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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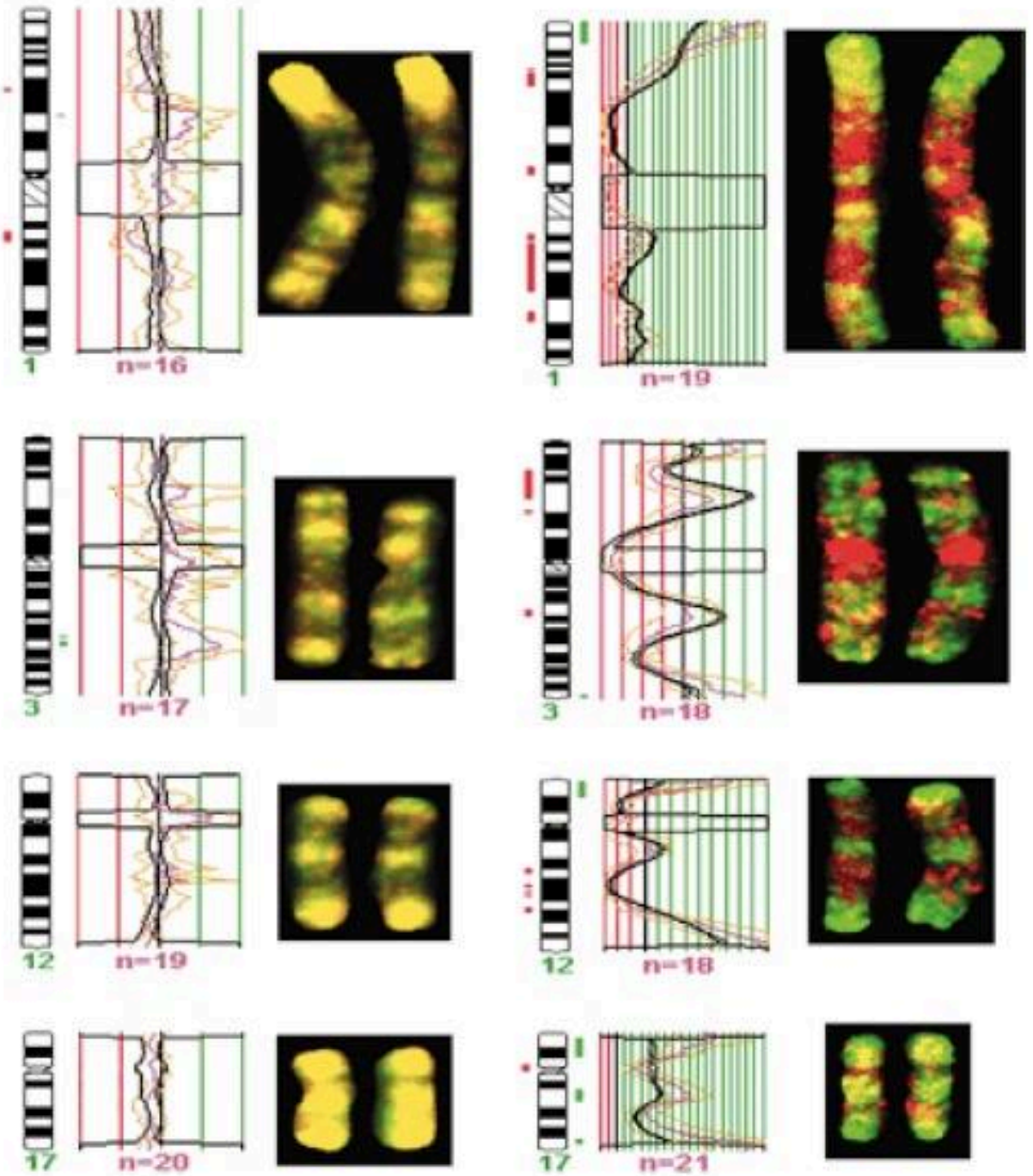
Summary

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.

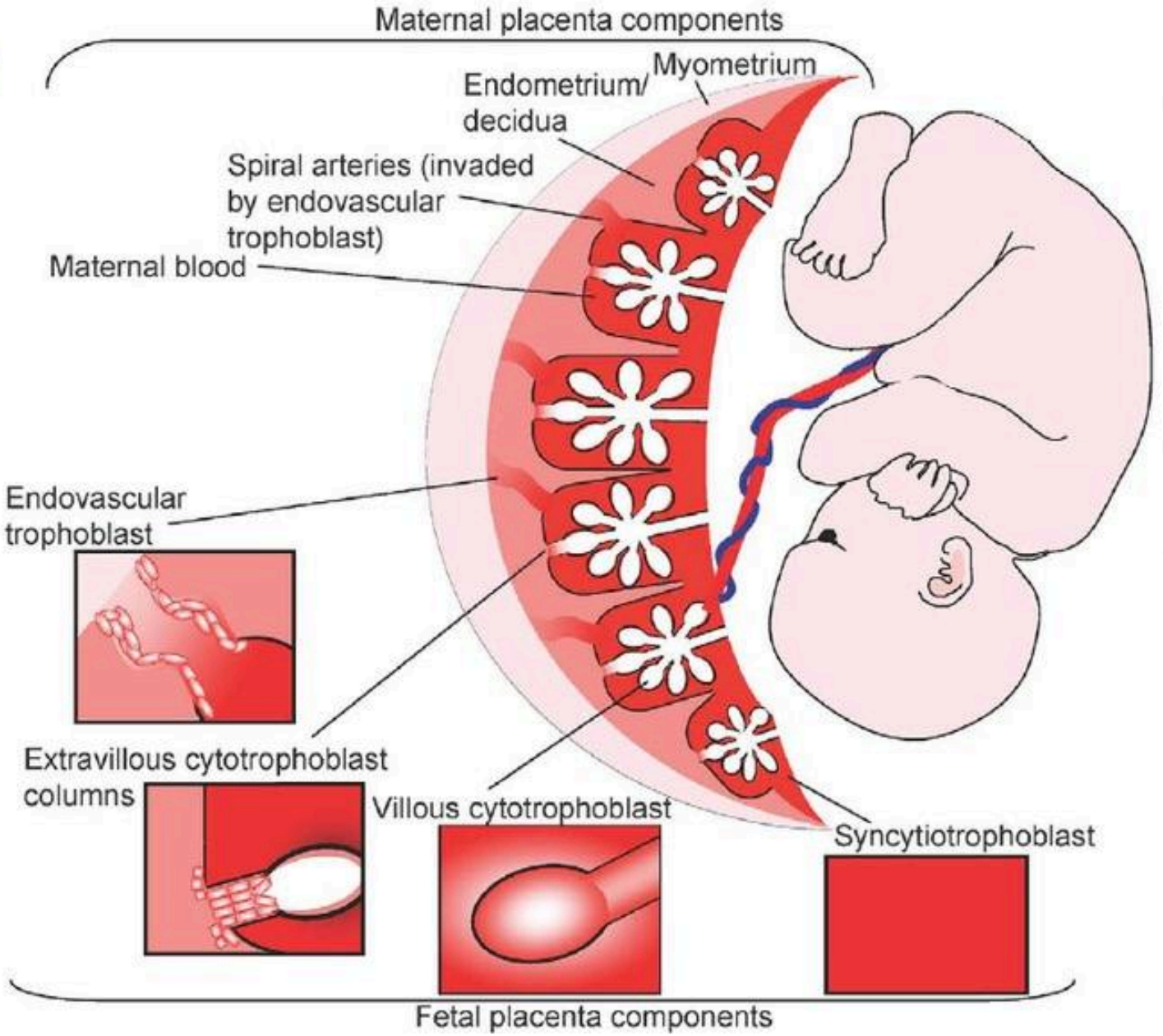
--So, the argument goes, imprinting will silence growth enhancing genes in the female and silence growth suppressing mutations in the male.

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

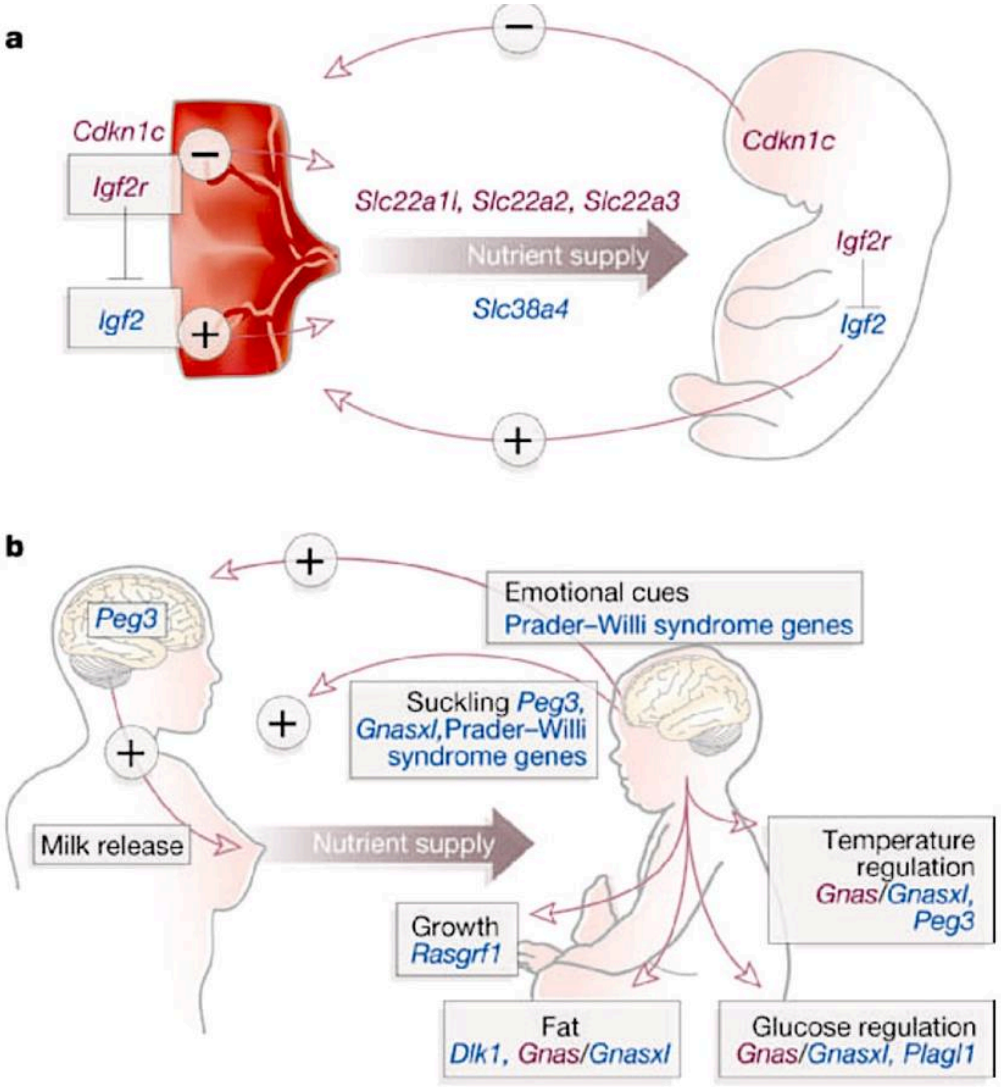


Figure 2 **Effects of imprinted genes on resource acquisition by offspring[6]. Imprinted genes that are expressed from the maternally derived copy are in purple; those expressed from the paternally derived copy are in blue.** a, **Growth of the fetus — promoted by, for example, *Igf2*, a paternally expressed 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.**

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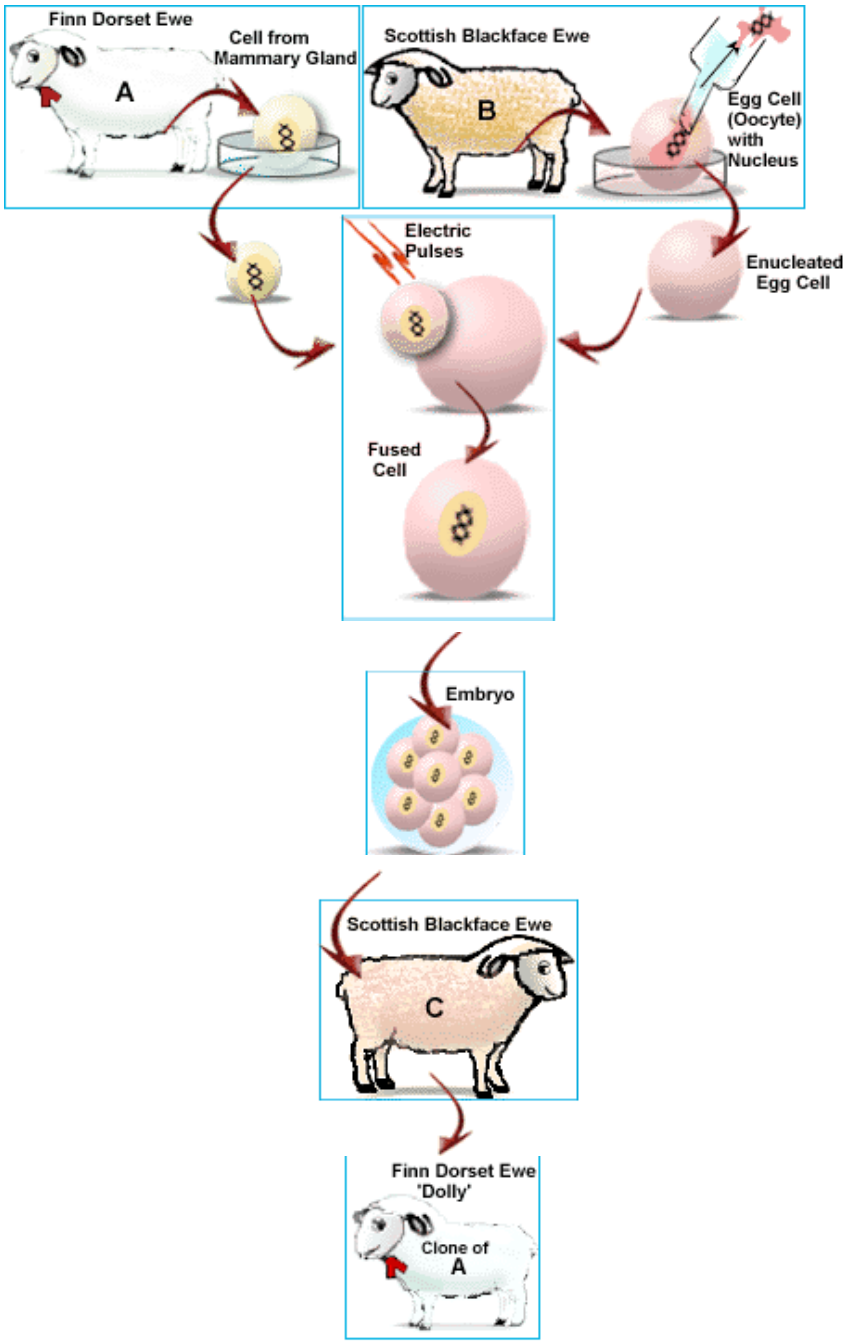
Relatively few genes (~100) have previously been shown to be imprinted such that their expression in progeny derives from either the maternal or paternal copy. Two recent studies by Gregg et al. (2010a, 2010b) in *Science* expand this list by an order of magnitude, revealing complex patterns of parent-of-origin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

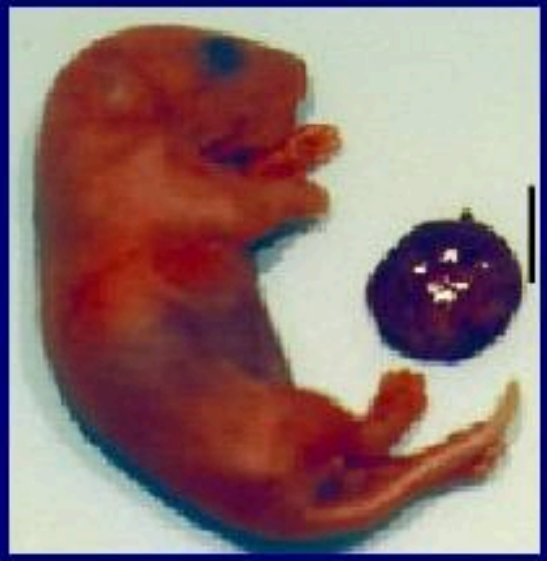
Birth of clone of Sheep A (Dolly)

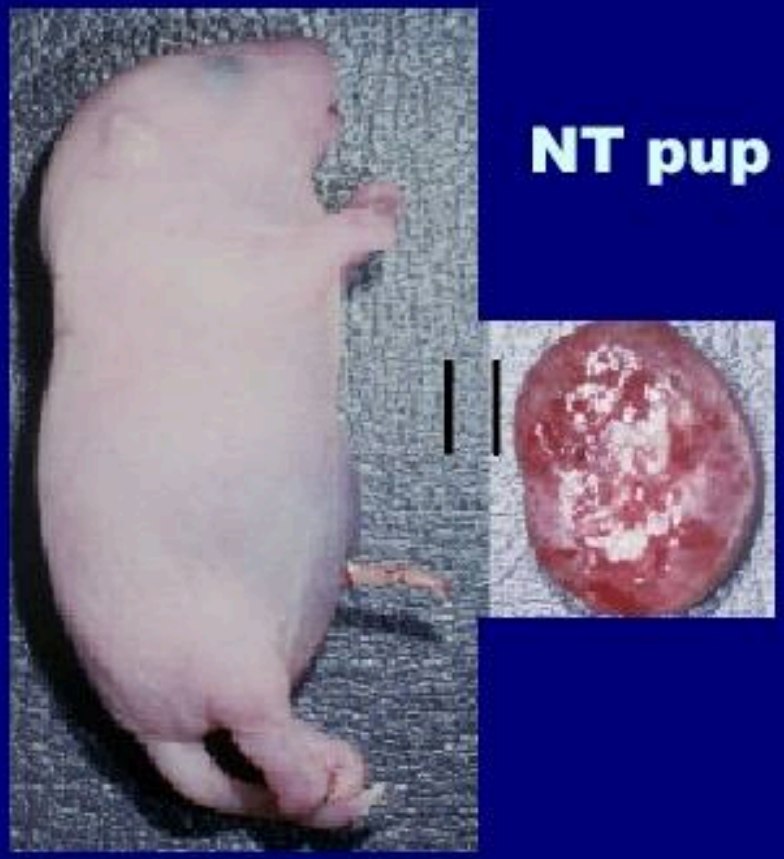
Although the clone of sheep A (Dolly) could have been created using only sheep A, sheep B and C were used in the cloning process in order to ensure that Dolly was truly a clone and the not the result of unexpected mating.

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:

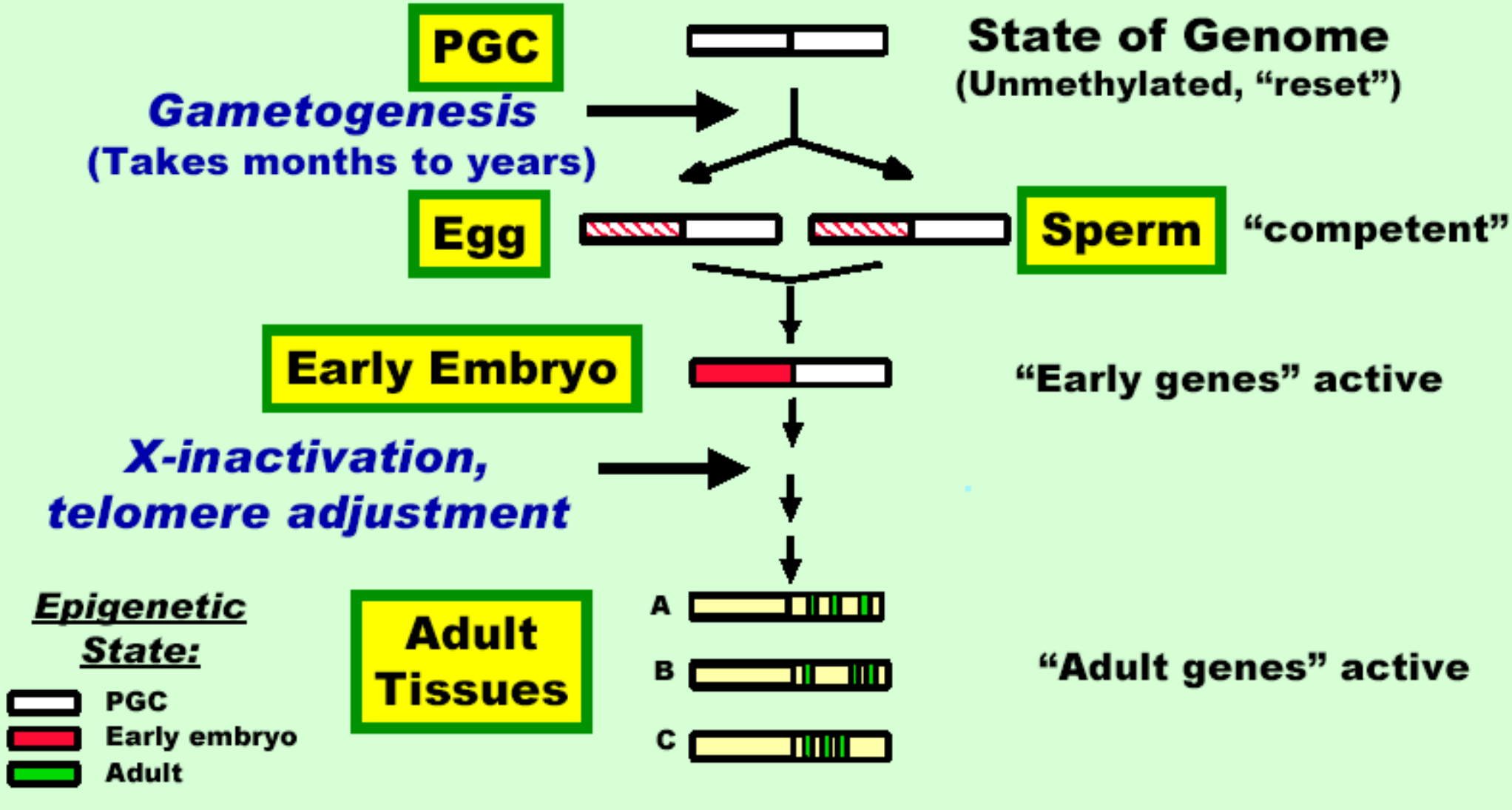
A. During gametogenesis

-- 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:

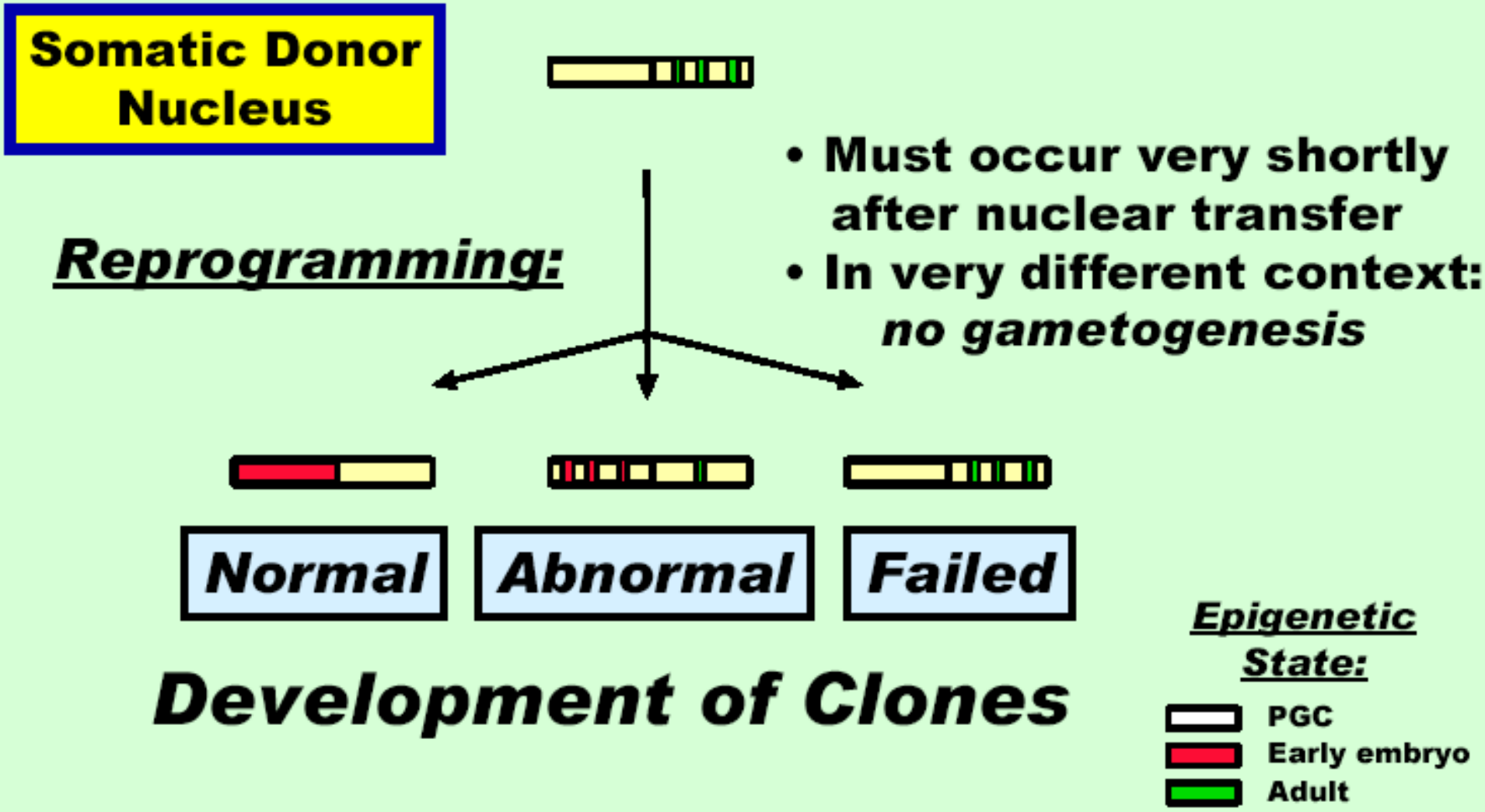
- the genome is reset during egg and sperm maturation:
  → ***this takes weeks, months, years***

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?

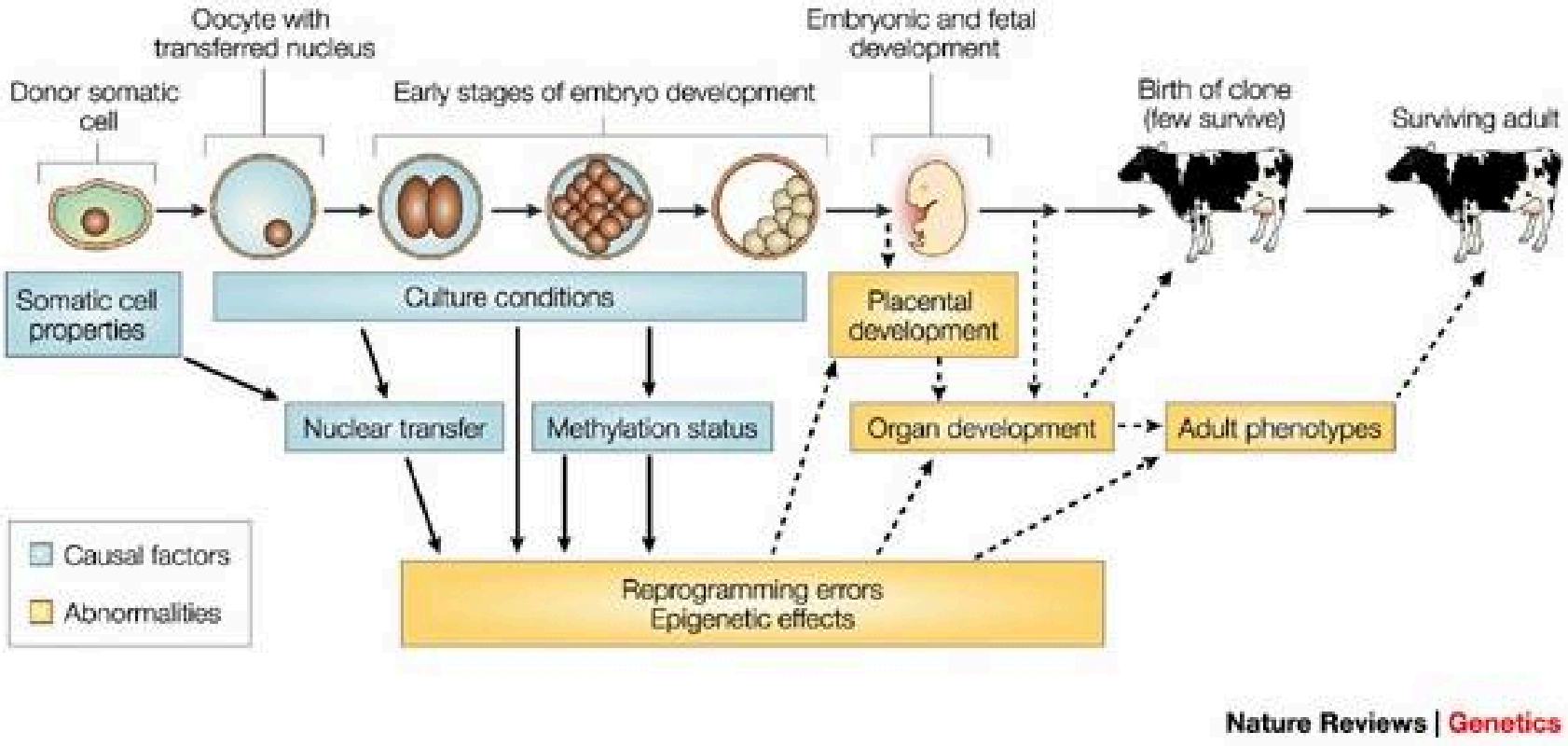
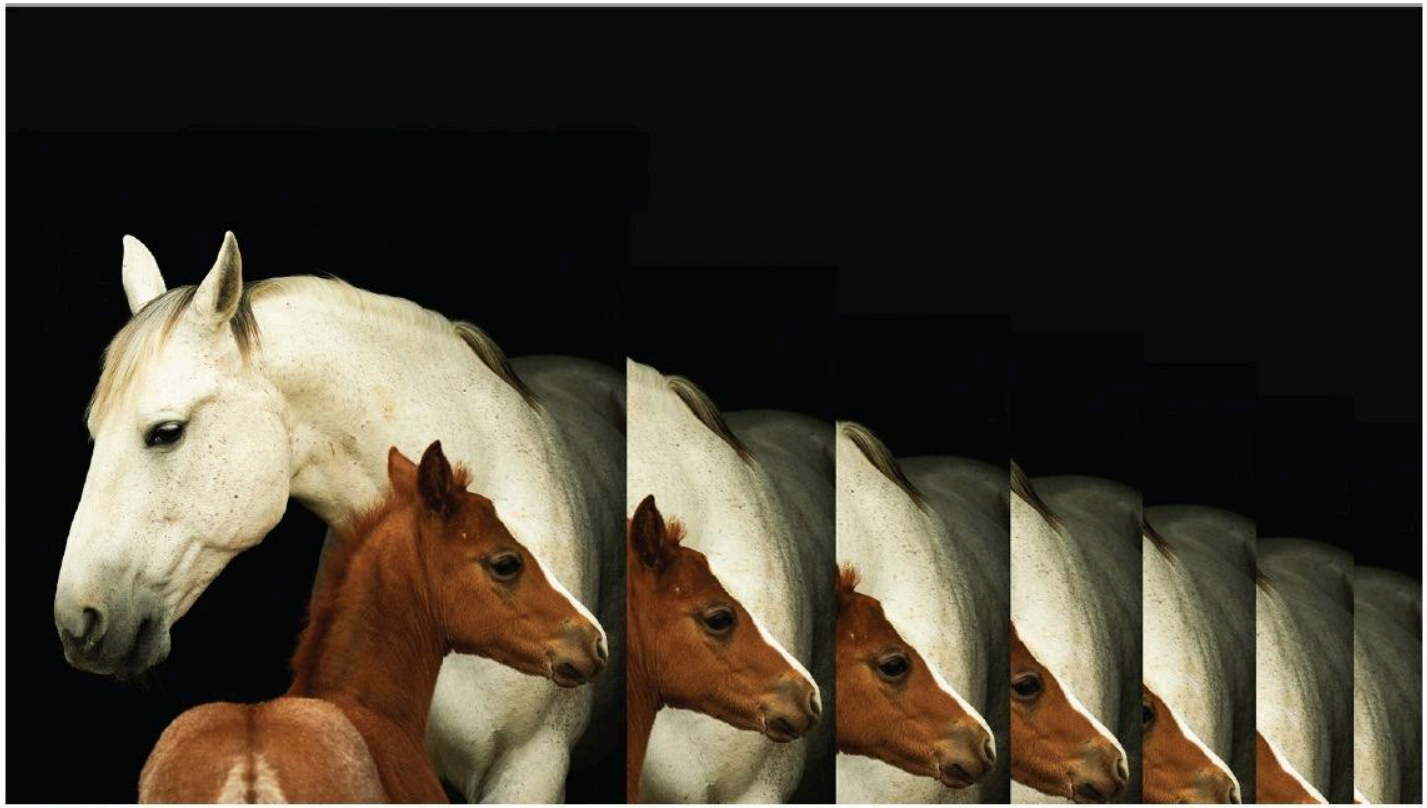


Figure 2 | **Overview of clone development showing the factors that contribute to clone failure.** Factors that affect clone development include the nature of the donor cell and features of the methodology that interfere with reprogramming and epigenetic mechanisms. Aberrant methylation also causes gene 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.



