

Pili-receptor interactions determine which species a bacteria can conjugate with

ock-McGrane et al.

Page 31

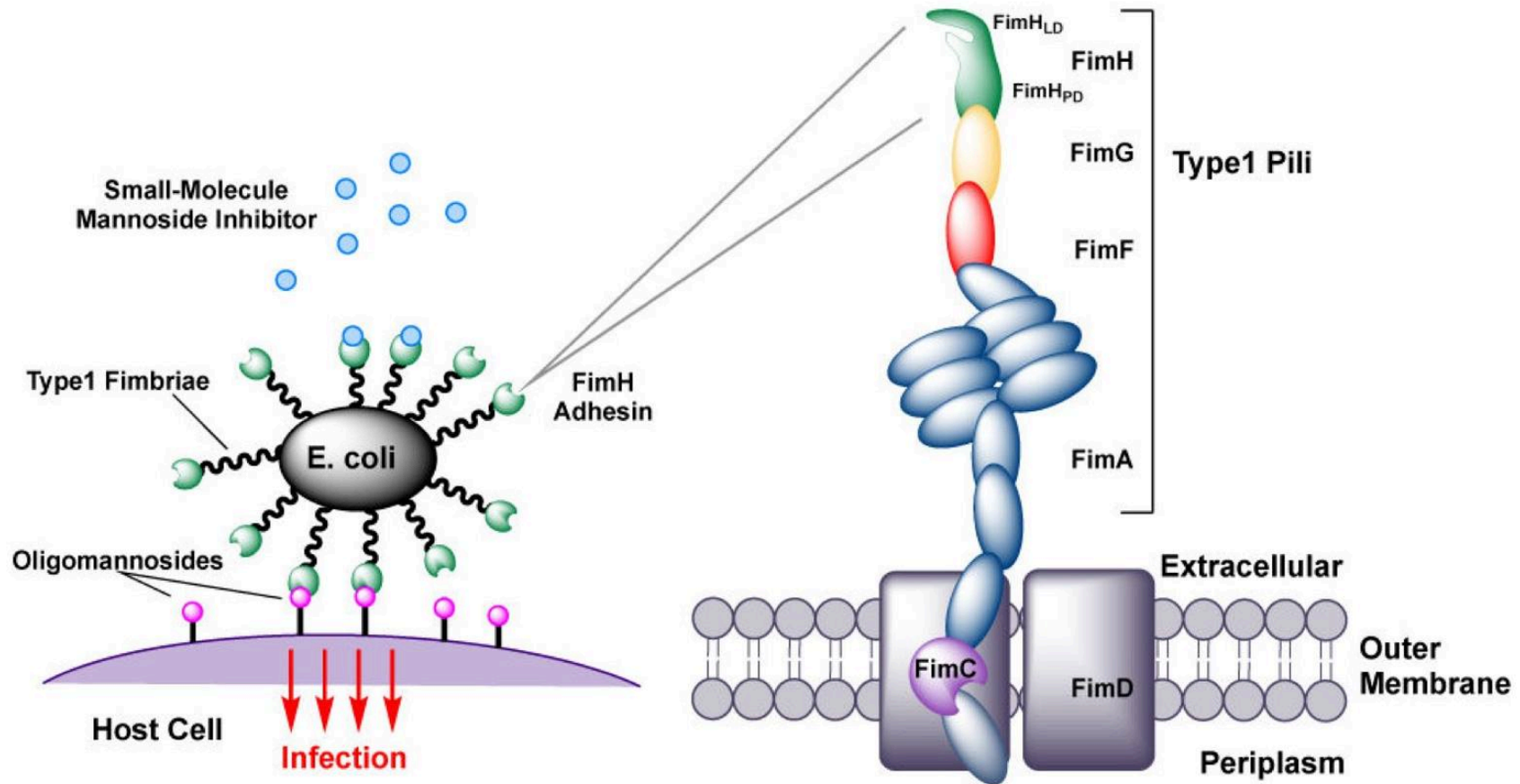


Figure 1.

Molecular recognition of mannosylated receptors on the bladder surface by FimH adhesin of UPEC, residing on the outer tips of type 1 pili. Therapeutic rationale for FimH mannose ligands in UTI and CD is to block adherence and invasion of bacteria.

ORIGINAL ARTICLE

Broad host range plasmids can invade an unexpectedly diverse fraction of a soil bacterial community

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Conjugal plasmids can provide microbes with full complements of new genes and constitute potent vehicles for horizontal gene transfer. Conjugal plasmid transfer is deemed responsible for the rapid spread of antibiotic resistance among microbes. While broad host range plasmids are known to transfer to diverse hosts in pure culture, the extent of their ability to transfer in the complex bacterial communities present in most habitats has not been comprehensively studied. Here, we isolated and characterized transconjugants with a degree of sensitivity not previously realized to investigate the transfer range of IncP- and IncPromA-type broad host range plasmids from three proteobacterial donors to a soil bacterial community. We identified transfer to many different recipients belonging to 11 different bacterial phyla. The prevalence of transconjugants belonging to diverse Gram-positive Firmicutes and Actinobacteria suggests that inter-Gram plasmid transfer of IncP-1 and IncPromA-type plasmids is a frequent phenomenon. While the plasmid receiving fractions of the community were both plasmid- and donor- dependent, we identified a core super-permissive fraction that could take up different plasmids from diverse donor strains. This fraction, comprising 80% of the identified transconjugants, thus has the potential to dominate IncP- and IncPromA-type plasmid transfer in soil. Our results demonstrate that these broad host range plasmids have a hitherto unrecognized potential to transfer readily to very diverse bacteria and can, therefore, directly connect large proportions of the soil bacterial gene pool. This finding reinforces the evolutionary and medical significances of these plasmids.

The ISME Journal (2015) **9**, 934–945; doi:10.1038/ismej.2014.191; published online 21 October 2014

Broad host range conjugative plasmids can enter and replicate in many different bacterial species

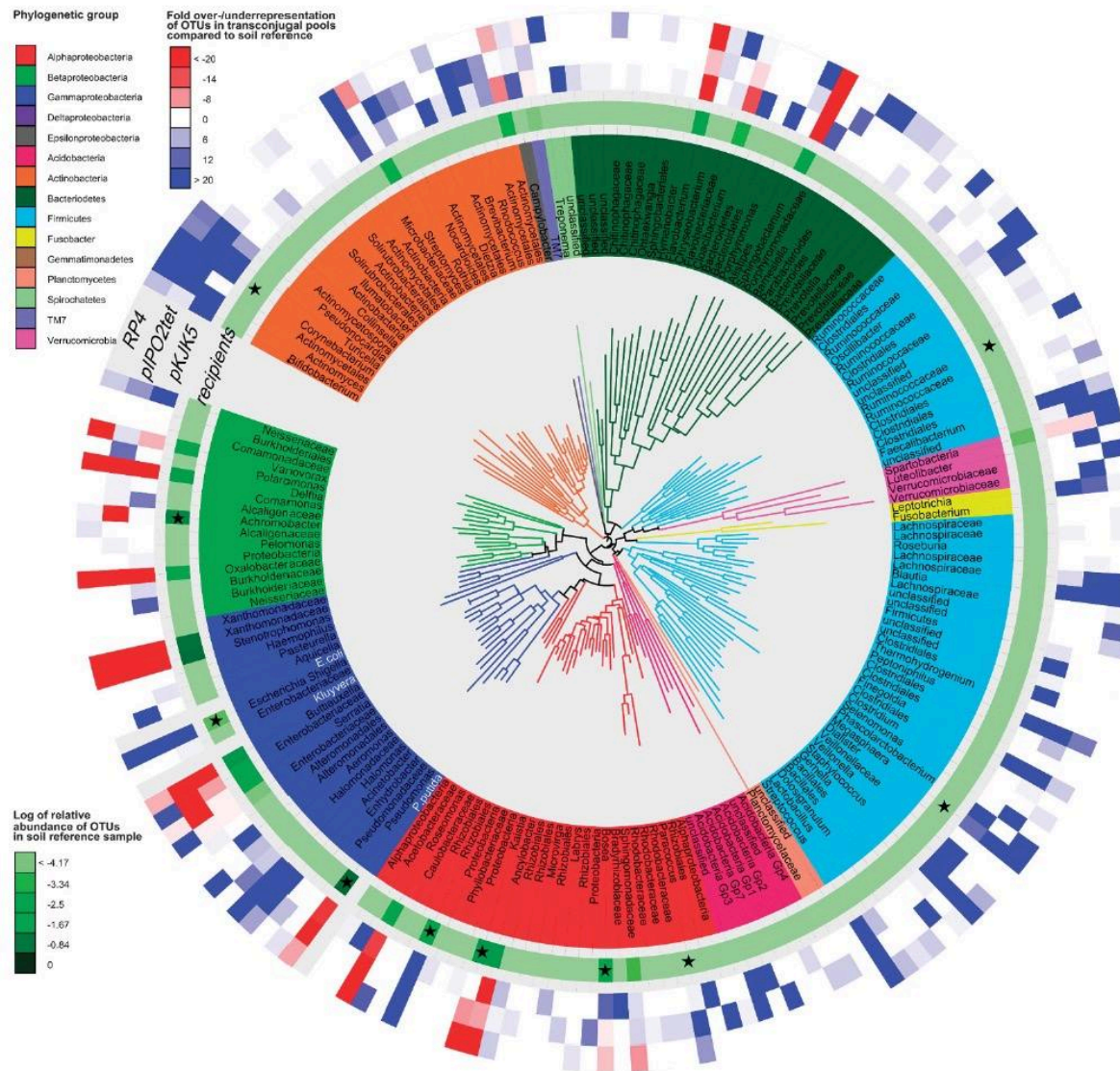
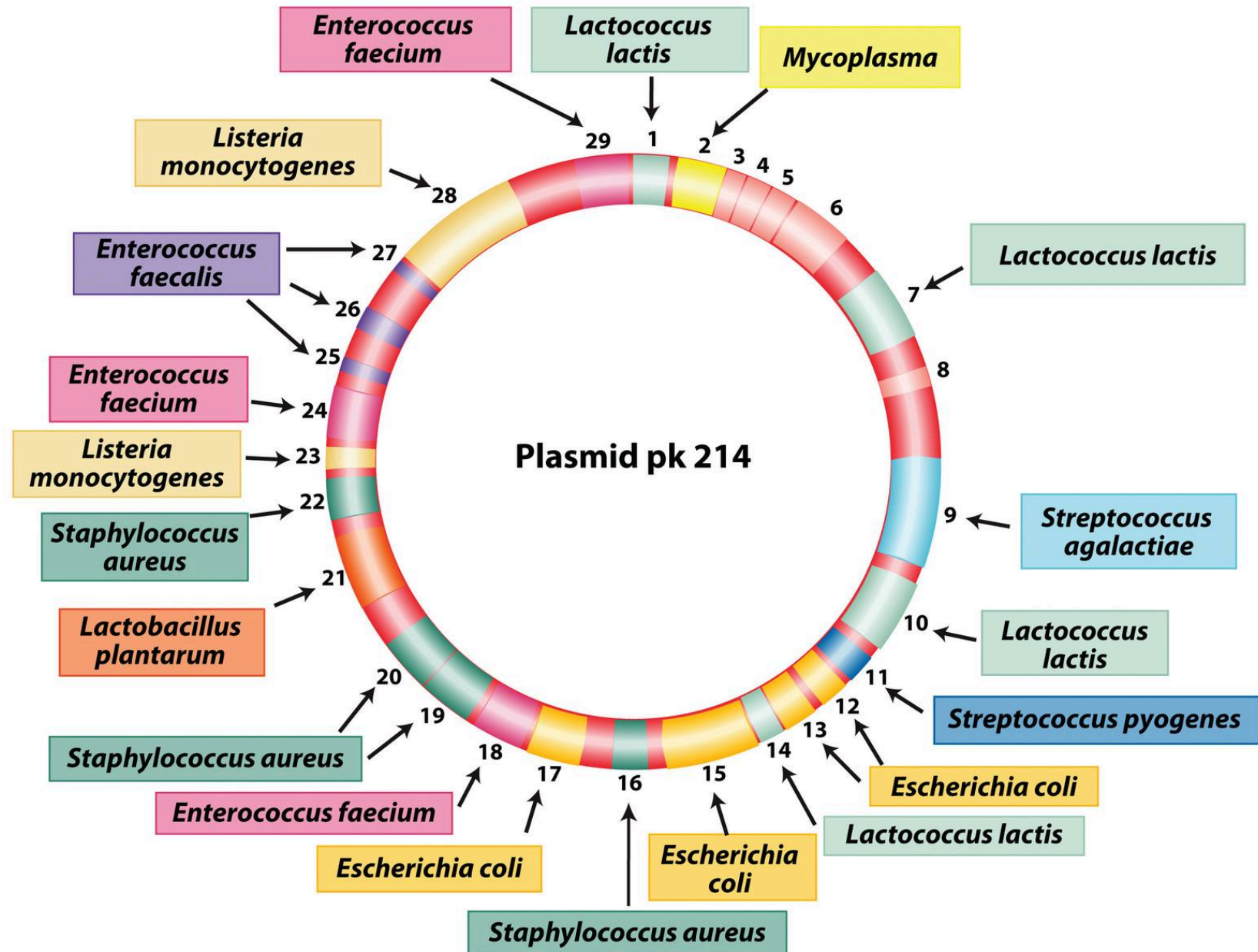


Figure 4 Phylogenetic tree showing all identified transjugant OTUs for three different plasmids (pKJK5, RP4 and pIPO2tet) from the same donor (*P. putida*). The colors of the branches mark different phylogenetic groups. The three donor strains are shown in white letters in the trees. One green heatmap circle around the tree represents the log-transformed relative OTU abundance in the soil reference-recipient community. Three heatmap circles in blue and red display the x-fold over- and underrepresentation of the OTU in the respective transconjugal pool in comparison with the abundance in the reference soil sample. Stars mark the shared (present in all three transconjugal pools) and abundant (present at more than 1% relative sequence abundance) transjugant OTUs, which constitute the core super-permissive community fraction. Sample size was normalized to 30 000 sequences per transconjugal pool.

A broad host range plasmid picks up genomic DNA from many former bacterial hosts; **these are common in our microbiome, in soil, in hospitals**



Data from Table 1 in V. Perreten, F. Schwarz, L. Cresta, M. Boeglin, G. Dasen, and M. Teuber, *Nature* 389, 801–802

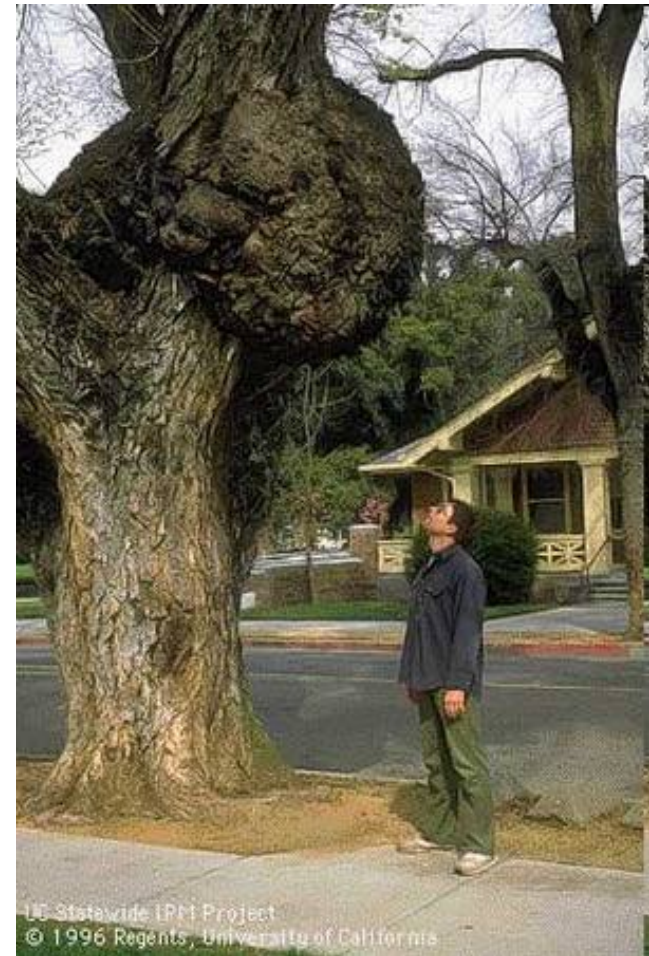
Figure 6-19
Introduction to Genetic Analysis, Twelfth Edition
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Agrobacterium

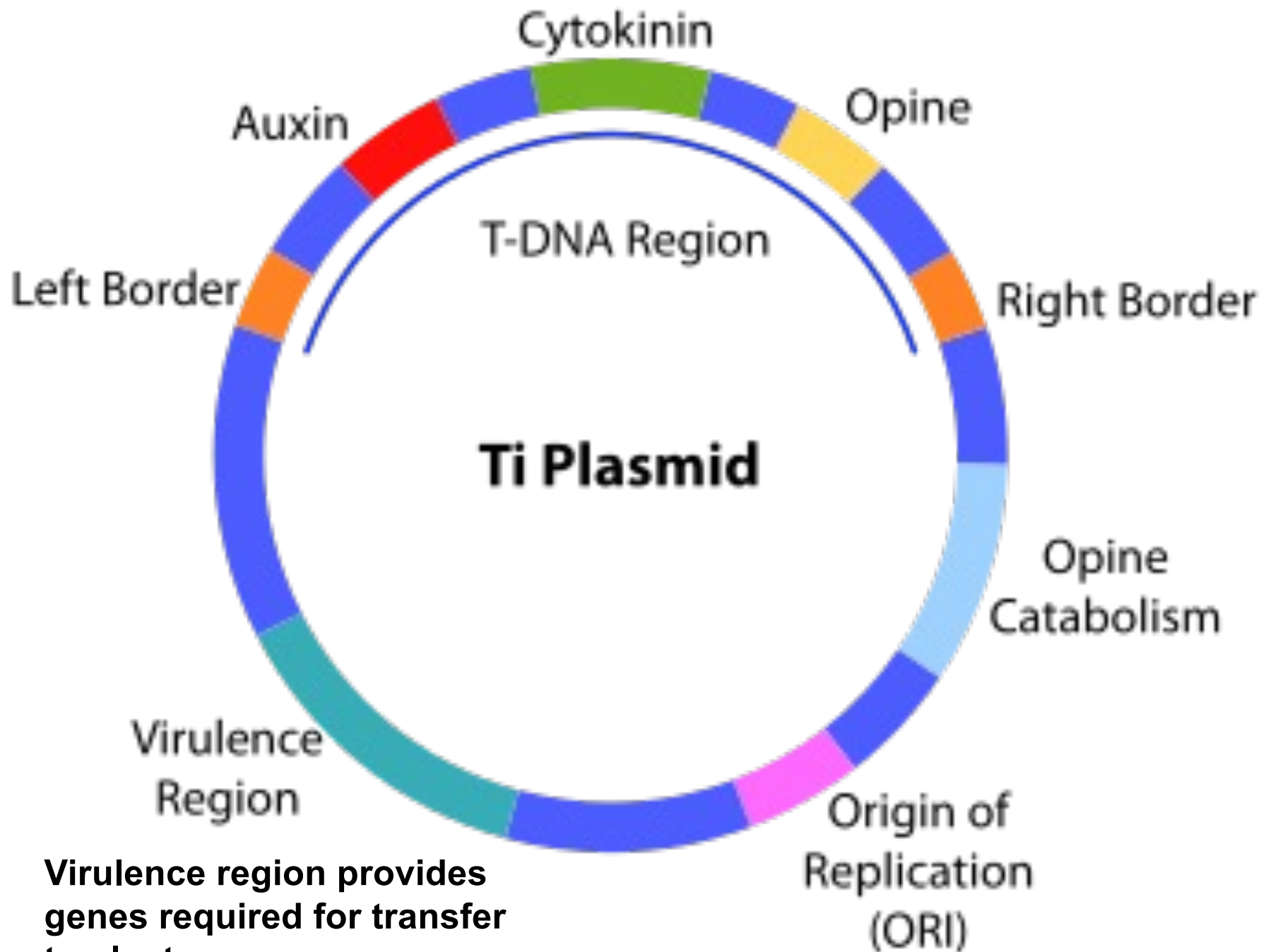
Turning a tumor forming bacteria into a powerful tool
for plant genetic engineering



Fig. 1. Crown gall tumor on an oak tree.



Agrobacterium



Virulence region provides genes required for transfer to plant

(a) Hybrid plasmid

T-DNA

Sticky ends

Hybrid plasmid cut open by

Sticky ends

Foreign gene

Recombinant plasmid

(b)

enzyme

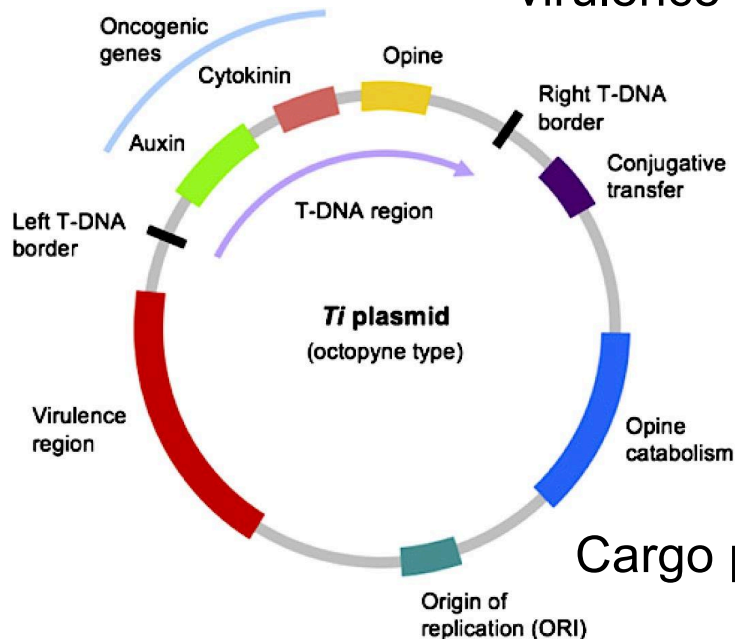
Recombinant plasmid

Transferred into *Agrobacterium*

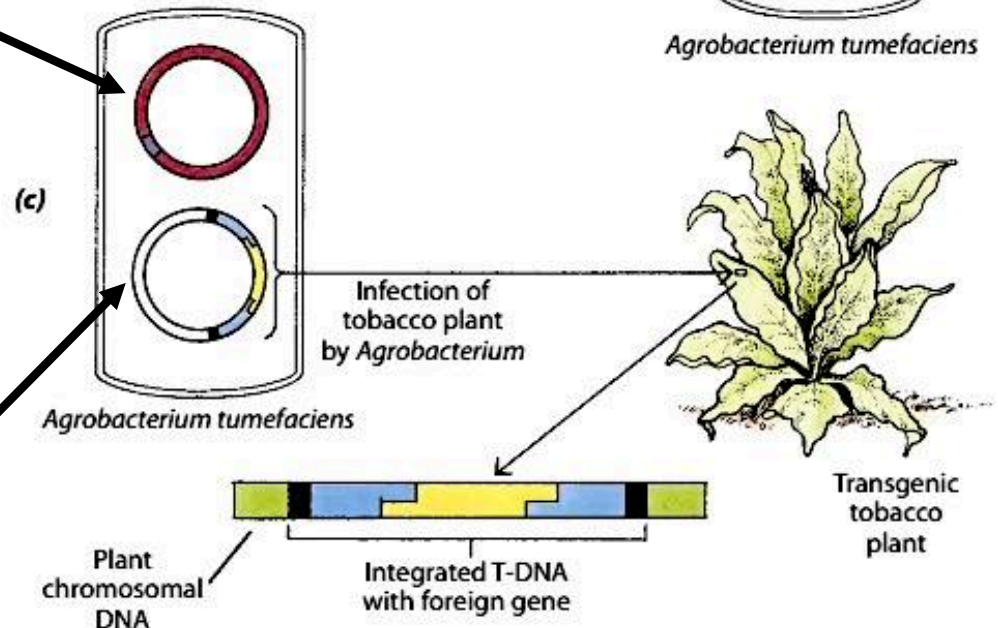
Modified Ti plasmid containing *vir* gene but no T-DNA

Agrobacterium tumefaciens

virulence genes



Cargo plasmid



Arabidopsis Floral Dip



Papaya ringspot virus

transgenic

Non-transgenic



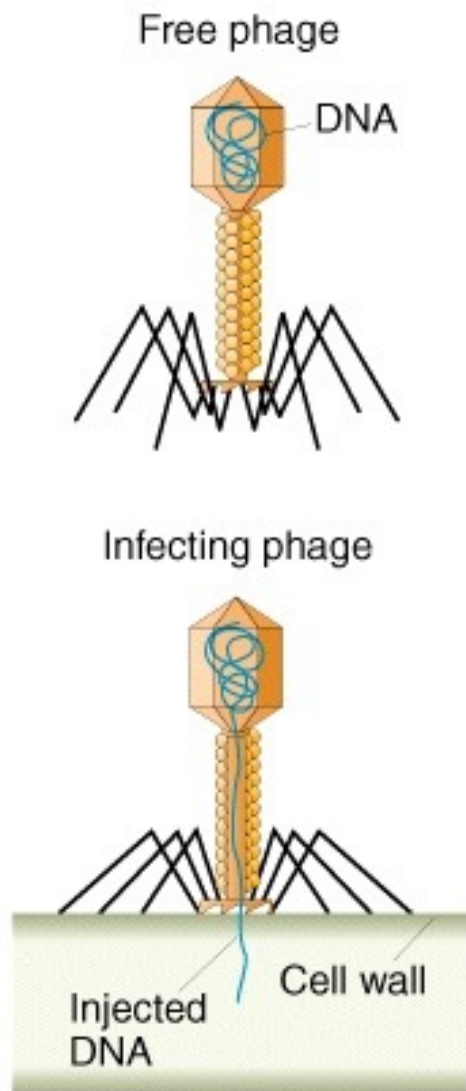
Image credit: istockphoto.com/design56



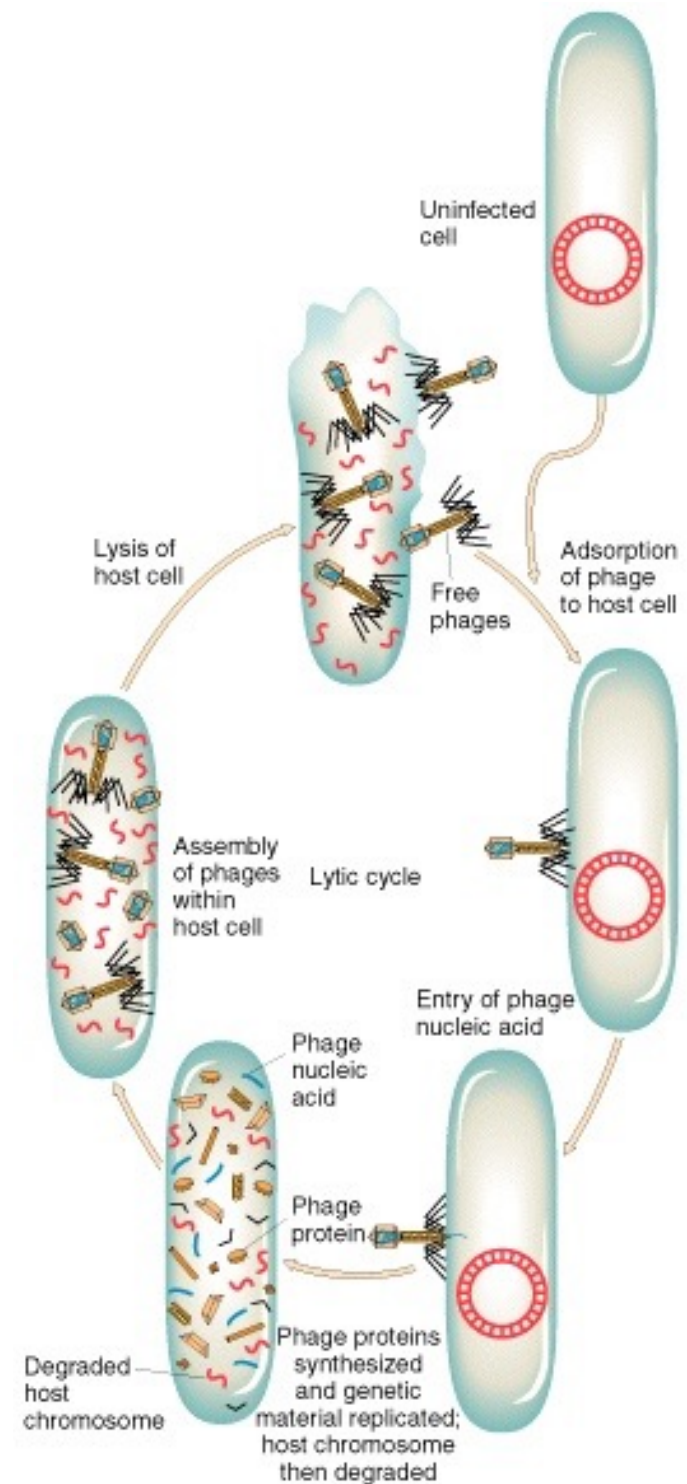
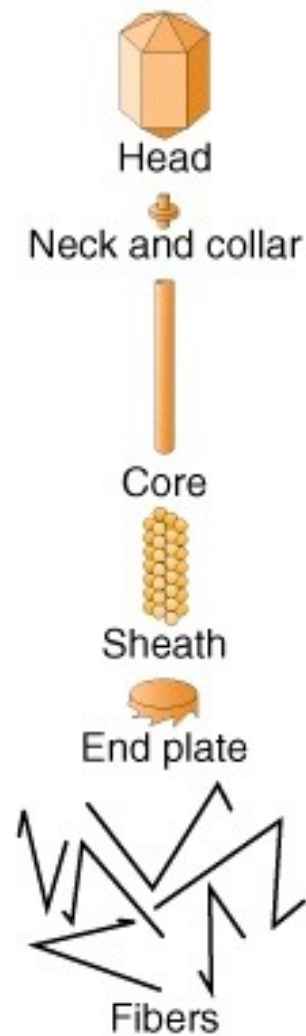
Genetics without Sex:

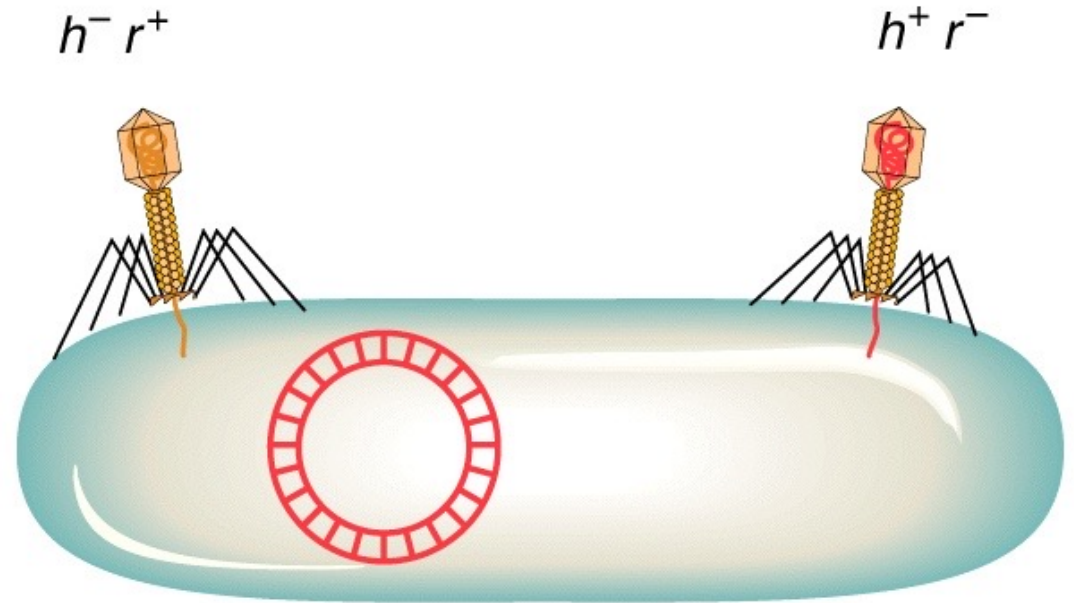
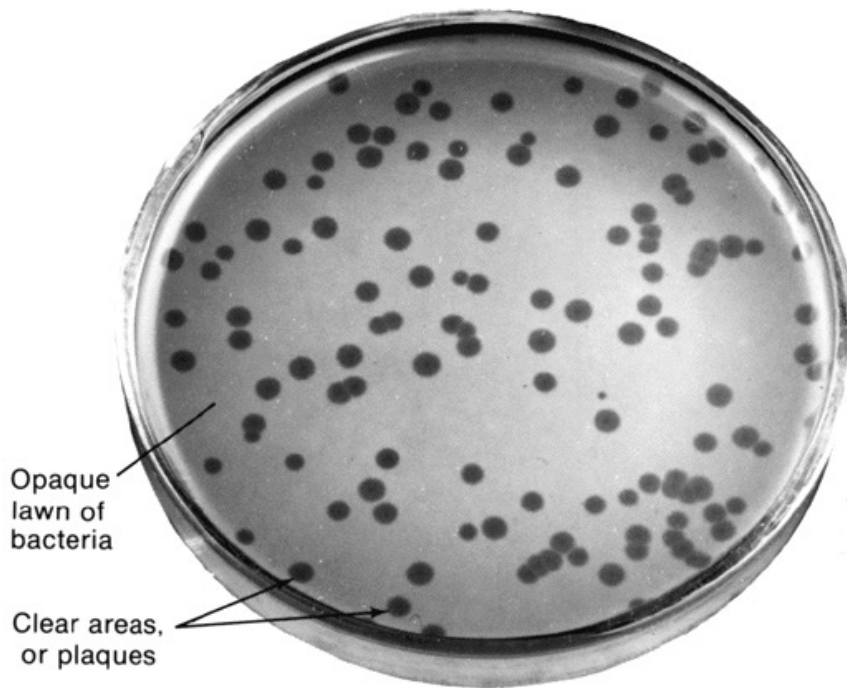
Gene transfer in Prokaryotes

