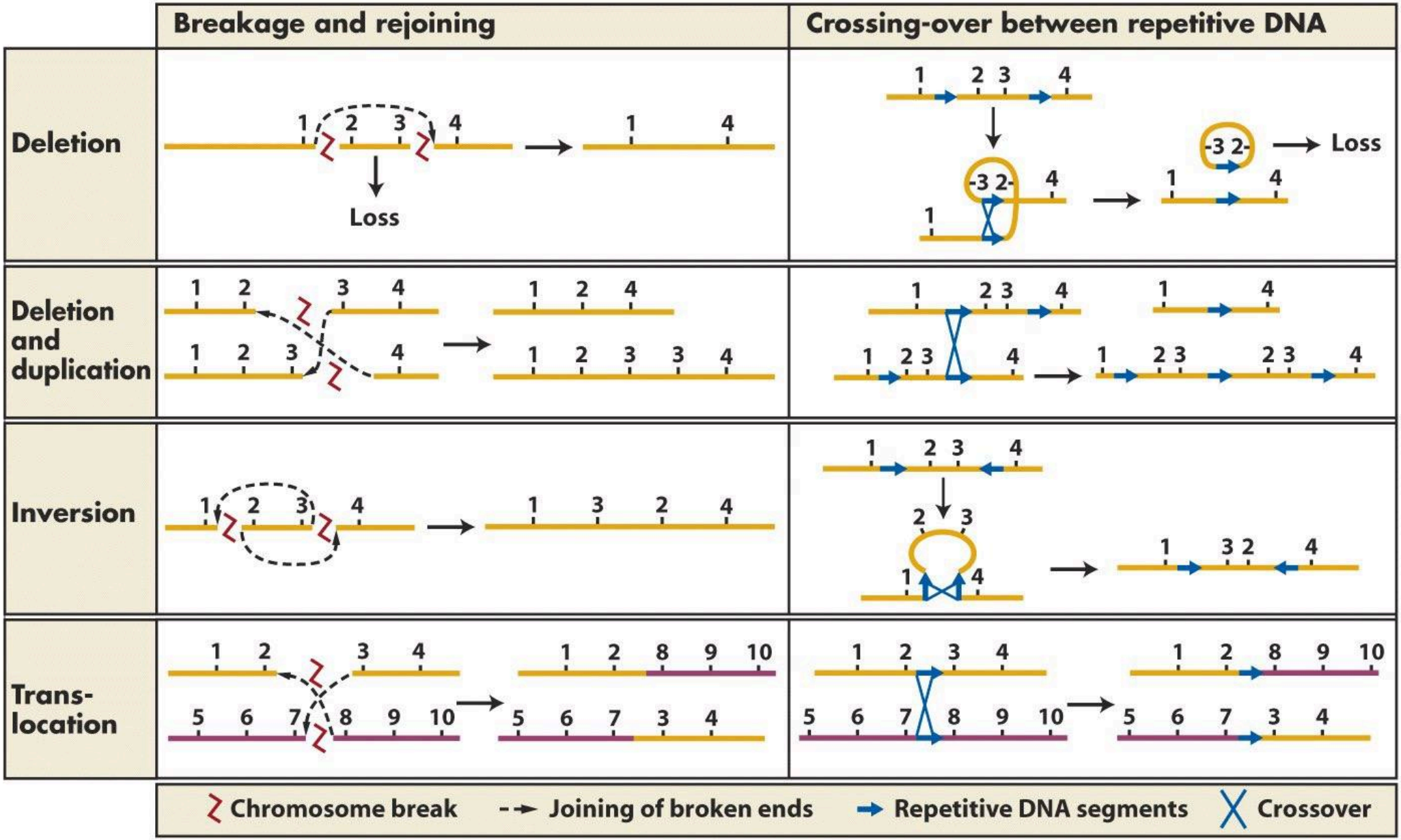

Breakage and rejoining
Crossing-over between repetitive DNA
Deletion
Deletion and duplication
Inversion
Trans-location
Loss
Chromosome break
Joining of broken ends
Repetitive DNA segments
Crossover

Using a circuit and natural bacterial transformation to detect colorectal cancer

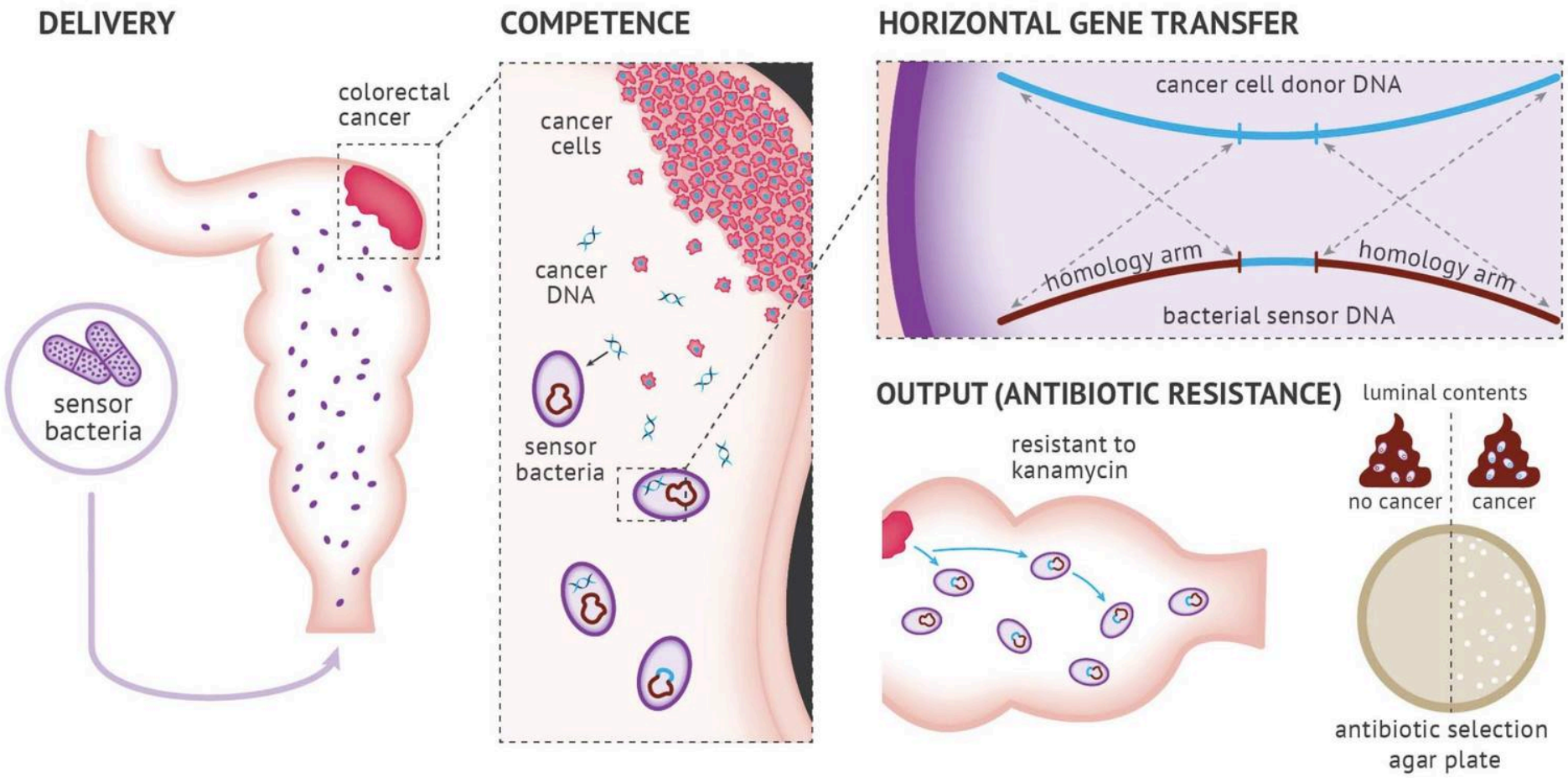
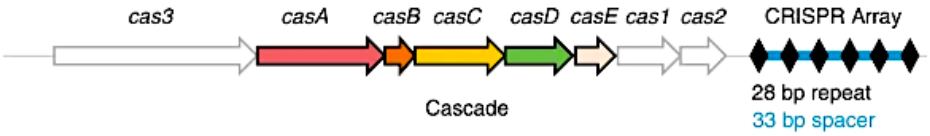
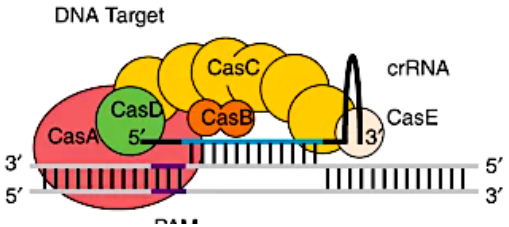
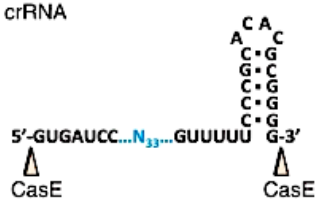


Fig. 1. Engineered bacteria to detect tumor DNA. Engineered *A. baylyi* bacteria are delivered rectally in an orthotopic mouse model of CRC. The naturally competent *A. baylyi* take up tumor DNA shed into the colorectal lumen. The tumor donor DNA is engineered with a *kanR* cassette flanked by *KRAS* homology arms. The sensor bacteria are engineered with matching *KRAS* homology arms that promote homologous recombination. Sensor bacteria that undergo HGT from tumor DNA acquire kanamycin resistance and are quantified from luminal contents by serial dilution on antibiotic selection plates.

doi: 10.1126/science.adf3974.

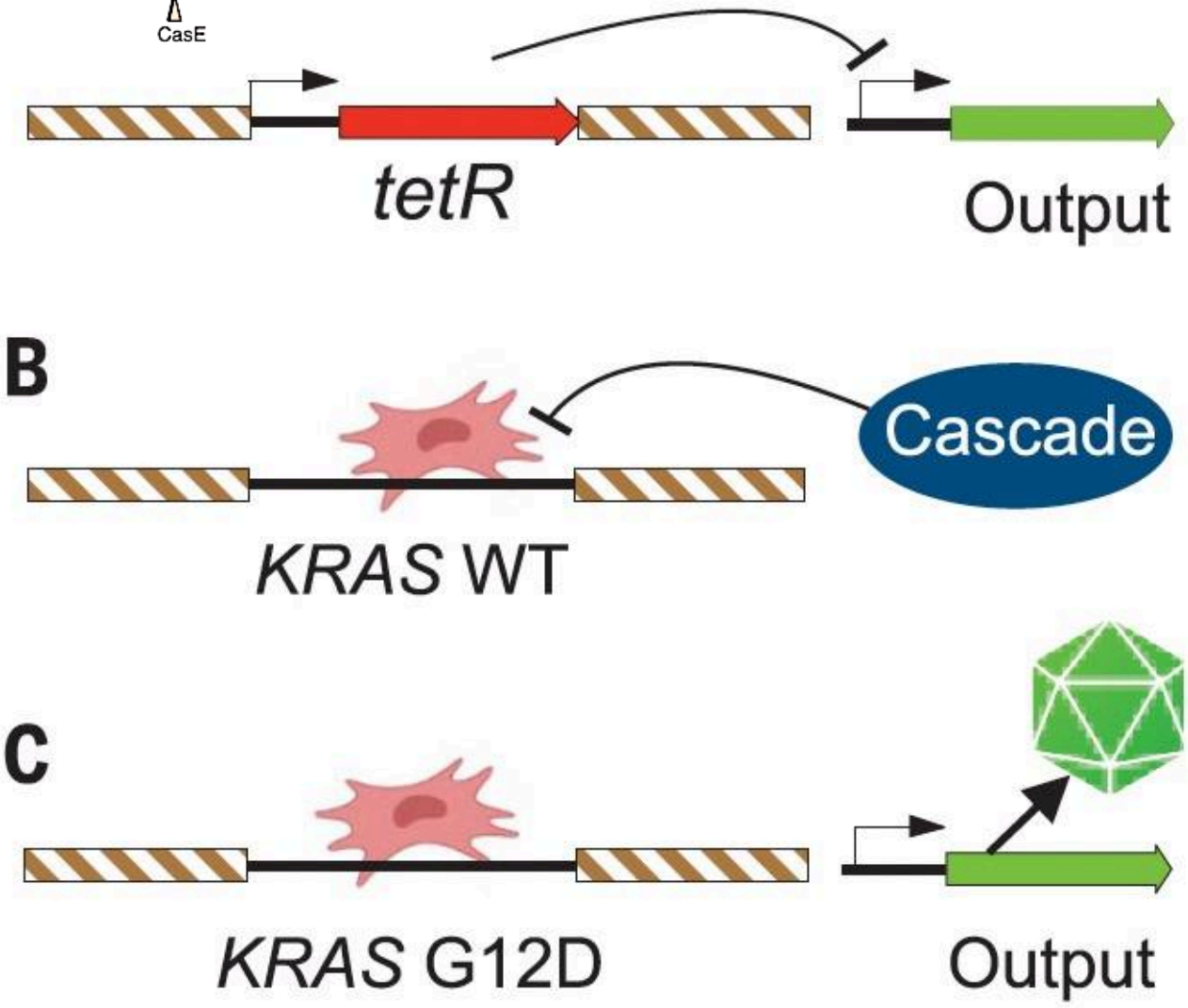






A circuit that allows detection of a cancer-specific mutation

KRAS, mutated in a large fraction of cancers, differs from WT by a single nucleotide.



A, *tetR* located between the homology arms on the *A. baylyi* genome represses expression of the output gene. **B,** Target DNA with wild-type *KRAS* sequence is recognized and degraded by the Type I-F CRISPR-Cas effector complex, Cascade. **C,** Target DNA with the *KRASG12D* mutation avoids degradation, replaces *tetR* in the biosensor genome, and relieves repression of the output gene.

3. Gene targeting

This is the golden age of genetics.

1. As discussed in the first lecture, we have the ability to move entire genomes from one cell to another. This provides a tool by which we can easily create genetically identical organisms down through the generations.

2. The genomes of many organisms have been sequenced. The pace of this work will only accelerate in the next 5 years. Thus the genetic blueprint for many, many, many organisms will be available for study. This information will provide an incredible resource for trying to understand how life does the many amazing things it does.

3. However, sequence information by itself is of very limited value. It allows us to form hypotheses as to what a gene or group of genes does, but testing these predictions requires that we go in and manipulate the genome in question to test our predictions. Specifically, we need a tool that allows us to cut out and replace pieces of genomic DNA with DNA of our choice. In this way we can truly engineer the genomes of organisms. This tool serves both a basic research goal, and it provides the foundation for much of the current revolution in biotechnology.

4. Homologous recombination provides the basis for such a tool. Below we go through the basic steps required to cut out and replace a piece of the mouse genome with a particular piece of DNA we are interested in. This technology is known generally as gene targeting.

Outline of several important considerations for gene targeting

1. Most DNA introduced into the zygote integrates randomly.

2. In order to carry out gene targeting, the selective alteration of homologous sequences, many integration events must be screened for those that have integrated homologously. This is most conveniently done in embryonic stem cells. These cells are derived from early mouse embryos and are pluripotent (they can differentiate into many different cell types when reintroduced into a mouse embryo).

3. Gene targeting requires a set of selection procedures to identify those clones that have integrated homologously.

4. Once such cells have been identified they can be introduced into embryos of a different genotype. Chimeric adults are then tested through mating for germ line transmission of the integrated gene. Progeny of these adults that carry the recombinant chromosome (usually identified through PCR or Southern blotting) are heterozygous for the altered gene.

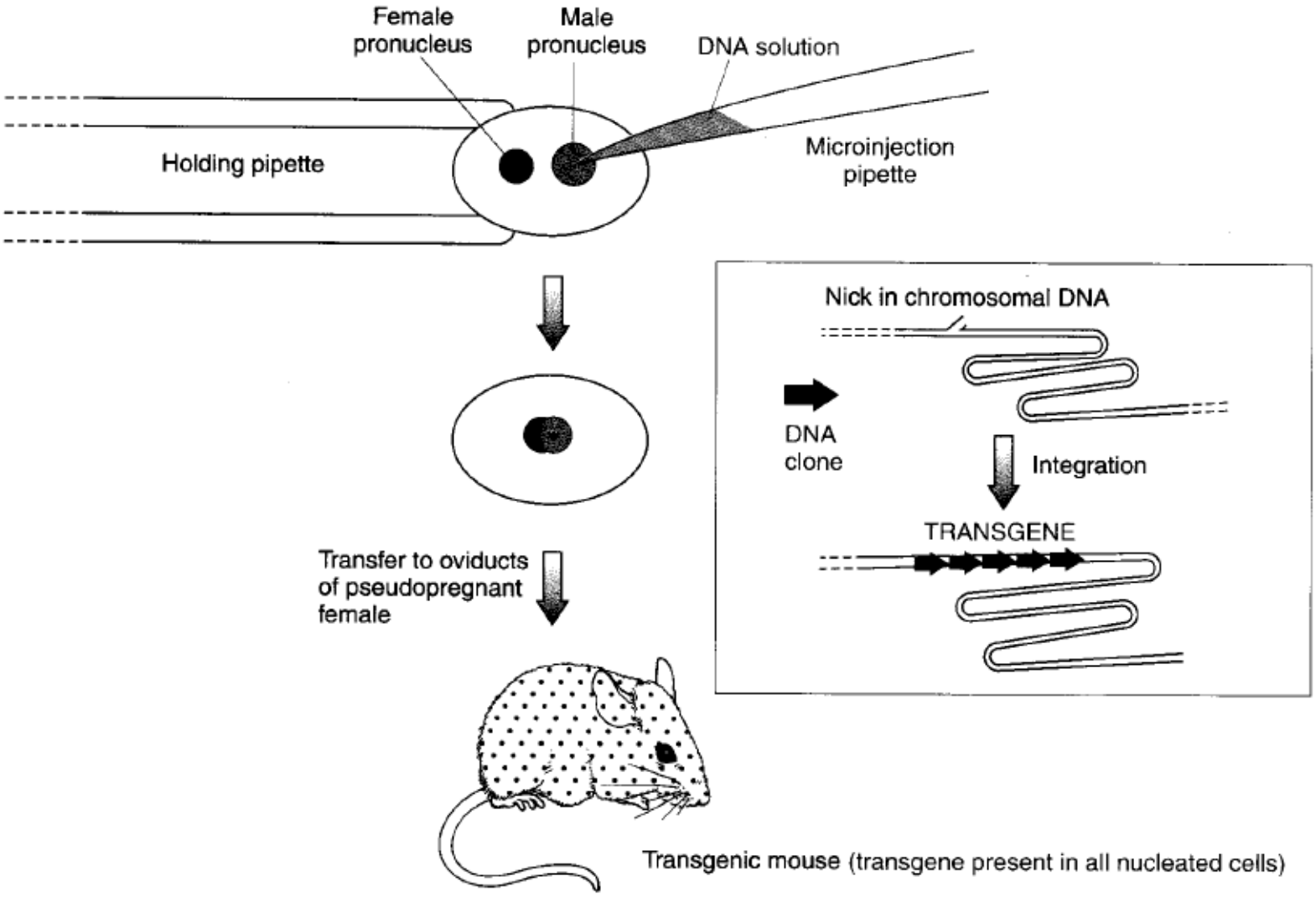
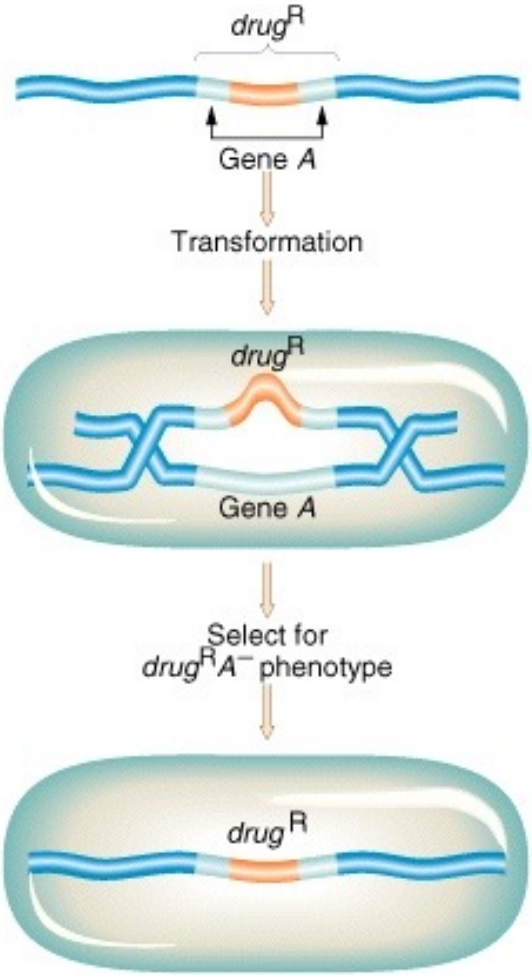


Figure 21.1: Construction of transgenic mice by pronuclear microinjection.

Very fine glass pipettes are constructed using specialized equipment: one, a holding pipette, has a bore which can accommodate part of a fertilized oocyte, and thereby hold it in place, while the microinjection pipette has a very fine point which is used to pierce the oocyte and thence the *male* pronucleus (because it is bigger). An aqueous solution of the desired DNA is then pipetted directly into the pronucleus. The introduced DNA clones can integrate into chromosomal DNA at nicks, forming transgenes, usually containing multiple head-to-tail copies. Following withdrawal of the micropipette, surviving oocytes are reimplanted into the oviducts of *pseudopregnant* foster females (which have been mated with a vasectomized male; the mating act can initiate physiological changes in the female which stimulate the development of the implanted embryos). Newborn mice resulting from development of the implanted embryos are checked by PCR for the presence of the desired DNA sequence (Gordon, 1992).



Recombinant vector carrying $drug^R$ gene within disrupted gene *A*

Nucleus

Disrupted gene in chromosome

The Nobel Prize in Physiology or Medicine 2007

"for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells"

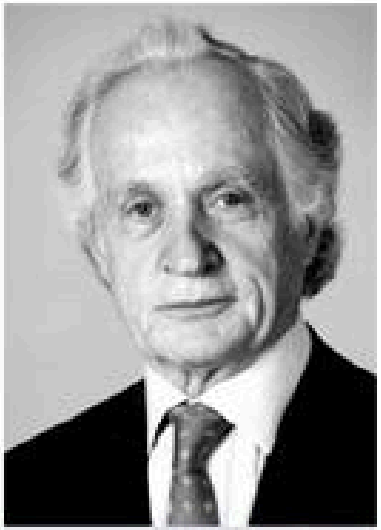


Photo: U. Montan

Mario R. Capecchi

1/3 of the prize

USA

University of Utah
Salt Lake City, UT, USA;
Howard Hughes Medical Institute

b. 1937
(in Italy)

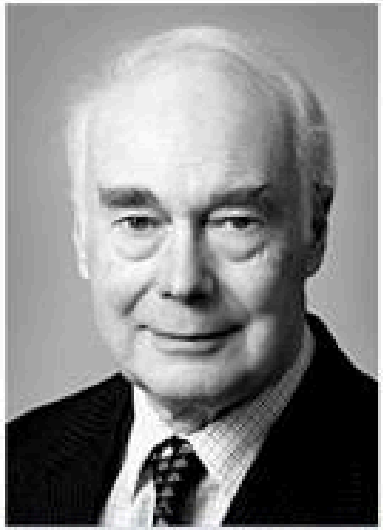


Photo: U. Montan

Sir Martin J. Evans

1/3 of the prize

United Kingdom

Cardiff University
Cardiff, United Kingdom

b. 1941

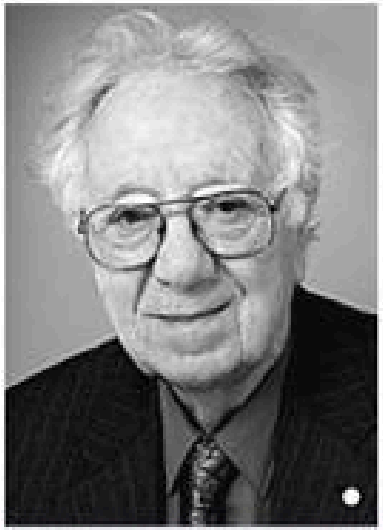


Photo: U. Montan

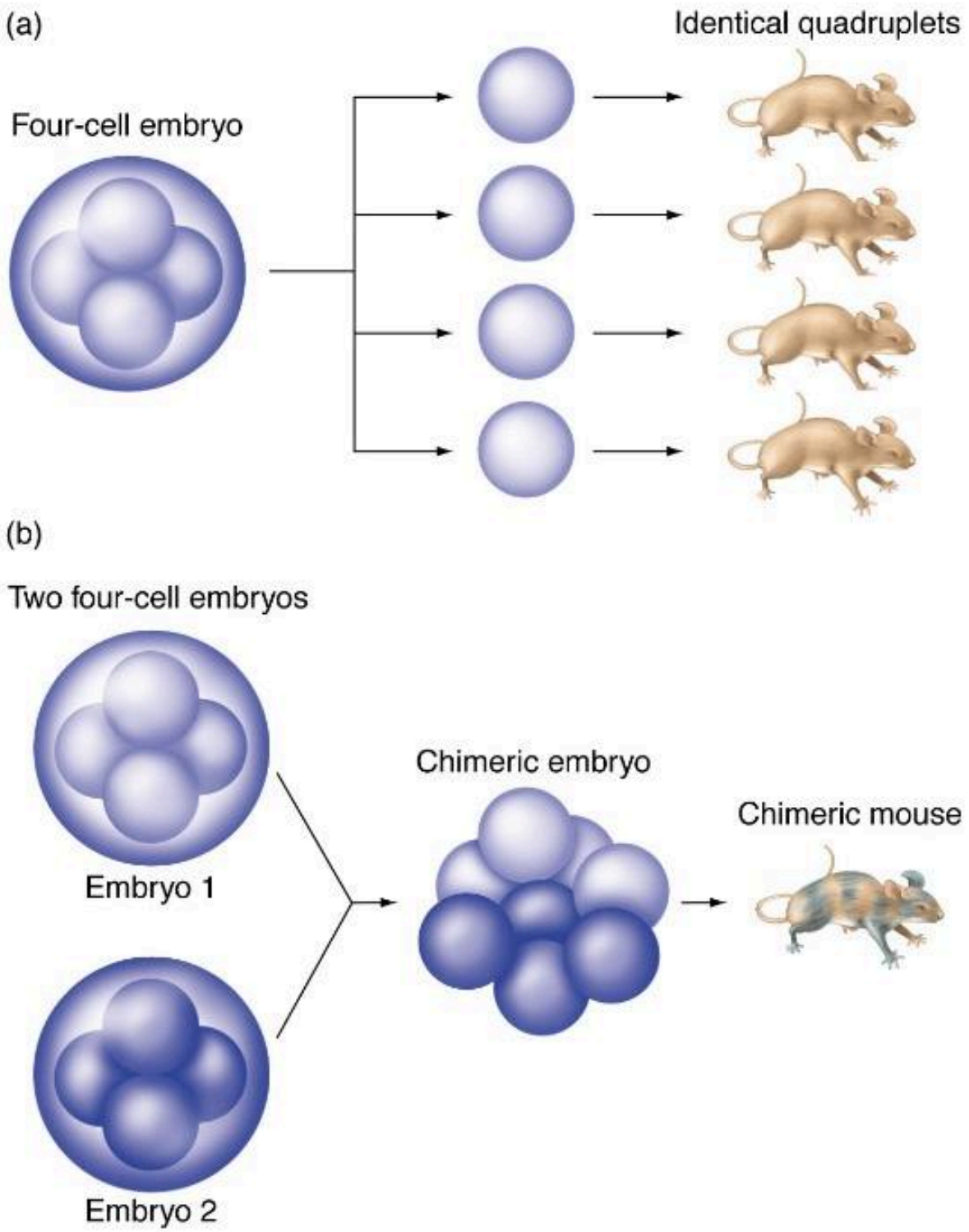
Oliver Smithies

1/3 of the prize

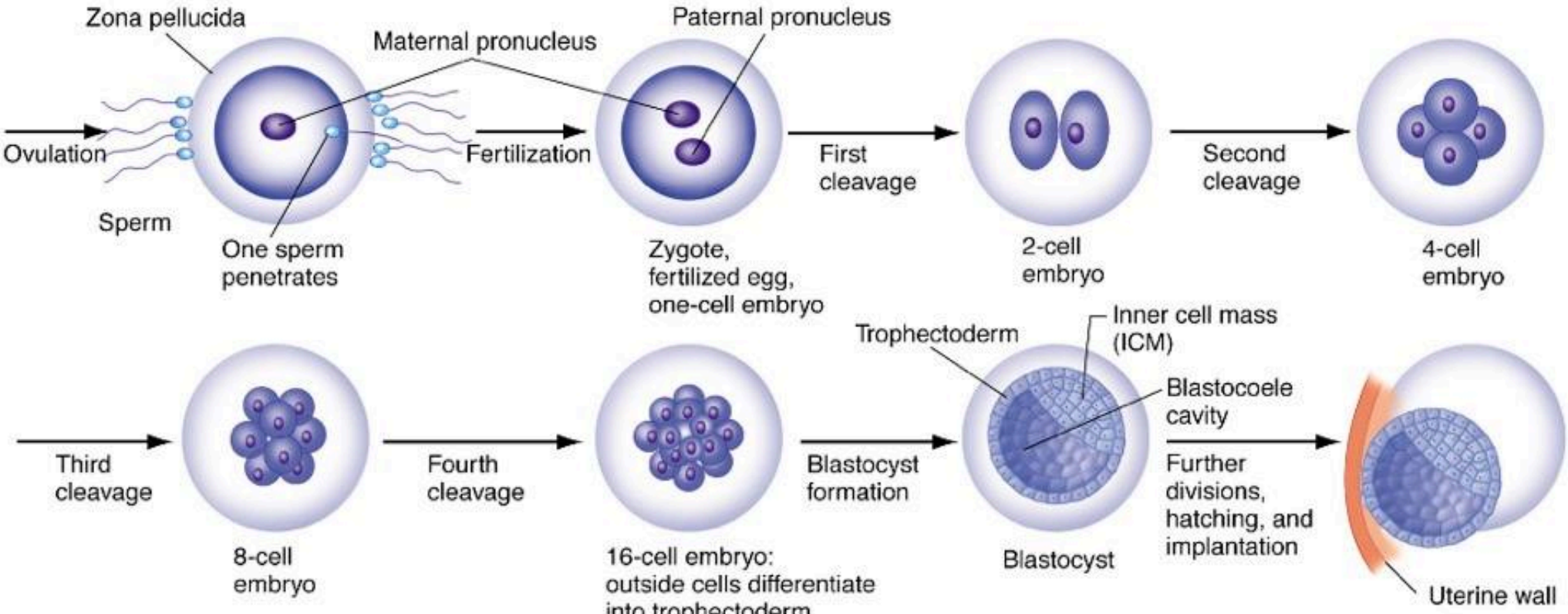
USA

University of North Carolina at Chapel Hill
Chapel Hill, NC, USA

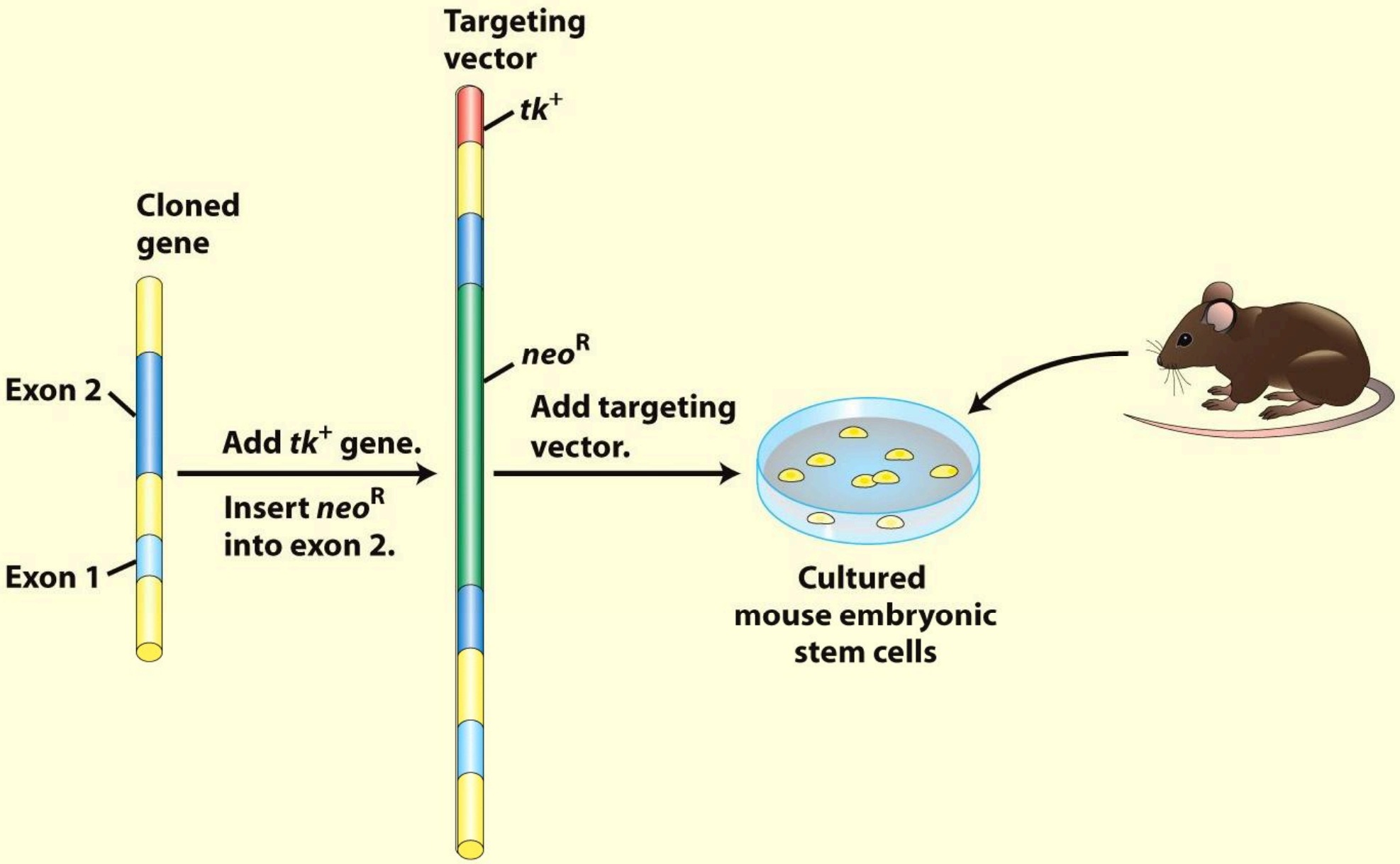
b. 1925
(in United Kingdom)

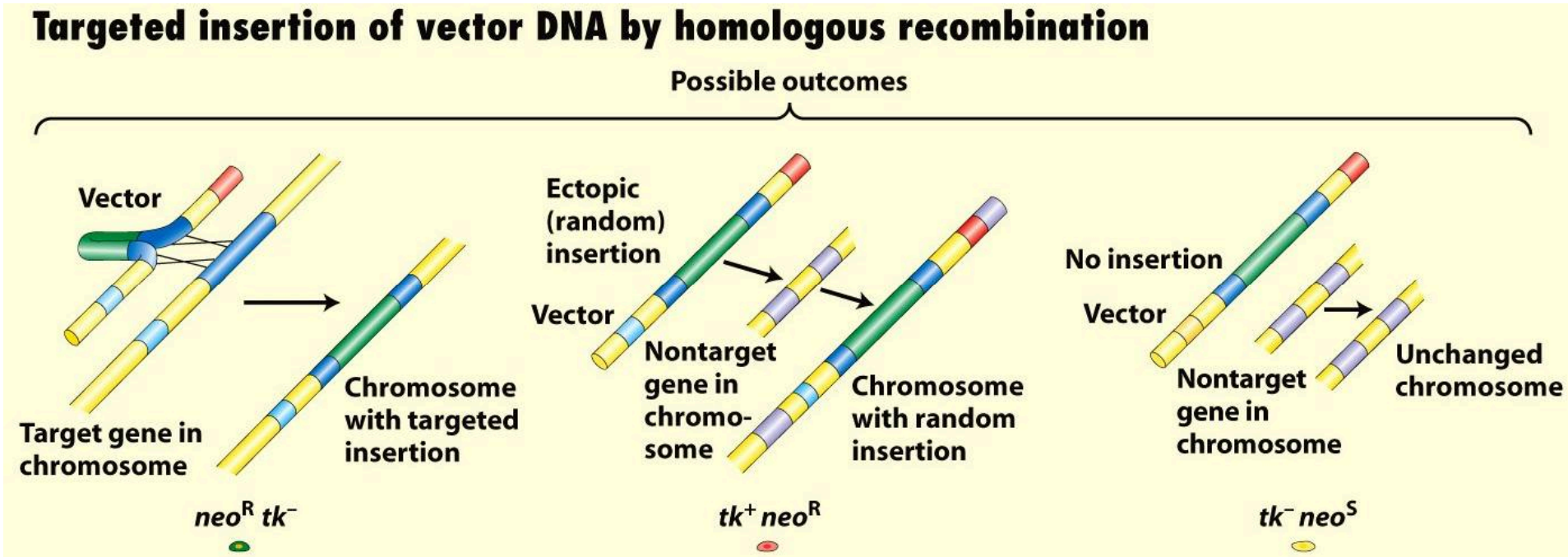

(a)
Identical quadruplets
Four-cell embryo
(b)
Two four-cell embryos
Chimeric embryo
Chimeric mouse
Embryo 1
Embryo 2

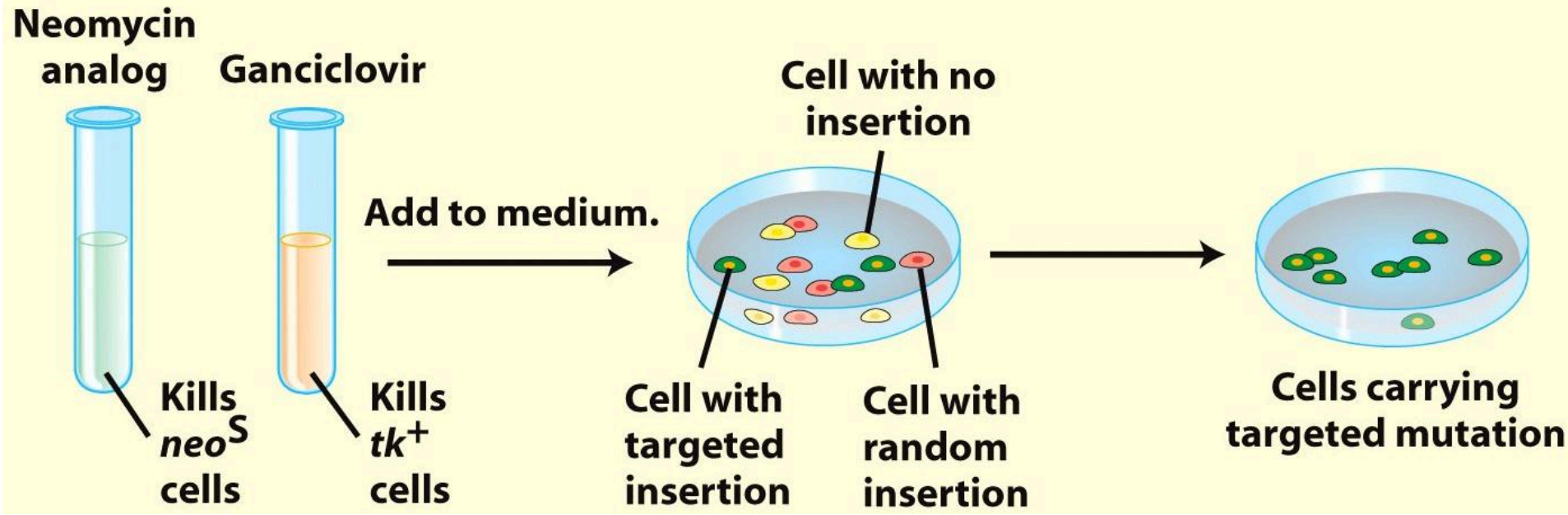
Preimplantation development and Implantation