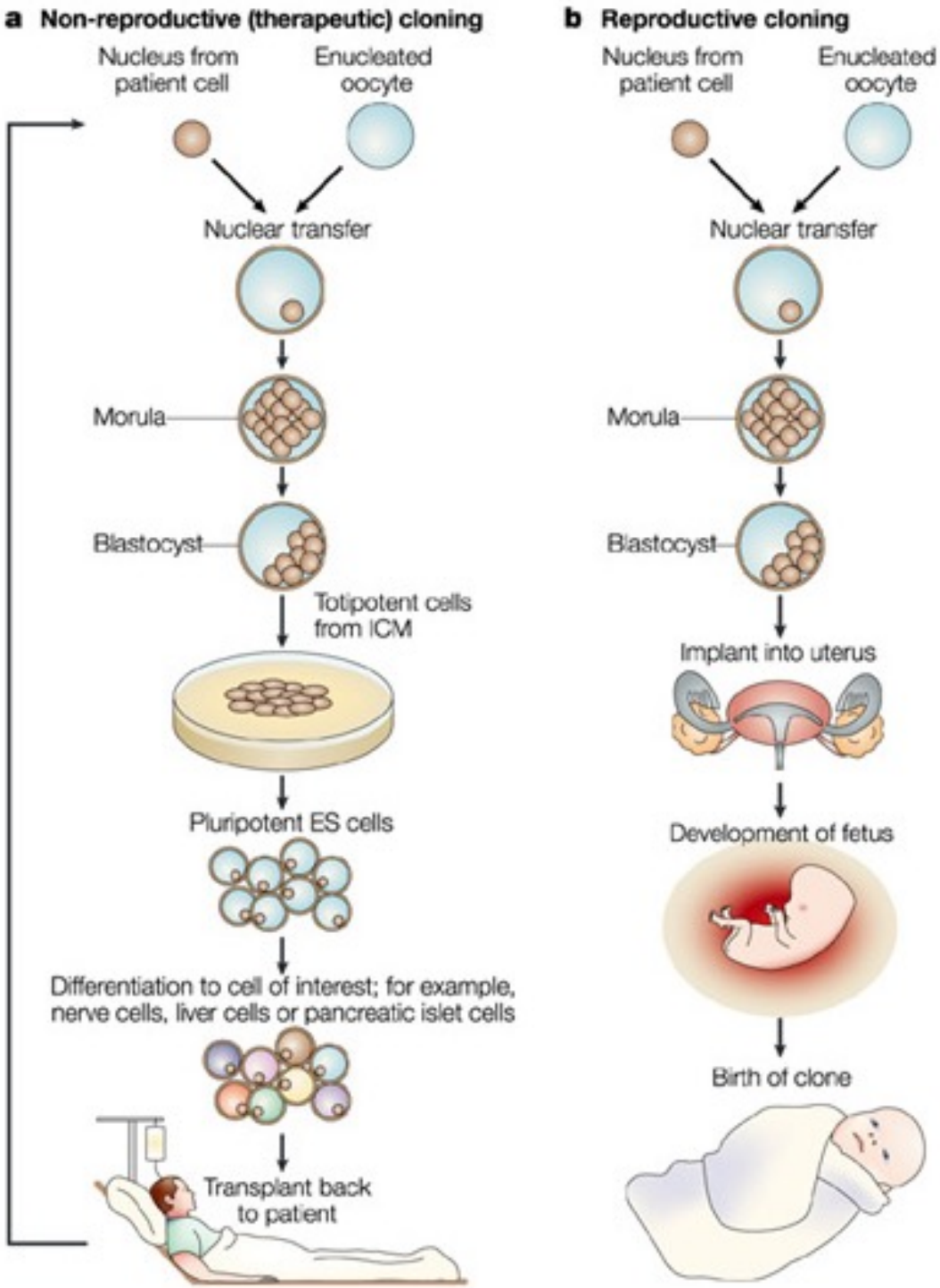
IDEAS

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

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**

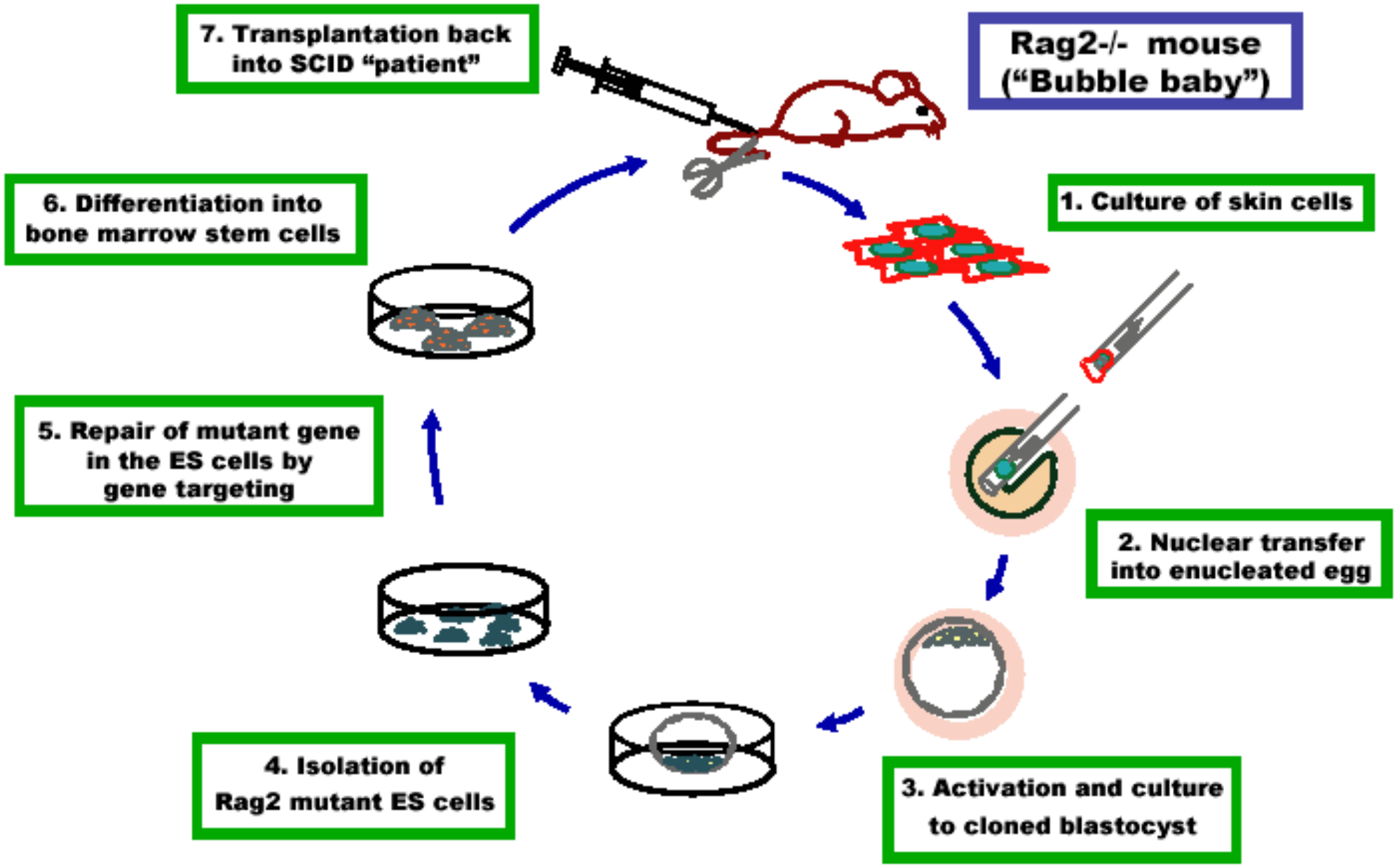
(No immuno-suppressive treatment required)

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



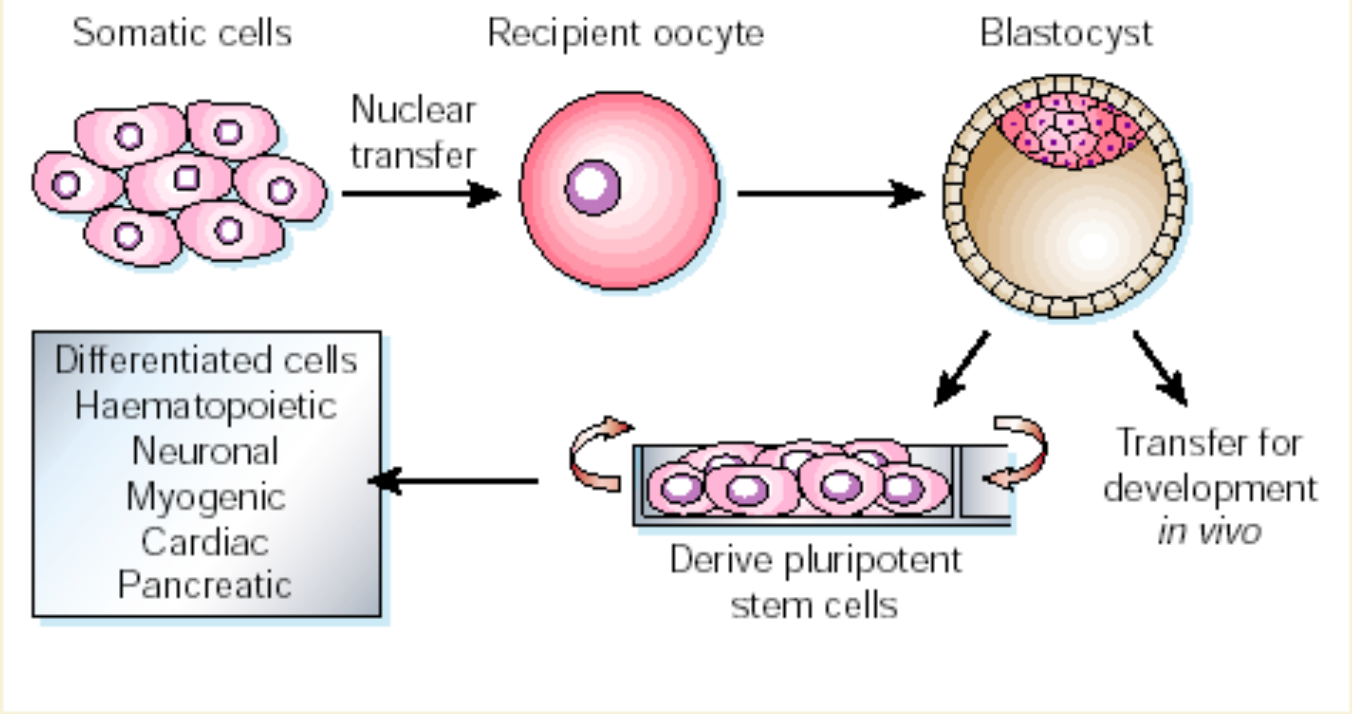


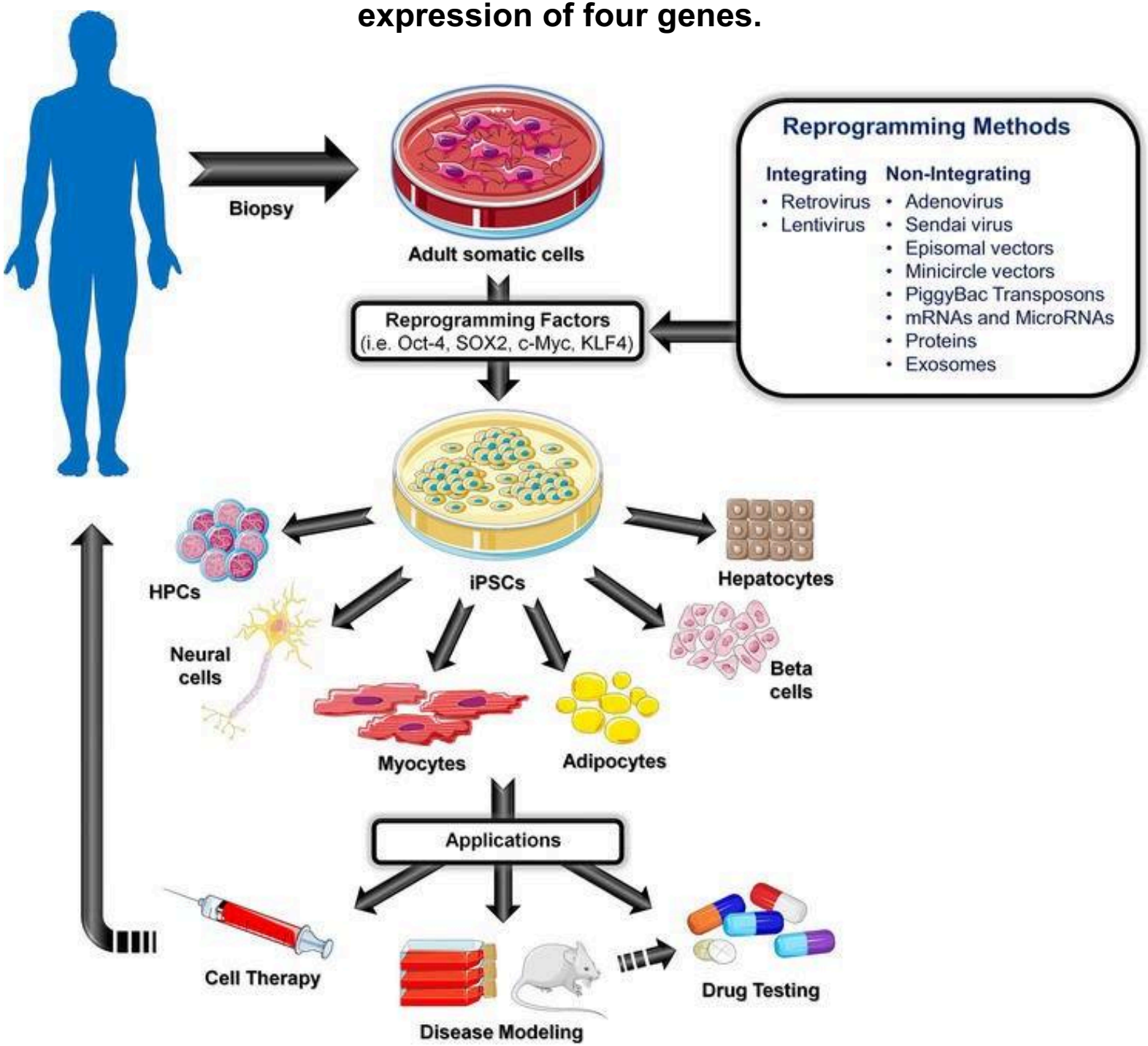
Figure 5 Reprogramming a somatic nucleus. When transplanted into an oocyte, a somatic nucleus may respond to the cytoplasmic factors and be reprogrammed back to totipotency. These cytoplasmic factors must be capable of erasing the 'molecular memory' that gives somatic cells their characteristic properties. 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

In-Hyun Park,[1,7] Natasha Arora,[1,7] Hongguang Huo,[1,7] Nimet Maherali,[2,3,7] Tim Ahfeldt,[2,5,7] Akiko Shimamura,[4] M. William Lensch,[1,7,9] Chad Cowan,[2,6,7] Konrad Hochedlinger,[2,7] and George Q. Daley[1,7,8,9,*]

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SUMMARY

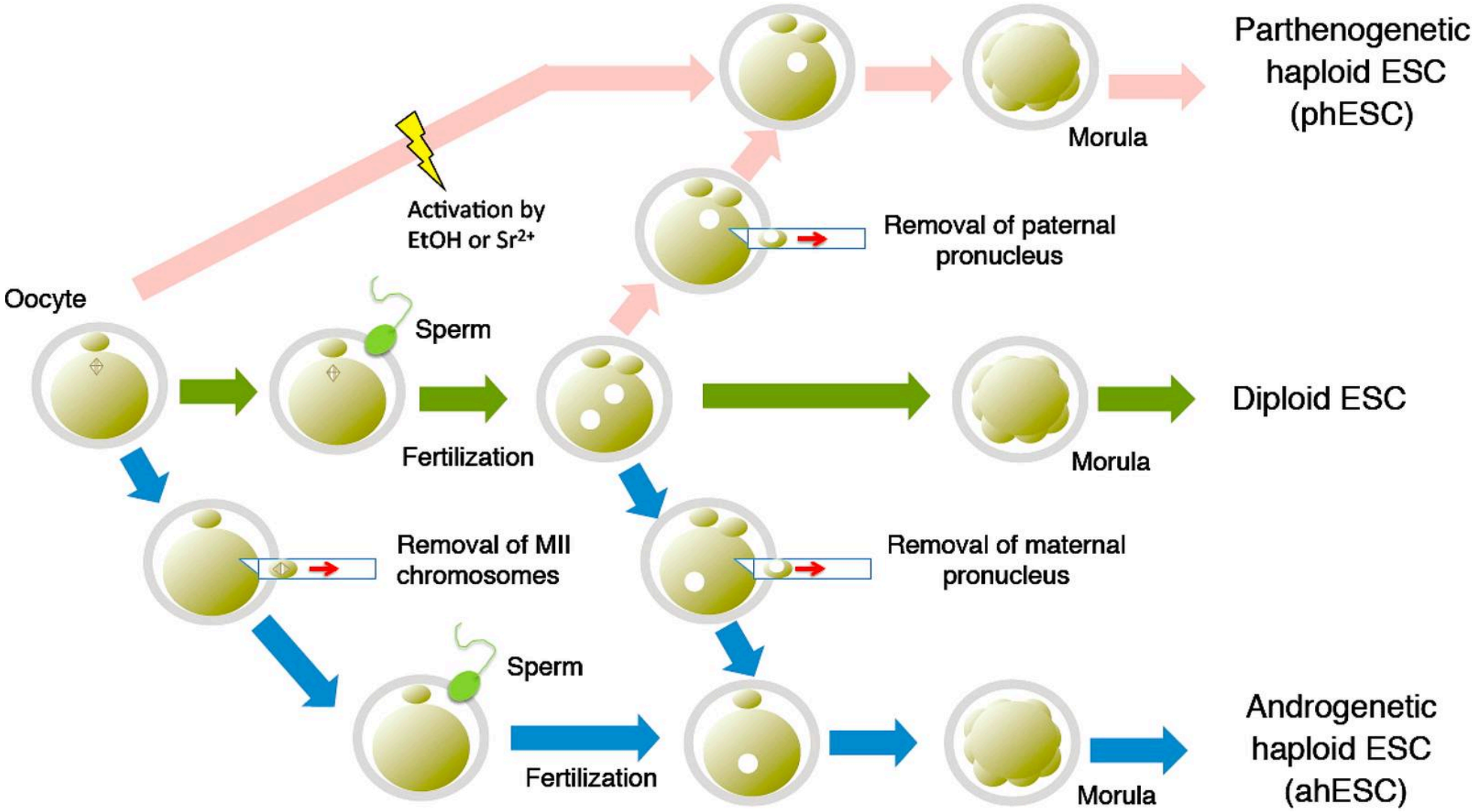
Tissue culture of immortal cell strains from diseased patients is an invaluable resource for medical research but is largely limited to tumor cell lines or transformed derivatives of native tissues. Here we describe the generation of induced pluripotent stem (iPS) cells from patients with a variety of 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.

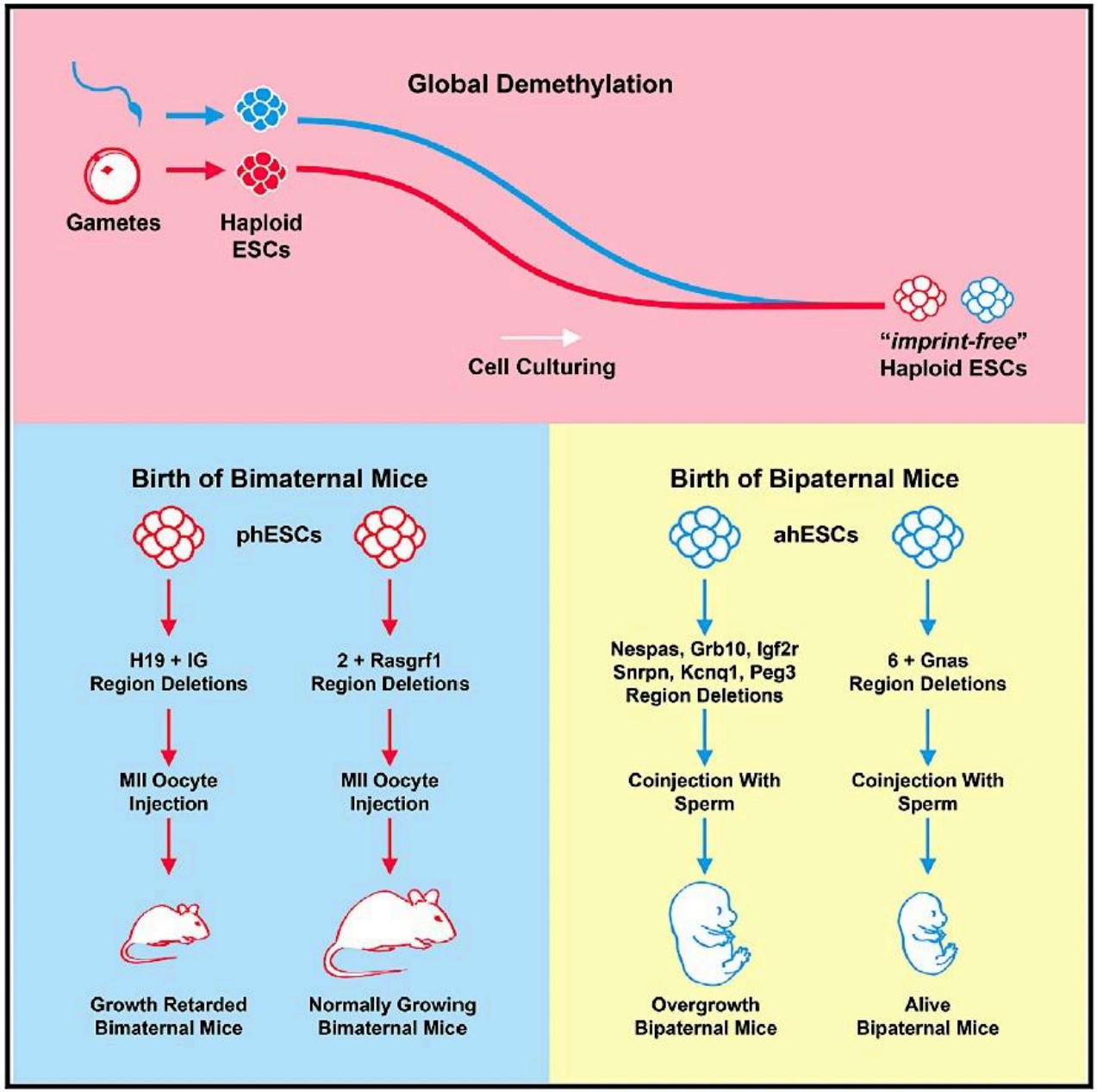
Human embryonic stem cells isolated from excess embryos from in vitro fertilization clinics represent an immortal propagation of pluripotent cells that theoretically can generate any cell type within the human body (Lerou et al., 2008; Murry and Keller, 2008). Human embryonic stem cells allow investigators 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.

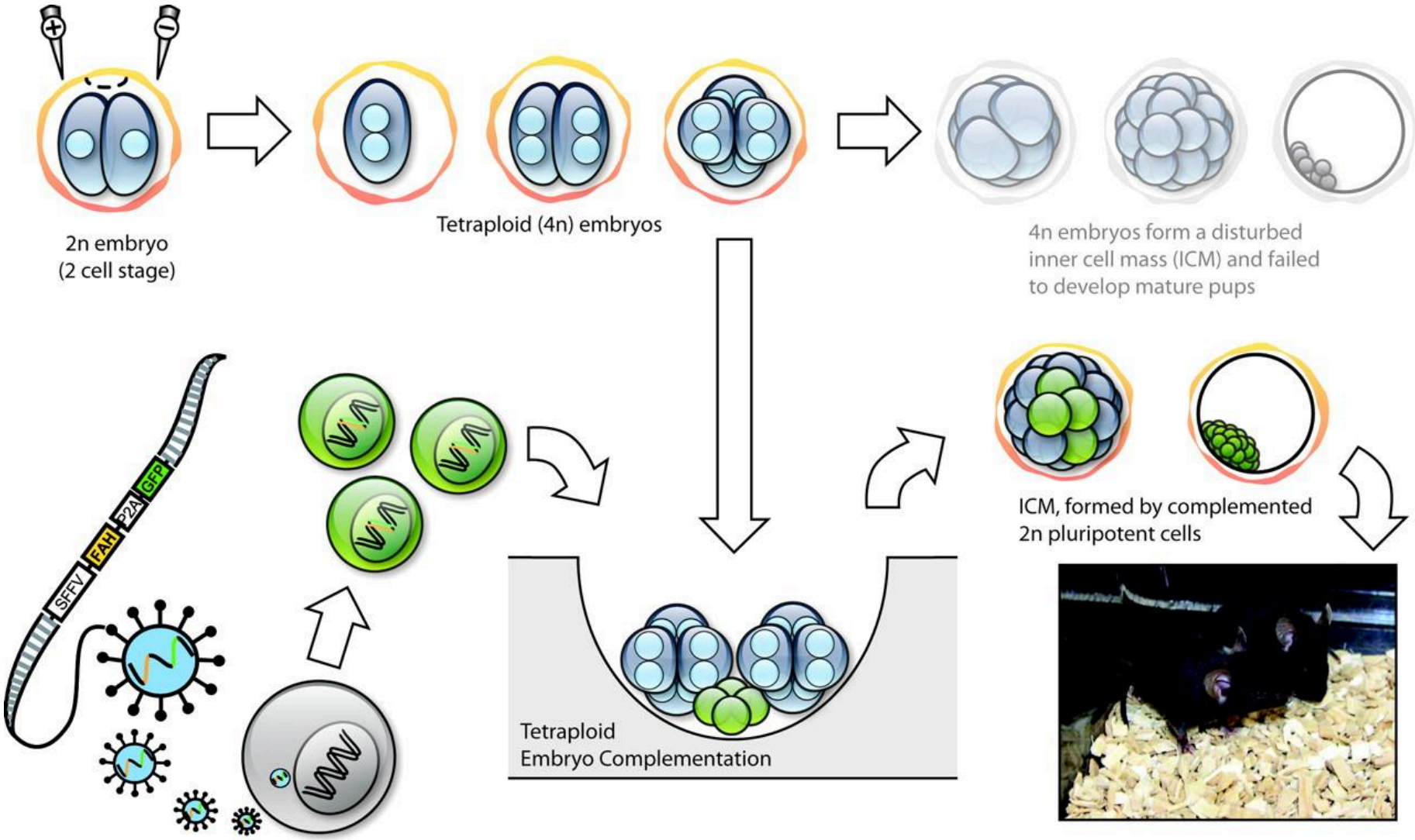
A tractable method for establishing immortal cultures of pluripotent stem cells from diseased individuals would not only facilitate disease research but also lay a foundation for producing

Generating haploid embryonic stem cells (ESCs)

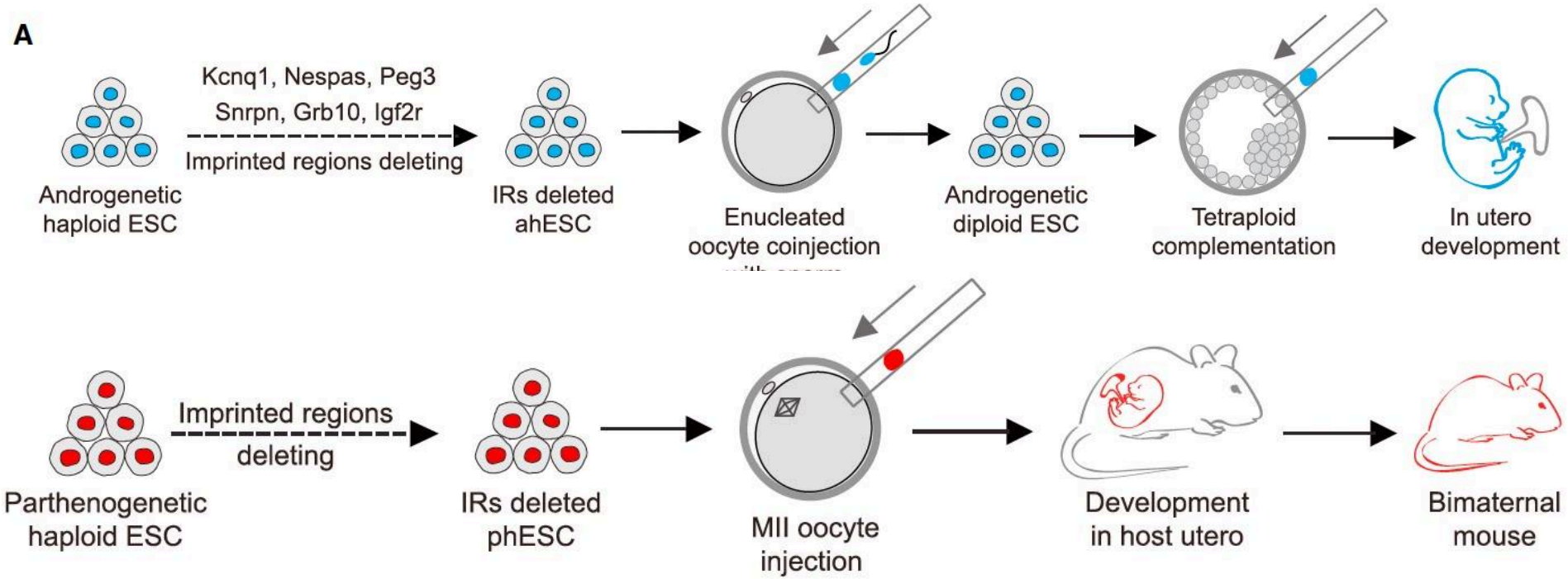


Mutation of specific imprinted genes allows survival of single parent offspring




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.

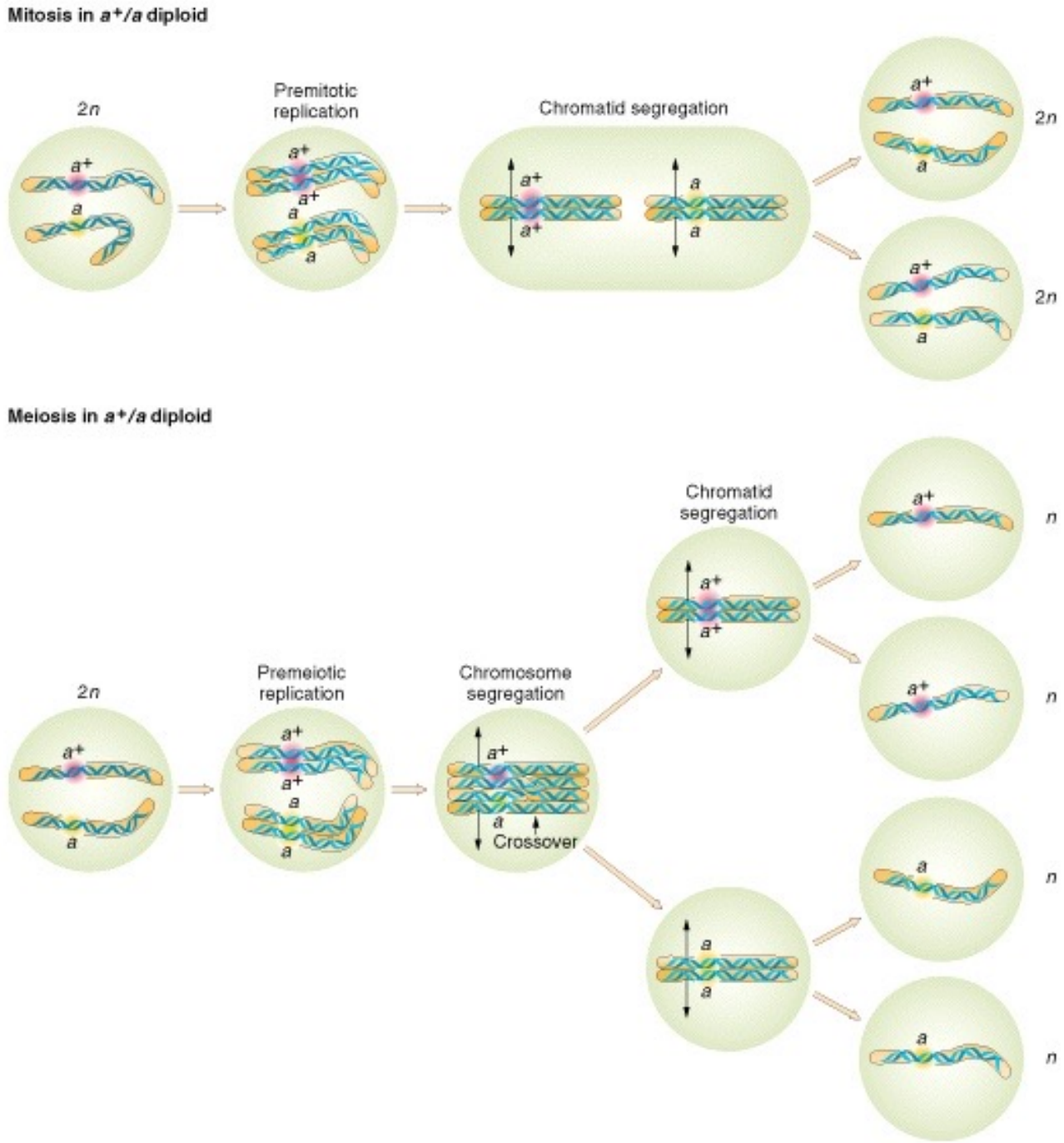


Interesting implication to think about. The progeny are viable but are heterozygous for LOF mutations in multiple genes involved in imprinting. What does this mean for the viability and phenotypes of their progeny? Would you use this technique in humans?

Organelles and prions

Random segregation

Centromeres ensure the proper segregation of genes during mitosis and meiosis.



Molecules not linked to chromosomes bearing nuclear centromeres behave differently during mitosis and do not obey Mendel's rules.