- **Transformation** - cells take up DNA directly from the environment
- **Conjugation** - cell-to-cell transfer of genetic material
- **Transduction** - Transfer of genetic material by phage or phage-like particles



T4 phage components

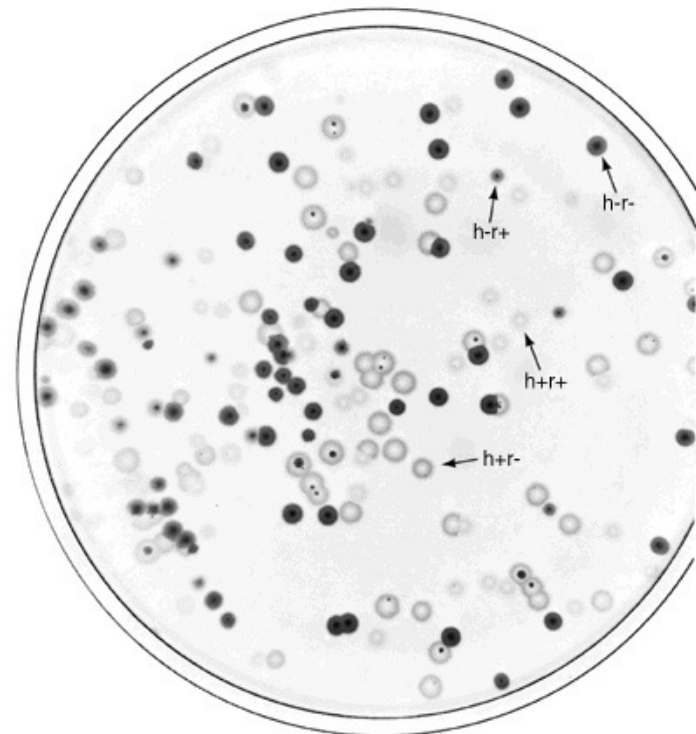




1. Phage have a single chromosome.
2. Thus they are haploid and genotype = phenotype.
3. Multiple phage can infect the same cell.
4. Recombination can occur between these genomes.

$$RF = \frac{(h+r+) + (h-r-)}{\text{Total plaques}}$$

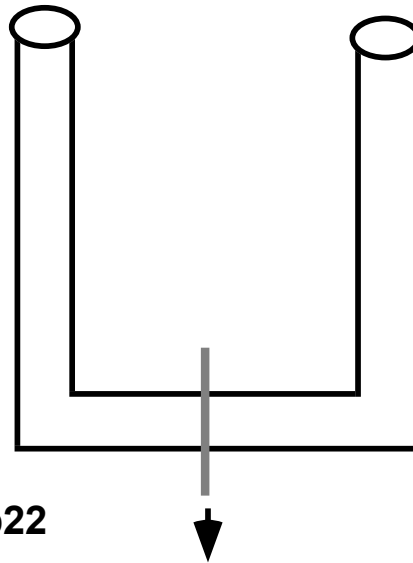
E. coli strain 1



Generalized transduction

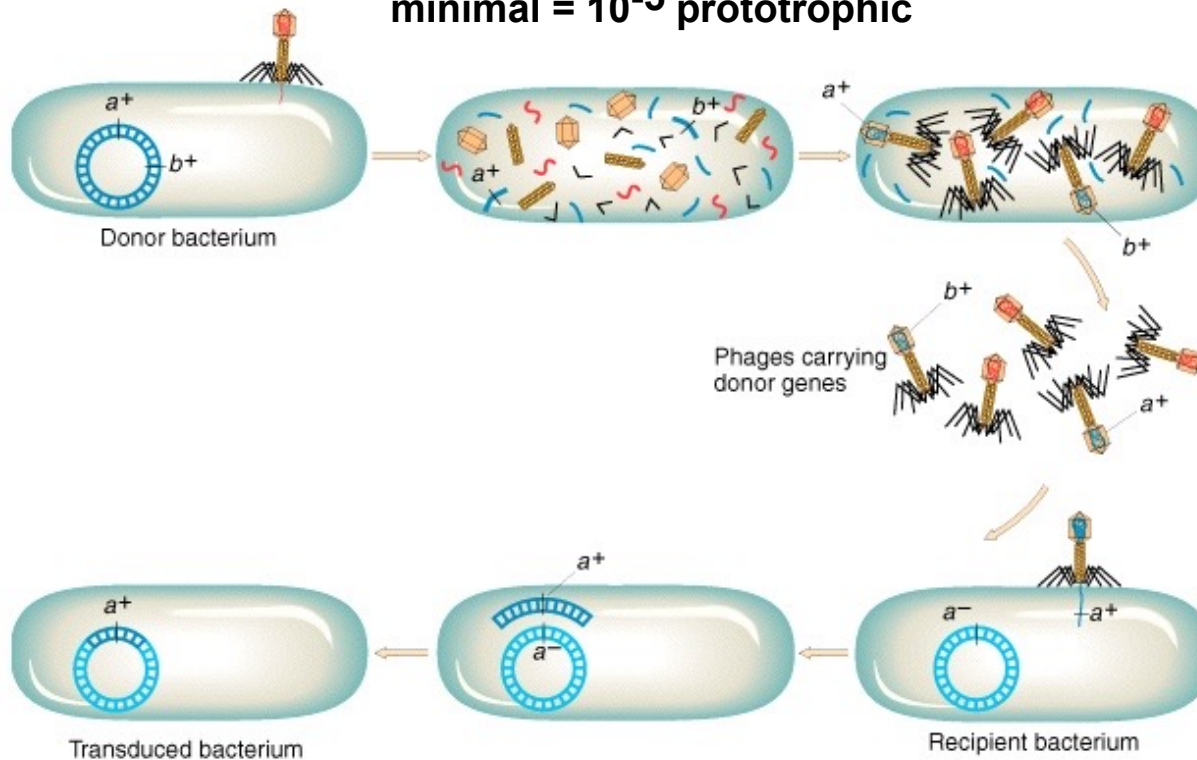
m⁺ h⁺ phe⁻ trp⁻ tyr⁻

m⁻ his⁻ phe⁺ trp⁺ tyr⁺

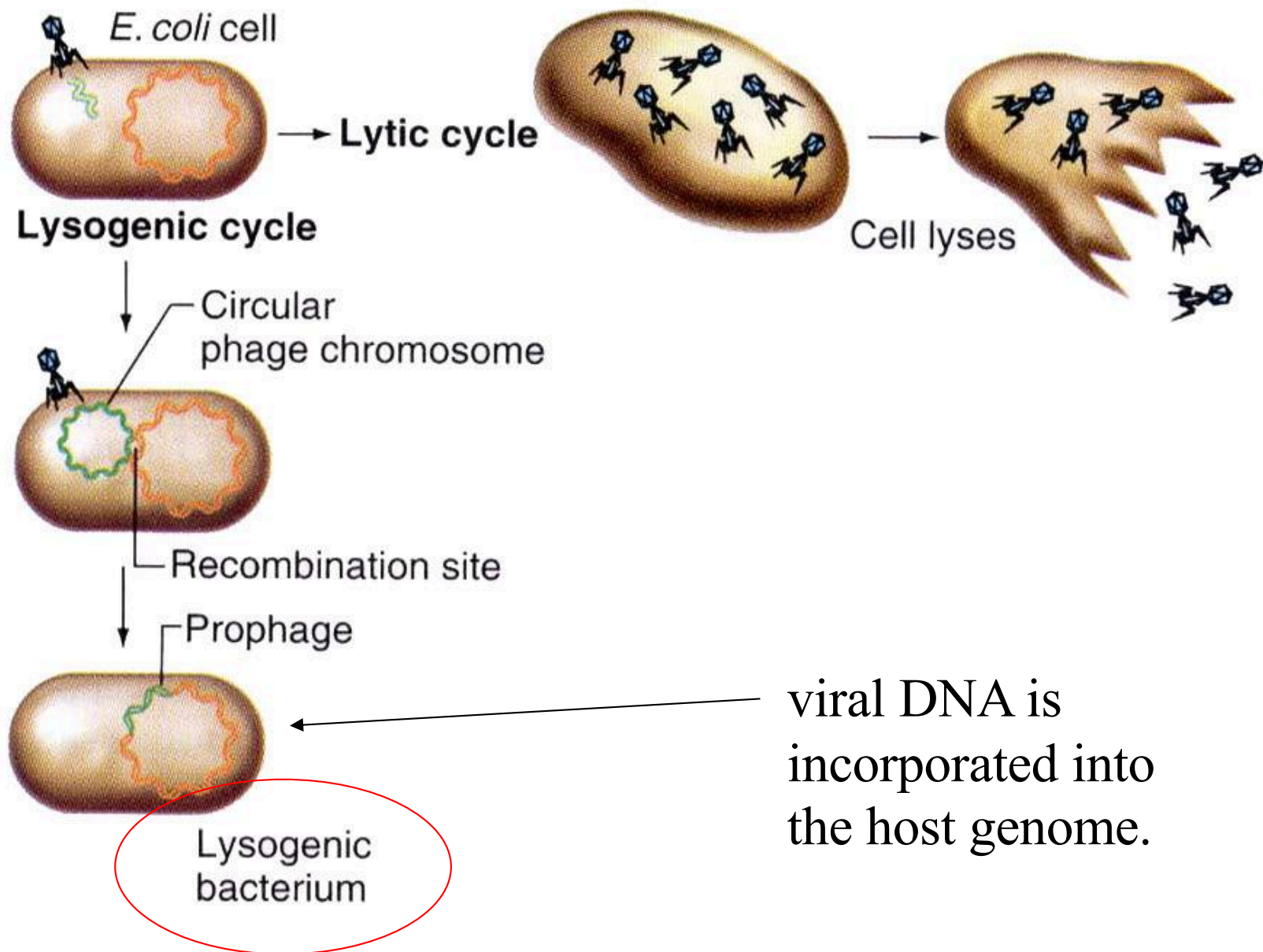


1. can pass through a filter
2. unidirectional
3. not inactivated by DNase
4. Strain on left lysogenic for phage p22

minimal = 10^{-5} prototrophic



Lytic vs Lysogenic



Lysogeny

...the integration of viral DNA into the bacterial genome,

- a virus that can integrate into the genome is termed **temperate**,
- an integrated phage is termed a **prophage**.

Prophage

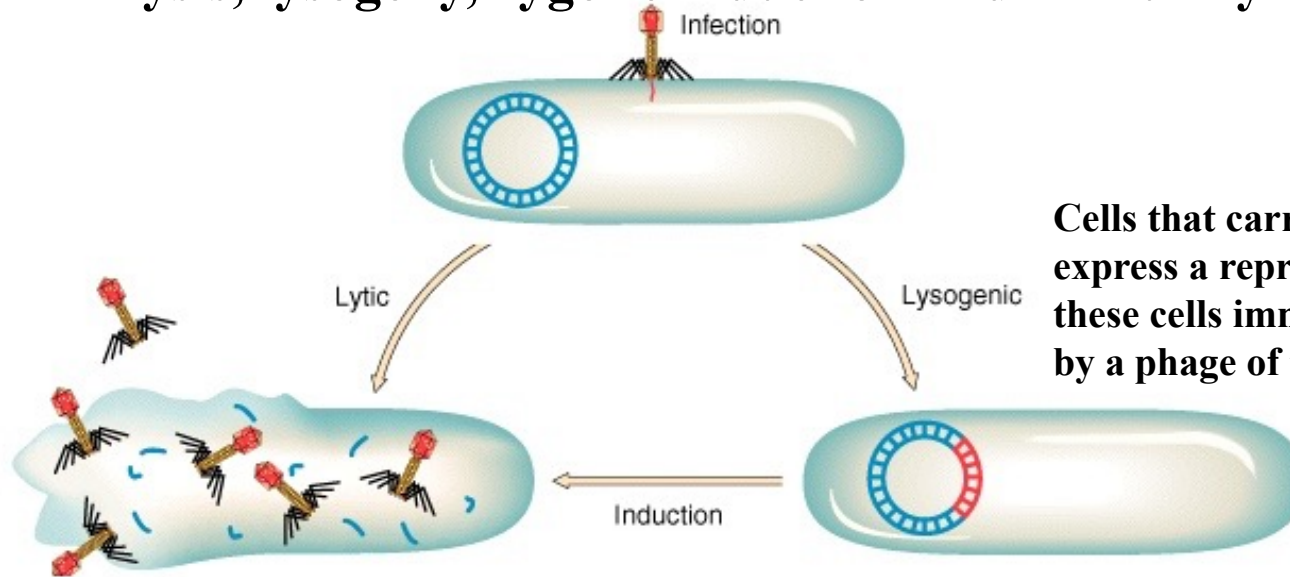
...non-virulent units that are inserted in the host chromosome, and multiply via binary fission along with the host DNA,

...prophage can re-enter the lytic cycle to complete the virus life cycle.

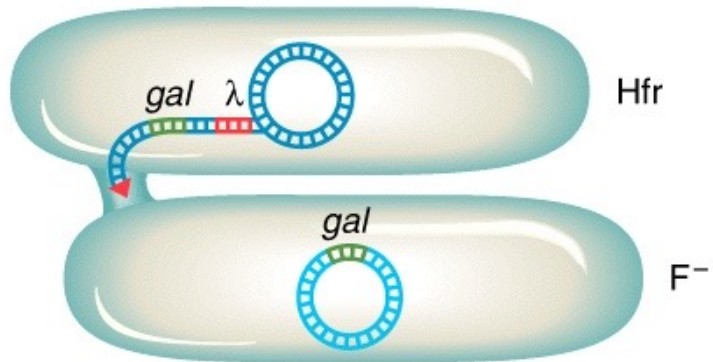
Phage Induction

- ...prophage express a repressor protein that inhibits further infection,**
 - also inhibits prophage DNA excision genes, and genes used during the lytic cycle,**
- ...environmental cues (especially events that damage DNA) block the expression of the repressor protein,**
 - prophage excises and enters a lytic cycle.**

Lysis, lysogeny, zygotic induction and immunity

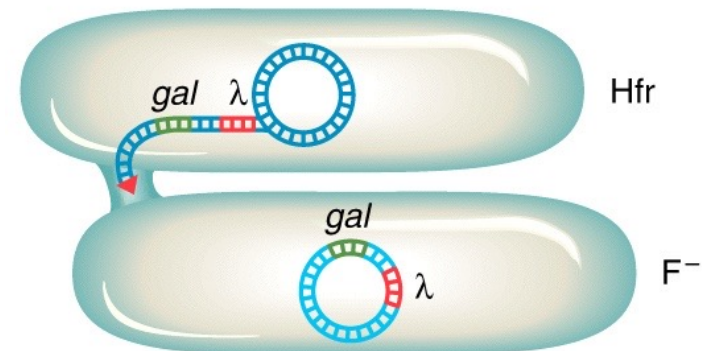


Cells that carry a lysogenic phage express a repressor protein that makes these cells immune to lytic infection by a phage of the same type.



(a)

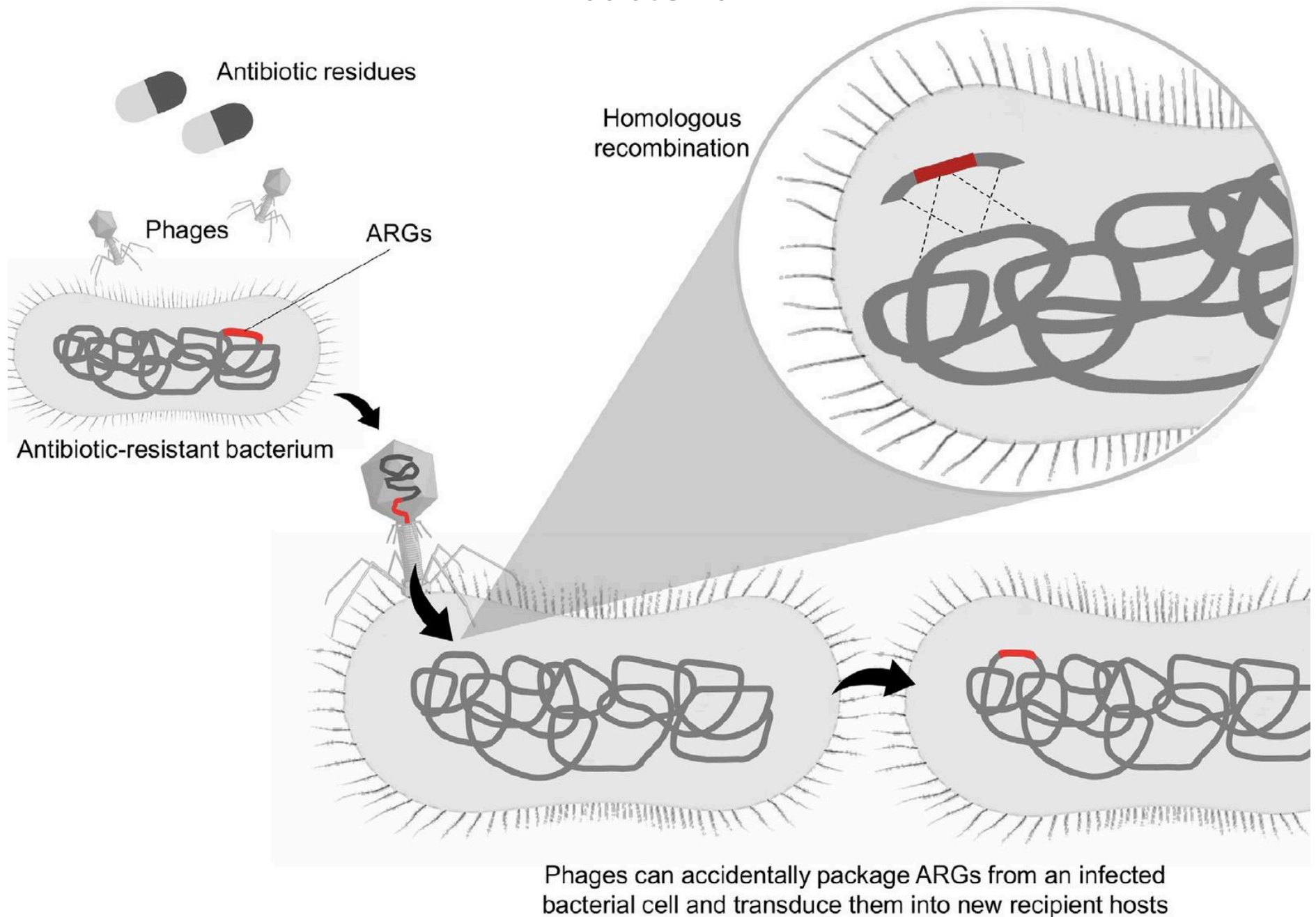
Nonimmune → lysis
(zygotic induction)



(b)

Immune → no lysis

Phage can also transfer antibiotic resistance between bacteria



Phage therapy for antibiotic resistant infections

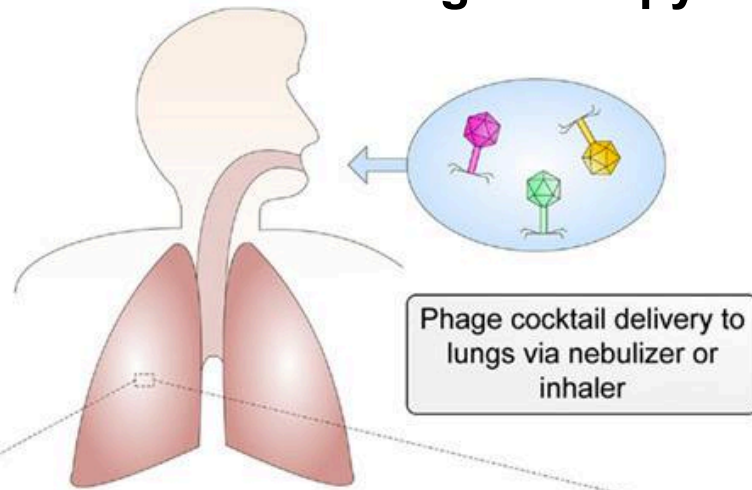
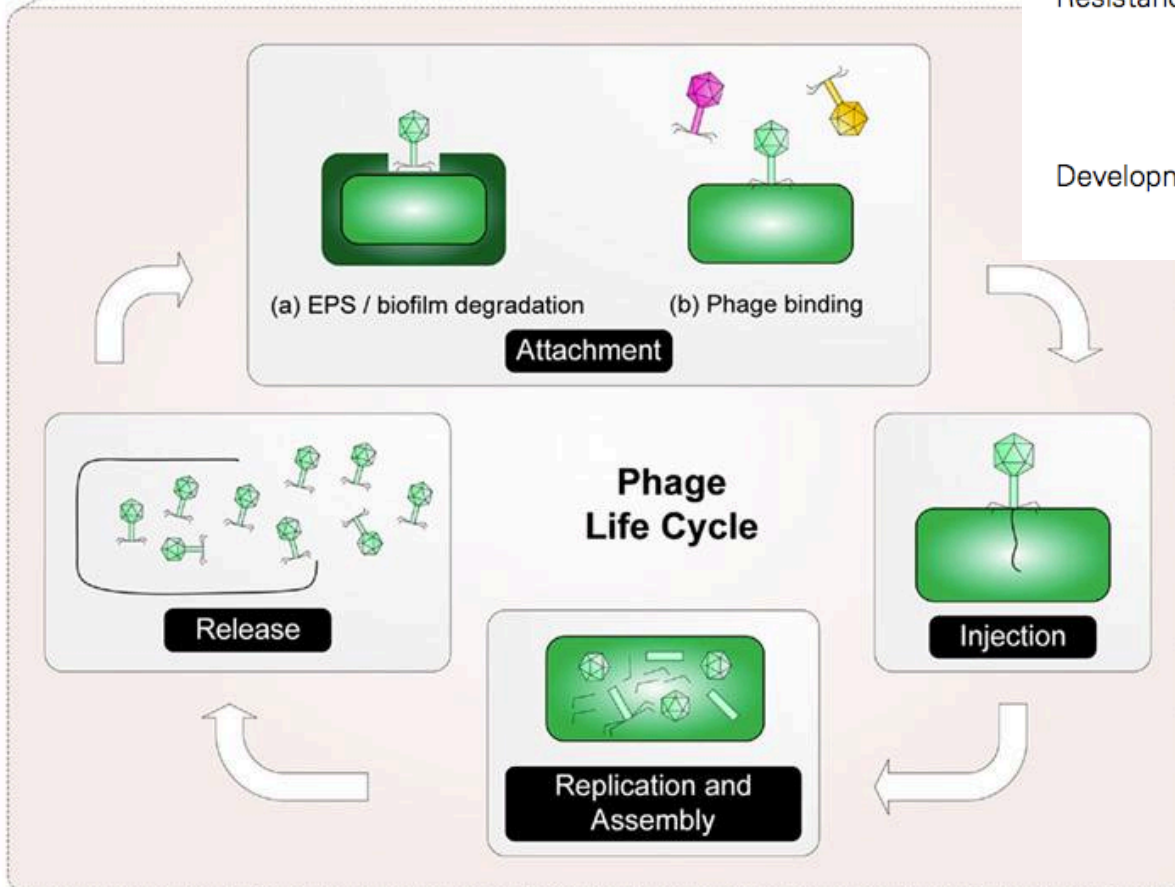
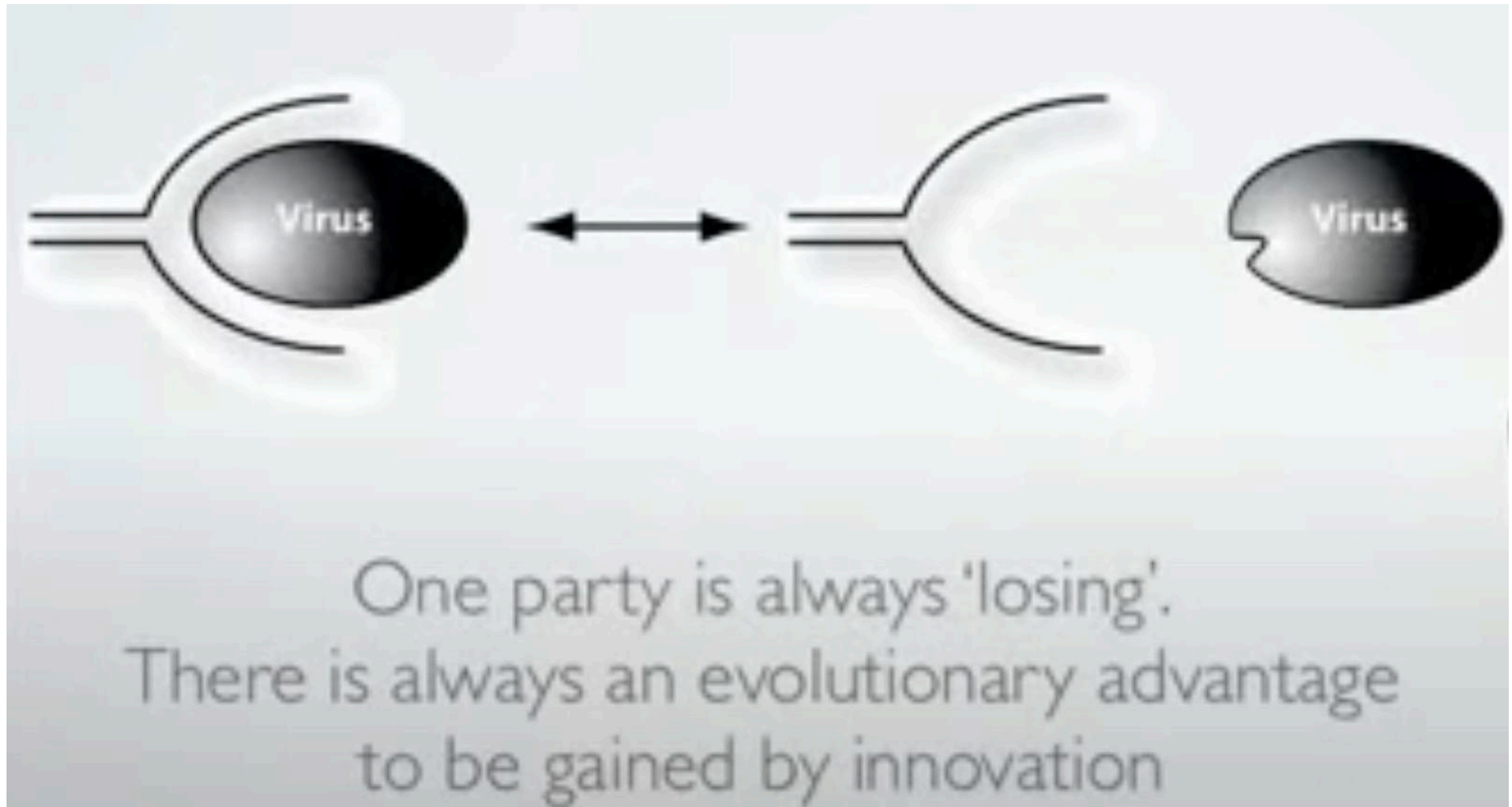


Table 1 | A comparison of some of the main advantages and disadvantages of antibiotics and phage therapy.