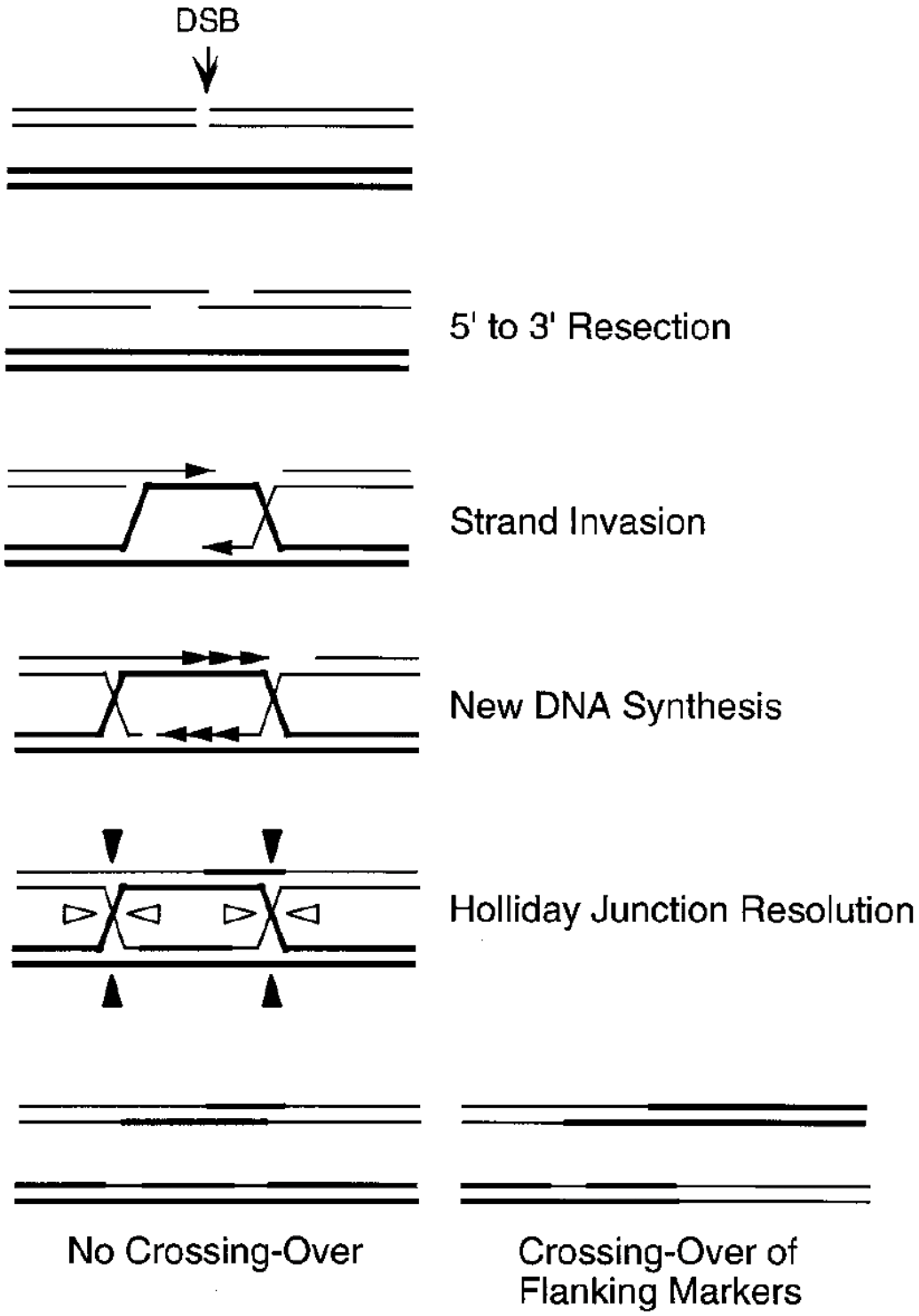
Producing cells containing a targeted gene knockout

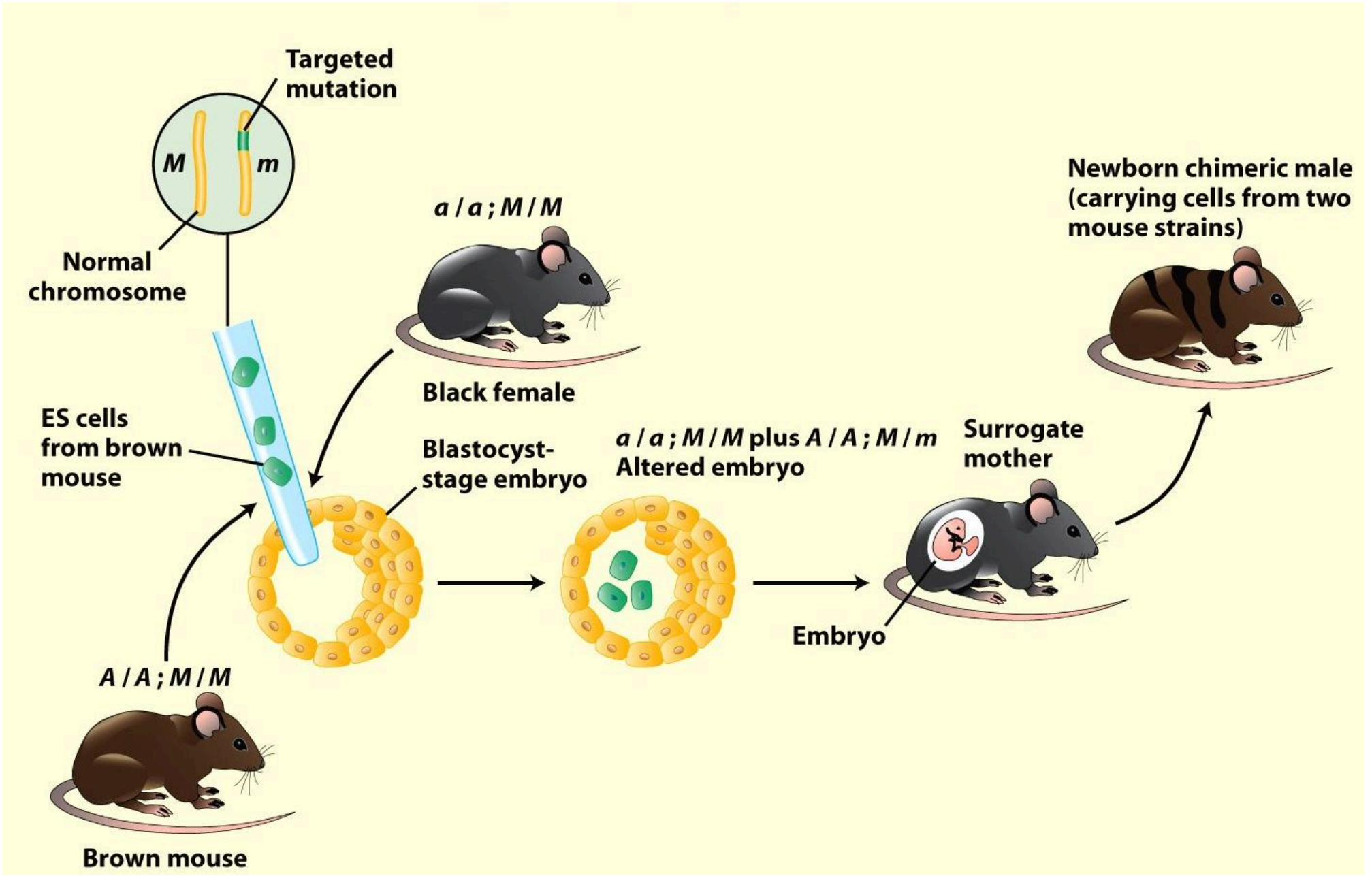


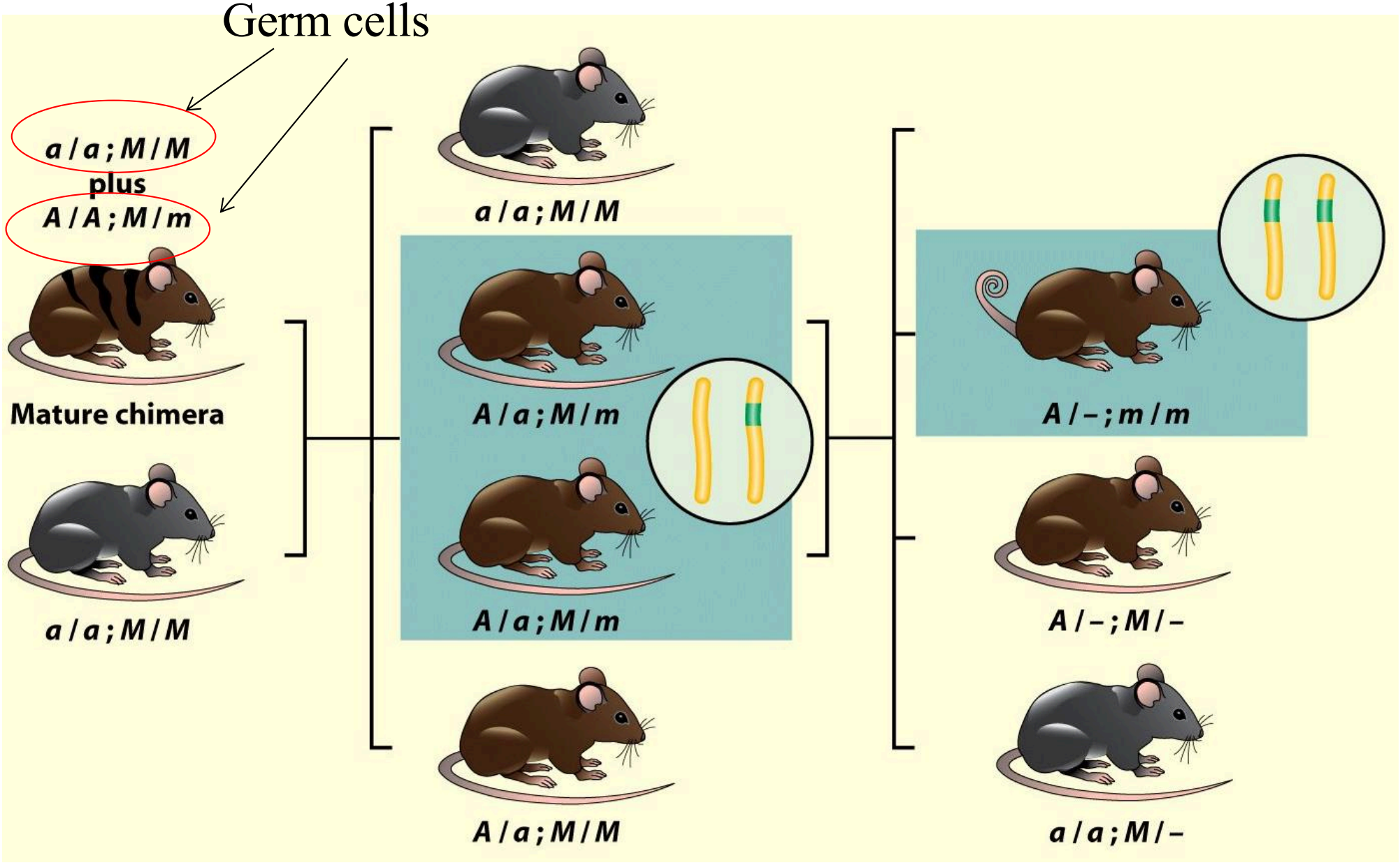

Targeted insertion of vector DNA by homologous recombination
Possible outcomes
Vector
Target gene in chromosome
Chromosome with targeted insertion
neoR tk−
Ectopic (random) insertion
Vector
Nontarget gene in chromo-some
Chromosome with random insertion
tk+ neoR
No insertion
Vector
Nontarget gene in chromosome
Unchanged chromosome
tk− neoS


Selection of cells with gene knockout
Neomycin analog
Ganciclovir
Add to medium.
Cell with no insertion
Kills neoS cells
Kills tk+ cells
Cell with targeted insertion
Cell with random insertion
Cells carrying targeted mutation

DSB formation is followed by 5'-to-3' resection of the ends. The resulting 3' ends are recombinogenic and can invade a homologous template, to initiate new DNA synthesis. Two HJs are formed and are resolved independently by cutting the crossed (open arrowhead) or noncrossed (closed arrowhead) strands, resulting in crossover or noncrossover products.

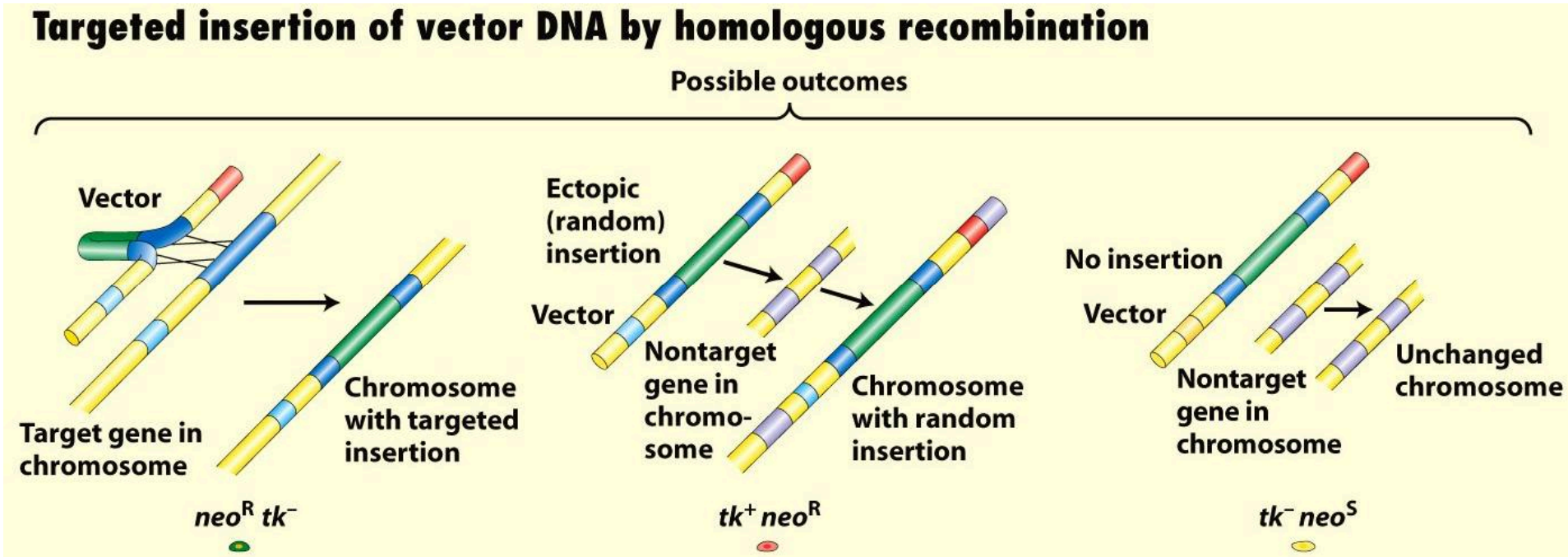


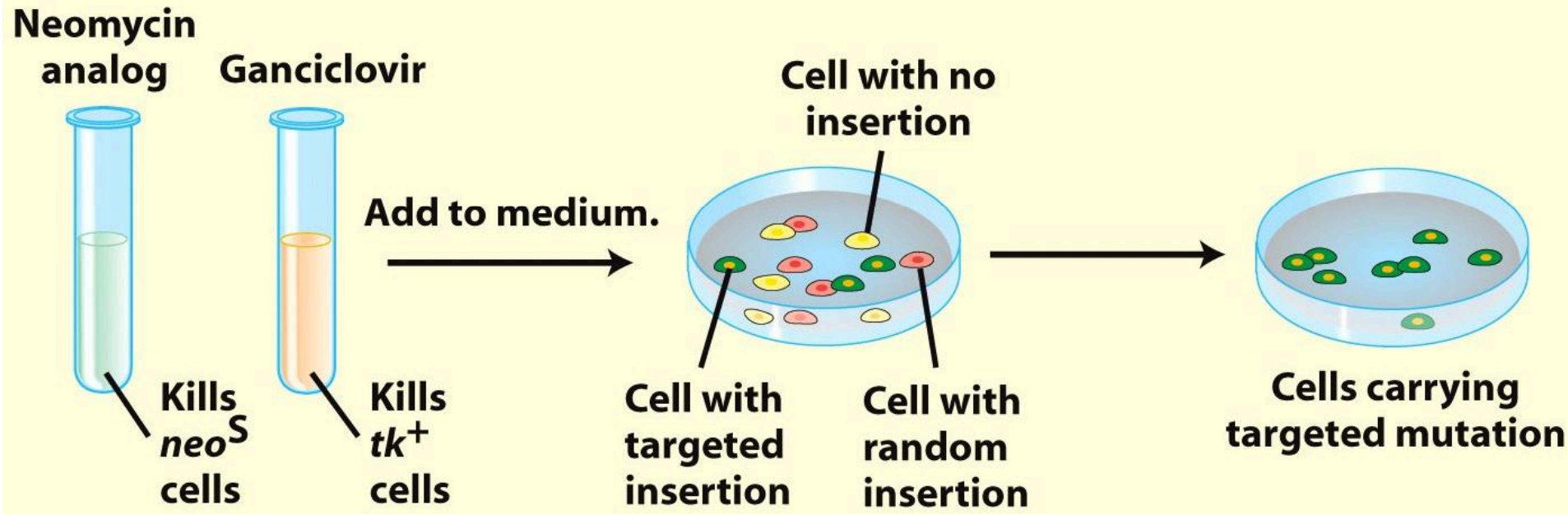

Targeted mutation
M
m
Normal chromosome
a / a ; M / M
Black female
Newborn chimeric male (carrying cells from two mouse strains)
ES cells from brown mouse
Blastocyst-stage embryo
a / a ; M / M plus A / A ; M / m Altered embryo
Surrogate mother
Embryo
A / A ; M / M
Brown mouse


Germ cells
a / a ; M / M
plus
A / A ; M / m
Mature chimera
a / a ; M / M
a / a ; M / M
A / a ; M / m
A / a ; M / m
A / a ; M / M
A / – ; m / m
A / – ; M / –
a / a ; M / –

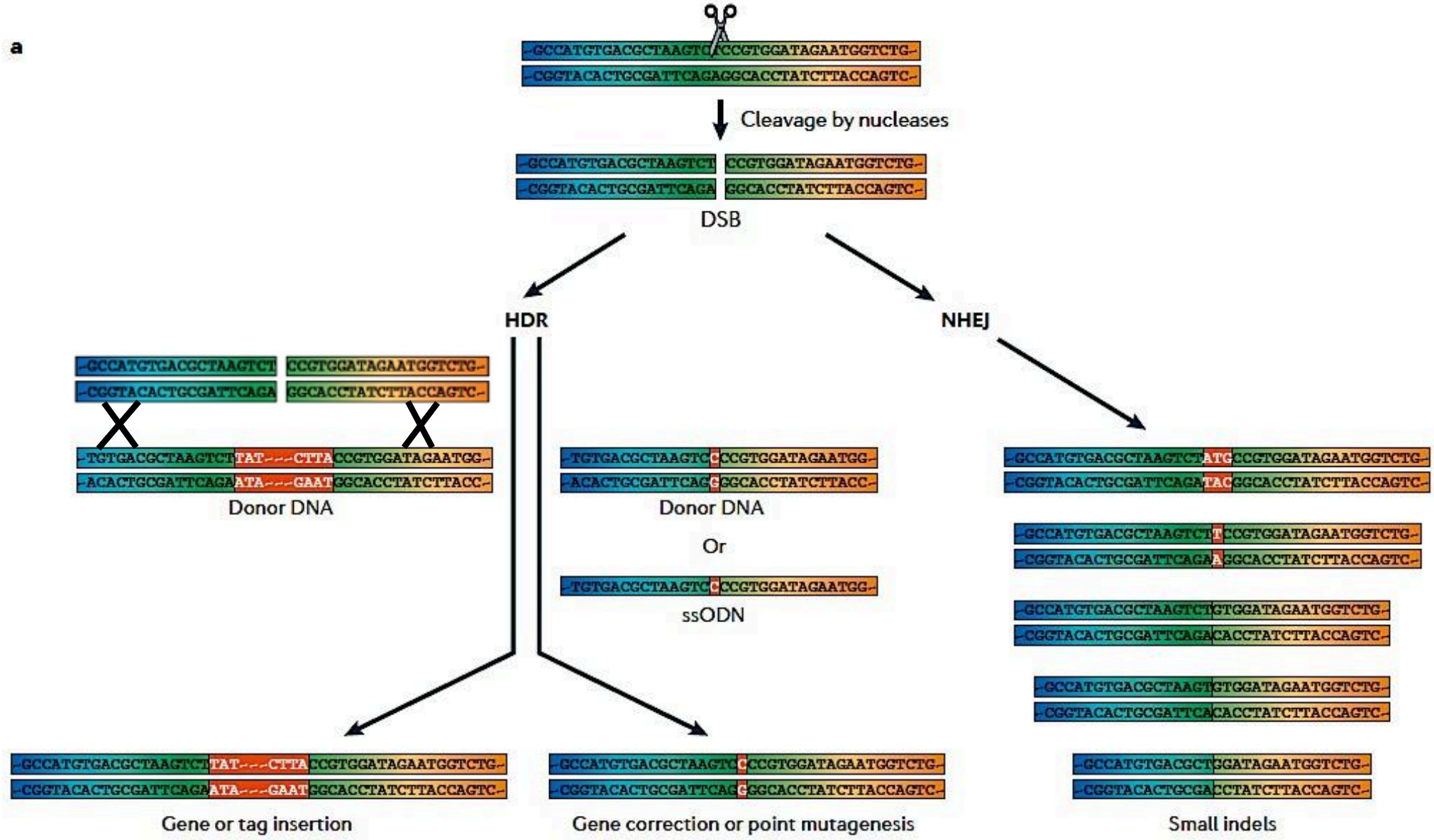
What drives the choice of homologous integration versus random integration?

Homologous recombination/integration is stimulated by the presence of a DNA break in one partner


Targeted insertion of vector DNA by homologous recombination
Possible outcomes
Vector
Target gene in chromosome
Chromosome with targeted insertion
neoR tk−
Ectopic (random) insertion
Vector
Nontarget gene in chromo-some
Chromosome with random insertion
tk+ neoR
No insertion
Vector
Nontarget gene in chromosome
Unchanged chromosome
tk− neoS


Selection of cells with gene knockout
Neomycin analog
Ganciclovir
Add to medium.
Cell with no insertion
Kills neoS cells
Kills tk+ cells
Cell with targeted insertion
Cell with random insertion
Cells carrying targeted mutation

Targeted generation of double-stranded DNA breaks can be used to introduce any desired mutation into a cell or organism



A breakthrough in genome engineering (called **genome editing**) uses engineered site-specific DNA binding proteins that have nuclease activity. The key steps are:

1. introduction of a site-specific nuclease;

2. generation of a double-strand break;

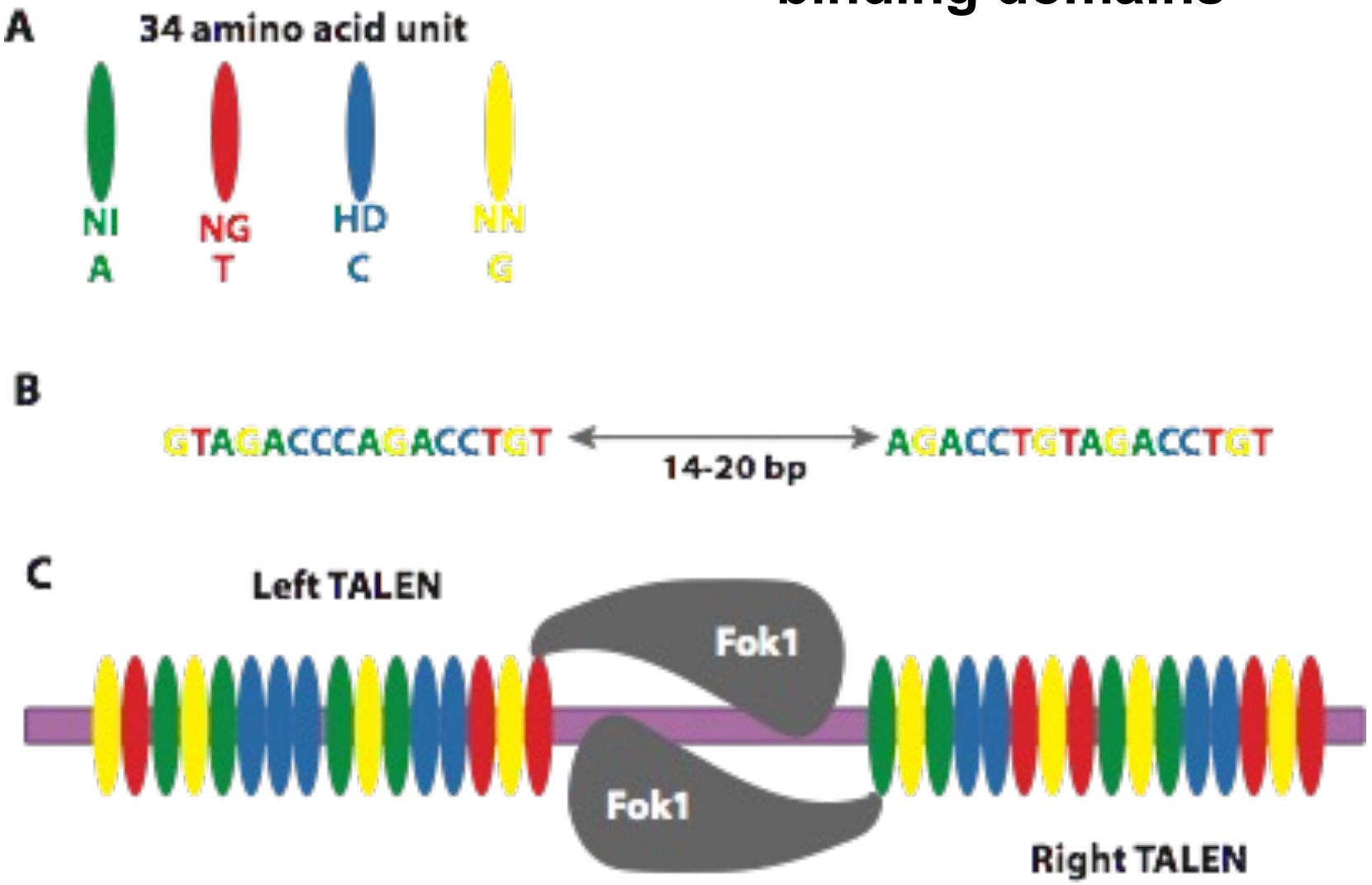
3. repair of the break can introduce a mutation by

a. non-homologous end joining, which is error-prone (for example, DNA is chewed back by endonucleases.

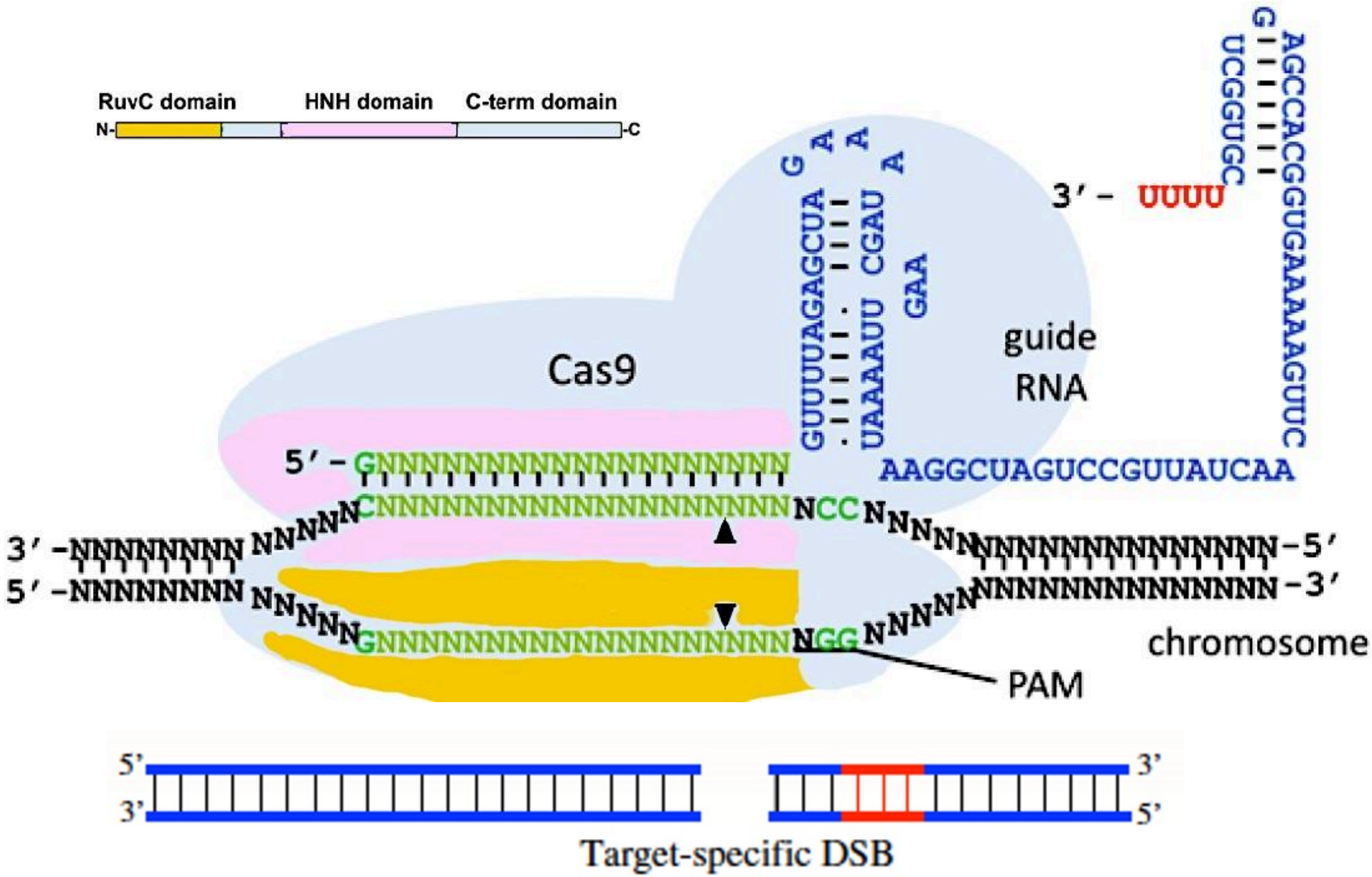
b. Homology directed repair converts the allele that has been cut.

TALENs

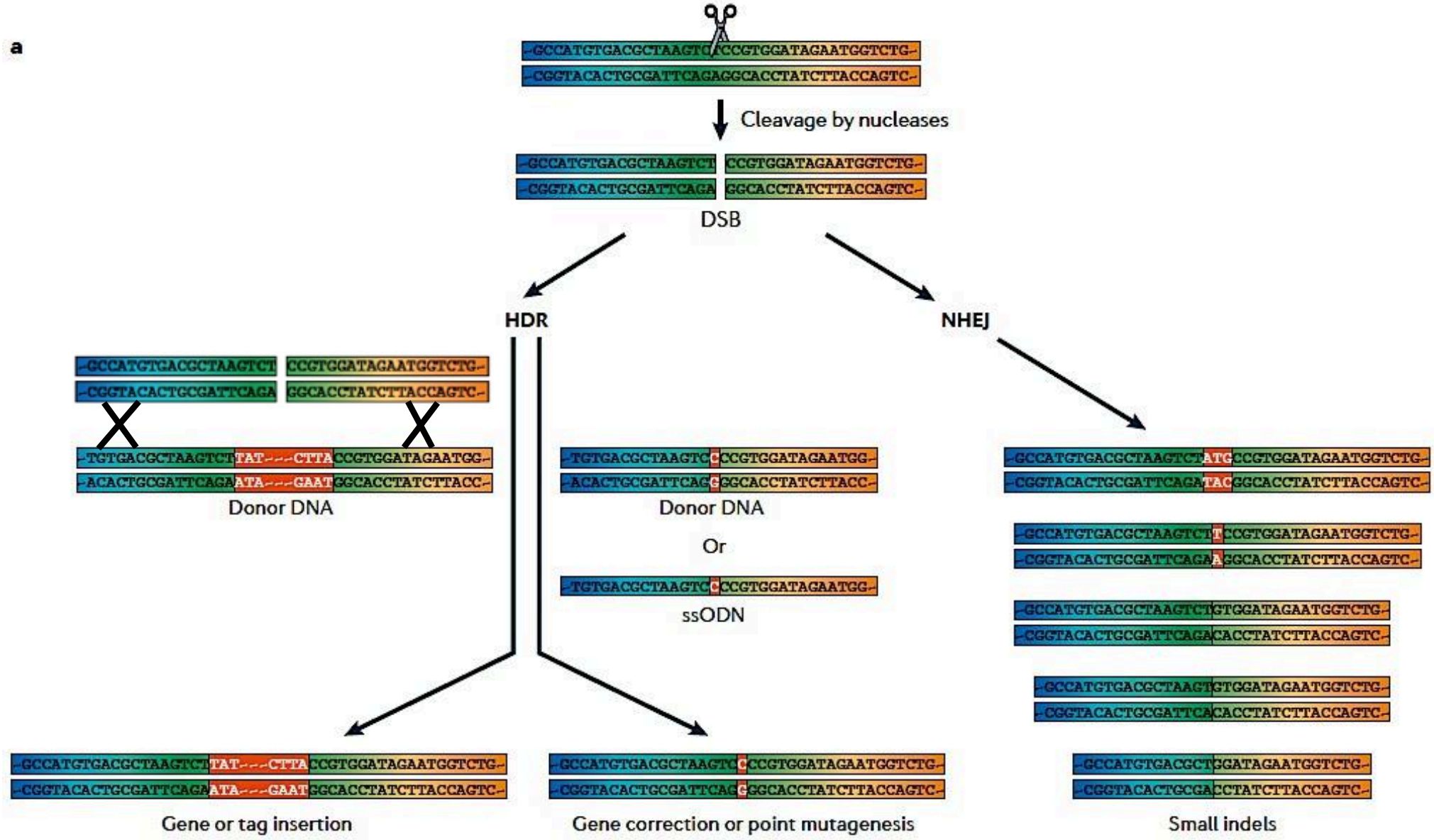
or Zinc Finger DNA binding domains

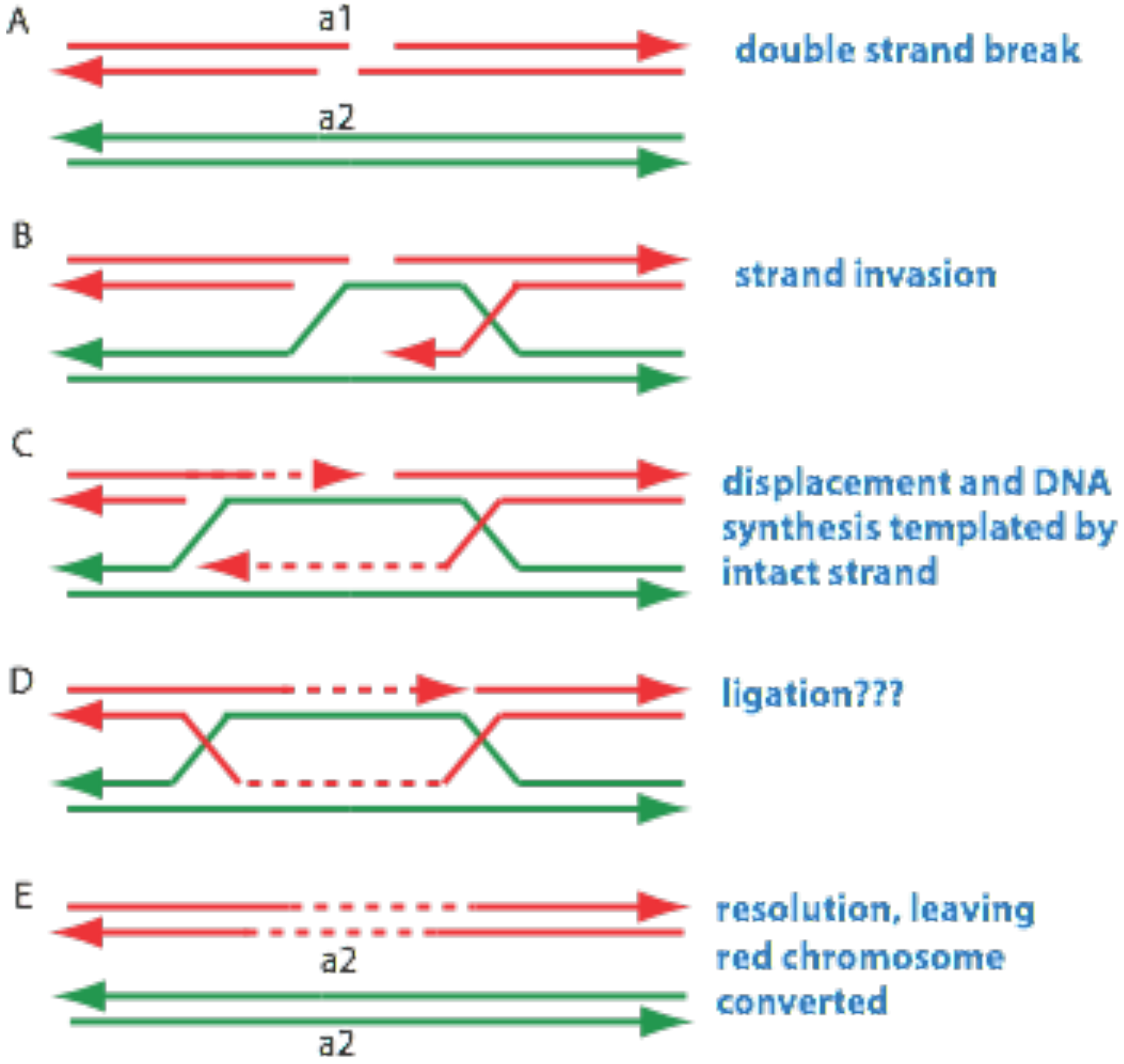


Programmable DNA cleavage using Cas9 and one or more guide RNAs

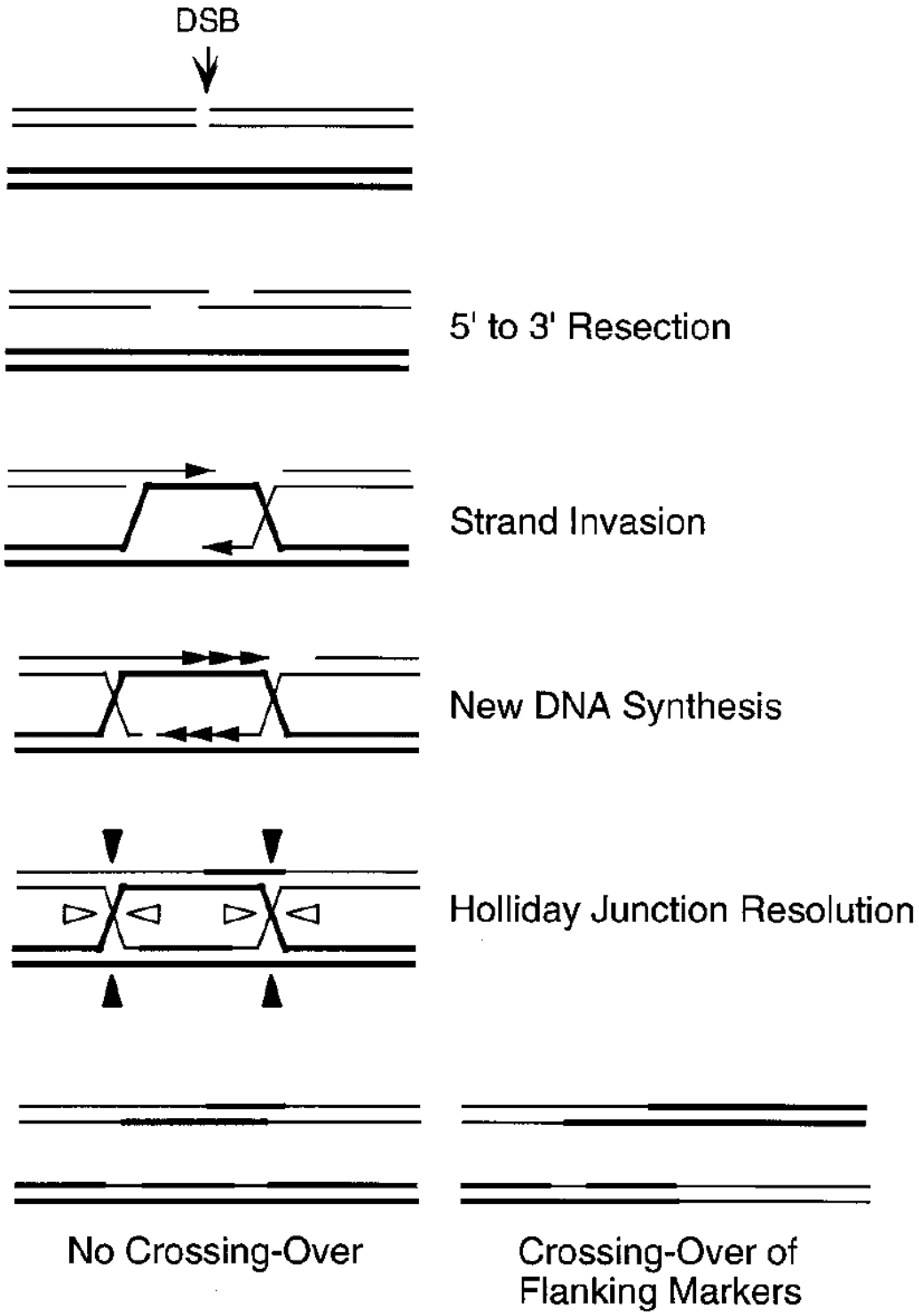


Targeted generation of double-stranded DNA breaks can be used to introduce any desired mutation into a cell or organism