- **Organelle chromosomes**
- DNA and RNA plasmids
- Protein structure

Organelle Genetics

Mitochondria and Chloroplasts
Involved in producing energy for the cell though either oxidative phosphorylation or photosynthesis

Each contain their own small genome

Nuclear vs Mitochondrial DNA

Nuclear DNA

3×10^6 kbp

Linear

>30,000 genes

Cell contains two copies of each gene

Encodes all cellular functions, including mitochondrial

Mitochondrial DNA

17 kbp

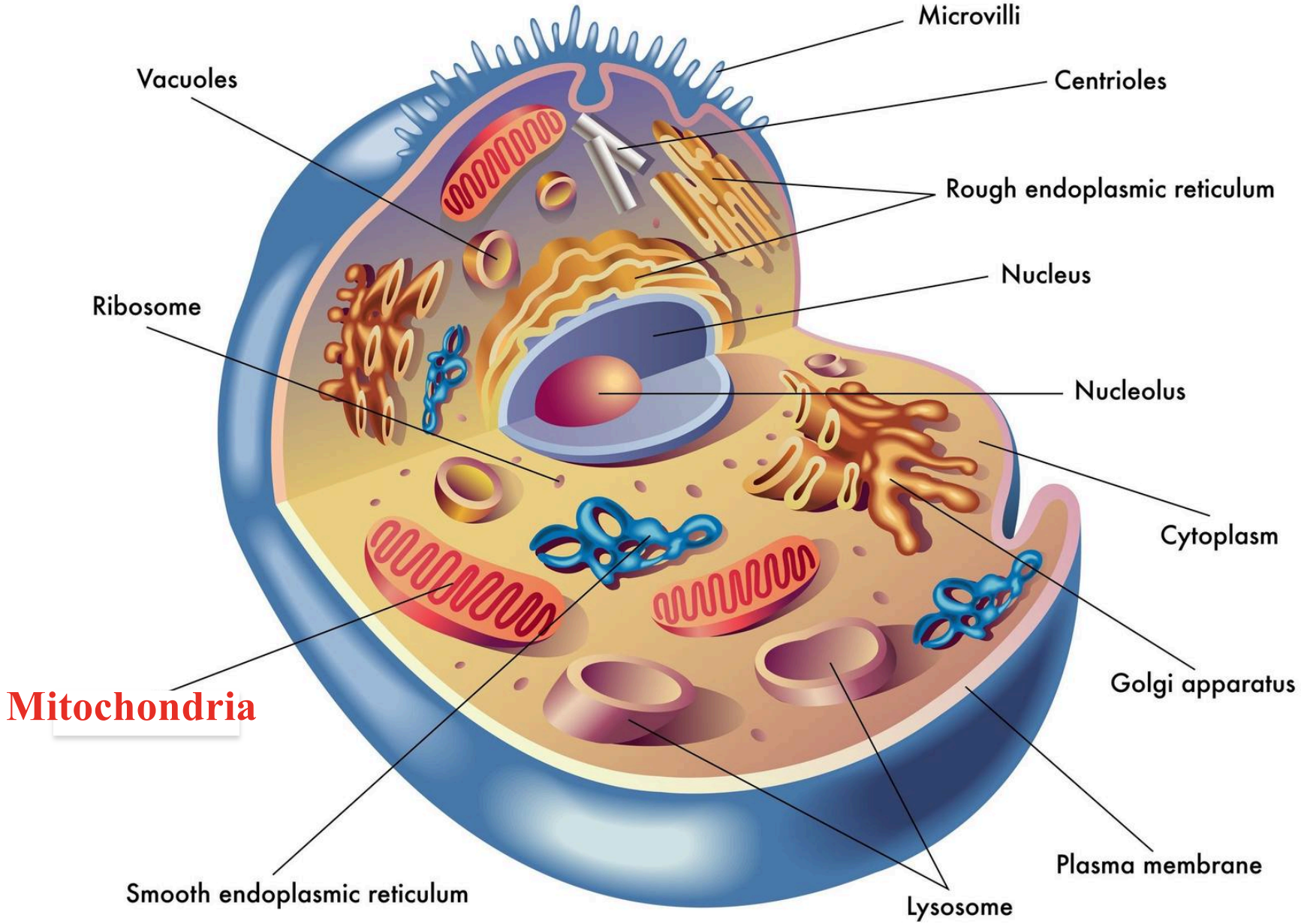
Circular

37 genes

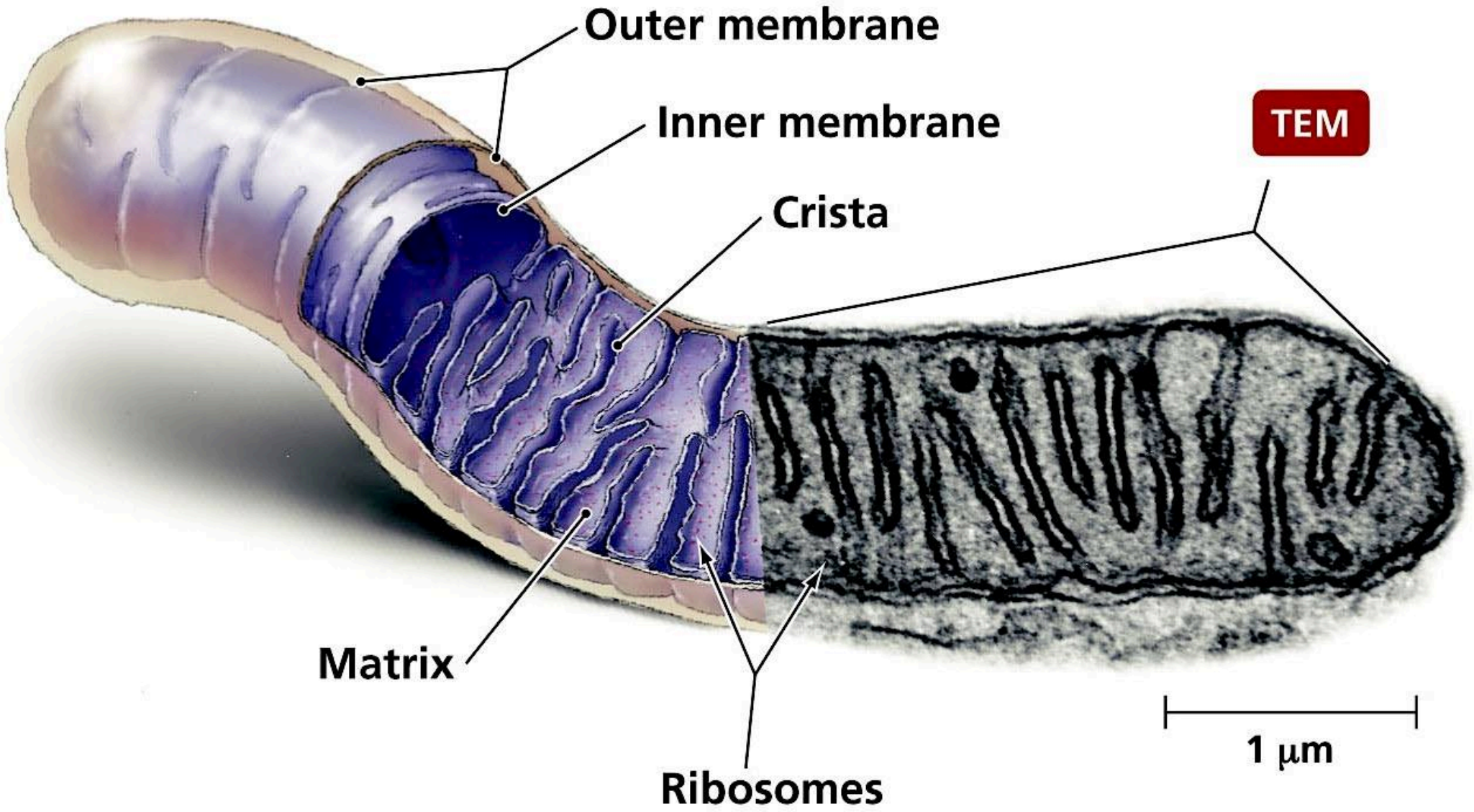
Cell contains 1000s of copies

Encodes only mitochondrial functions

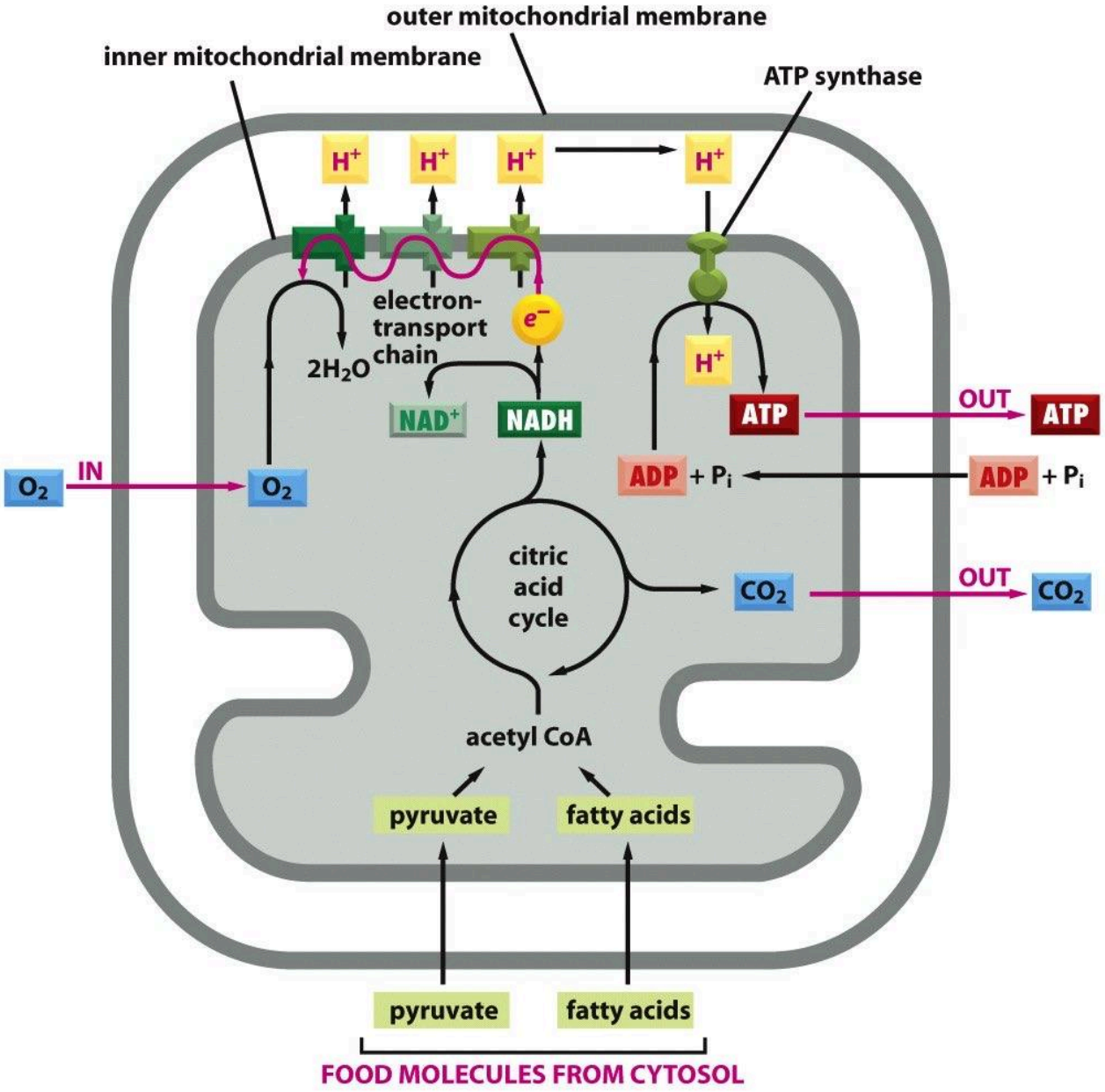
Mitochondria generate >90% of the energy (ATP) we use each day through oxidative phosphorylation (OXPHOS)

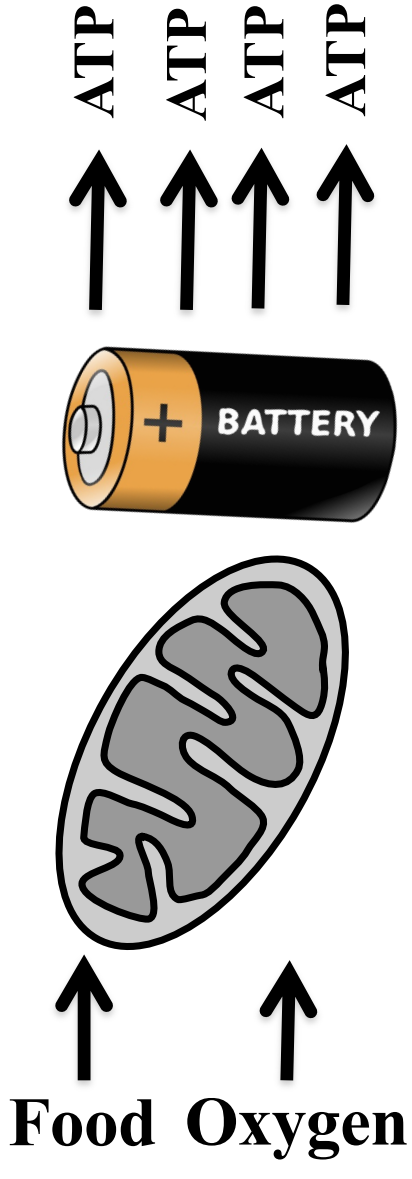


Mitochondria have an inner matrix bounded by inner and outer membranes. The inner membrane is highly convoluted, and has a large surface area

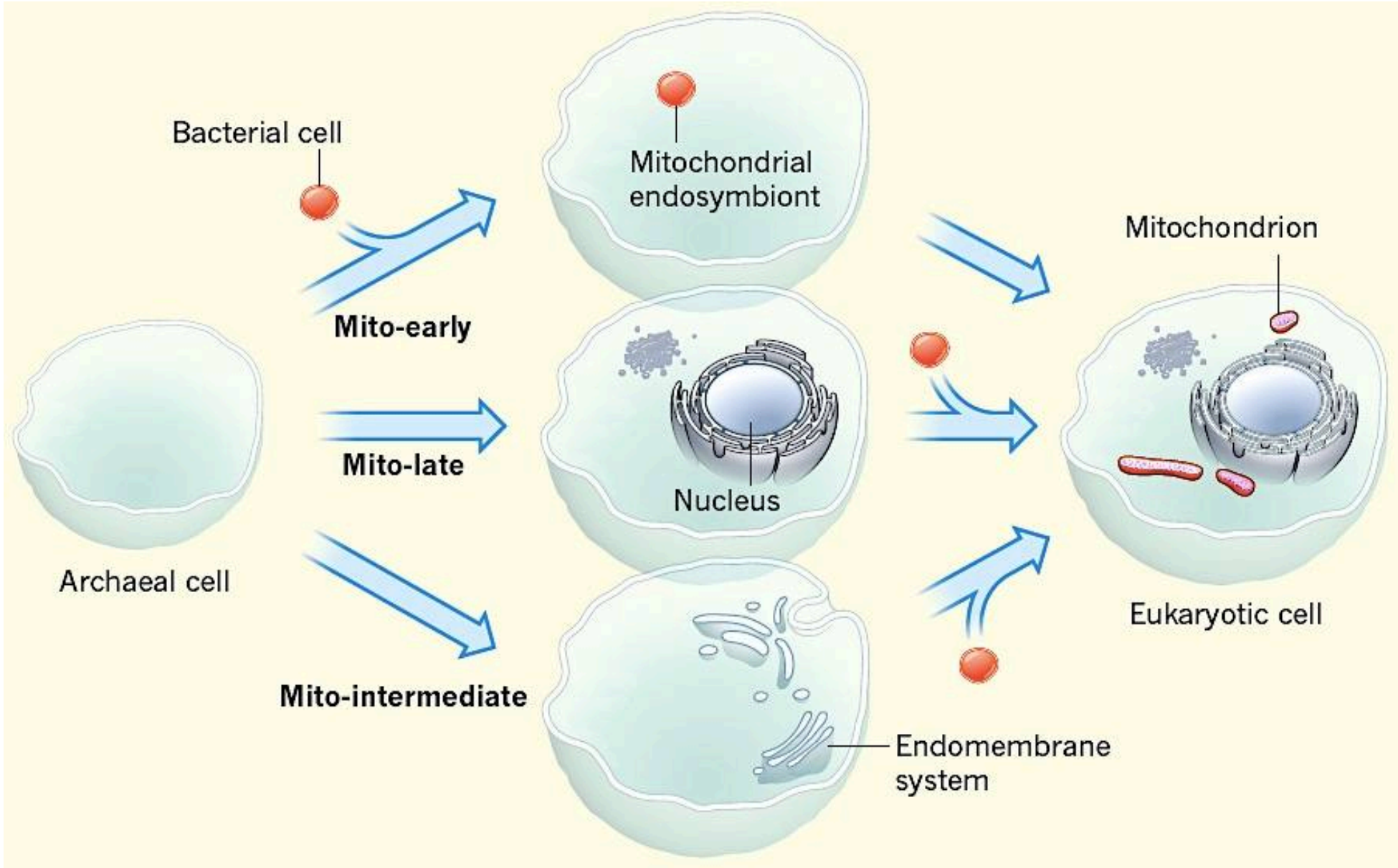


In OXPHOS, electron transfer is used to convert food and O_2 into a proton gradient across the inner membrane that powers ATP synthesis





Mitochondria derive from an ancestral symbiont



Evidence for Endosymbiont Theory

Similar lipid compositions in membranes of bacteria and mitochondria.

Bacterial and mitochondrial genomes circular and lack associated histones.

Transcription and protein synthesis in bacteria and mitochondria similar.

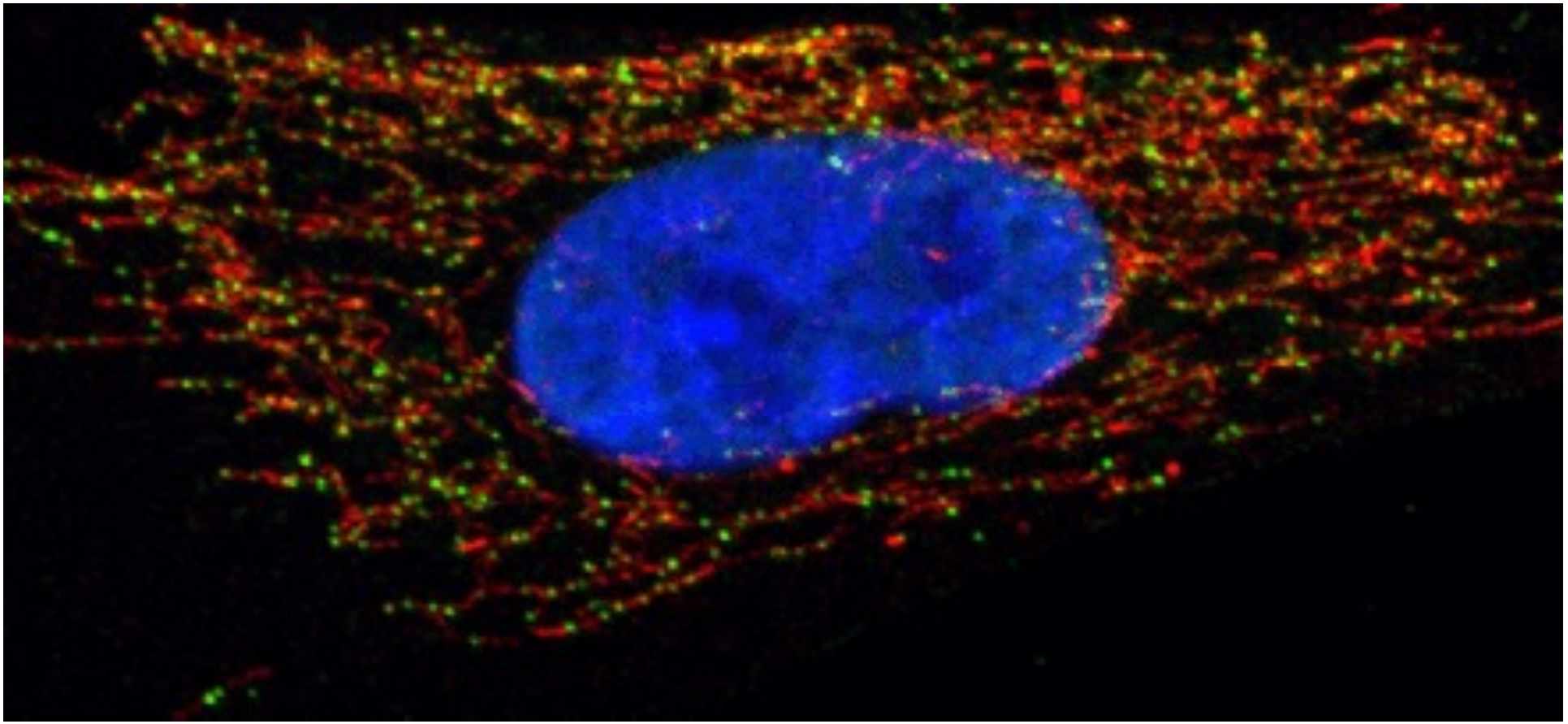
rRNAs similar in bacteria and mitochondria

Each mitochondria carries multiple copies of the mitochondrial genome (mtDNA)

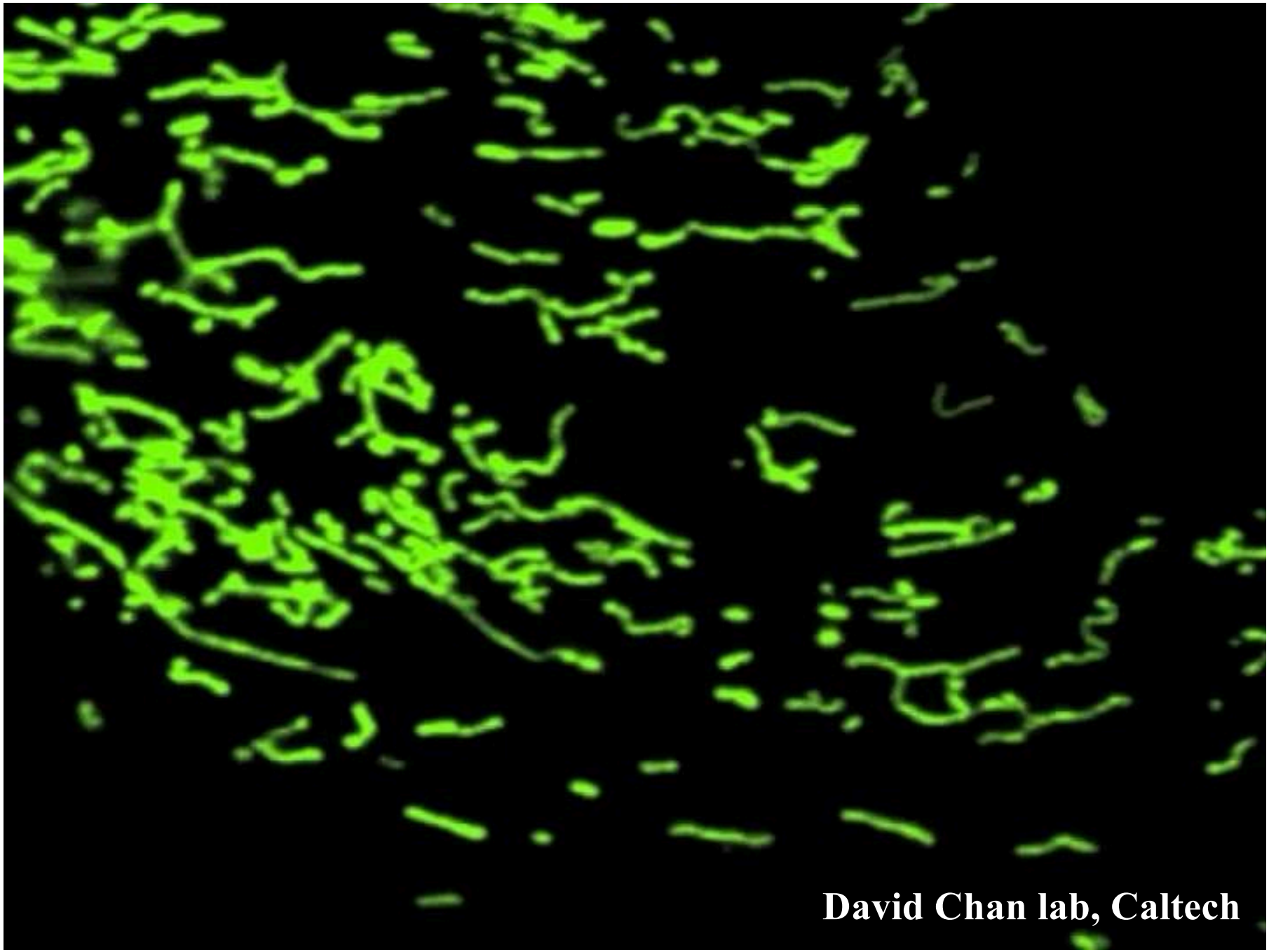
There are 100s to 1000s of mitochondria per cell

Mitochondria mtDNA

Nuclear DNA



Mitochondria go through dynamic cycles of fusion and fission, which promote content mixing and periodic isolation, respectively



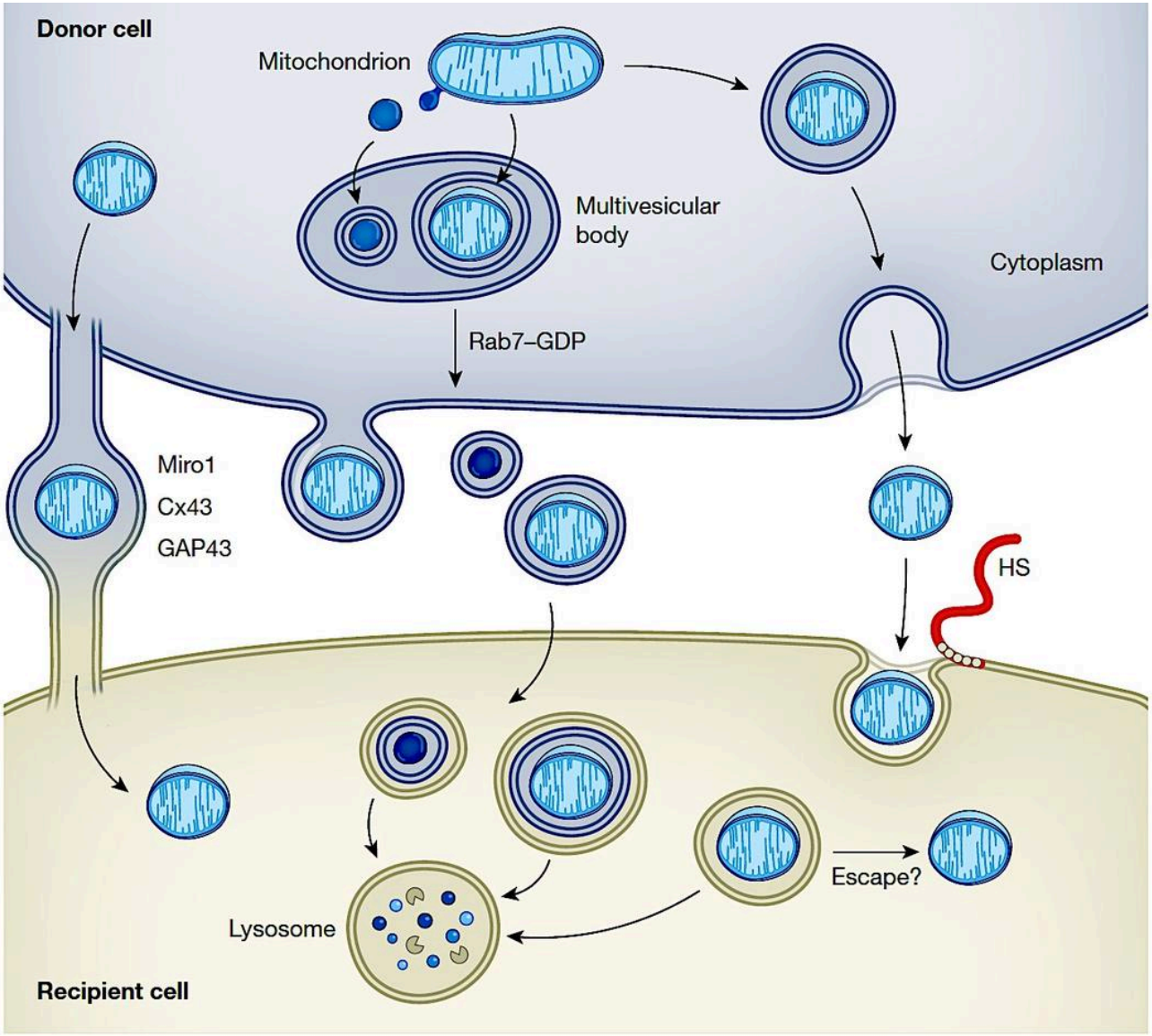


Fig. 1 | Mechanisms of mitochondria transfer. Cells transfer mitochondria through transient cellular connections, such as tunnelling nanotubes, that depend on Cx43, GAP43 and the Rho-GTPase Miro1 shuttle. In addition, mitochondria are exported from cells via multivesicular bodies. Mitochondria-derived vesicles and whole mitochondria are packaged in vesicles studded with CD9, CD63, CD81 and/or LC3, which are released as extracellular vesicles in a Rab7–GDP-dependent manner for capture by recipient cells. Mitochondria are also released in extracellular vesicles via budding off the plasma membrane. The recipient cell degrades mitochondria captured in extracellular vesicles via the lysosome. Finally, free mitochondria are released from cells and then captured by recipient cells in a heparan sulfate (HS)-dependent manner. Captured mitochondria can then be degraded via the lysosome, with some evidence suggesting that they might escape into the cytoplasm.

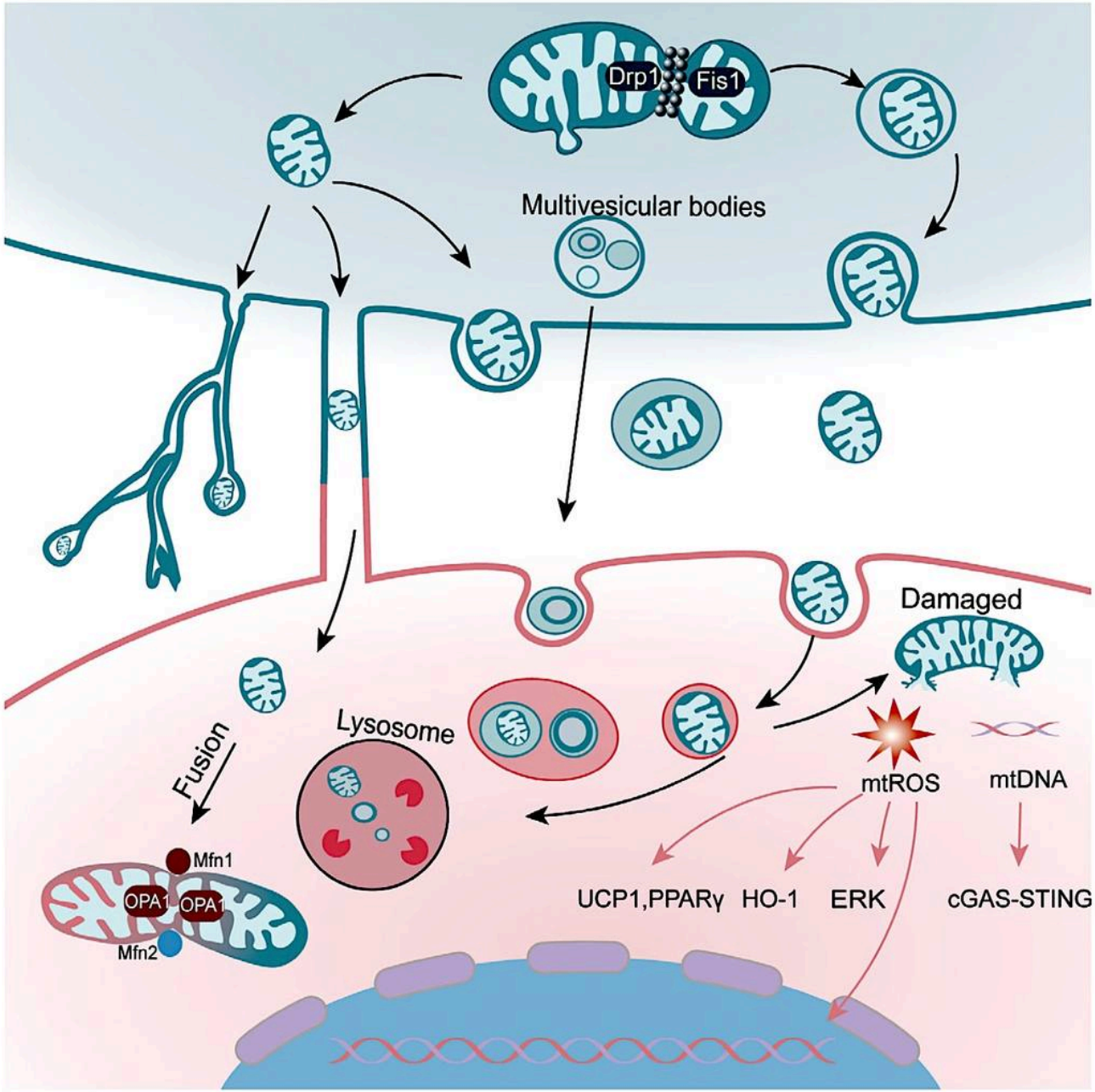


Fig. 2. Mitochondria can be transferred through open channels, such as tunneled nanotubes. In addition, mitochondria can be released as EVs through mitocytosis, MDVs and through budding from the plasma membrane, encapsulated in multivesicular bodies. Vesicles containing mitochondria also fuse with the plasma membrane and are released in free form. Recipient cells can degrade the captured mitochondrial components via lysosomes. Some evidence suggests that mitochondria escape into the cytoplasm. Mitochondria that escape the endosomal pathway may fuse with the recipient cell's mitochondrial network to resist oxidative stress. On the other hand, transferred damaged mitochondria carry certain ROS and mtDNA. A small amount of ROS promotes cell proliferation and stress resistance, whereas excess ROS is harmful and can promote oxidative damage and inhibition of proliferation in recipient cells. mtDNA can stimulate innate immune signaling.