	Antibiotics	Phage therapy
Specificity	Broad spectrum, affecting more than the targeted organism	Generally species or strain specific
Side effects	Many, including allergies and intestinal disorders	No side effects (Bruttin and Brüssow, 2005; Merabishvili et al., 2009; Rhoads et al., 2009; Wright et al., 2009)
Resistance	Occurs and is not limited to targeted bacteria	Occurs, but can be linked to host virulence attenuation (Zahid et al., 2008). Also, phage can co-evolve with host
Development	Time-consuming and expensive	Rapid



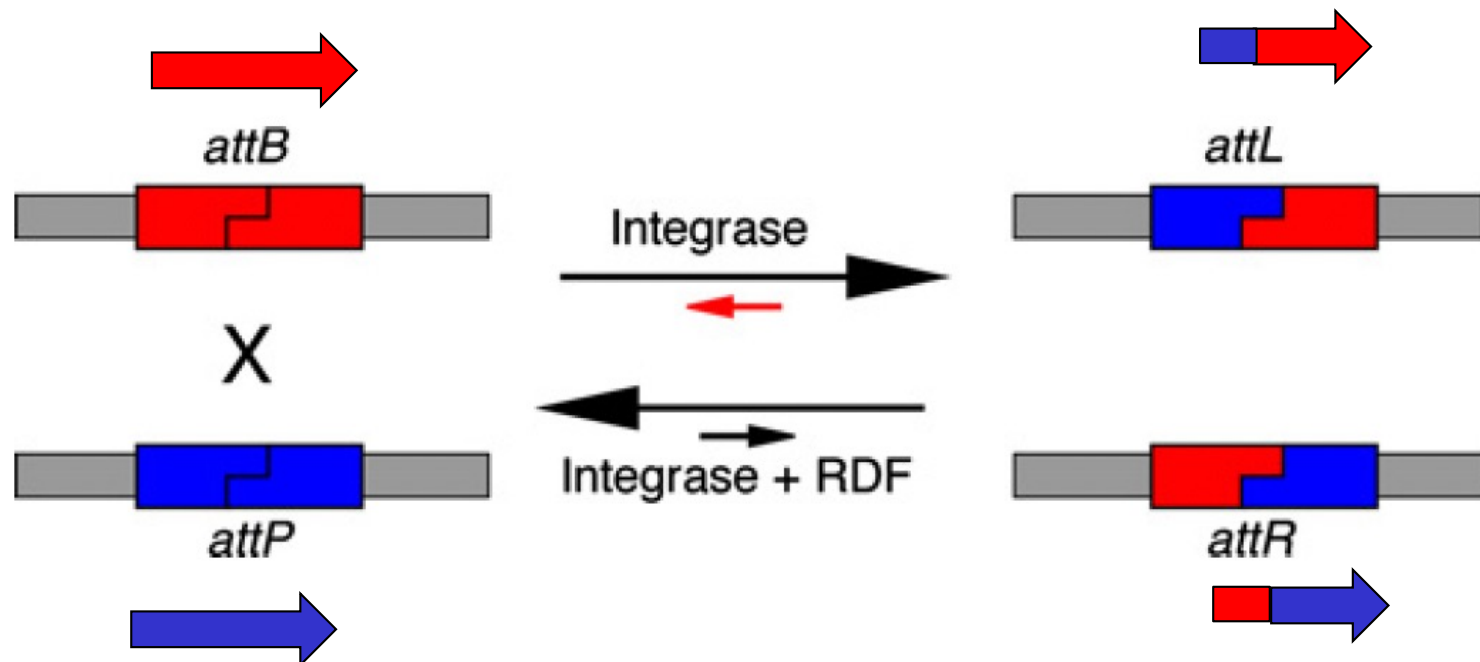
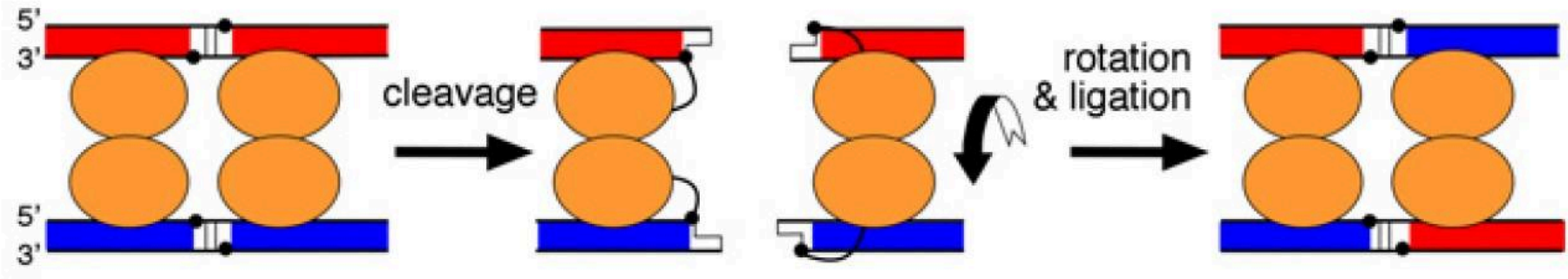
How do species keep winning? Specialized mechanisms for creating genetic diversity at specific loci (genes)



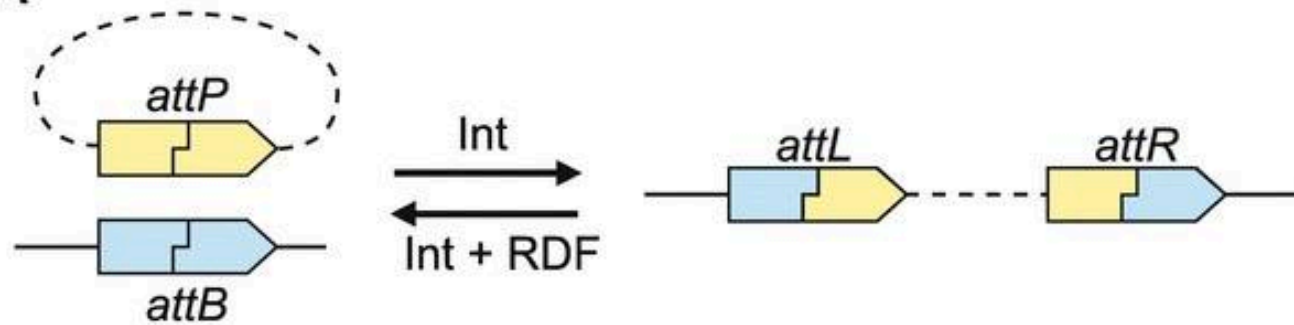
https://www.youtube.com/watch?v=y6rqugj_mVk

Site-specific recombination between two sequences to change DNA orientation

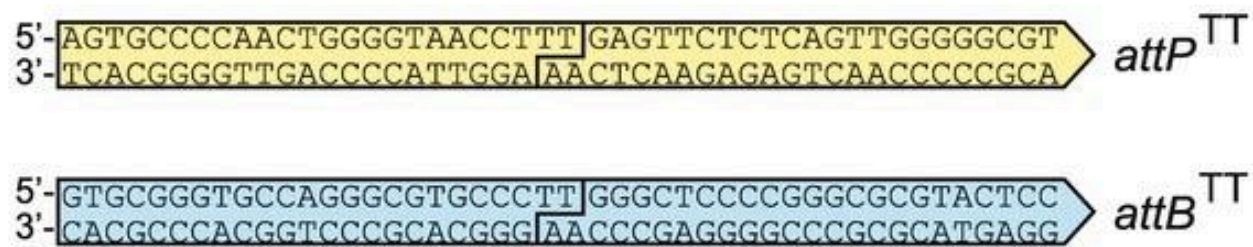
A Serine recombinases



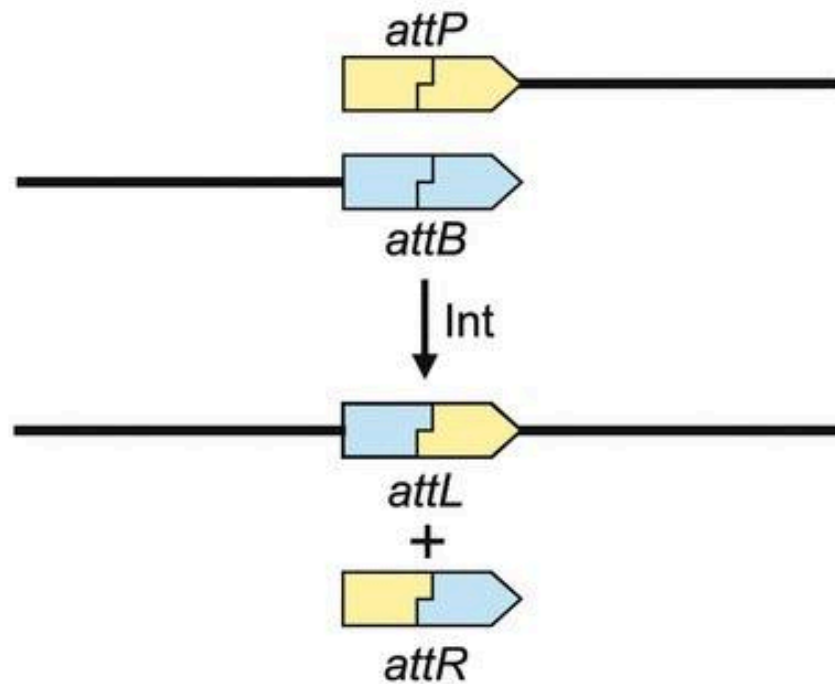
A



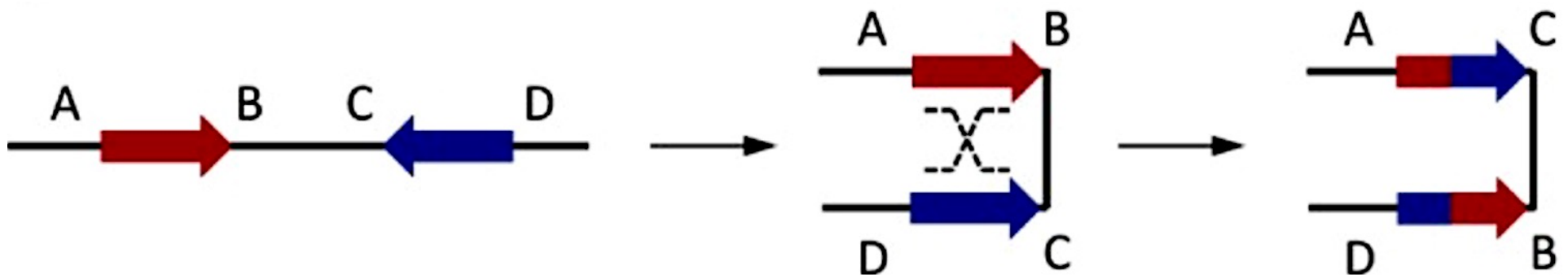
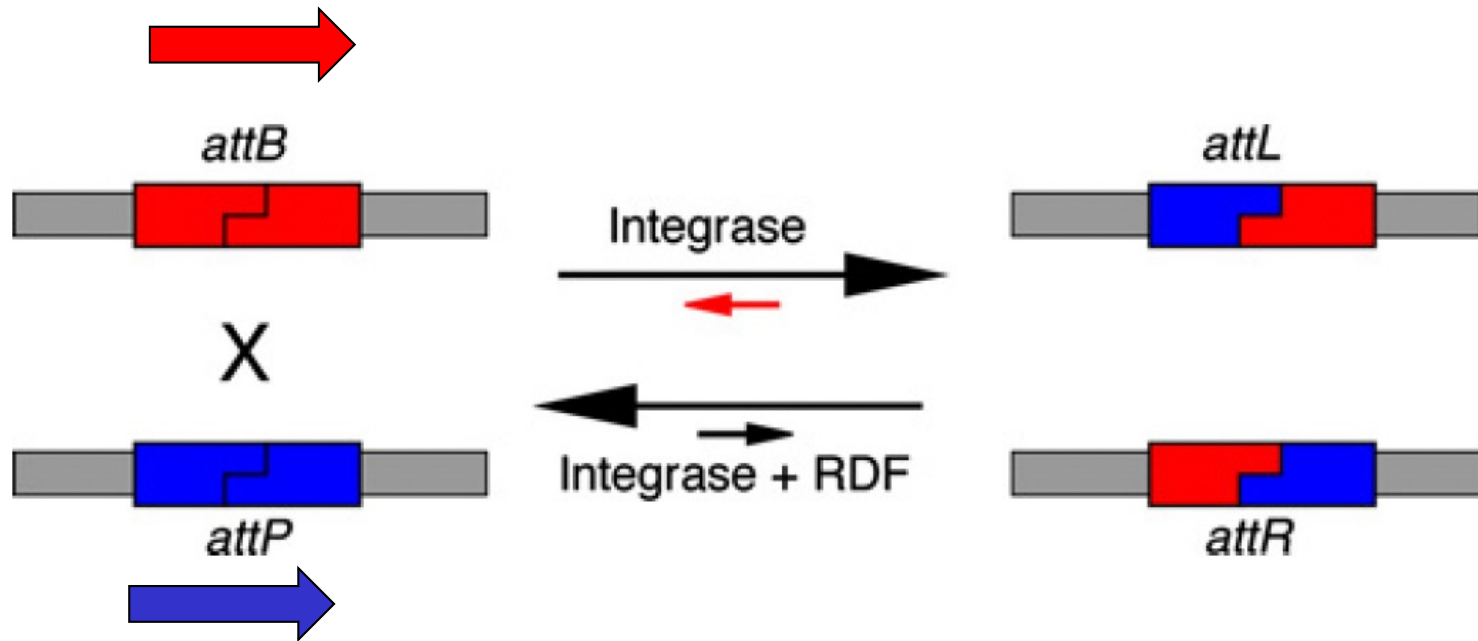
B

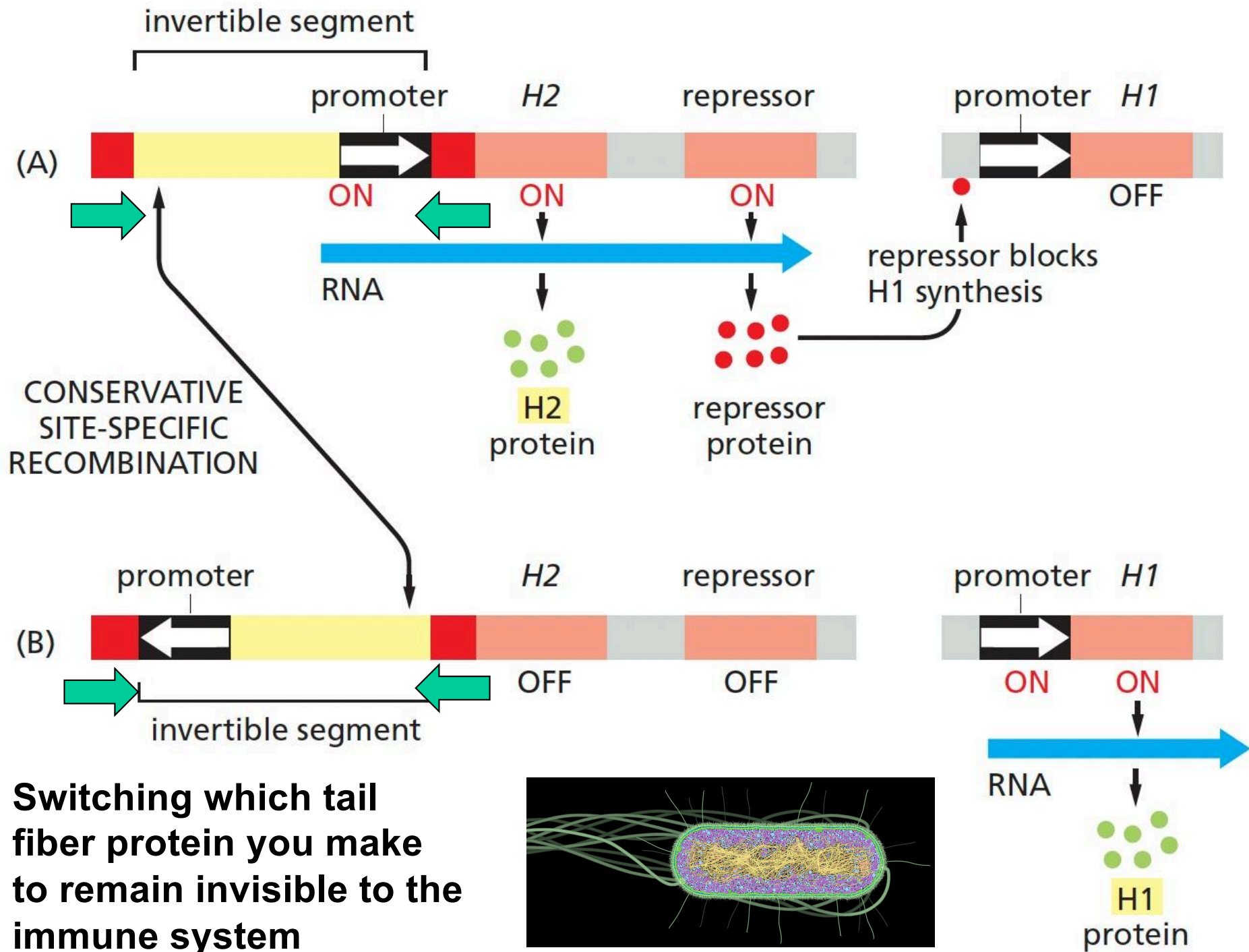


C

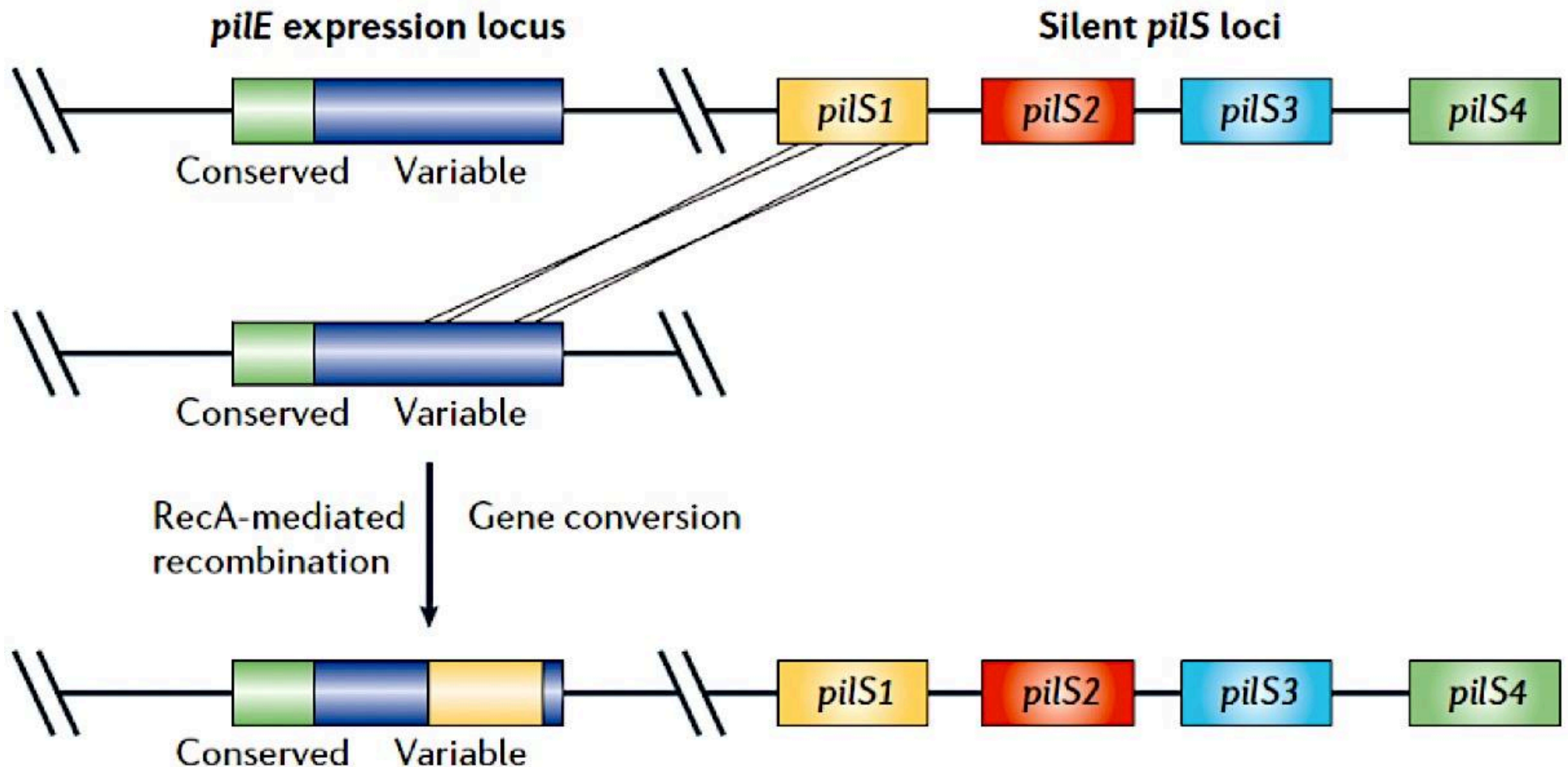


When recombinase target sites are inverted with respect to each other the intervening DNA is inverted



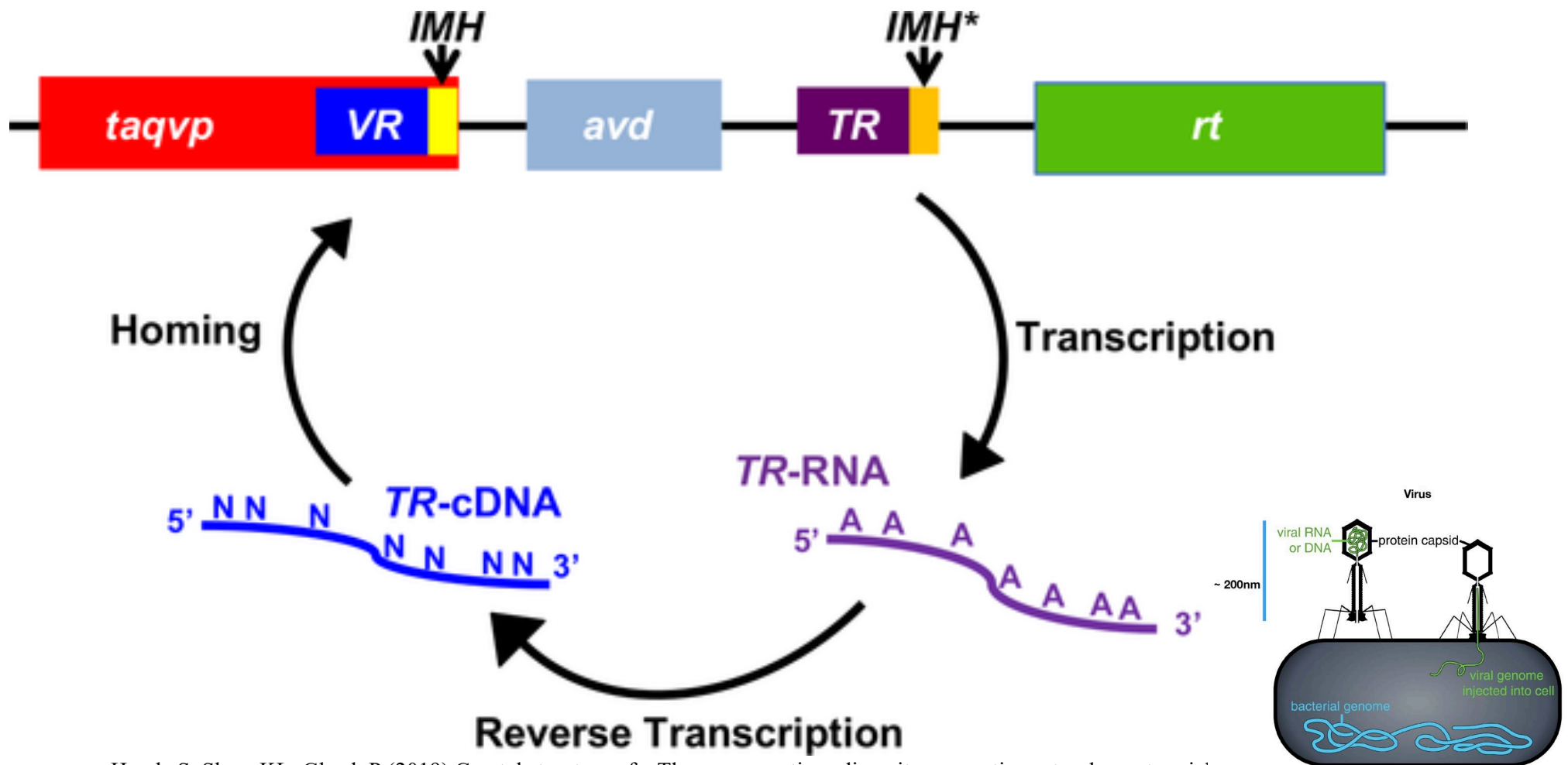


Swapping between a number of possible protein sequences using homologous recombination



Diversity generating retroelement

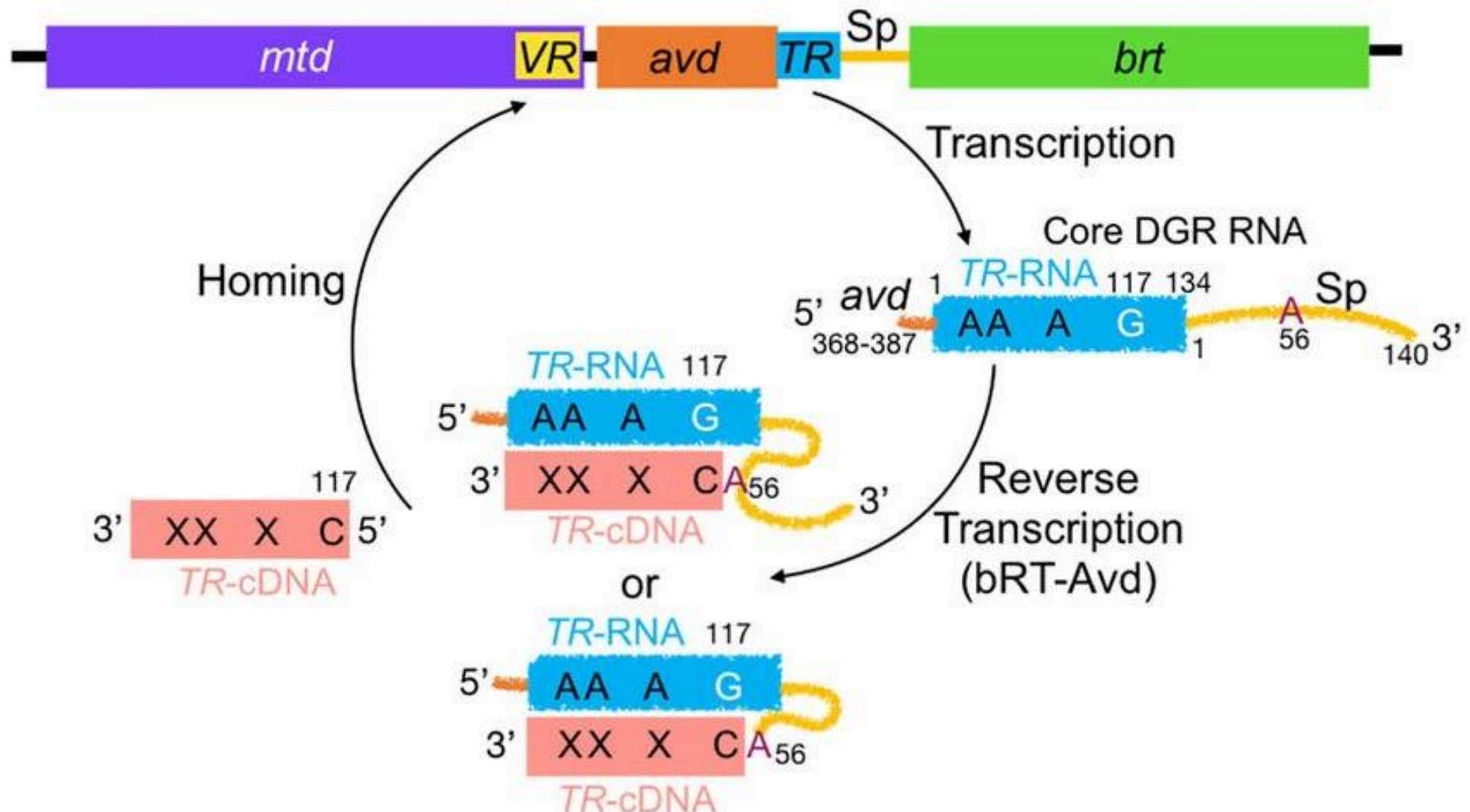
Reverse transcriptase copies the TR-RNA into DNA. It takes every A residue and randomizes it. This generates the TR c (copy) DNA. The TR cDNA inserts itself in place of the VR region, creating a gene that carries a novel protein sequence that can bind to other receptors. 100 positions randomized over 20 amino acids. More diversity than stars in the universe. **Infinite diversity (targeted mutagenesis) in a very small package**

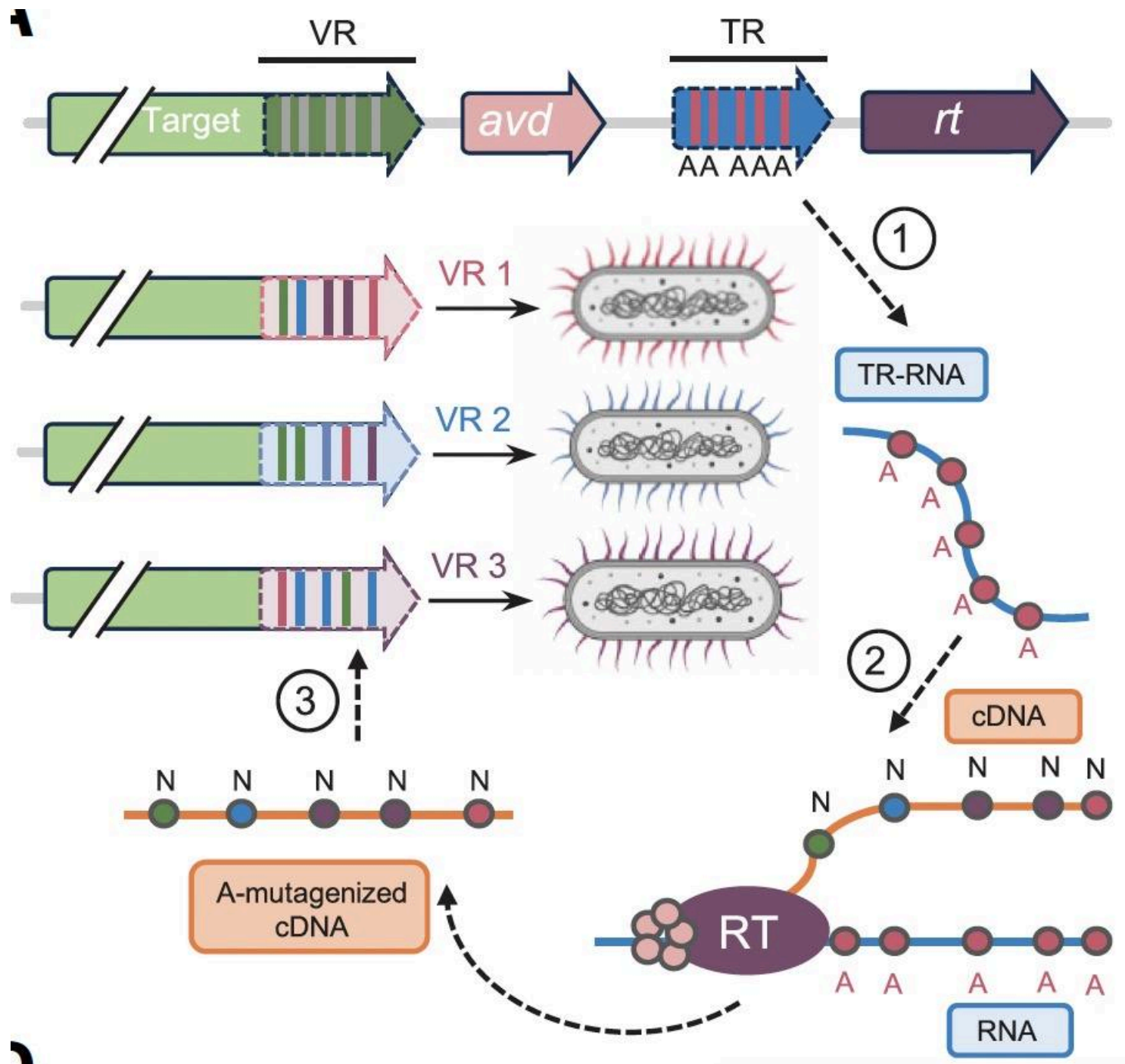


Handa S, Shaw KL, Ghosh P (2019) Crystal structure of a *Thermus aquaticus* diversity-generating retroelement variable protein. *PLoS ONE* 14(1): e0205618. <https://doi.org/10.1371/journal.pone.0205618>

Diversity generating retroelement

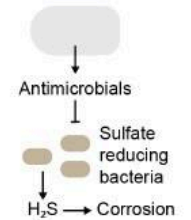
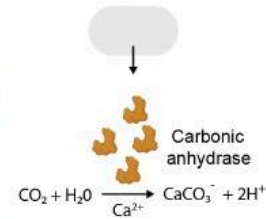
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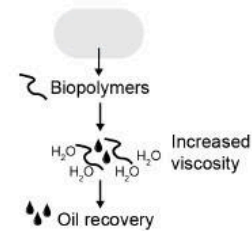


Engineered microbes for environmental release (EMER)

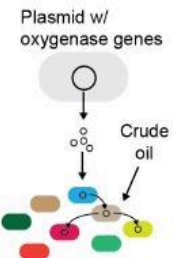
1. Corrosion \$2.5 trillion



2. Enhanced oil recovery \$90 billion



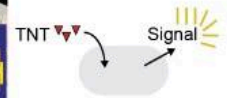
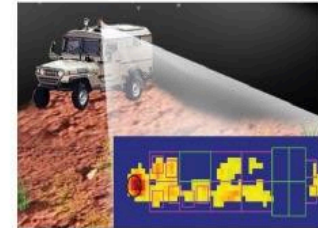
3. Oil spill cleanup \$10 billion



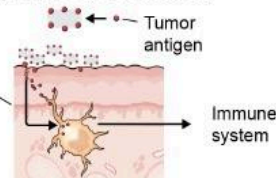
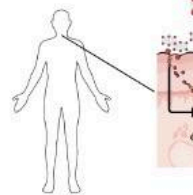
4. Fertilizer \$207 billion



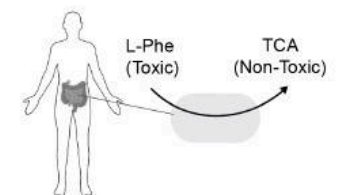
5. Landmine removal \$50-100 billion



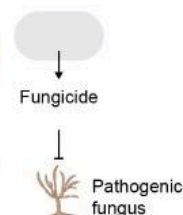
6. Skin therapeutics \$180 billion



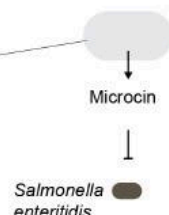
7. GI disease therapeutics \$210 billion



8. Endangered species protection \$76 billion



9. Livestock therapeutics \$17.2 billion



Policy Recommendations for the Regulation of Engineered Microbes for Environmental Release

June 2024

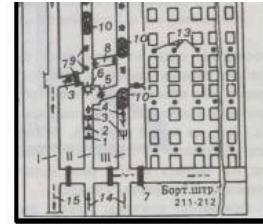
Engineering Bacteria for Environmental Release: Regulatory Challenges and Design Strategies

Yonatan Chemla¹, Connor J. Sweeney¹, Christopher A. Wozniak², and Christopher A. Voigt¹

¹Synthetic Biology Center, Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA

²(Retired) Office of Pesticide Programs, U.S. Environmental Protection Agency, Alexandria, VA, USA

Phenol pollution remediated with *Pseudomonas putida*. Phenol is a potent carcinogen that was released due to an 81-day fire at the world's largest oil shale mine in the U.S.S.R. (now Estonia) in 1988. Engineered *P. putida* containing a recombinant phenol-degrading pathway was spread to remediate the pollution^{32,33}. The bacteria were inoculated into mine enclosures where it reduced phenol levels 700-fold. Image showing the mine fire diagram (6; location where fire started), adapted from ref³⁴.