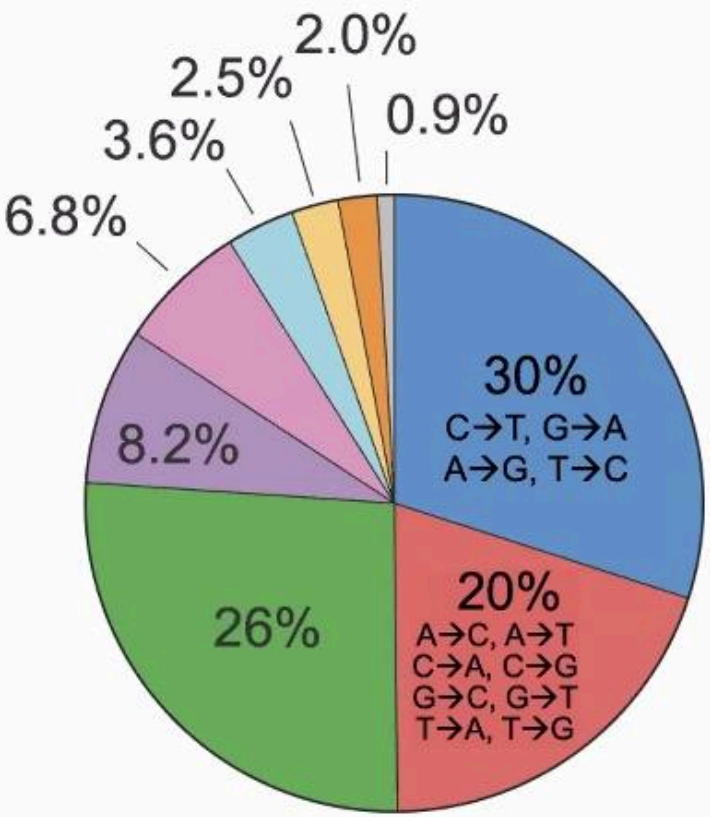



A
a1
a2
double strand break
B
strand invasion
C
displacement and DNA synthesis templated by intact strand
D
ligation???
E
a2
a2
resolution, leaving red chromosome converted

DSB formation is followed by 5'-to-3' resection of the ends. The resulting 3' ends are recombinogenic and can invade a homologous template, to initiate new DNA synthesis. Two HJs are formed and are resolved independently by cutting the crossed (open arrowhead) or noncrossed (closed arrowhead) strands, resulting in crossover or noncrossover products.



Pathogenic Human Genetic Variants



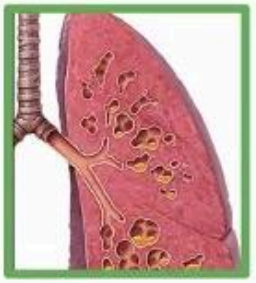
Total variants > 75,000

- Transition point mutation
- Transversion point mutation
- Deletion
- Duplication
- Copy number loss
- Copy number gain
- Insertion
- Insertion and deletion
- Other

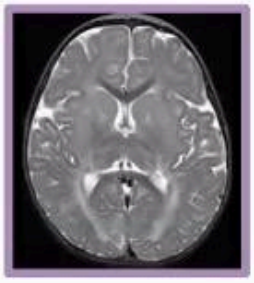
LMNA c.1824C→T

HBB E6V(A→T)

CFTR ΔF508



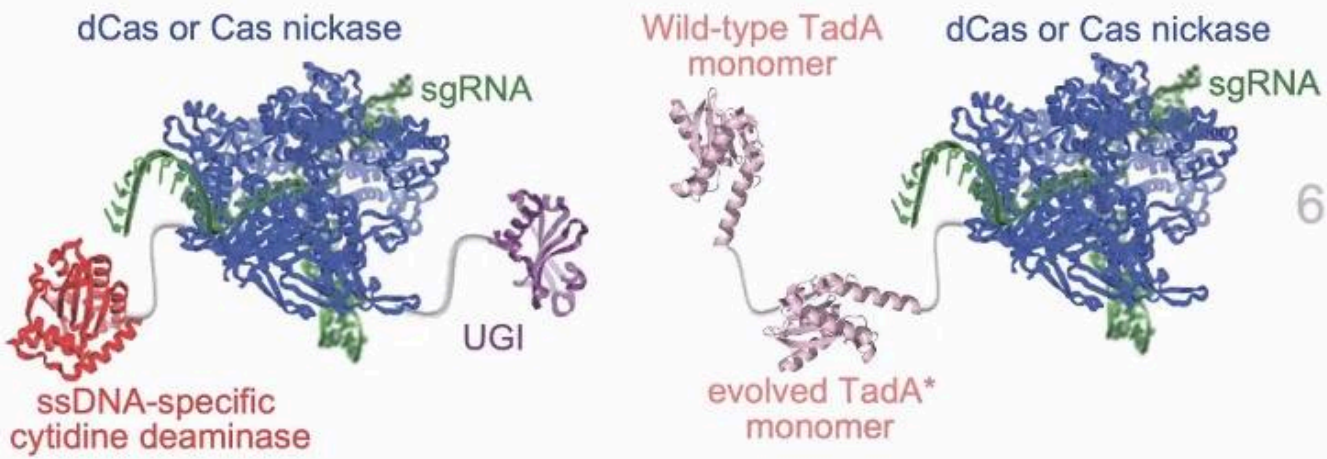
HEXA c.1278+TATC



- Programmable nucleases (DSBs → indels or HDR) are well-suited to install or correct only a minority of pathogenic alleles in most cell types

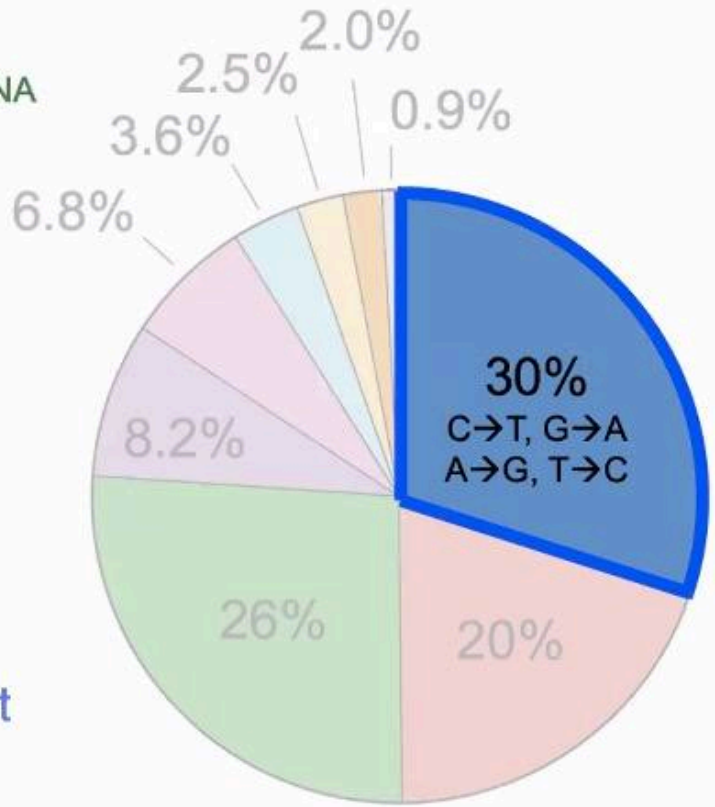
ClinVar database: Landrum et al., *Nucl. Acids Res.* **44**, D862 (2016), inDelphi: Shen, Arbab, Liu, Gifford, Sherwood *et al. Nature* **563**, 646 (2018

Base Editors Make Targeted Transition Point Mutations



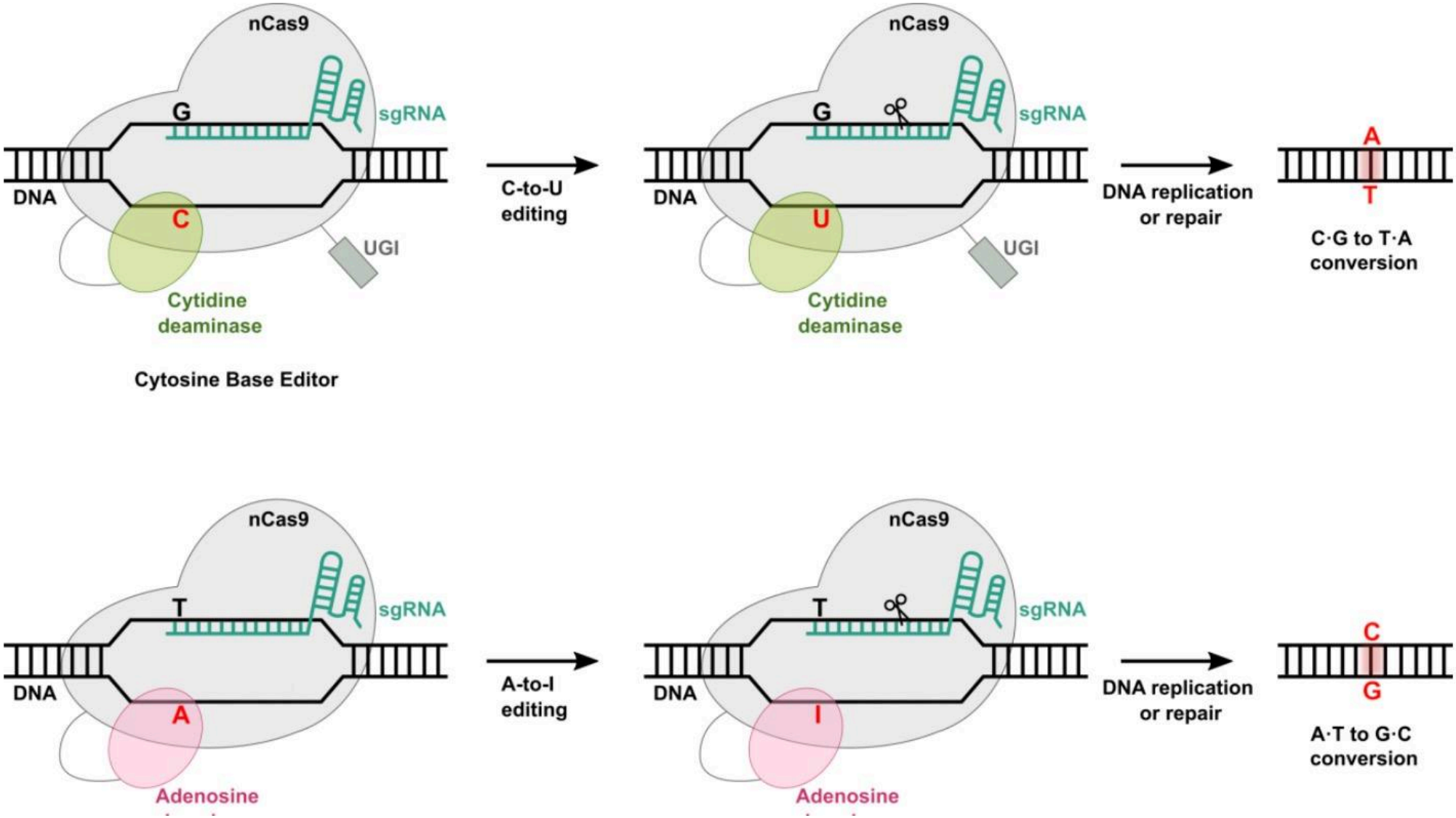
Cytosine base editor (CBE)
C → T
G → A

Adenine base editor (ABE)
A → G
T → C



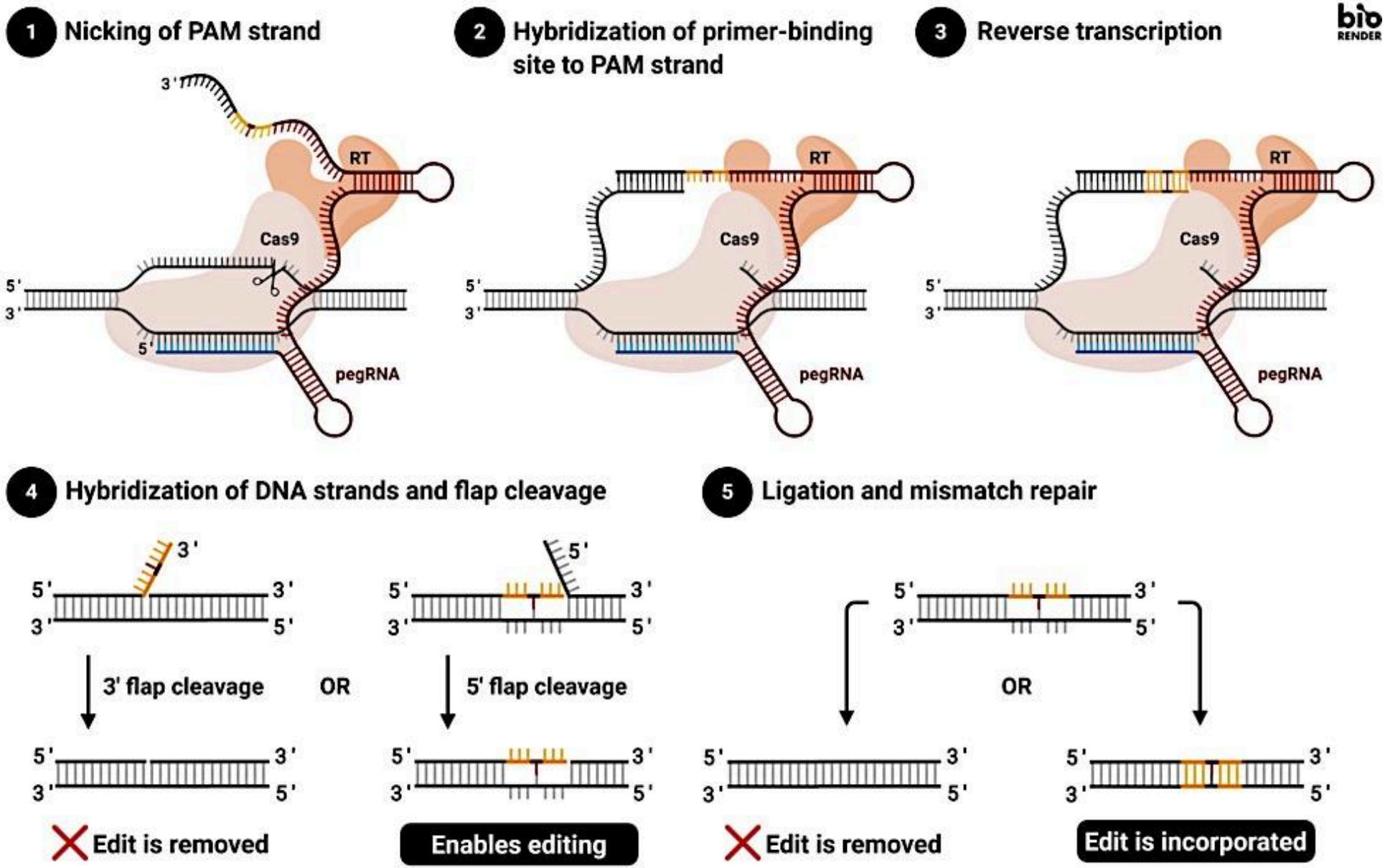
- Base editors mediate all four transition mutations at target sites without requiring DSBs or donor DNA templates

Komor, Kim, Packer, Zuris, Liu *Nature* **533**, 420 (2016); Nishida, Kondo *et al. Science* **353**, aaf8729 (2016);
Gaudelli, Komor, Rees, Packer, Badran, Bryson, Liu *Nature* **551**, 464 (2017); Rees & Liu, *Nat. Rev. Genet.* **19**, 770 (2018)



Inosine read as a G

Prime editing: Reverse transcription of pegRNA followed by incorporation new DNA into target DNA



Chromosome rearrangements

and

Changes in chromosome number

Rearrangements and ploidy

Up until now we have assumed that the arrangement or order of genes on chromosomes in any species is fixed--and that the number and type of chromosomes is unchanging. This is usually true, but sometimes in nature, and more frequently in the laboratory (due to the use of mutagens such as X-rays) changes in the arrangements of genes and chromosomes does occur.

<u>Examples of some types of chromosomal arrangements are :</u>
1. Inversions

2. Deficiencies or deletions

3. Duplications

4. Translocations

5. Changes in number chromosome number, or ploidy.

<u>These changes are important for a number of reasons.</u>
1. They result in alterations in apparent map distances between genetic units.

2. They may lead to altered segregation ratios that manifest themselves as semisterility or a high incidence of miscarriage.

3. They may lead to phenotypic changes resulting from changes in the spatial and temporal patterns of gene expression, as well as alterations in gene balance.

The Nobel Prize in Physiology or Medicine 2010

Robert G. Edwards

The Nobel Prize in Physiology or Medicine 2010

Summary

Prize Announcement

Press Release

Advanced Information

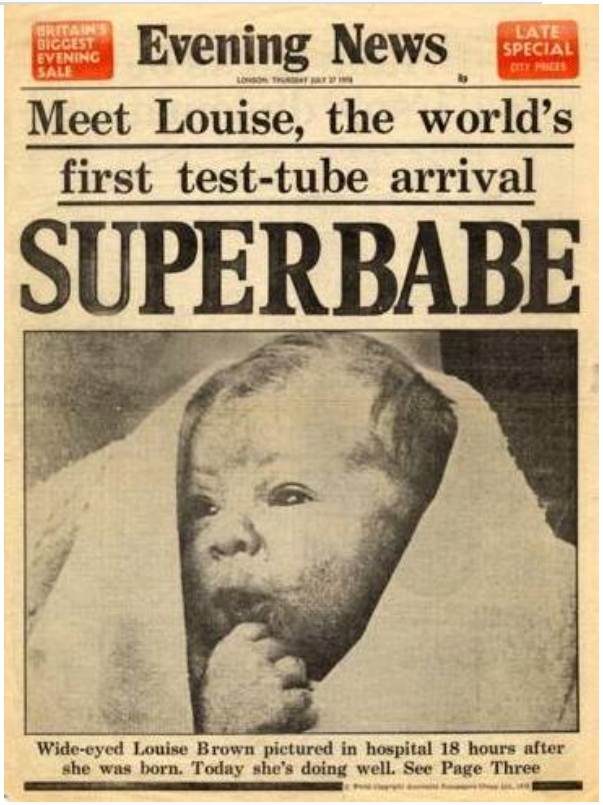
Speed Read

Robert G. Edwards

~2% of all babies born since 1981

Photo: Sheikh Hamdan Bin Rashid Al Maktoum Award for Medical Sciences

Robert G. Edwards



Evening News

Meet Louise, the world's first test-tube arrival

SUPERBABE

Wide-eyed Louise Brown pictured in hospital 18 hours after she was born. Today she's doing well. See Page Three

The Nobel Prize in Physiology or Medicine 2010 was awarded to Robert G. Edwards *"for the development of in vitro fertilization"*.

% surviving since birth, humans

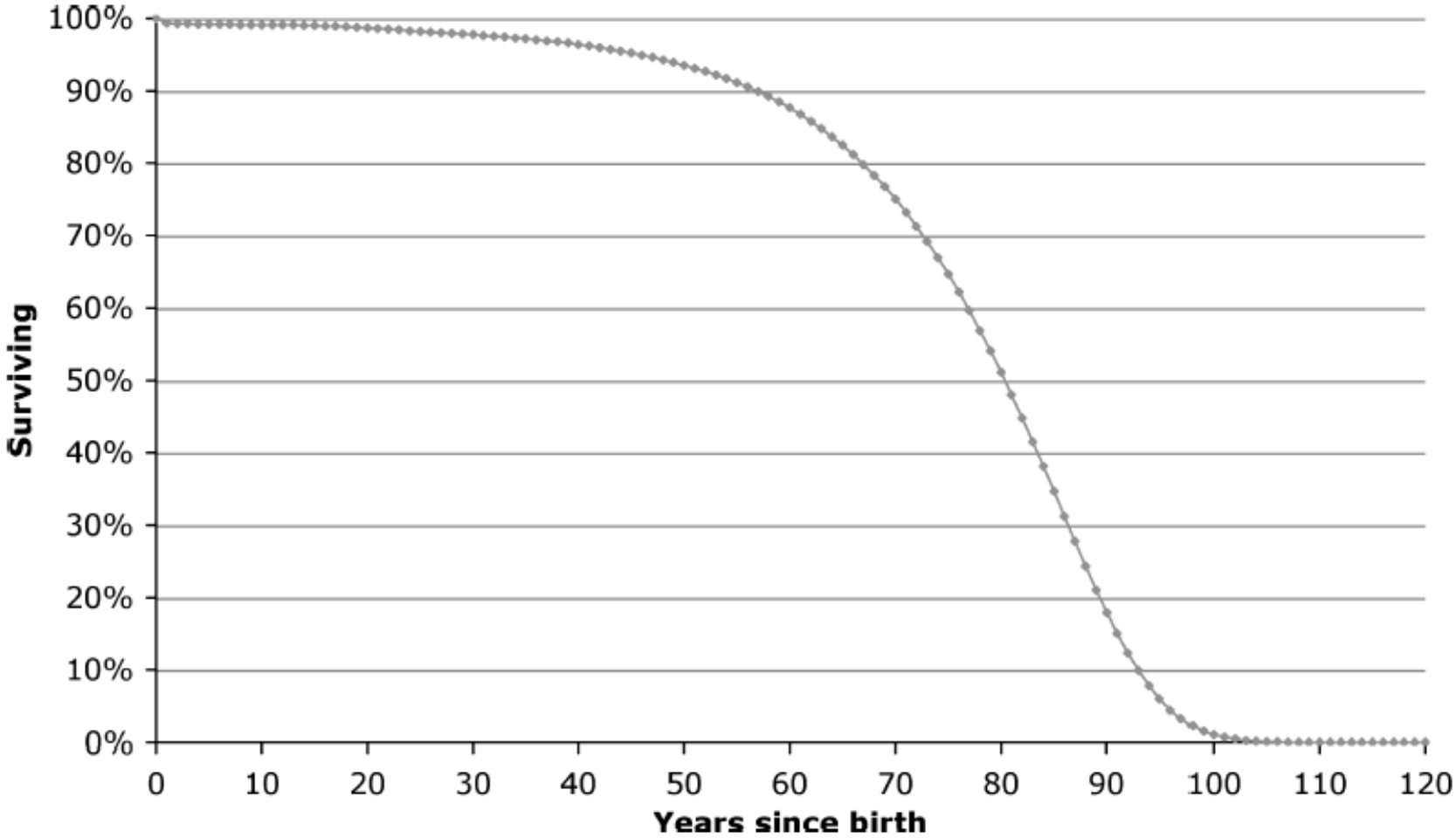
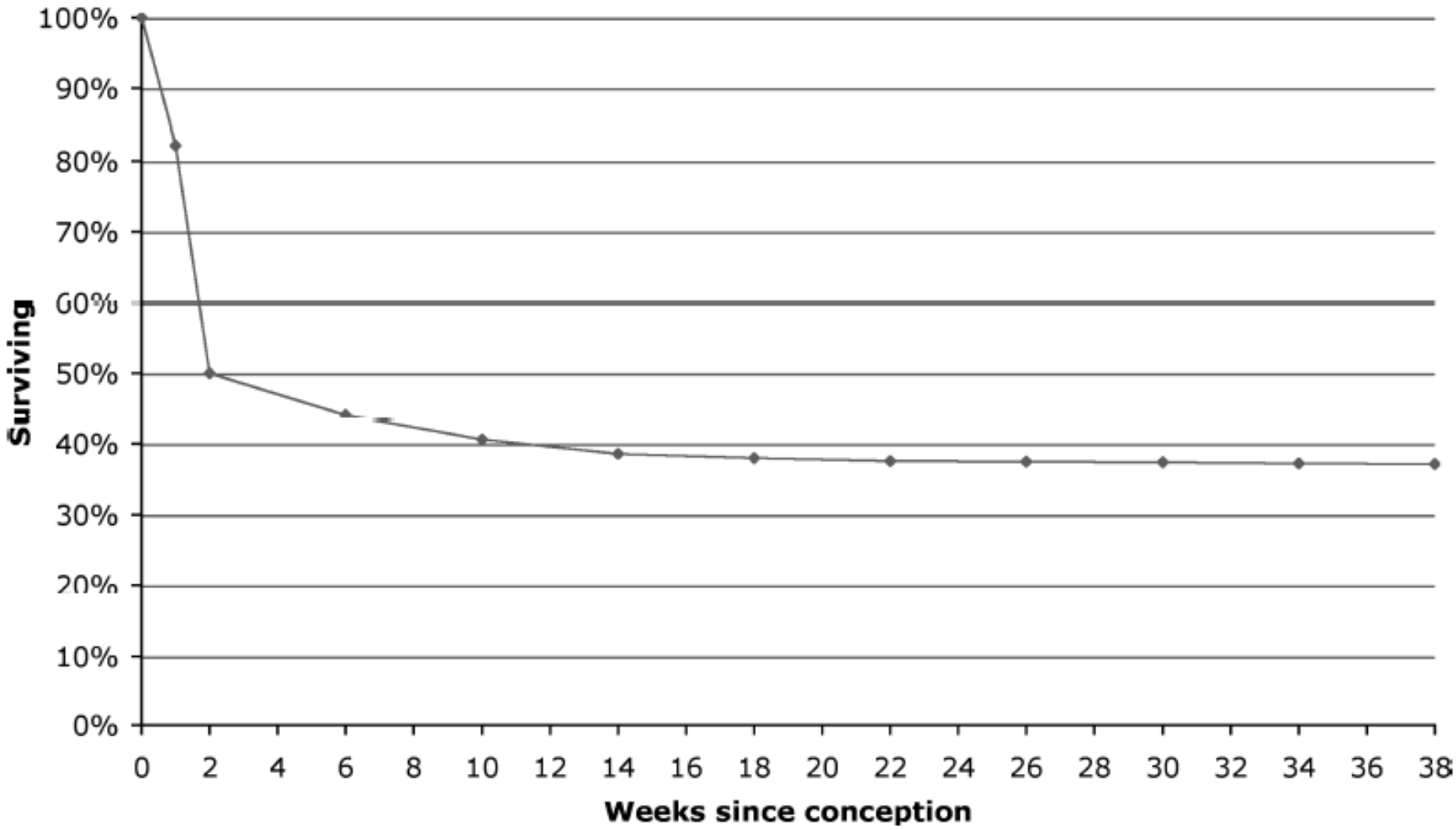


Figure 2. Graph of survival and years since birth for the United States population (US Social Security Administration 2002).

% human embryo survival as a function of time.



gure 1. Intrauterine life: Graph of survival and weeks since conception.

% human survival, from conception onwards

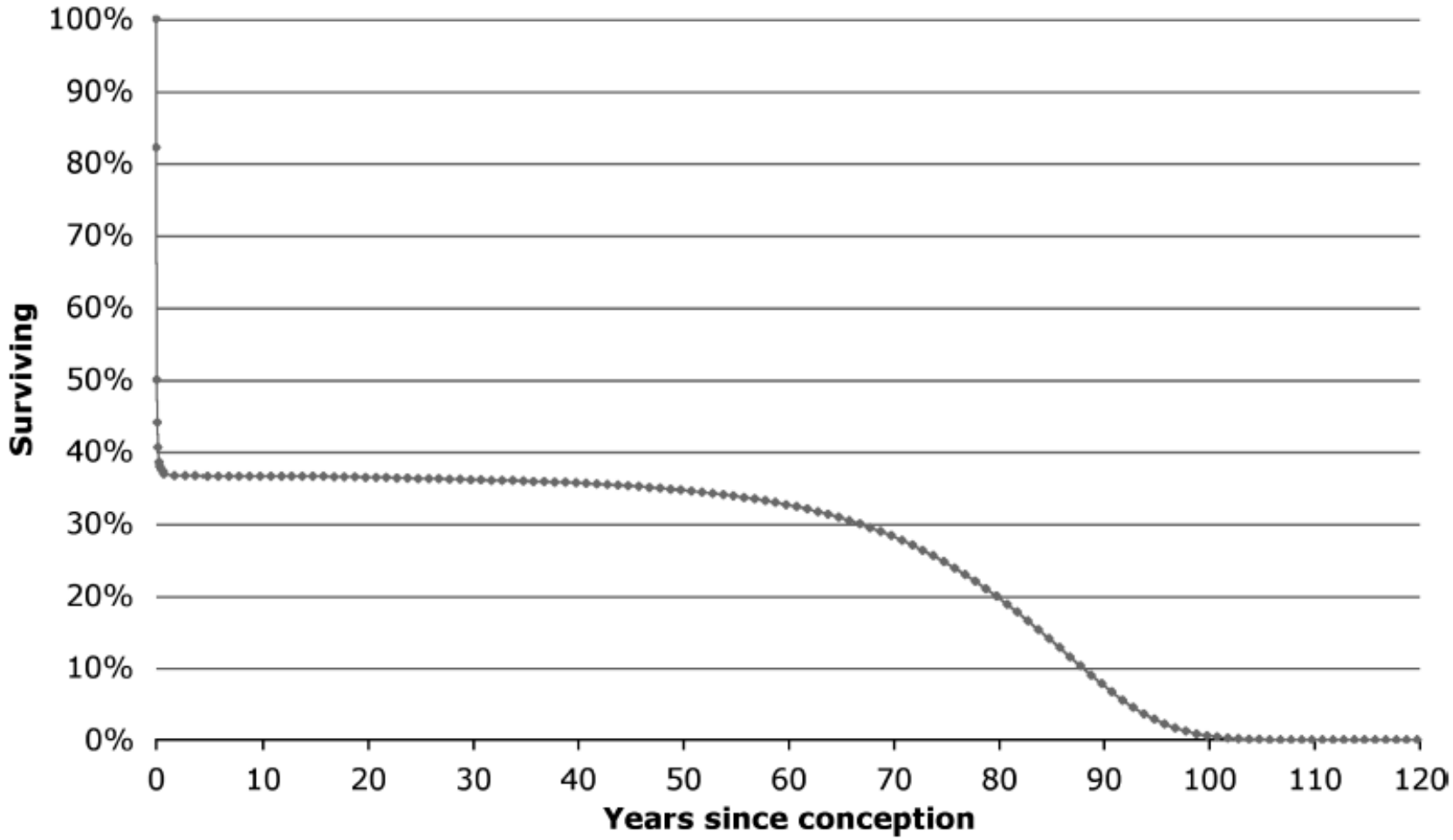


Figure 3. Graph of mortality in the United States from conception through to death.

Larsen et al. BMC Medicine 2013, 11:154
http://www.biomedcentral.com/1741-7015/11/154

BMC Medicine

REVIEW Open Access

New insights into mechanisms behind miscarriage

Elisabeth Clare Larsen[1], Ole Bjarne Christiansen[1], Astrid Marie Kolte[1] and Nick Macklon[1,2,3,4,5*]

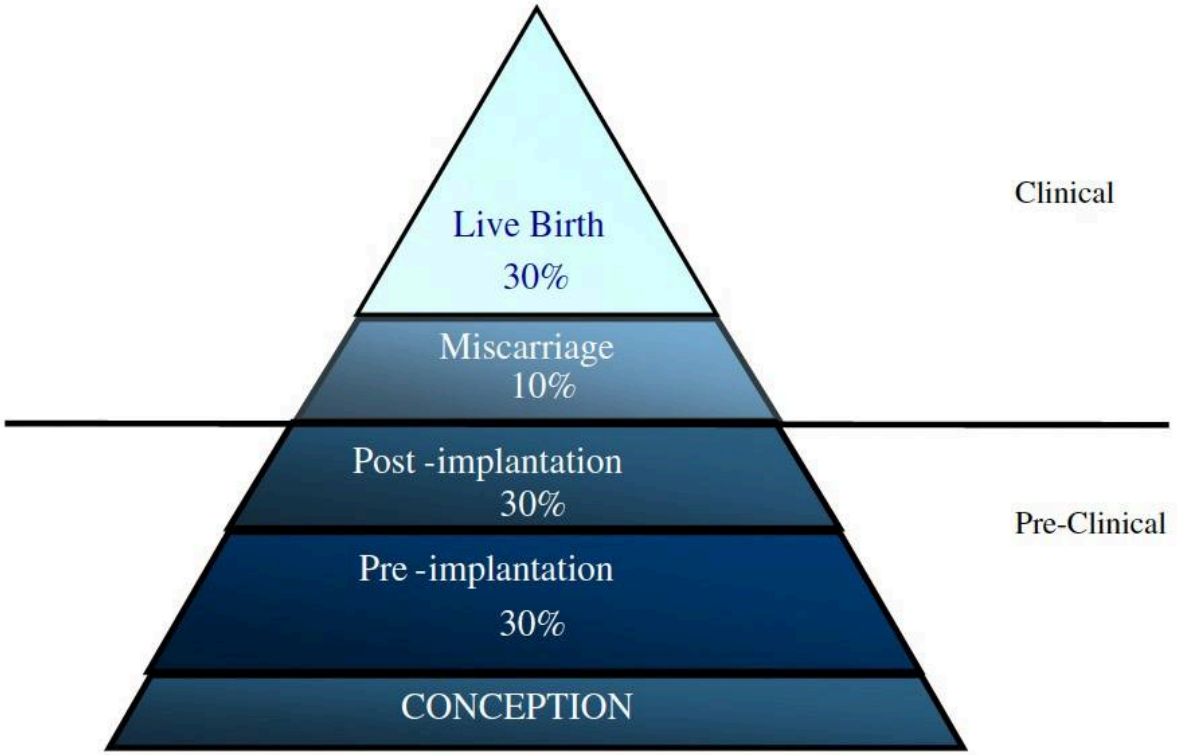


Figure 1 The pregnancy loss iceberg: an overview of the outcome of spontaneous human conceptions. It is estimated that 70% of conceptions are lost prior to live birth. The majority of these losses occur prior to implantation or before the missed menstrual period, and since they are not revealed to the woman they are termed preclinical. In the pregnancy loss 'iceberg', they are therefore below the 'waterline'. Figure reproduced with permission from Oxford University Press [37].

More than 66% of fertilized human eggs (embryos) have chromosome abnormalities

Reprod Biomed Online. 2007 May;14(5):628-34.

Maternal age, morphology, development and chromosome abnormalities in over 6000 cleavage-stage embryos.

Munné S, Chen S, Colls P, Garrisi J, Zheng X, Cekleniak N, Lenzi M, Hughes P, Fischer J, Garrisi M, Tomkin G, Cohen J.

Reprogenetics, LLC, 3 Regent Street, Livingston, NJ 07039, USA. munne@embryos.net

Abstract

Previous studies assessing the relationship between embryo development, maternal age and chromosome abnormalities were either small or analysed mostly embryos not suitable for replacement. The present study includes >6000 embryos, including many suitable for replacement. Embryos with the best morphology and development were 44% euploid in patients younger than 35, decreasing to 21% in patients 41 and older. The worst morphology group had only 30% normal embryos from patients younger than 35, and 12% in embryos from patients 41 and older. Thus morphological analysis was able to improve the population of normal embryos only from 30 to 44% in the best of cases. Regarding specific abnormalities, 20% of embryos were aneuploid, 32% aneuploid plus other abnormalities, and the rest had post-meiotic abnormalities. Of those, only aneuploidy increased with maternal age. There were no big differences in the frequency of chromosome abnormalities depending on patient indication, within a similar age group. In summary, previous trends detected in suboptimal embryos were also confirmed in the best embryos for replacement. Although dysmorphism and advanced maternal age are both related to chromosome abnormalities, these parameters can yield at most <50% euploid embryos, and other techniques such as preimplantation diagnosis are required to ensure that only euploid embryos are replaced.

