in Nuclear Cloning

- ***Donor cells:***
  - Express tissue specific genes (= genes for milk production in case of Dolly)
  - But NOT genes needed for embryonic development
- ***Transplanted Nucleus*** **must:**
  - Activate embryonic genes
  - Inactivate tissues specific genes

The Main Issue of Nuclear Cloning: Reprogramming of the Genome

In normal development:

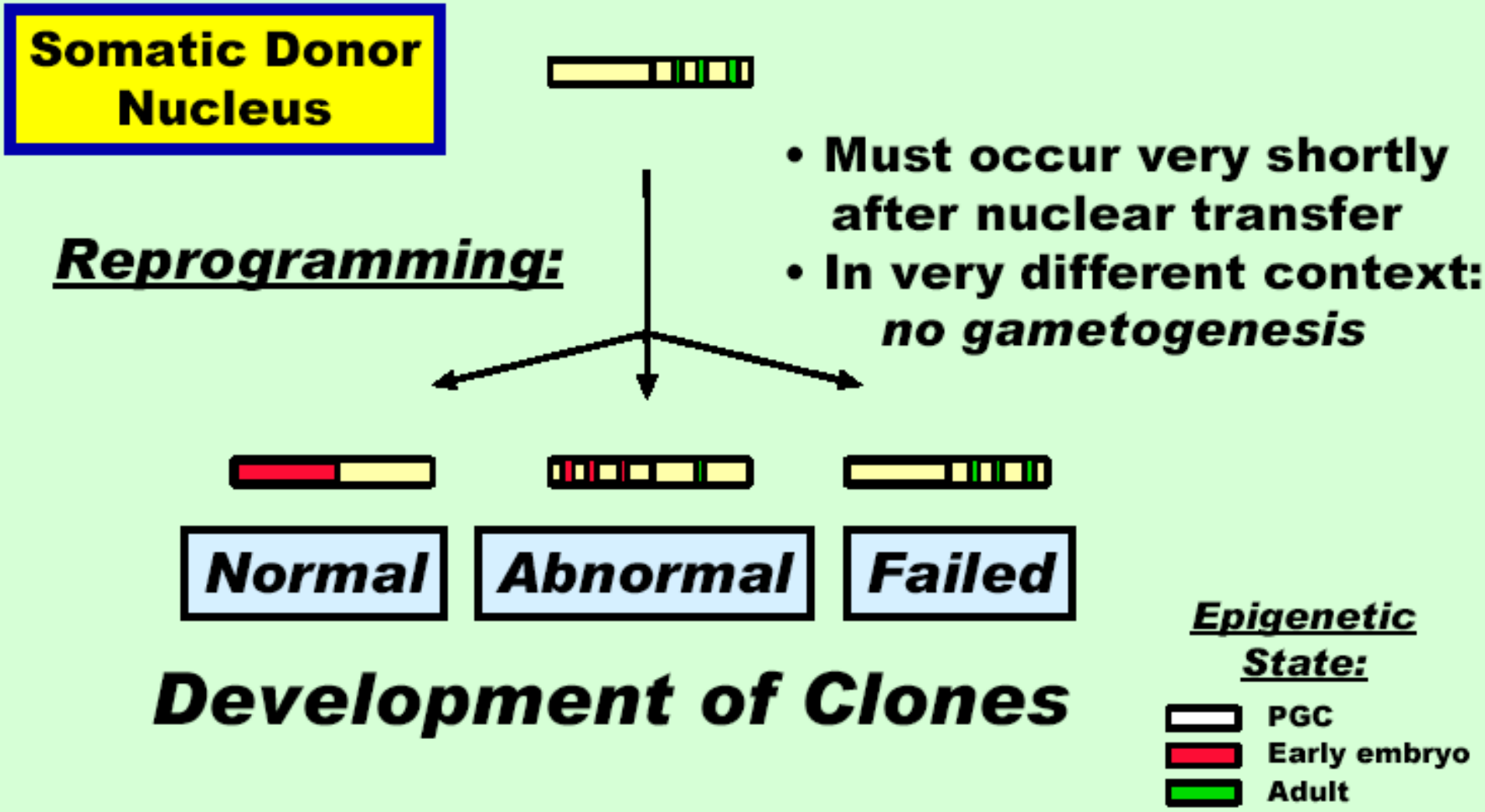
- the genome is reset during egg and sperm maturation:
  → ***this takes weeks, months, years***

In nuclear cloning:

- The egg needs to divide: the genome must be reset
  → ***within a short time and in a radically different cellular context***

- This is an error prone process -

Reprogramming in Nuclear Cloning



Reprogramming of Transplanted Nucleus: *Predictions*

- Little or no problems of reprogramming events that occur *after* fertilization
  - X chromosome inactivation
  - Telomere length adjustment
- Problems for reprogramming events that occur *prior* to fertilization
  - In principle all genes in genome
    - Non-imprinted genes
    - Imprinted genes

Cloned Mice Show Pronounced Dysregulation of Imprinted Genes

- None of 50 cloned pups expressed all 6 tested imprinted genes correctly
- Dysregulation of a given gene did not correlate with expression of any other gene (stochastic problem)
- Most cloned pups and placentas were oversized *("Large Offspring Syndrome")*

Imprinting may Limit Nuclear Cloning

- **Epigenetic imprinting marks are established only during gametogenesis**

  → and this is circumvented in cloning

- **Error prone maintenance of marks during life of donor *cannot* be repaired in nuclear clones**

→ This may lead to unbalanced imprinting and fetal growth abnormalities

Nature Reviews Genetics **4**; 855-864 (2003); doi:10.1038/nrg1205

HUMAN CLONING: CAN IT BE MADE SAFE?