Bio-insecticides based on *Bacillus thuringiensis* with targeted pest control. *B. thuringiensis* produces proteins that kill insects and wild-type strains are the most used bio-insecticide globally^{35,36}. These strains disrupt different pests through variations in the endotoxins that they produce (Cry proteins), but a wild-type strain may not have an optimal combination of toxins for a particular pest³⁶. Mycogen Corporation developed M-Cap by cloning the Bt toxin from *B. thuringiensis* into *Pseudomonas fluorescens*, to facilitate its use in maize crops³⁷. The engineered bacteria are killed before use on the field. M-Cap was released for commercial use in 1990. Other similar products have been commercially developed, including products where the bacteria are not killed prior to release^{22,38-40}. Image of Stem borers, sensitive to M-Cap, by the International Maize and Wheat Improvement Center, creative commons license.



Nitrogenous fertilizer delivery to maize with consortium. Nitrogenous fertilizer consumes 2% of global energy and is a major contributor to runoff pollution and greenhouse gases⁴¹. Microbes do not produce fixed N when used in conjunction with synthetic fertilizer unless they are genetically engineered. In the 1990s, recombinant *Rhizobium meliloti* got EPA approval for legumes, but was never commercialized. In 2019, Pivot Bio commercialized an engineered *Klebsiella variicola* to deliver nitrogen to corn, and later a consortium by adding engineered *Kosakonia sacchari*. Their product is used on millions of acres and replaced an estimated 32,000 tons of synthetic fertilizer in 2022⁴². Image courtesy of Pivot Bio.



Engineered *Bacillus subtilis* probiotic for treating hangovers. Enzyme-based therapeutics often suffer from inactivation when stored or consumed. Activity could be improved if produced at the site needed in the body. Zbiotic is an over-the-counter liquid drink that contains a genetically engineered *Bacillus subtilis* designed to degrade gut-derived acetaldehyde that builds up in the colon after consuming alcohol. The product was released for sale in 2019, and nearly 4 million bottles containing their genetically engineered bacteria are in circulation as of April 2024. Image courtesy of Zbiotics.



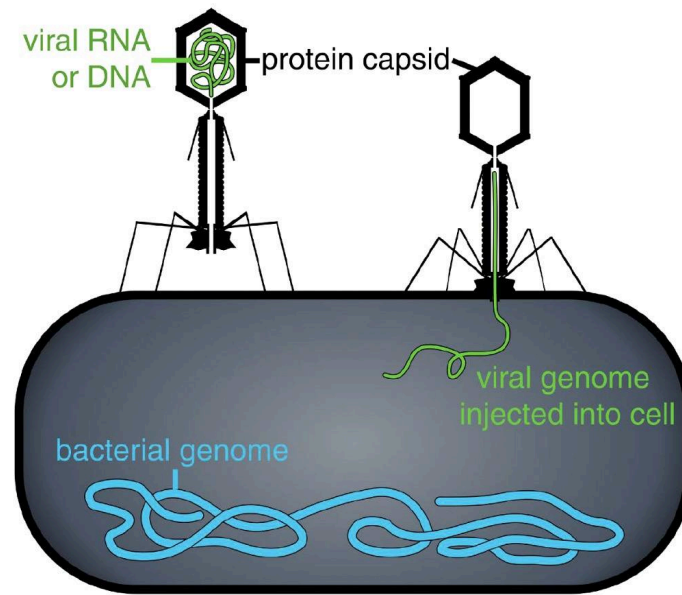
Oral cavity-reducing *Streptococcus mutans* engineered to not produce lactic acid. Biofilms can cause dental cavities by producing lactic acid. An isolate of *S. mutans* was found to produce the antibiotic mutacin 1140, increasing its ability to outcompete native strains and colonize teeth⁴³. In this strain, the lactate dehydrogenase gene was replaced with an alcohol dehydrogenase gene from *Zymomonas mobilis*, using allelic exchange. The resulting strain stopped producing lactic acid⁴⁴. It is used by one-time application to teeth to reduce dental cavity formation. While trying to approve the product in the early 2000s as an oral therapeutic, the FDA put constraints on the trial which never completed. In 2023, Latern Bioworks brought the product to market as a "cosmetic" to avoid the need for an FDA permit. Image of dental decay by bacteria. By Shaimaa Abdellatif, under creative commons license.



Bacteria in oceans 1.3×10^{29}

Virus infections in ocean each day 1×10^{23} /second

Evolutionary arms race



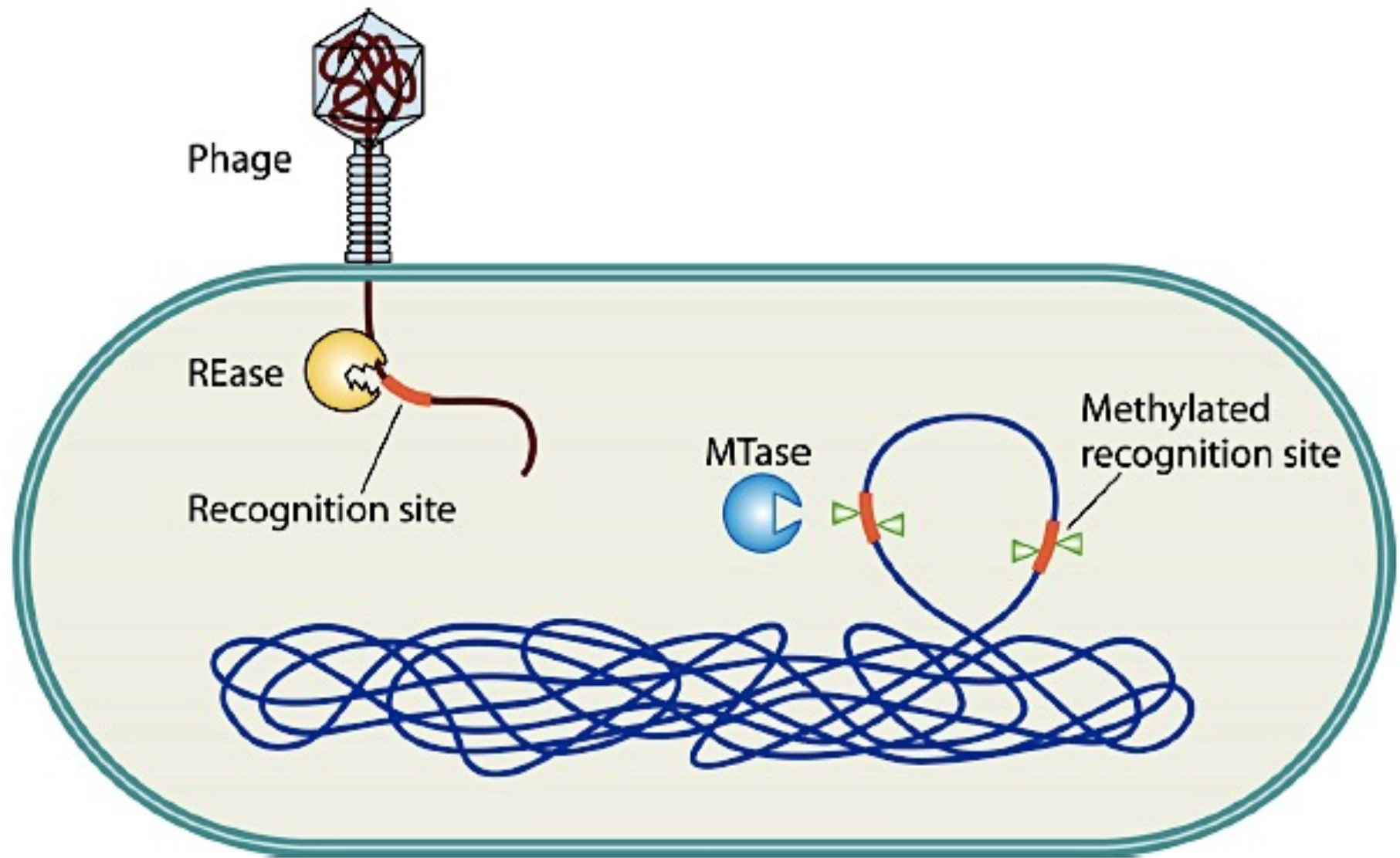
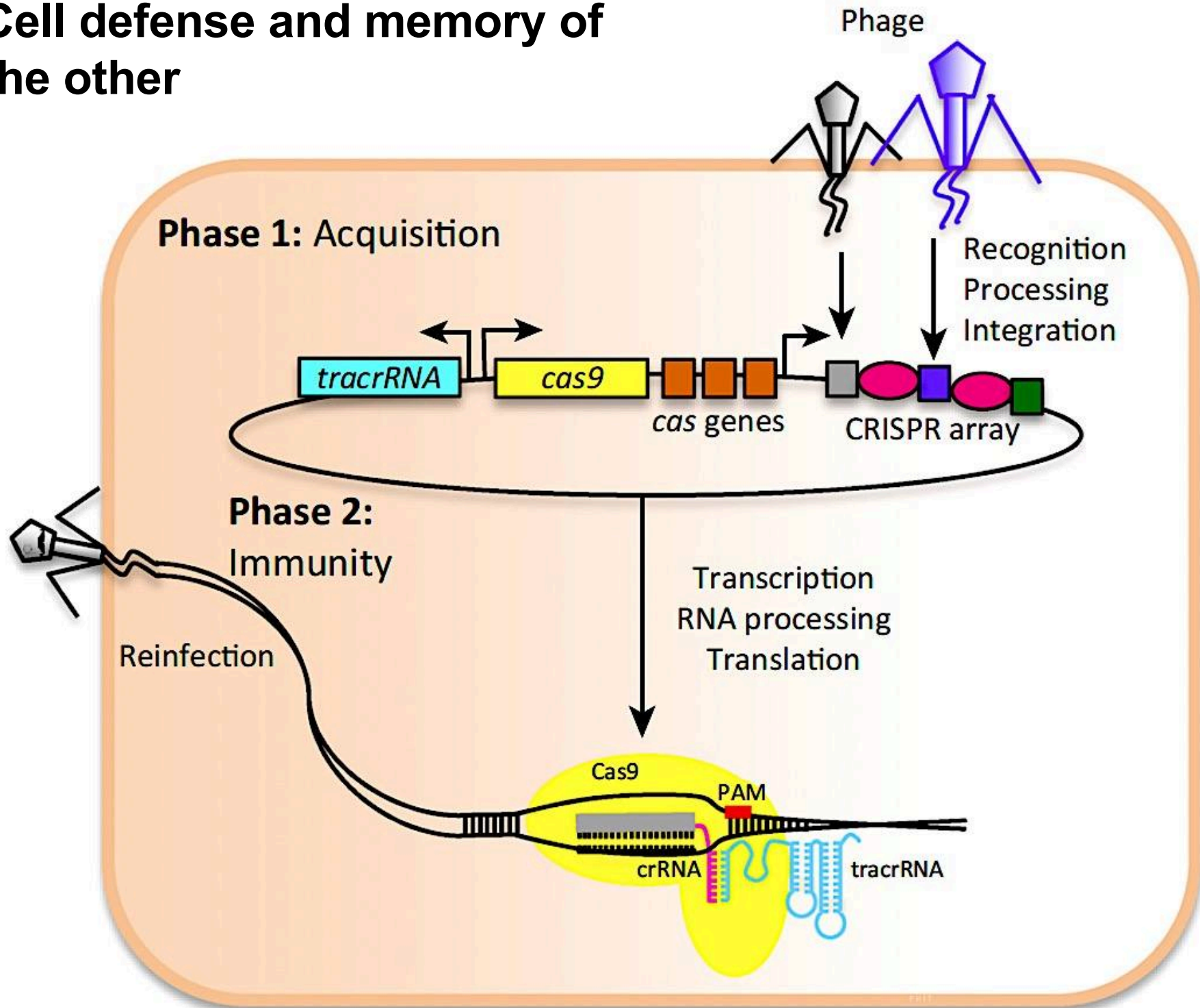
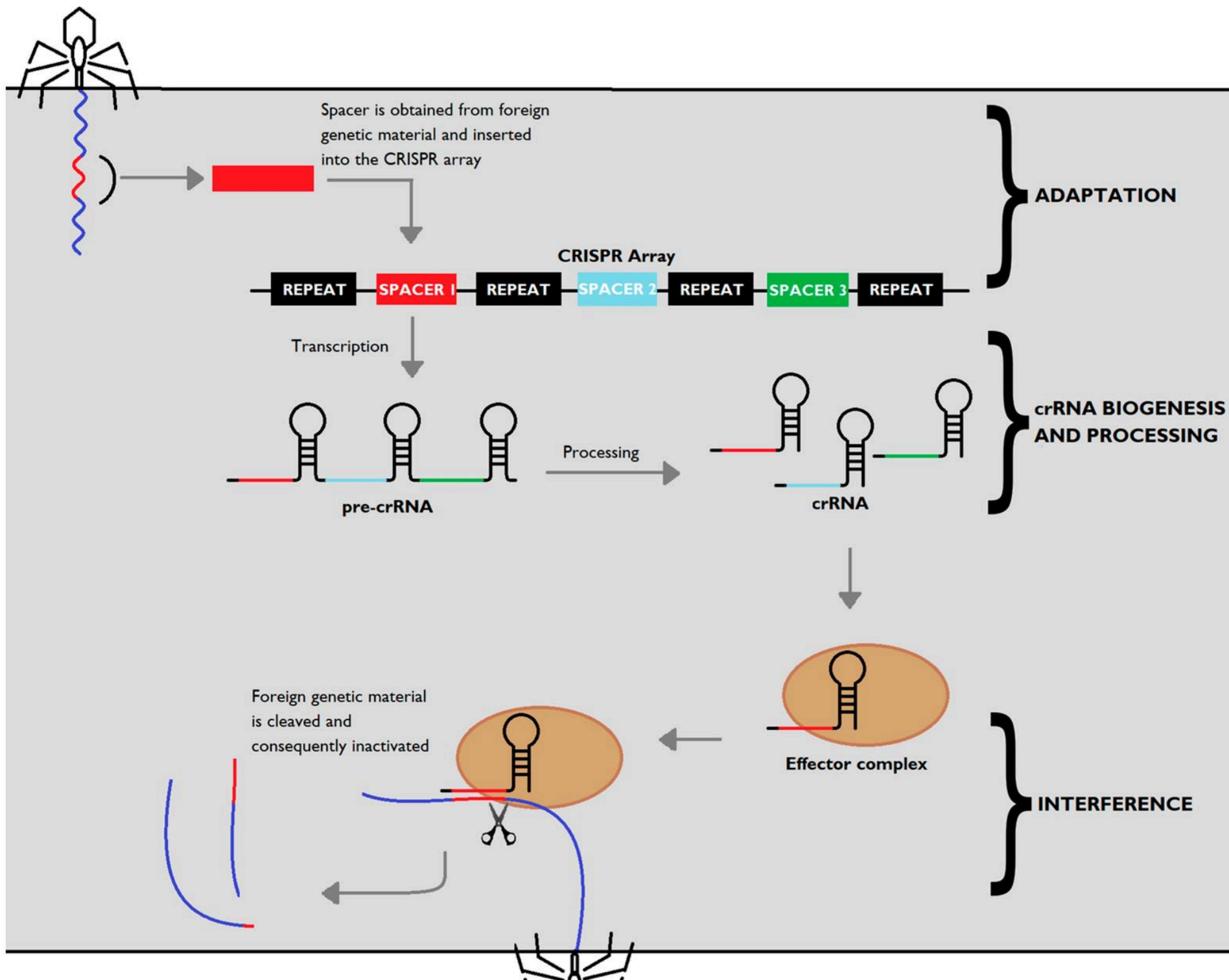


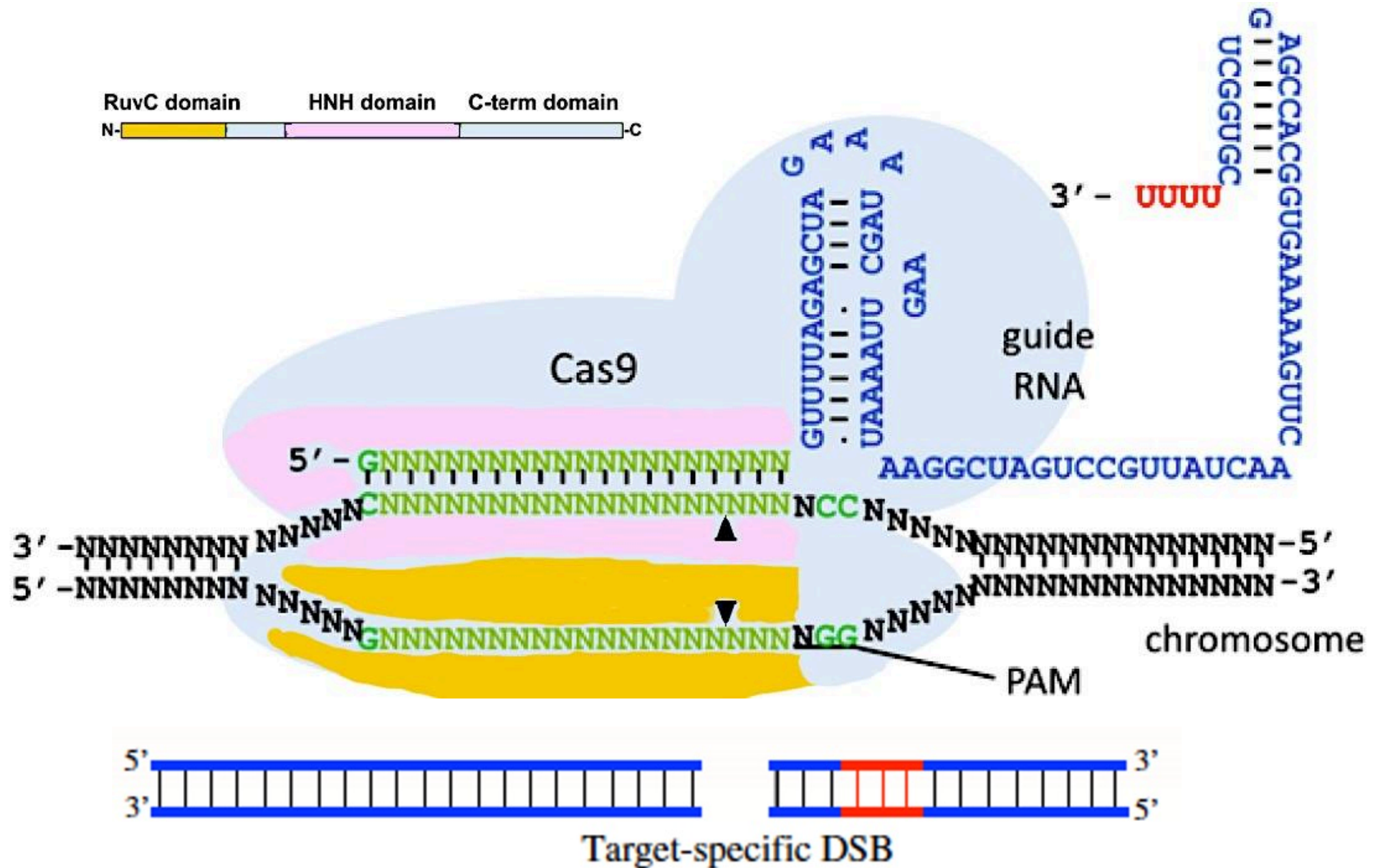
FIG 1 Restriction-modification (R-M) systems as defense mechanisms. R-M systems recognize the methylation status of incoming foreign DNA, e.g., phage genomes. Methylated sequences are recognized as self, while recognition sequences on the incoming DNA lacking methylation are recognized as nonself and are cleaved by the restriction endonuclease (REase). The methylation status at the genomic recognition sites is maintained by the cognate methyltransferase (MTase) of the R-M system.

Cell defense and memory of the other

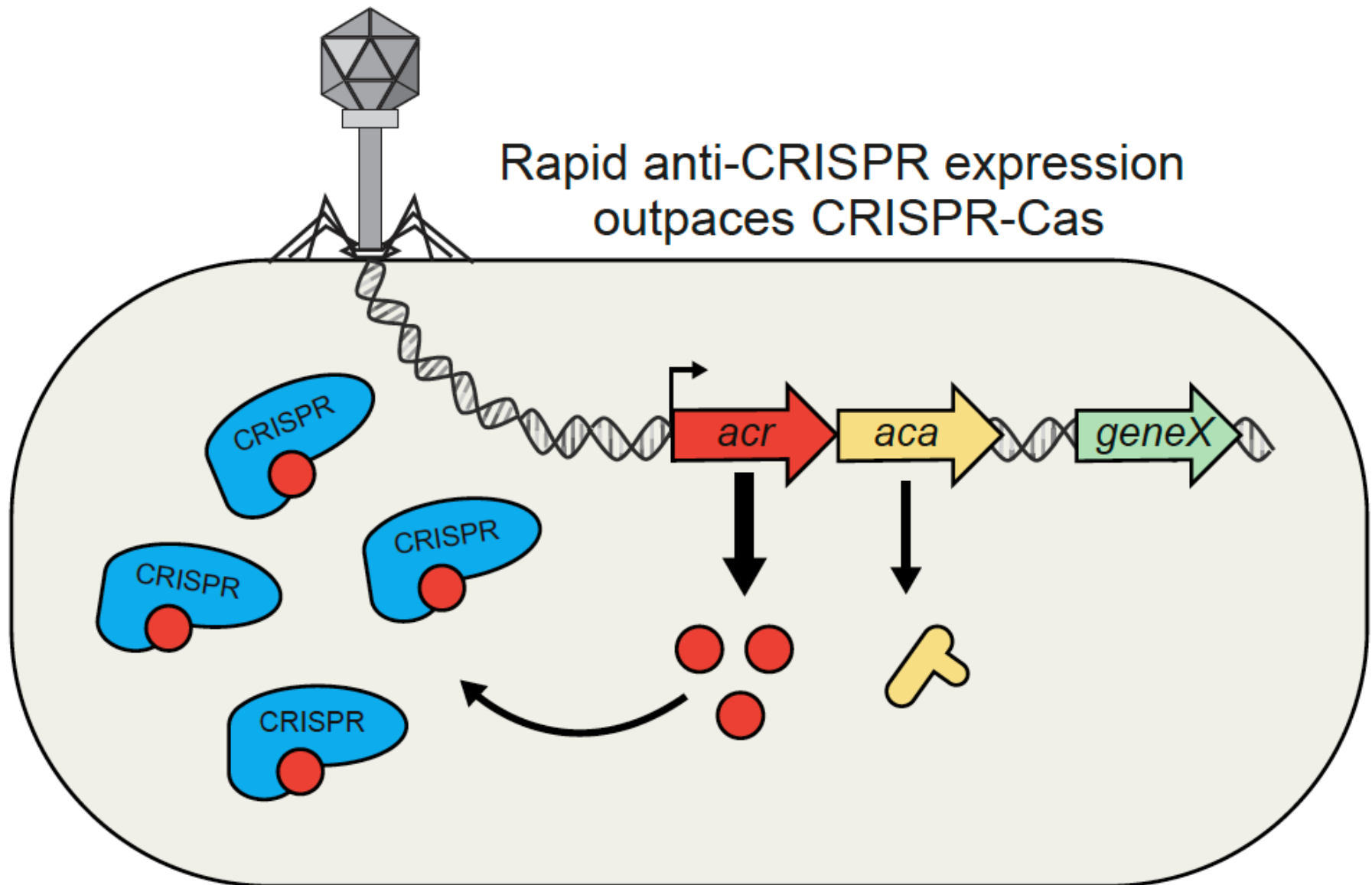




Programmable DNA cleavage using Cas9 and one or more guide RNAs

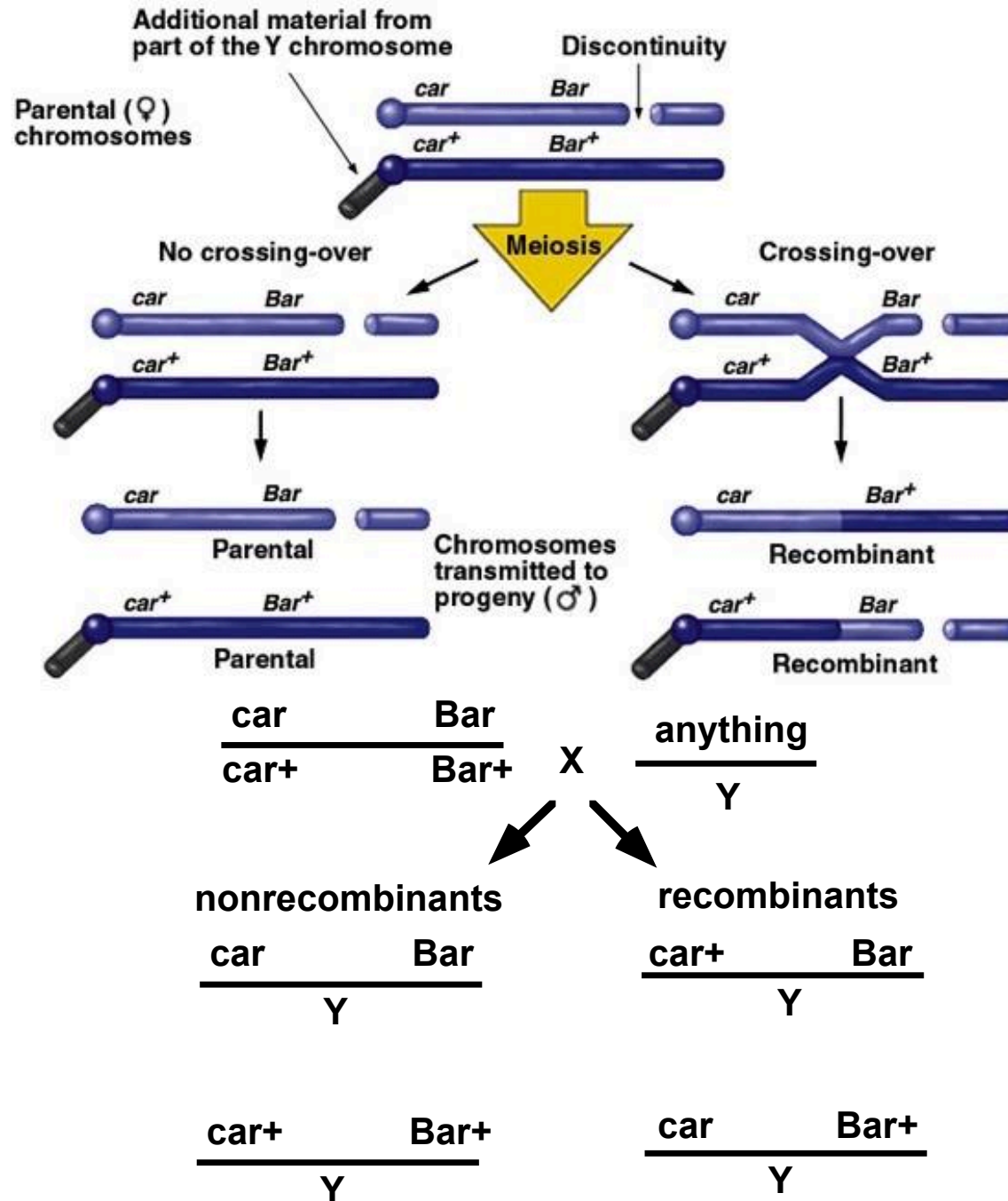


**Some phage encode anti-CRISPR proteins that prevent
CRISPR-based destruction of incoming phage
(the arms race continues)**



Mechanisms of recombination and gene targeting

Evidence for recombination



Allele segregation

The fungus *Neurospora*

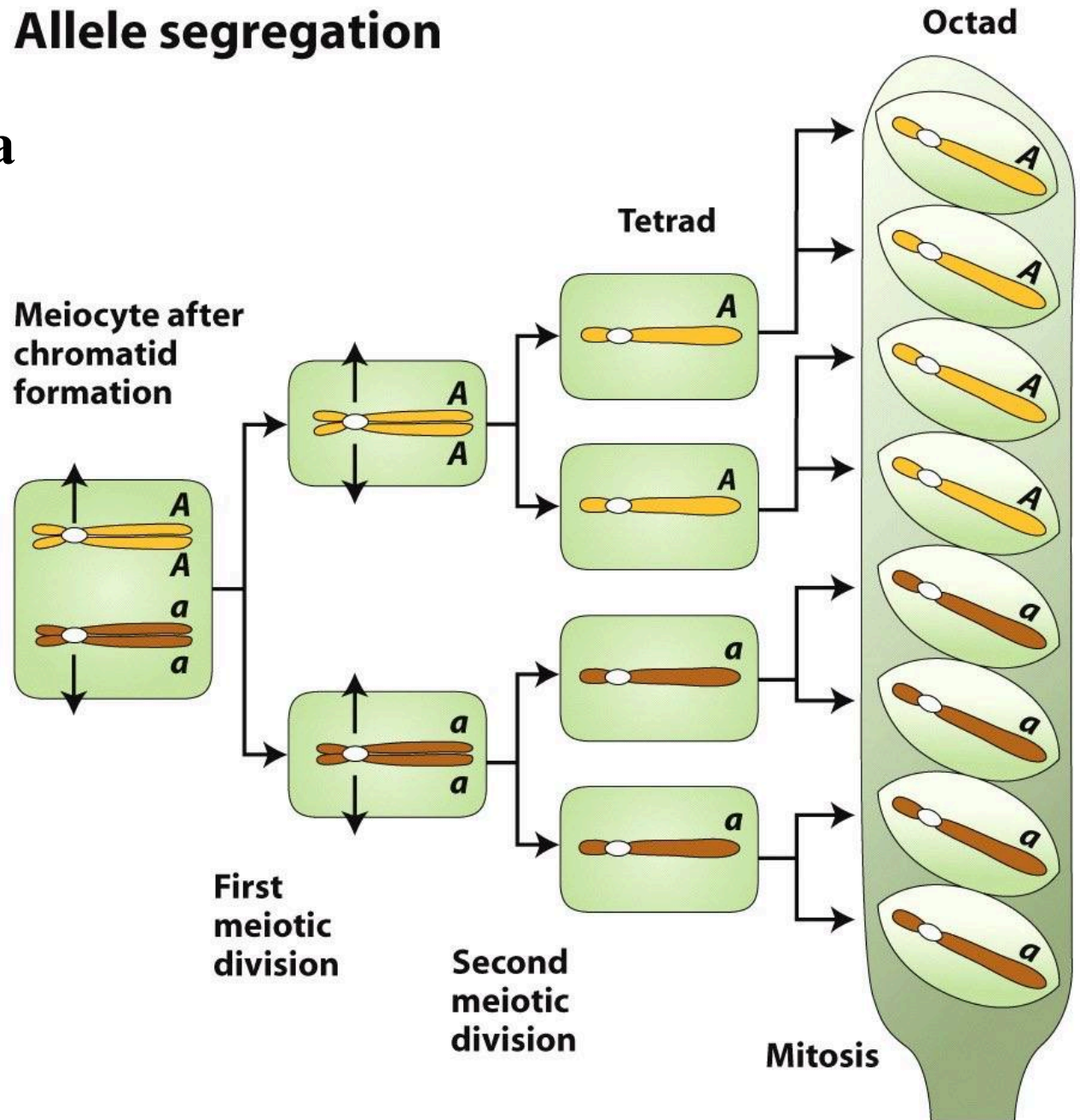
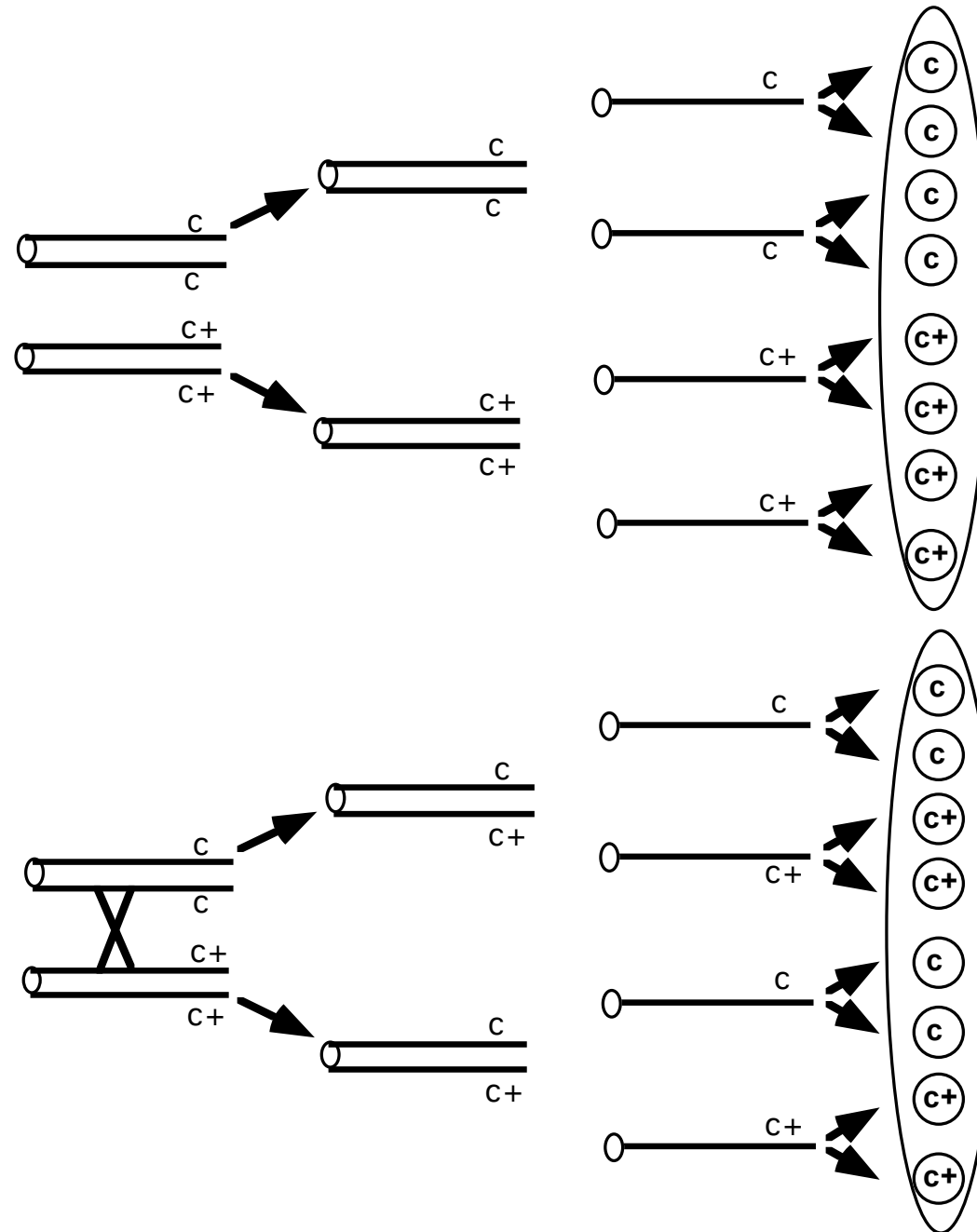