OPEN ACCESS Freely available online

PLoS one

Aneuploidy and Confined Chromosomal Mosaicism in the Developing Human Brain

Yuri B. Yurov[1,2☯*], Ivan Y. Iourov[1,2☯], Svetlana G. Vorsanova[1,2☯], Thomas Liehr[3], Alexei D. Kolotii[2], Sergei I. Kutsev[4], Franck Pellestor[5], Alfia K. Beresheva[1,2], Irina A. Demidova[1,2], Viktor S. Kravets[2], Viktor V. Monakhov[1], Ilia V. Soloviev[1]

1 National Research Center of Mental Health, Russian Academy of Medical Sciences, Moscow, Russia, **2** Institute of Pediatrics and Children Surgery, Roszdrav, Moscow, Russia, **3** Institute of Human Genetics and Anthropology, Jena, Germany, **4** Rostov State Medical University, Roszdrav, Rostov-on-Don, Russia, **5** Institute of Human Genetics, Montpellier, France

Background. Understanding the mechanisms underlying generation of neuronal variability and complexity remains the central challenge for neuroscience. Structural variation in the neuronal genome is likely to be one important mechanism for neuronal diversity and brain diseases. Large-scale genomic variations due to loss or gain of whole chromosomes (aneuploidy) have been described in cells of the normal and diseased human brain, which are generated from neural stem cells during intrauterine period of life. However, the incidence of aneuploidy in the developing human brain and its impact on the brain development and function are obscure. ***Methodology / Principal Findings.*** To address genomic variation during development we surveyed aneuploidy/polyploidy in the human fetal tissues by advanced molecular-cytogenetic techniques at the single-cell level. Here we show that the human developing brain has mosaic nature, being composed of euploid and aneuploid neural cells. Studying over 600,000 neural cells, we have determined the average aneuploidy frequency as 1.25–1.45% per chromosome, with the overall percentage of aneuploidy tending to approach 30–35%. Furthermore, we found that mosaic aneuploidy can be exclusively confined to the brain. ***Conclusions / Significance.*** Our data indicates aneuploidization to be an additional pathological mechanism for neuronal genome diversification. These findings highlight the involvement of aneuploidy in the human brain development and suggest an unexpected link between developmental chromosomal instability, intercellural/intertissular genome diversity and human brain diseases.

Citation: Yurov YB, Iourov IY, Vorsanova SG, Liehr T, Kolotii AD, et al (2007) Aneuploidy and Confined Chromosomal Mosaicism in the Developing Human Brain. PLoS ONE 2(6): e558. doi:10.1371/journal.pone.0000558

SCIENTIFIC AMERICAN®

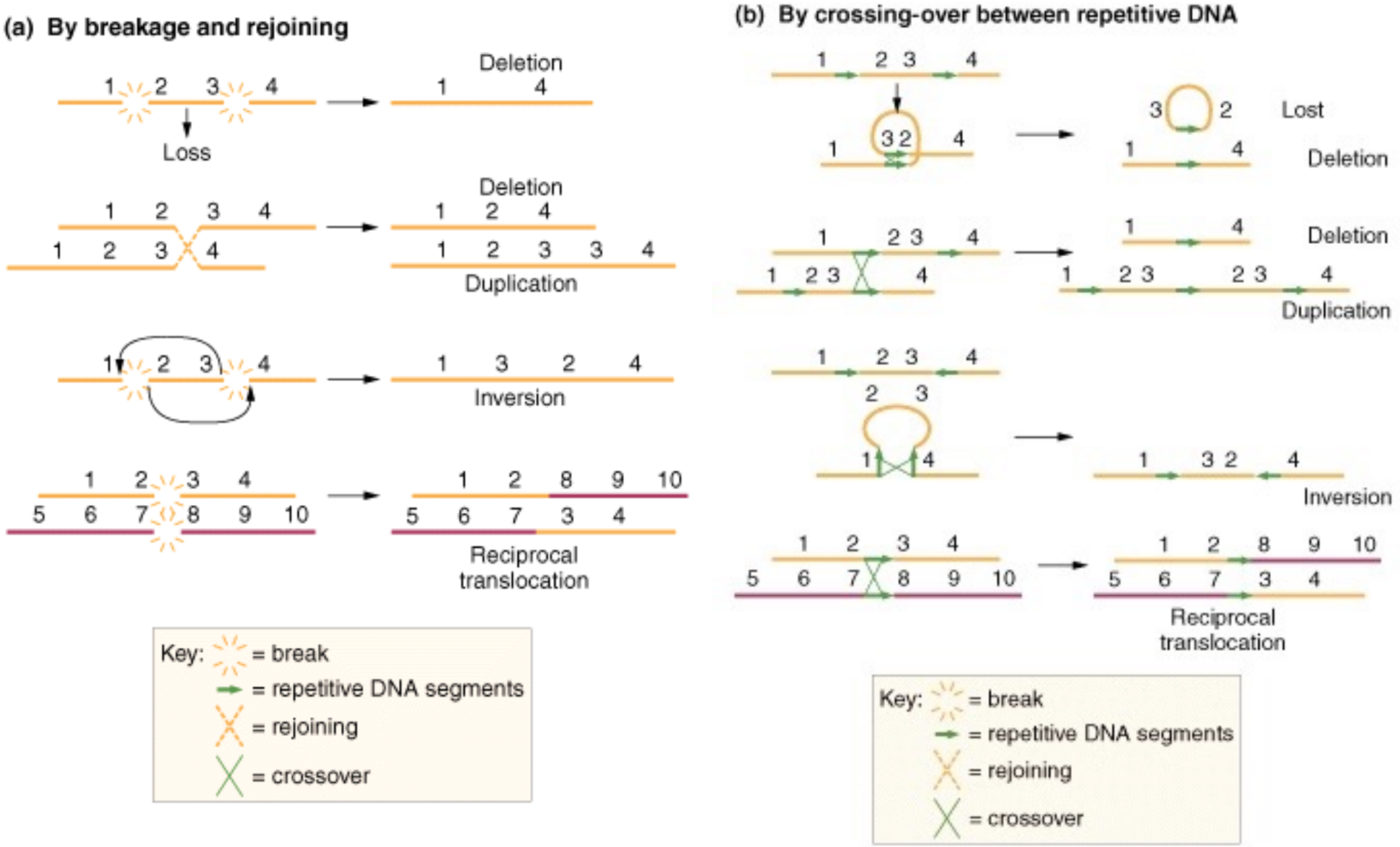
NEUROSCIENCE

Scientists Surprised to Find No Two Neurons Are Genetically Alike

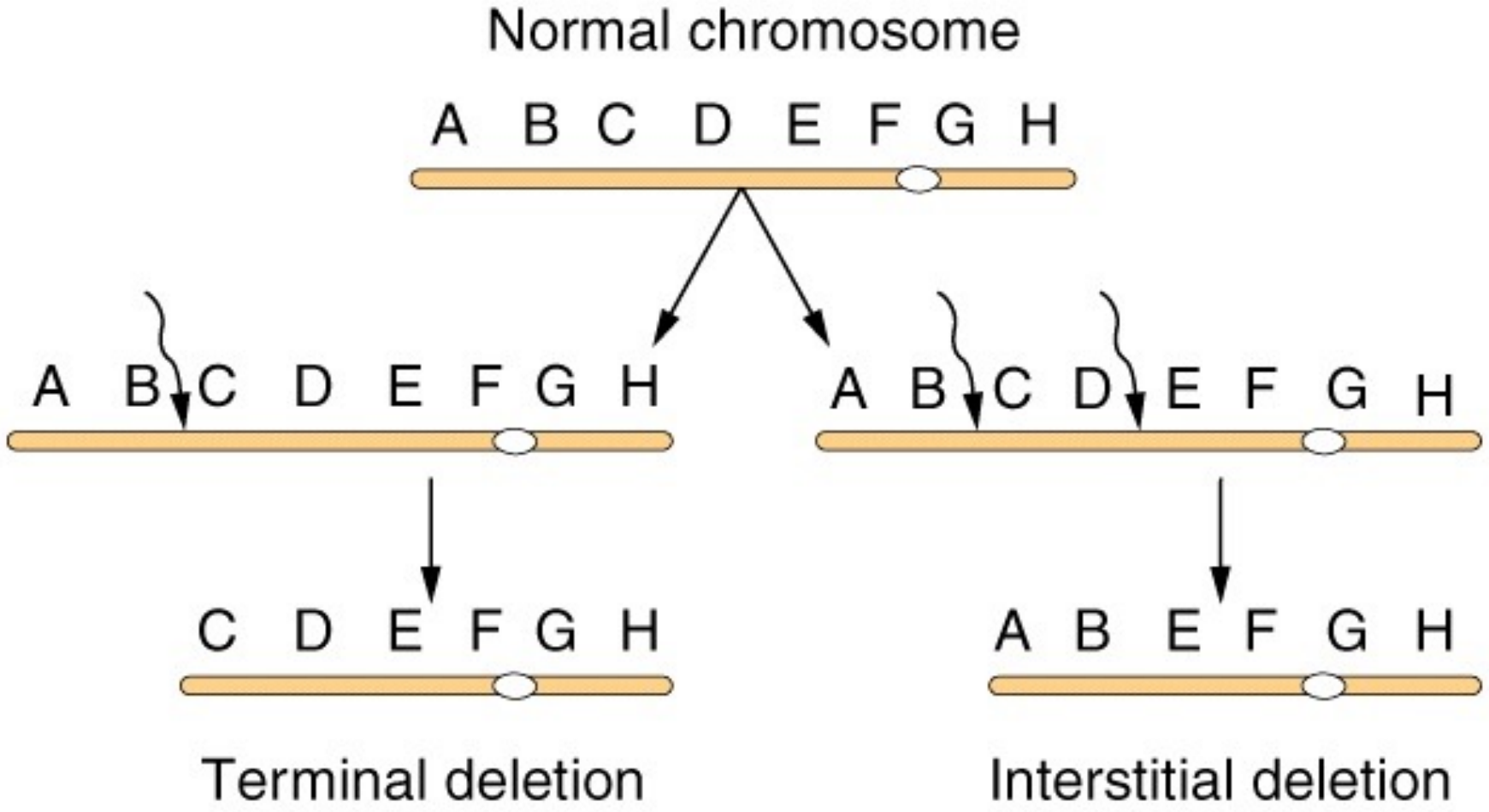
The genetic makeup of any given brain cell differs from all others. That realization may provide clues to a range of psychiatric diseases

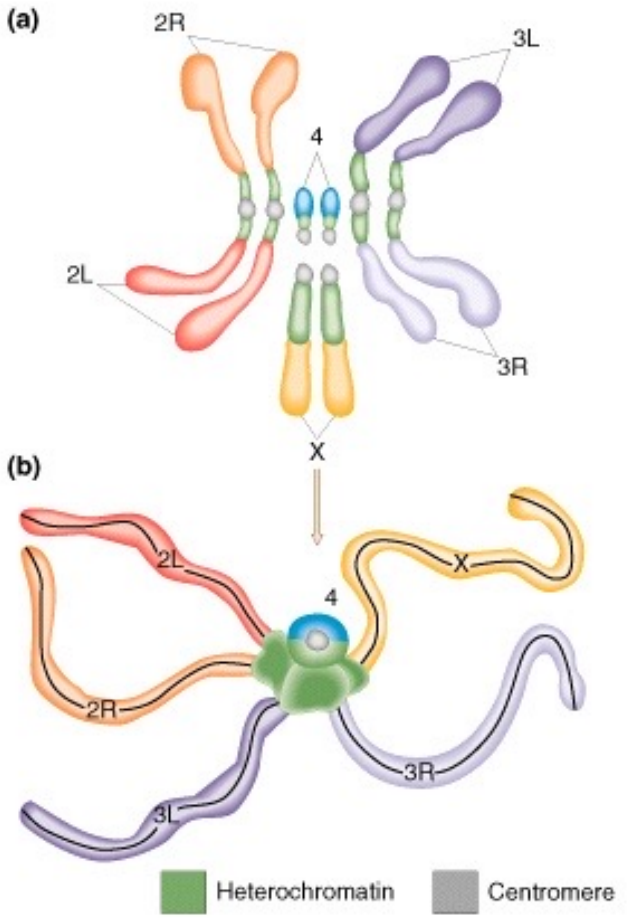
By Simon Makin on May 3, 2017

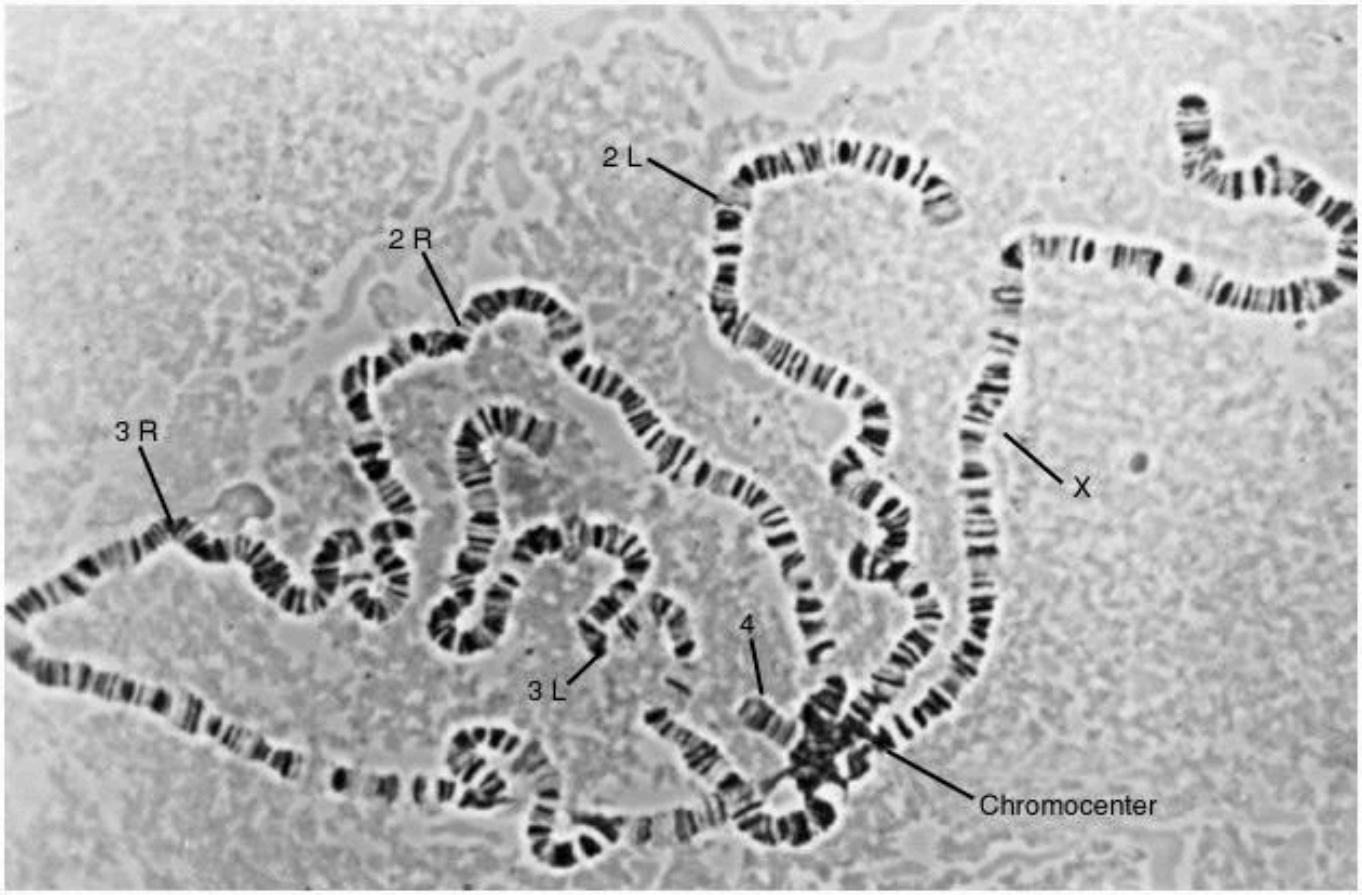
Chromosome rearrangements: how do they happen?



Deletions


Normal chromosome
A B C D E F G H
A B C D E F G H
A B C D E F G H
C D E F G H
A B E F G H
Terminal deletion
Interstitial deletion





The Drosophila larval salivary gland has been a great help for the analysis of chromosomal abnormalities, even though these are somatic, not meiotic cells.

1. Each salivary gland chromosome is polytene, composed of 500-2000 side by side chromatids aligned in register and held parallel to each other. These result from 8-10 (2^{10} = 1024) replications of the salivary gland chromosomes without intervening cell division events.

2. The two chromosome homologs are also held in pairing configuration. All of the chromosomes are joined at their centromeres by a structure known as the chromocenter.

3. Positions on the chromosomes can be identified by a chromosome-specific series of bands. These can be shown to correspond to positions on the genetic map.

Deficiencies/deletions

Deficiencies (alternatively called deletions) remove a segment of a chromosome. Thus they result in the loss of many genes. In polytene chromosome spreads the wildtype chromosome appears as a loop when present in a deletion heterozygote.

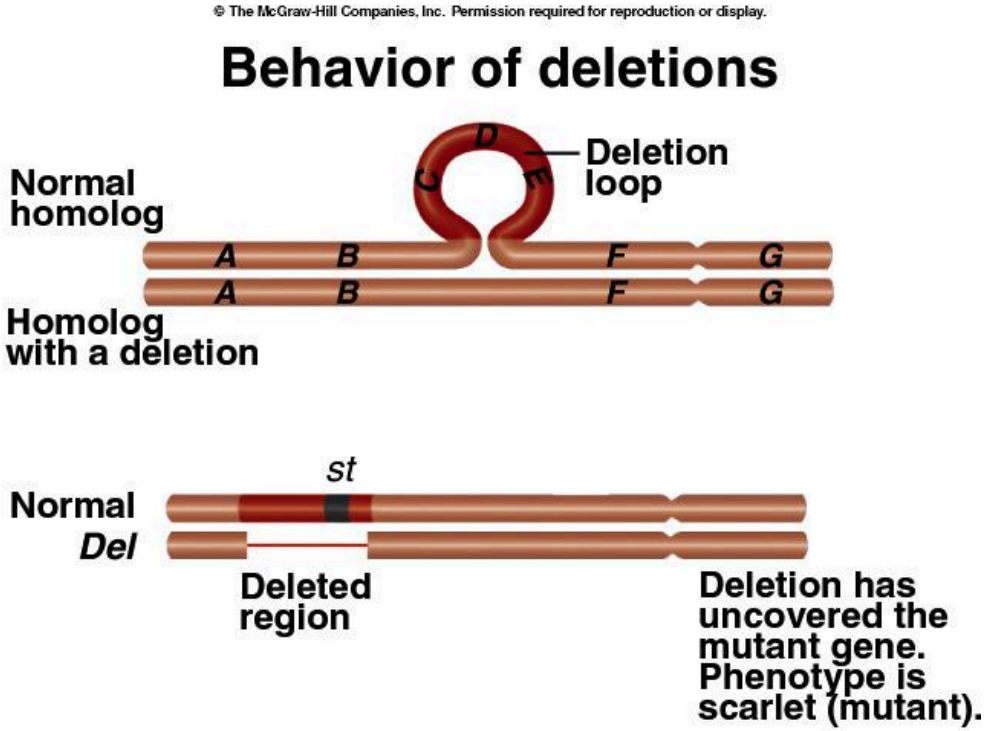
Deficiency size can vary greatly, though large ones are heterozygous lethal because they alter the genetic balance.

Deficiencies show pseudodominance. This simply means that the presence of a deficiency that removes a wildtype copy of a gene will display the mutant phenotype when present in conjunction with a homolog which is specifically mutant in that gene.

Deficiencies are most useful for gene mapping. By recording which bands are missing, and which genes are removed by making Df/mutant heterozygotes one can generate a map of the relative order, and rough position of genes on a chromosome.

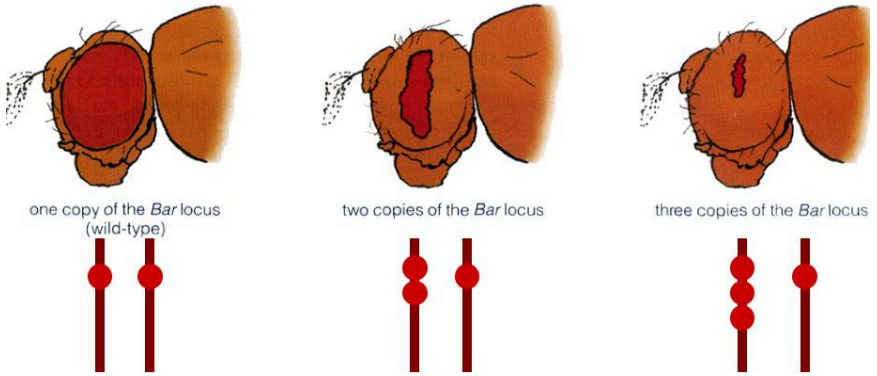
Other features of deficiencies:

1. There is no crossing over in the deficiency region.
2. There is no mutational reversion.
3. Deficiency homozygotes are usually dead.



Duplications

1. Duplications can be anywhere in the genome.
2. Cytologically duplications look like deficiencies. But an analysis of the banding pattern can distinguish the two.
3. Short duplications usually do not have much of a phenotype.
4. When a duplication is heterozygous with a deficiency for the same region the animals are phenotypically wildtype
5. In contrast to deficiencies, duplications do show reversion.



Bar is a tandem duplication of a region of the X chromosome.

+ = no duplication
Bar = duplication

$\frac{+}{+}$ large, normal eye

$\frac{+}{B}$ small eye

$\frac{B}{B}$ or $\frac{B}{Y}$ very small eye

In 1921 Zelany scored 85,008 progeny of the cross $\frac{B}{B} \times \frac{+}{Y}$

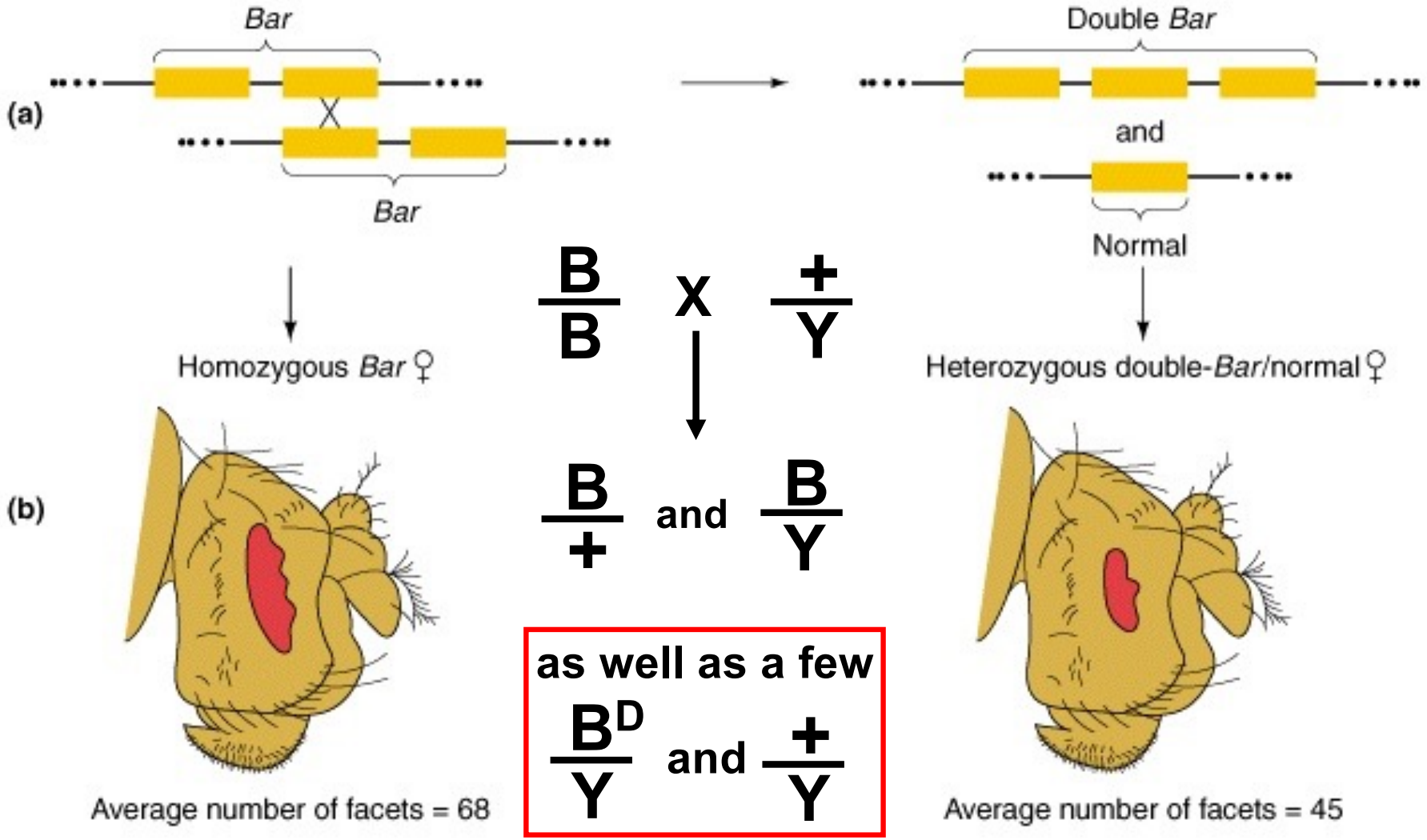
Among the Bar/+ and Bar/Y progeny he observed

52 F1 + eyes
3 F1 ultra small eyes (ultrabar)

Ultrabar females crossed to wildtype males reverted to Bar at a low frequency

In 1923 Sturtevant and Morgan observed that Bar reverts only in females. Crossing over does not occur in Drosophila males. This provided a strong clue that this phenomenon involved crossing over.

Asymmetric pairing and crossing over can generate multiple copies of DNA segments.



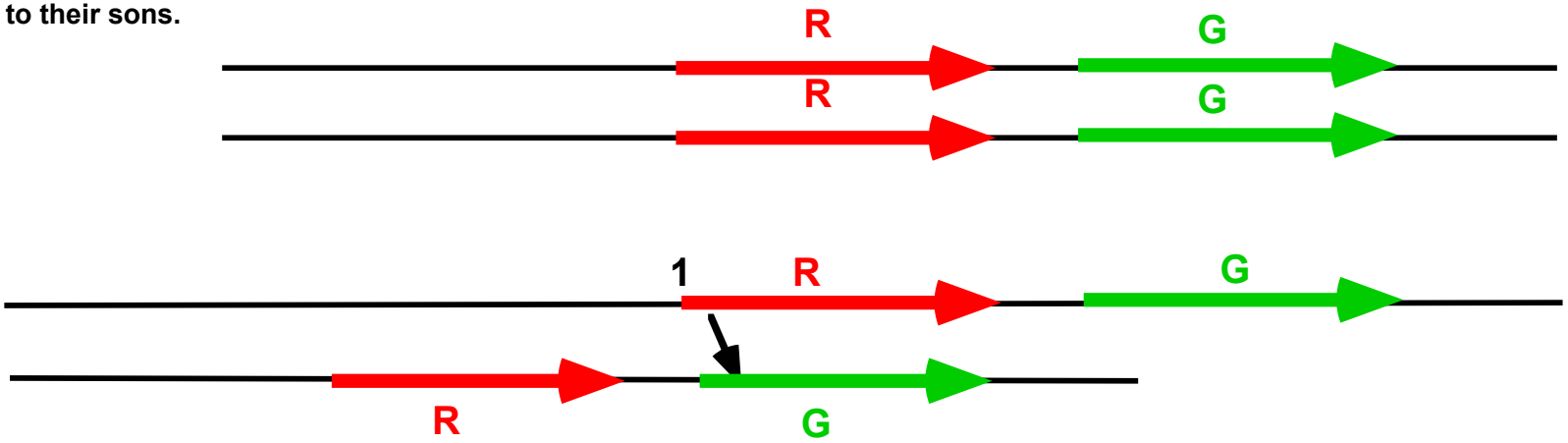
Red-green colorblindness

5% in males

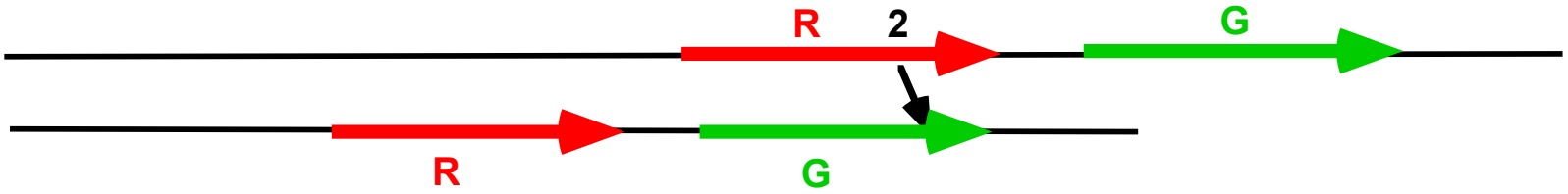
Red-green colorblindness offers an example of the role recombination between duplicated segments of DNA plays in shaping the human genome. Light is received in the retina by rhodopsins, light sensitive proteins. One class of rhodopsin is red sensitive, while a second is green sensitive. These genes are arranged as a tandem duplication on the X chromosome. The genes are very highly related to each other. Thus, mispairing can sometimes occur during meiosis. When this occurs some progeny males will end up with only one rhodopsin gene. Which light wavelengths they are most sensitive to depends on where the recombination happens. If the recombinant protein is mostly green in character they will be most sensitive to green light but be unable to see red, and visa versa.

Males that end up with the reciprocal product of recombination will have three rhodopsin genes, two of which will have either more red or green character, depending on where the recombination occurred. These individuals have no phenotype (they have normal vision). However, it is interesting to note that the third copy of the rhodopsin gene is now free to mutate without any adverse effect on the human's vision. Duplications of this sort are thought to provide a substrate for evolution. For example, if it was advantageous to be sensitive to ultraviolet light, one might expect that the twin forces of mutation and selection could lead to the appearance of individuals in which the third rhodopsin was sensitive to UV light.

Red-green color blindness is rare in females since they have two X chromosomes. However, they may act as carriers of the trait, which is passed on to their sons.



1. The rhodopsin pigment is most like the intact green-sensitive rhodopsin. Recombinant individuals cannot see red.



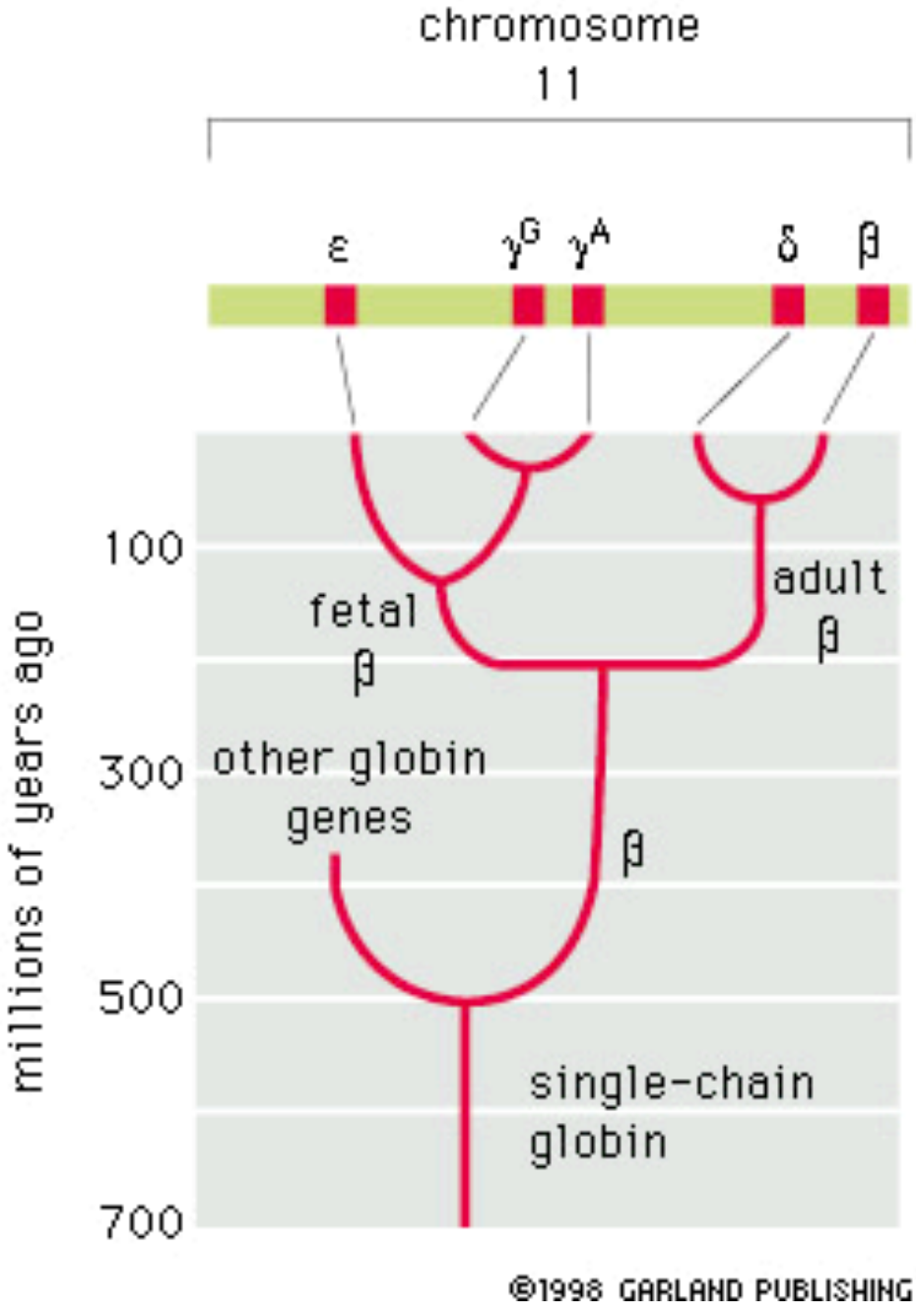
2. The rhodopsin pigment is most like the intact red-sensitive rhodopsin. Recombinant individuals cannot see green.

Random DNA duplications create families of related genes.

Ex. Different forms of actin expressed in different types of muscle cells and in other cell types.

There are 5 beta-globin genes, each produced at a different time during embryonic, fetal, and adult development. Each with a different oxygen-binding and -releasing characteristic appropriate for the time it is expressed. And each under independent regulation.

Comparing the sequences of genes like the beta-globin gene leads to models like this which may reflect the evolution of the gene through millions of years.