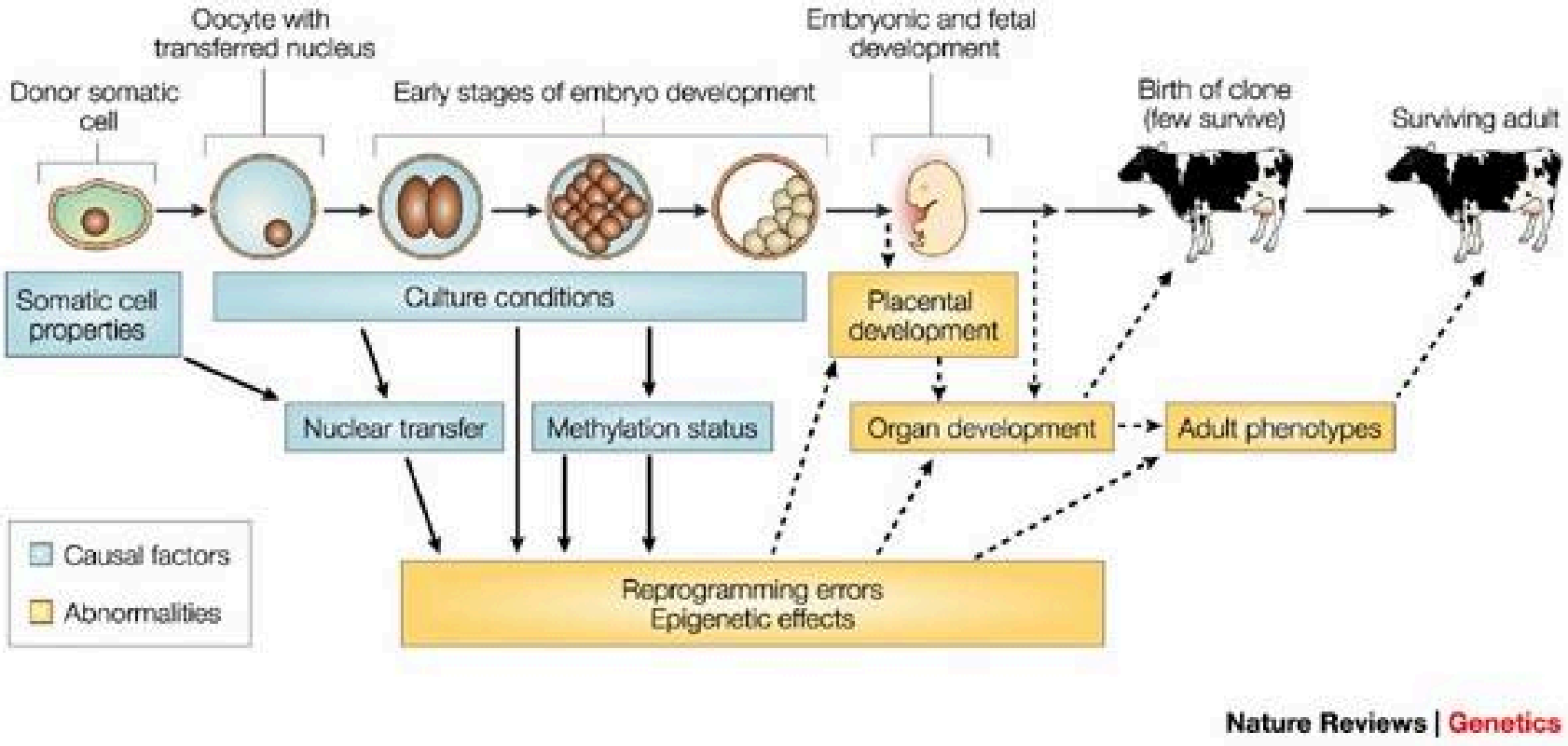
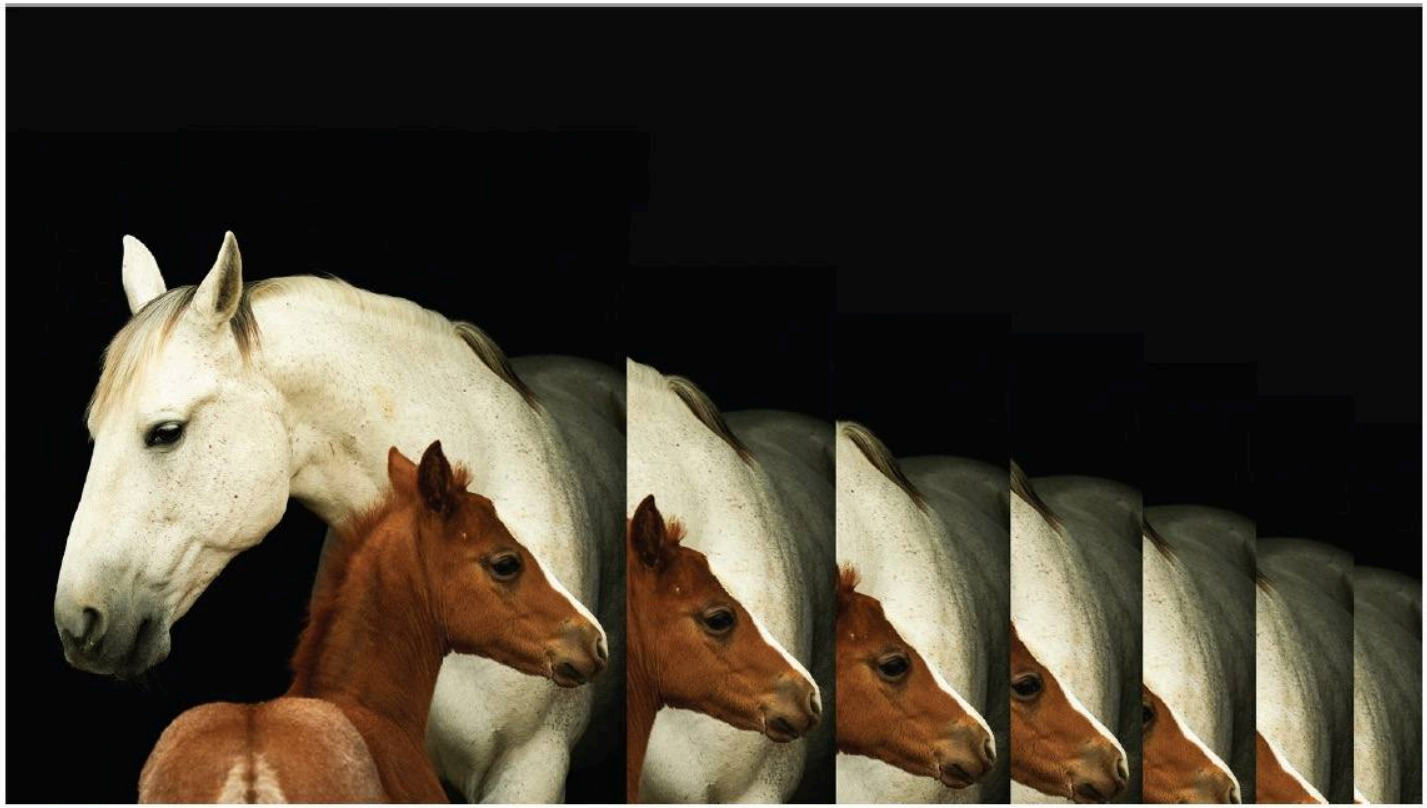