Figure 3-10b

Introduction to Genetic Analysis, Tenth Edition

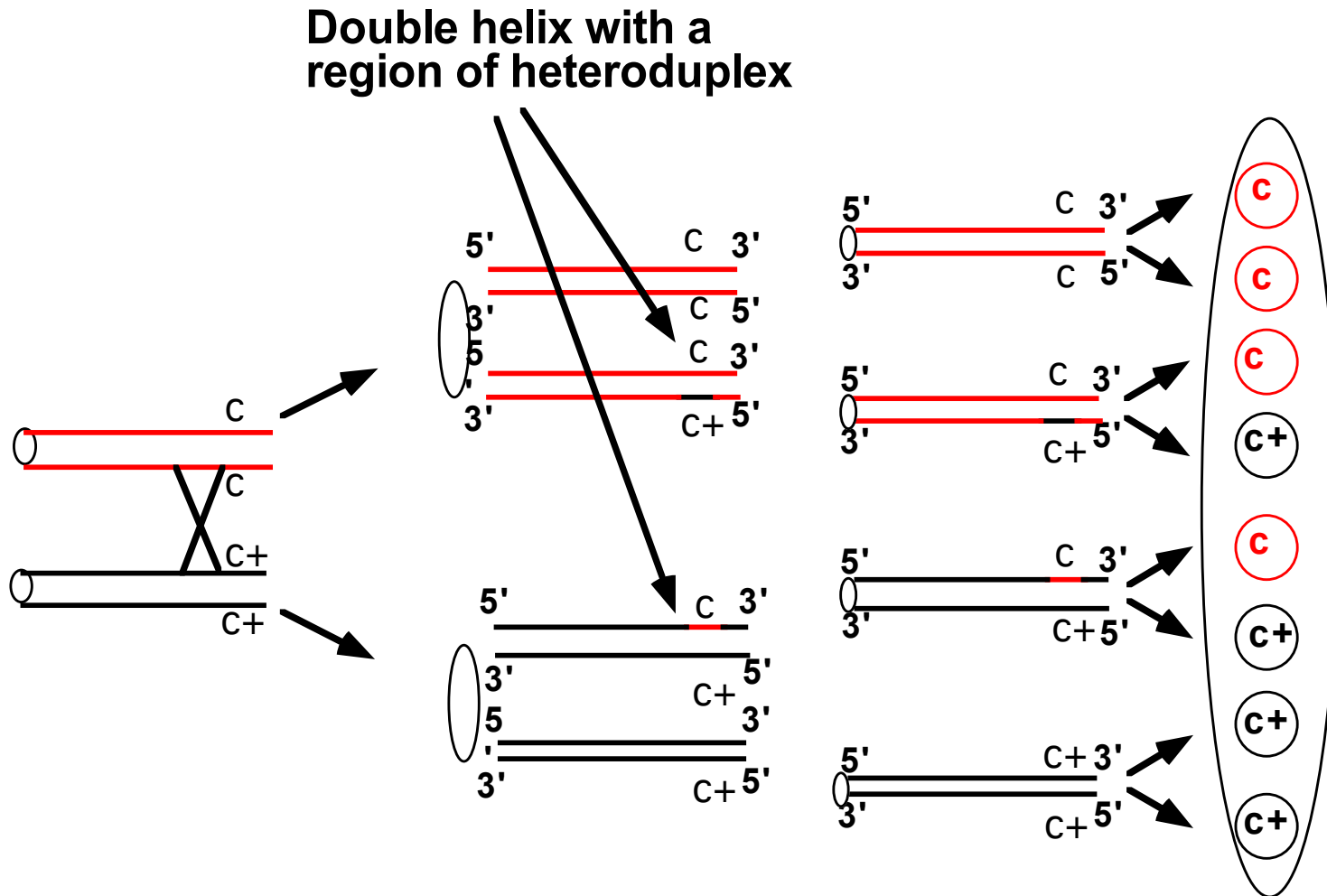
© 2012 W. H. Freeman and Company

Recombination occurring far away from the *c* gene in *Neurospora* Leads to a 2: 2: 2: 2 ratio of spores



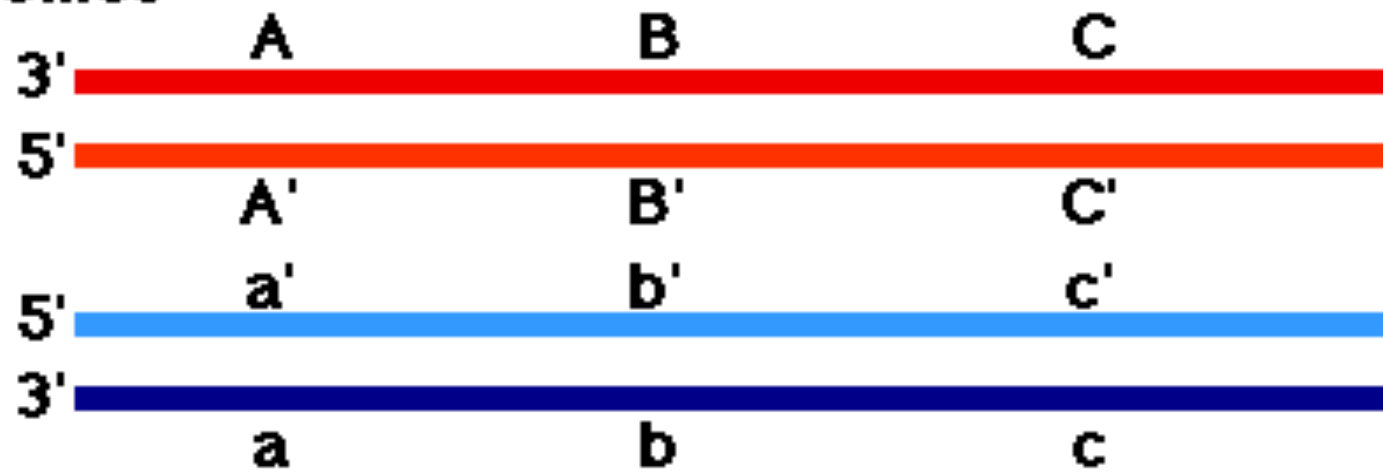
Recombination occurring near the *c* gene in *Neurospora* can lead to the formation of heteroduplex DNA near the site of crossing over. This can lead to aberrant ratios of spores.

When the site of recombination is close to a gene (*c*) it can lead to heteroduplex formation.

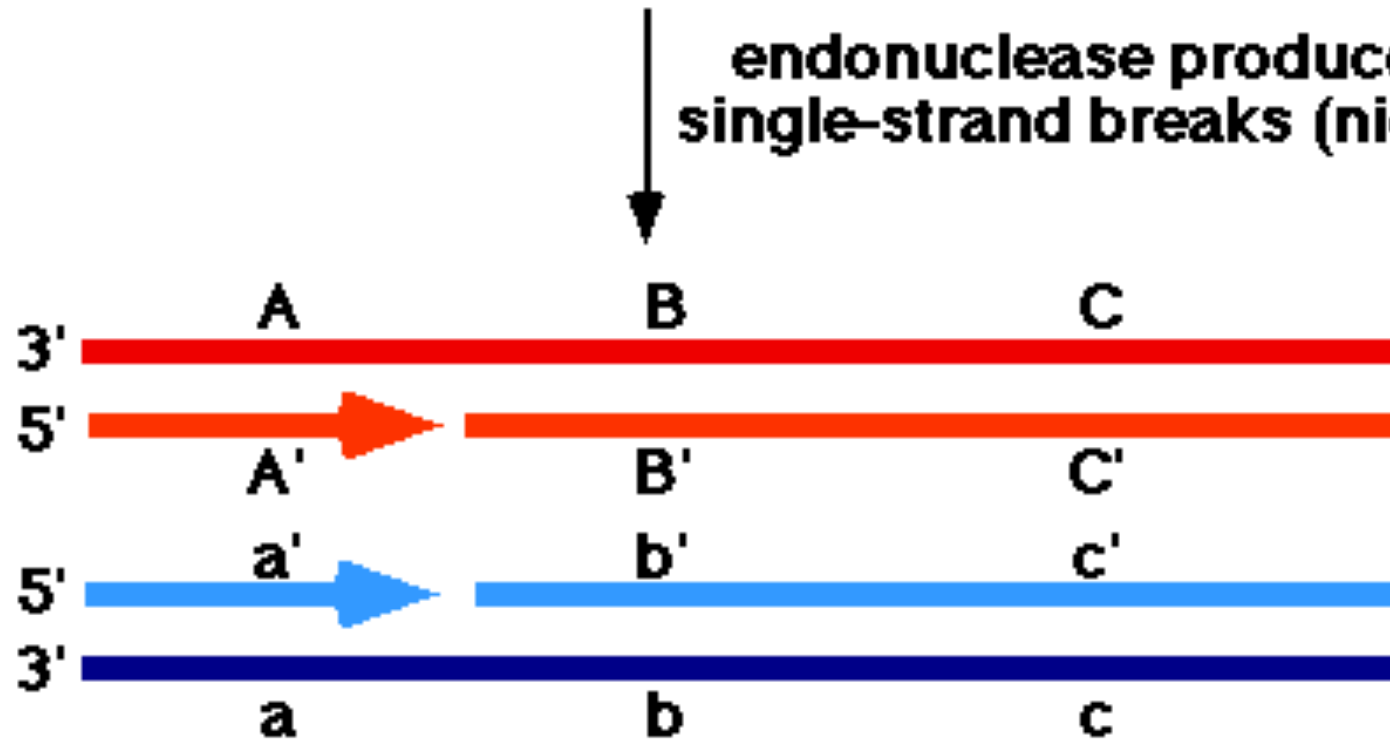


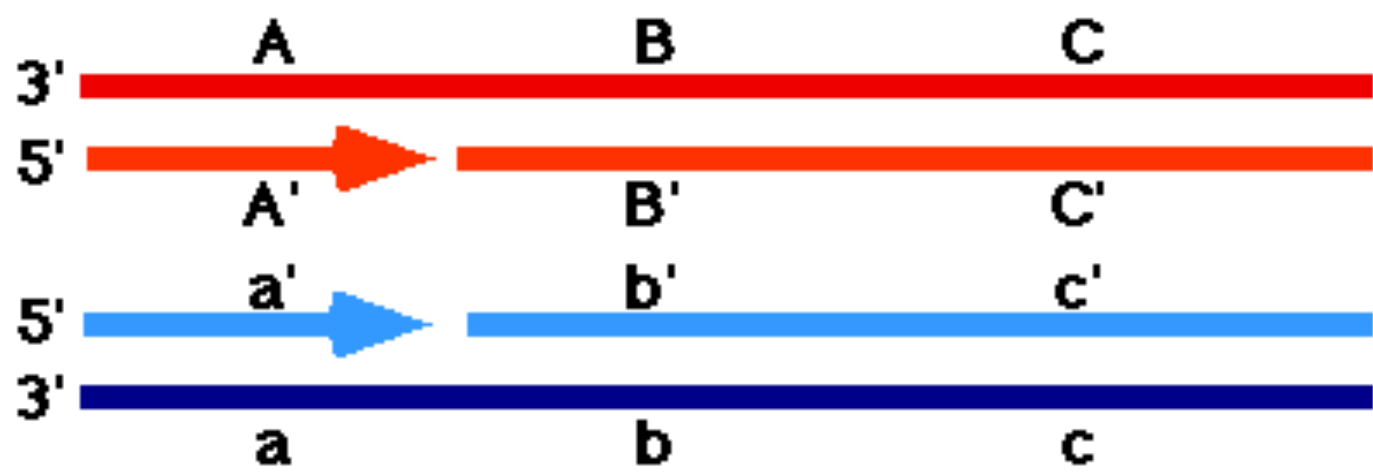
In the example shown recombination of markers distal to *c* has not occurred (see discussion of gene conversion several slides below).

homologous chromosomes

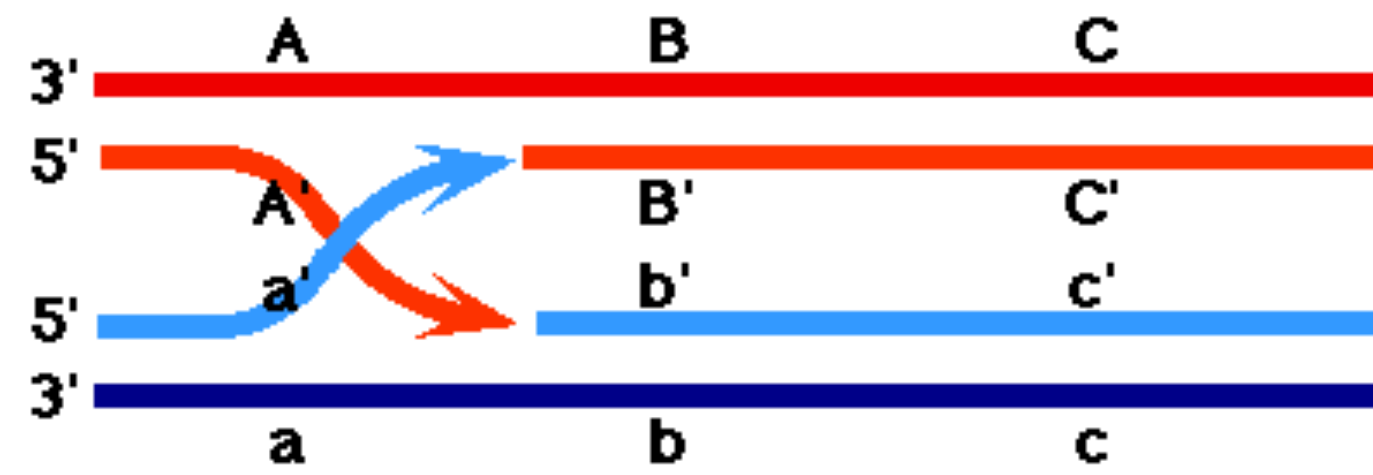


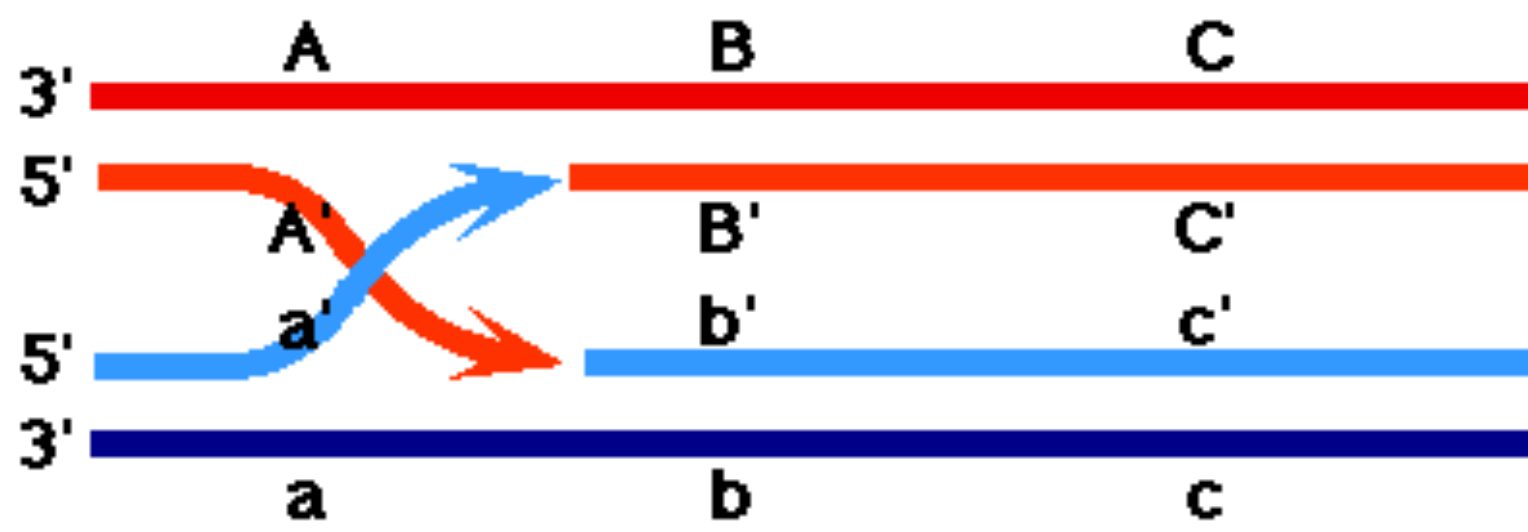
endonuclease produces single-strand breaks (nicks)