Horizontal transfer of whole mitochondria restores tumorigenic potential in mitochondrial DNA-deficient cancer cells

Mitochondrial Genome Acquisition Restores Respiratory Function and Tumorigenic Potential of Cancer Cells without Mitochondrial DNA

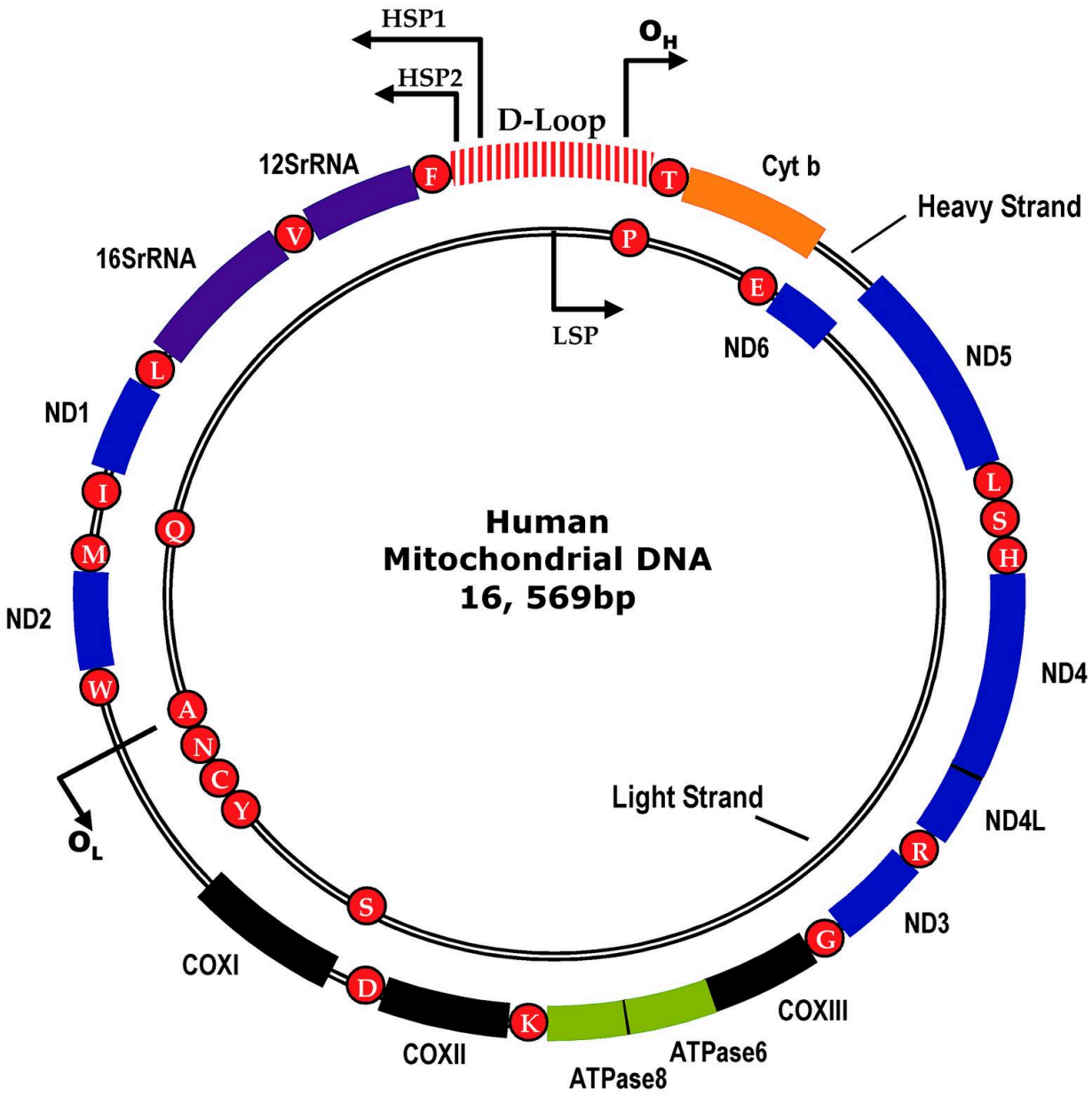
An S. Tan,[1,6] James W. Baty,[1,6] Lan-Feng Dong,[2,6] Ayenachew Bezawork-Geleta,[2] Berwini Endaya,[2] Jacob Goodwin, Martina Bajzikova,[3] Jaromira Kovarova,[3] Martin Peterka,[3] Bing Yan,[2] Elham Alizadeh Pesdar,[2] Margarita Sobol,[4] Anatolyj Filimonenko,[4] Shani Stuart,[5] Magdalena Vondrusova,[3] Katarina Kluckova,[3] Karishma Sachaphibulkij,[2] Jakub Rohlena,[3] Pavel Hozak,[4] Jaroslav Truksa,[3] David Eccles,[1] Larisa M. Haupt,[5] Lyn R. Griffiths,[5] Jiri Neuzil,[2,3,7,*] and Michael V. Berridge[1,7,*]

www.impactjournals.com/oncotarget/ Oncotarget, 2017, Vol. 8, (No. 9), pp: 15539-15552

Research Paper

Tunneling nanotubes promote intercellular mitochondria transfer followed by increased invasiveness in bladder cancer cells

mtDNA is dense with genes, all of which are required for oxidative phosphorylation



Mitochondrial genome sizes

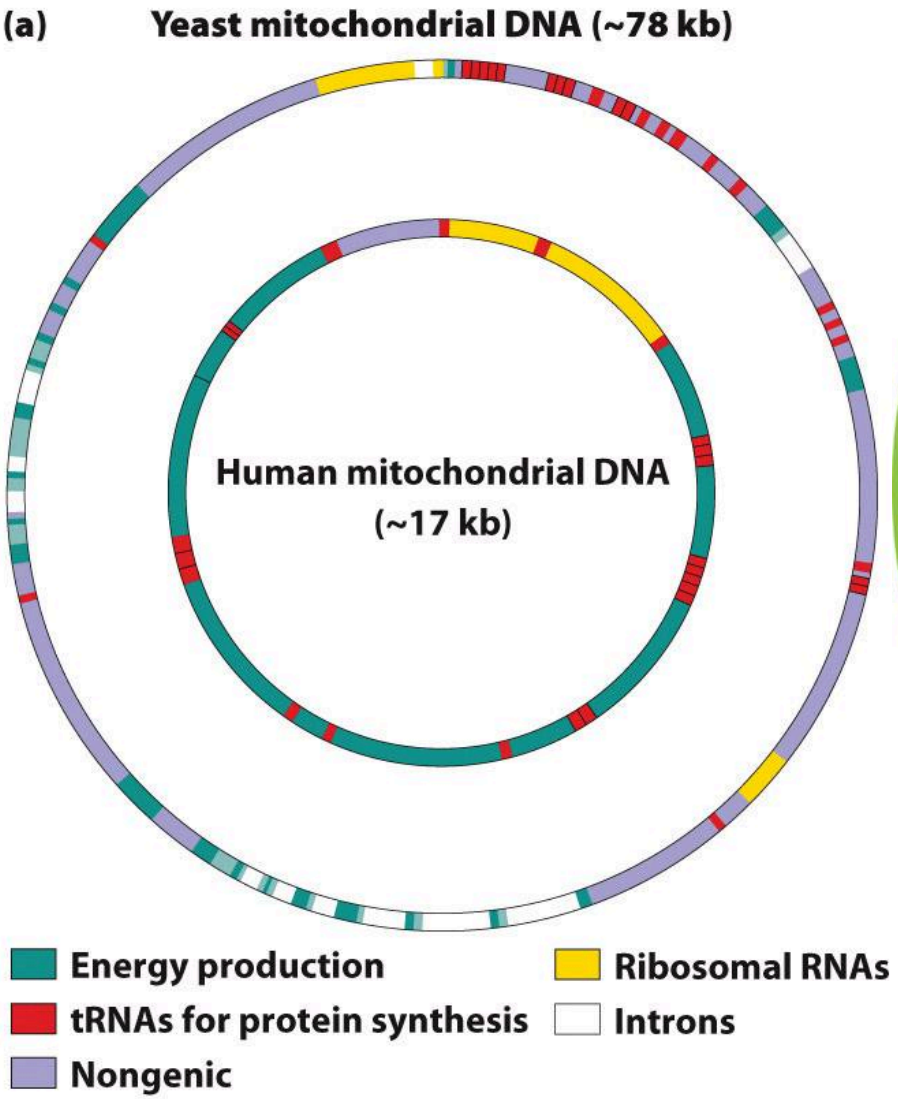


Figure 3-18
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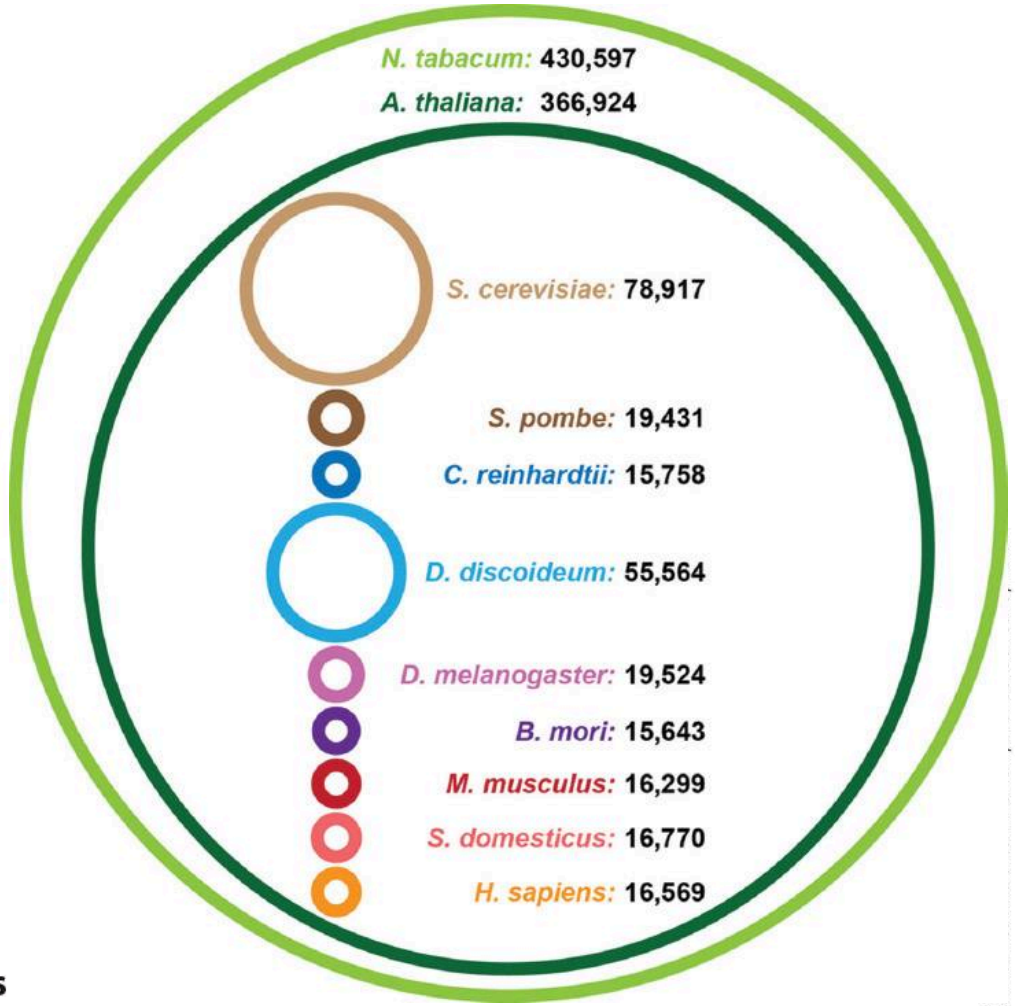
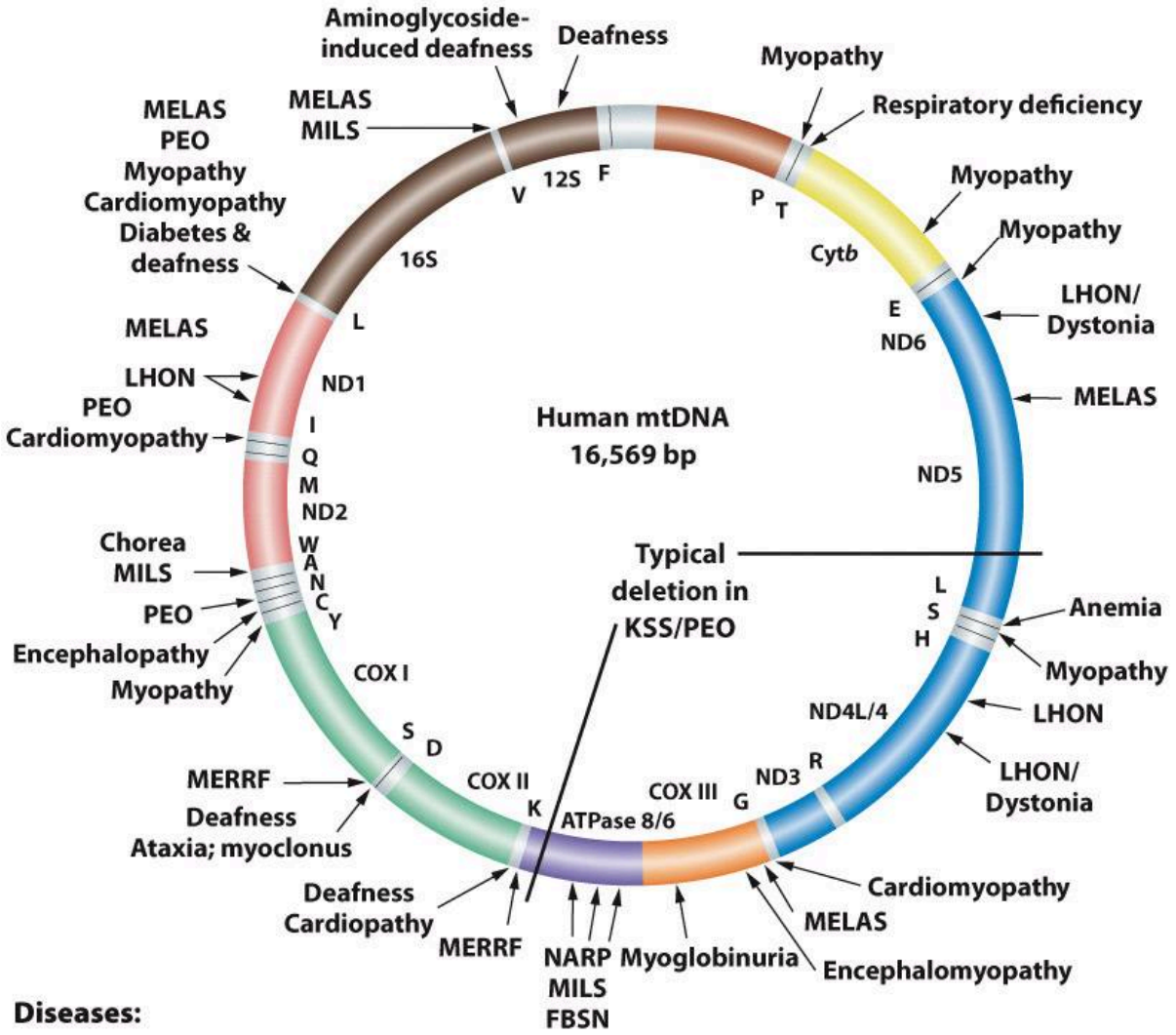


Data from

tabacum and thaliana are Plant mtDNA genomes



Diseases:

MERRF	**Myoclonic epilepsy and ragged red fiber disease**
LHON	**Leber hereditary optic neuropathy**
NARP	**Neurogenic muscle weakness, ataxia, and retinitis pigmentosum**
MELAS	**Mitochondrial encephalomyopathy, lactic acidosis, and strokelike symptoms**
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PEO	**Progressive external opthalmoplegia**
KSS	**Kearns–Sayre syndrome**
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Data from S. DiMauro et al., "Mitochondria in Neuromuscular Disorders," *Biochim. Biophys. Acta* 1366, 1998, 199–210

Figure 3-22

Introduction to Genetic Analysis, Twelfth Edition

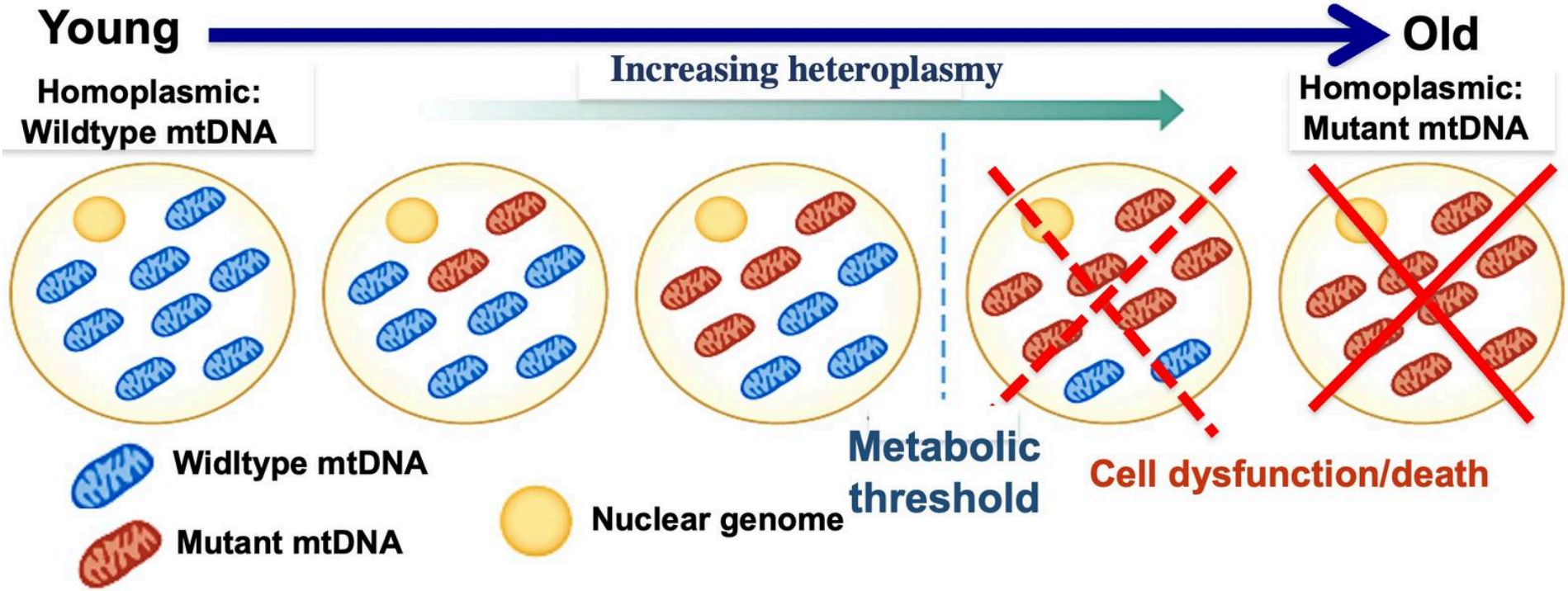
Mitochondria, heteroplasmy and Muller's ratchet

- mtDNA has a high mutation rate and limited repair capacity

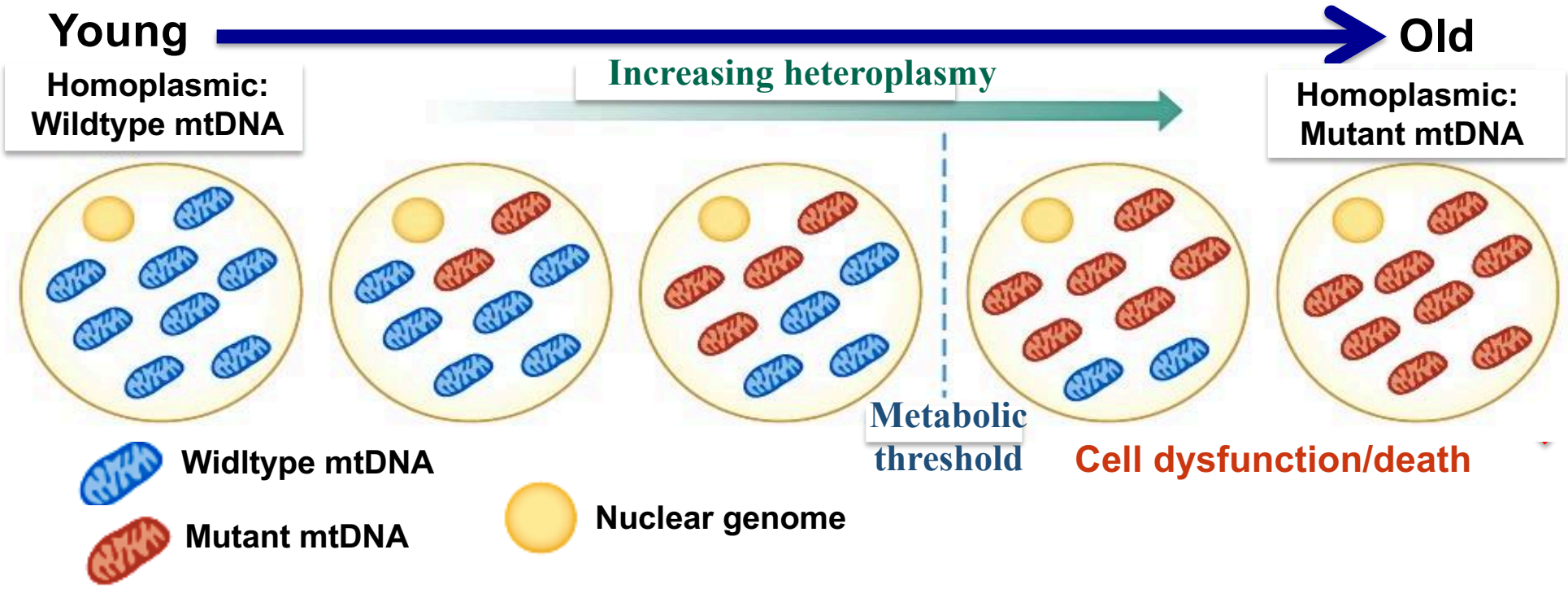
- 90% of people are born with some level of heteroplasmy, 20% carry lethal lethal alleles at >1%

- Deleterious mtDNA accumulates throughout life

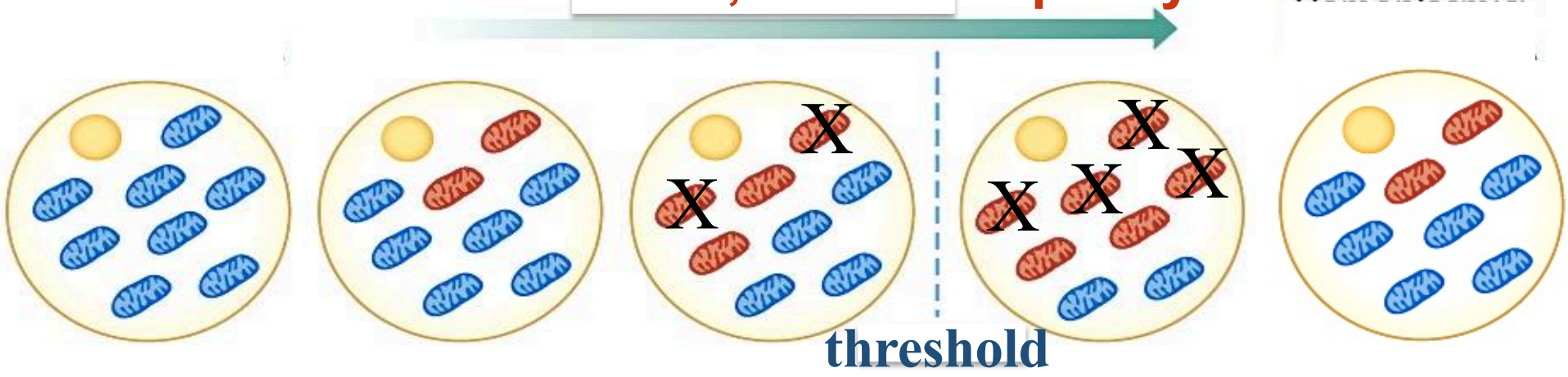
- Leads to cell dysfunction and death



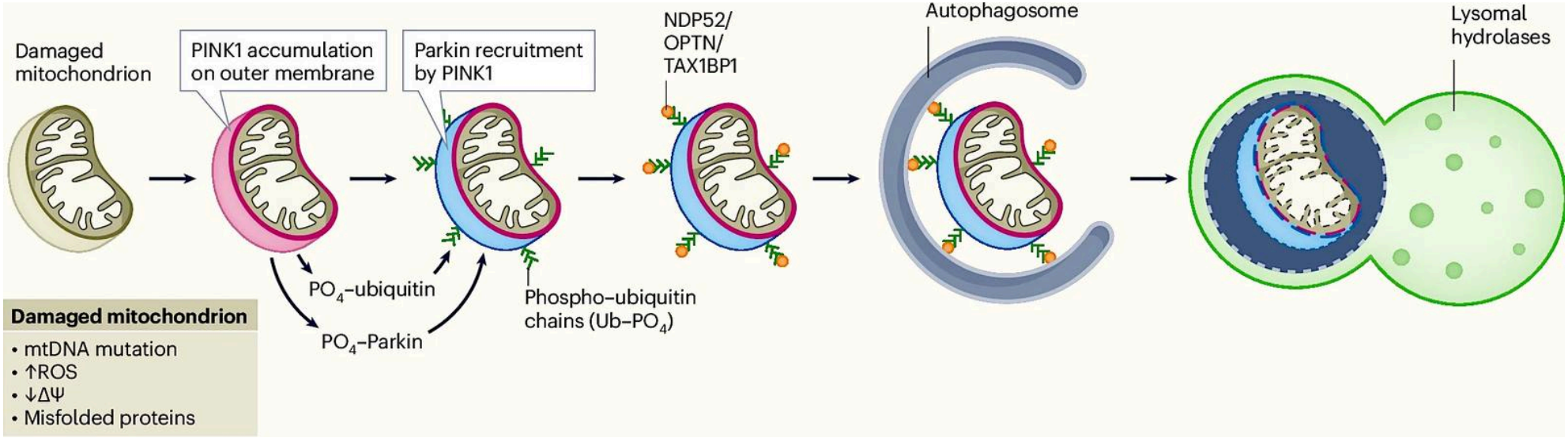
Can the component of aging due to accumulation of mutant mtDNA be slowed or reversed?

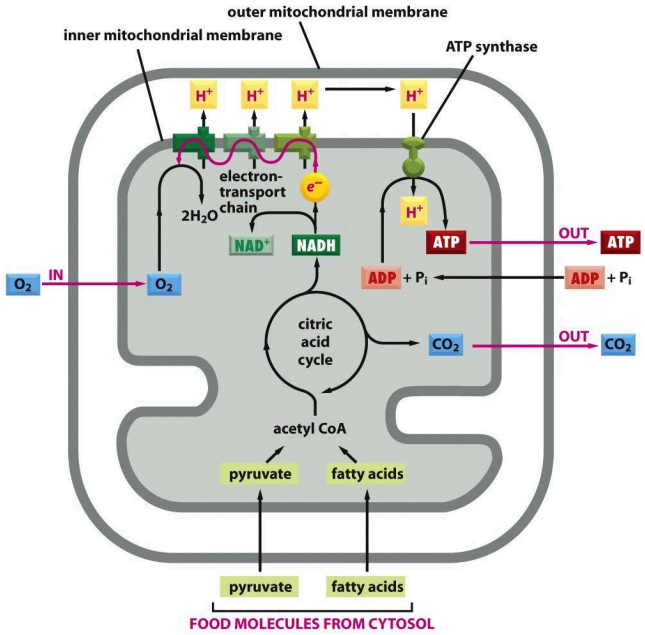


Can we promote the selective elimination of mutant mtDNA, a form of quality control????



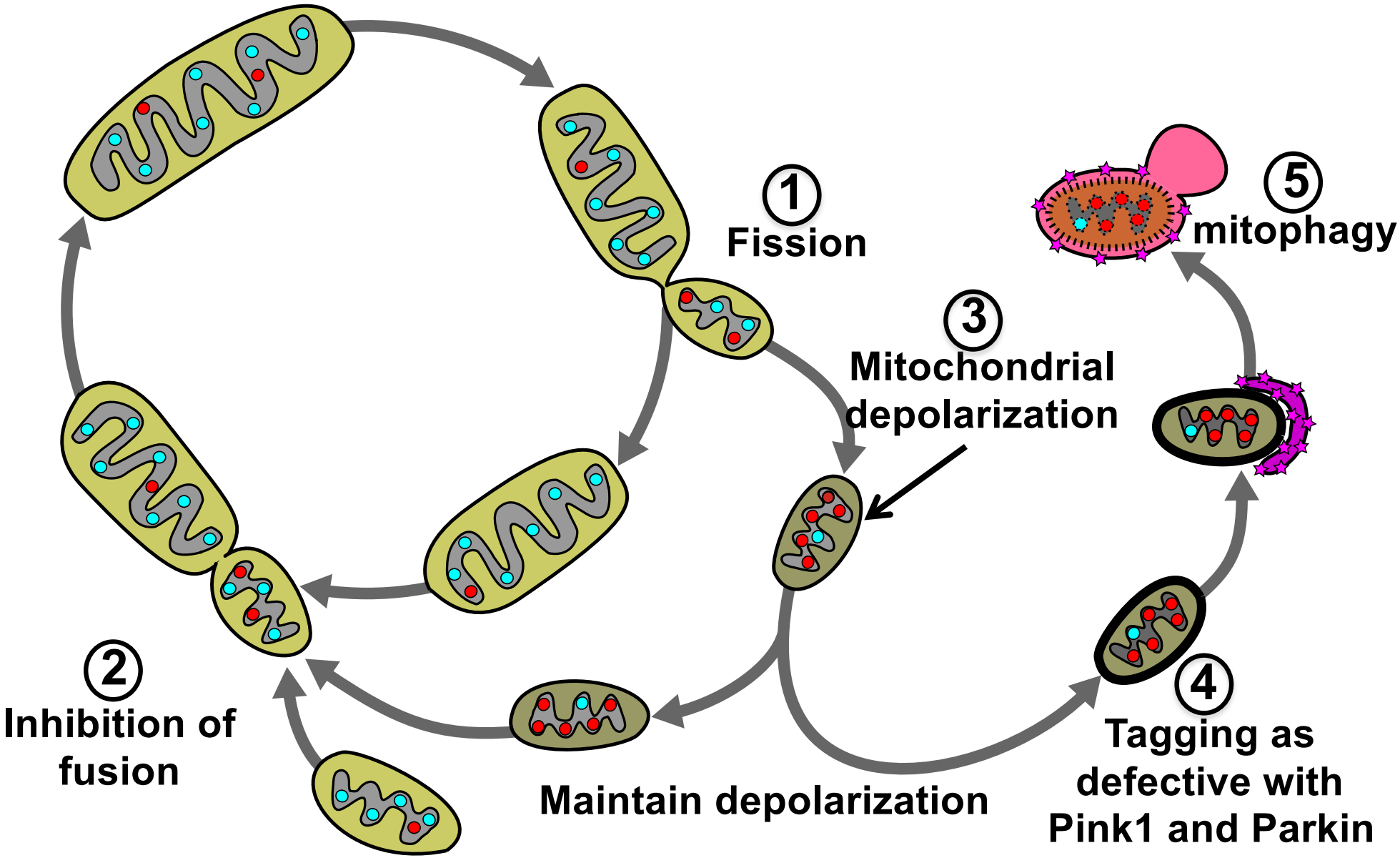
Some pathways, like the Pink1 Parkin pathway, promote the removal of mitochondria damaged mitochondria – those that have lost their membrane potential





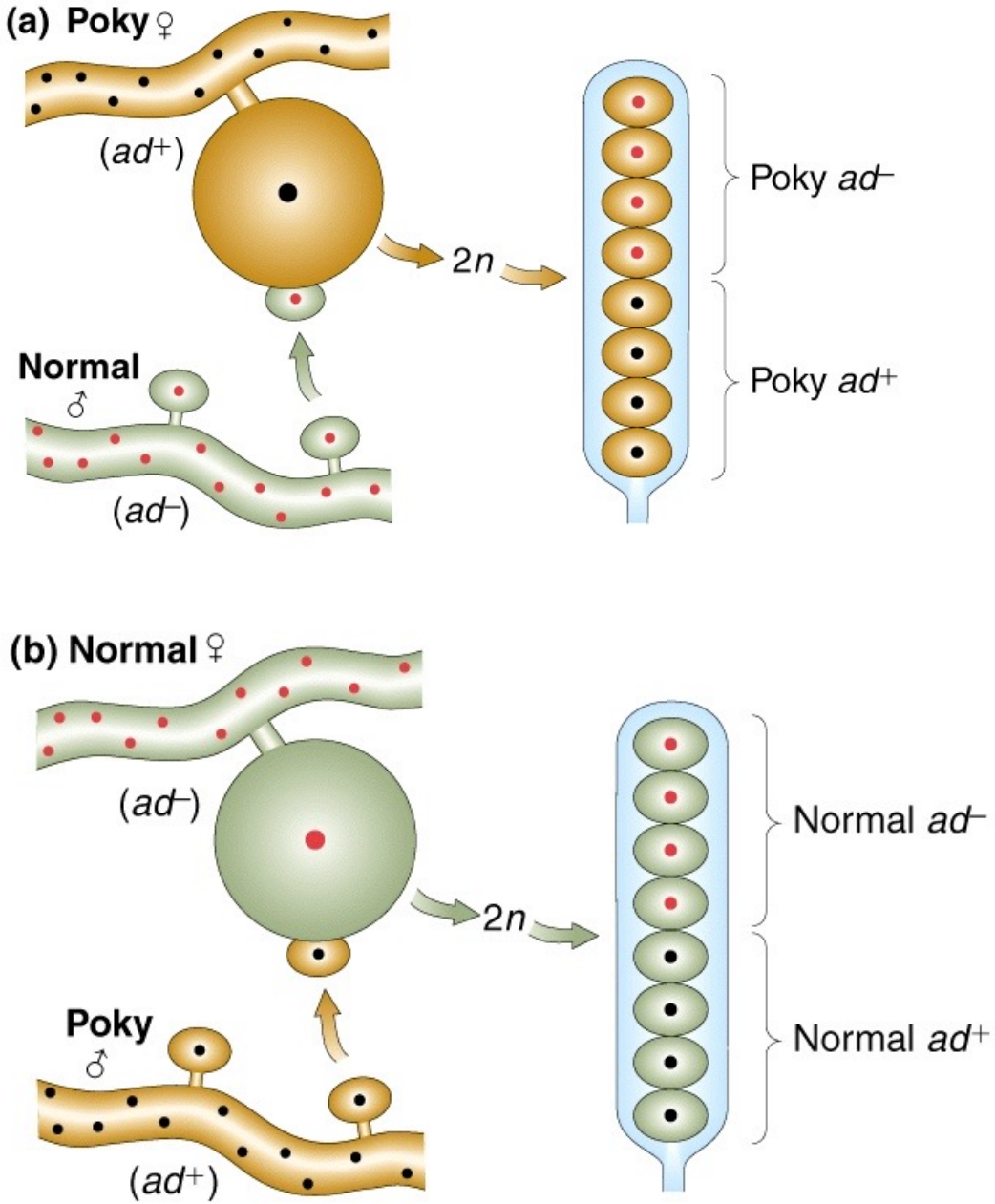
But yet.....you and I are WT with respect to Pink1 and Parkin, and mutant mtDNA still accumulates. So, there must be other factors at play

Key steps in mtDNA quality control: Isolation, incompetence, tagging and removal

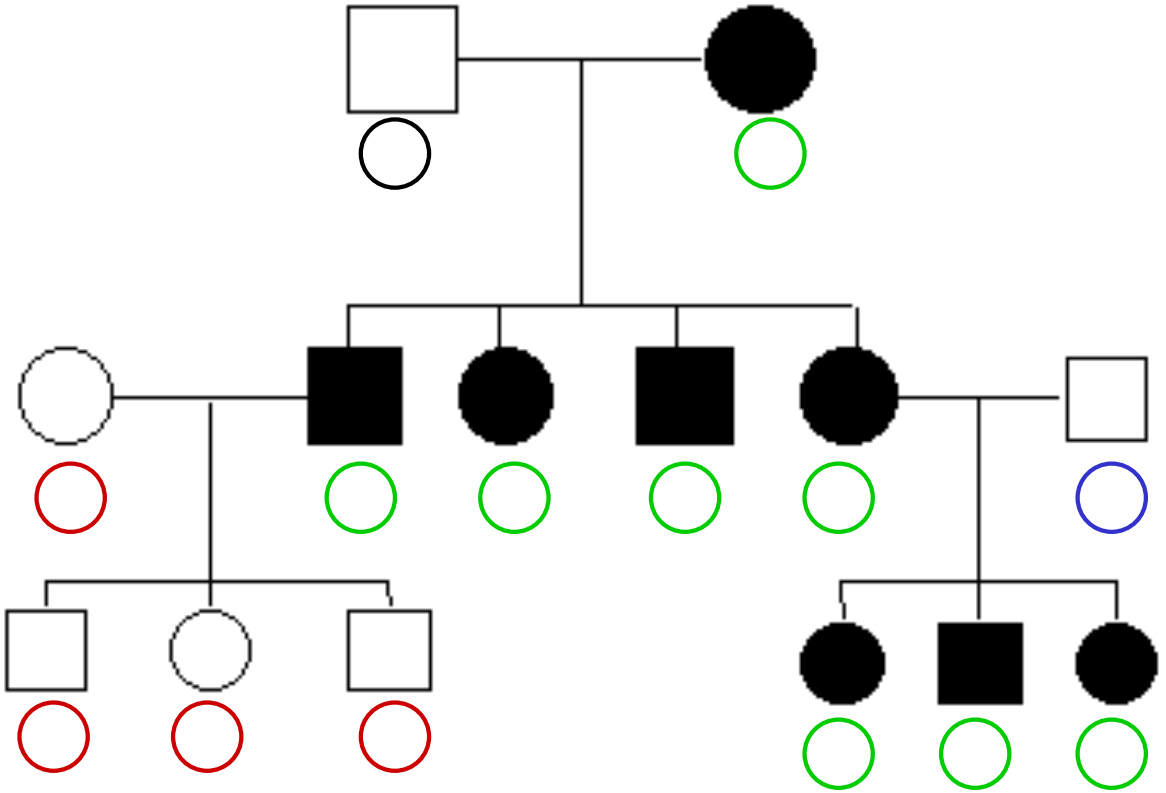


In many organisms mitochondrial and chloroplast contribution to the zygote is uniparental, usually maternal.