Figure 2 | **Overview of clone development showing the factors that contribute to clone failure.** Factors that affect clone development include the nature of the donor cell and features of the methodology that interfere with reprogramming and epigenetic mechanisms. Aberrant methylation also causes gene dysregulation. These errors have the potential to impact on placental and organ development, resulting in failure during embryogenesis and fetal development, or around birth/during the neonatal period. Animals that survive into adulthood might have more subtle abnormalities that are compatible with life, or might be phenotypically normal. Solid arrows represent recognized links; broken arrows representative speculative links.



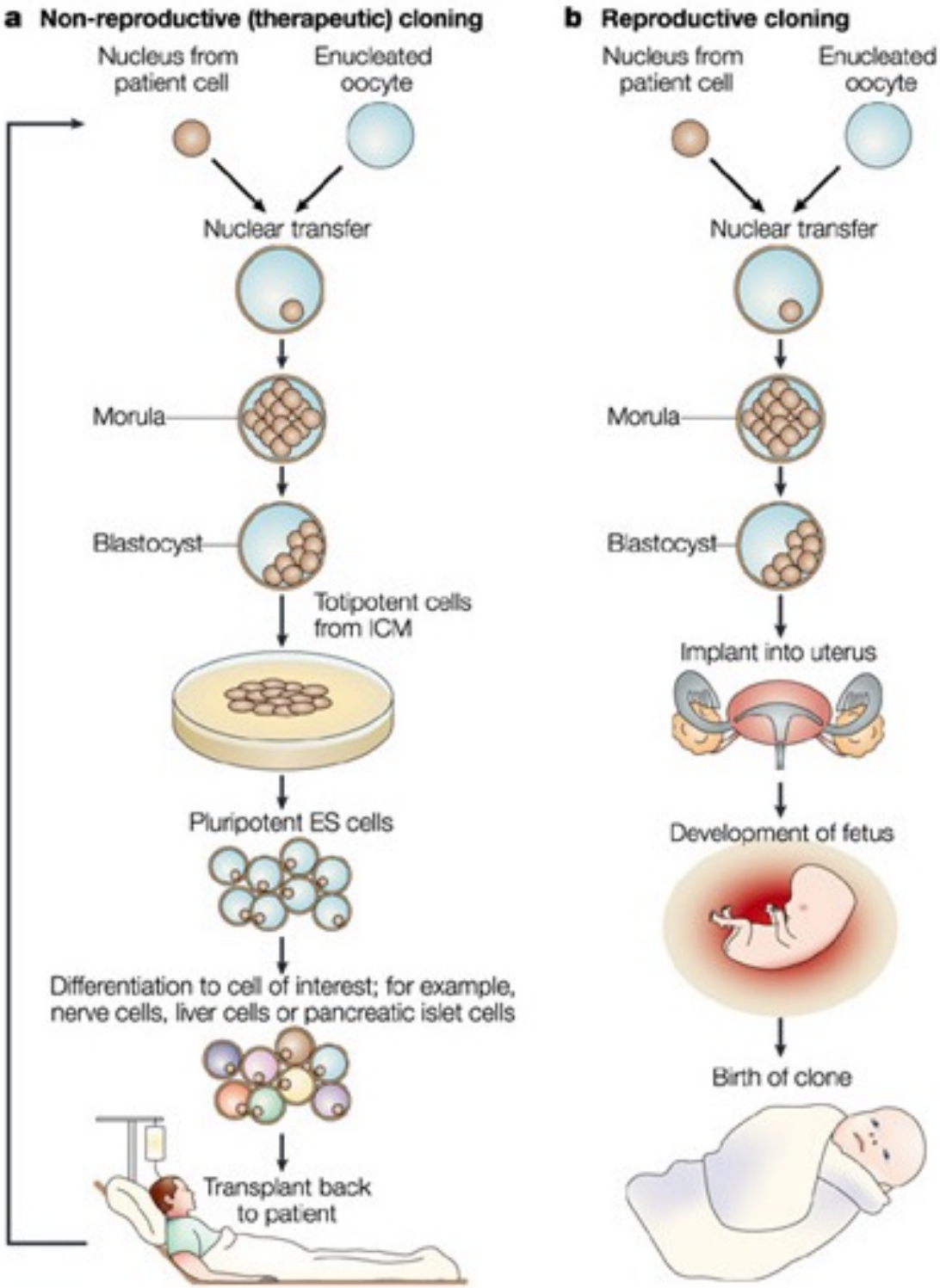
IDEAS

INSIDE THE BIG BUSINESS OF CLONING ANIMALS

Faster horses, superior cattle, immortal pets

By Bianca Bosker

Photographs by Brian Finke


a Non-reproductive (therapeutic) cloning
Nucleus from patient cell
Enucleated oocyte
Nuclear transfer
Morula
Blastocyst
Totipotent cells from ICM
Pluripotent ES cells
Differentiation to cell of interest; for example, nerve cells, liver cells or pancreatic islet cells
Transplant back to patient
b Reproductive cloning
Nucleus from patient cell
Enucleated oocyte
Nuclear transfer
Morula
Blastocyst
Implant into uterus
Development of fetus
Birth of clone

The problem

In order to generate ES cells, one has to destroy an early human embryo

Six to fourteen embryos per birth – healthy ones frozen, and then discarded (=medical waste)

Transplantation Medicine

Solid evidence:

- **Transplantation of normal cells represents a valid therapy for diseases such as Diabetes, Parkinson, blood-, liver disorders.....**
  - **Donor cells: from aborted fetuses, cadavers**

Problems:

- **Immune rejection**
- **Availability and quality control of donor cells**

SCNT ("Therapeutic Cloning"):