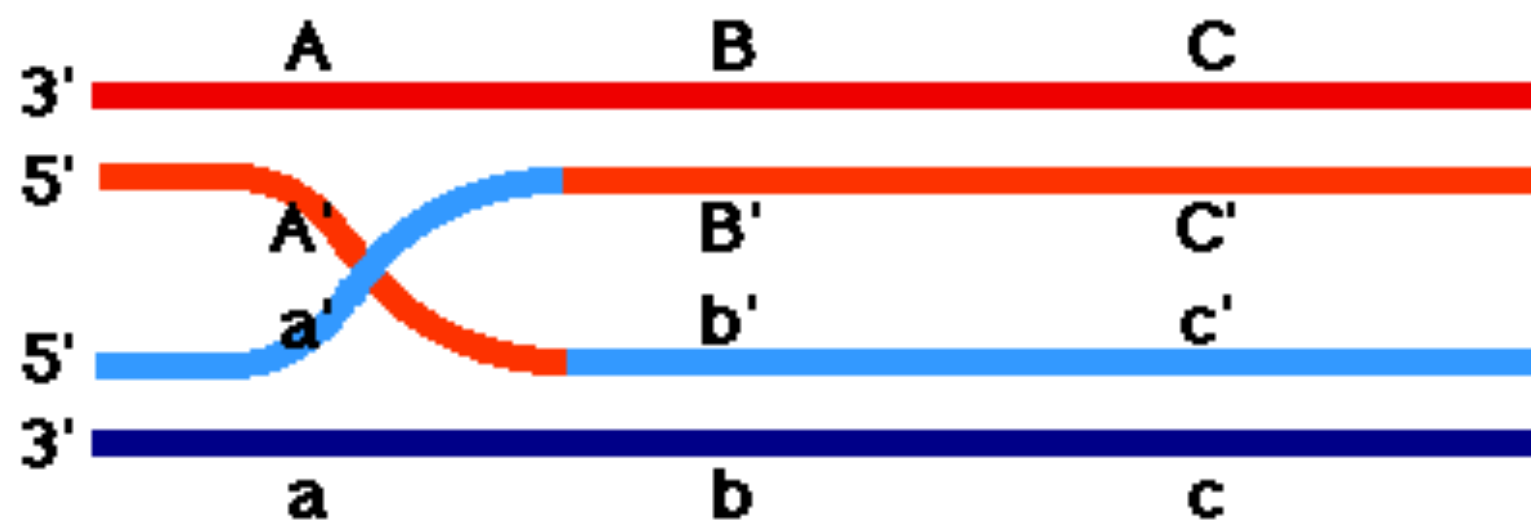


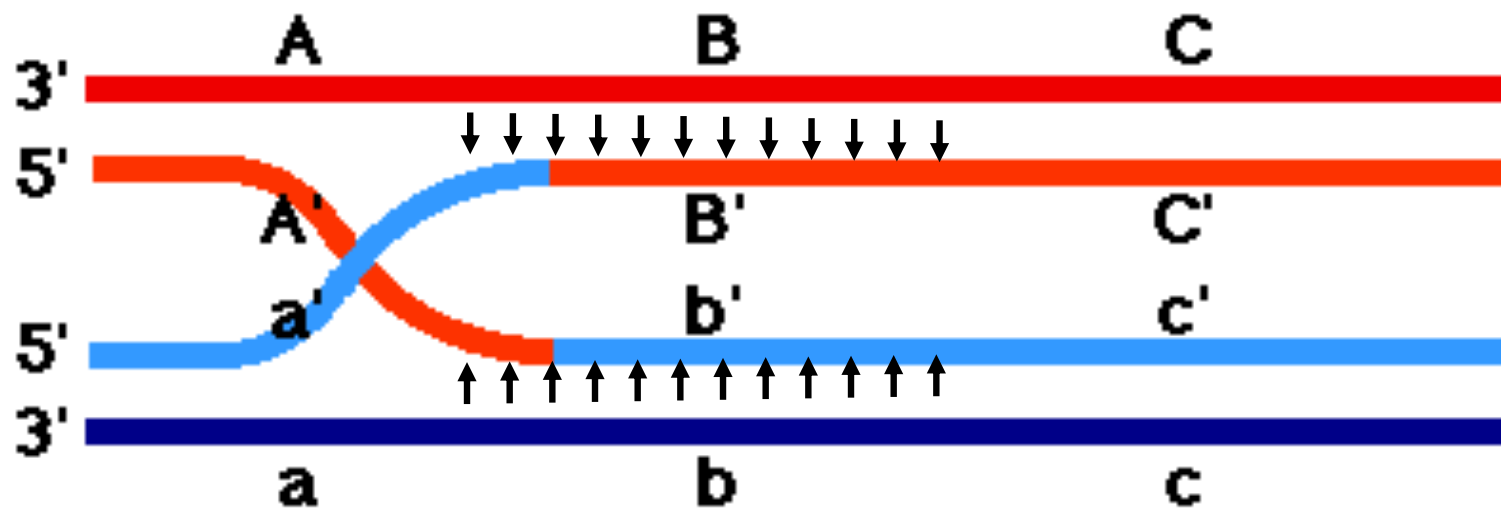
strand invasion



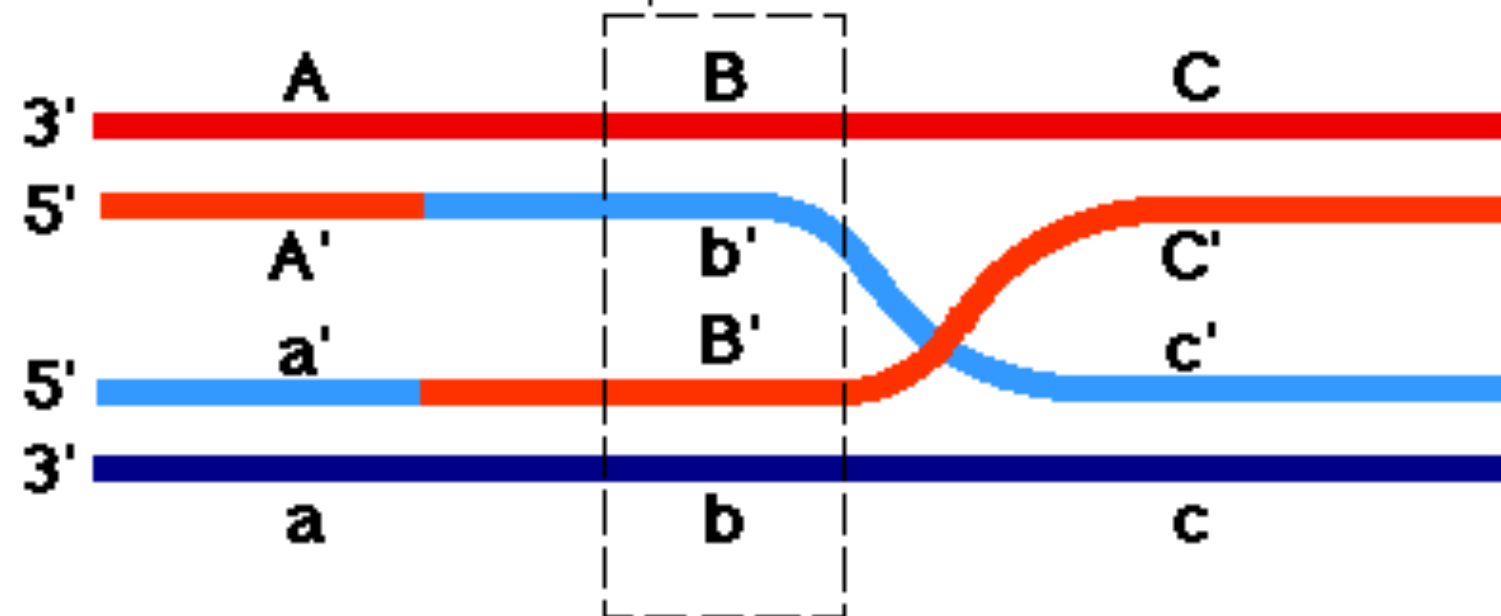


ligation

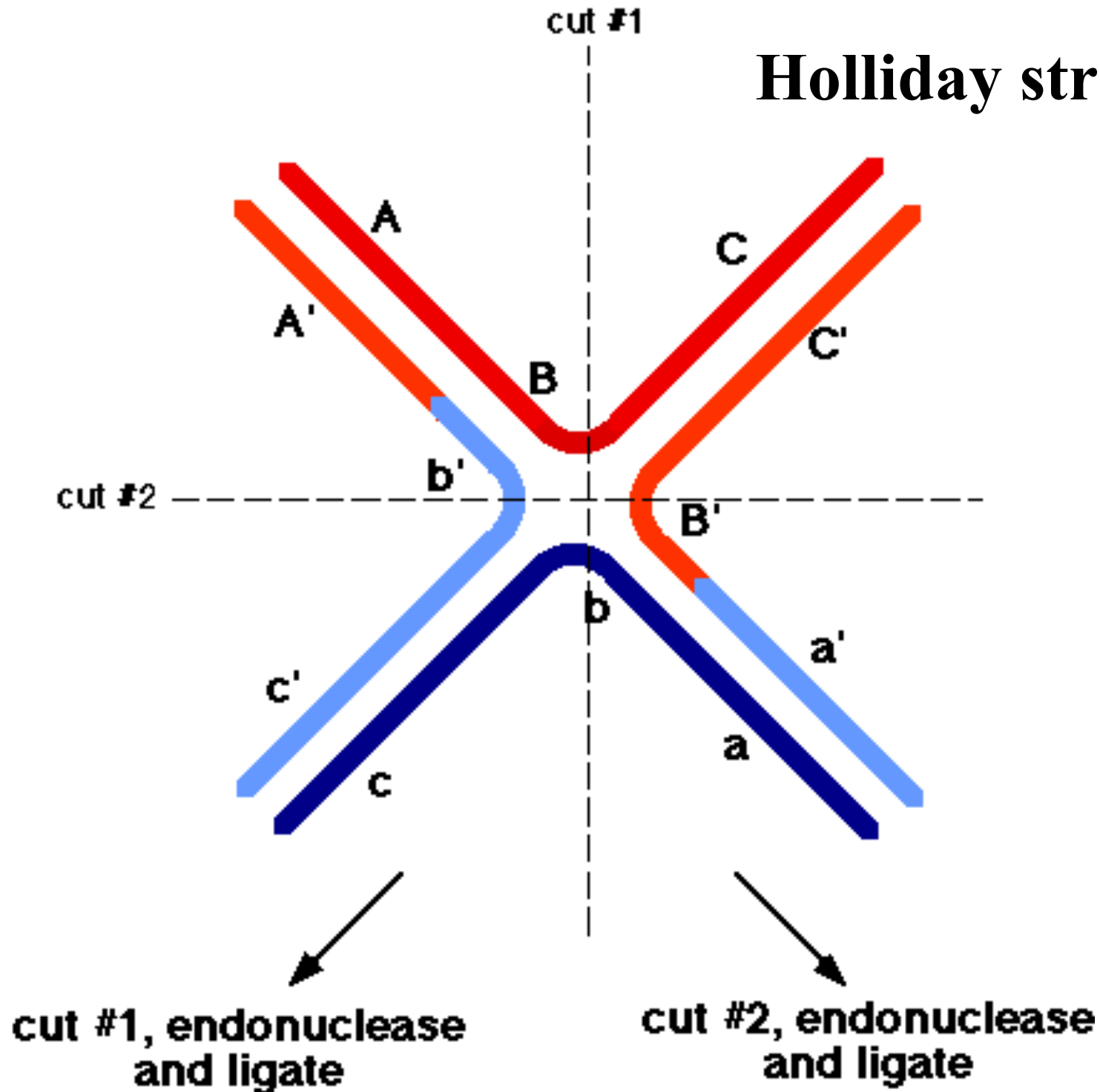


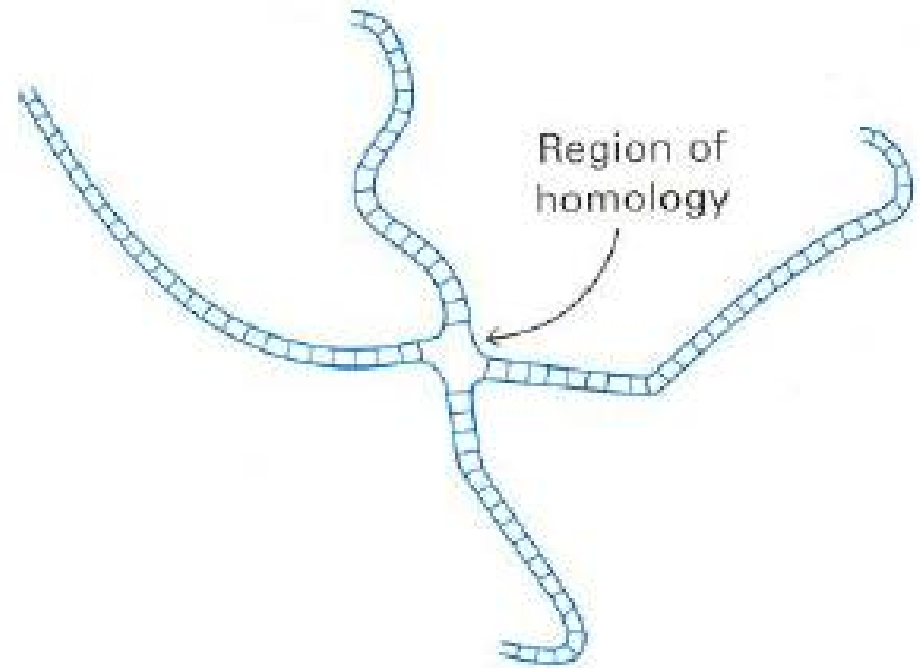


**branch migration
(heteroduplex formation)**

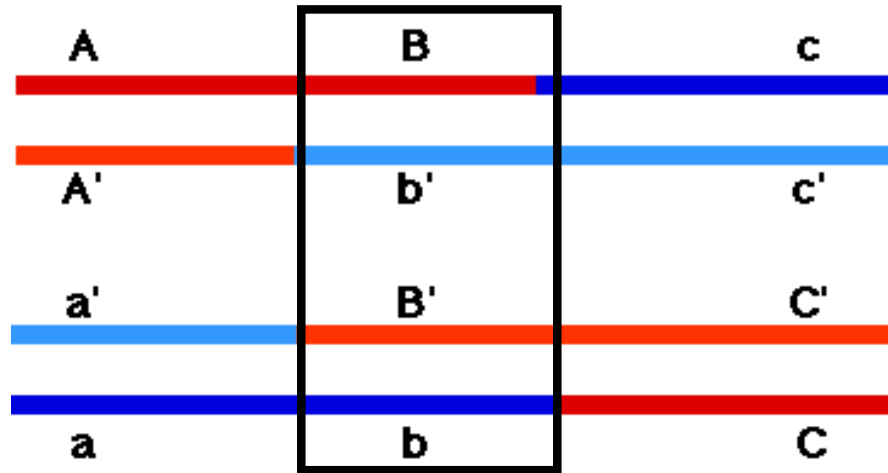


Holliday structure

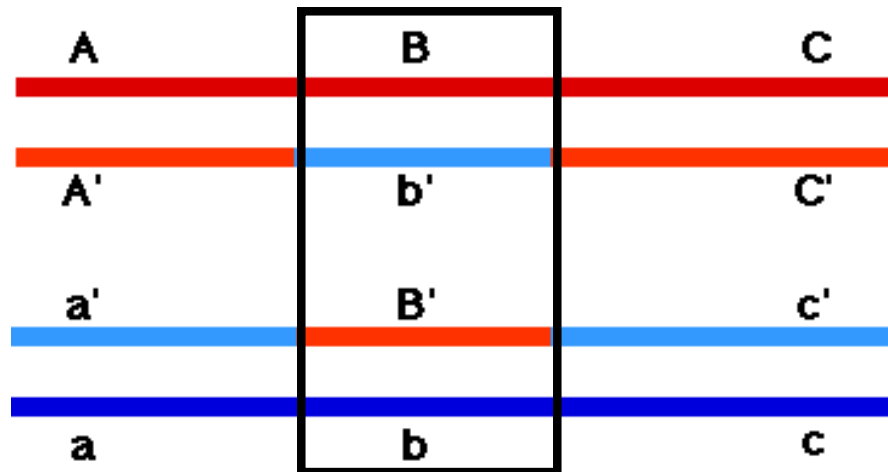




cut #1, endonuclease
and ligate



heteroduplexes and recombinants
cut #2, endonuclease
and ligate

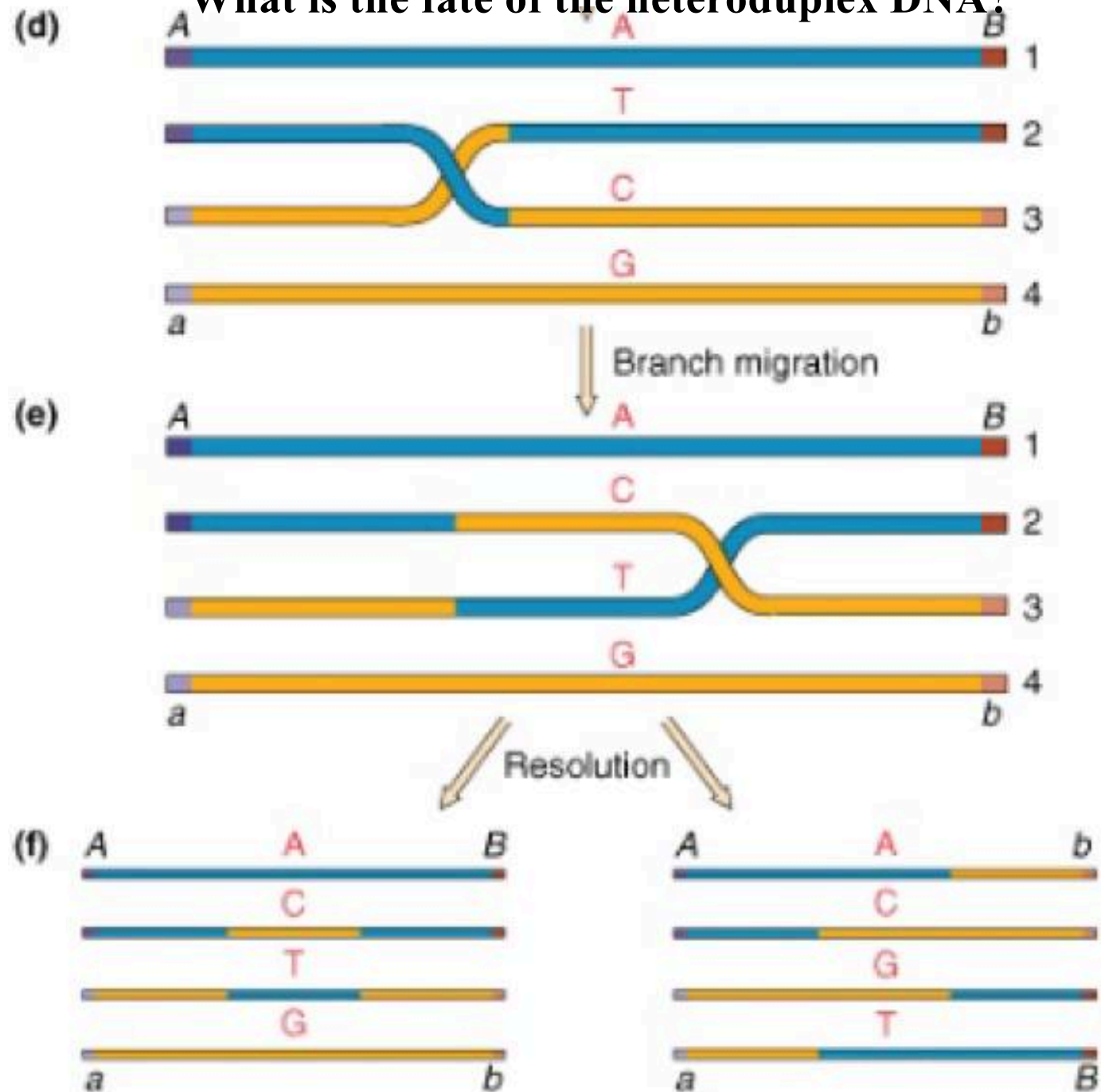


heteroduplexes but no recombinants

Because the Holliday junction is symmetric, predicts equivalent numbers of recombinant and nonrecombinant tetrads associated with heteroduplex DNA and gene conversion.

Also notice that the heteroduplex DNA is symmetric or is present on both chromatids.

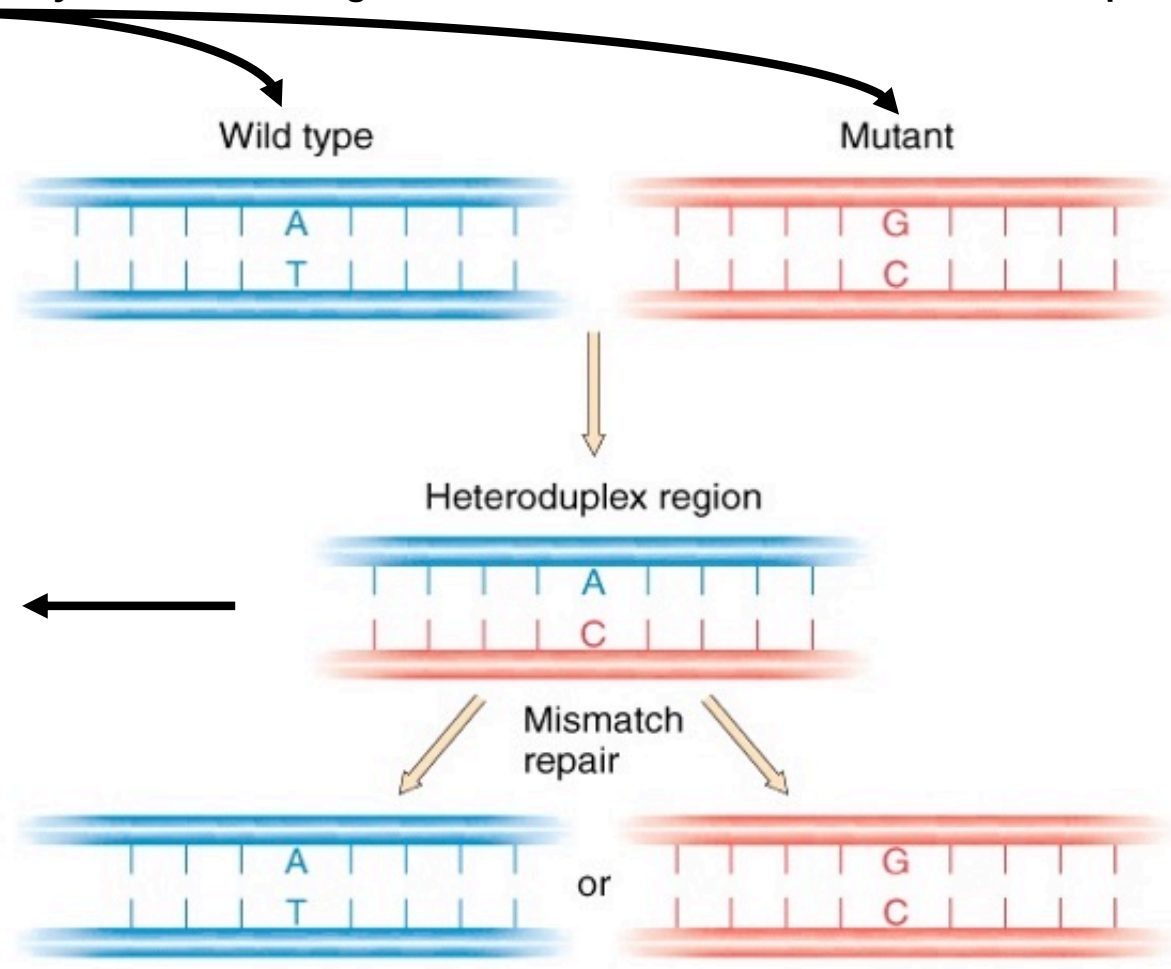
What is the fate of the heteroduplex DNA?



Gene conversion in a heteroduplex

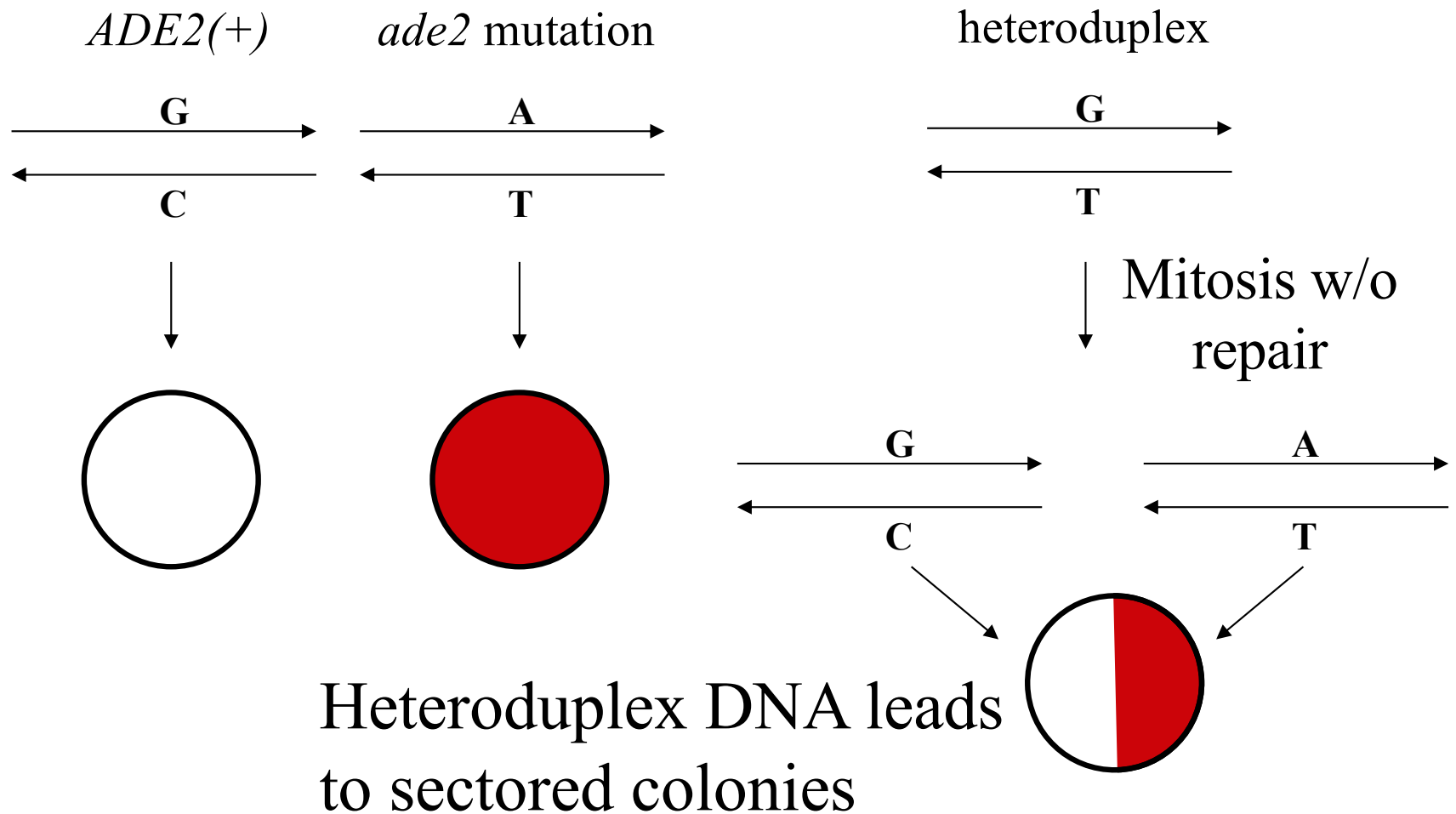
1. Gene conversion results from the repair of heteroduplex DNA by the mismatch repair system. Repair can occur in either direction.
2. Gene conversion can result in the loss of allelic information near the site of crossover.
3. Gene conversion only occurs in the direction of branch migration. That is the direction in which heteroduplex DNA is formed.
4. 50% of gene conversions are associated with crossing over.
5. But crossing over is only rarely associated with gene conversion since the amount of heteroduplex DNA is typically small.

No repair. The duplexes segregate following DNA replication reproducing the two original alleles



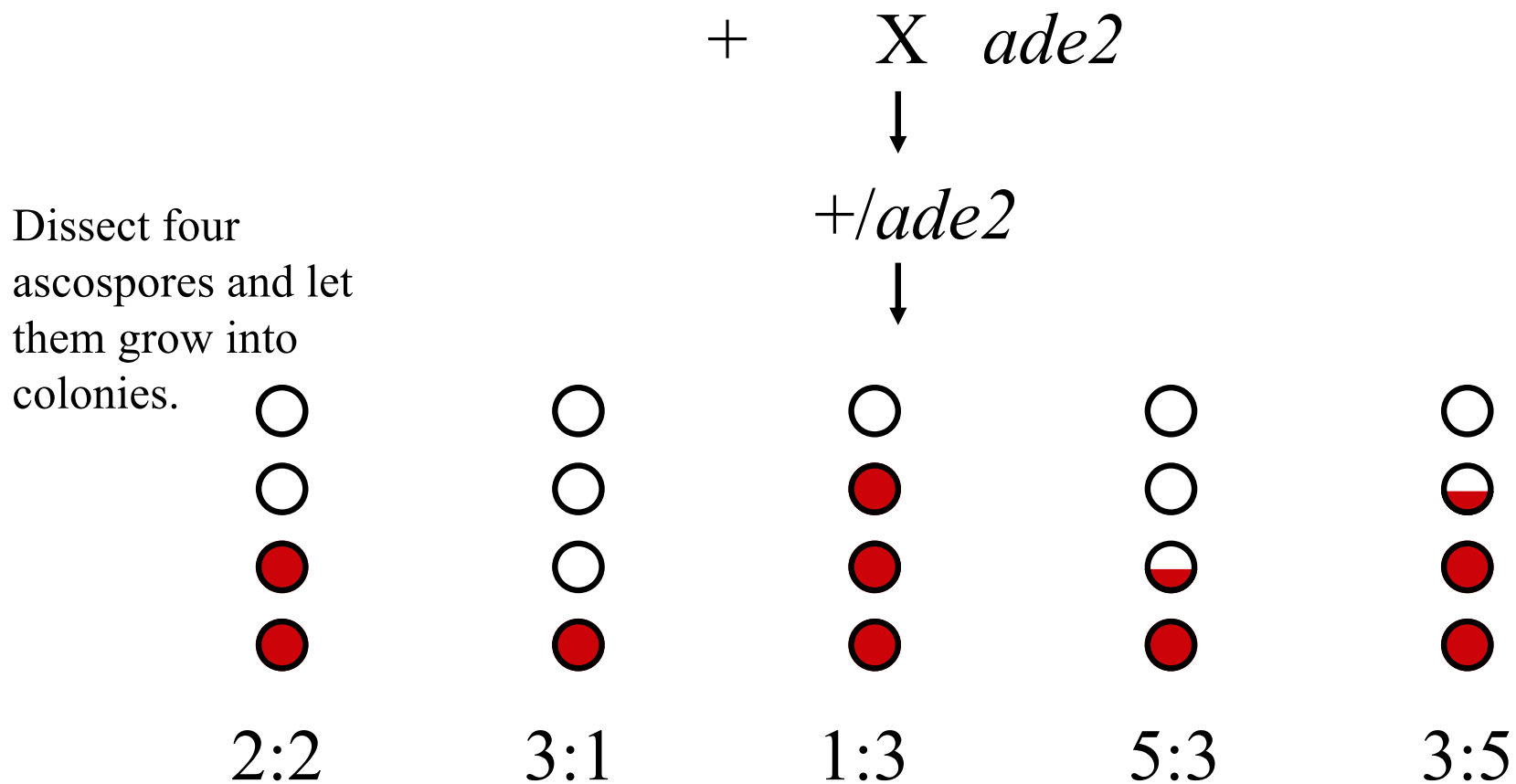
What is going on?

Magnify DNA and consider both DNA strands



Sectored colonies

e.g., yeast *ADE2* gene involved in adenine biosynthesis
red intermediate accumulates in *ade2* mutants



5:3 and 3:5 segregation patterns also exhibit parity.

If we now consider each strand of DNA

2:2 is 4:4 segregation

3:1 is 6:2

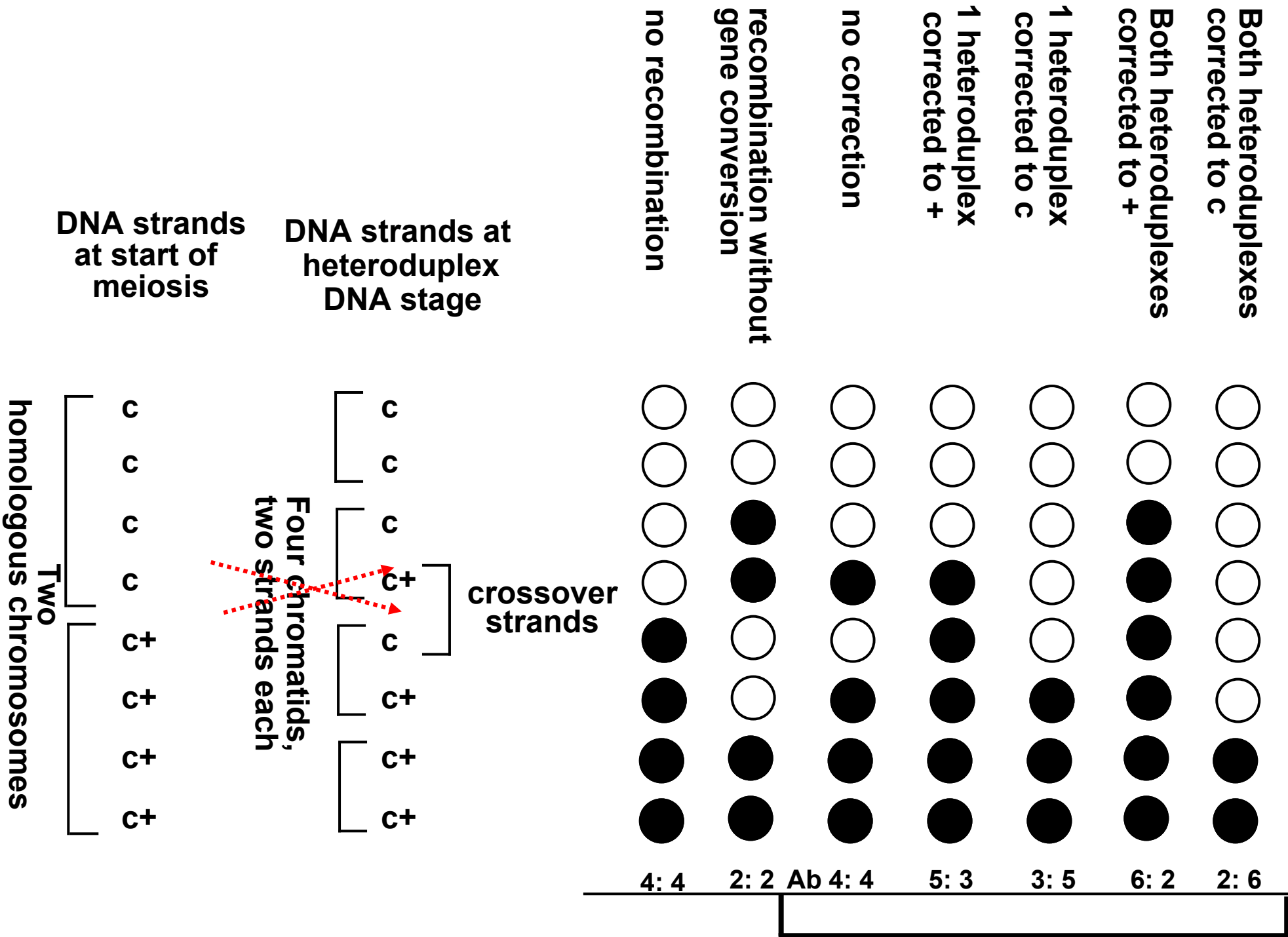
1:3 is 2:6

One way of explaining 3:1 and 1:3 segregation patterns is to propose that they were derived from 5:3 and 3:5 by mismatch repair.

5:3 could be repaired to 3:1 or 4:4

3:5 could be repaired to 1:3 or 4:4

Gene conversion in Neurospora



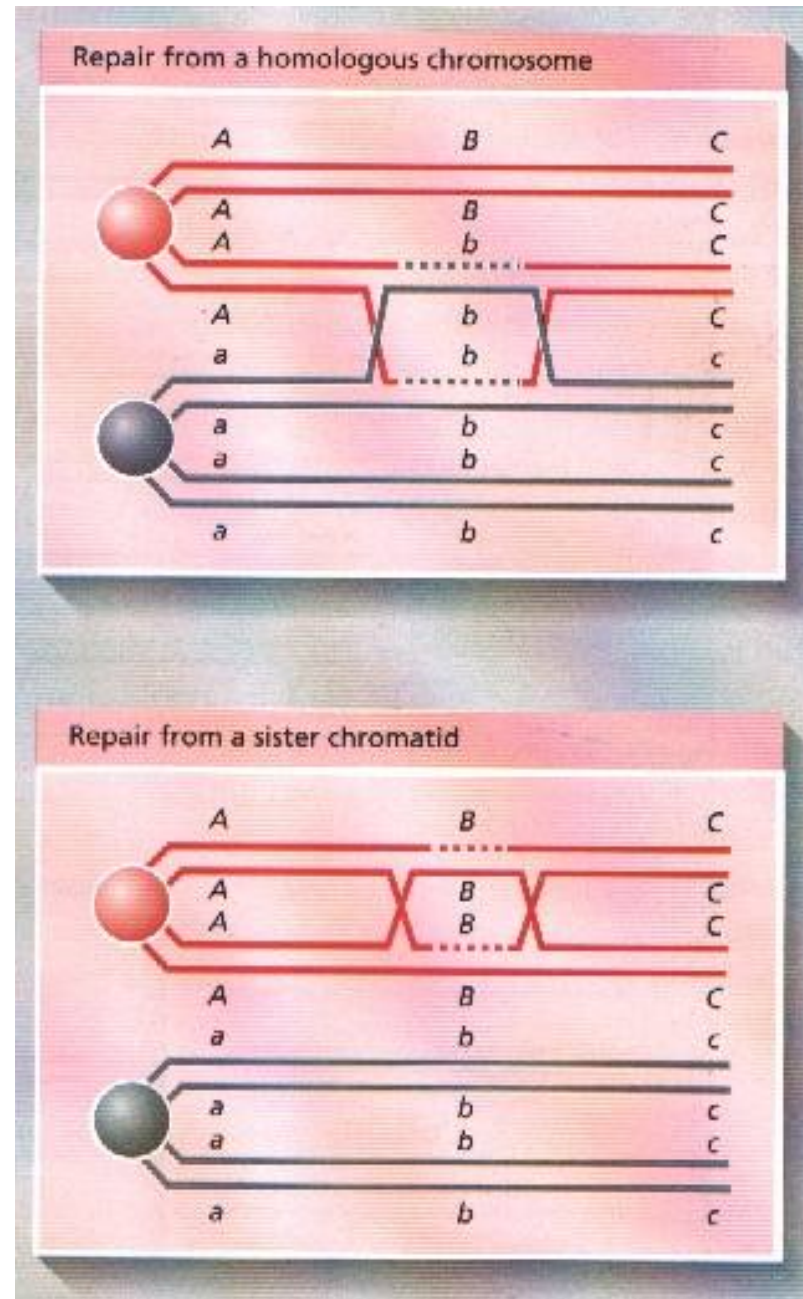
Homologous recombination

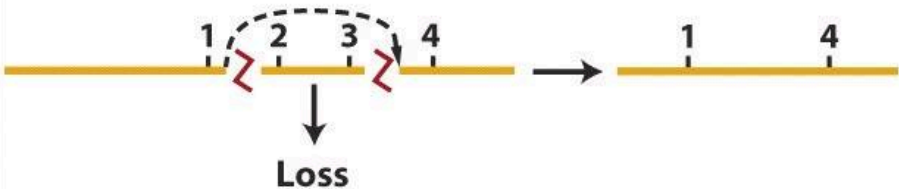
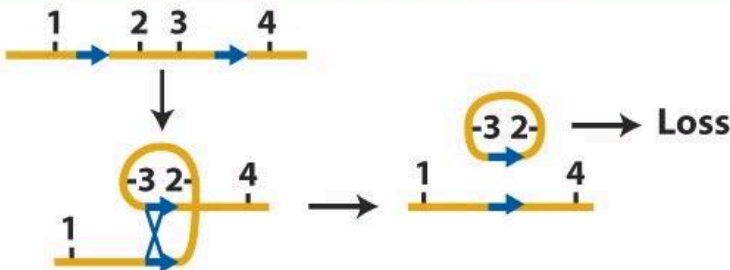
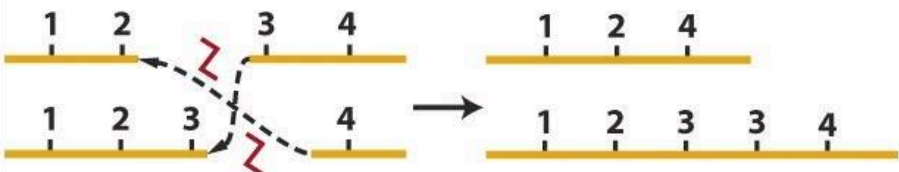
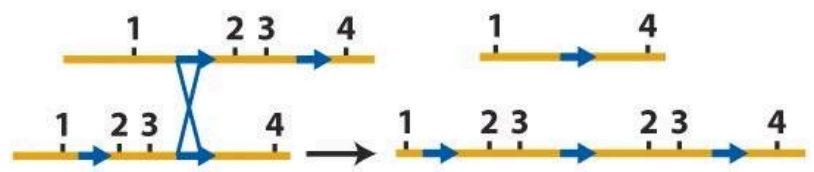
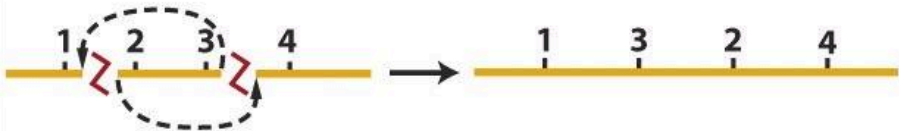
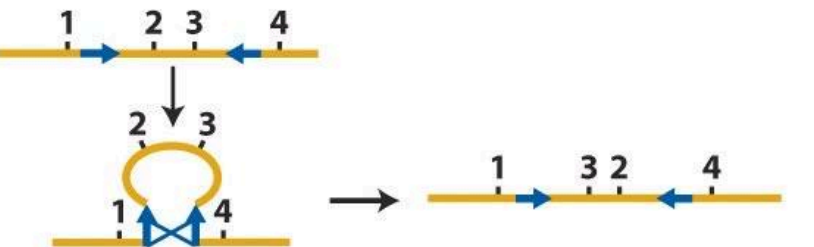
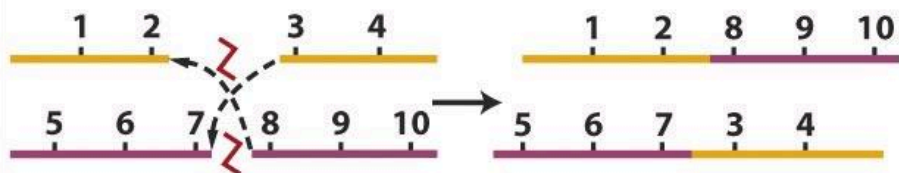
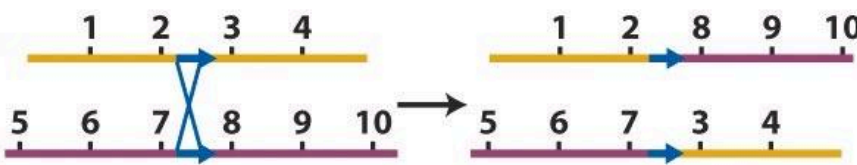
Meiotic recombination occurs when chromosomes pair during meiosis. The basis for this pairing is not understood. It does not involve Watson-Crick base pairing since the chromatids are already replicated. Nonetheless, it is clear that during meiosis the cell makes a powerful effort to line up regions of homology.

Homologous pairing and recombination of local or distant regions of homologous duplexes occurs in number of other situations as well, and is a major force for both genomic stability as well as for various forms of instability.