1. Poky in Neurospora



In animals, mtDNA is inherited maternally.



mtDNA A mtDNA C

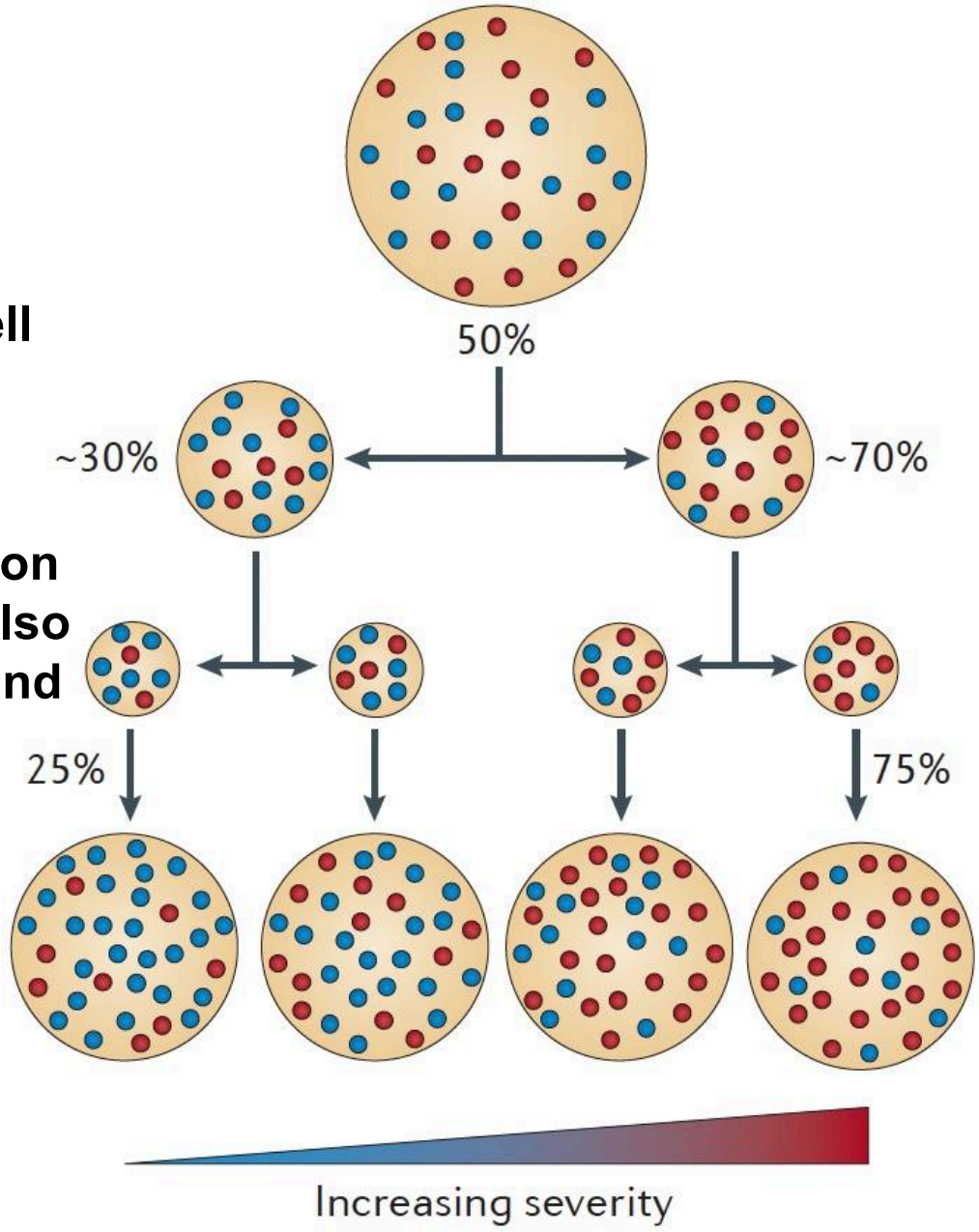
mtDNA B mtDNA D

Homoplasmy: All mitochondrial DNA molecules are identical.

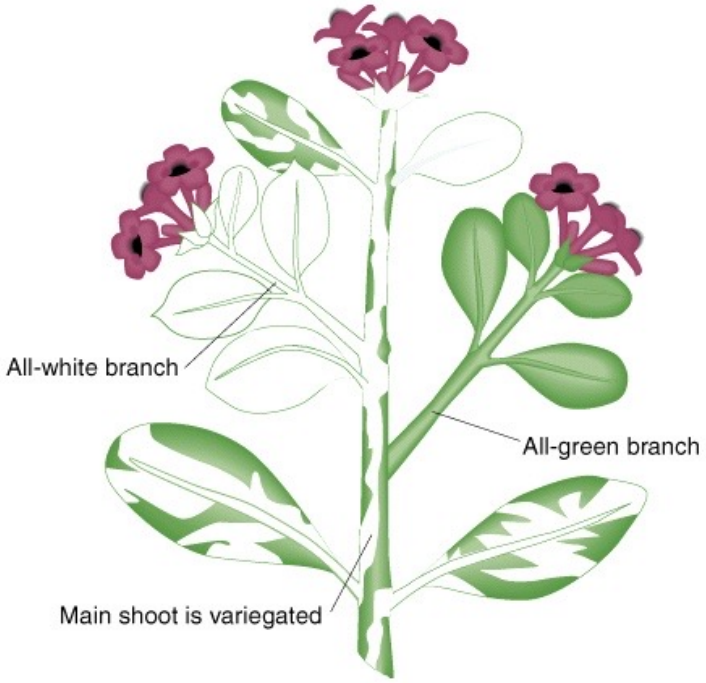
Heteroplasmy: Different mitochondrial genomes are present in the same cell

Different levels of heteroplasmy arise through random segregation during cell division.

In non-dividing cells mitochondria continue to undergo cycles of replication and degradation, a process which is also stochastic in terms of which are lost, and which are replicated

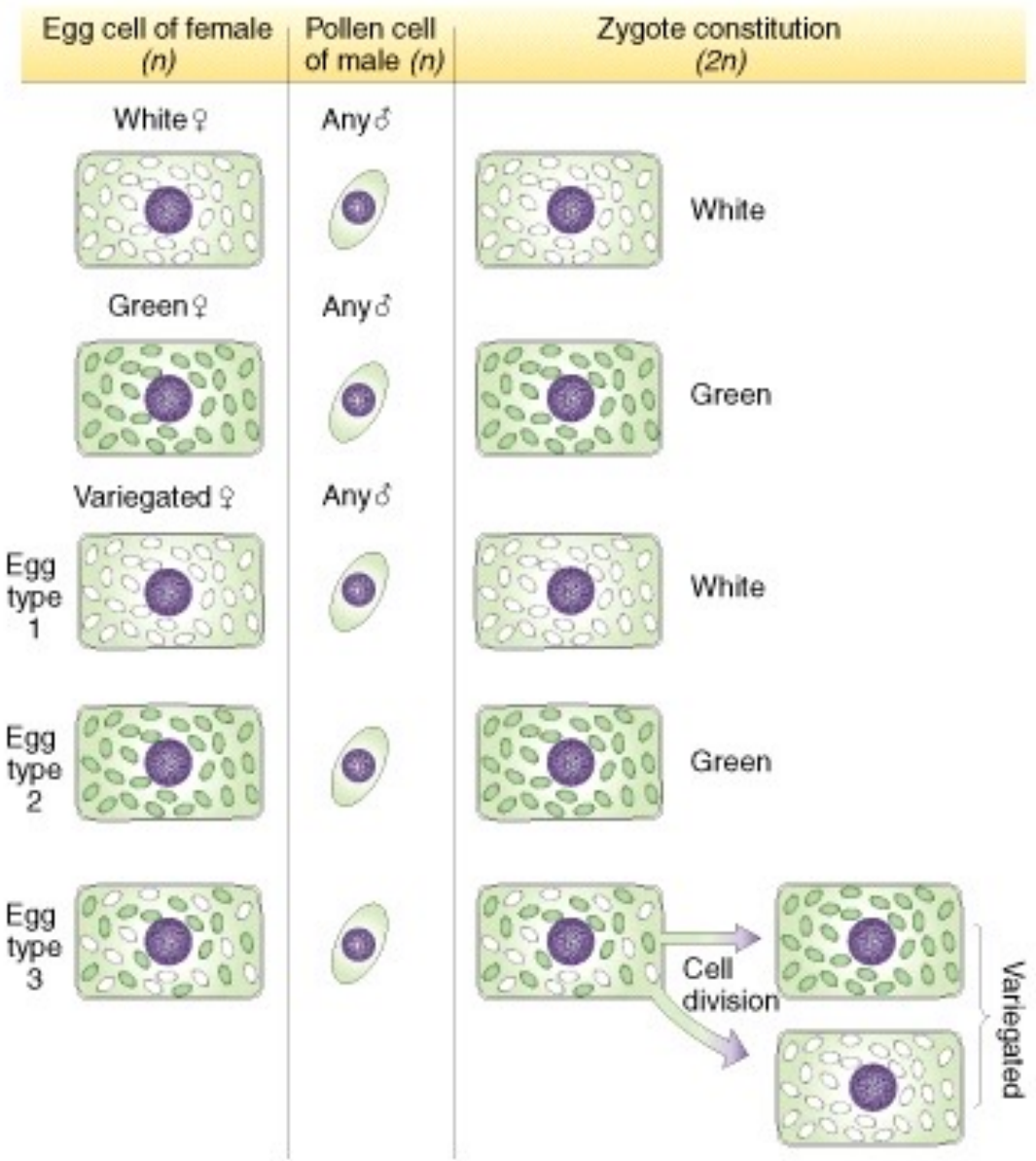


Chloroplast inheritance also involves random segregation

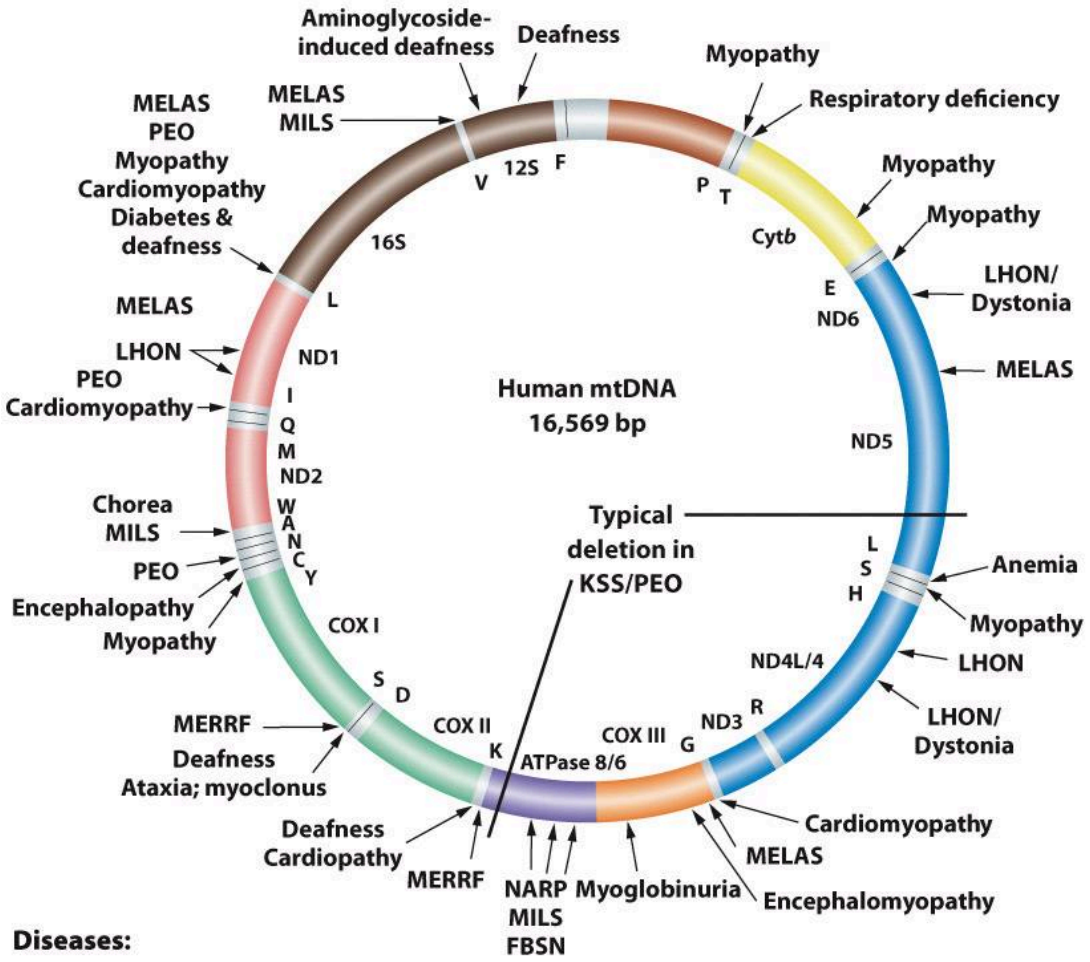


21-1 TABLE Results of Crossing Flowers on Variegated Four-o'clock Plants

Phenotype of branch bearing egg parent (♀)	Phenotype of branch bearing pollen parent (♂)	Phenotype of progeny
White	White	White
White	Green	White
White	Variegated	White
Green	White	Green
Green	Green	Green
Green	Variegated	Green
Variegated	Green	Variegated, green, or white
Variegated	Green	Variegated, green, or white
Variegated	Variegated	Variegated, green, or white



mtDNA mutations that eliminate function not tolerated unless wildtype DNA is also present (heteroplasmy)



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Data from S. DiMauro et al., "Mitochondria in Neuromuscular Disorders," *Biochim. Biophys. Acta* 1366, 1998, 199–210

Figure 3-22

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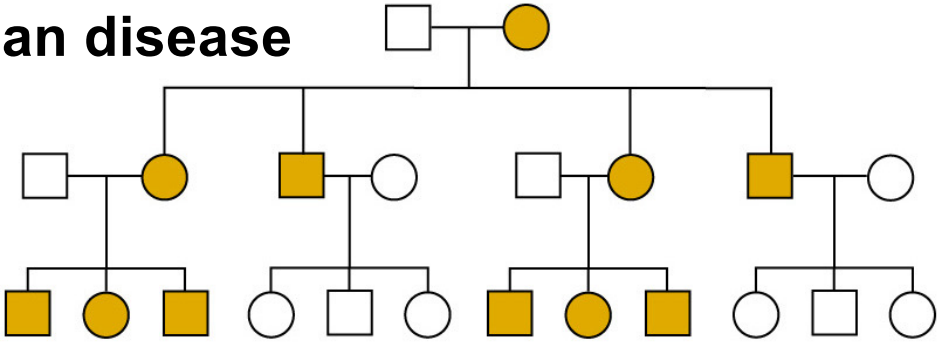
Mitochondria and human disease

1. Maternal inheritance
2. Variable penetrance and expressivity
3. Variable age of onset



All offspring of diseased mother can express the disease phenotype, while none of the offspring of the diseased fathers do.

Individual mtDNA genotypes		Tissues Affected				
				Skeletal Muscle		
		Brain	Heart	Type I	Type II	Skin
I	20% mutant mtDNAs	+	–	–	–	–
II	40% mutant mtDNAs	+	+/–	–	–	–
III	60% mutant mtDNAs	+	+	+	–	–
IV	80% mutant mtDNAs	+	+	+	+/–	+/–

Problems with determining maternal inheritance in mtDNA diseases.

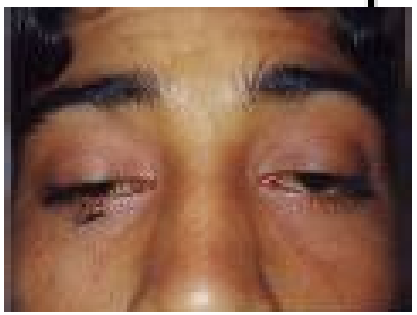
- Individuals within the pedigree will differ in phenotype (variable penetrance and expressivity) because of variability in mutant:wild type mtDNA ratio (heteroplasmy).
- Different organs within an individual can have different degrees of pathology because of heteroplasmy.

The proportion of mutant mitochondria determines the severity of the MERF phenotype and the tissues that are affected. Tissues with higher energy requirements (e.g., brain) are least Tolerant of mutant mitochondria. Tissues with low energy Requirements (e.g., skin) are only affected when the proportion Wildtype mitochondria is greatly reduced.

CPEO

Chronic Progressive External Ophthalmoplegia

Slow paralysis of the extraocular muscles



$tRNA^{Leu}$ mutation

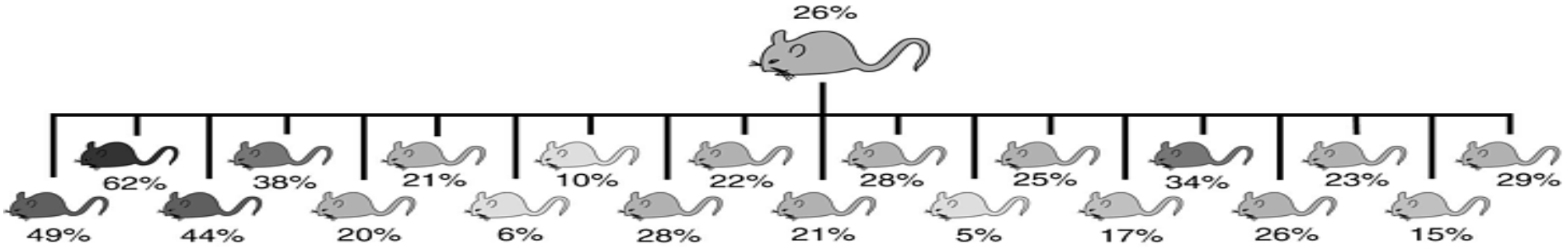
Both cardiomyopathy, diabetes and MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke) patients have the same tRNA lesion!!

Cardiopathy and MELAS can be associated with other mt mutations.

Transmission of mitochondrial DNA polymorphisms from heteroplasmic mother to offspring

(Chinnery et al Trends Genet 16:500)

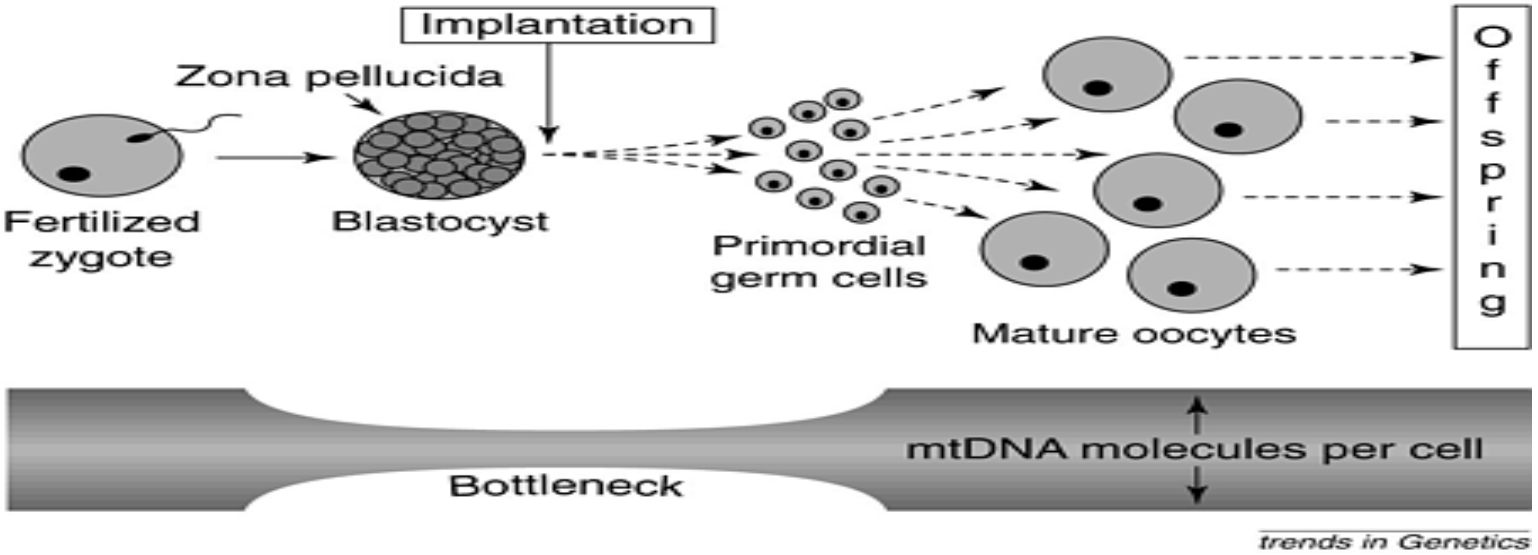
FIGURE 1. The transmission of heteroplasmic mtDNA polymorphisms in mice



trends in Genetics

Random genetic drift in a mouse pedigree transmitting heteroplasmic mitochondrial DNA (mtDNA) polymorphisms. Heteroplasmic mice were generated by karyoplast transfer from a NZB donor to a C57BL6 recipient (see text). The pedigree shown here includes all the offspring of an F1 female mated with a C57BL6 male. The percentage level of NZB is indicated below a single mother and her offspring. The varying grey scale illustrates the relative proportion of NZB genotype in each mouse.

FIGURE 2. The mitochondrial genetic bottleneck



trends in Genetics

Preimplantation embryonic development and the formation of the female germ line. Diagram showing a reduction in the number of mitochondrial DNA (mtDNA) molecules per cell — the mitochondrial genetic 'bottleneck'.

Possible mechanisms for implementing maternal bottleneck

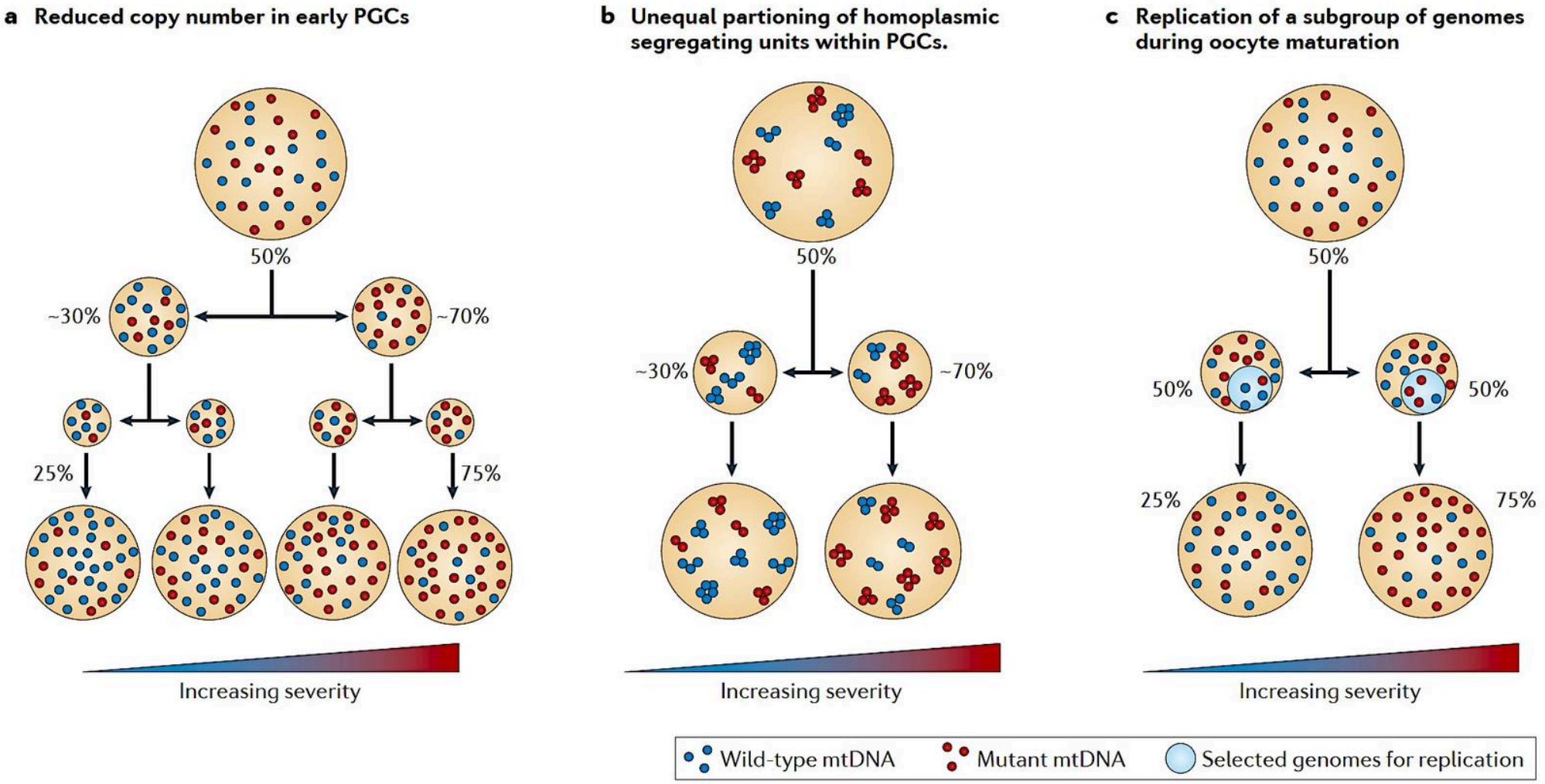


Figure 4 | **Models for the mtDNA genetic bottleneck.** **a** | The rate of vegetative segregation is faster if the mitochondrial DNA (mtDNA) content of the cells is smaller. Unequal partitioning (segregation) of mutated (red) and wild-type (blue) genotypes will lead to shifts in heteroplasmy in oocytes as a consequence of the reduction in mtDNA copy number immediately prior to expansion of the primordial germ cell (PGC) population, as observed in mice[66,78]. **b** | If mtDNA molecules are packaged into homoplasmic nucleoids, or simply kept separate within discrete homoplasmic mitochondria, this reduces the number of segregating units and accelerates the rate of drift, as proposed in REFS 79,123. **c** | The replication of a subpopulation of mitochondrial genomes would also lead to a bottleneck effect. There is some evidence that this might occur during postnatal oocyte maturation[78]. Adapted with permission from REF. 124, Elsevier.

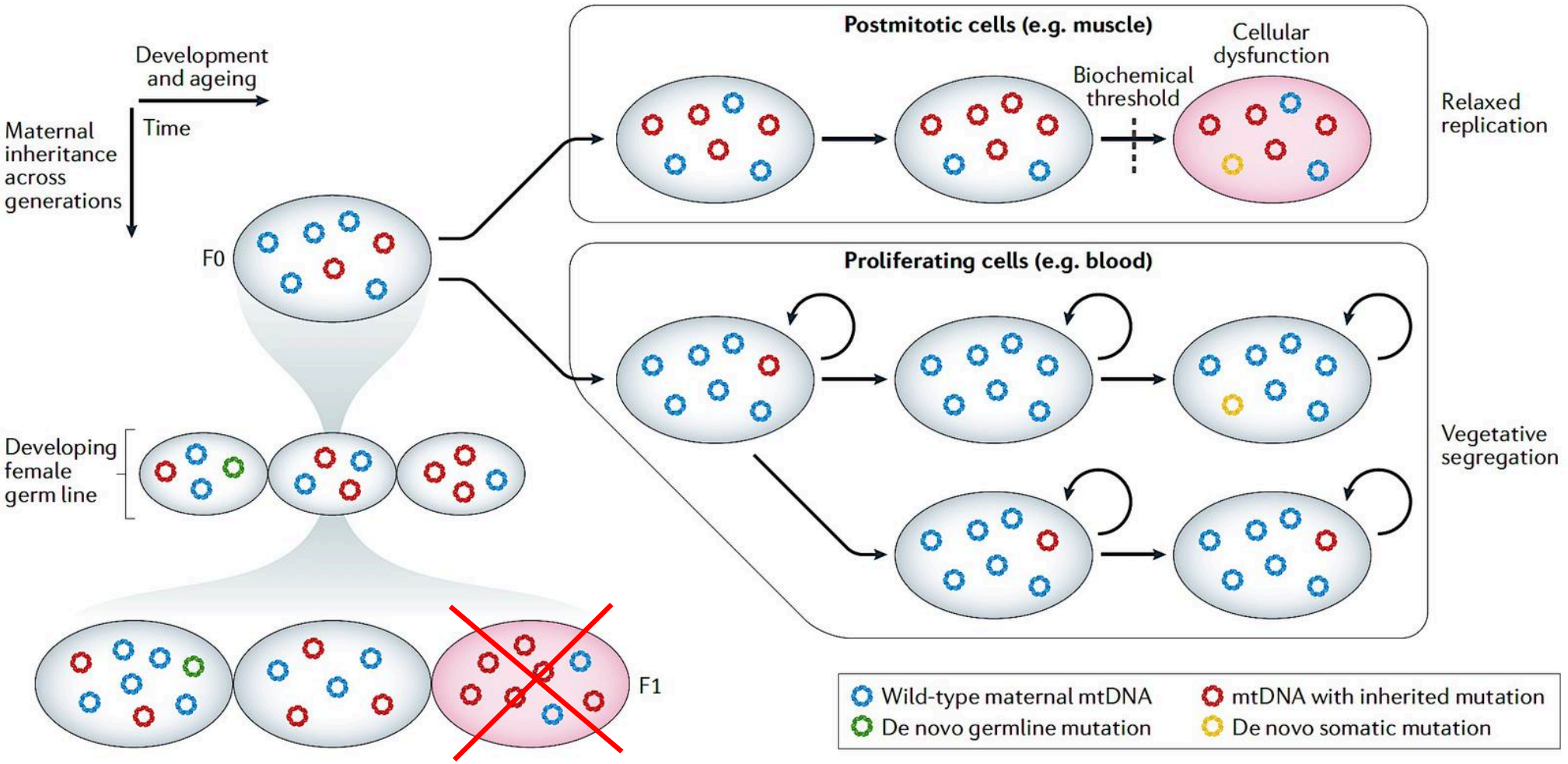
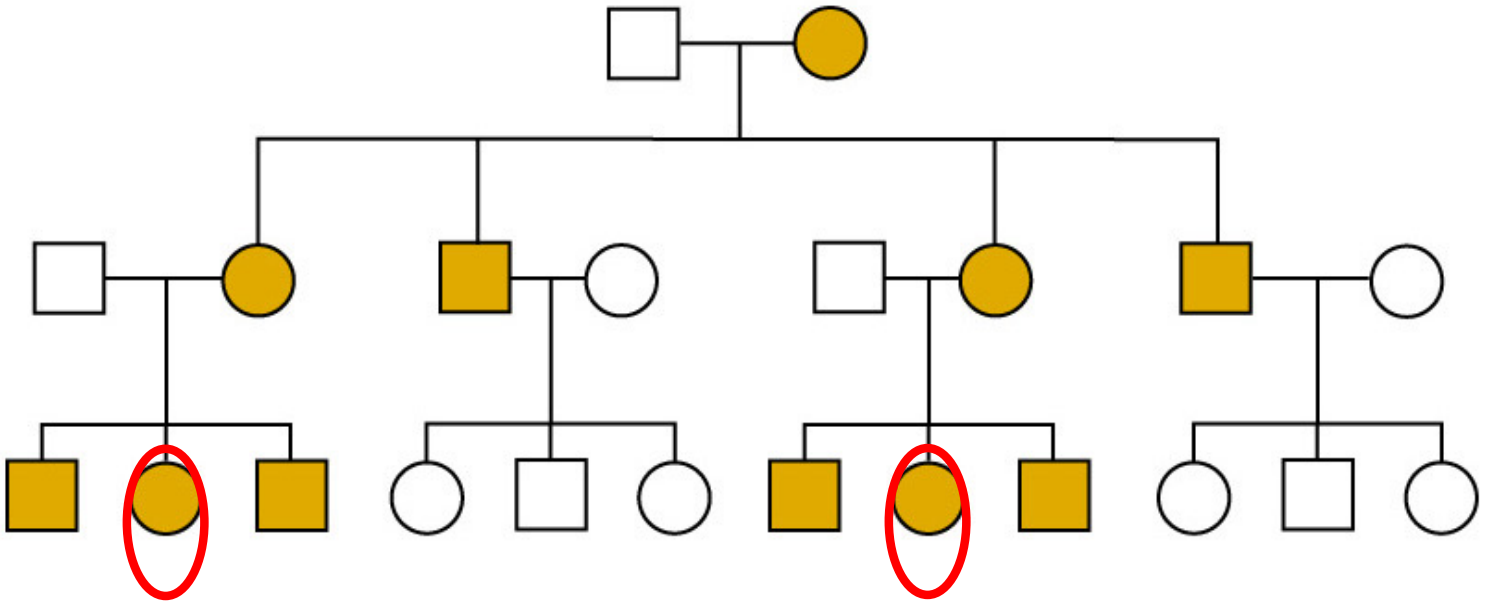


Fig. 3 | **How mitochondrial DNA heteroplasmy levels change.** Mitochondrial DNA (mtDNA) heteroplasmy levels can change during life through vegetative segregation in dividing cells and through relaxed replication in non-dividing and dividing cells. In non-dividing (postmitotic) cells, relaxed replication during life can lead to an increase in the proportion of mutant mtDNA, which if it exceeds a critical threshold leads to a biochemical defect of oxidative phosphorylation. The germline genetic bottleneck accelerates segregation between the generations, leading to major changes in the heteroplasmy level. In both contexts, high levels of a pathogenic mutation can cause a cellular biochemical defect of adenosine triphosphate (ATP) synthesis, but there is emerging evidence that even very low heteroplasmy levels can have physiological consequences. Heteroplasmy can also be introduced through somatic and germline de novo mutations. Adapted with permission from REF.[143], Elsevier.