Potential Solution for some Problems of Transplantation Medicine

Cloned embryonic stem cells

- **Inexhaustible source of any tissue type**
- **Tailored to needs of Patient**

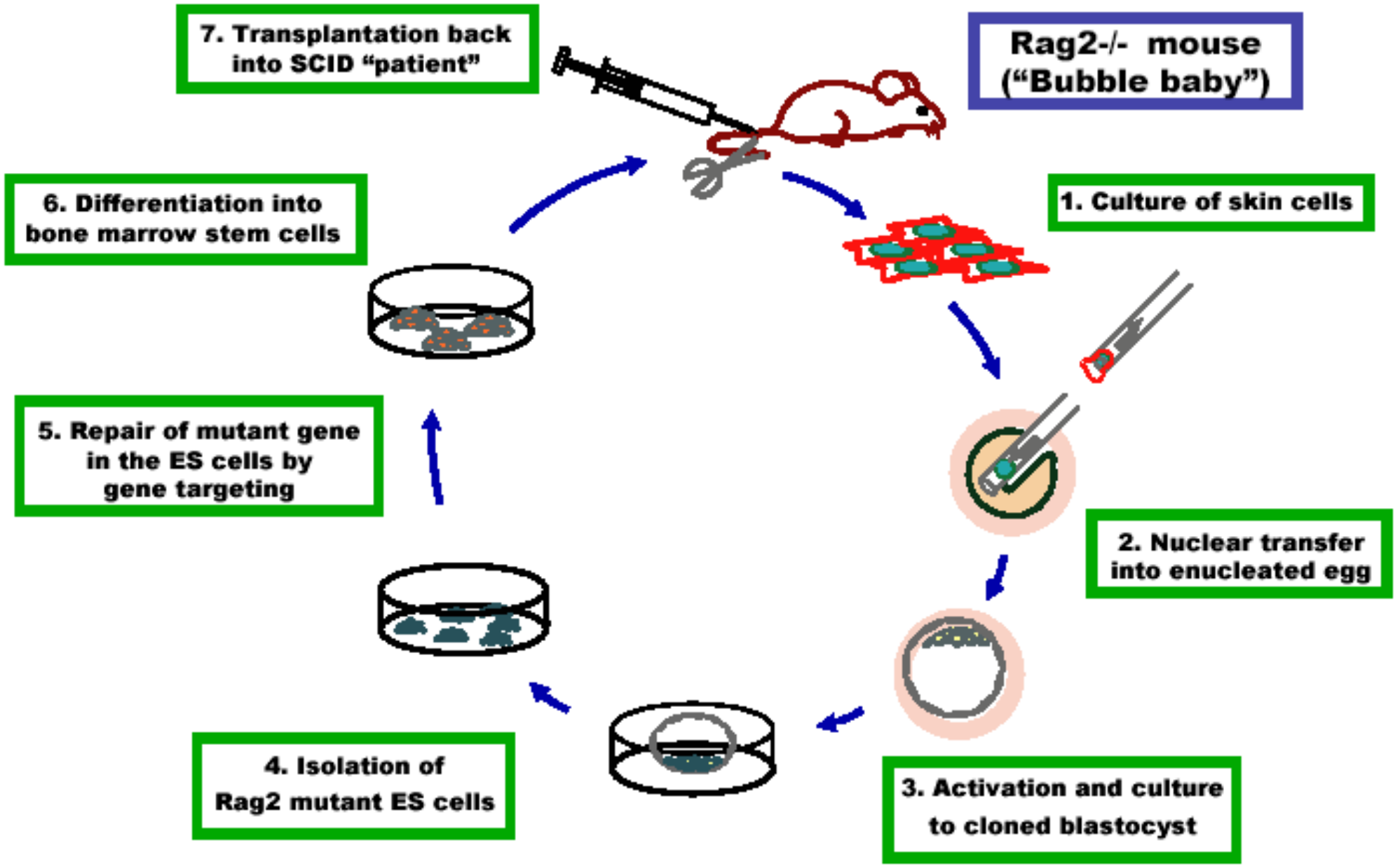
(No immuno-suppressive treatment required)

Does it work?

Proof of Principle Experiment: Correction of *Severe Immune Deficiency* in Mutant Mice

Condition equivalent to "Bubble Babies"

Correction of a Genetic Defect by Combination of Therapeutic Cloning and Gene Therapy