- 1. DNA repair, both of single strand lesions, and of double strand breaks. (Stability)**
- 2. The genome is full of repeated sequences. These repeats are able to recombine with each other based on shared homology. This can lead to many different kinds of chromosome abnormalities, including inversions, duplications and deletions. (Instability)**
- 3. Exogenous DNA, designed by the experimenter, can be introduced into cells where it may undergo homologous recombination with chromosomal sequences. This technique can be used to “knock out” or modify chromosomal genes in cells of the germline.**

Homologous recombination: repair of a double strand DNA break



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

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.**

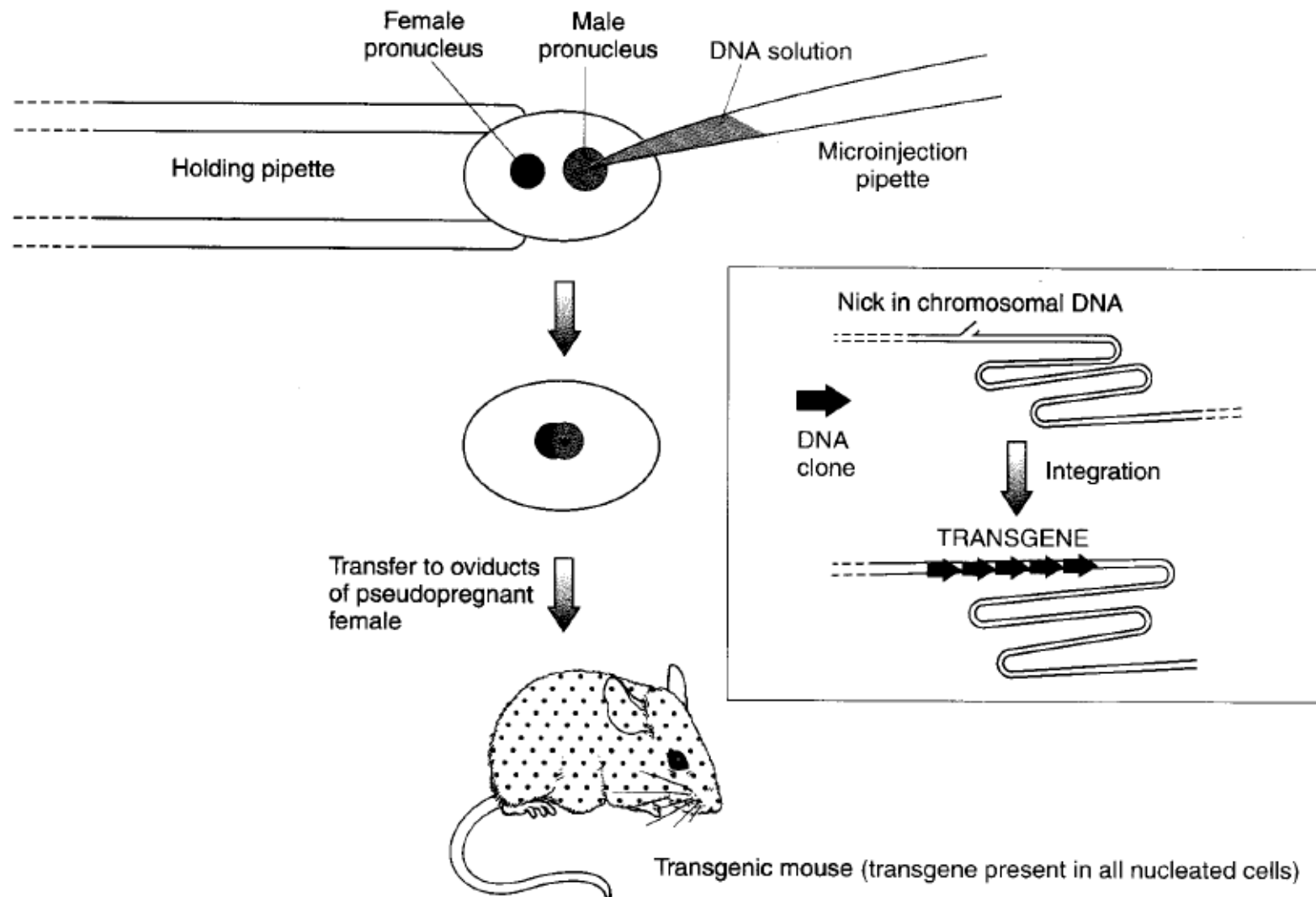


Figure 21.1: Construction of transgenic mice by pronuclear microinjection.

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).



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"

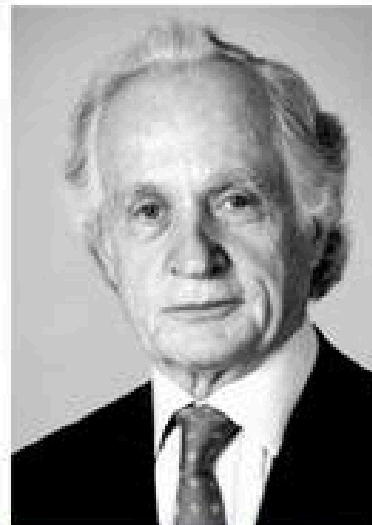
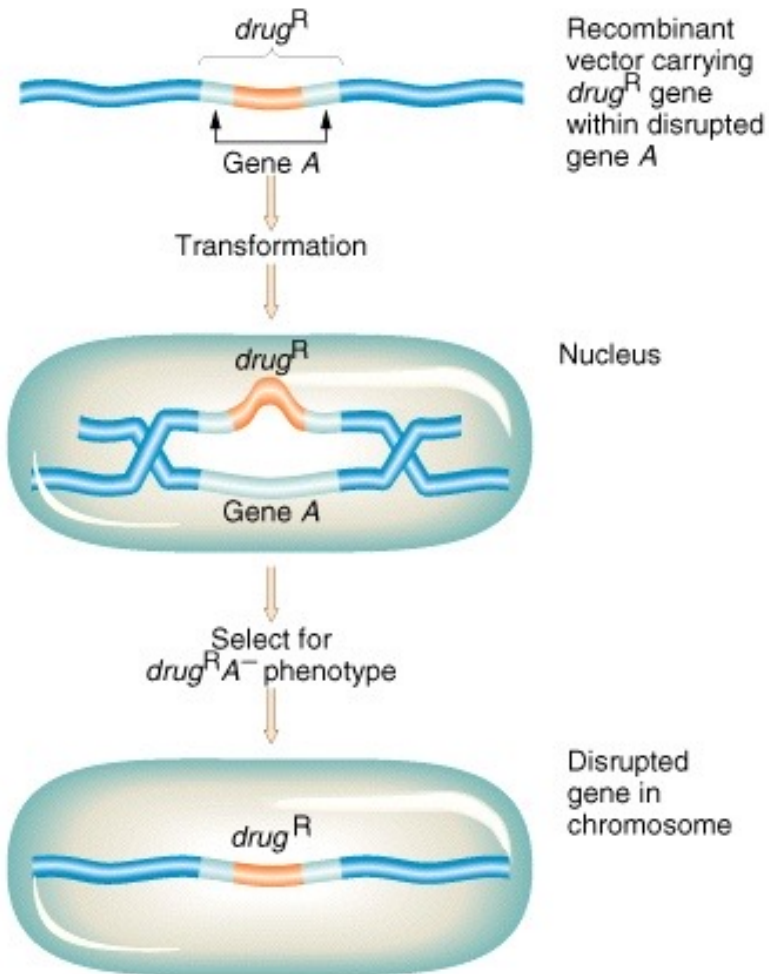


Photo: U. Montan

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)

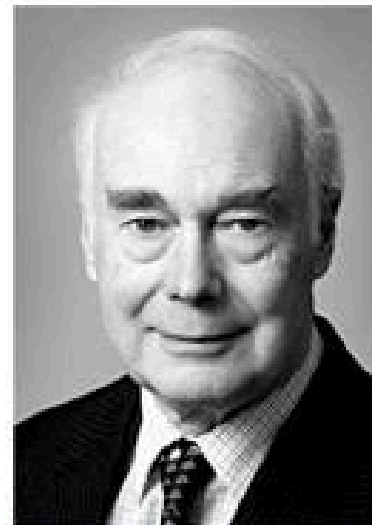


Photo: U. Montan

Sir Martin J. Evans

🕒 1/3 of the prize

United Kingdom

Cardiff University
Cardiff, United Kingdom

b. 1941

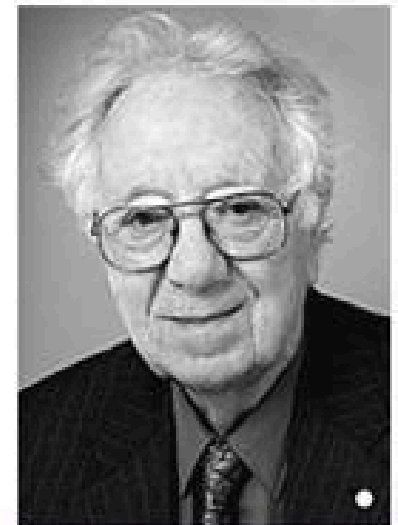


Photo: U. Montan

Oliver Smithies

🕒 1/3 of the prize

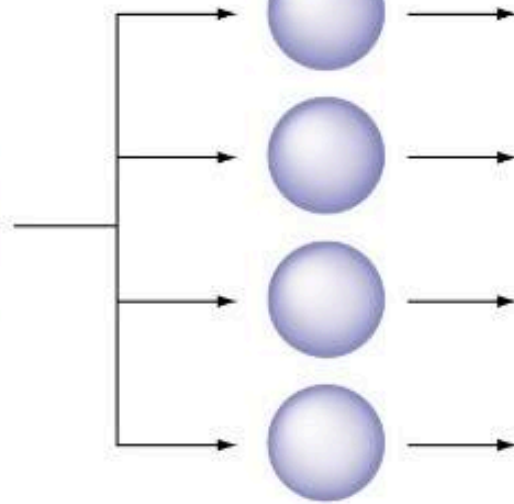
USA

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

b. 1925
(in United Kingdom)

(a)

Four-cell embryo



Identical quadruplets



(b)

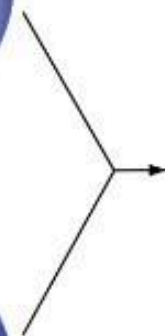
Two four-cell embryos



Embryo 1



Embryo 2



Chimeric embryo

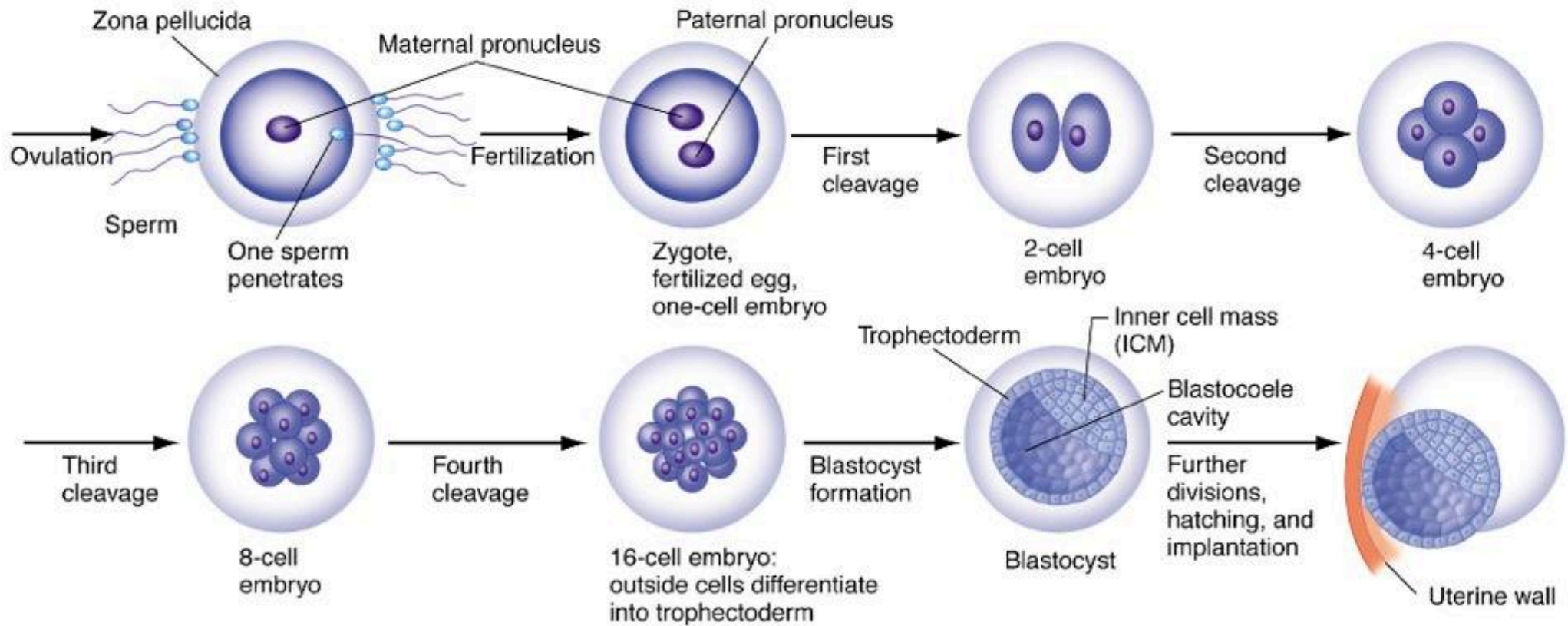


Chimeric mouse



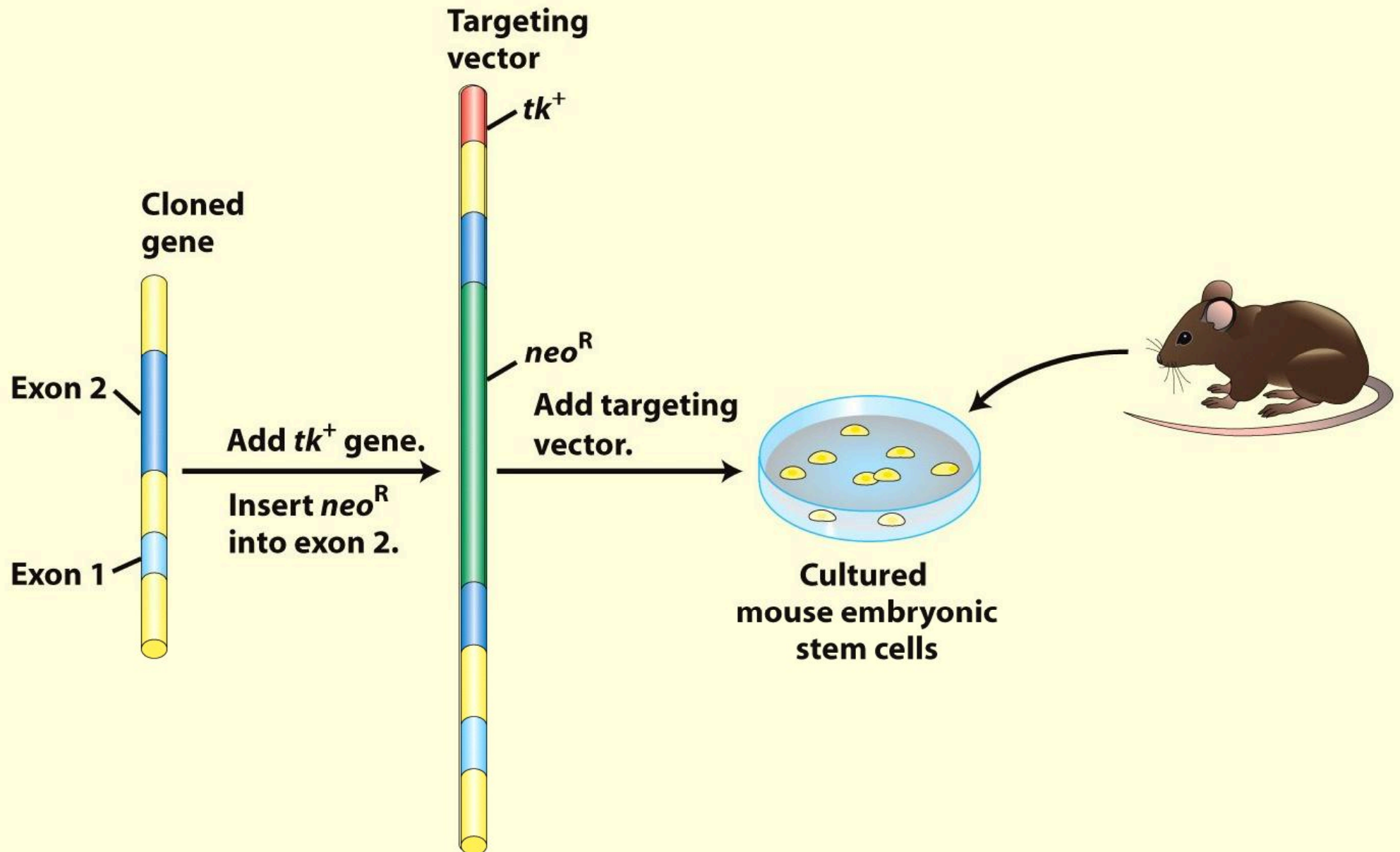
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Preimplantation development and Implantation



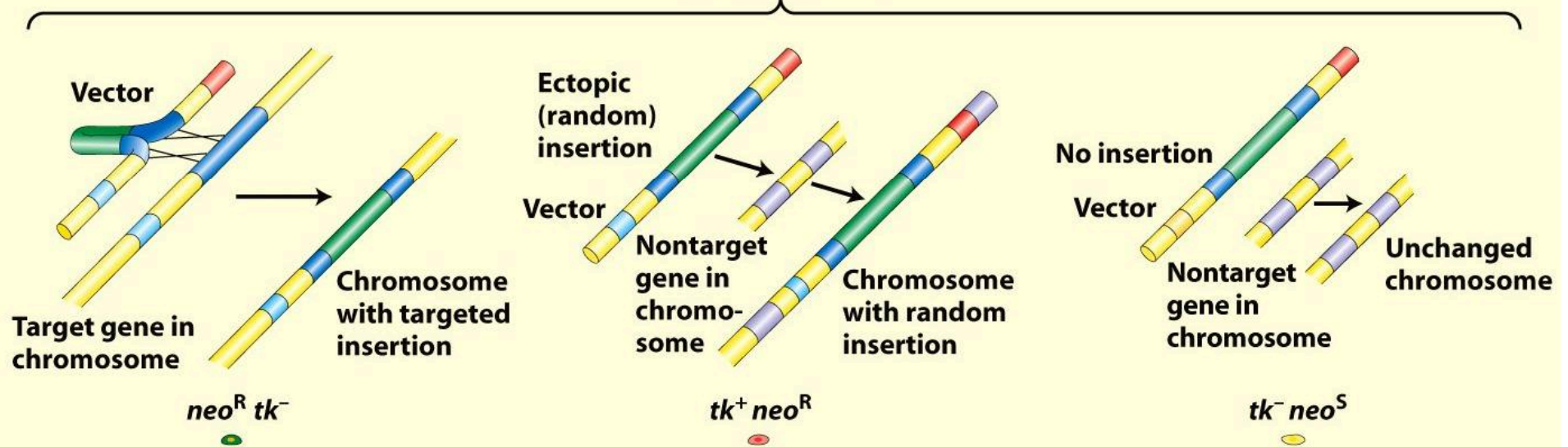
Producing cells containing a targeted gene knockout

Production of ES cells with a gene knockout



Targeted insertion of vector DNA by homologous recombination

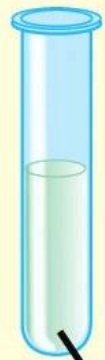
Possible outcomes



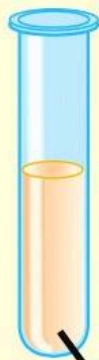
Selection of cells with gene knockout

Neomycin
analog

Ganciclovir



Kills neo^S cells



Kills tk^+ cells

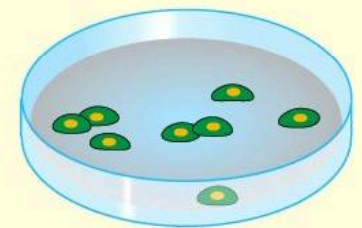
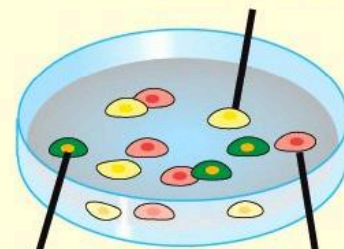
Add to medium.

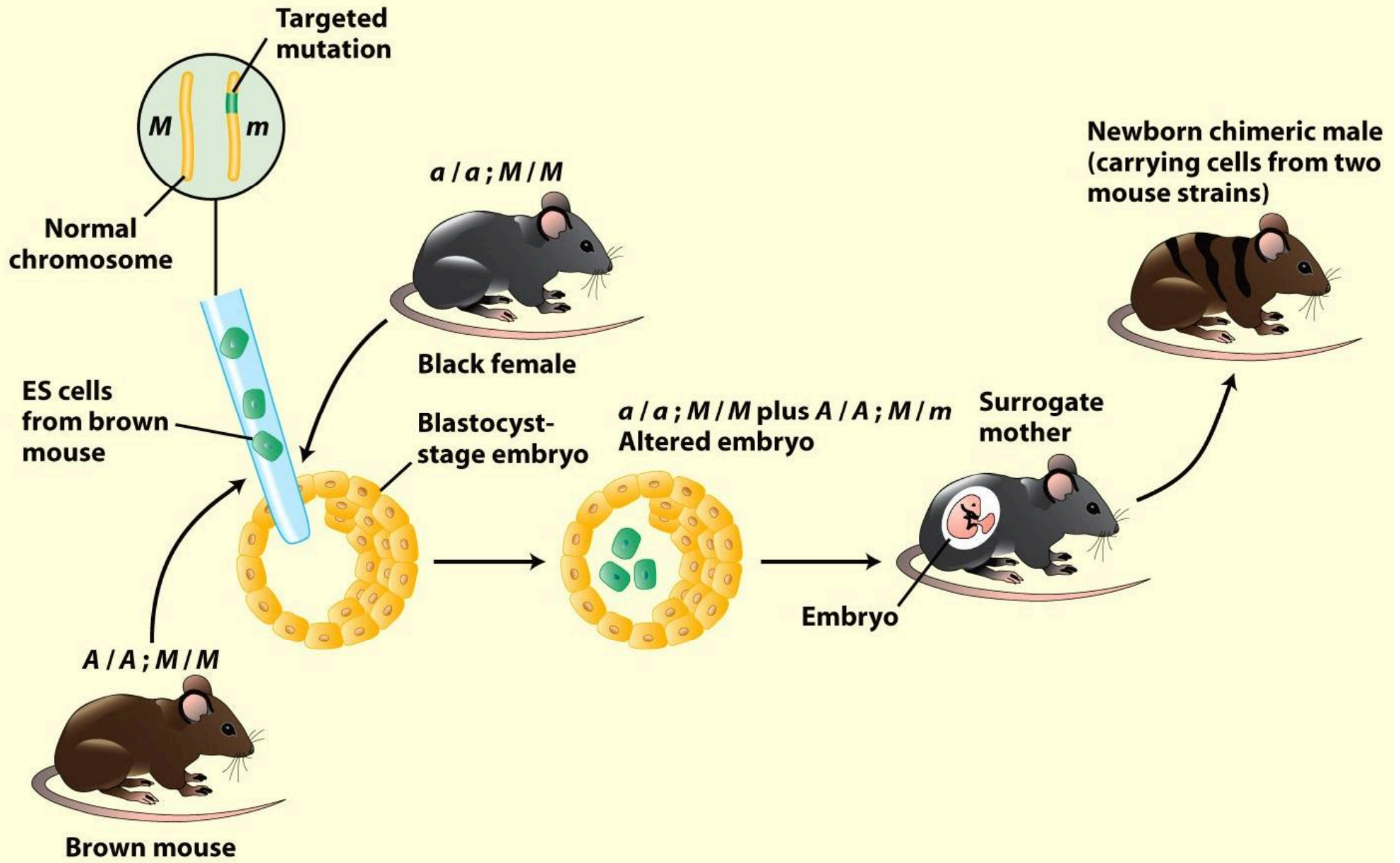
Cell with no
insertion

Cell with
targeted
insertion

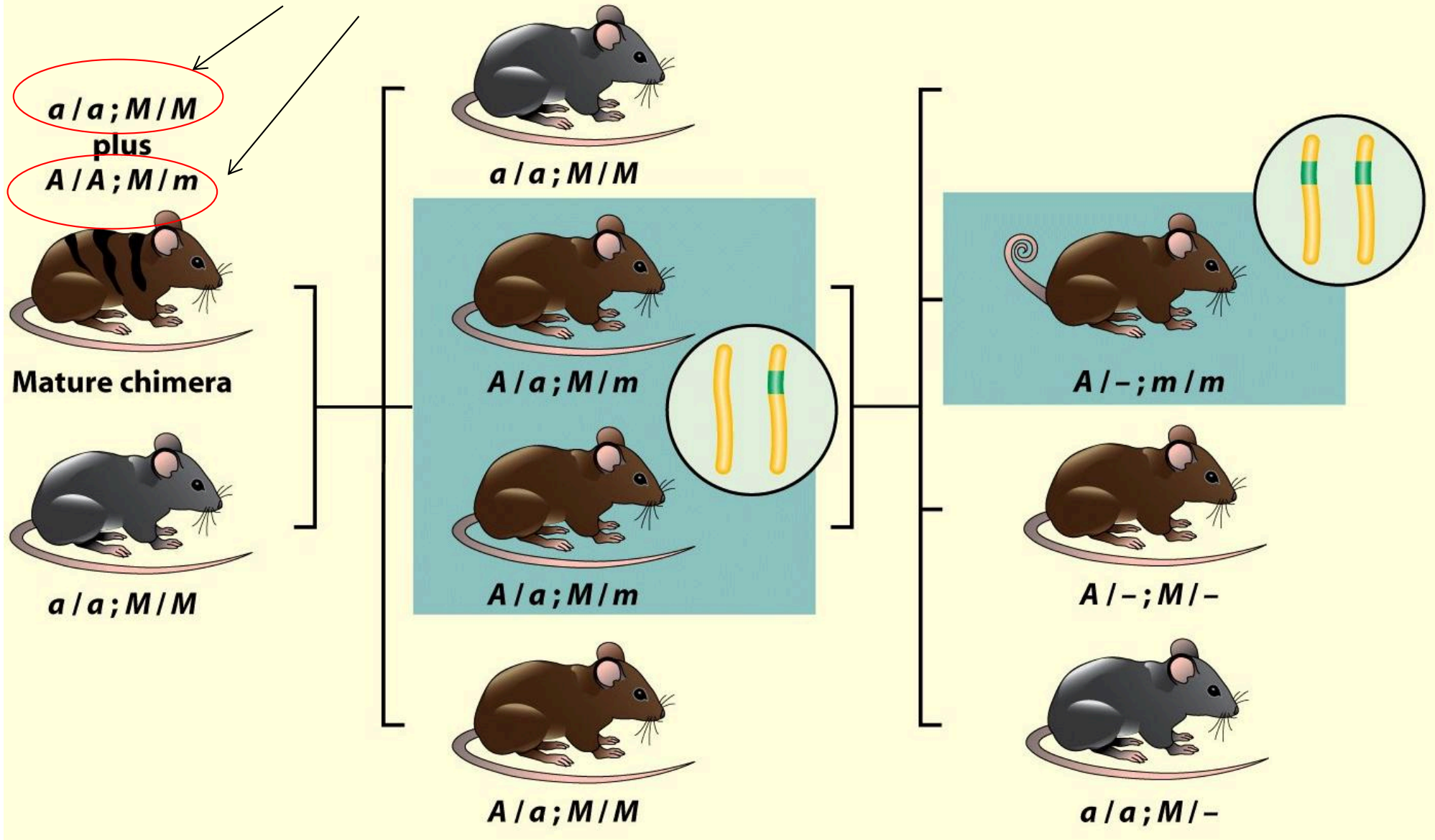
Cell with
random
insertion

Cells carrying
targeted mutation



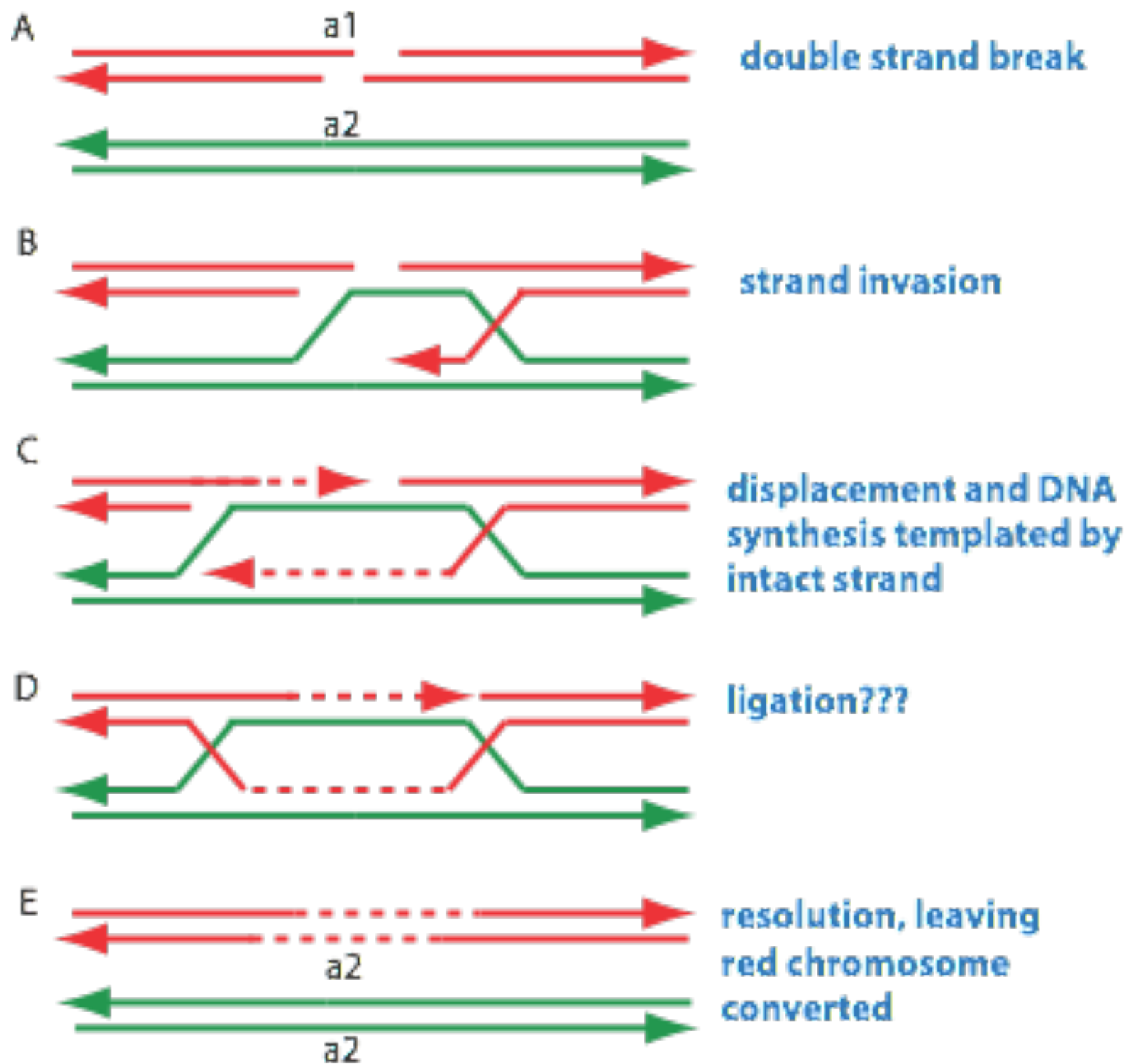


Germ cells



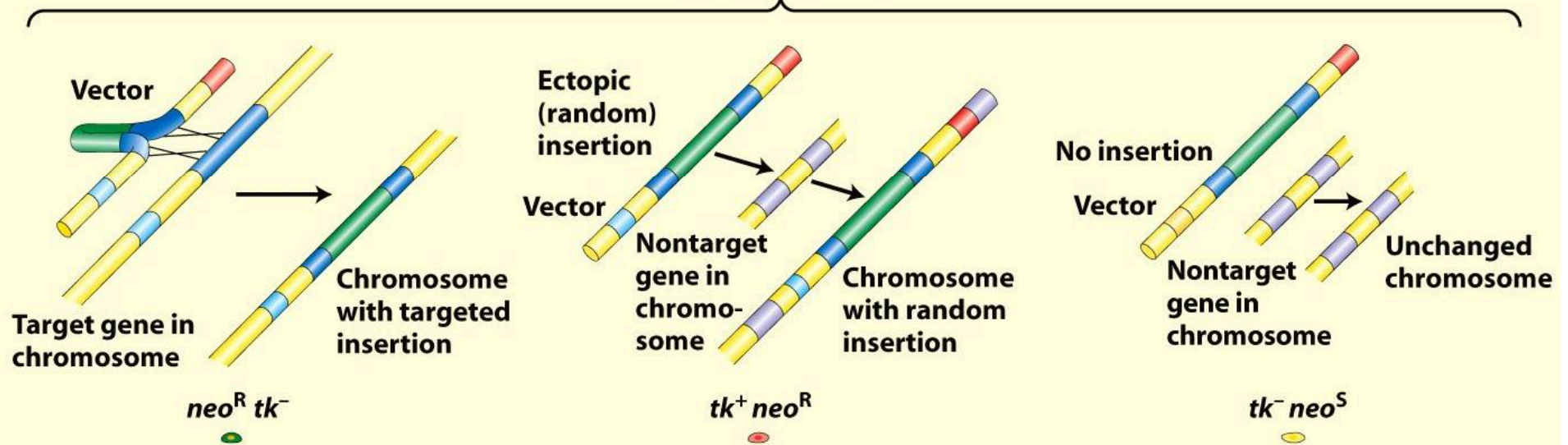
What drives the choice of homologous integration versus random integration?