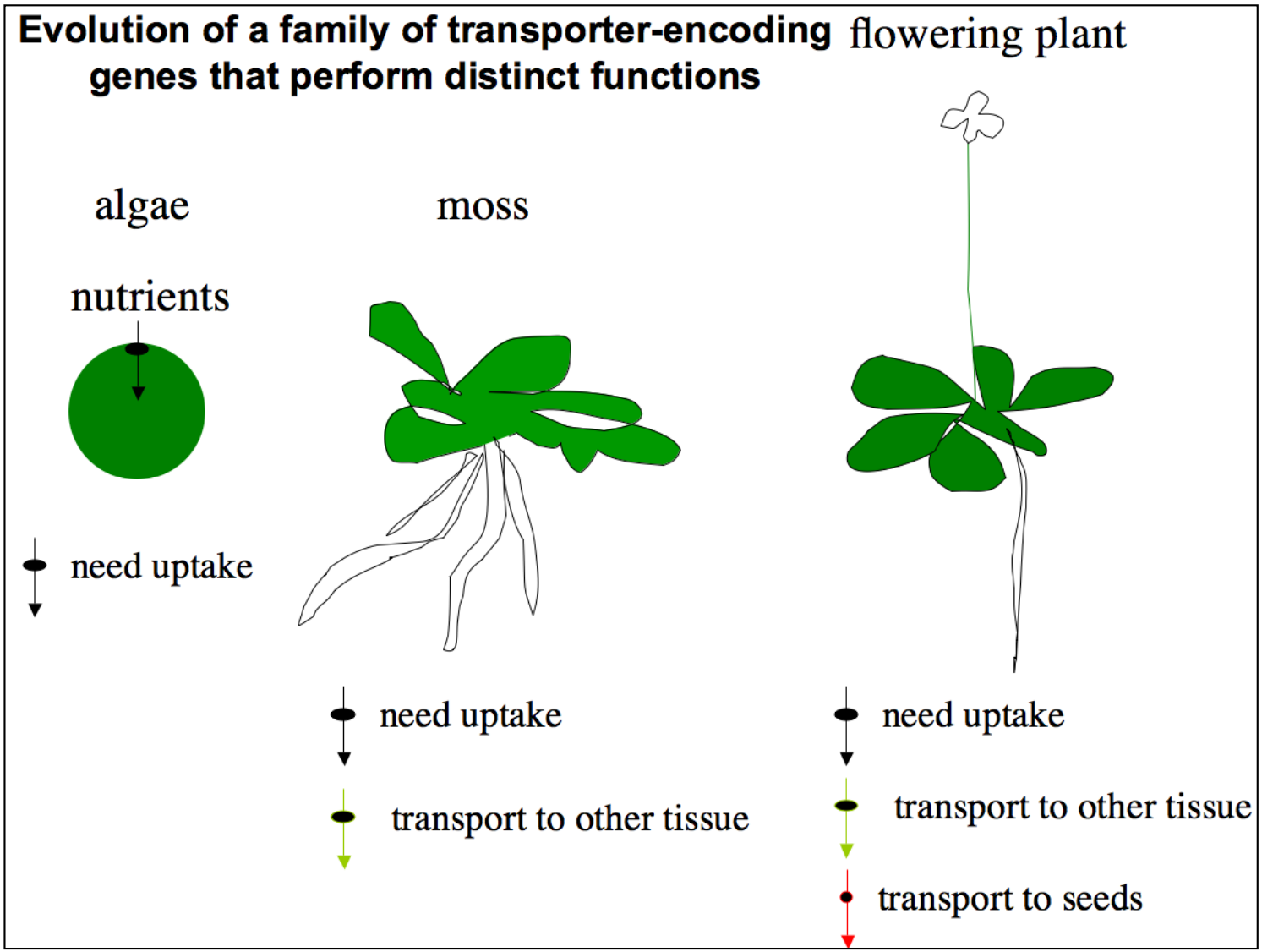


Duplication in Evolution

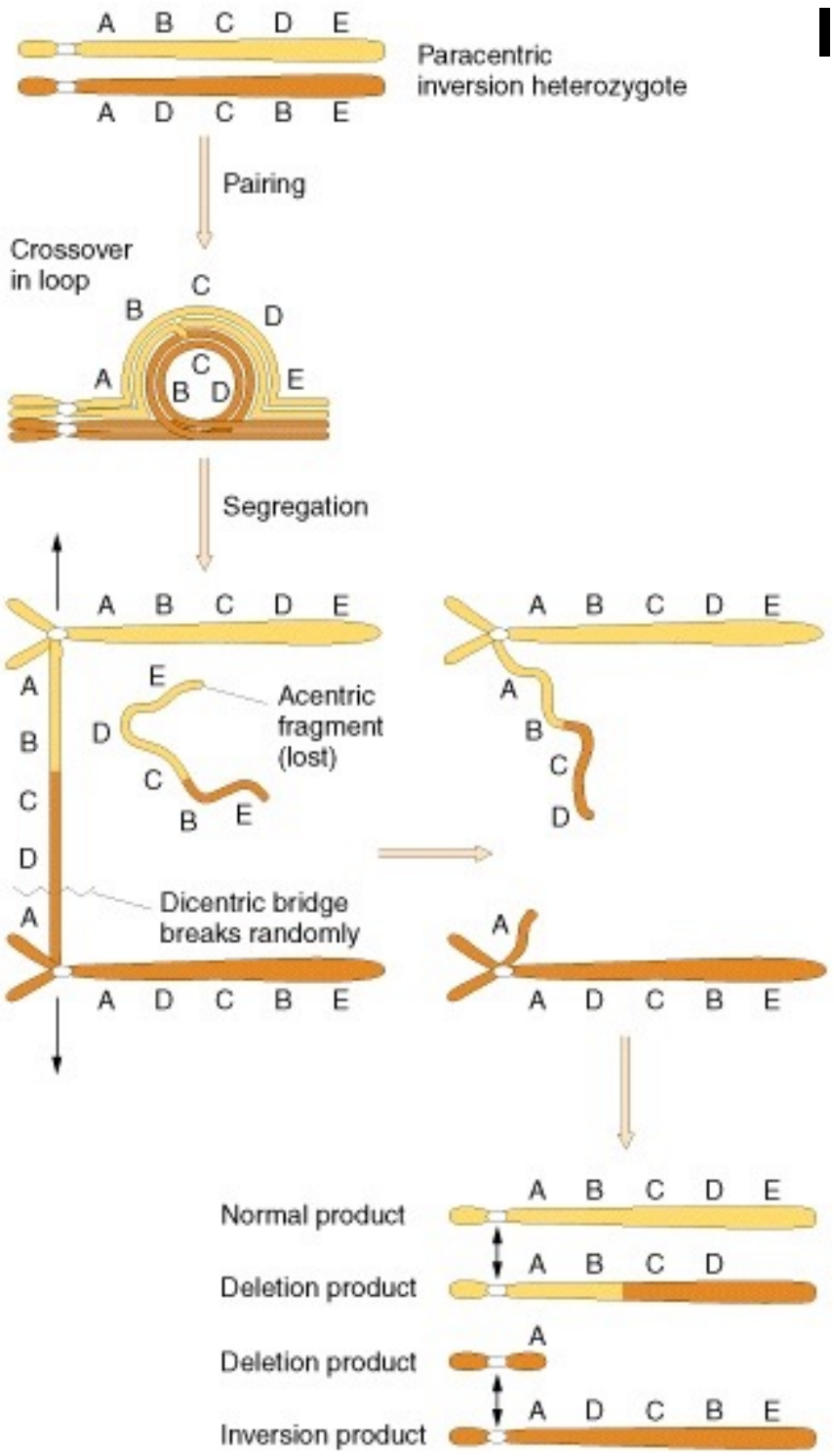
…essential genes do not tolerate mutation,

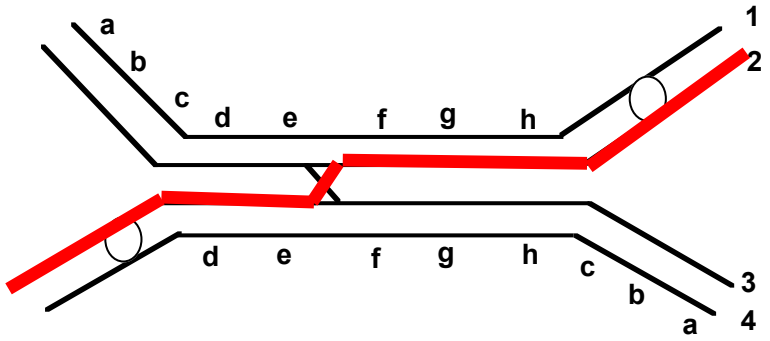
…duplications of essential genes, then subsequent mutations, confers adaptive potential to the organism,

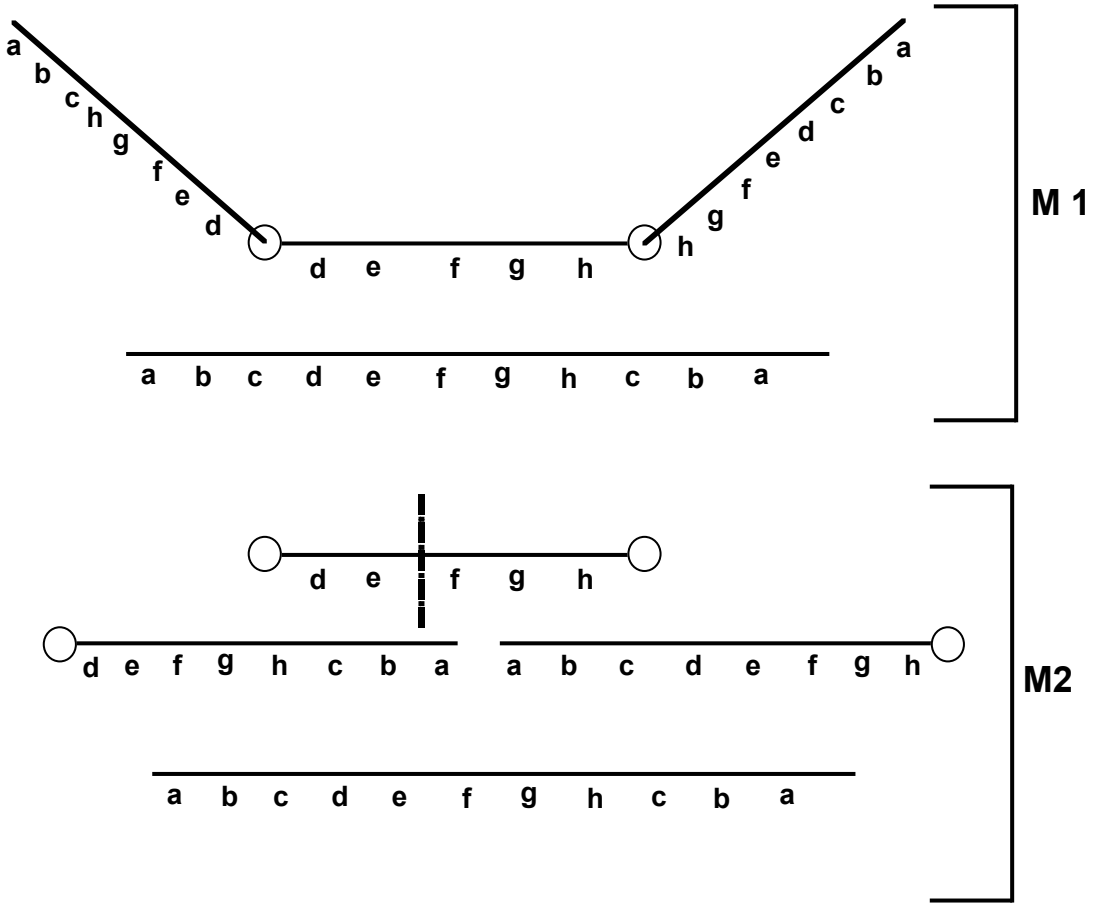
…new gene family members are ‘recruited’ to perform new functions.



Inversions: Paracentric







During meiosis 1 the centromeres pull to opposite sides of the cell. During meiosis 1 the homologs normally move to opposite sides of the cell. The sister chromatids are still attached at this point. In the case of a inversion heterozygote this leads to the formation of two products:

A. One fragment with two centromeres that contains the chromatids not involved in the inversion as well as a chromatid that has two centromeres, and is thus dicentric.

B. A second product that has no centromere. It just sits in the nucleus and goes nowhere because it cannot attach to the spindle microtubules.

During meiosis 2 the centromeres associated with the paired sister chromatids pull apart.

A. The two intact chromatids that did not participate in the inversion move to opposite poles. They can form functional gametes.

B. The dicentric chromosome is pulled apart somewhere in the middle. it does not contributer sufficient genetic information to produce a functional gamete.

C. The acentric fragment, which contains some duplicated material, stays in the middle.

Only the chromatids that did not participate in the crossover in the inversion contribute functional gametes. Of course, crossovers occurring outside the inversion would proceed normally.

What happens to the gametes from an inversion heterozygote?

In plants they die. The haploid nuclei of the gametes are required in the male for pollen tube growth, and in the female for growth of the embryo sac. Thus a piece of corn from an inversion heterozygote parent would look ugly, because many of the kernals would be dead.

In the fly the situation is a little different.

1. In the male there is no meiotic recombination. So essentially all the gametes are nonrecombinant.

2. In the female the development of the egg causes them not to contribute. The plane of the cell divisions during meiosis 1 and meiosis 2 is such that only the chromatids that migrate to one pole of the egg are able to contribute to the zygote. The others end up as polar bodies. Because the acentric and dicentric fragments are unable to do this they do not participate in gamete formation.

Proof that single crossovers do occur within an inversion

1. Chromosome bridges can be seen in meiotic spreads

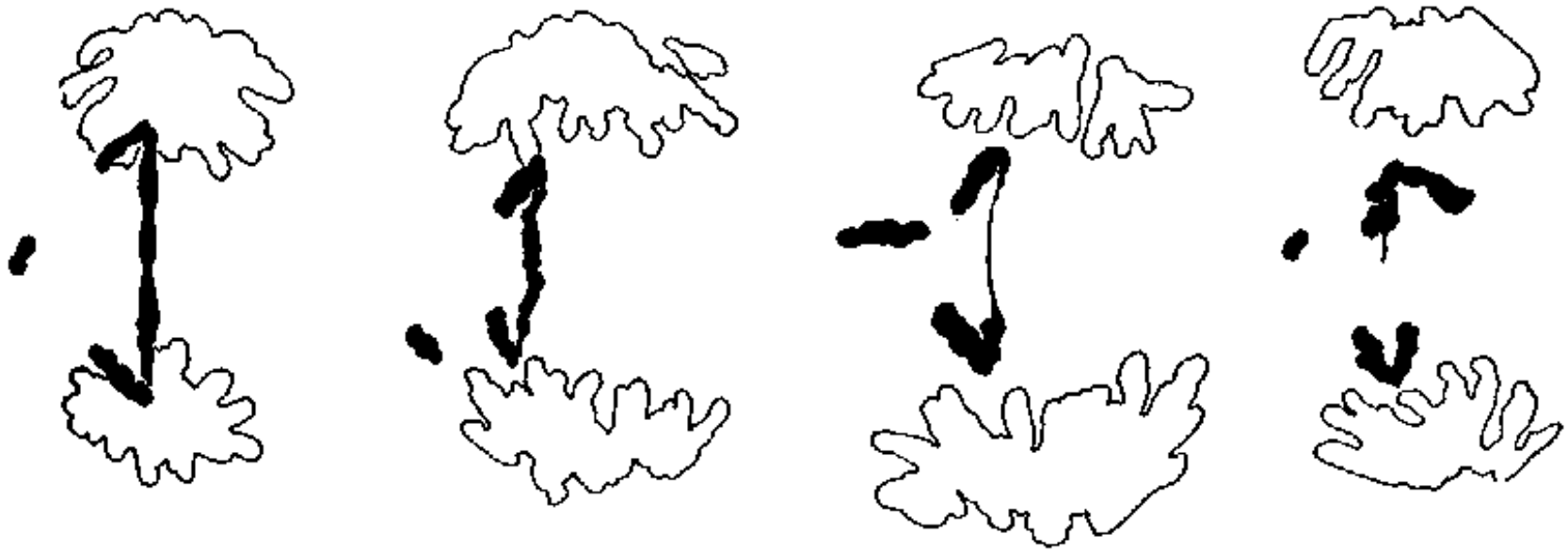
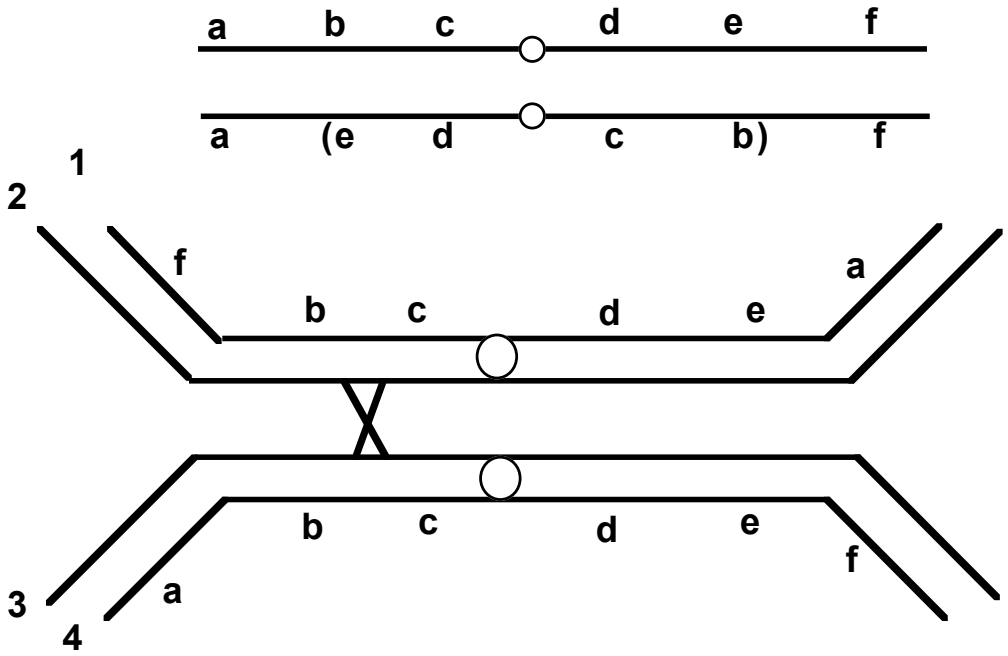


Fig. 51.—Telophase of first meiotic division in Fritillaria showing chromatin bridges and acentric fragments resulting from crossing over between segments inverted with respect to each other. The difference in the size of the fragments results from differences in positions of the different inversions involved. (After Bennett.)

Pericentric inversions

Pericentric inversions involve the centromere. Recombination within the inversion leads to the formation of gametes that are either inversion parental or wildtype, or that contain deletions and duplications for specific chromosomal regions. The fate of the deletion/duplication chromosomes depends on their size. They are usually not recovered. Thus, as with paracentric inversions, gametes recombinant within the inversion are usually not observed.



1 f b c d e a **inversion parental**

2 f b c d e f **deletion for a duplication of f**

3 a b c d e a **deletion of f duplication of a**

4 a b c d e f **wildtype parental**

Inversion summary

Forming inversions:

1. Breakage and rejoining of internal chromosome fragment.

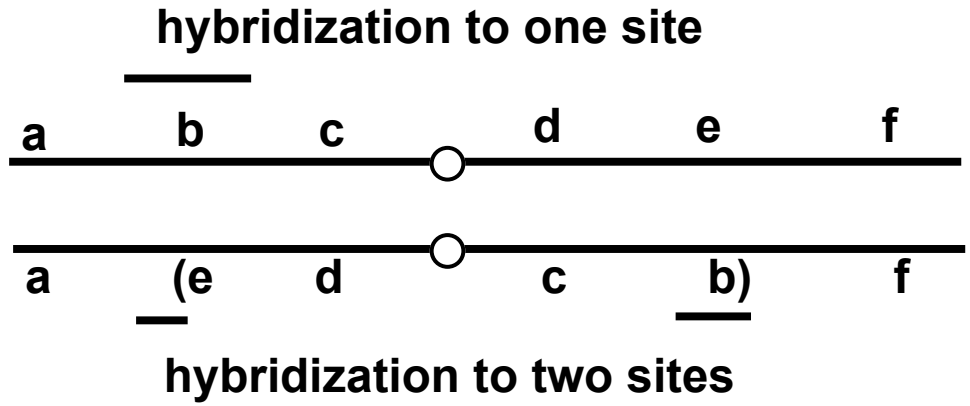
2. Recombination between inverted repeats of homologous DNA

Properties of inversions:

1. Inversion homozygotes are normal. They just have a different gene order.
2. The products of meiotic recombination within an inversion do not contribute.
3. Thus genes within an inversion appear to be tightly linked.

Detecting inversions:

1. Cytologically.
Band order and the presence of loops in polytene chromosomes.
Chromatin bridge fragments at anaphase of meoisis.
2. Genetics. Gene map order will be altered. There will be a difference between maps. Generated from inversion homozygotes and heterozygotes.
3. Physically. If you take a piece of DNA that spans an inversion breakpoint (the point at Which the inversion initiates), and hybridize it to an inversion chromosome, you will see Two spatially distinct signals. If this DNA probe was hybridized to a wildtype chromosome there would be only one signal.



Inversions and evolution

If loci inside an inversion affect a single trait (or suite of related traits), this means they'll be inherited together and allele combinations won't be broken up by recombination.

A suite of tightly linked loci that affect a single trait is collectively known as a SUPERGENE.

- mimicry coloration in some species of butterflies
- snail shell color pattern (some species)

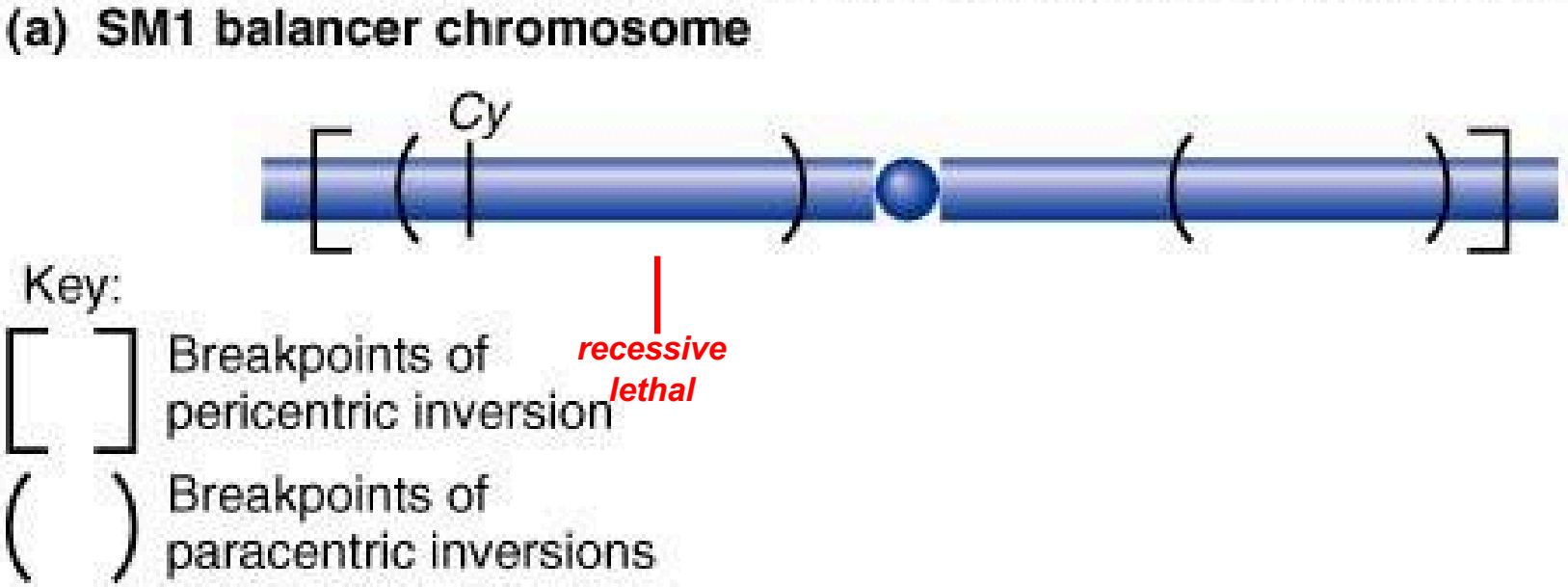
Inversions, a genetic tool for preserving embryonic lethal mutations

- ***kni* is embryonic lethal**
- **What will happen to this cross:**
  - ***kni-/+ X kni-/+***
    - ***1 kni-/kni- (all die)***
    - ***2 kni-/+ (viable)***
    - ***1 +/+ (viable)***
  - ***kni-/+ X +/+***
    - ***1 kni-/+ (viable)***
    - ***1 +/+***

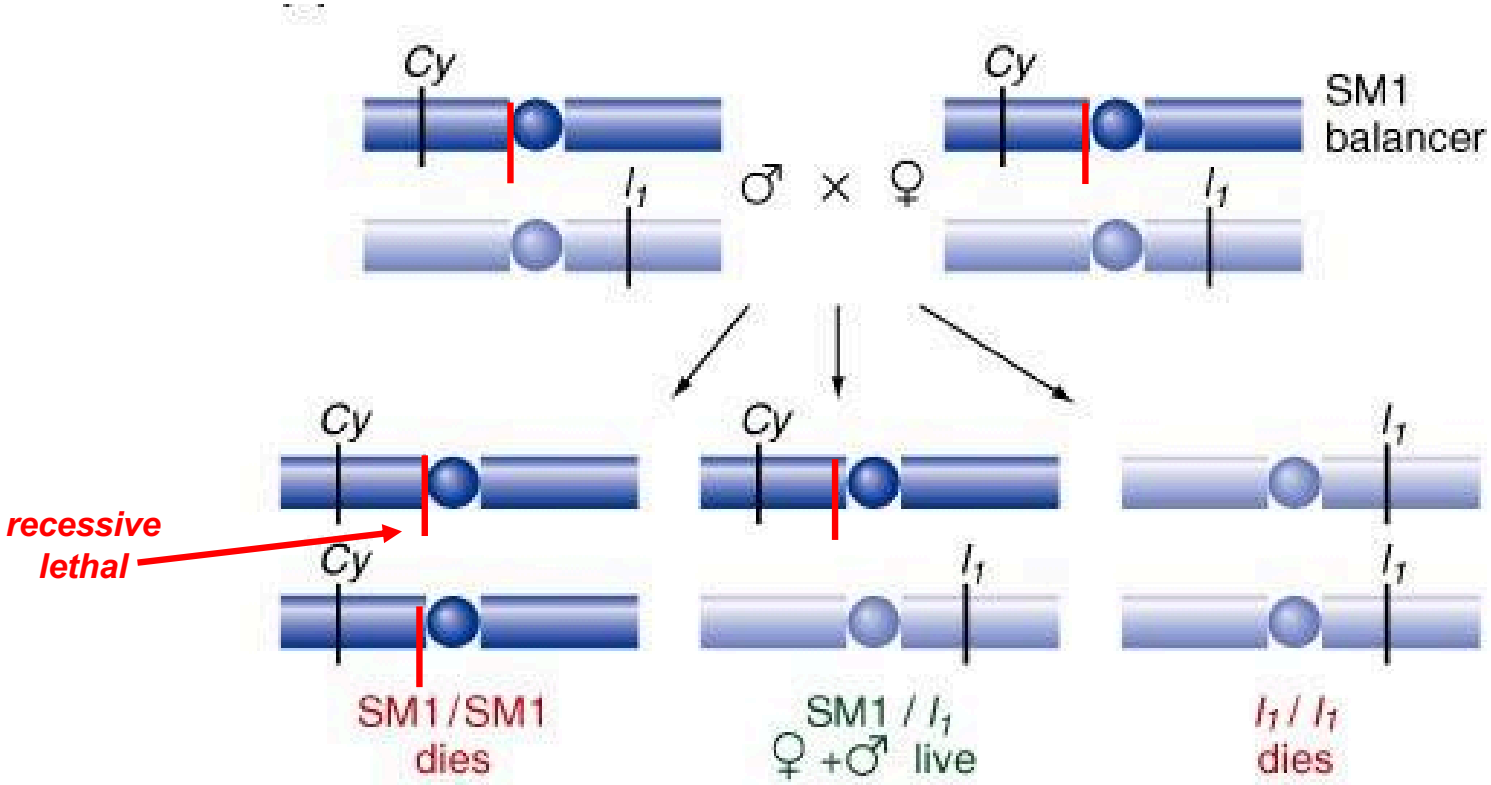
Inversions, a genetic tool for preserving embryonic lethal mutations

- **Balancer chromosomes allow the maintenance of alleles that are lethal when homozygous**
  - **Carries multiple, overlapping inversions to prevent recombination**
  - **Carries recessive lethal mutation to prevent survival of homozygotes**
  - **Carries dominant marker (e.g. *Curly*) to allow research to follow balancer chromosome in crosses**

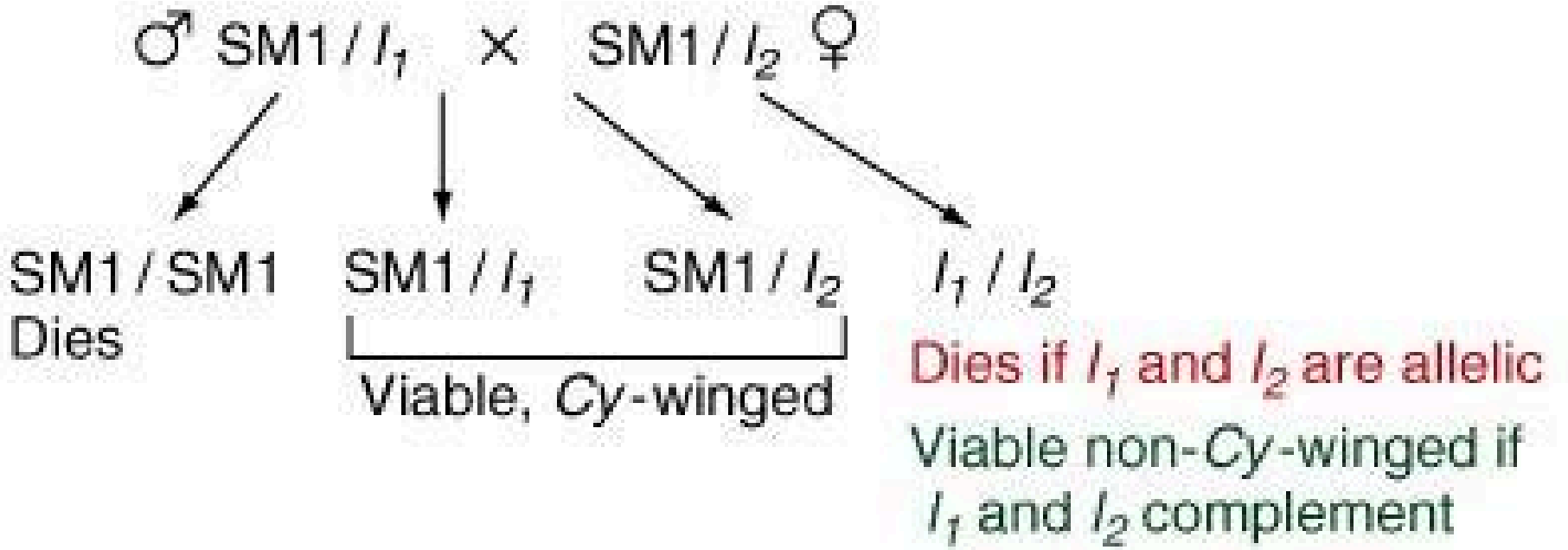
SM1 balancer chromosome



In a balanced lethal stock, the only adult survivors are identical to their parents



Are two independently isolated lethal mutations (l_1 and l_2) viable?



Balancers and mutagenesis

One important function of balancers is in carrying out mutagenesis screens. Because you can identify a balancer chromosome due to the presence of a dominant mutation, and recombination cannot occur between it and its homolog, you can use the balancer to follow the fate of the non-balancer homolog.

As an example, imagine that you are interested in creating new lethal mutations on a sn chromosome in Drosophila.

1. Mutagenize male flies. At this point each sperm has received a unique mutagenic event. The goal of a screen is to isolate each of these mutagenized chromosomes and determine if a lethal mutation is now present.

2. Cross these males to sn/FM7 females. FM7 is an X chromosome balancer that carries the dominant mutation Bar, but is not homozygous (or hemizygous) lethal.A number of progeny types are possible. You isolate a number of unmated sn*/FM7 females.

3. Each sn*/FM7 female is mated to a male that is FM7/Y. Notice that at this point the only unknown X chromosome is the mutagenized sn chromosome. All the other X chromosomes are FM7.

4. If no new lethal has been generated on the sn chromosome then there will be sn/Y male progeny.

5. If a new lethal mutation has been induced on the sn chromosome then only sn*/FM7 and FM7/Y and FM7/FM7 progeny will be produced. This is now a balanced stock which can be stably passaged from generation to generation. FM7/FM7 females are essentially sterile and do not genetically contribute.

$$\frac{sn^*}{Y} \times \frac{sn}{Bal} \rightarrow \frac{sn^*}{sn} ; \frac{sn^*}{Bal} ; \frac{Y}{Bal} ; \frac{sn}{Y}$$

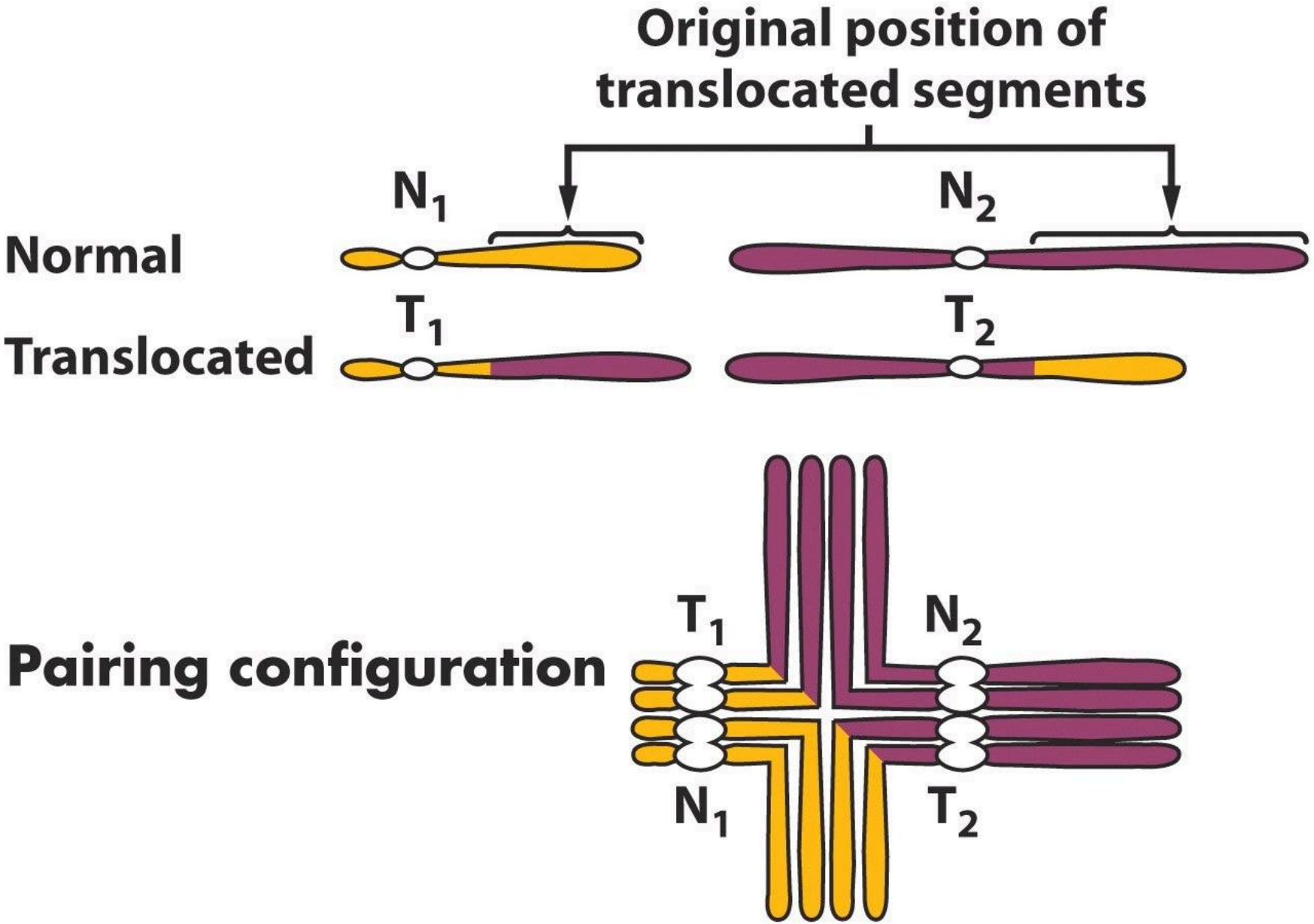
$$\frac{sn^*}{Bal} \times \frac{Y}{Bal} \rightarrow \frac{sn^*}{Bal} ; \boxed{\frac{sn^*}{Y}} ; \frac{Y}{Bal} ; \frac{Bal}{Bal}$$

very sickly

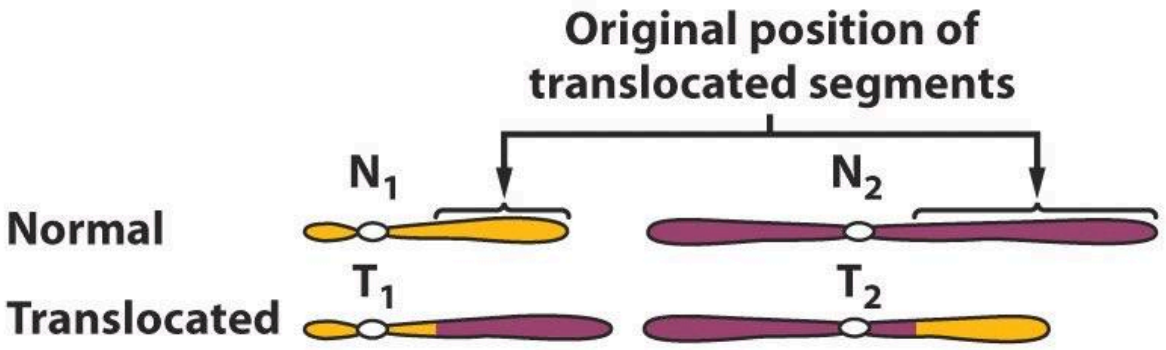
hemizygous male

Are there hemizygous males? If not then the sn* chr. is lethal.

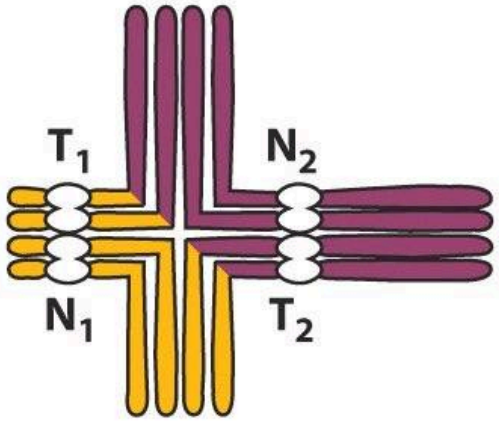
Translocation heterozygote



Translocation heterozygote



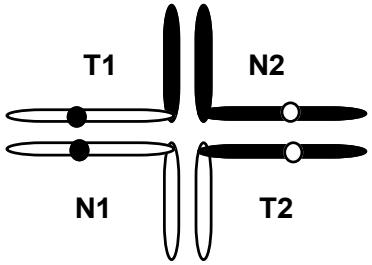
Pairing configuration



Two types of segregations:

Adjacent-1		Final meiotic products	
Up	$T_1 + N_2$	Duplication of purple, deletion of orange translocated segment	Often inviable
Down	$N_1 + T_2$	Duplication of orange, deletion of purple translocated segment	
Alternate			
Up	$T_1 + T_2$	Translocation genotype	Both complete and viable
Down	$N_1 + N_2$	Normal	

In a cross between translocation heterozygotes, only a small fraction of the zygotes are viable. In plants most translocation heterozygotes are viable, while in Drosophila they are lethal. There is a simple explanation for this. In plants translocations must be transmitted through a haploid multicellular gametophyte to be transmitted at all. Those that would be lethal in a diploid homozygote are also lethal to the haploid gametophyte. In flies, which have a very short haploid stage that is unicellular, the gametes are fertile and viable even when the chromosome constitution is grossly abnormal. Thus they can be transmitted even if they would be lethal in the homozygous state.



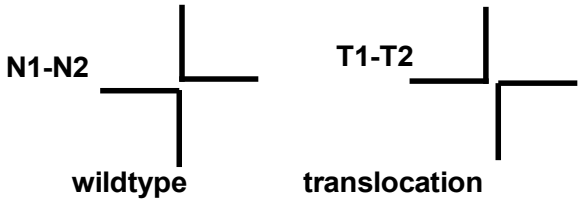
A cross between translocation heterozygotes, <u>in animals</u>, = 6/16.

	T1-N2	T2-N1	T1-T2	N1-N2
T1-N2	–	+	–	–
T2-N1	+	–	–	–
T1-T2	–	–	+	+
N1-N2	–	–	+	+

Plants 1: 2: 1 wildtype: het: transl. homozygote

<u>Adjacent-1 segregation</u>
Dupication + deficiency

<u>Alternate segregation</u>
Viable



<u>Adjacent-2 segregation</u>
Homologous centromeres go to the same pole. These are rare and inviable.

In plants the pattern simplifies because unbalanced translocation gametes are inviable in the gametophyte stage. Thus certainzygote genotypes are not produced.

One common way in which chromosome rearrangements contribute to human disease occurs when genes that promote cancer come under the control of powerful promoters that drive their expression in specific cells of the immune system. This can lead to leukemia and lymphoma, forms of cancer

Deletions
Inversions
Translocations

move genes to new locations

This may alter the protein product or the pattern of gene expression.