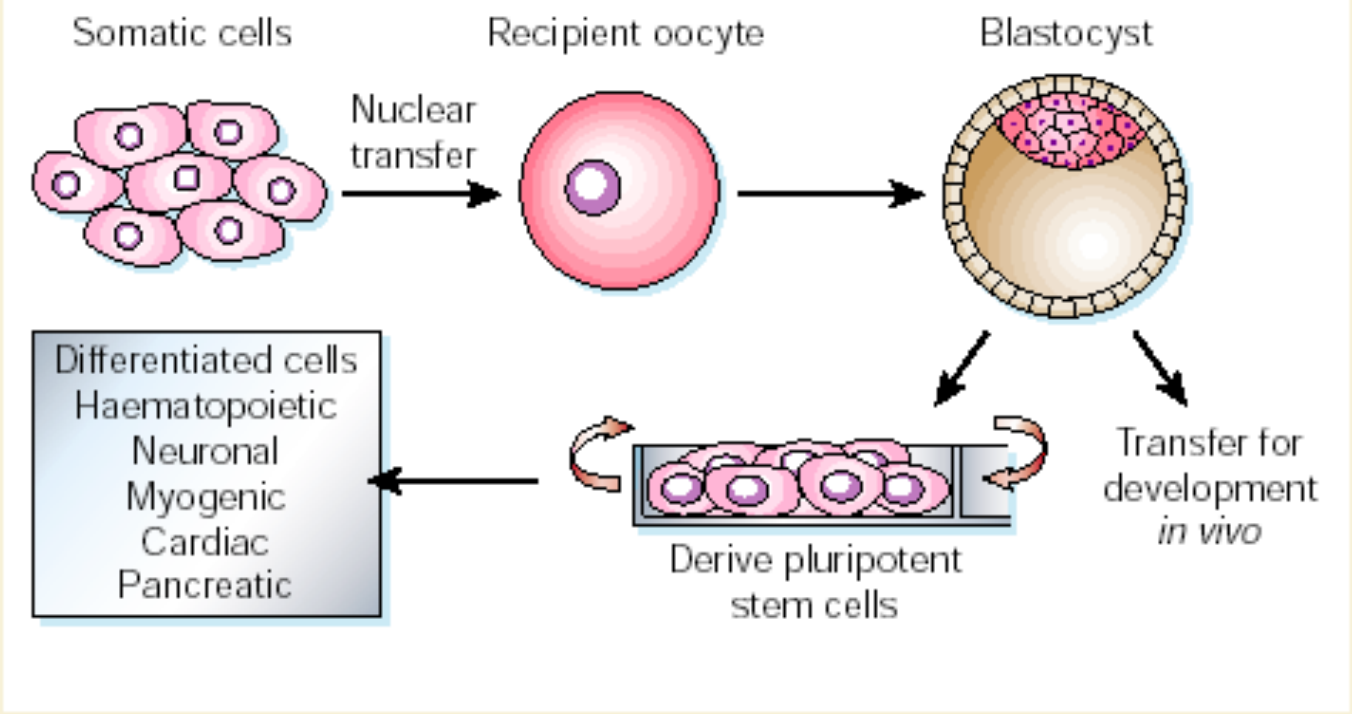


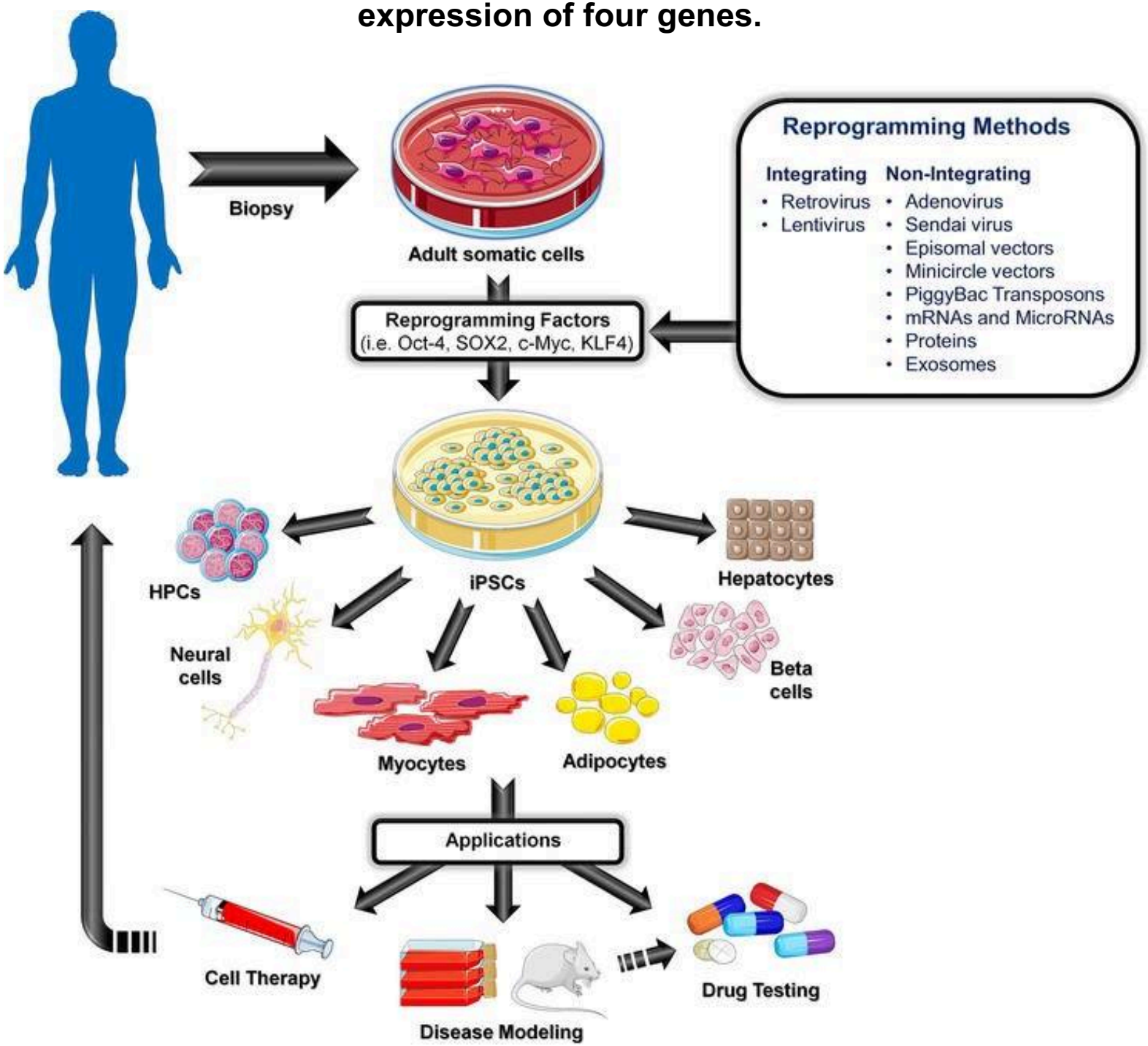
Figure 5 Reprogramming a somatic nucleus. When transplanted into an oocyte, a somatic nucleus may respond to the cytoplasmic factors and be reprogrammed back to totipotency. These cytoplasmic factors must be capable of erasing the 'molecular memory' that gives somatic cells their characteristic properties. It would also be necessary for the reprogrammed nucleus to switch off specific genes that are expressed by the somatic nucleus and initiate embryo-specific genes at the two-cell stage in the mouse. The reprogrammed genome generates pluripotent epiblast cells, and undergoes rapid transdifferentiation to generate trophectoderm cells. The extent of reprogramming can be judged by the development of the conceptus *in vivo*, as well as by the derivation of ES cells from blastocysts. These ES cells can potentially be induced to differentiate to generate the entire repertoire of adult cell types and germ cells.

Sir John B. Gurdon

Shinya Yamanaka

The Nobel Prize in Physiology or Medicine 2012

Adult somatic cells can be made to differentiate into pluripotent stem cells through the expression of four genes.



Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

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SUMMARY

Successful reprogramming of differentiated human somatic cells into a pluripotent state would allow creation of patient- and disease-specific stem cells. We previously reported generation of induced pluripotent stem (iPS) cells, capable of germline transmission, from mouse somatic cells by transduction of four defined transcription factors. Here, we demonstrate the generation of iPS cells from adult human dermal fibroblasts with the same four factors: Oct3/4, Sox2, Klf4, and c-Myc. Human iPS cells were similar to human embryonic stem (ES) cells in morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity. Furthermore, these cells could differentiate into cell types of the three germ layers in vitro and in teratomas. These findings demonstrate that iPS cells can be generated from adult human fibroblasts.

issues is to induce pluripotent status in somatic cells by direct reprogramming (Yamanaka, 2007).

We showed that induced pluripotent stem (iPS) cells can be generated from mouse embryonic fibroblasts (MEF) and adult mouse tail-tip fibroblasts by the retrovirus-mediated transfection of four transcription factors, namely Oct3/4, Sox2, c-Myc, and Klf4 (Takahashi and Yamanaka, 2006). Mouse iPS cells are indistinguishable from ES cells in morphology, proliferation, gene expression, and teratoma formation. Furthermore, when transplanted into blastocysts, mouse iPS cells can give rise to adult chimeras, which are competent for germline transmission (Maherali et al., 2007; Okita et al., 2007; Wernig et al., 2007). These results are proof of principle that pluripotent stem cells can be generated from somatic cells by the combination of a small number of factors.

In the current study, we sought to generate iPS cells from adult human somatic cells by optimizing retroviral transduction in human fibroblasts and subsequent culture conditions. These efforts have enabled us to generate iPS cells from adult human dermal fibroblasts and other human somatic cells, which are comparable to human ES cells in their differentiation potential in vitro and in teratomas.

Disease-Specific Induced Pluripotent Stem Cells

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SUMMARY

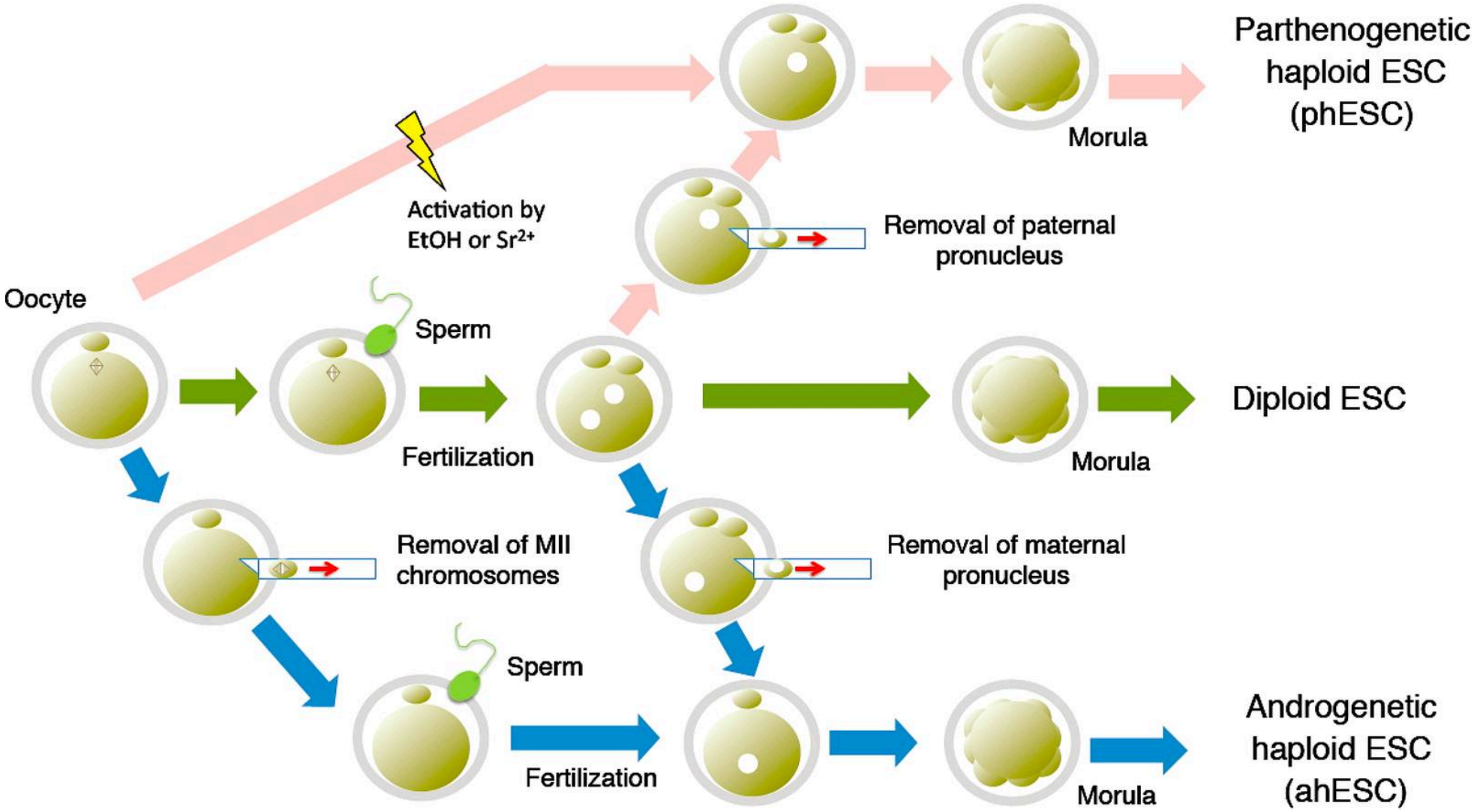
Tissue culture of immortal cell strains from diseased patients is an invaluable resource for medical research but is largely limited to tumor cell lines or transformed derivatives of native tissues. Here we describe the generation of induced pluripotent stem (iPS) cells from patients with a variety of genetic diseases with either Mendelian or complex inheritance; these diseases include adenosine deaminase deficiency-related severe combined immunodeficiency (ADA-SCID), Shwachman-Bodian-Diamond syndrome (SBDS), Gaucher disease (GD) type III, Duchenne (DMD) and Becker muscular dystrophy (BMD), Parkinson disease (PD), Huntington disease (HD), juvenile-onset, type 1 diabetes mellitus (JDM), Down syndrome (DS)/trisomy 21, and the carrier state of Lesch-Nyhan syndrome. Such disease-specific stem cells offer an unprecedented opportunity to recapitulate both normal and pathologic human tissue formation in vitro, thereby enabling disease investigation and drug development.

tissues or are genetically modified to drive immortal growth (Grimm, 2004). Primary human cells have a limited life span in culture, a constraint that thwarts inquiry into the regulation of tissue formation, regeneration, and repair. Indeed, many human cell types have never faithfully been adapted for growth in vitro, and the lack of accessible models of normal and pathologic tissue formation has rendered many important questions in human development and disease pathogenesis inaccessible.