But quality control is not perfect and roughly 1/4000 people have disease associated with mutation of mtDNA



If you have the disease, there are no treatments. But, ...
can we prevent future transmission from mothers to offspring?

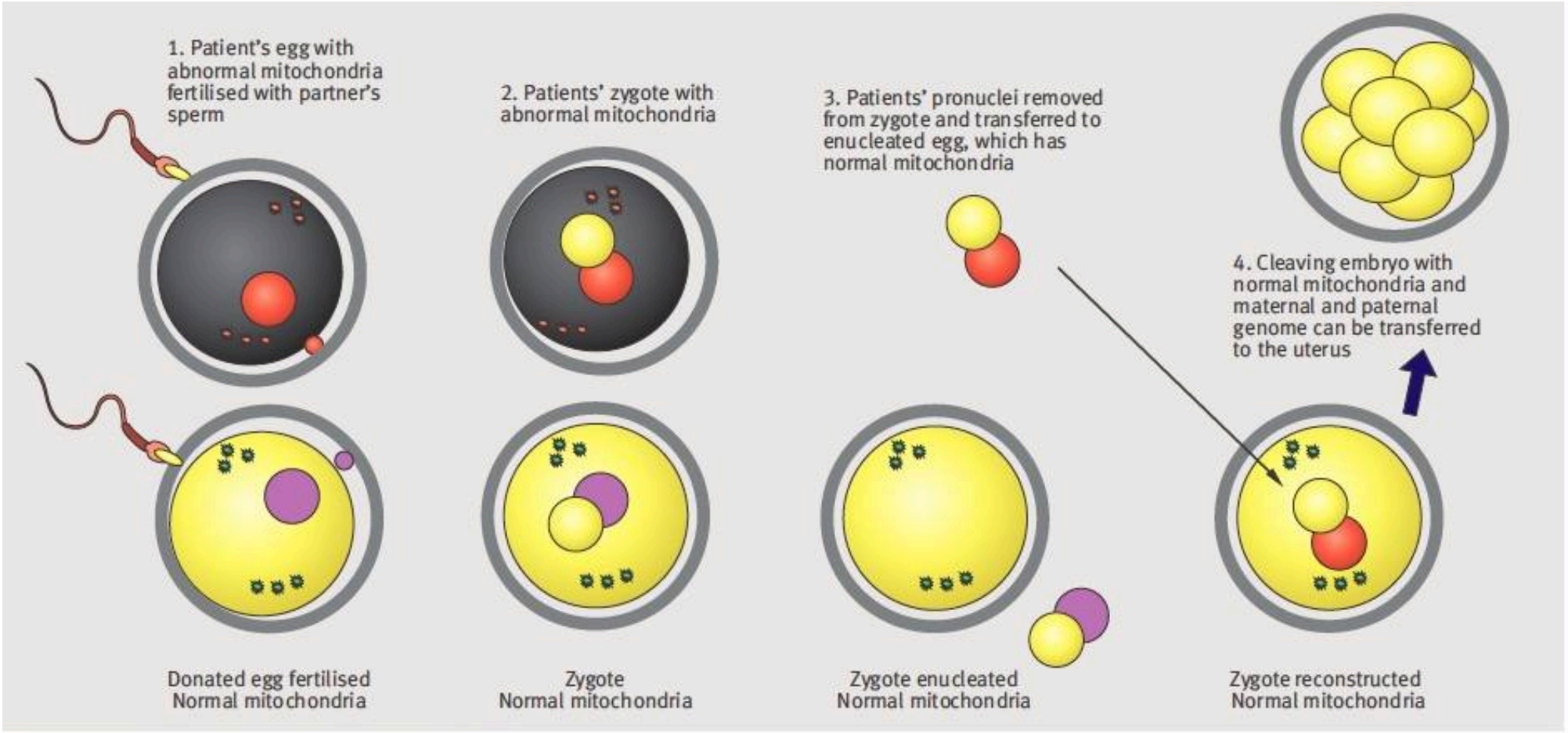
UK Government backs mitochondrial replacement

01 July 2013

By Dr Rosie Morley

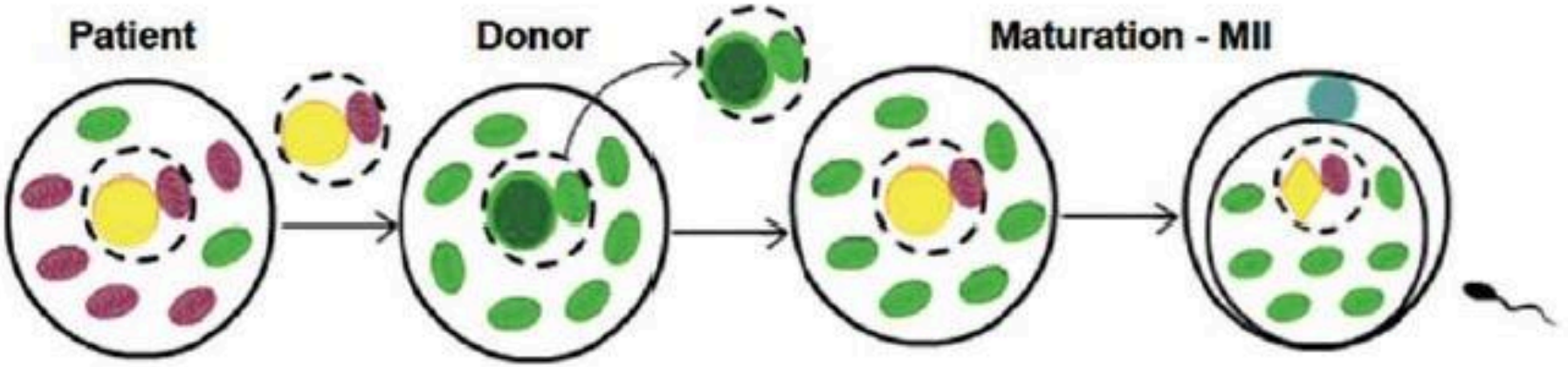
Appeared in BioNews 711

The UK Government is to support the introduction of mitochondrial replacement therapy. The IVF-based procedure could allow women with mitochondrial disease the opportunity to have healthy children, by replacing their own, faulty mitochondria with healthy mitochondria from a donor.

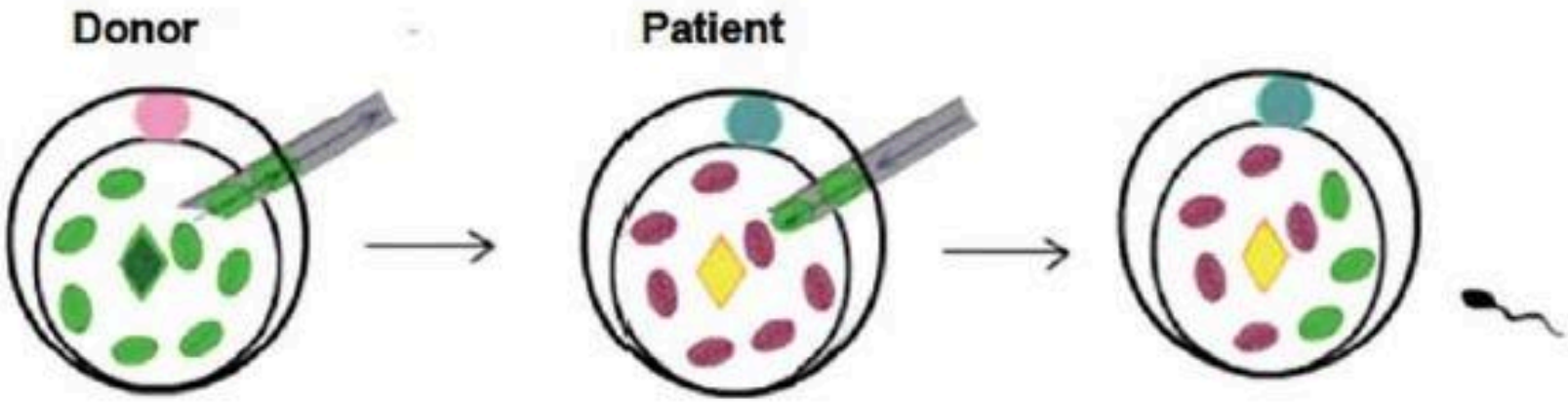


Mitochondrial gene replacement (pronuclear stage)

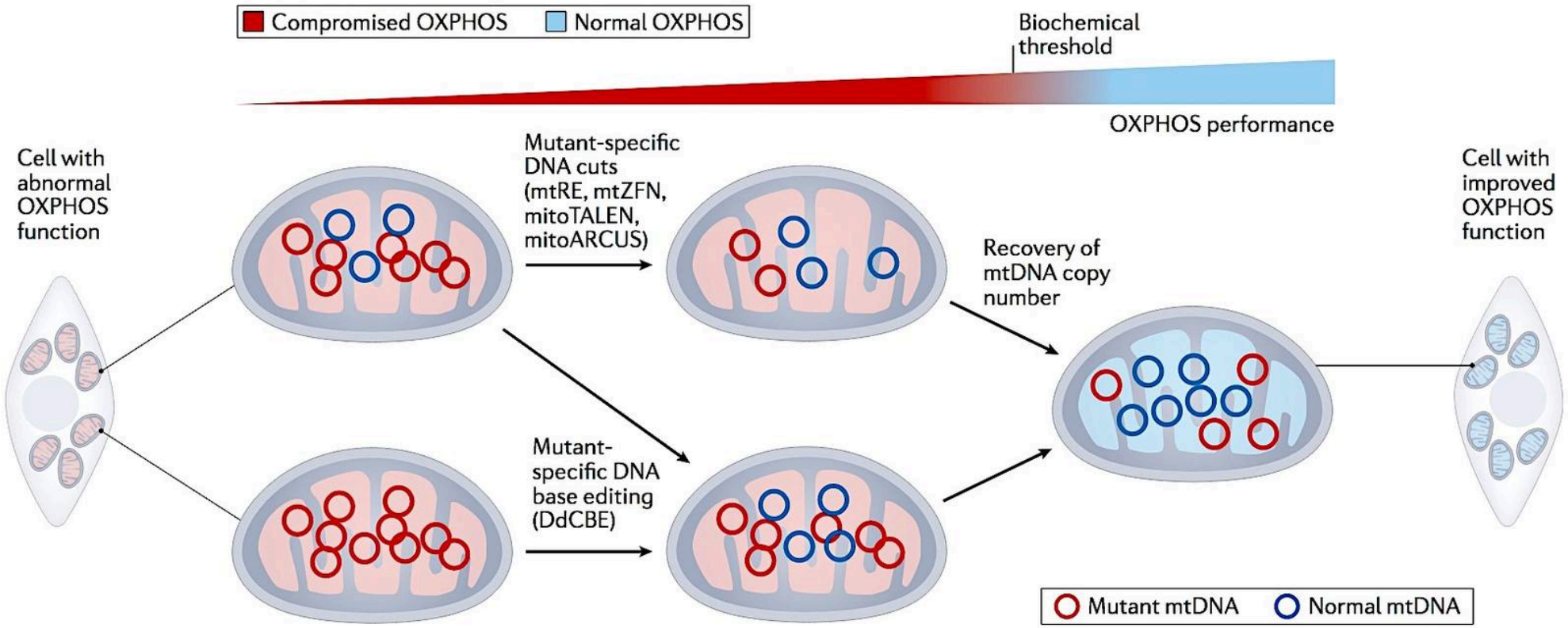
A- GERMINAL VESICLE TRANSFER



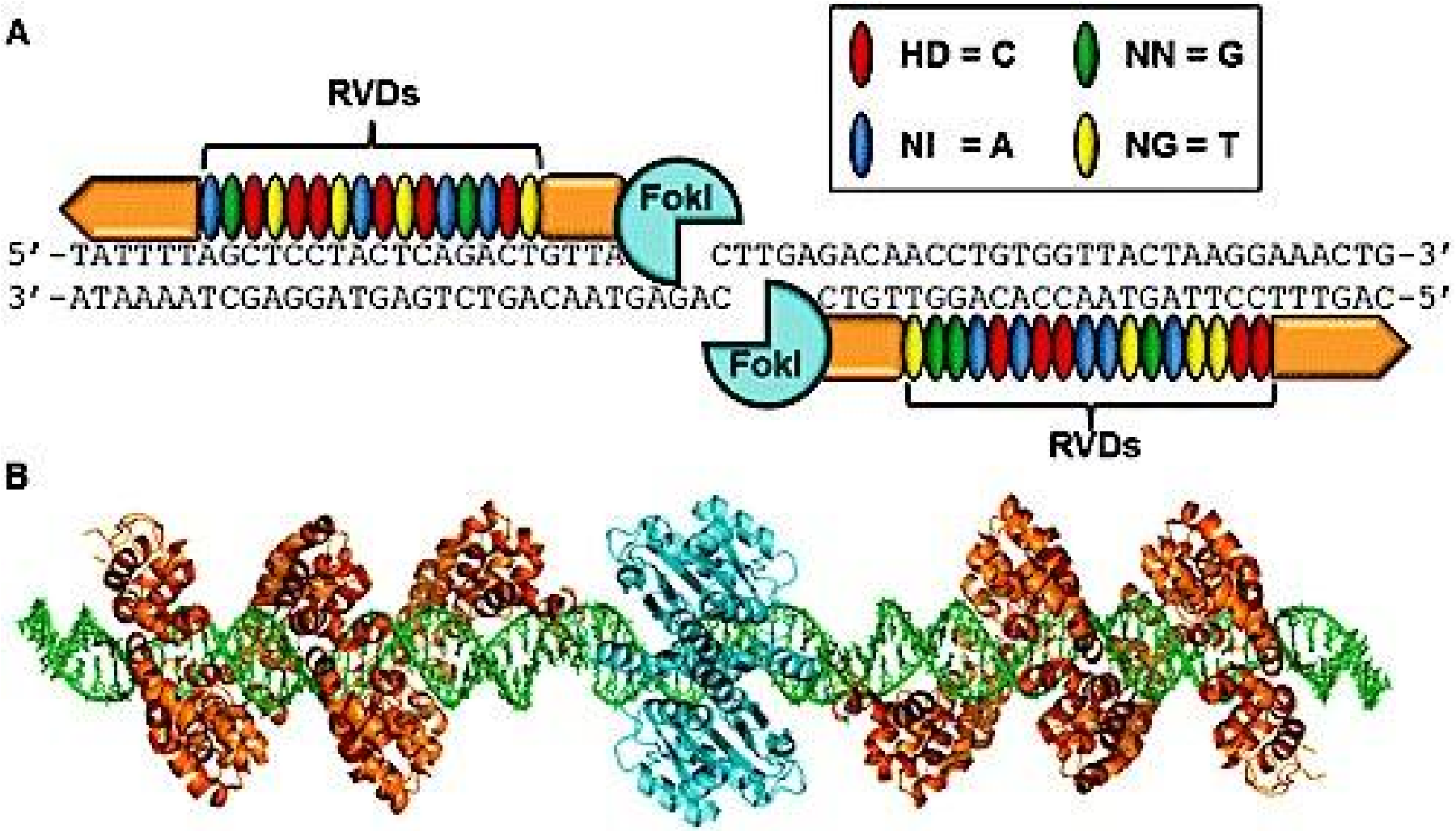
B- OOPLASM TRANSFER



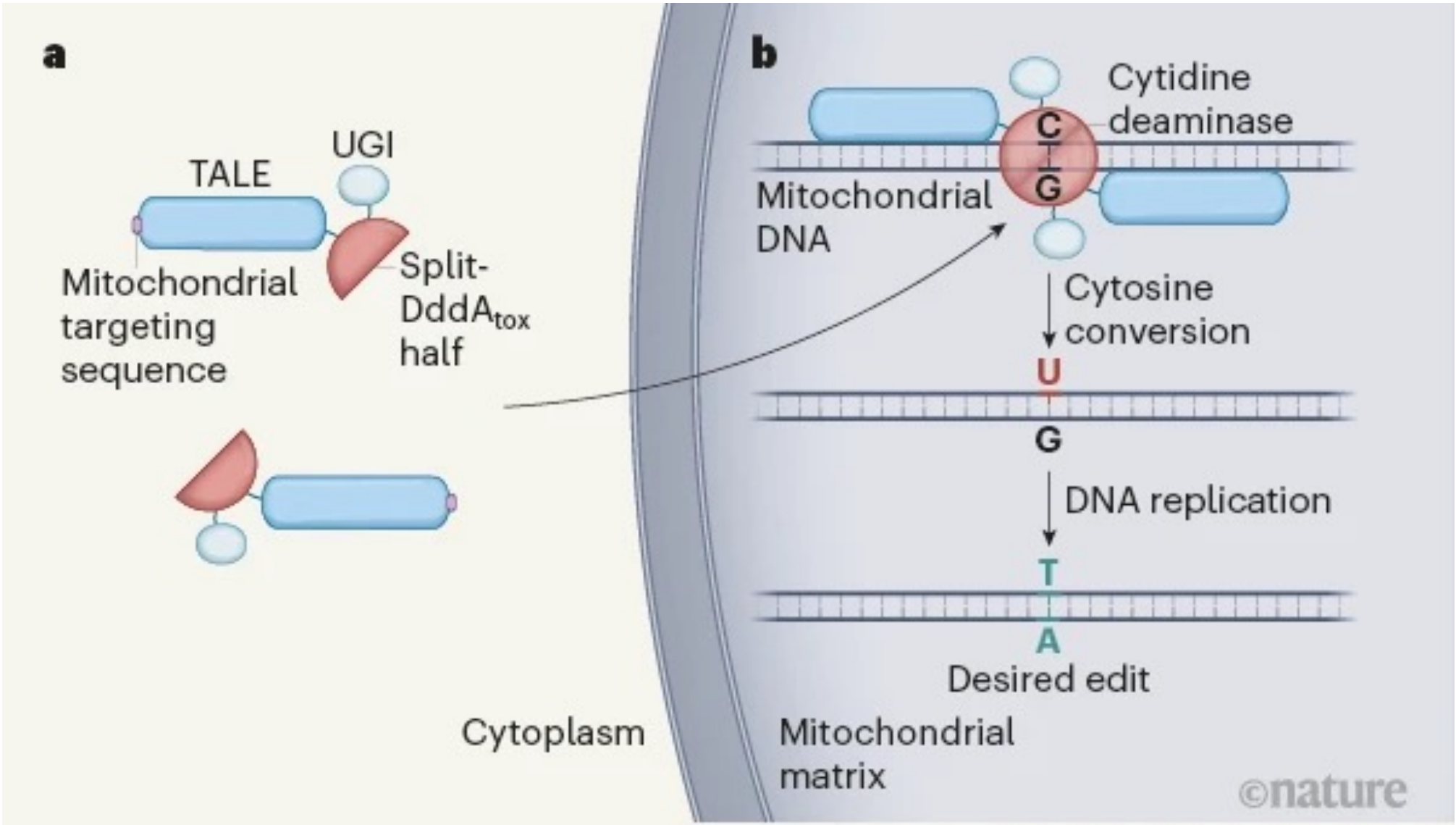
Deleting or modifying specific mutant mtDNA genotypes using site-specific cleavage or base editing.



Site-specific DNA cleavage occurs when two FokI nuclease domains are brought near each other through TALE-mediated DNA binding



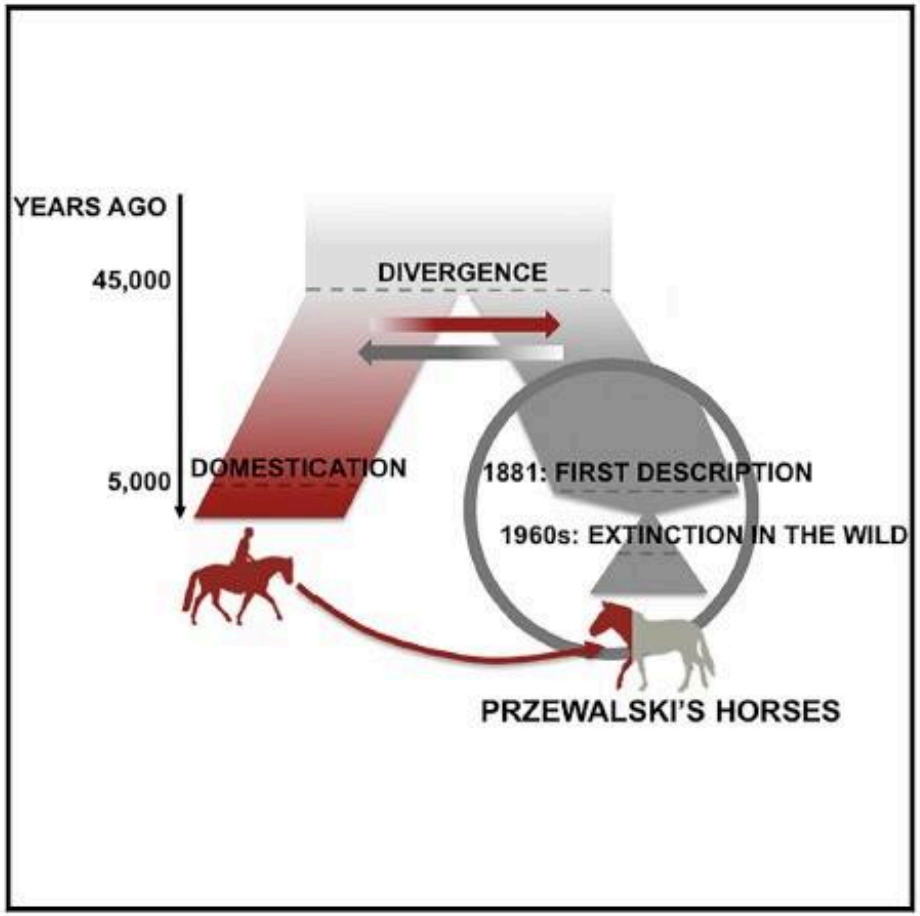
Site-specific DNA base editing occurs when deaminase domains are brough near each other through site-specific TALE binding



Current Biology

Evolutionary Genomics and Conservation of the Endangered Przewalski's Horse

Graphical Abstract



Authors

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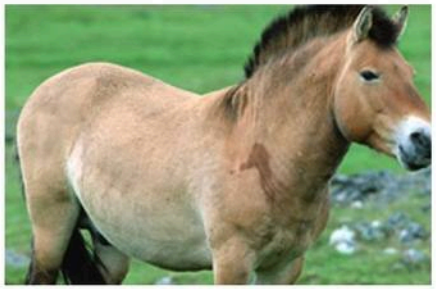
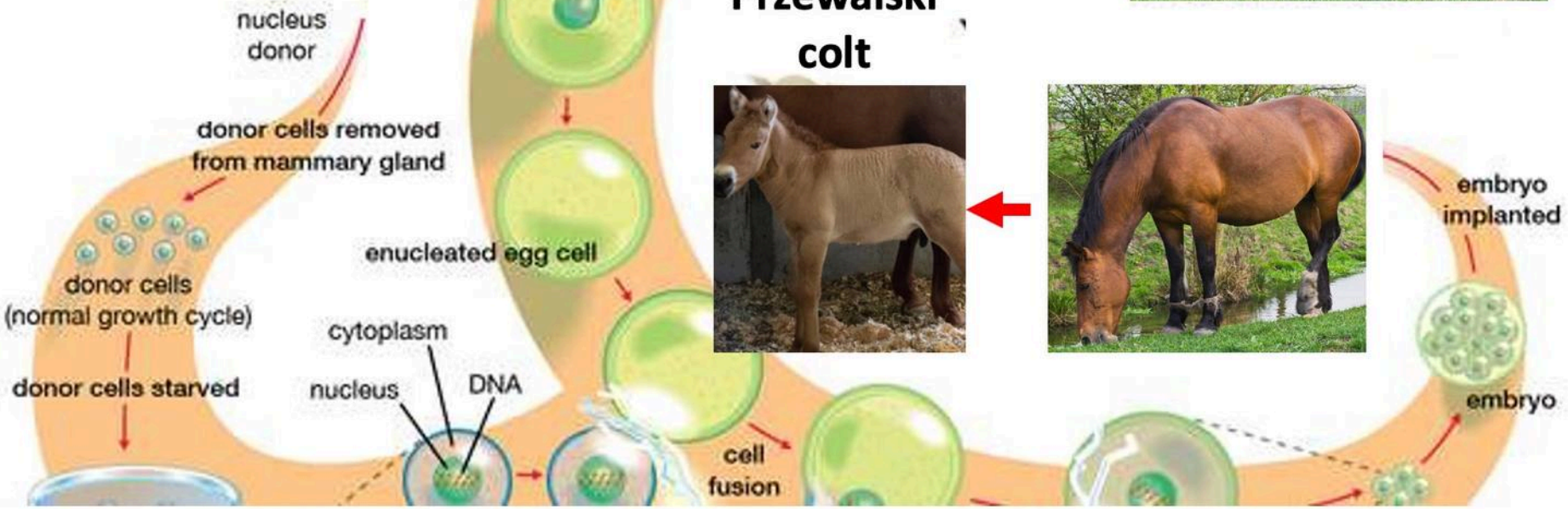
Trans-species cloning. What could go wrong?

40 year old cryopreserved somatic cells from Prewalski horse



Modern domestic horse

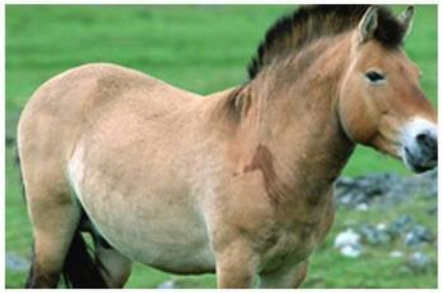


Przewalski colt

Trans-species cloning. What could go wrong?

The nuclear genome of one species and the mitochondrial genome of a second

40 year old cryopreserved somatic cells from Prewalski horse



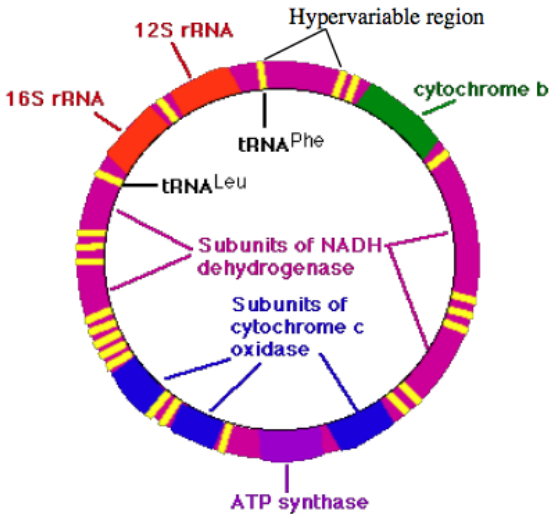
Dear Genetic Rescue Colleagues —

With great enthusiasm,
we are announcing today the birth of a Przewalski's foal, cloned from a 40-year-old cryopreserved specimen. Formerly extinct in the wild, the Przewalski's horse (pronounced "shuh-VAL-skee") has survived for the past 40 years almost entirely in zoos around the world, and all of the surviving horses are related to 12 Przewalski's horses born in the wild. The colt's birth revives genetic diversity that had been lost to the world and has now been recovered due to this important partnership between Revive & Restore, ViaGen Equine, and San Diego Zoo Global.

What problems might this horse have later in life?

Could HE pass those defects on to his progeny?

Hypervariable region can often be used to distinguish individuals



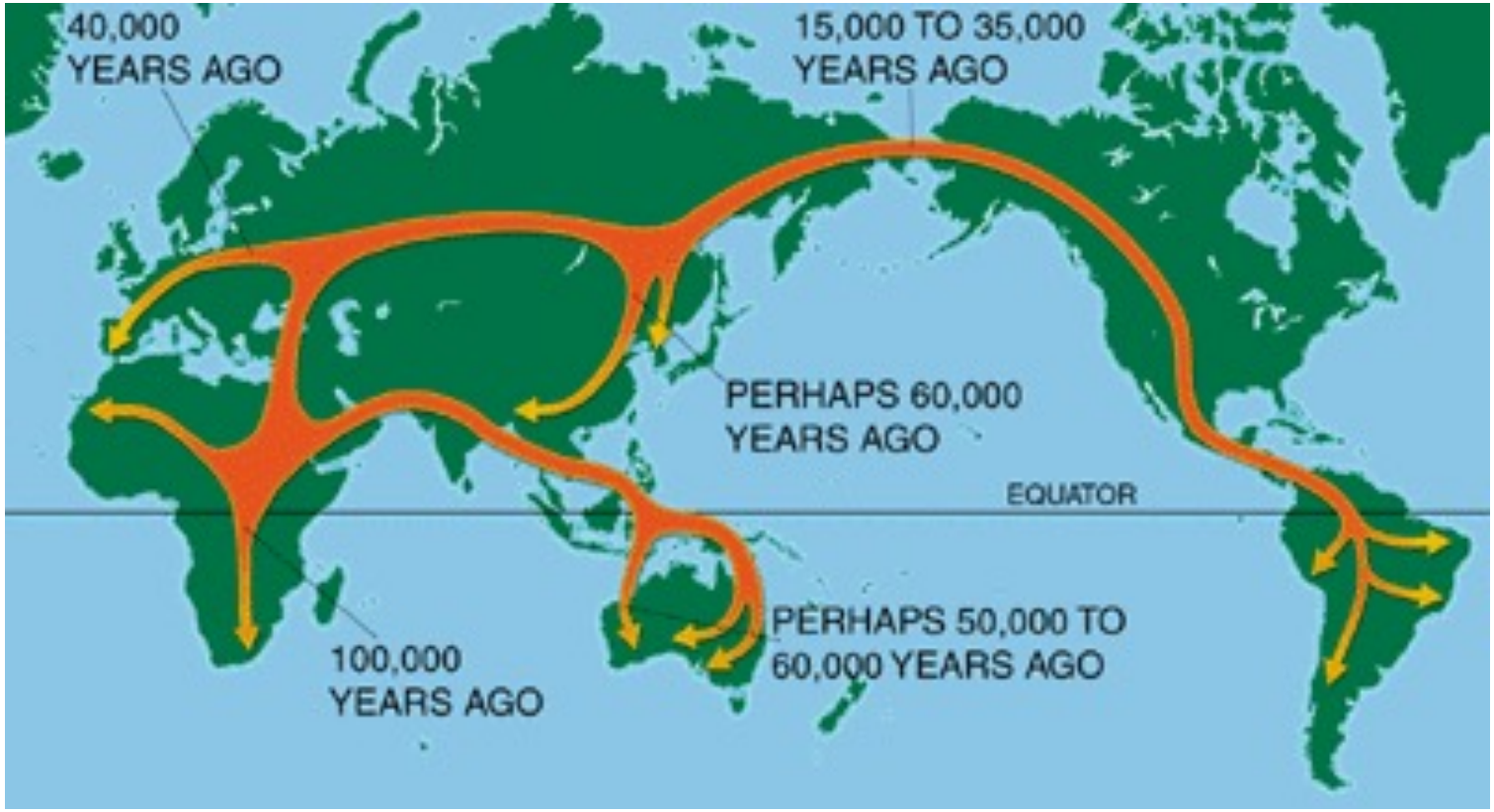
Between 1976 and 1983, the military of Argentina kidnapped, jailed, and killed more than 10,000 dissidents. Along with their parents, many infants and toddlers disappeared. In 1977, the grandmothers of these children began to hold vigils in the main square of Buenos Aires to inform others that about the disappearance of their grandchildren. This group became known as the "Grandmothers of the Plaza de Mayo." They contacted many organizations for help, including the American Association for the Advancement of Science (AAAS).