An example from human biology is the Philadelphia chromosome

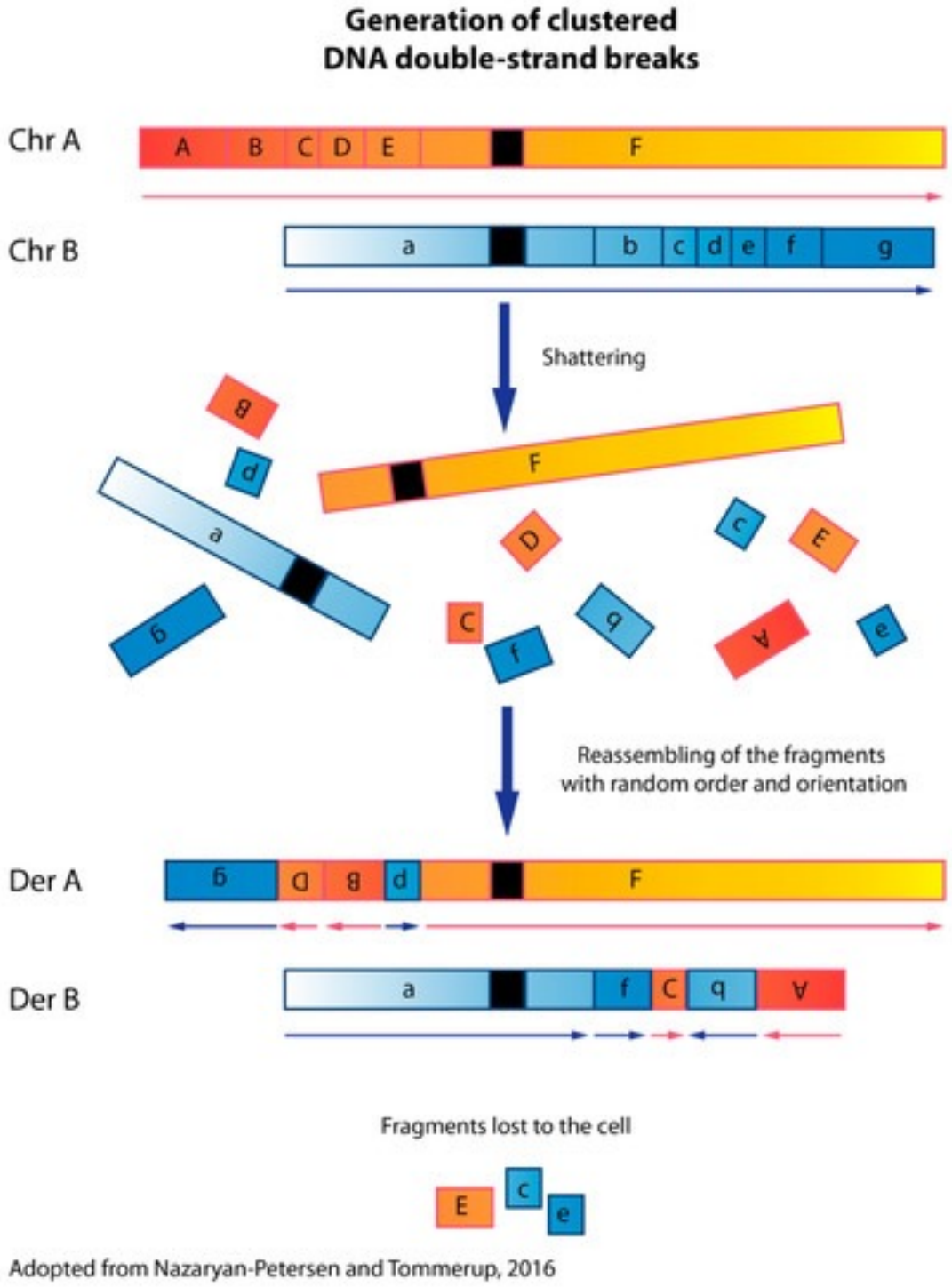
9: 22 translocation → Leukemia Inappropriate activation of a gene, c-abl

Burkitt's lymphoma 8: 14 translocation → Inappropriate activation of c-myc

Chromothripsis (chromosome shattering)

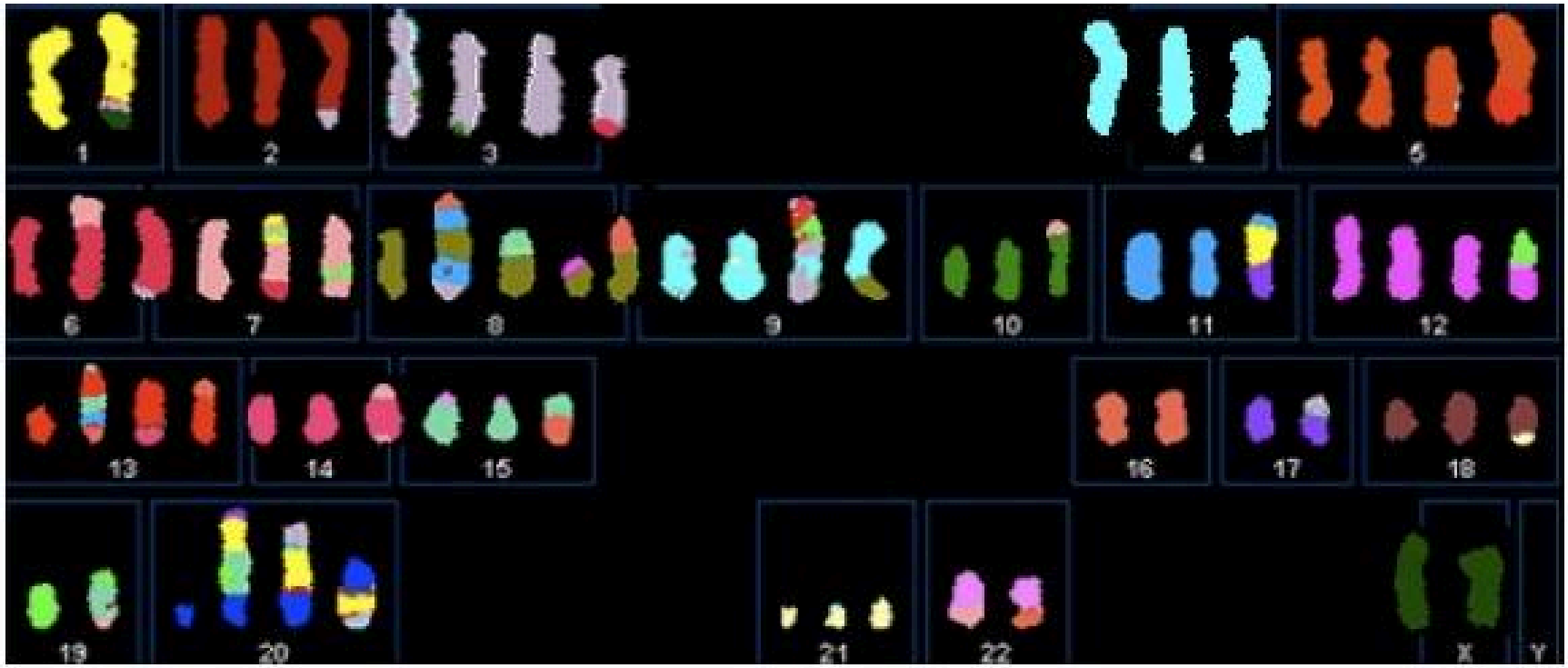
Breakage-fusion-bridge is one mechanism.

Others, involving defects in DNA repair exist as well



The breakage-fusion-bridge cycle can give rise to gross aneuploidy. This will include chromosomes that have lost homozygosity in specific regions

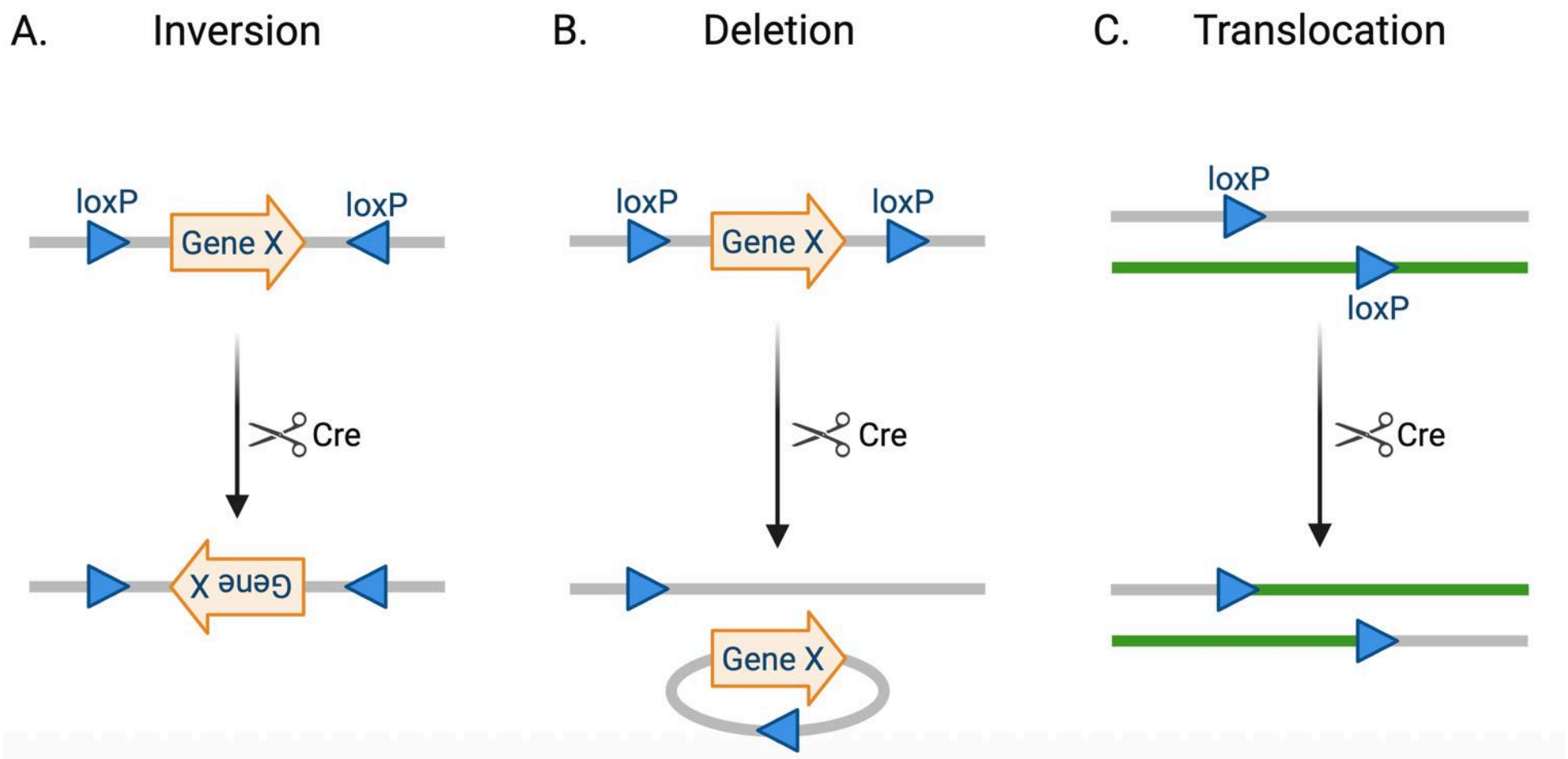
Gross Chromosome Instability



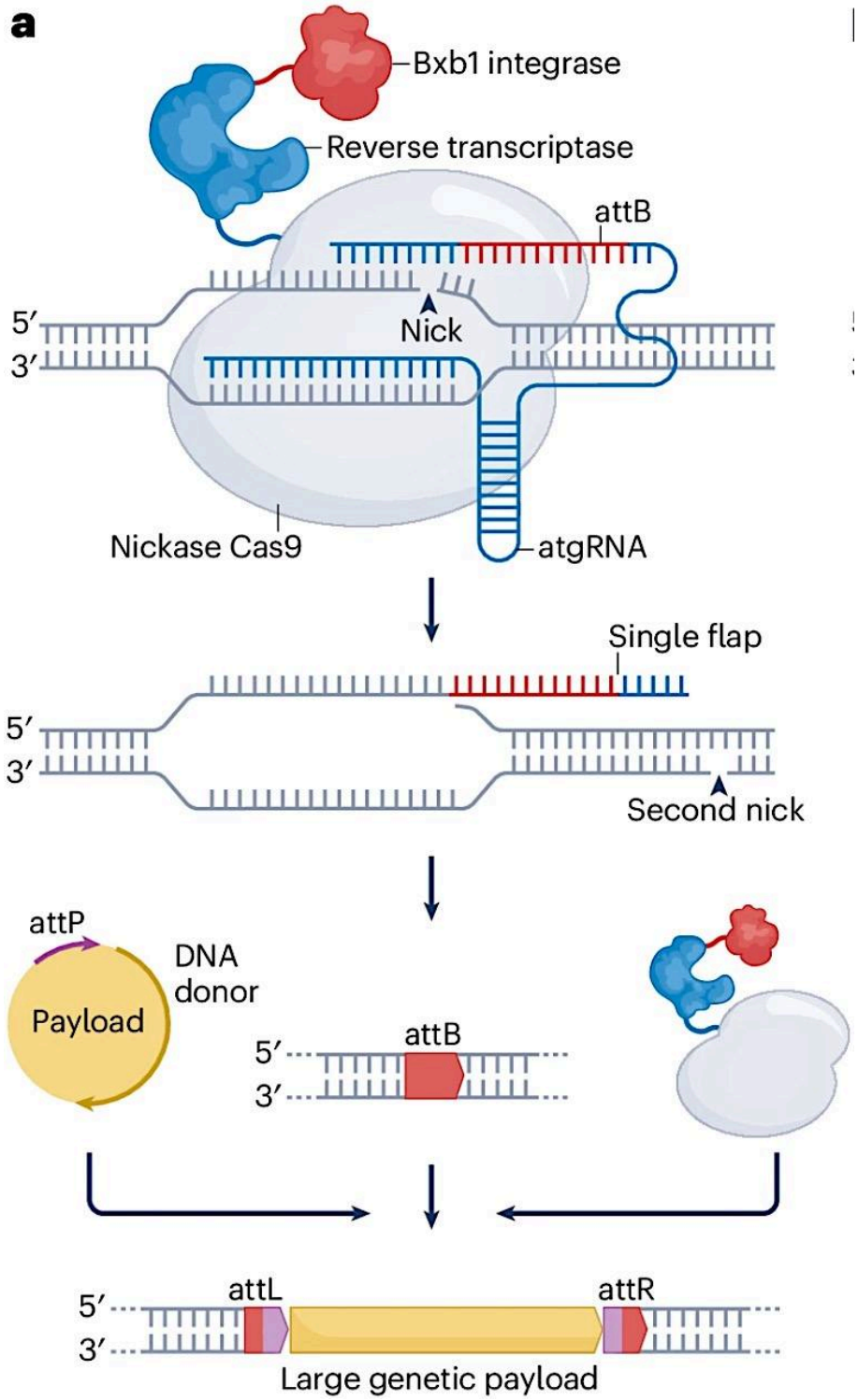
http://www.path.cam.ac.uk/

SKY Paint of MCF7 Breast Tumor Cell Line

Site-specific recombination between two sequences to change DNA orientation



Prime editing to introduce a site-specific recombination site and (in this case) a transgene payload

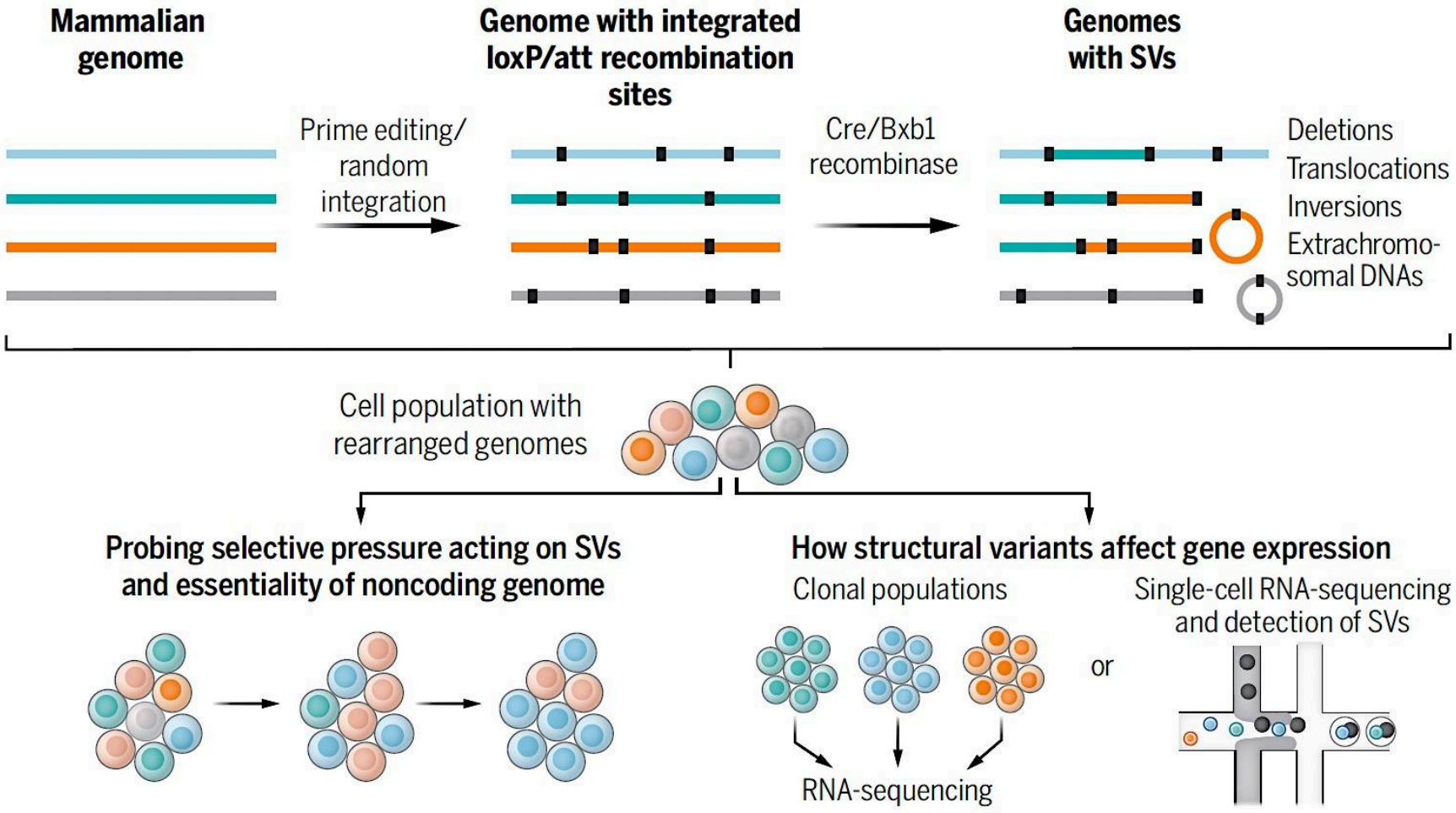


Screening for rearrangements with specific phenotypes: random generation followed by selection

doi: 10.1126/science.adv1570

Rearranging genomes

Thousands of loxP or attB sites can be inserted into the mammalian genome through prime editing or random integration. Cre or Bxb1 recombinases induce recombination between these sites, generating structural variants (SVs). Tracking the resulting heterogeneous cell population over time allows for probing selective pressure on SVs and the essentiality of the noncoding genome. Transcriptome sequencing reveals how SVs influence gene expression.



Changes in chromosome number

Ploidy: **the number of chromosome sets**

Monoploid: **Organism with one chromosome set (described for organisms from essentially diploid taxa).**

Euploid: **An organism that contains some multiple of the basic chromosome set.**

Polyploid: **An organism that contains more than 2 chromosome sets.**

The basic chromosome number, X. This is the number of distinct chromosomes that an organism contains. In a normal diploid x = n, the haploid chromosome number. However, some organisms such as wheat are hexaploid. Wheat has 42 chromosomes, but the basic set is 42/6 = 7 = X. In contrast, n = 42/2 = 21.

Haploid number, n. n is the number of chromosomes in gametes.

Autopolyploids: **Polyploid organisms created by chromosome duplication within a species.**

Allopolyploids: **polyploids created by hybridization between different (but usually related) species. In this situation the related chromosomes are usually described as being homeologous, rather than homologous.**

Polyploidy Generalities

de novo

- **rare in most animal species,**
- **known in lizards, fish and amphibians,**
- **fairly common in plants,**
- **odd numbers of ploidy are not usually maintained,**
  3n, 5n, etc
  rarely found in organisms that rely on sexual propagation.

Monoploidy

…a haploid of a diploid is monoploid,

…has one chromosome set

- **male wasps, bees and ants have only 1 haploid genome,**
  - **males develop from unfertilized eggs,**
    - **gametes are formed by mitosis.**

Monoploids.

In some organisms, such as bees and wasps, the males are monoploid. They develop from an unfertilized egg. Being monoploid should present a problem during meiosis since the individual chromosomes have no pairing partners. Males in these species get around this problem by using mitosis rather than meiosis during gamete production.

If a monoploid cell (or as we will get to in a moment, any cell with an odd number of homologs that must be dealt with at meiosis) does undergo meiosis there is a problem. The movement towards one pole or the other is essentially random and independent for each group of homologs. In a monoploid some cells get none of each chromosome while others get one. Similarly, in a triploid some cells would get one homolog while other cells would get two. In either situation this creates a genetic imbalance in the gamete, and thus almost certainly a genetic imbalance in the fertilized zygote.

$$\left(\frac{1}{2}\right)^{x-1}$$

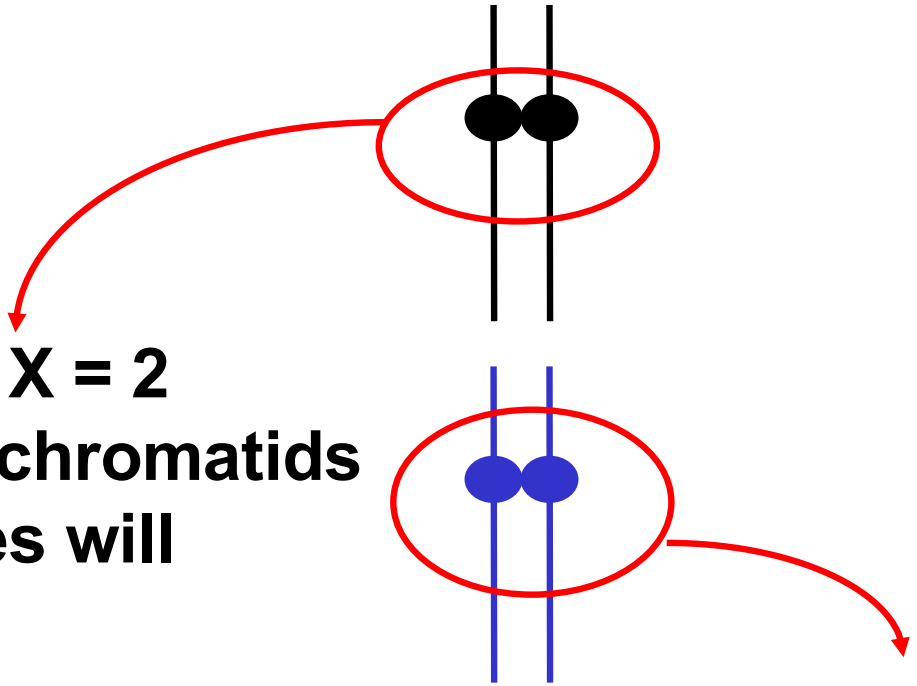
X = the number of chromosomes (in monoploids), or X, the basic chromosome set number, in polyploids such as triploids.

Triploids have problems during meiosis that lead to sterility

Triploids arise from a cross between a tetraploid and a diploid. Diploid and haploid gametes fuse to produce a triploid embryo. The triploid offspring can develop. However, during meiosis a similar problem occurs to that present in a monoploid (figure below). It is very unlikely [p = (1/2) x-1] that a similar number of the different chromosomes will all go to the same gamete during meiosis. This leads to genome imbalance.

Take as an example the banana. It has 11 chromosomes and is a triploid (3x = 33 chromosomes). This means that at meiosis there is a 1/1024 chance of producing a gamete with a full haploid or diploid complement of chromosomes. The frequency of two such gametes getting together to produce a viable seed is extremely low. Triploidy is used to create seedless versions of fruits such as bananas and watermelon. So, you might ask, if they are seedless, how do they propagate. This is done artificially, using cuttings, grafts or bulbs. Essentially, a piece of the original triploid is used to start a new plant.

In meiosis 1 in a monoploid the duplicated chromatids go one way or the other. They have no pairing partner. In the example, X = 2 There is a 50% chance pairs of chromatids from two different chromosomes will go to the same side.



Monoploids.

In some organisms, such as bees and wasps, the males are monoploid. They develop from an unfertilized egg. Being monoploid should present a problem during meiosis since the individual chromosomes have no pairing partners. Males in these species get around this problem by using mitosis rather than meiosis during gamete production.

If a monoploid cell (or as we will get to in a moment, any cell with an odd number of homologs that must be dealt with at meiosis) does undergo meiosis there is a problem. The movement towards one pole or the other is essentially random and independent for each group of homologs. In a monoploid some cells get none of each chromosome while others get one. Similarly, in a triploid some cells would get one homolog while other cells would get two. In either situation this creates a genetic imbalance in the gamete, and thus almost certainly a genetic imbalance in the fertilized zygote.

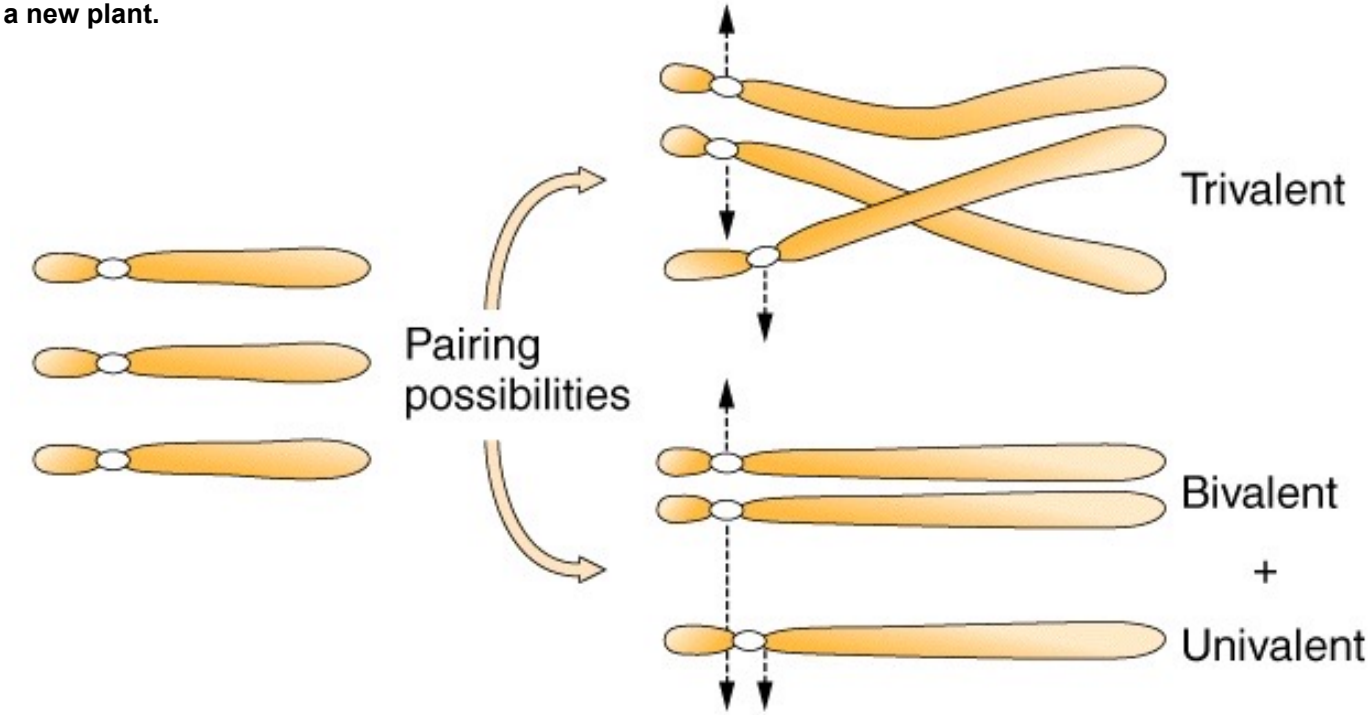
$$\left(\frac{1}{2}\right)^{x-1}$$

X = the number of chromosomes (in monoploids), or X, the basic chromosome set number, in polyploids such as triploids.

Triploids have problems during meiosis that lead to sterility

Triploids arise from a cross between a tetraploid and a diploid. Diploid and haploid gametes fuse to produce a triploid embryo. The triploid offspring can develop. However, during meiosis a similar problem occurs to that present in a monoploid (figure below). It is very unlikely [p = (1/2) x-1] that a similar number of the different chromosomes will all go to the same gamete during meiosis. This leads to genome imbalance.

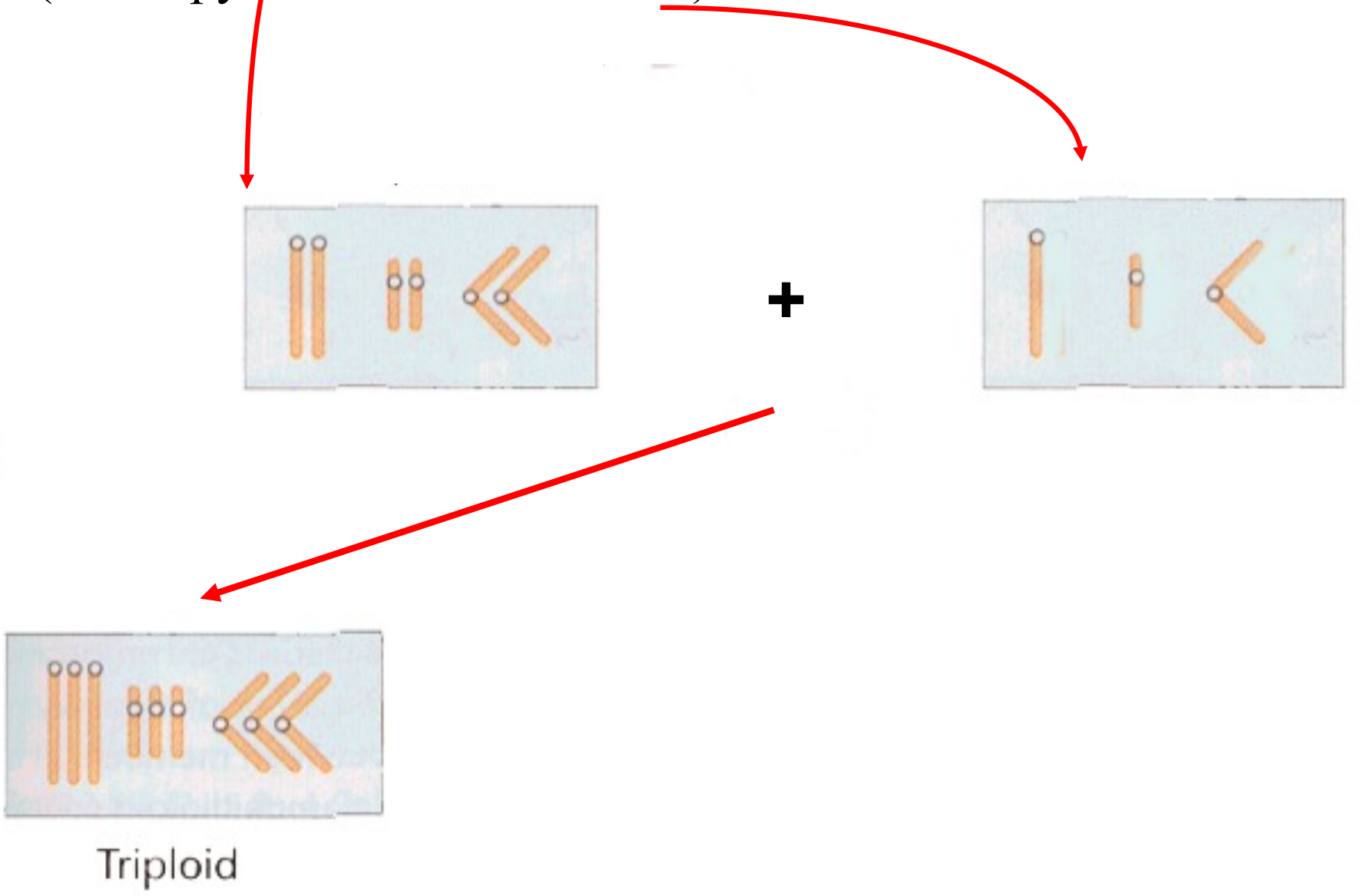
Take as an example the banana. It has 11 chromosomes and is a triploid (3x = 33 chromosomes). This means that at meiosis there is a 1/1024 chance of producing a gamete with a full haploid or diploid complement of chromosomes. The frequency of two such gametes getting together to produce a viable seed is extremely low. Triploidy is used to create seedless versions of fruits such as bananas and watermelon. So, you might ask, if they are seedless, how do they propagate. This is done artificially, using cuttings, grafts or bulbs. Essentially, a piece of the original triploid is used to start a new plant.



Triploids are sterile.

Bananas, seedless watermelons are seedless because they are triploid

Triploids are generated by combining gametes from a tetraploid (2 copies of each chromosome) with those of a diploid (one copy of each chromosome).



Production of Trout Offspring from Triploid Salmon Parents

Tomoyuki Okutsu,[1] Shinya Shikina,[1] Megumi Kanno,[1] Yutaka Takeuchi,[1] Goro Yoshizaki[1,2]*

In recent decades, the number of salmonid species has declined markedly, and several species have become extinct or endangered. Because cryopreservation of fish eggs is difficult due to their large size and high fat content, we investigated the potential of surrogate broodstock technologies as a new method of genetic resource preservation for fish. Surrogate broodstock technologies involve the transplantation of primordial germ cells (PGCs) (*1*) or spermatogonia (*2*) from a target fish species into a related species for which rearing techniques are well developed. In doing so, the recipient species can produce sperm and eggs of the target species (*3*). Furthermore, because PGCs and spermatogonia are sufficiently small for cryopreservation, animals can be generated via the transplantation of thawed PGCs or spermatogonia into recipients, even if the target species becomes extinct. In prior work, we demplanting spermatogonia into sterile xenogeneic recipients.

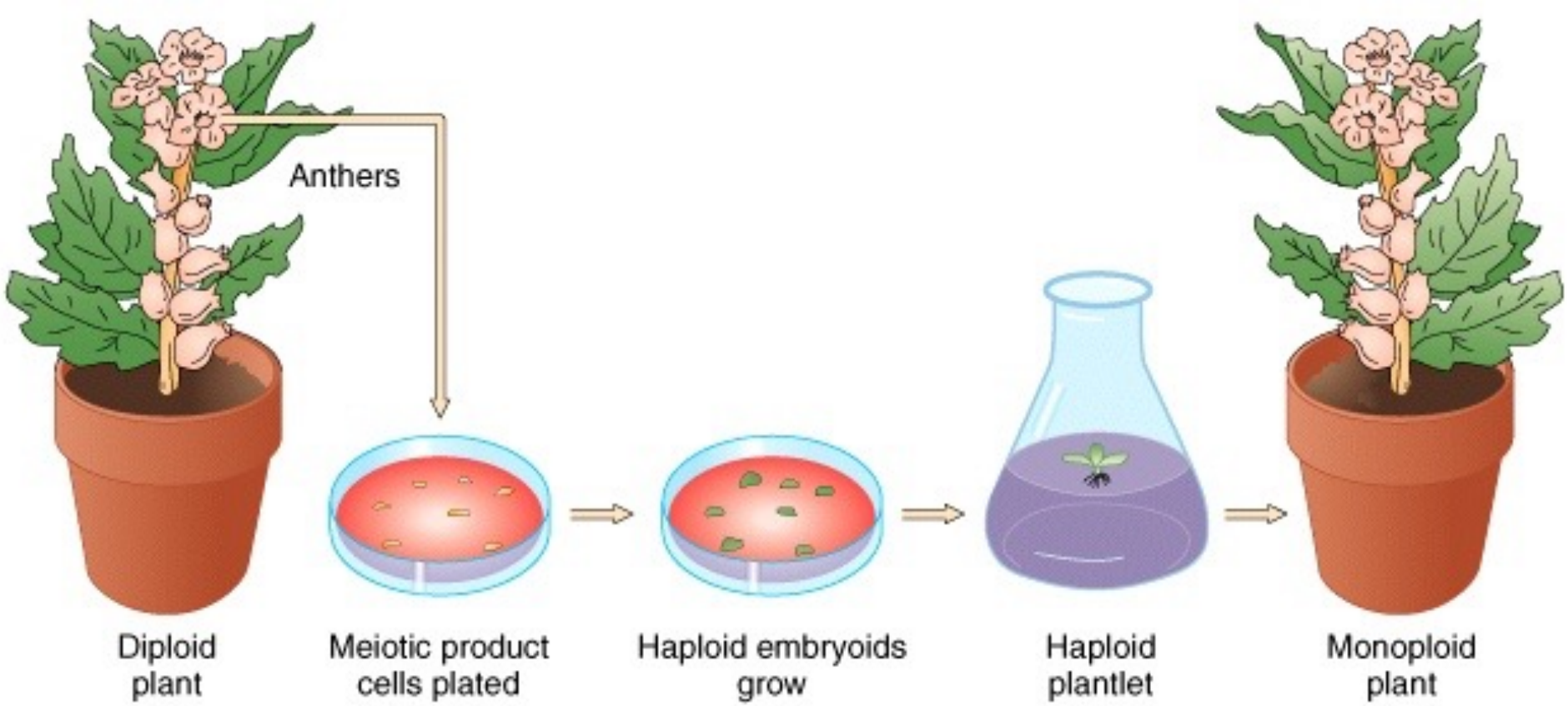
In this study, spermatogonia of p*vasa-Gfp* (where Gfp represents green fluorescent protein) hemizygous (p*vasa-Gfp*/–) and dominant orange-colored mutant heterozygous [*OR/wild type (WT)*] adult rainbow trout (*Oncorhynchus mykiss*) were intraperitoneally microinjected into newly hatched embryos of triploid sterile masu salmon (*O. masou*). Hybrids of these two species do not survive. Histological examination showed that, whereas the testes of 2-year-old triploid salmon in the control group (no transplantation) were immature and contained mostly spermatogonia, testes of recipients appeared normal (Fig. 1A). Ten of the 29 male triploid salmon recipients produced milt. Offspring produced with milt from these 10 recipients and wild-type trout eggs developed normally (fig. S1 and table S1). Five F_1 progeny were collected from

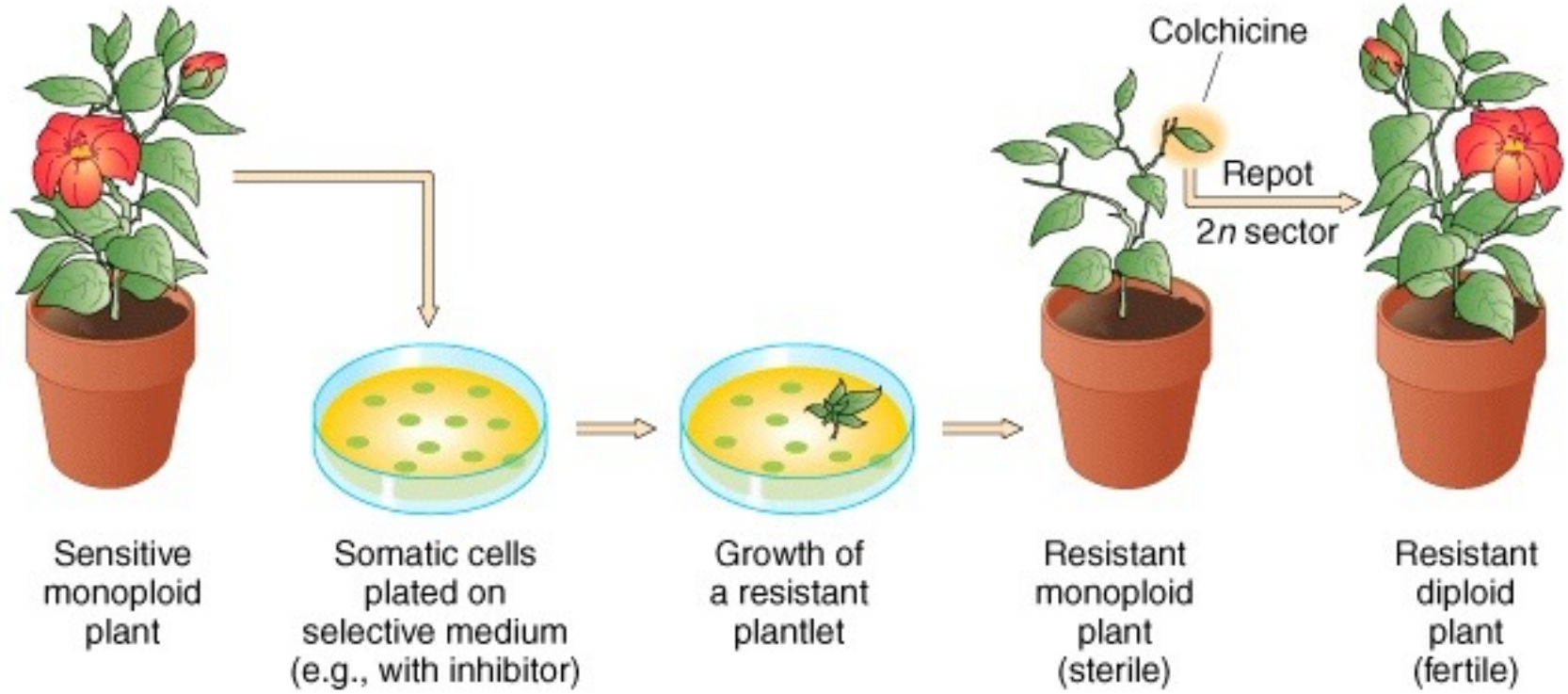
Monoploid plants in approaches to plant breeding

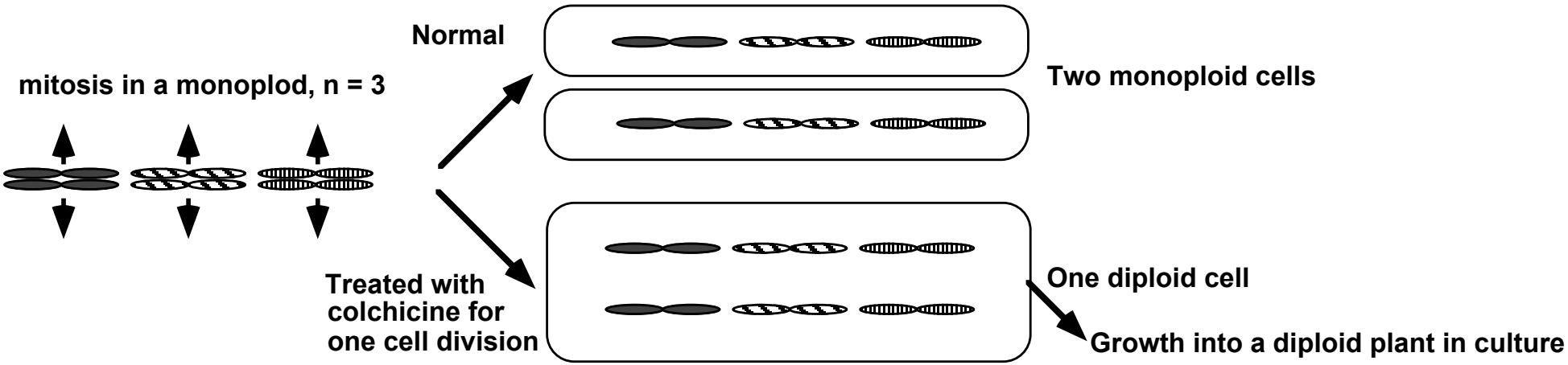
Diploidy is a pain in the ass if one is trying to create organisms that have particular genetic characteristics through mutagenesis. This is because in order to generate organisms that are homozygous recessive for some trait it is necessary to go through multiple generations in order to mutagenize, generate F1s, and then self these F1s and test them in the next generation for the desired characteristic. See for example the mutagenesis screen described at the end of lecture 2 in which we were trying to carry out a screen for plants with white flowers.