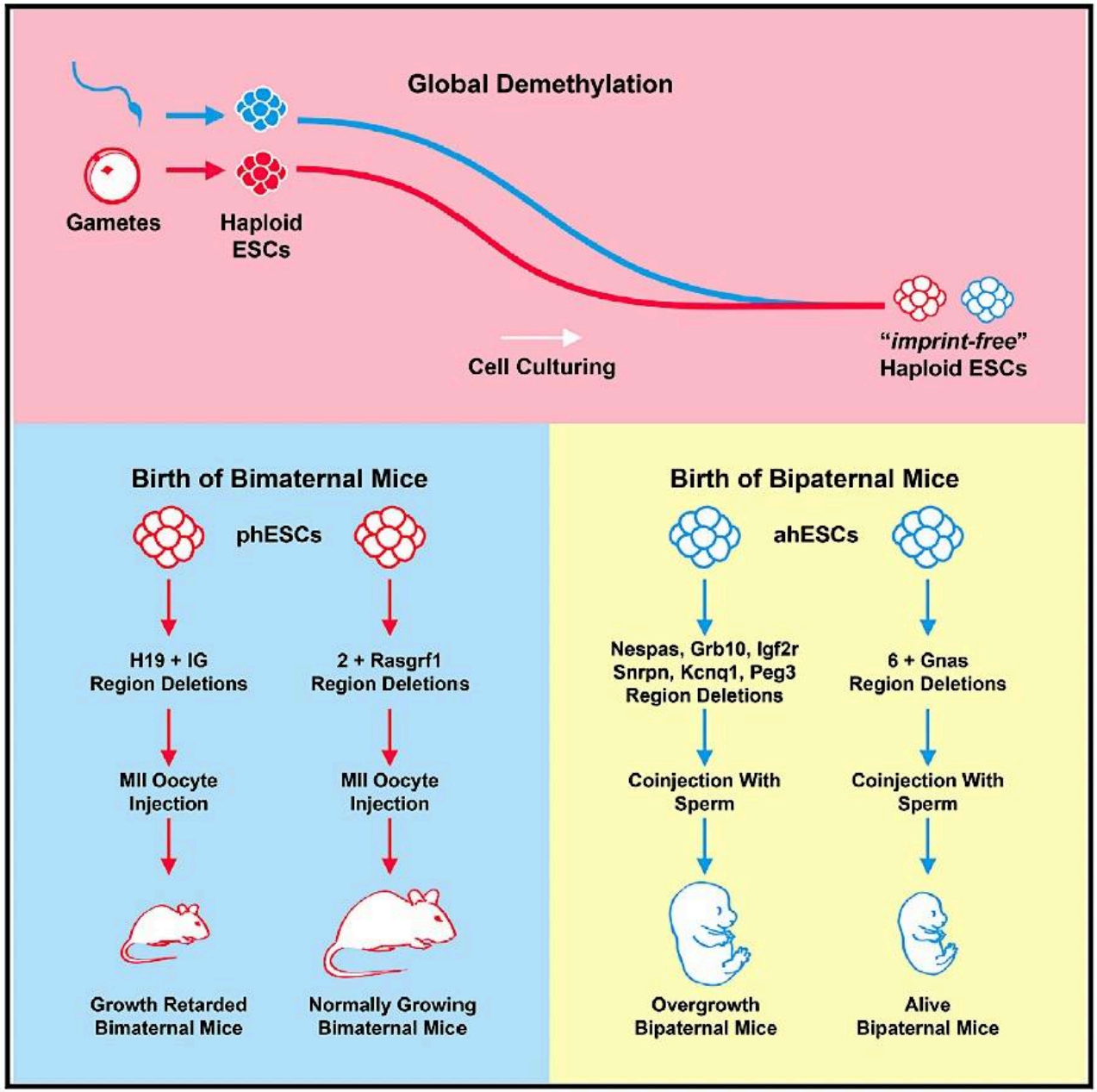
Human embryonic stem cells isolated from excess embryos from in vitro fertilization clinics represent an immortal propagation of pluripotent cells that theoretically can generate any cell type within the human body (Lerou et al., 2008; Murry and Keller, 2008). Human embryonic stem cells allow investigators to explore early human development through in vitro differentiation, which recapitulates aspects of normal gastrulation and tissue formation. Embryos shown to carry genetic diseases by virtue of preimplantation genetic diagnosis (PGD; genetic analysis of single blastomeres obtained by embryo biopsy) can yield stem cell lines that model single-gene disorders (Verlinsky et al., 2005), but the vast majority of diseases that show more complex genetic patterns of inheritance are not represented in this pool.