First, different alleles of the HLA locus (nuclear) were used. Compared the human lymphocyte antigens (HLAs) on white blood cells. Children should share one allele with one of the paternal grandparents and the other with one of the maternal grandparents. Mary Claire King, then a geneticist at Cal, taught Argentine medical workers to analyze HLA markers on white blood cells, and the workers typed HLAs from family members of the missing children.
The probability that a tested child belonged to the family claiming him or her on the basis of eye-witness accounts of abduction varied from 75-99%.

Then, mtDNA analysis was used.

By the mid-1980s, the simpler cases had been solved. Professor King turned to her former mentor and colleague Allan Wilson, a molecular biologist at Cal who was studying mtDNA. PCR amplification and sequencing of the highly variable region of mtDNA made it possible to match a child with his or her maternal grandmother. The probability that two unrelated individuals would be identical for this 131 bp region is almost nil. In addition, the high copy number of the mtDNA aided in the identification of the remains of individuals killed by the military regime.

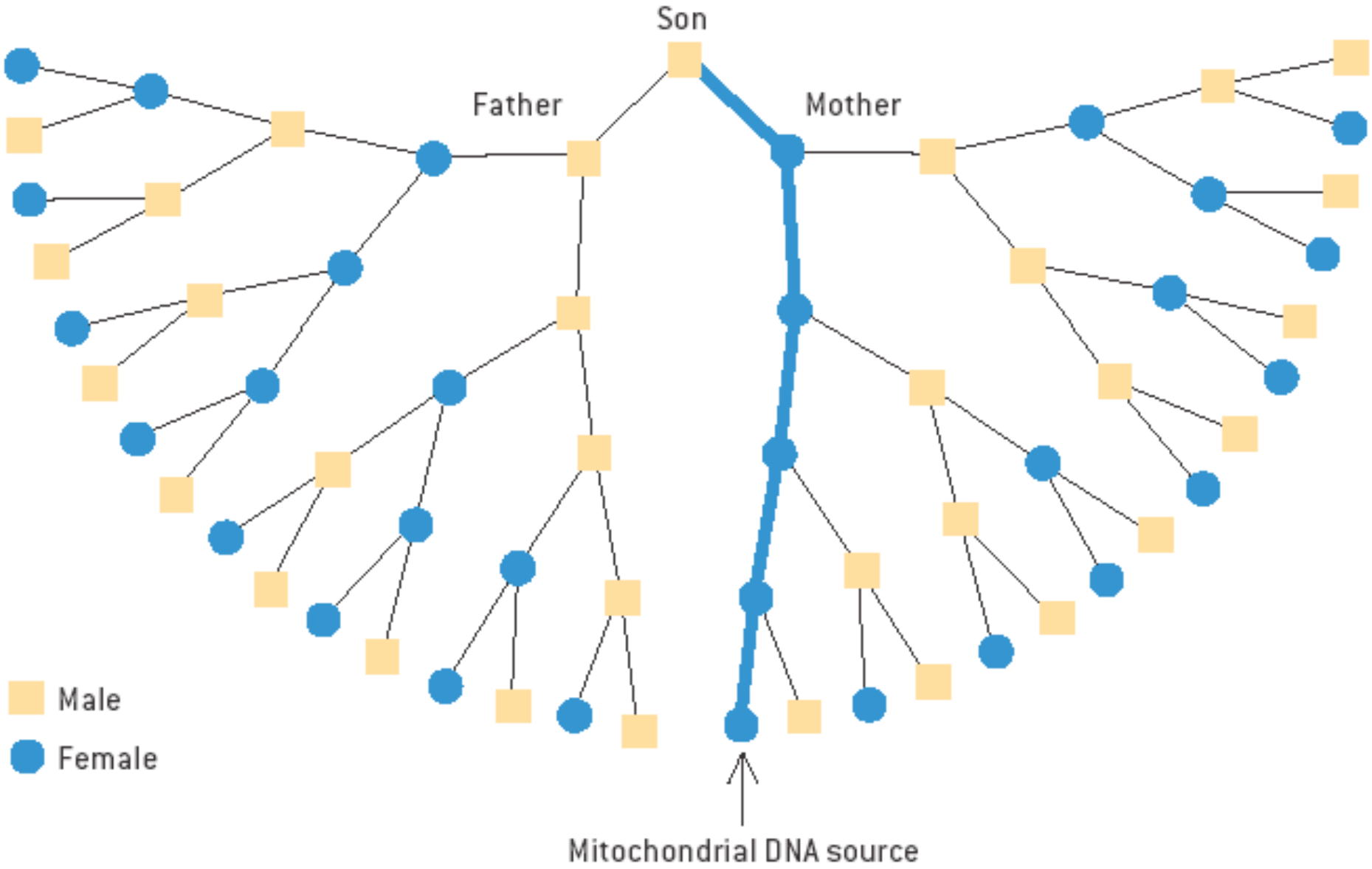
Human migrations can be studied using Y chromosome and mtDNAs



Analysis of human mtDNA led to the Mitochondrial Eve Hypothesis

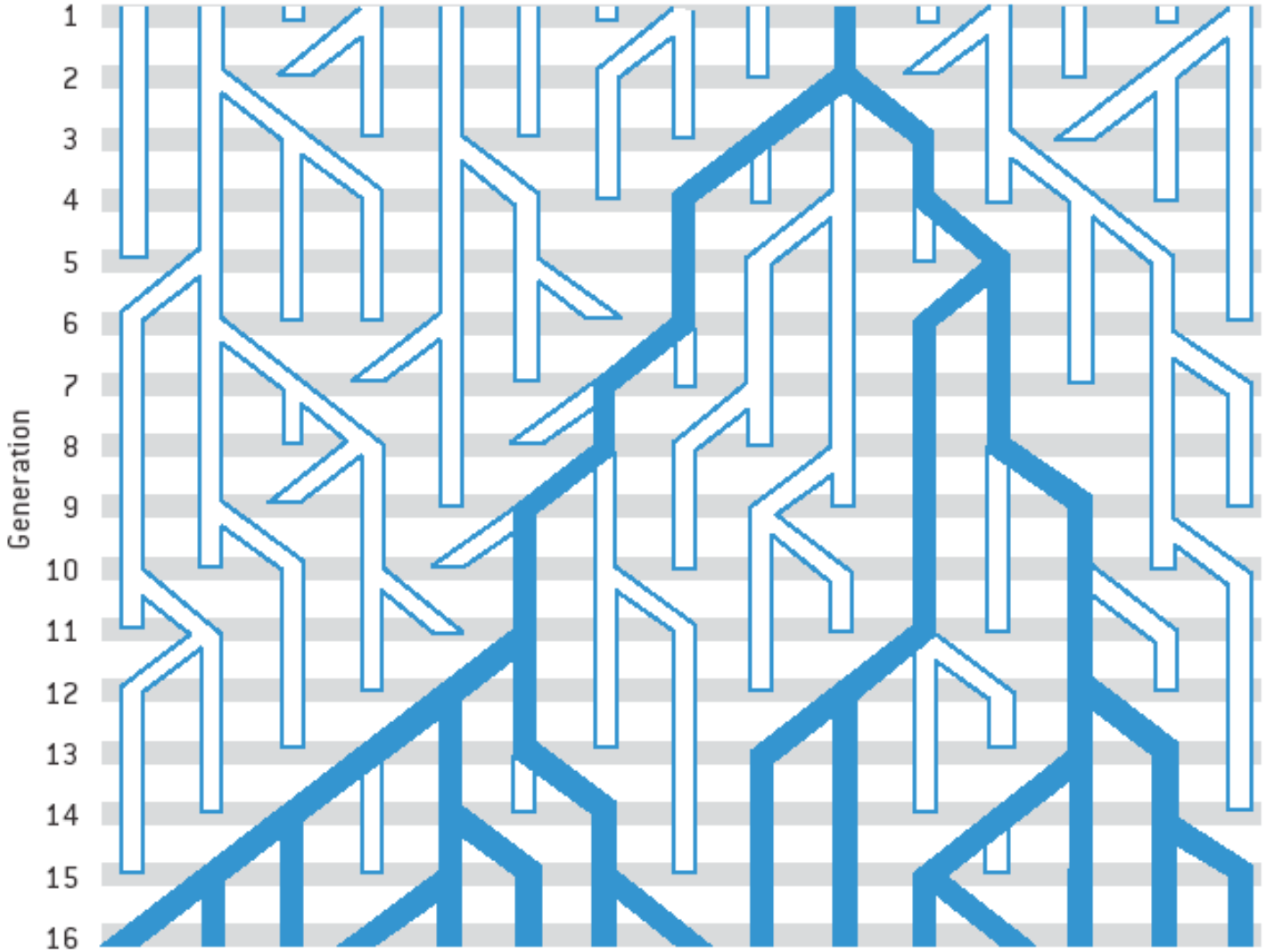
In the 1980s, Allan Wilson pioneered the use of mtDNA to study human evolution.

In two papers published in 1987 and 1991, he and his colleagues at Cal proposed that we all come from a population of humans that lived in Africa approximately 200,000 years ago.



PEDIGREE of one individual illustrates the difference between the patterns of nuclear and mitochondrial inheritance. All 32 ancestors from five generations ago contributed equally to his nuclear DNA. His mitochondrial lineage (*blue line*) leads back to only one person in every generation.

Every daughter has a mother, but not every mother has a daughter. If we go back far enough all mtDNA lineages but one are eliminated.



UNIVERSAL MATERNAL ANCESTOR can be found for all the members of any population. The example shown here traces the lineages of 15 females in a stable population. In each generation, some maternal lineages proliferate and others become extinct. Eventually, by chance, one maternal lineage (*dark blue*) replaces all the others.

Evidence for Mitochondrial Eve hypothesis

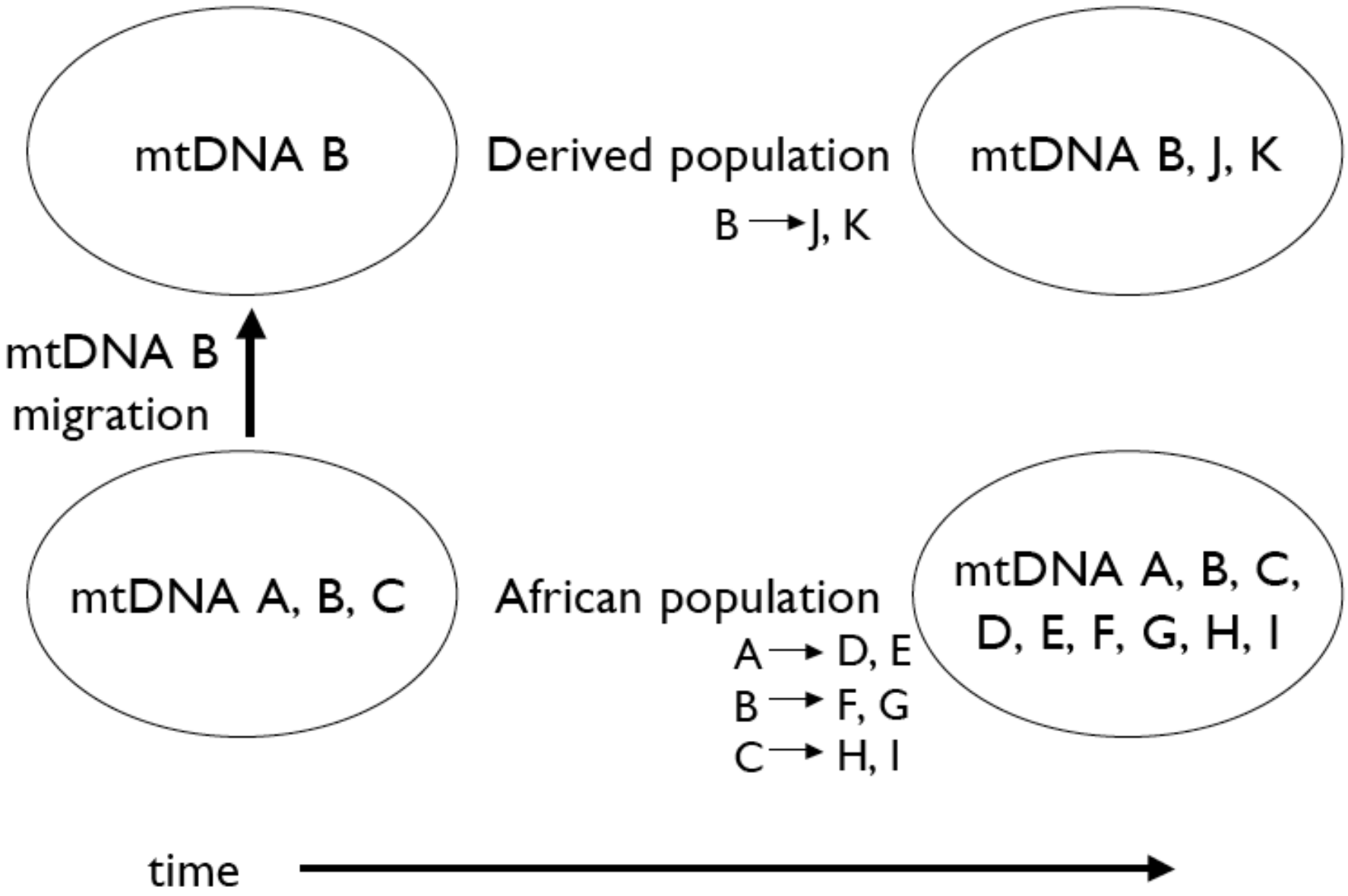
Compared sequences from the highly variable region of mtDNA.

Greater sequence differences among native Africans than any other group, which included Europeans, American Indians, Papua New Guineans, Asians, aboriginal Australians and individuals of Middle Eastern origin.

The diversity of Africans is equivalent to the diversity of all groups combined.

These observations led Wilson to propose that the African population has had the longest time to evolve variation and thus humans originated in Africa.

Model for understanding mtDNA diversity argument



But when did Mitochondrial Eve live?

2.8% of the mitochondrial base pairs differed in the population of sub-Saharan Africans studied.

Chimpanzees and humans diverged about 5 million years ago, and human and chimpanzee mtDNAs differed at 15% of the base pairs of the mtDNA.

Adjusting the data to account for multiple substitutions at the same base pair, they calculated that mtDNA has been diverging at a rate of 13.8% per million years.

Assuming that this "molecular clock" is ticking at a constant rate, they determined that a "Mitochondrial Eve" from whom we are all derived lived 2.8/13.8 or 0.20 million years ago. Although there has been much argument about the assumptions and statistical methods used, most evolutionary geneticists accept that the women carrying our ancestral mtDNA lived in sub-Saharan Africa approximately 200,000 years ago.

Mitochondrial inheritance versus maternal effect

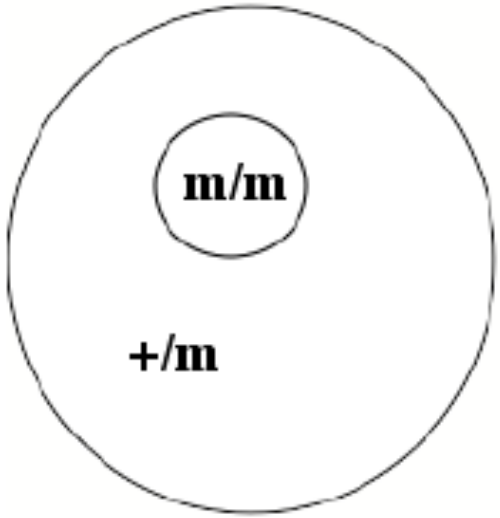
Two sources of information to direct development

Maternal component: Stored in egg as mRNA, protein and organization.

Embryonic (zygotic) component: Newly transcribed from the embryonic genome.

Maternal genotype determines progenyπs phenotype

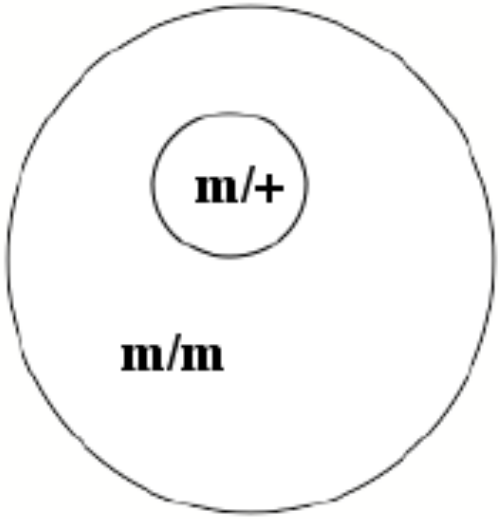
m/+ parents



Embryo lives because of + product in egg

m/m mother x +/+ father

Embryo dies because it lacks + product in cytoplasm



Maternal effect mutations vs zygotic mutations

Zygotic mutation

z/+ x z/+

↓

z/z z/+ +/+

Show phenotype

Maternal effect mutation

m/+ x m/+

↓

m/m m/+ +/+

m/m x +/+ (or m/m or m/+)

↓

m/+ (or m/m)

Show phenotype

Maternal genotype determines progeny's phenotype

Maternal effect genes also display a form of maternal inheritance.

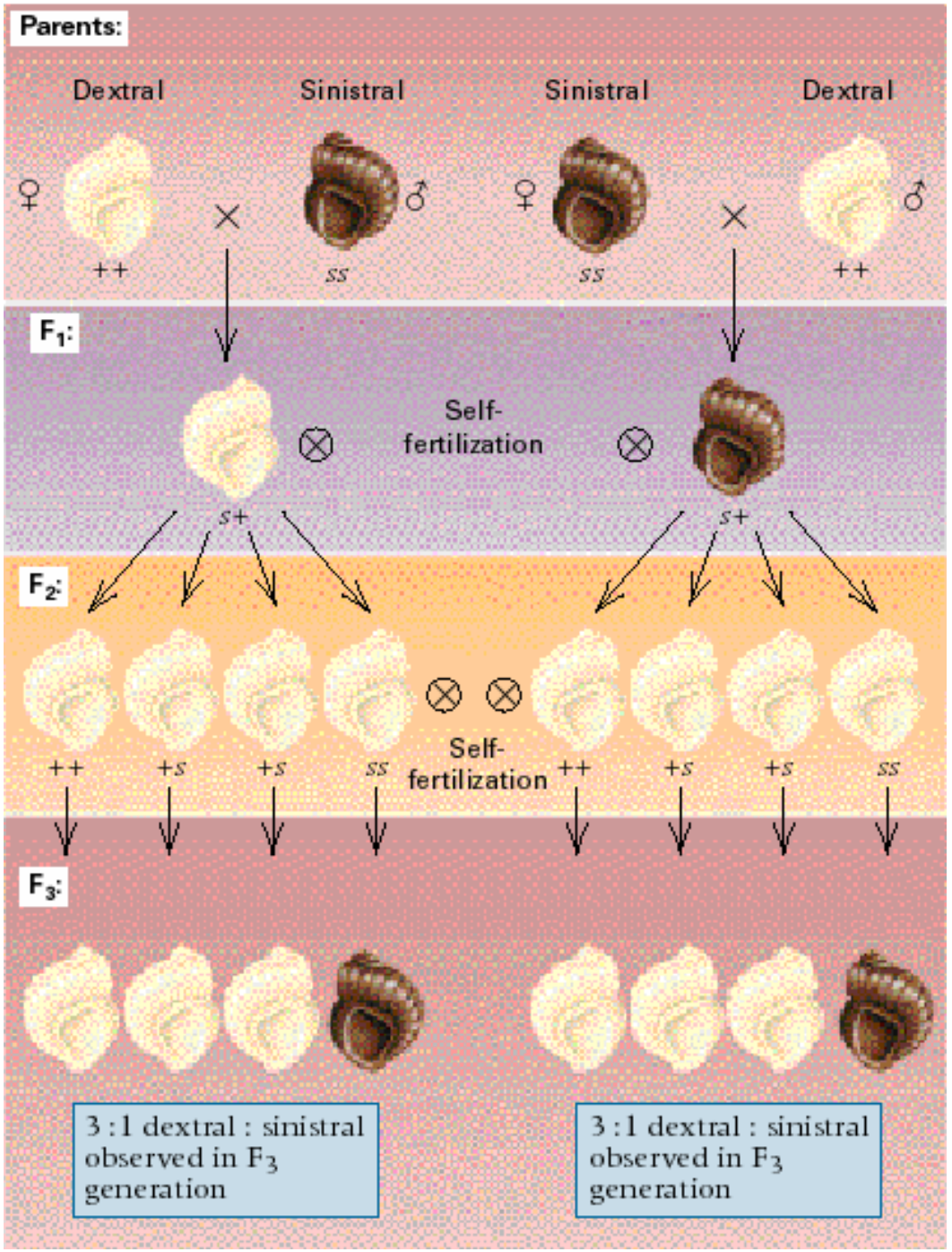
Mitochondrial mutations display maternal inheritance, in which the progeny always have the maternal phenotype. In maternal effect the progeny have the phenotype specified by the maternal nuclear genotype. Maternal effect does not involve extranuclear genes.

In many organisms the development of the embryo is prominently regulated by molecules that are deposited in the oocyte/egg. Since these molecules derive from the mother their composition is dependent on the maternal genotype. Mutations that disrupt the function of these genes in the female, but not the male, lead to developmental abnormalities.

A classic example comes from the study of snail shell coiling. The direction of shell coiling is determined by a pair of nuclear alleles, D (for dextral or rightward coiling), and d (for coiling in the sinistral or leftwards direction). That the phenotype is dependent on the maternal genotype is shown through the results of the reciprocal crosses diagramed below.

Cytological analysis of the developing eggs shows that the orientation of the mitotic spindle during the first division determines the direction of shell rotation. This led to the idea that perhaps the D gene product either was, or indirectly influenced the formation of something that determined mitotic spindle orientation. Experiments demonstrating that this is in fact the case involve taking cytoplasm from D/- eggs and introducing it into d/d eggs. The recipient eggs now develop with a dextral orientation. in contrast cytoplasm from d/d eggs is unable to influence the development of D/- eggs. These observations are consistent with the idea that the D gene specifies a product that is deposited in the egg and promotes a particular mitotic spindle orientation. The d product produces no product or a defective product. These embryos coil in the sinistral direction by default.

There are many examples of important developmental regulators that are maternal effect genes. we will discuss some of these when we talk about developmental genetics.



Help 'lefty' snail Jeremy find a mate, please

Updated: Saturday, October 22nd 2016, 8:41 pm PDT

By Marvin Harris CONNECT

(RNN) - Jeremy, a rare "lefty" garden snail, needs your help in finding a mate.

Your successful matchmaking might advance evolutionary research and benefit humans.

Perhaps your garden, compost heap or plant pot contains a rarity you'd kindly pack safely and ship alive overseas.

Unable to find a love interest in Great Britain, Jeremy is depending on the public worldwide to help him find another one-in-a million snail like himself.

Jeremy has left-handed spirals. Most snails of his species have right-handed spirals, and Jeremy's genitals mismatch with theirs.

A scientist at the University of Nottingham in England is issuing appeals on behalf of Jeremy, the first such snail the professor has seen in his more than 20 years of studying mollusks.

"We are very keen to study the snail's genetics to find out whether this is a result of a developmental glitch or whether this is a genuine inherited genetic trait," Angus Davison, a professor in evolutionary genetics, said in a news release.

Davison believes research using snails can help explain how internal organs in other animals, including humans, can sometimes be placed out of their normal positions.

Jeremy was found in a compost heap in London. The retired scientist who found him mailed the snail to Davison.

Preferring to reproduce with a mate, Jeremy, if the public fails him, being a hermaphrodite, can reproduce alone.

Conclusions

To identify contributions to embryonic development provided during oogenesis, one must identify maternal effect mutations.

To identify contributions dependent upon embryonic gene expression, one must identify zygotic mutations.

1 Scrapie is a highly infectious disease of sheep that is caused by neurotoxic prion protein aggregation.

LANDMARK: NEURODEGENERATION

Shifts and drifts in prion science

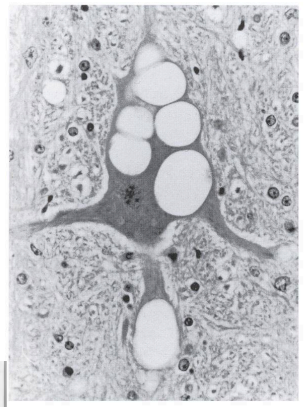
Important questions remain unanswered since prions were discovered four decades ago

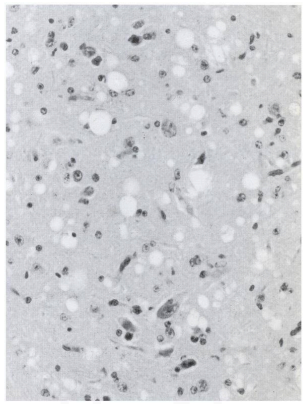
ist without its respective gene, discovered in hamsters the gene encoding the cellular prion protein (PrP^{C}), whose misfolding yields tightly packed aggregates called PrP^{Sc}. It is generally believed that prion replication occurs when coalesced PrP^{Sc} is broken down into smaller species. Those species then accrue further PrP^{Sc}, in a process akin to the growth of crystals, and

What are Prions?

Proteinaceous infectious particles that resist inactivation by procedures that modify nucleic acids.

Prion diseases are often called spongiform encephalopathies because of the post mortem appearance of the brain with large vacuoles in the cortex and cerebellum.

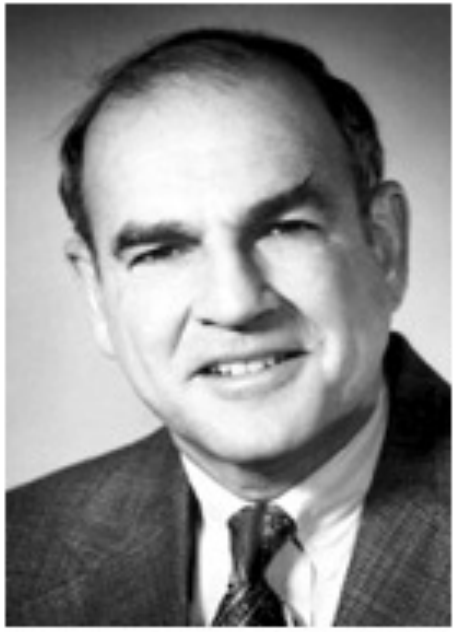




Animals prion diseases
Scrapie: sheep
TME (transmissible mink encephalopathy): mink
CWD (chronic wasting disease): muledeer, elk
BSE (bovine spongiform encephalopathy): cows

Human prion diseases
CJD: Creutzfeld-Jacob Disease
GSS: Gerstmann-Straussler-Scheinker syndrome
FFI: Fatal familial Insomnia
Kuru
Alpers Syndrome

The Nobel Prize in Physiology or Medicine 1976



Baruch S. Blumberg

D. Carleton Gajdusek

The Nobel Prize in Physiology or Medicine 1976 was awarded jointly to Baruch S. Blumberg and D. Carleton Gajdusek ***"for their discoveries concerning new mechanisms for the origin and dissemination of infectious diseases"***

D. Carleton Gajdusek dies at 85; Nobel Prize winner identified exotic disease, was unrepentant pedophile

By Thomas H. Maugh II
December 18, 2008

Dr. D. Carleton Gajdusek, the brilliant yet deeply flawed pediatrician, virologist and anthropologist who won the 1976 Nobel Prize in medicine for his identification and description of kuru, the exotic disease of a remote tribe in New Guinea that was caused by a family of mysterious agents called prions, died Dec. 12 at the hotel where he lived in Tromso, Norway. He was 85.

Kuru is a rare and fatal brain disorder that occurred at epidemic levels during the 1950s-60s among the Fore people in the highlands of New Guinea. The disease was the result of the practice of ritualistic cannibalism among the Fore, in which relatives prepared and consumed the tissues (including brain) of deceased family members. Brain tissue from individuals with kuru was highly infectious, and the disease was transmitted either through eating or by contact with open sores or wounds. Government discouragement of the practice of cannibalism led to a continuing decline in the disease, which has now mostly disappeared.

Kuru belongs to a class of infectious diseases called transmissible spongiform encephalopathies (TSEs), also known as prion diseases. The hallmark of a TSE disease is misshapen protein molecules that clump together and accumulate in brain tissue. Scientists believe that misshapen prion proteins have the ability to change their shape and cause other proteins of the same type to also change shape. Other TSEs include Creutzfeldt-Jakob disease and fatal familial insomnia in humans, bovine spongiform encephalopathy in cattle (also known as mad cow disease), scrapie in sheep and goats, and chronic wasting disease in deer and elk

Kuru

- **Prion disease in Fore region of Papua New Guinea**
- **Epidemic in 1950s**
- **Transmitted by endocannibalism**
  - **Originated from sporadic case?**

- **Mainly women and children affected**
- **Mean incubation time ~ 12 years**

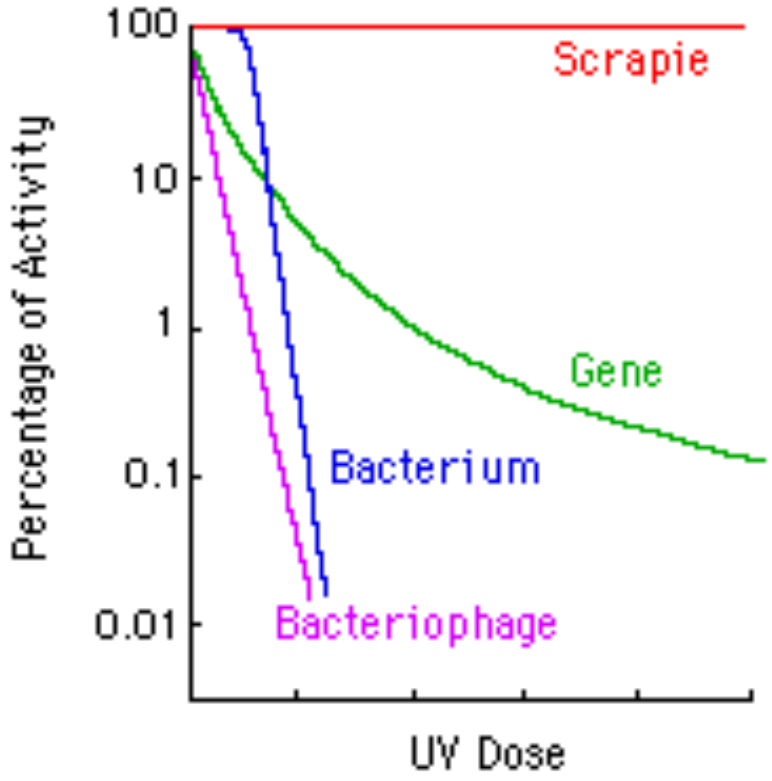
- **Model for vCJD?**

Iatrogenic CJD

- Intracerebral electrodes
- corneal grafts
- dura mater grafts
- pituitary derived human growth hormone

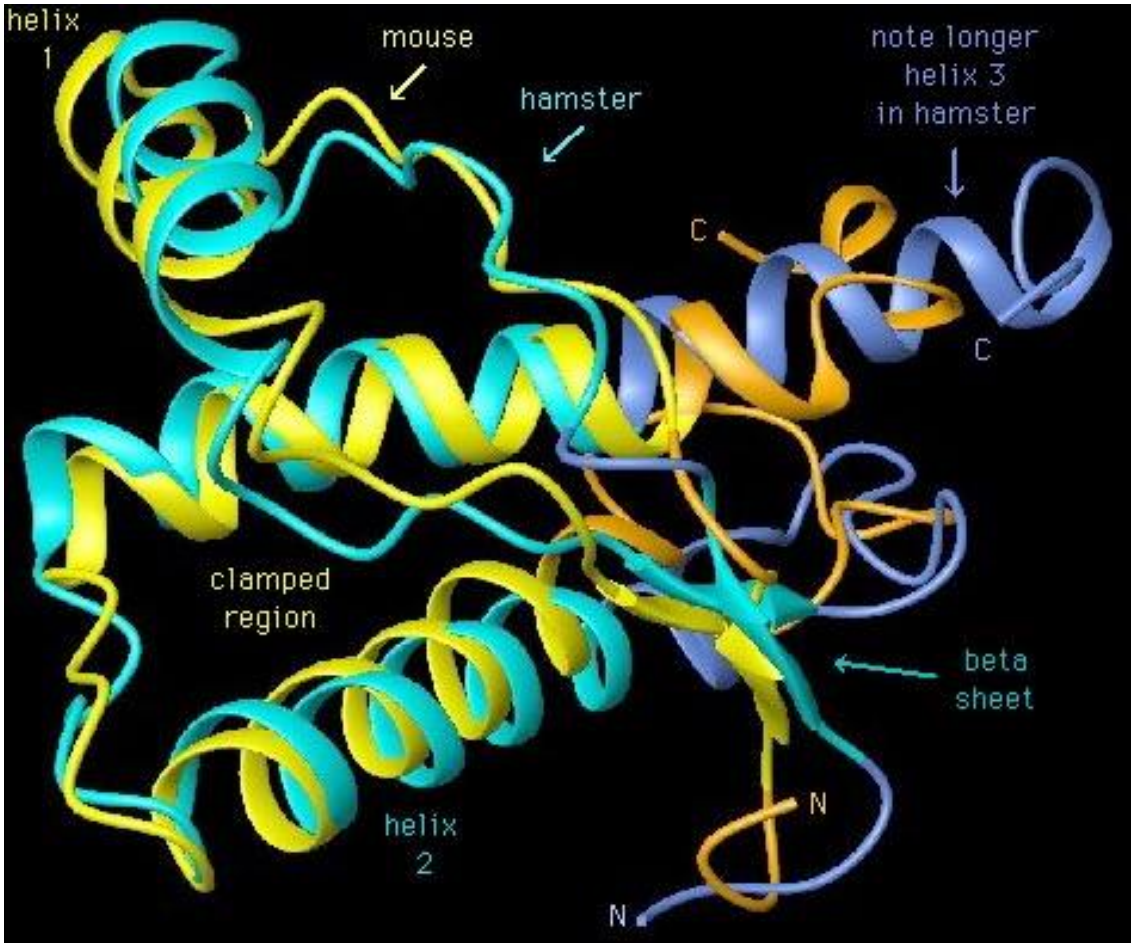
What is the infectious agent?