1. Cells from a plant can be dissociated, placed in culture, and used to generate an entirely new plant. Thus essentially all cells in a plant are pluripotent (able to contribute to all cell types).

2. The ploidy state of a plant can be easily manipulated in tissue culture.
A. Monoploid plants can be created from gametes grown in culture.
B. Plants with increased ploidy can be generated by preventing the segregation of sister chromatids during mitosis using the microtubule disrupting drug colchicine.




Colchicine
Repot
2n sector
Sensitive monoploid plant
Somatic cells plated on selective medium (e.g., with inhibitor)
Growth of a resistant plantlet
Resistant monoploid plant (sterile)
Resistant diploid plant (fertile)

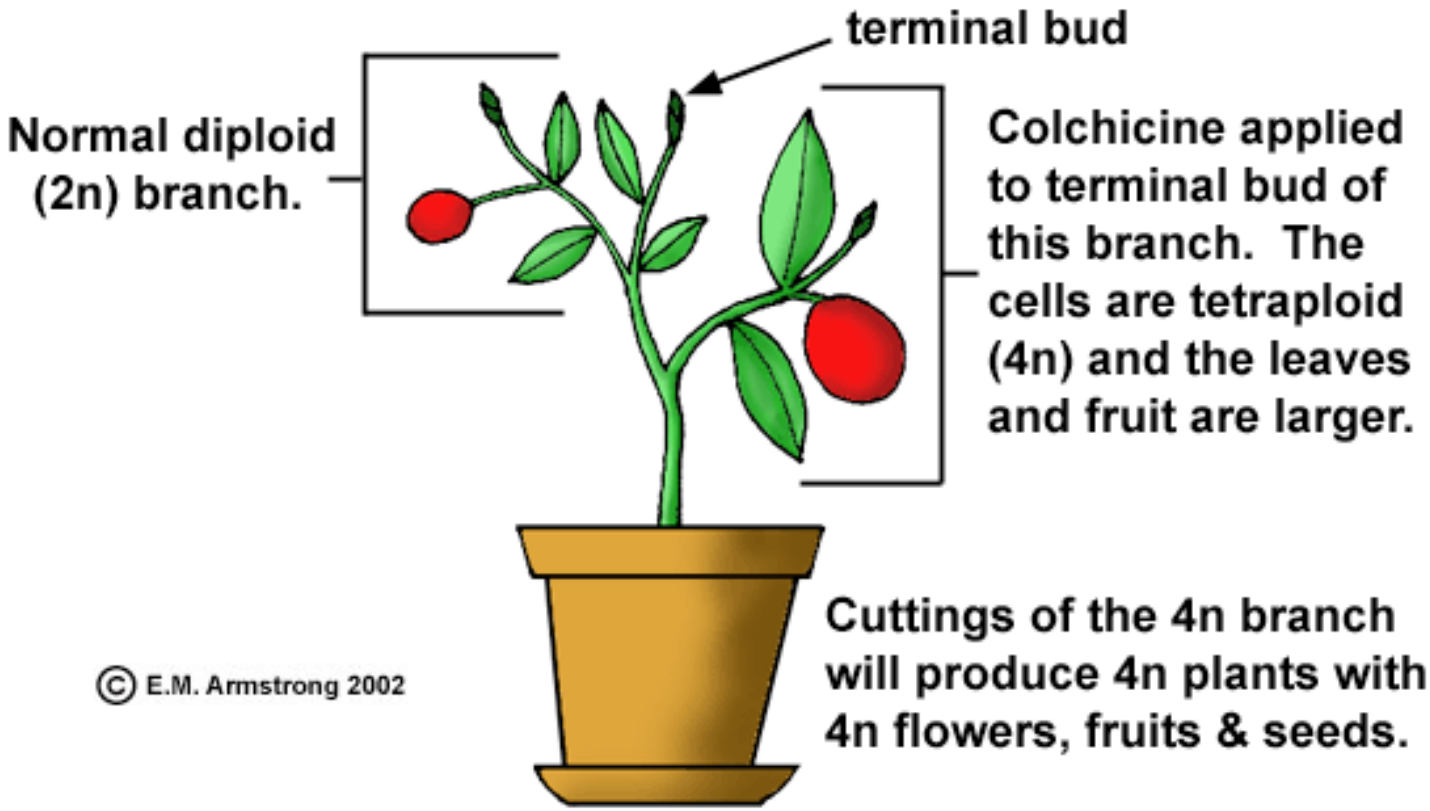

mitosis in a monoplod, n = 3
Normal
Two monoploid cells
Treated with colchicine for one cell division
One diploid cell
Growth into a diploid plant in culture

Autopolyploidy

...polyploidy resulting from the replication of one or more sets of chromosomes,

…the additional set of chromosomes is identical to the normal haploid complement of that species.

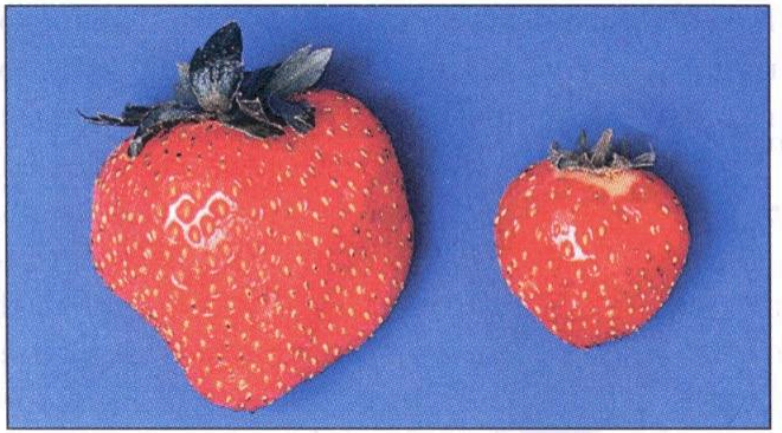
Can artificially use colchicine to increase ploidy.



Autopolyploidy Applications

- Treating a plant with colchicine often produces autopolyploidy, resulting in plants with larger flowers and/or fruit,

4n *2n*



8n *2n*

…And of course generating adults that can be crossed with diploids to generate sterile triploid progeny

(a)

(b)

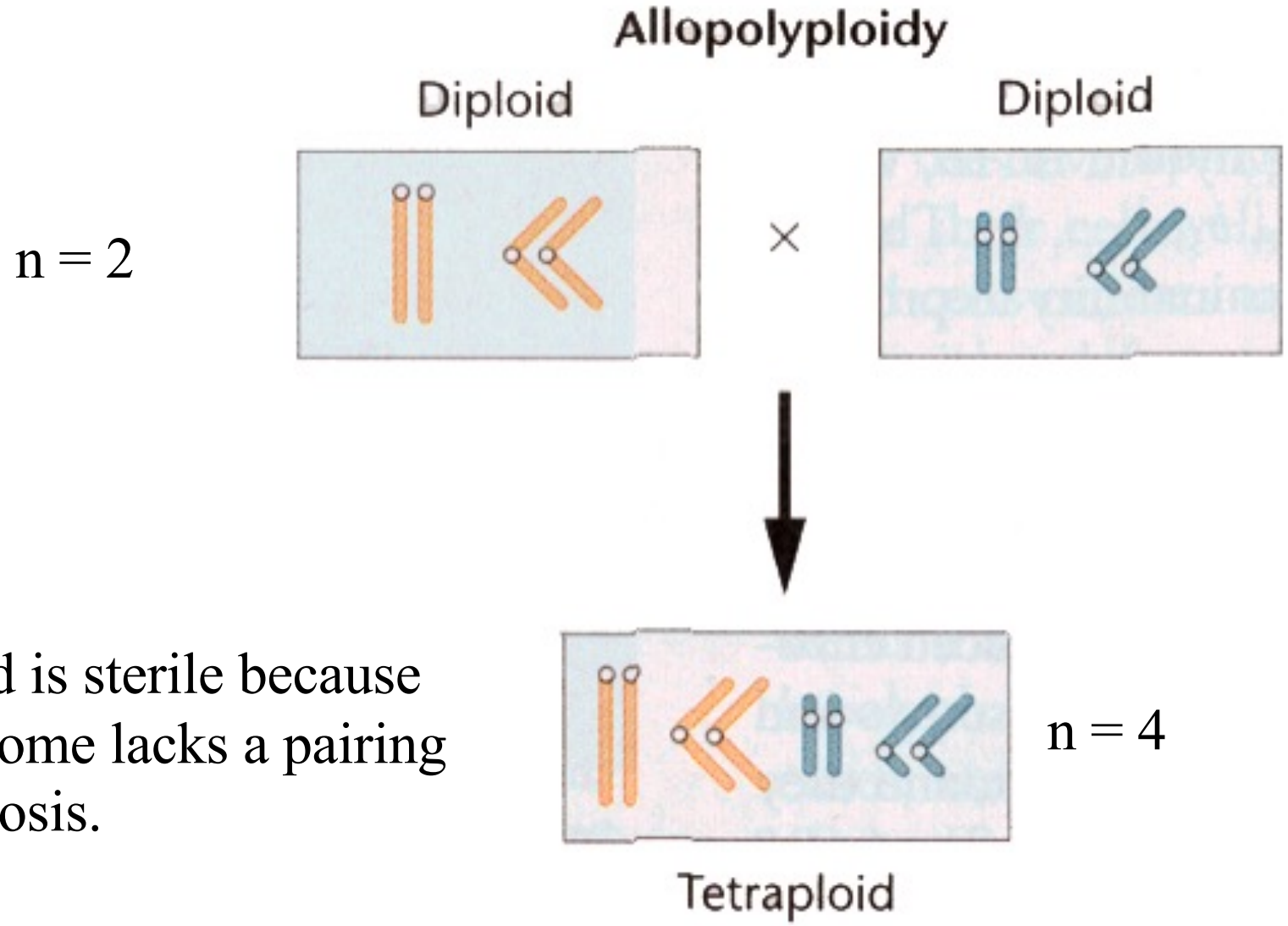
Diploid & Tetraploid *Hyla*

These are essentially the same frog - it is the *balance* between the number of gene copies that is important for proper development, not necessarily the *total number* of gene copies

Up until now we have been dealing with autopolyploids, which contain multiple sets of chromosomes from the same species. Allopolyploids are produced from sets of chromosomes of closely related species.

Closely related chromosomes from different species are not homolgous, but are referred to as homeologous.

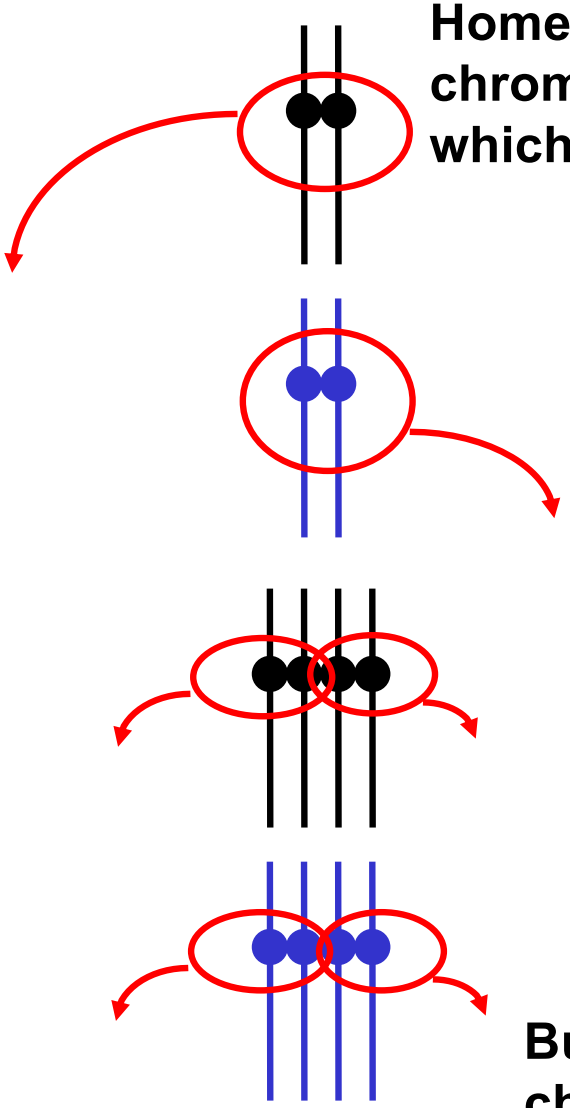
Homeologous chromosomes do not pair during meiosis and this can lead to problems.



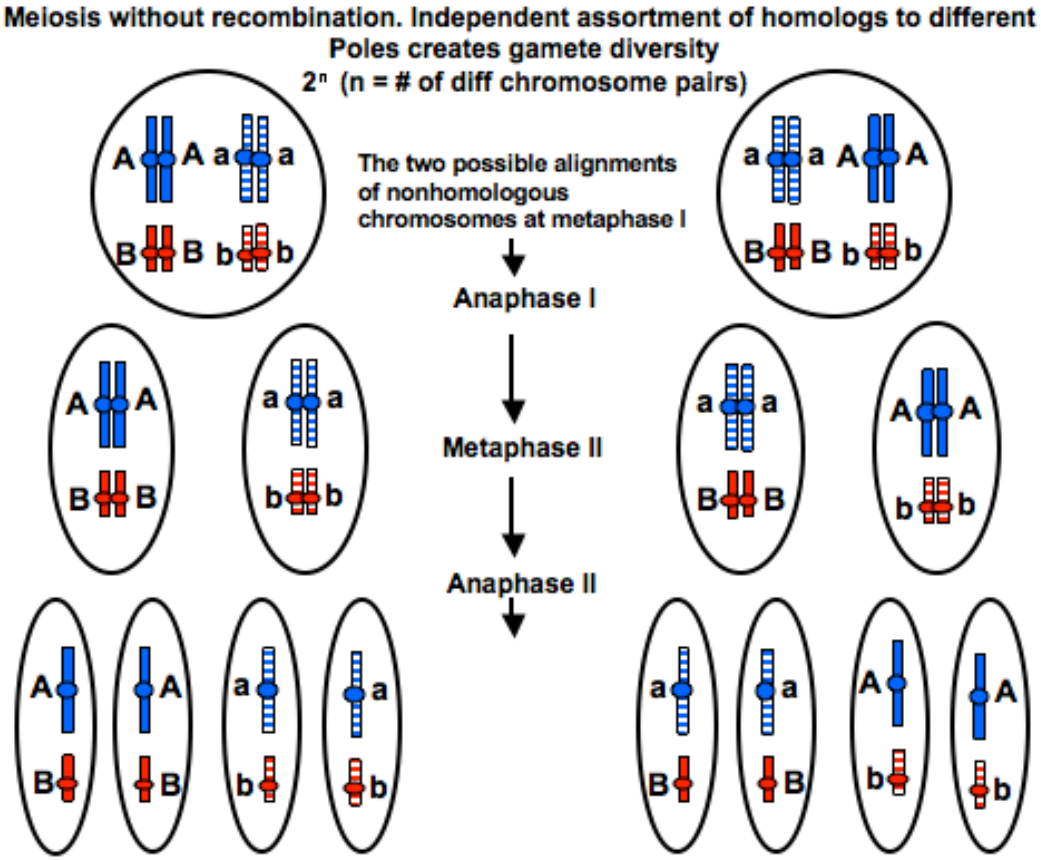
This tetraploid is sterile because each chromosome lacks a pairing partner at meiosis.

Solution: Duplicate each chromosome by using colchicine to double the number of chromosomes, thereby giving each chromosome a pairing partner.

If cross two related species, ok until meiosis.



Homeologous chromosomes don't pair, which leads to sterility.



But by doubling the chromosomes, generate an allopolyploid (amphidiploid) that is fertile

The Sad Tail of *Raphanobrassica*

Cast

Dr. G. Karpechenko, Russian Plant breeder,

Brassica oleracea (2*n* = 18),

- cabbage, large above ground food stock, worthless root,

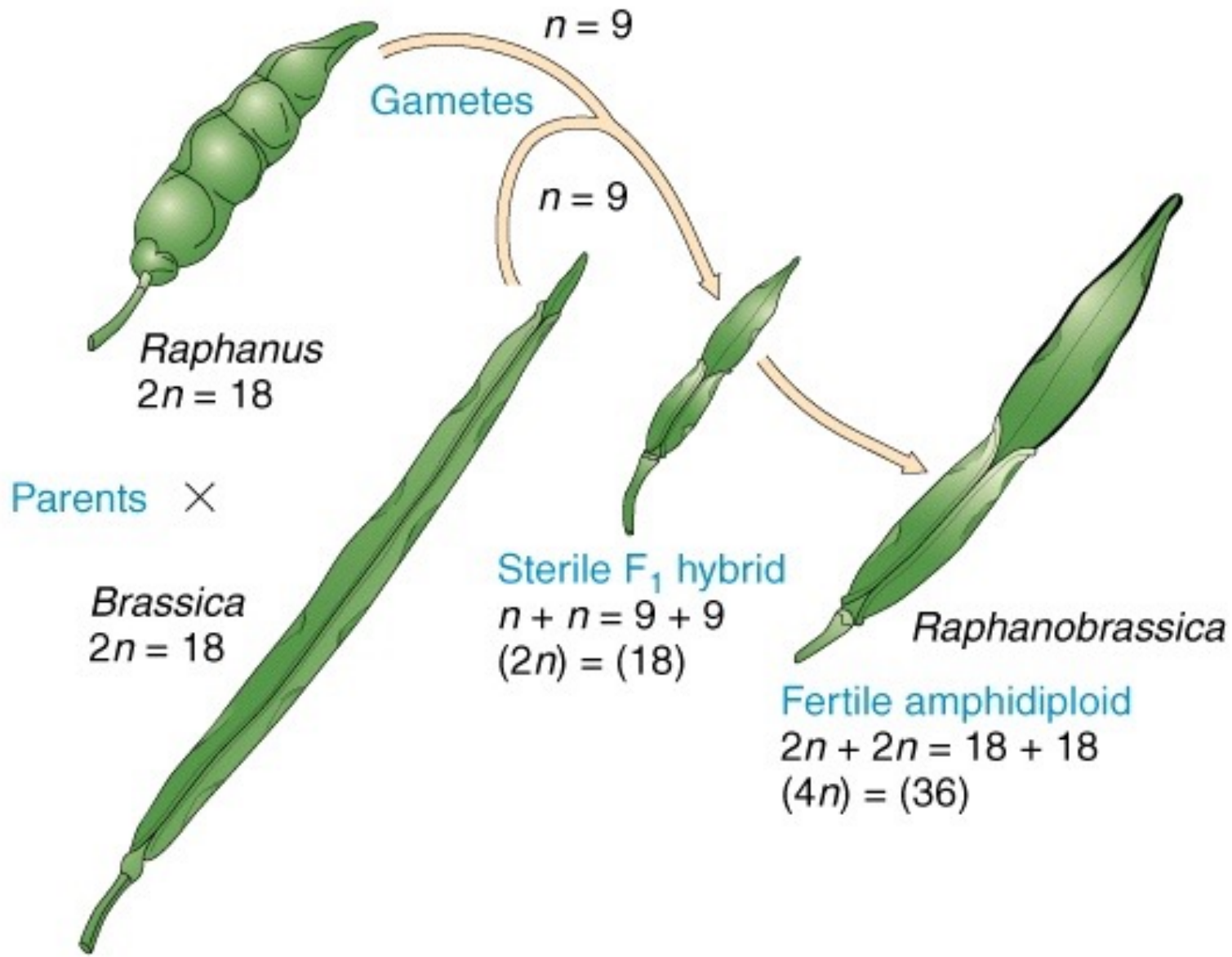
Raphanus sativus (2n = 18),

- the common radish, large root food stock, worthless leaves.

Georgi Karpechenko

wanted to generate a plant that had the leaves of a cabbage and the roots of a radish.

cabbage + radish = cabrad?


n = 9
Gametes
n = 9
Raphanus
2n = 18
Parents ×
Brassica
2n = 18
Sterile F1 hybrid
n + n = 9 + 9
(2n) = (18)
Raphanobrassica
Fertile amphidiploid
2n + 2n = 18 + 18
(4n) = (36)

and produced a …radcab!

leaves of a radish, roots of a cabbage. No good for people food. But.....

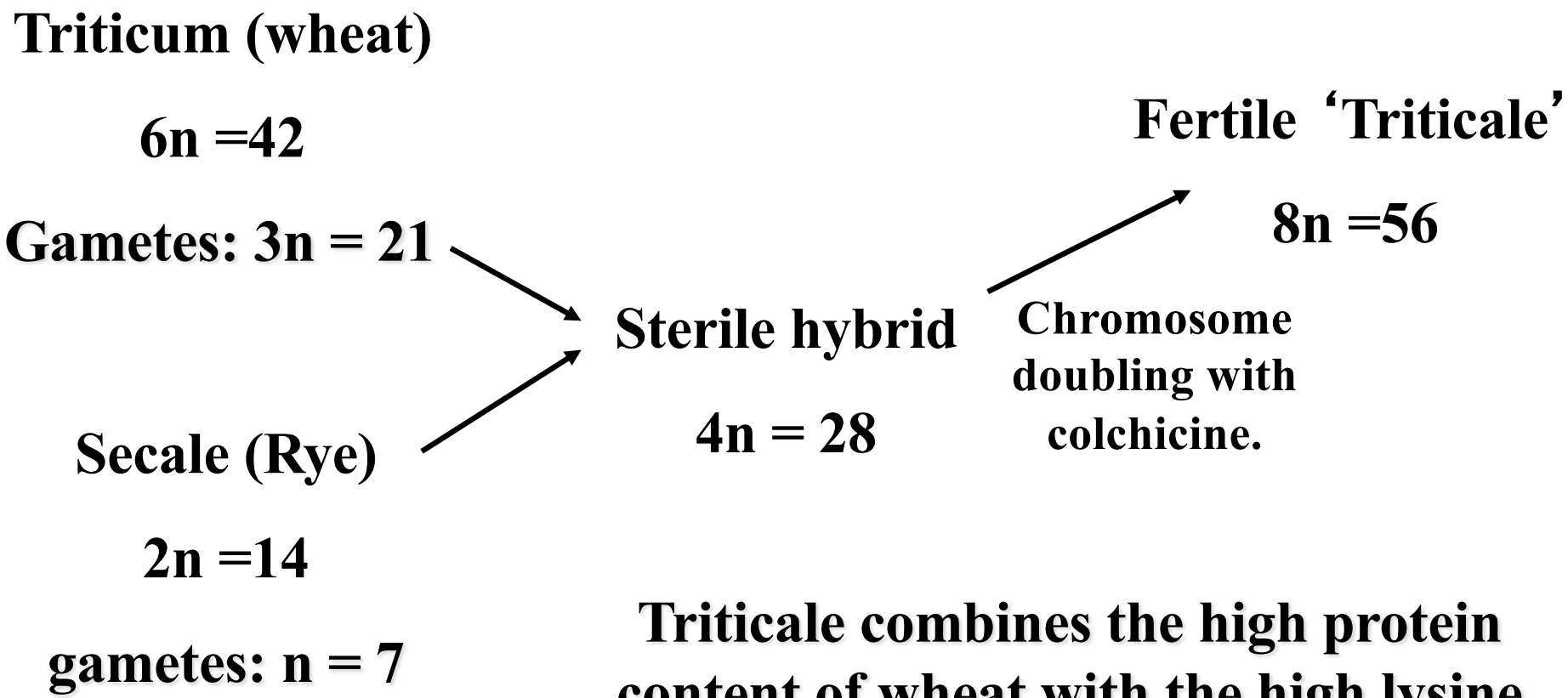
Revived by the Scottish Plant Breeding Station in Dundee, Scotland,

Raphanobrassica's high dry matter content makes it an excellent fodder for sheep and cattle.

Cannot breed with either parent = a new species!!!

While it is possible to produce allopolyploids, the outcomes are unpredictable.

One successful allopolyploid is Triticale



Triticale combines the high protein content of wheat with the high lysine levels and ability to adapt to marginal environments of rye.

Polyploidy Summary

- More than 2 whole sets of chromosomes,

- Autopolyploidy,
  - from the same genome,
  - naturally occurring, or induced,
  - often results in larger varieties,

- Allopolyploidy,
  - from different genomes,
  - naturally occurring, or induced,
  - often results in larger varieties,

- Autotriploids,
  - most often sterile
  - can produce beneficial traits.

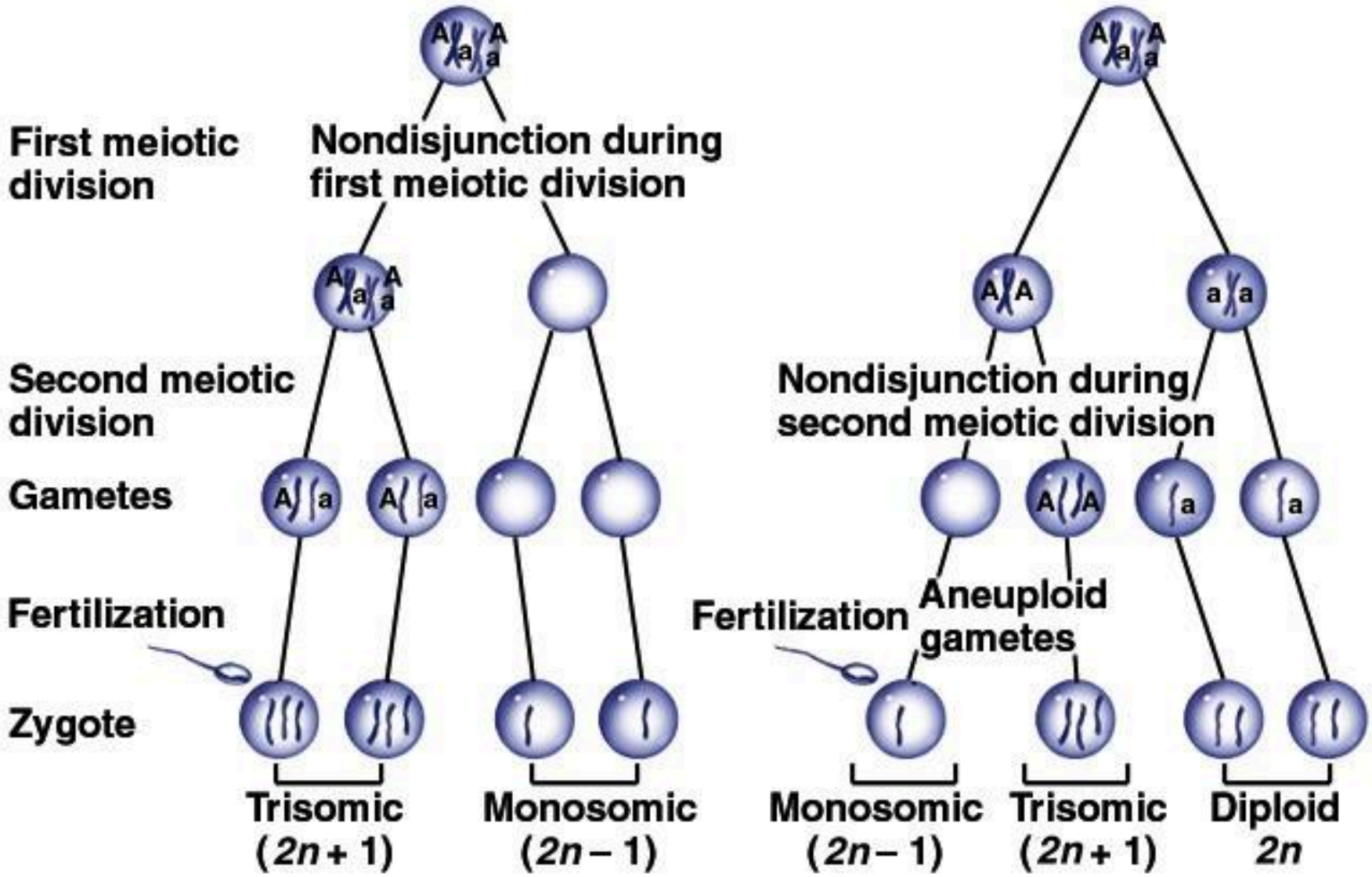
Aneuploidy

Nondisjunction in meiosis I or II results in gametes with an extra or missing chromosome.

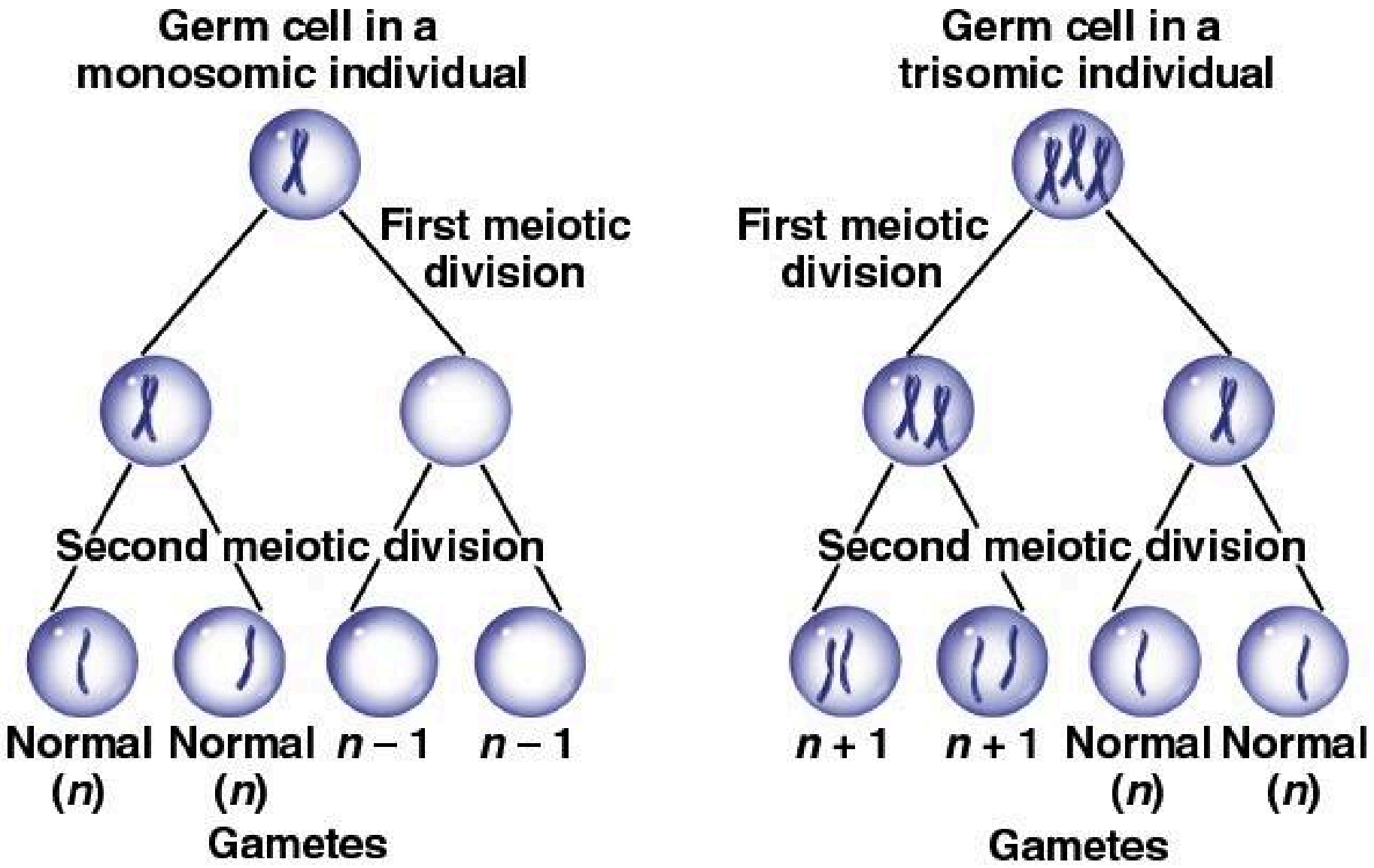
When these gametes fuse, the fusion results in zygotes with an extra or missing chromosome, a situation termed aneuploidy!

What are the consequences of aneuploidy?

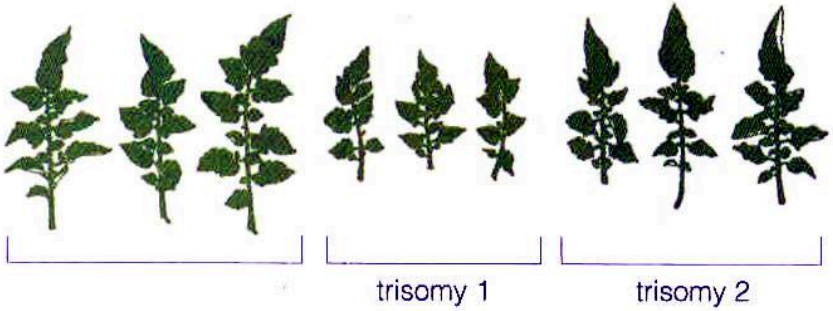
Aneuploidy

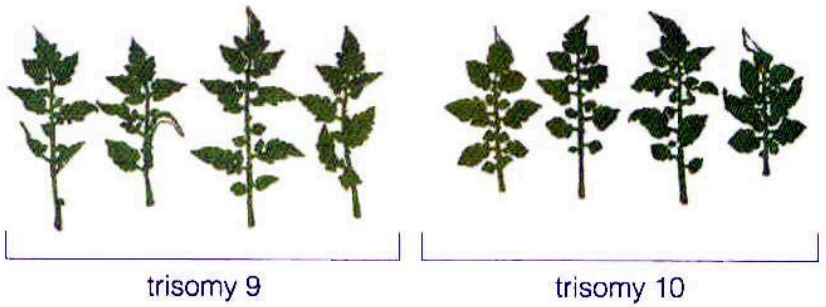


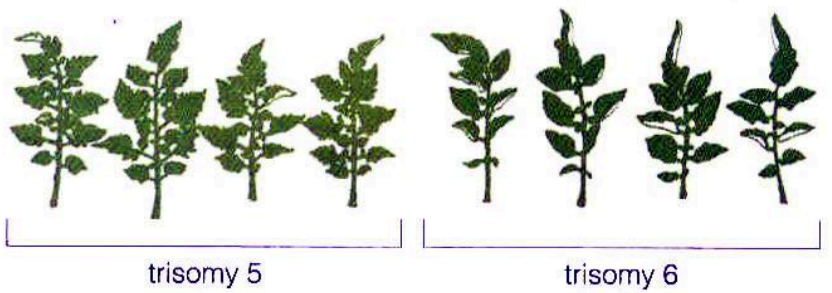
Aneuploidy

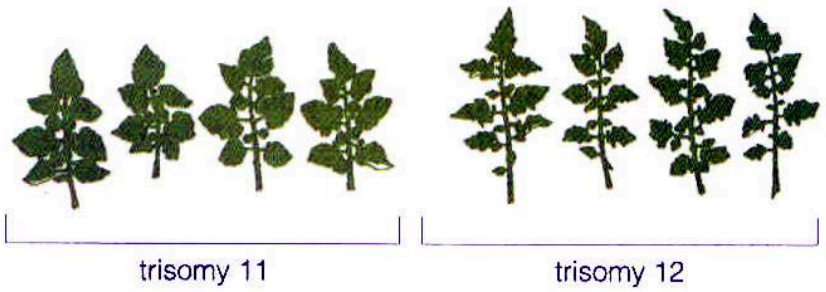


Plants just don't care that much: the tomato









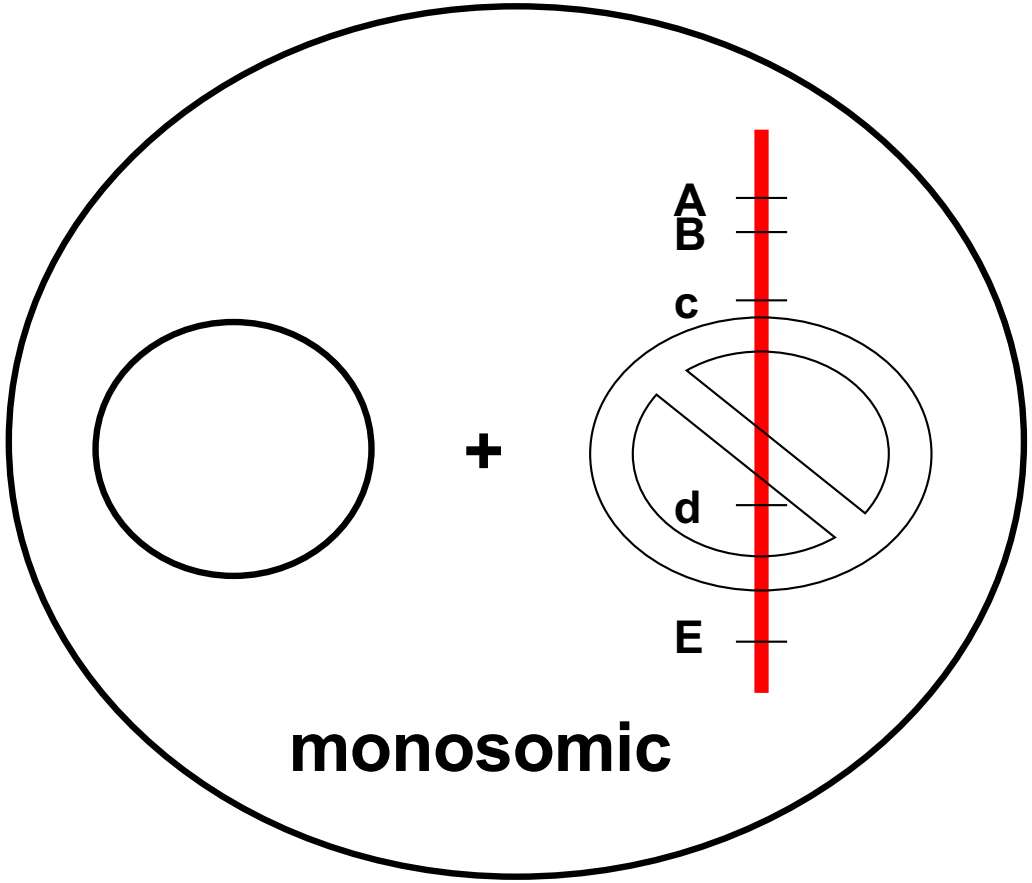
Human Autosomes first!