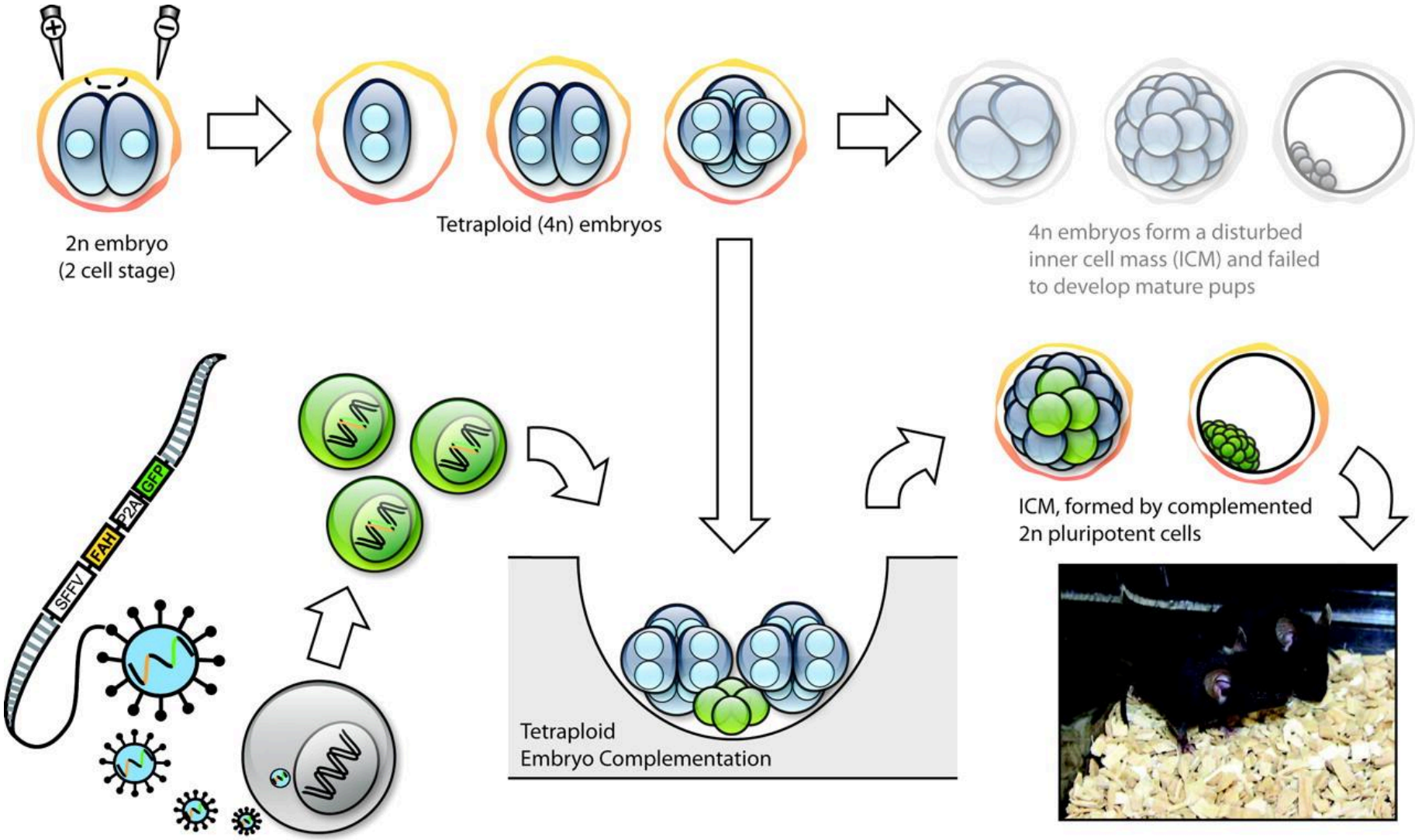
A tractable method for establishing immortal cultures of pluripotent stem cells from diseased individuals would not only facilitate disease research but also lay a foundation for producing

Generating haploid embryonic stem cells (ESCs)

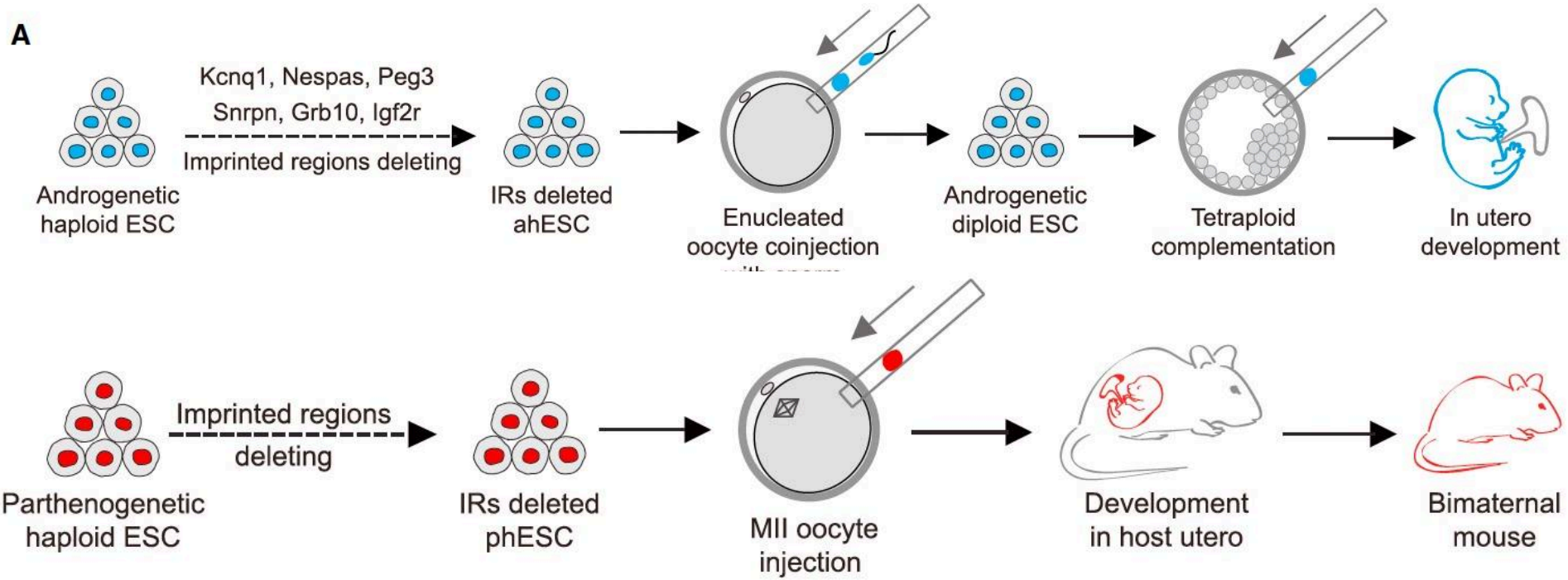


Mutation of specific imprinted genes allows survival of single parent offspring




2n embryo
(2 cell stage)
Tetraploid (4n) embryos
4n embryos form a disturbed
inner cell mass (ICM) and failed
to develop mature pups
SFFV
FAH
P2A
GFP
Tetraploid
Embryo Complementation
ICM, formed by complemented
2n pluripotent cells

Creating viable progeny by mutating (turning off) genes that would normally be silenced through imprinting in the gamete that no longer contributes.



Interesting implication to think about. The progeny are viable but are heterozygous for LOF mutations in multiple genes involved in imprinting. What does this mean for the viability and phenotypes of their progeny? Would you use this techni in humans?