1. UV treatment has no effect on activity of Scrapie infectious agent.

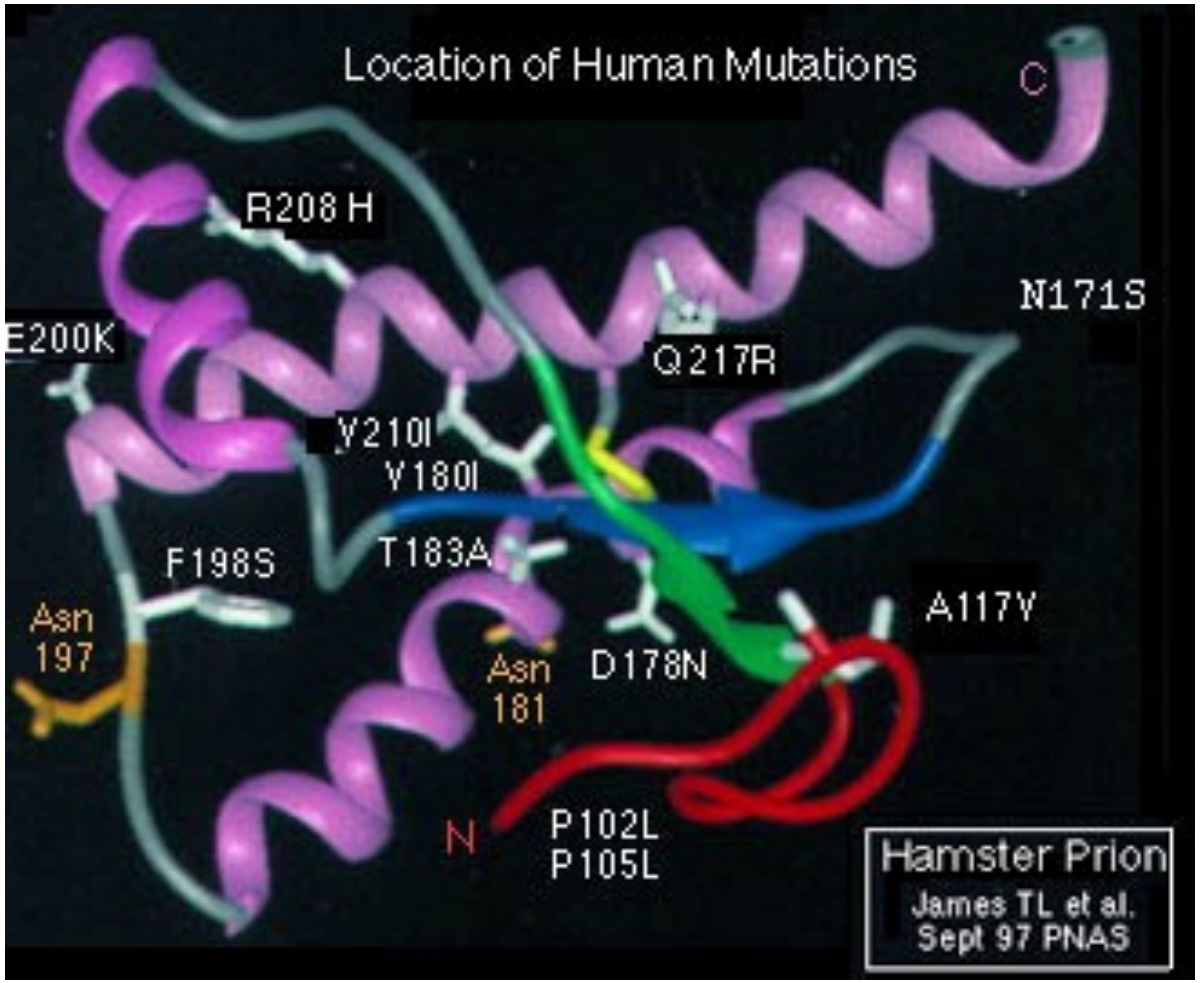


2. Infectious agent susceptible to proteases, but not nucleases.

3. Purified infectious material contains only a single protein called PrP^{Sc}. This protein has the same sequence as a normal noninfectious protein, called PrP^{C}, which is encoded by a nuclear gene.



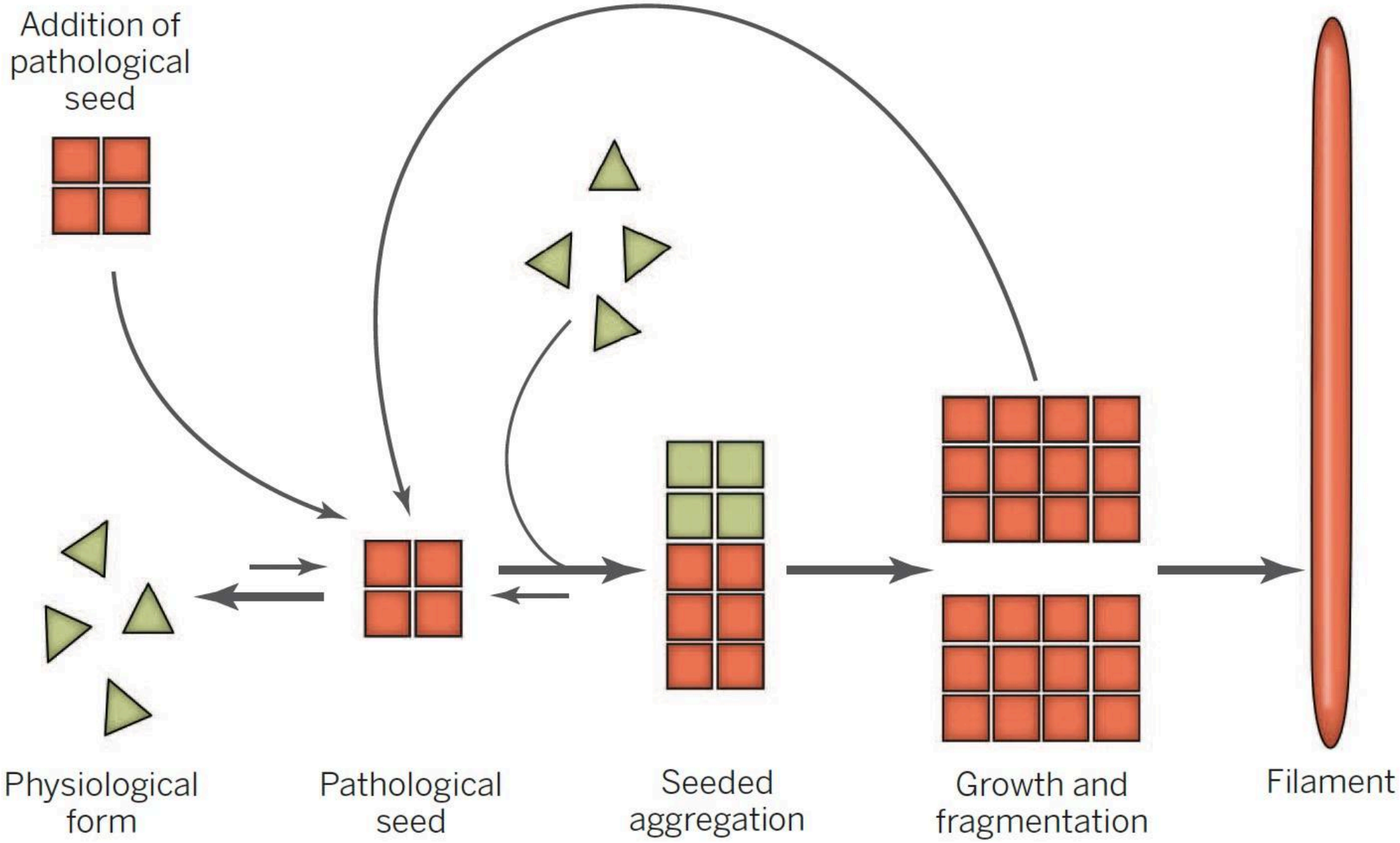
4. Individuals with inherited spongiform encephalopathies have PrP^C that are different from unaffected people. In one GSS pedigree, for example, the proline at position 102 is changed to a leucine.



5. Mice containing a transgene with the P102L change develop spongiform encepholopathy.

6. Knockout mice lacking the PrP gene function are resistant to infection.

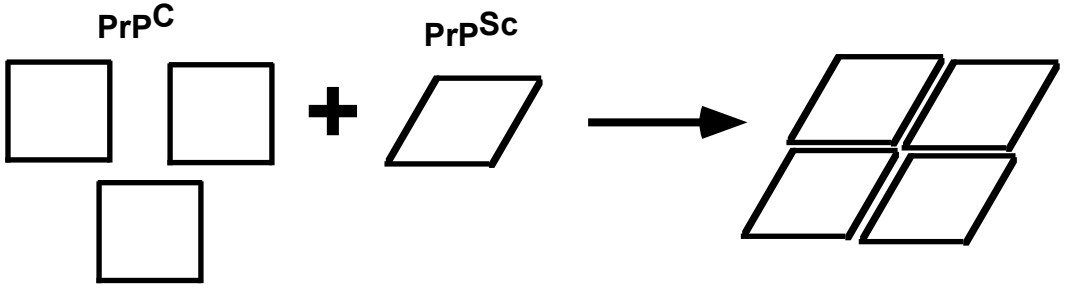
The model


Addition of pathological seed
Physiological form
Pathological seed
Seeded aggregation
Growth and fragmentation
Filament

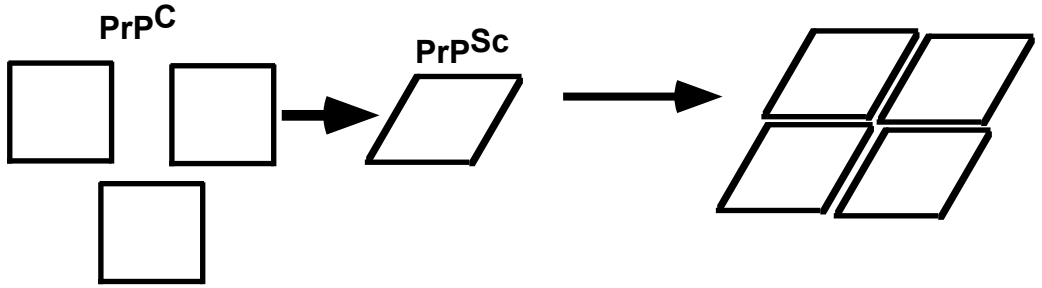
The infectious scrapie agent is highly resistant to procedures that destroy or modify nucleic acids, while being susceptible to procedures that hydrolyze or modify proteins. By biochemical purification to enrich for infectivity, a single 27-30kD protein was discovered. It was encoded chromasomally.

Normal individuals contain a protease-sensitive cellular form (PrPC). Infected individuals contain PrPSc. Aggregates of PrPSc induce the conversion of PrPC.

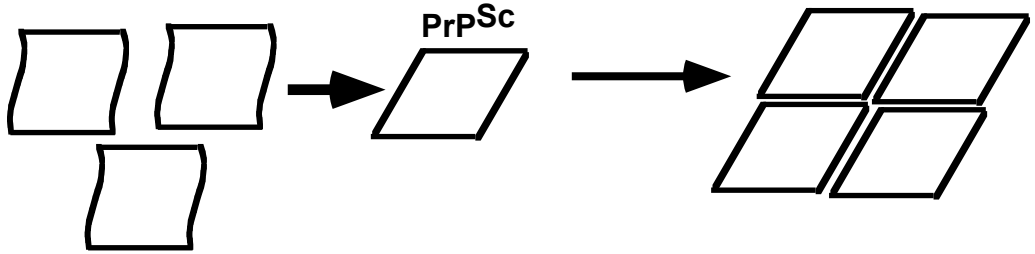
Lateral transmission through contamination with PrP^{Sc}



Sporadic conversion of PrP^{C} to PrP^{Sc} = rare



PrP^{C} mutation that predisposes to PrP^{Sc} conversion



Key experiments:

--Genetic: autosomal dominant

--Mice that express human PrP result in disease with the characteristics of the human disease.

--Mice that express PrPSc spontaneously develop scrapie

--Mice that lack PrPC are resistent to infection with PrPSc

Prions in yeast behave as dominant traits, but with non-Mendelian inheritance

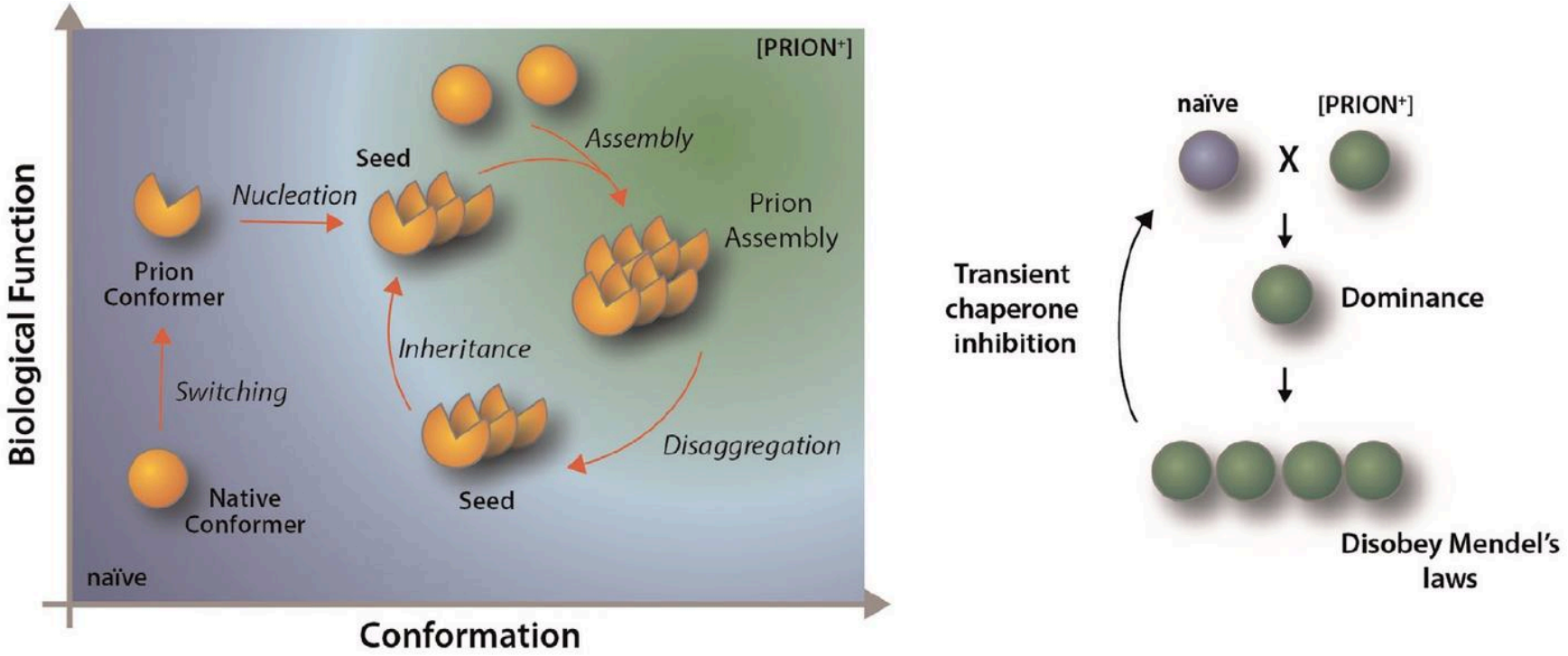


Fig. 2. Molecular and genetic hallmarks of prions. (Left panel) The *x*-axis represents the conformational space explored by a prion protein. On this axis, the multimeric prion assembly and native conformer depict two distinct and stable structural states of the same prion polypeptide. On the *y*-axis, the functional states of these two distinct structural states are mapped. The functional difference in these two distinct and stable structural states manifests as separate naïve and [*PRION*$^+$] traits, depicted in blue and green. The mechanism of prion formation and transmission to progeny fueling such epigenetic inheritance involves disaggregation by molecular chaperones to generate propagons that can be passed to the next generation. (Right panel) Accessing two distinct phenotypic states (naïve in blue and [*PRION*$^+$] in green) from the same polypeptide engenders distinctive behavior in genetic crosses. Prion-based traits are transmissible via cytoplasmic transfer, dominant in diploids, and passed to meiotic progeny in a non-Mendelian fashion. Most can also be permanently reverted to a naïve [*prion*$^-$] state from by transient chaperone inhibition. The unusual [*PRION*$^+$] nomenclature for these protein-based genetic elements is derived from these behaviors.

Asymptomatic deer excrete infectious prions in faeces

Gültekin Tamgüney[1,2], Michael W. Miller[3], Lisa L. Wolfe[3], Tracey M. Sirochman[3], David V. Glidden[4], Christina Palmer[1], Azucena Lemus[5], Stephen J. DeArmond[1,5] & Stanley B. Prusiner[1,2]

Infectious prion diseases[1]—scrapie of sheep[2] and chronic wasting disease (CWD) of several species in the deer family[3,4]—are transmitted naturally within affected host populations. Although several possible sources of contagion have been identified in excretions and secretions from symptomatic animals[5–8], the biological importance of these sources in sustaining epidemics remains unclear. Here we show that asymptomatic CWD-infected mule deer (*Odocoileus hemionus*) excrete CWD prions in their faeces long before they develop clinical signs of prion disease. Intracerebral inoculation of irradiated deer faeces into transgenic mice overexpressing cervid prion protein (PrP) revealed infectivity in 14 of 15 faecal samples collected from five deer at 7–11 months before the onset of neurological disease. Although prion concentrations in deer faeces were considerably lower than in brain tissue from the same deer collected at the end of the disease, the estimated total infectious dose excreted in faeces by an infected deer over the disease course may approximate the total contained in a brain. Prolonged faecal prion excretion by infected deer provides a plausible natural mechanism that might explain the high incidence and efficient horizontal transmission of CWD within deer herds[3,4,9], as well as prion transmission among other susceptible cervids.

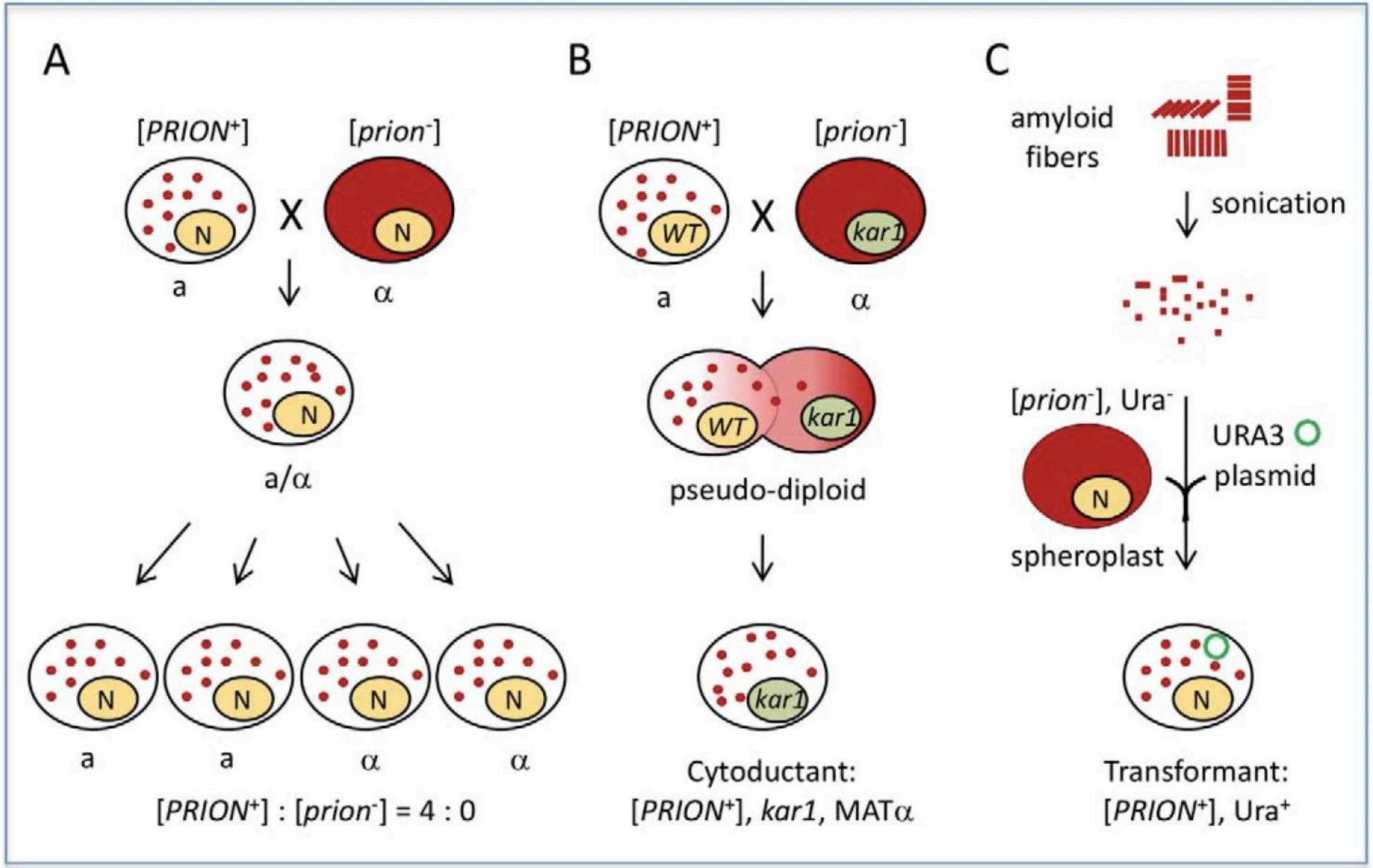
The Spokesman-Review, Spokane, Wash.

For the first time in a decade, Washington wildlife officials sample for chronic wasting disease on opening day of deer hunting season

Eli Francovich, The Spokesman-Review, Spokane, Wash.

Sun, October 17, 2021, 8:01 AM

Oct. 17—For the first time in a decade, Washington wildlife officials surveyed for a deadly neurological disease during Saturday's modern deer hunting season opener.


A
[PRION+]
[prion-]
N
X
N
a
α
N
a/α
N
N
N
N
a
a
α
α
[PRION+] : [prion-] = 4 : 0
B
[PRION+]
[prion-]
WT
X
kar1
a
α
WT
kar1
pseudo-diploid
kar1
Cytoductant:
[PRION+], kar1, MATα
C
amyloid
fibers
sonication
[prion-], Ura-
URA3
plasmid
N
spheroplast
N
Transformant:
[PRION+], Ura+

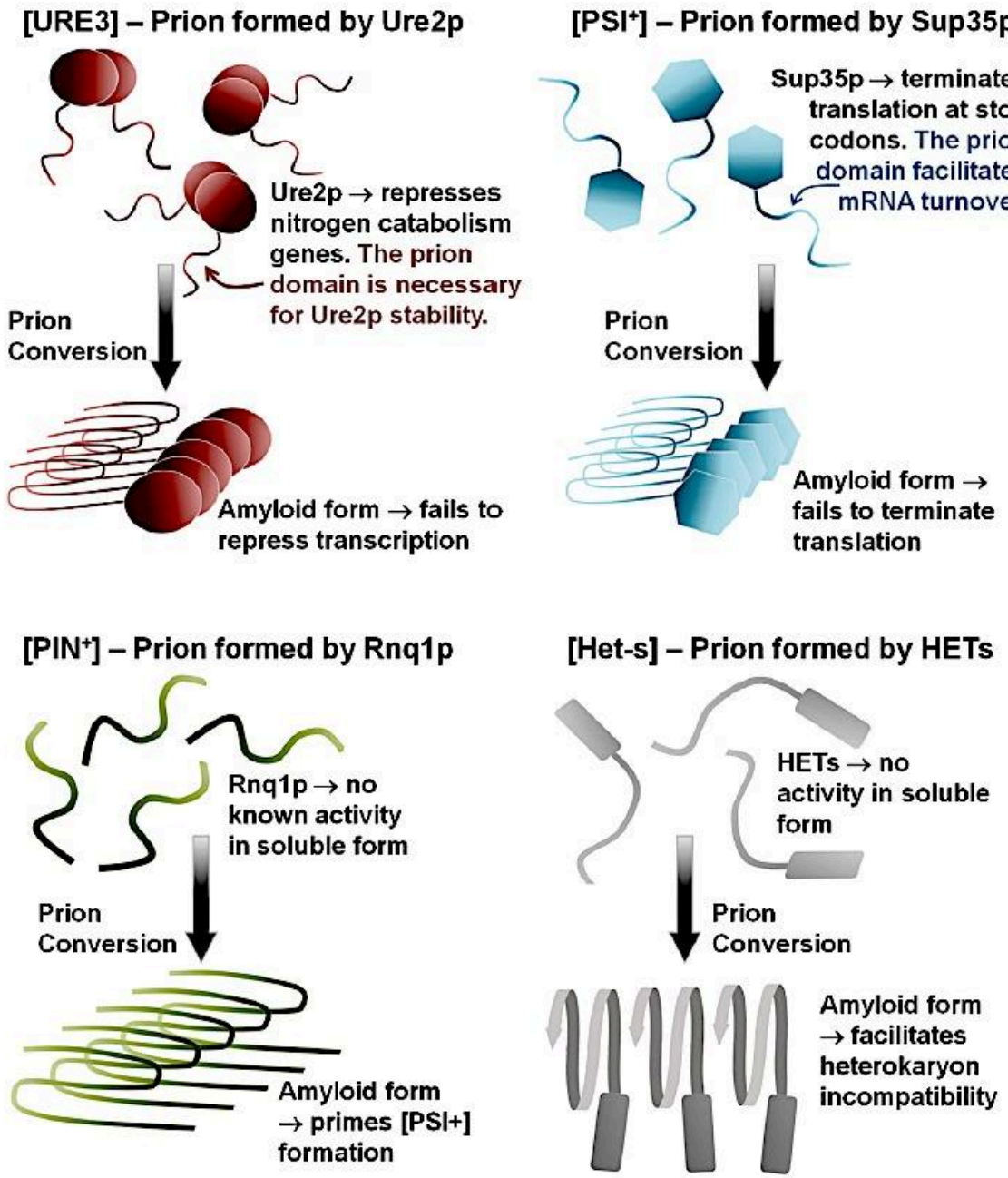


FIG 1 Prions [URE3], [PSI+], and [PIN+] of *S. cerevisiae* and [Het-s] of *Podospora anserina*. These prions are based on self-propagating amyloids of Ure2p, Sup35p, Rnq1p, and HET-s, respectively. The prion domains of Ure2p and Sup35p have nonprion functions, explaining their retention in evolution despite detrimental prion formation.

Prion formation is regulated by cellular signaling.
Heat shock chaperones in this case

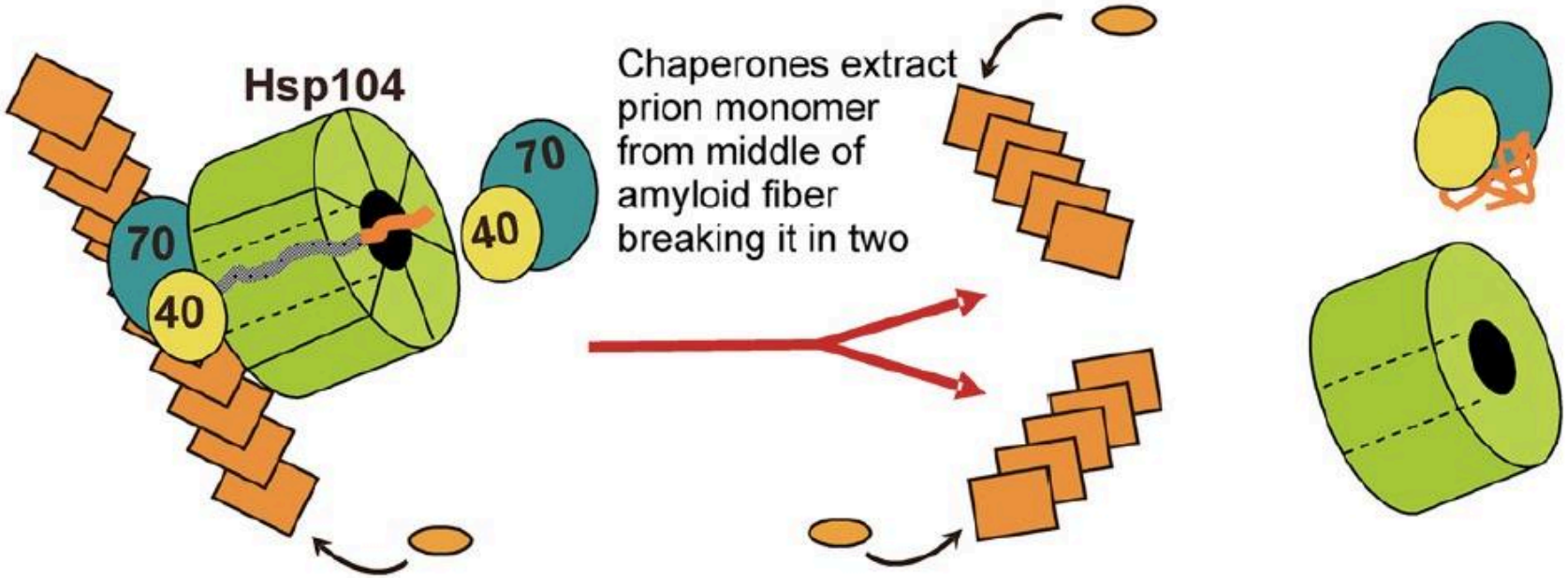


FIG 5 Mechanism of seed generation by Hsp104-Hsp70-Hsp40. Hsp104, working with Hsp40 (mainly Sis1p) and Hsp70 (Ssa proteins), extracts a monomer from an amyloid filament, resulting in the formation of two filaments, with two more growing ends. (Adapted from reference 217.)

Intrinsically disordered proteins/domains and prion formation.

The creation of unique epigenetic states that are reversible

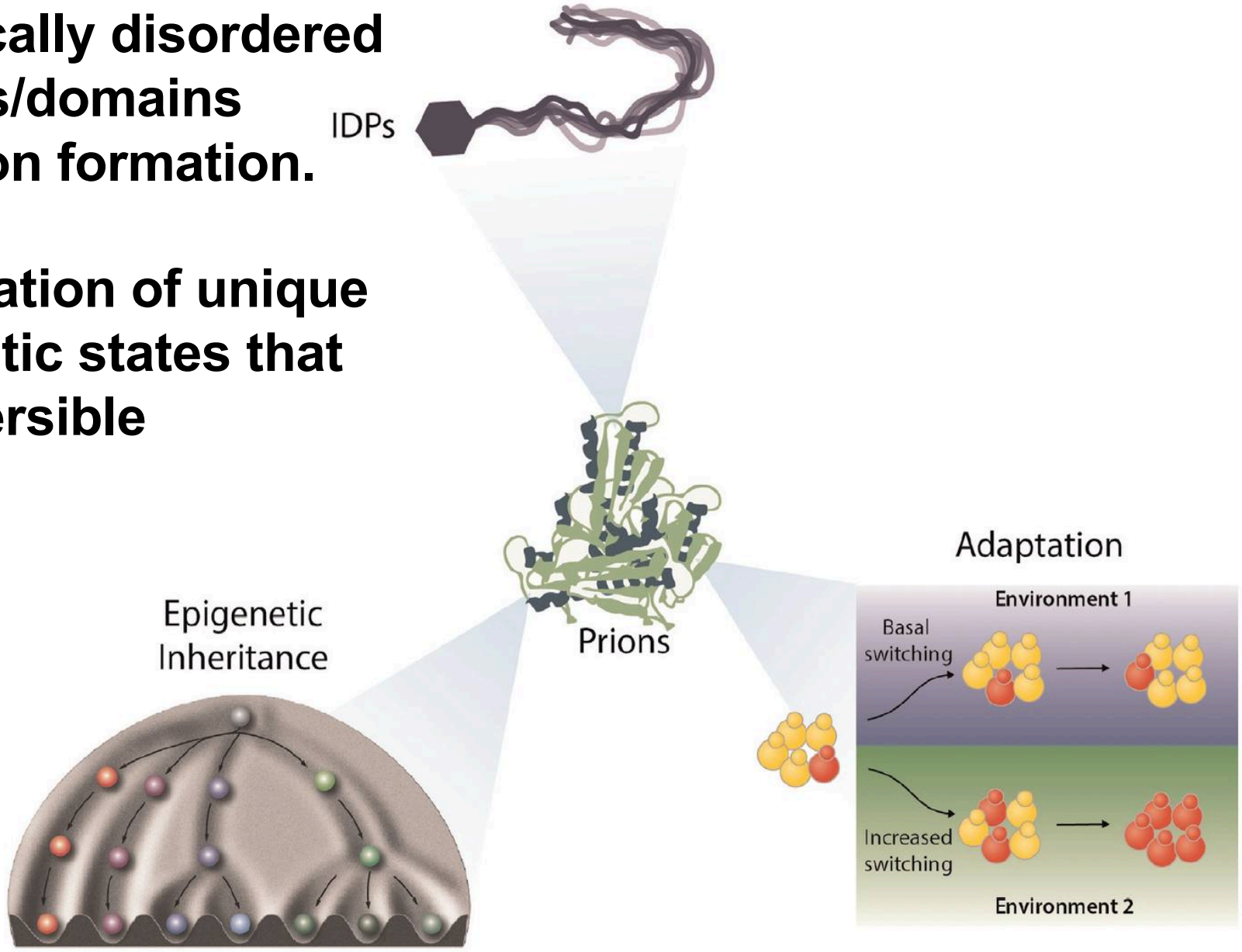


Fig. 1. Prions at the crossroads of three distinct ideas—IDPs, epigenetic inheritance, and evolutionary adaptation in fluctuating environments. IDRs in IDPs fuel the exploration of multiple conformational states. For prions, at least one such conformational state has the ability to self-template and is therefore heritable from one generation to the next. Epigenetic inheritance driven by prions produces different cell states in isogenic backgrounds. Such epigenetic states can be adaptive when faced with changing environments.