All monosomic (43 autosomes; missing an autosome) spontaneously abort.

Almost all trisomic (45 autosomes; an extra autosome) fetuses spontaneously abort!
There are three exceptions.

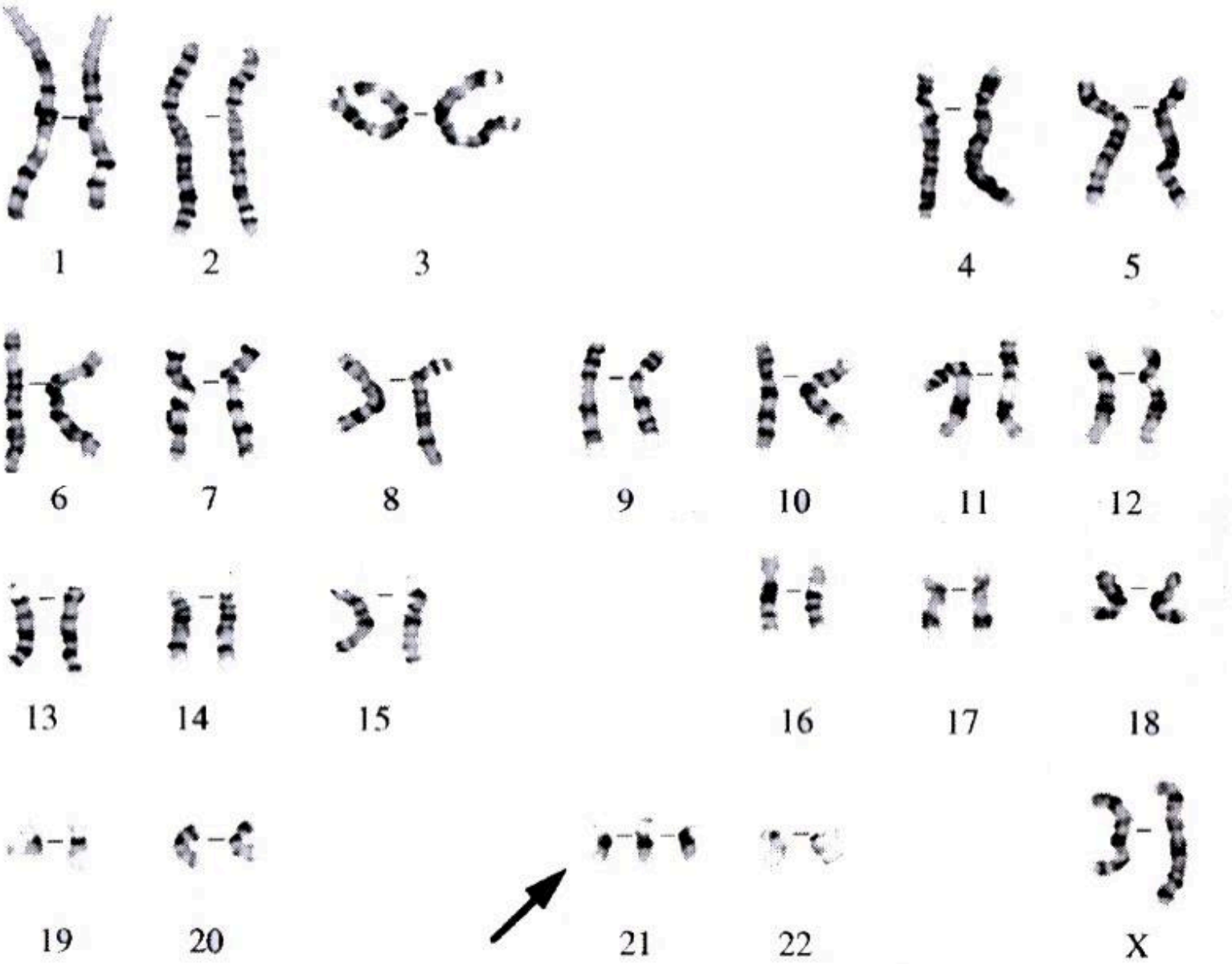
Autosomal Monosomy

- nearly always deleterious, usually lethal,
  - recessive lethal alleles are expressed,



c and d are recessive lethal alleles. Therefore, a monosomic individual that carries this chromosome is dead.

Down Syndrome (47, +21)



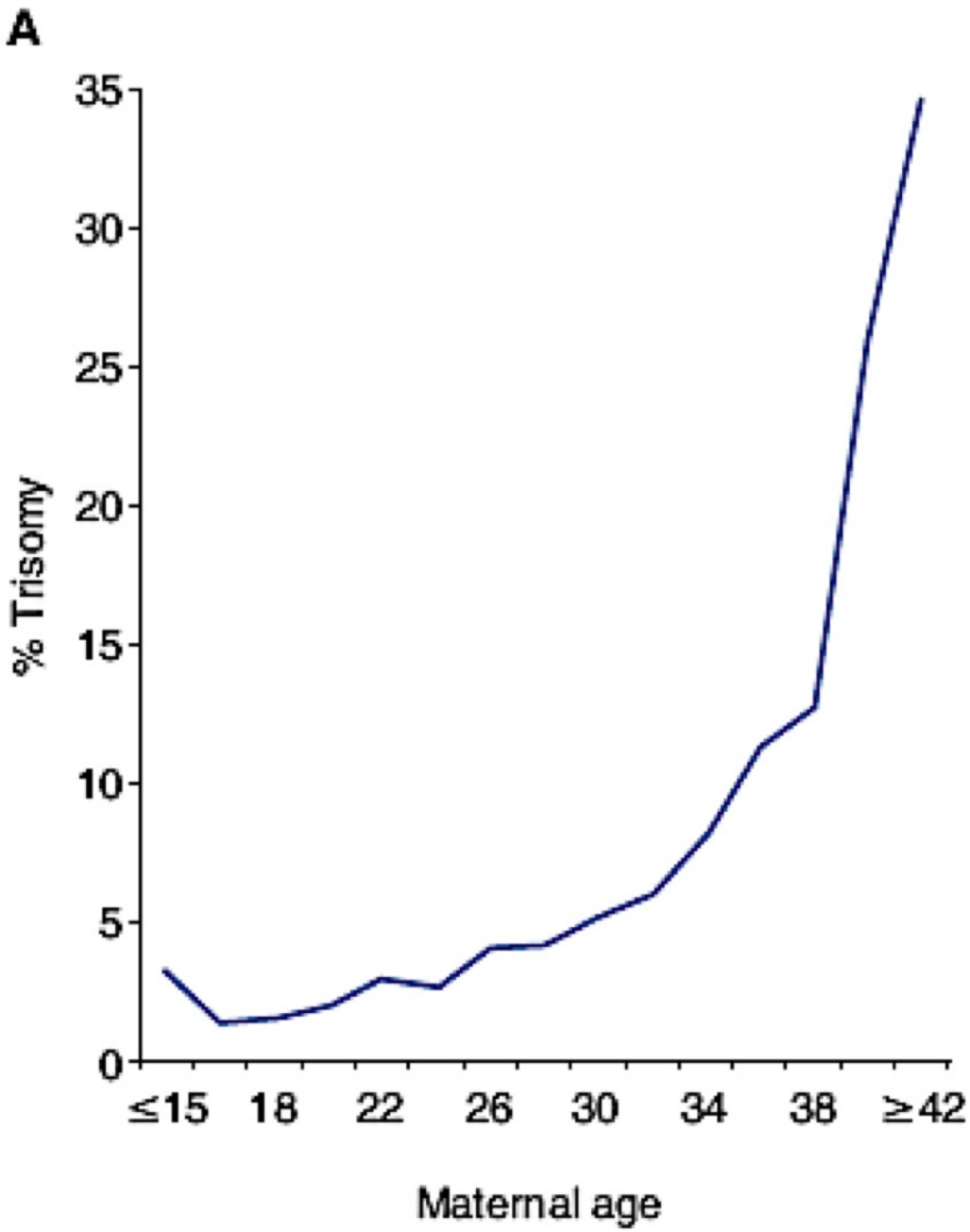
Trisomy in Humans

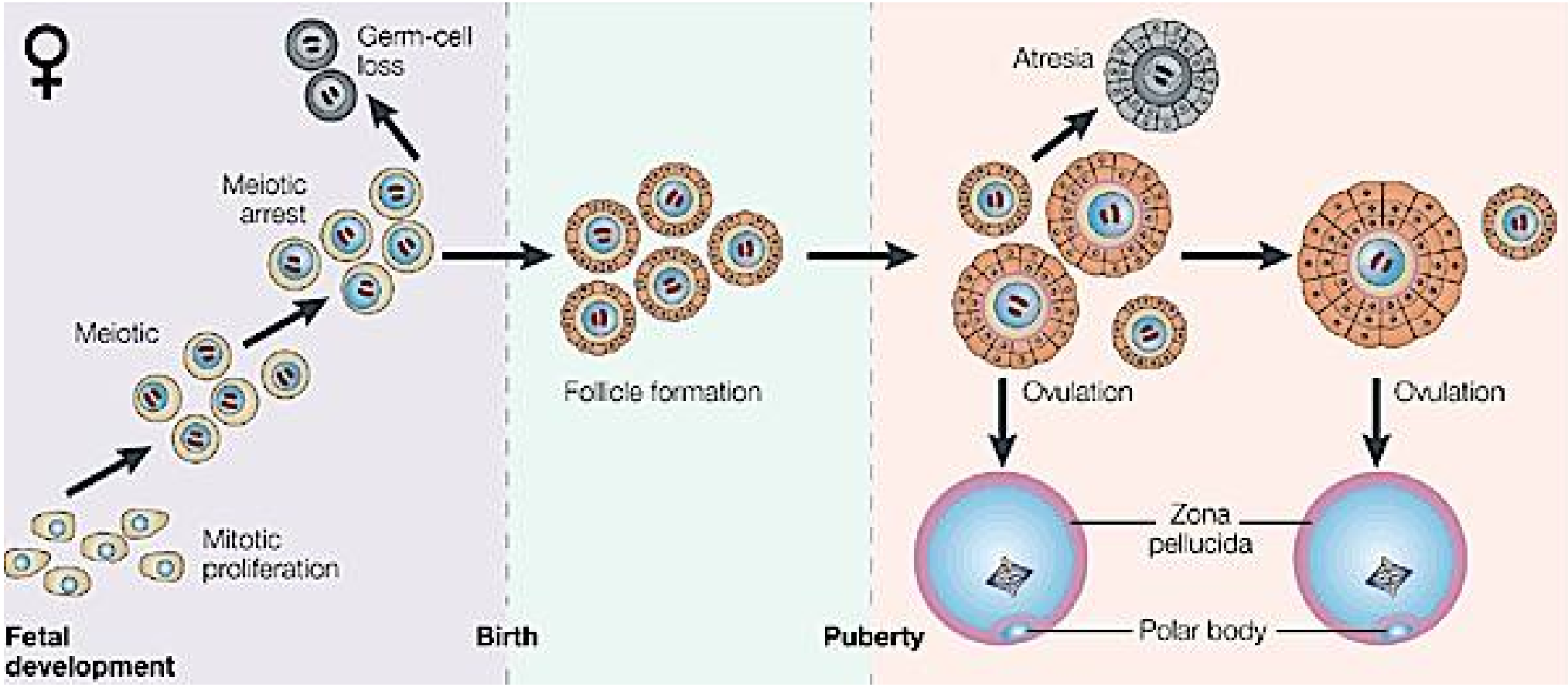
Down Syndrome

(47, +21)

- occurs at a frequency of about 0.15%,
- mental retardation (I.Q. 20 - 50),
- mean life expectancy is about 17 years,
- 47, +21 females are fertile,
- trisomy 21 risk factors, maternal age.

Maternal Age and trisomy

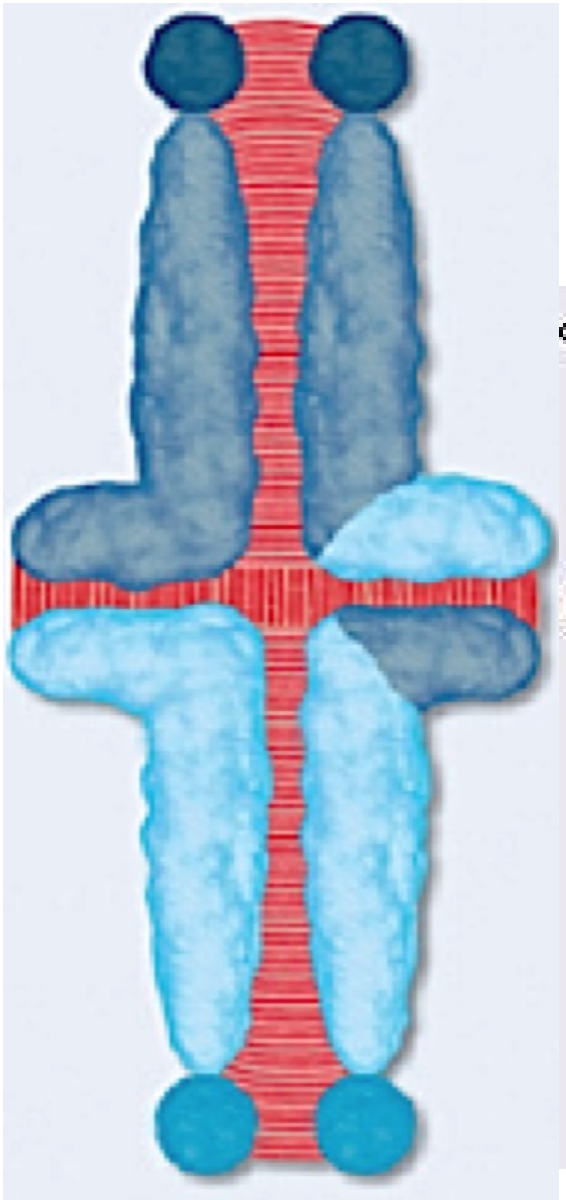




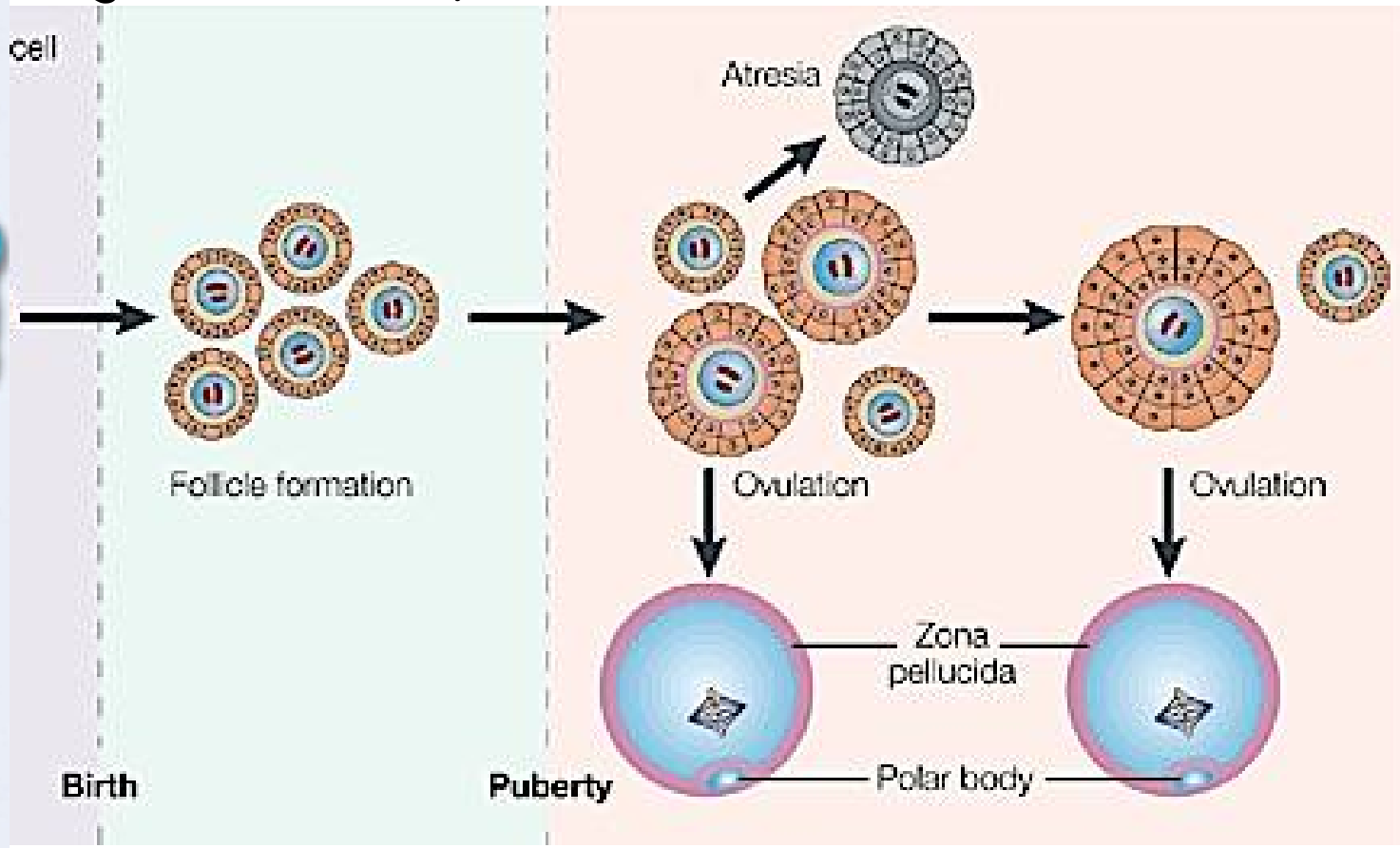
-In female humans, oocytes arrest in Prophase I before birth. Meiosis continues upon menstruation.

-Thus, tetrad formation and cellular function must be maintained for decades.

- The proteins mediating



-The cohesion molecules that maintain alignment of the homologs when you are born are literally the same protein molecules that maintain alignment when you are 40. Errors arise.



-In female humans, oocytes arrest in Prophase I before birth. Meiosis continues upon menstruation.

-Thus, tetrad formation and cellular function must be maintained for decades

Other Human Autosomal Trisomics

- **Edwards Syndrome (47, +18)**
  - **0.0125% live births,**
  - **mental abnormalities,**
  - **“faunlike ears”, “rockerbottom feet”, small jaw,**
  - **nearly all die within several weeks of birth,**

- **Patau Syndrome (47, +13),**
  - **0.005% live births,**
  - **mental abnormalities,**
  - **cleft lip, small mal-formed head, “rockerbottom feet”,**
  - **mean life expectancy is about 130 days.**

Somatic polyploidy

Drosophila salivary glands. 1024-2048 copies

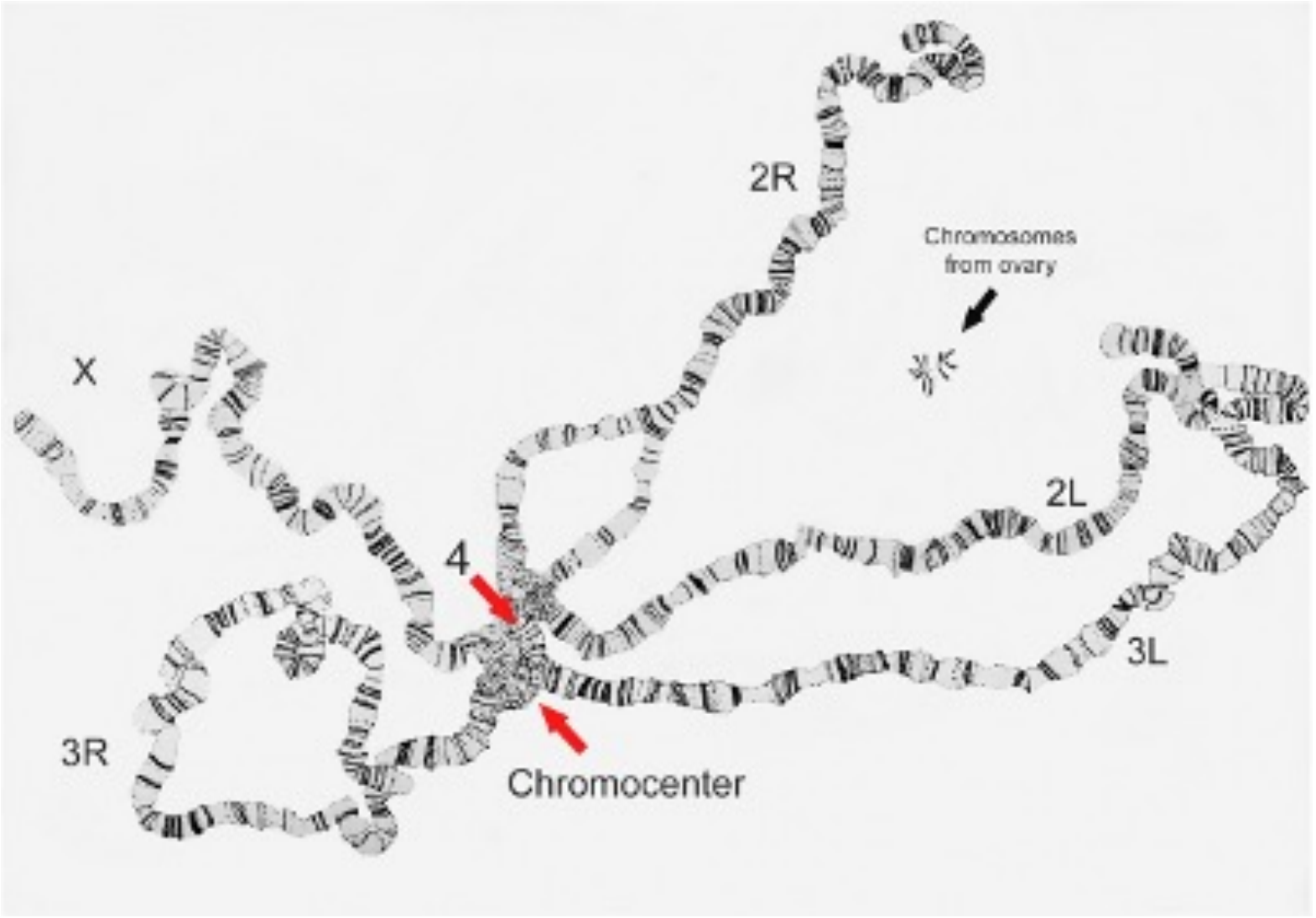
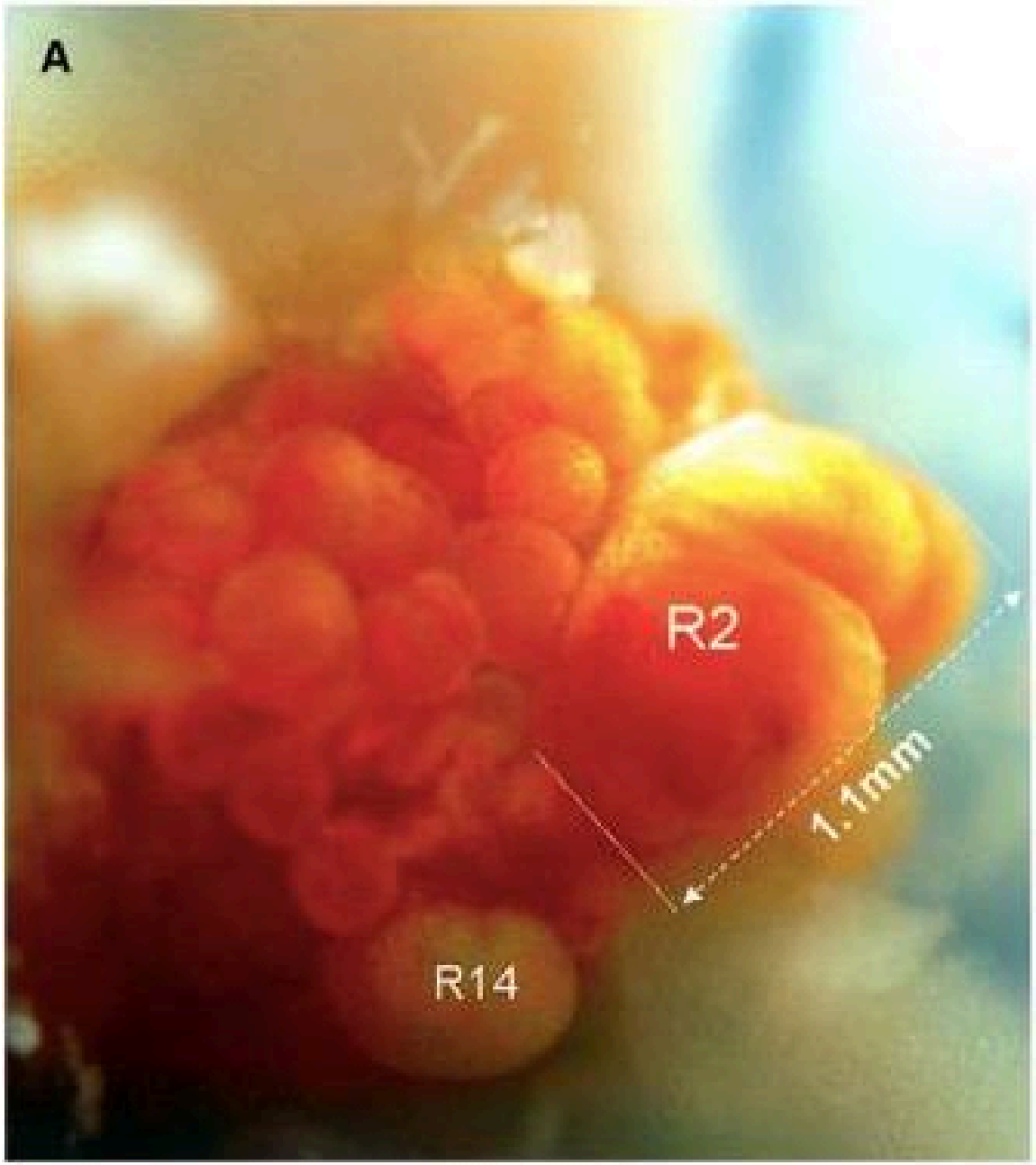


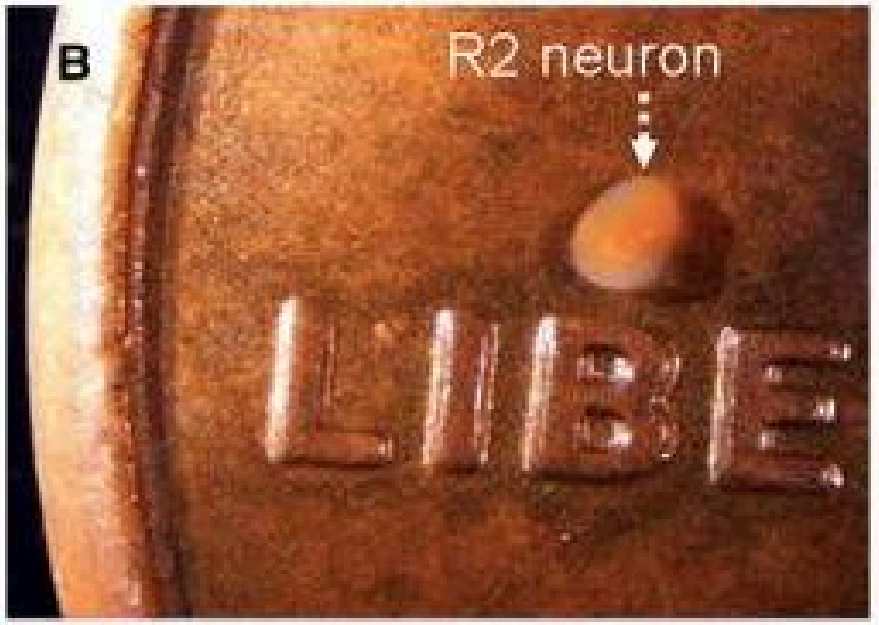
TABLE 1 - *High-level polyteny in animals and plants. C values are based on DNA measurements, n values on nuclear volumetry.*

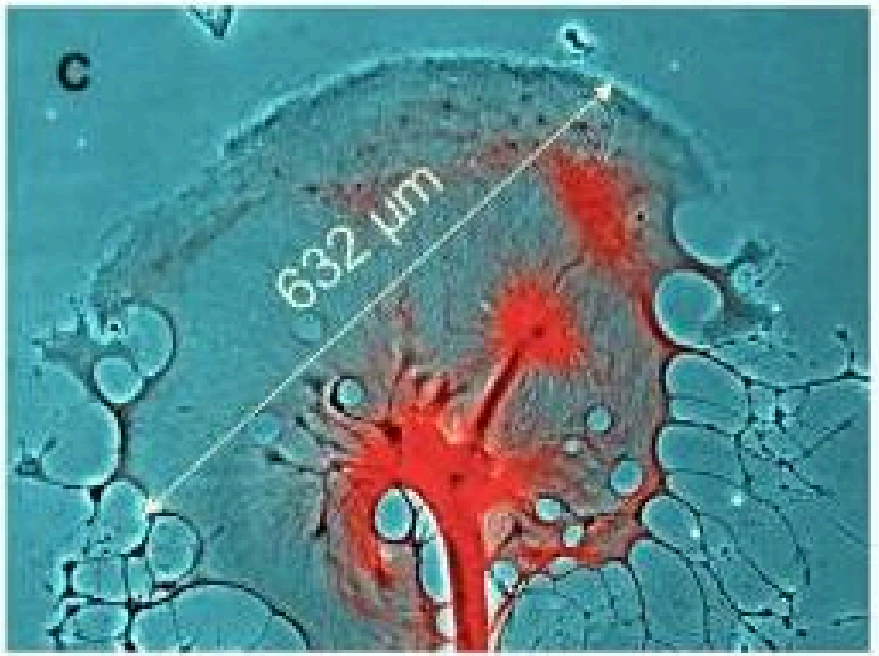
Species	Tissue or cell	Maximum level of polyteny	Reference
ANIMALS			
Aplysia californica	Giant neurons	200,000 C	LASEK and DOWER 1971 [101]
Ascaris suum	Esophageal gland	262,144 C	ANISIMOV 1976 [102]
Bombyx mori	Silk gland	524,288 C	GAGE 1974 [103]
	id, posterior zone	1,048,576 C	PERDRIX-GILOT 1978 [104]
Chironomus tentans	Salivary gland	32,768 n	BEERMANN 1952 [105]
Chironomus thummii	Salivary gland	16,384 C	RASCH 1970 [106]
Drosophila melanogaster	Salivary gland	2,048 C	RASCH 1970 [106]
Microtus arvalis	Trophoblast	2,048 C	ZYBINA *et al.* 1975 [107]
Mus musculus	Trophoblast	1,024 C	BARLOW and SHERMAN 1972 [108]
Rattus rattus	Trophonblast	4,096 C	NAGL 1972 [109]
Sciara coprophila	Salivary gland	8,192 C	RASCH 1970 [106]
PLANTS			
Arum maculatum	Endosperm haustorium *	24,576 n	ERBRICH 1965 [110]
Echinocystis lobata	Endosperm, central	3,072 n	TURALA 1966 [111]
Melampyrum nemorosum	Endosperm haustrium	1,536 n	ERBRICH 1965 [110]
Phaseolus coccineus	Suspensor	8,192 C	BRADY 1973 [112]
Phaseolus vulgaris	Suspensor	4,096 C	NAGL 1974 [113]
Scilla bifolia	Elaiosome	4,096 n	SPETA 1972 [114]
Scilla decidua	Antipodal **	1,024 C	FRISCH and NAGL 1979 [75]

* Endosperm cells are initially triploid ($3n$).
** Antipodal cells are initially haploid (n).

Aplysia R2 neuron







Region-specific amplification/underreplication

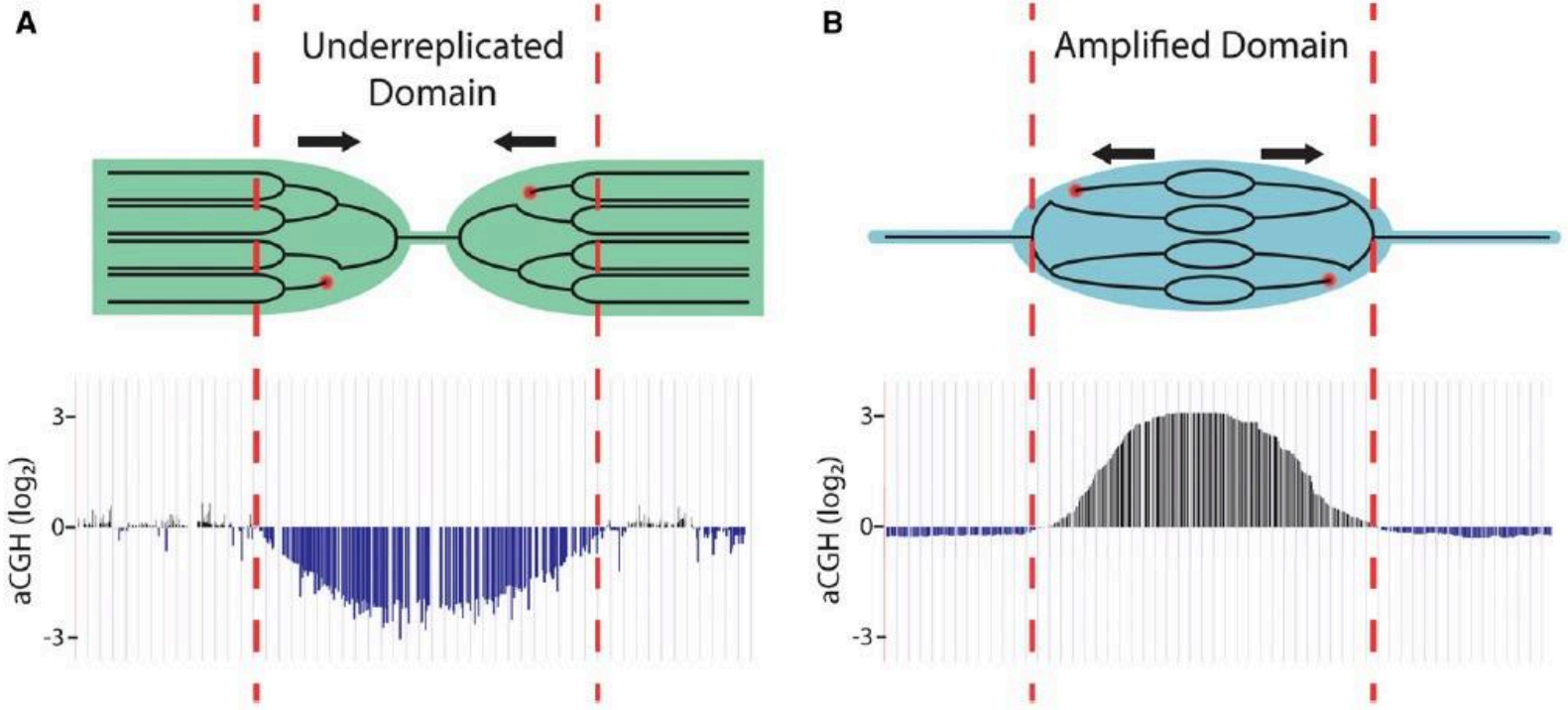


Figure 3 Differential DNA replication. (A) Underreplication results from two effects: absence of replication origins and initiation within a domain coupled with impaired progression of replication forks initiating from flanking origins (arrows indicate direction of fork progression). Replication forks are destabilized within underreplicated regions and can lead to double-stranded DNA breaks (red circles). These can lead to deletions or rearrangements in the region. Array-based comparative genome hybridization (aCGH) analysis of an underreplicated site indicates decreased copy number relative to overall ploidy. aCGH data are modified from Sher *et al.* (2012). (B) In endocycling follicle cells, developmentally programmed gene amplification occurs through repeated replication origin firing followed by bidirectional fork progression away from the origin (arrows indicate direction of fork progression). Rereplication can lead to fork collisions, resulting in double-strand DNA breaks (red circles). aCGH analysis of an amplified site indicates a gradient of increased copy number relative to overall ploidy spanning 100 kb, with the highest copy number at the origin of replication. aCGH data are modified from Kim *et al.* (2011).

Table 2 *Drosophila* amplicons in follicle cells

Cytological location	Max fold amplification	Stages of origin firing	Genes involved in egg shell function
7F	18–20	10B–11	*Cp7Fa*, *Cp7Fb*, *Cp7Fc*, *Cp36*, *Cp38*
22B	4	10B–13	None
30B	4	10B	*CG11381*, *CG13113*, *CG13114*[a]
34B	6	10B, 13	*Vm34Ca*
62D	4	10B, 13	*yellow-g*, *yellow-g2*
66D	60–80	10B–11	*Cp18*, *Cp15*, *Cp19*, *Cp16*

[a] Predicted chorion genes (Fakhouri *et al.* 2006; Tootle *et al.* 2011).