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



Targeted insertion of vector DNA by homologous recombination

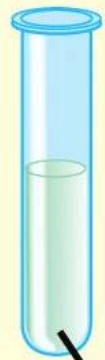
Possible outcomes



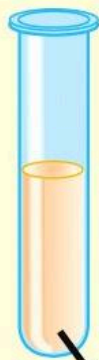
Selection of cells with gene knockout

Neomycin
analog

Ganciclovir



Kills neo^S cells



Kills tk^+ cells

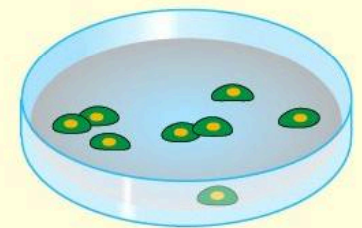
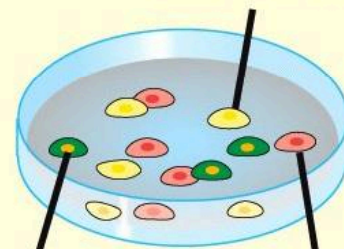
Add to medium.

Cell with no
insertion

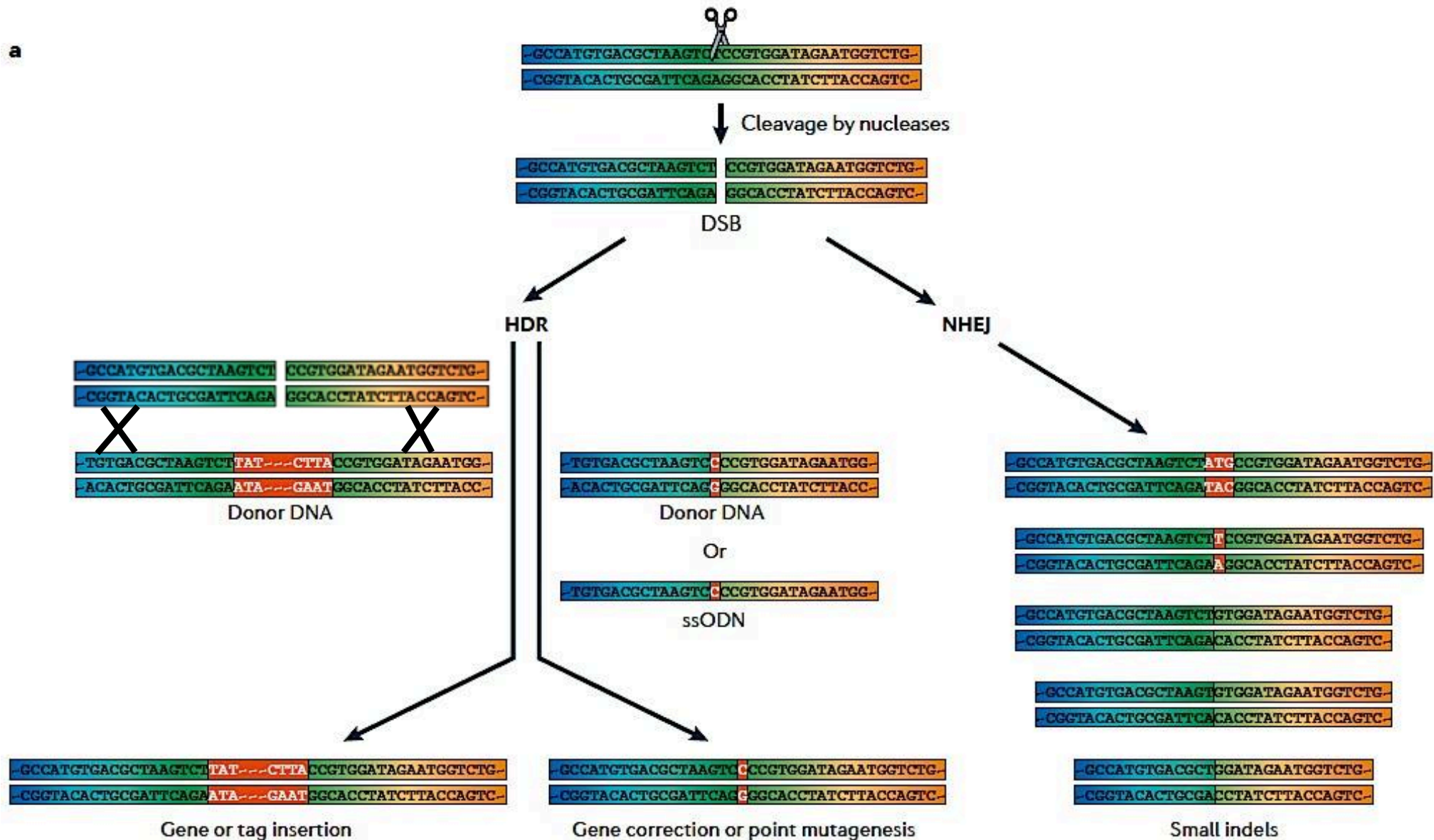
Cell with
targeted
insertion

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

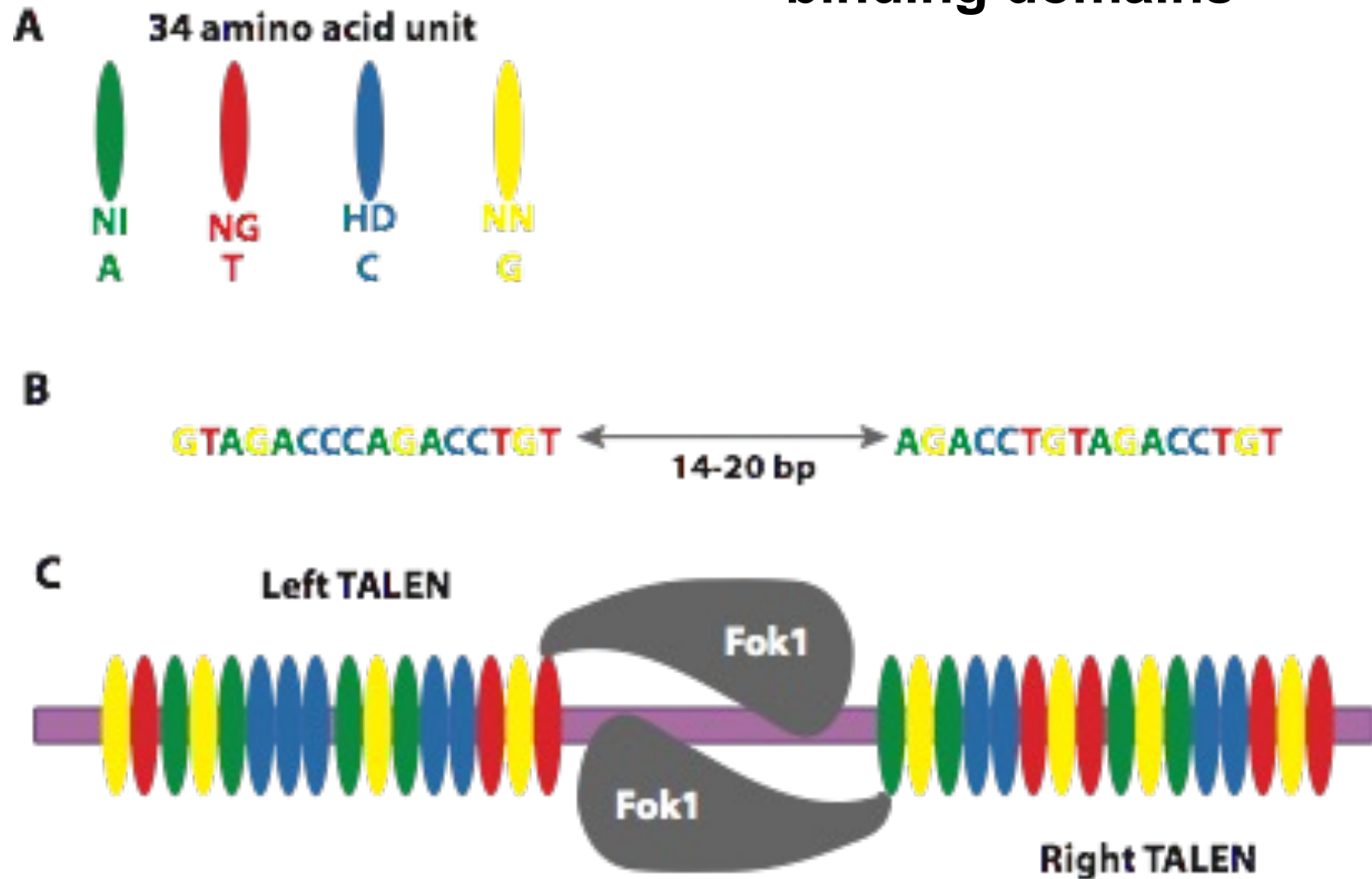


A breakthrough in genome engineering (called **genome editing**) uses engineered site-specific DNA binding proteins that have nuclease activity. The key steps are:

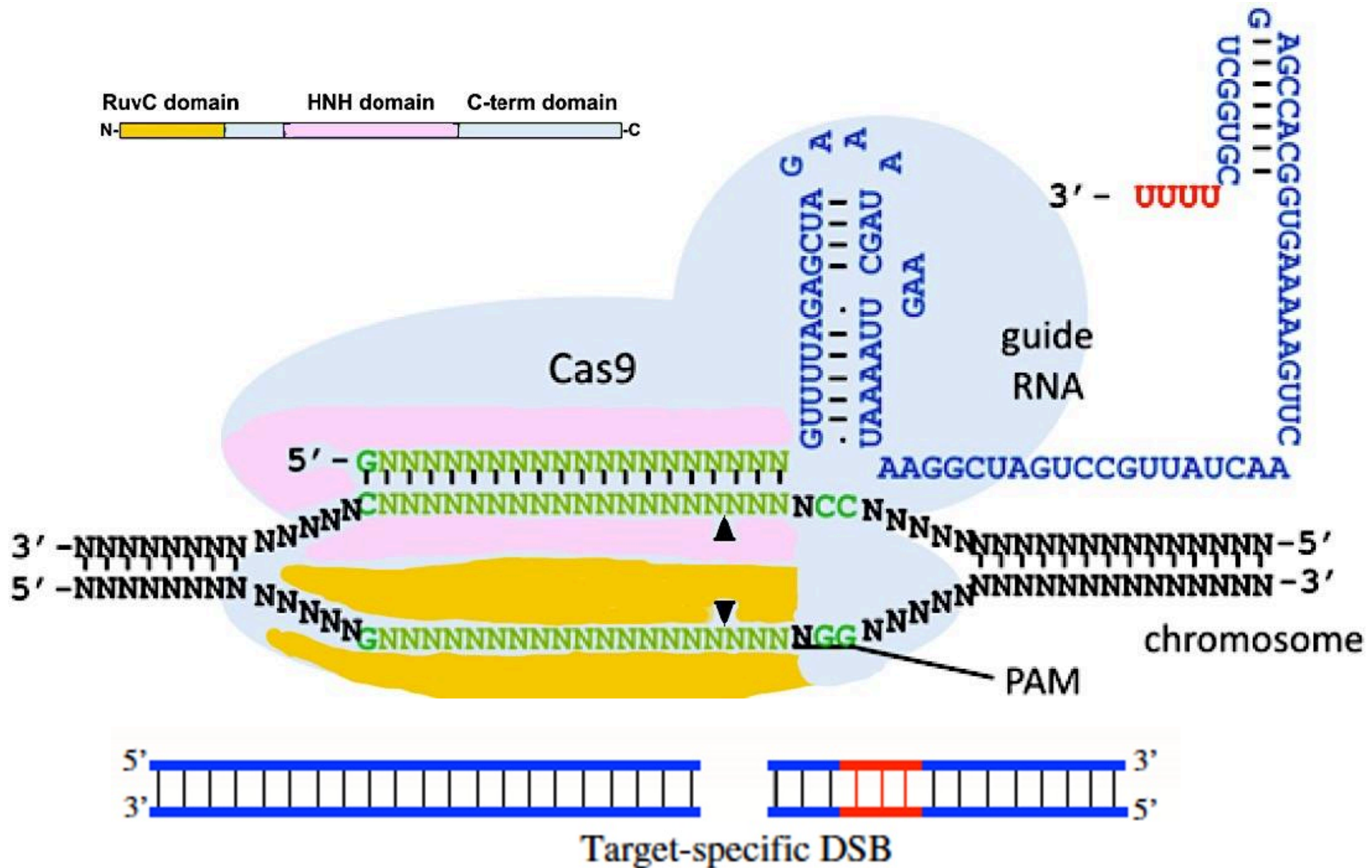
1. introduction of a site-specific nuclease;
2. generation of a double-strand break;
3. repair of the break can introduce a mutation by
 - a. non-homologous end joining, which is error-prone (for example, DNA is chewed back by endonucleases.
 - b. Homology directed repair converts the allele that has been cut.

TALENs

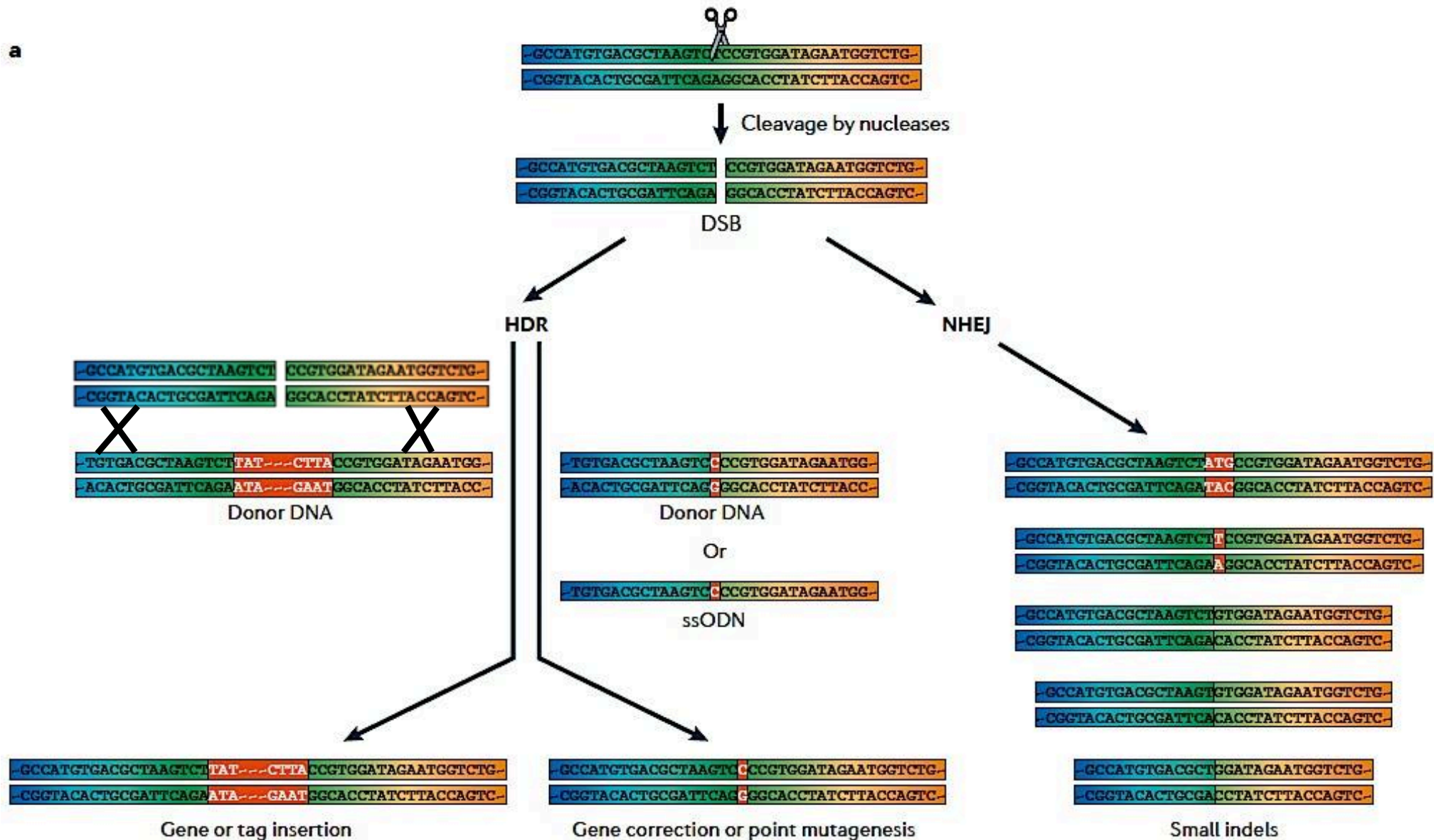
or Zinc Finger DNA
binding domains



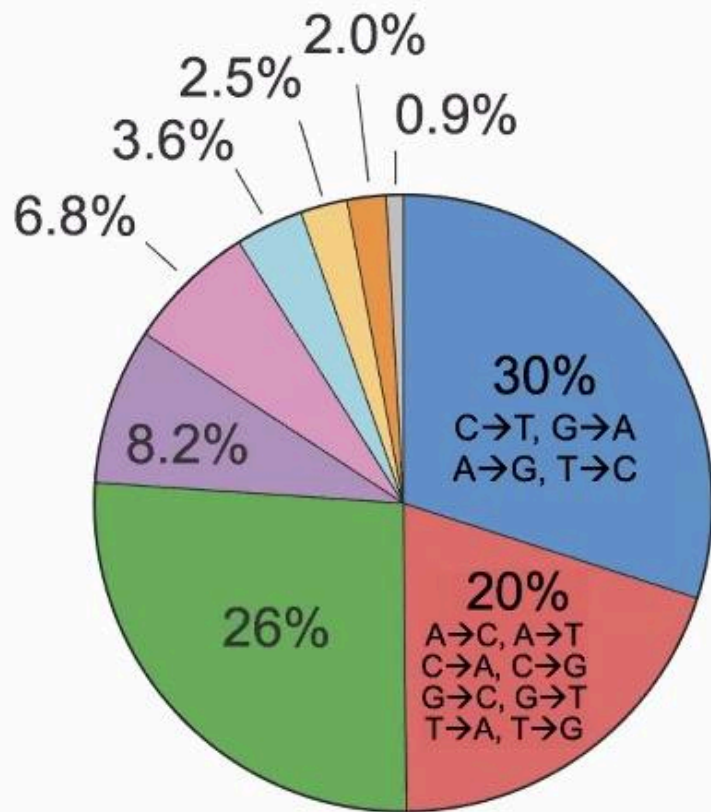
Programmable DNA cleavage using Cas9 and one or more guide RNAs



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



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

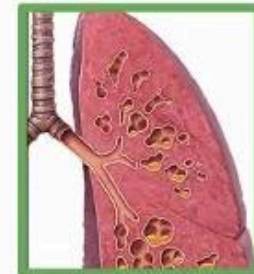
LMNA c.1824C→T



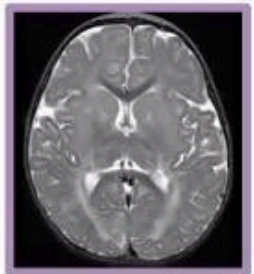
HBB E6V(A→T)



CFTR ΔF508

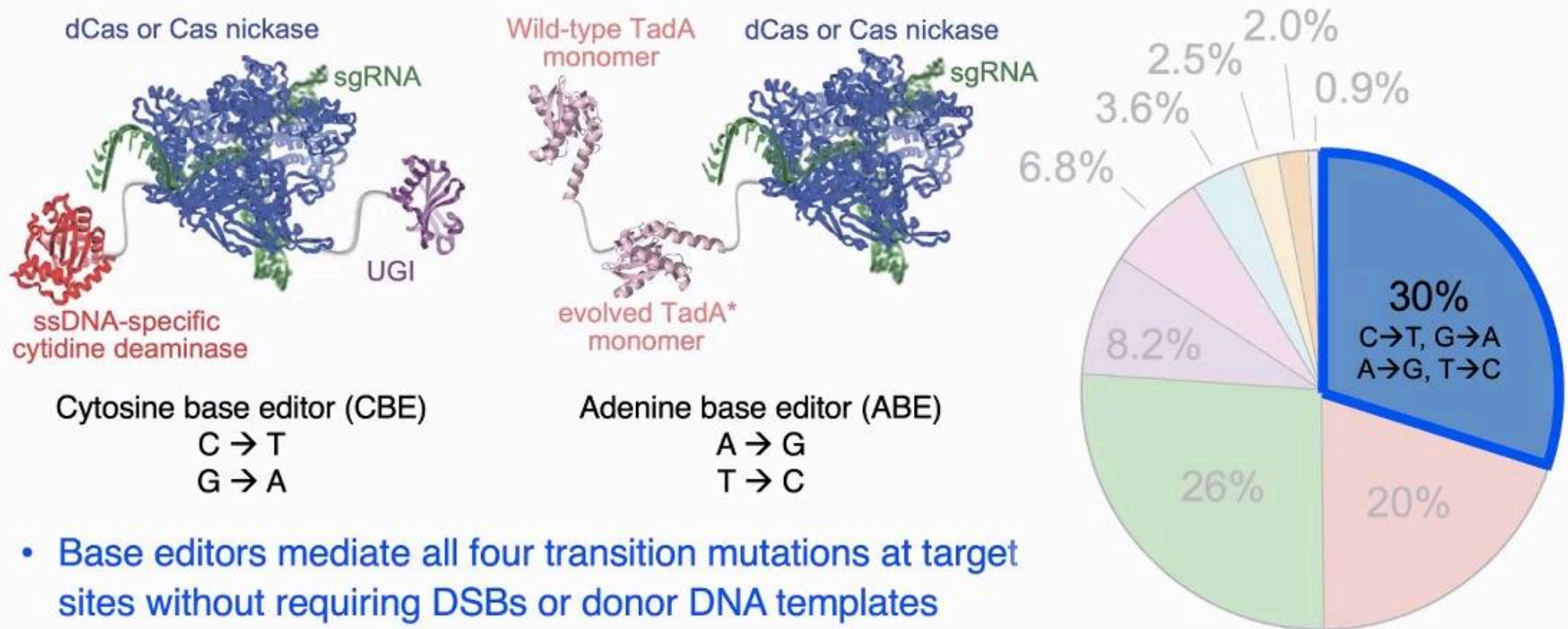


HEXA c.1278+TATC

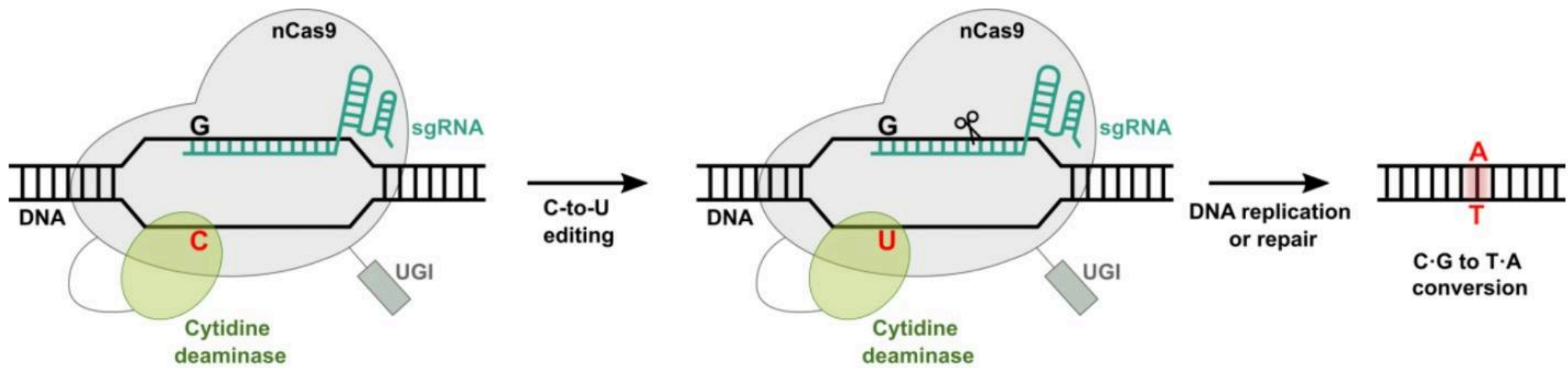


- Programmable nucleases (DSBs → indels or HDR) are well-suited to install or correct only a minority of pathogenic alleles in most cell types

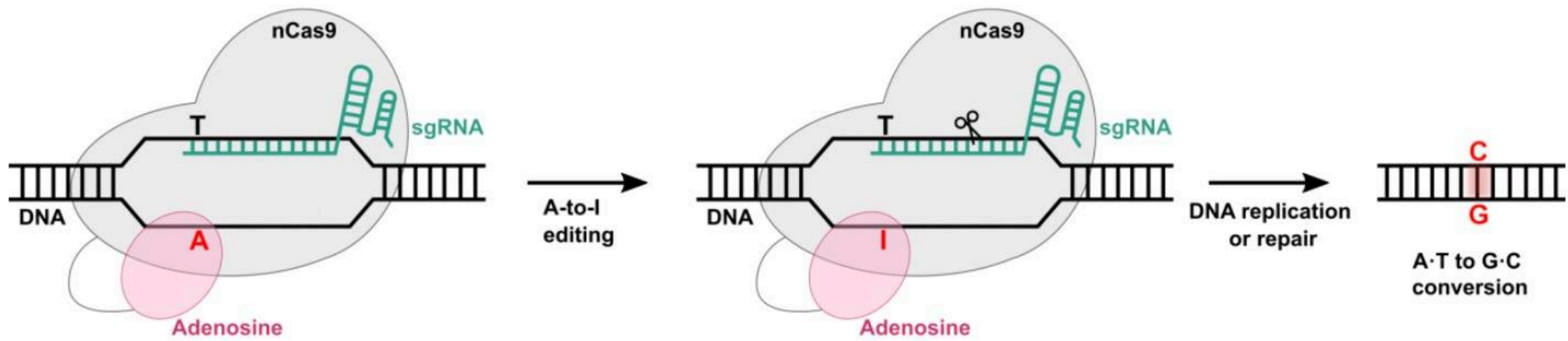
Base Editors Make Targeted Transition Point Mutations



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)



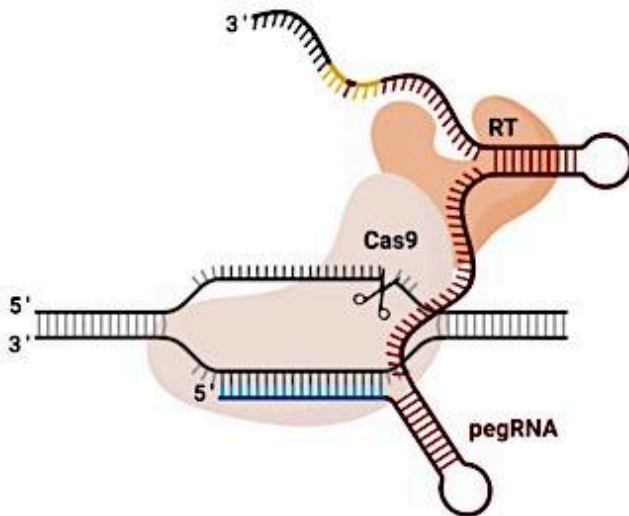
Cytosine Base Editor



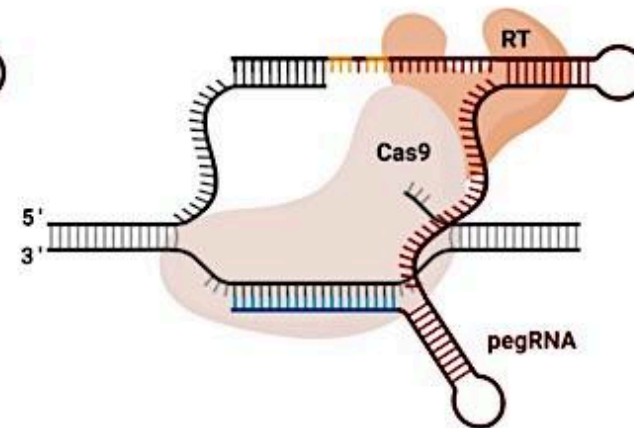
A·T to G·C conversion

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

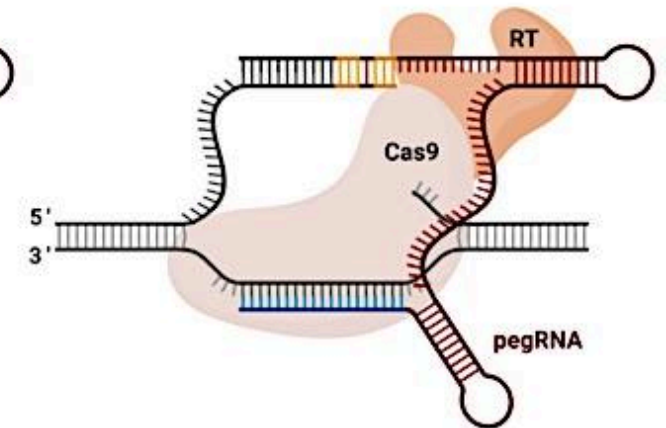
1 Nicking of PAM strand



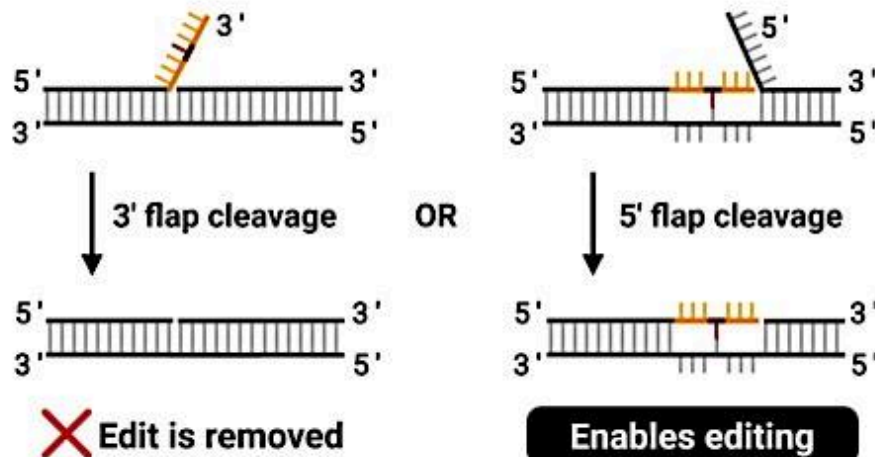
2 Hybridization of primer-binding site to PAM strand



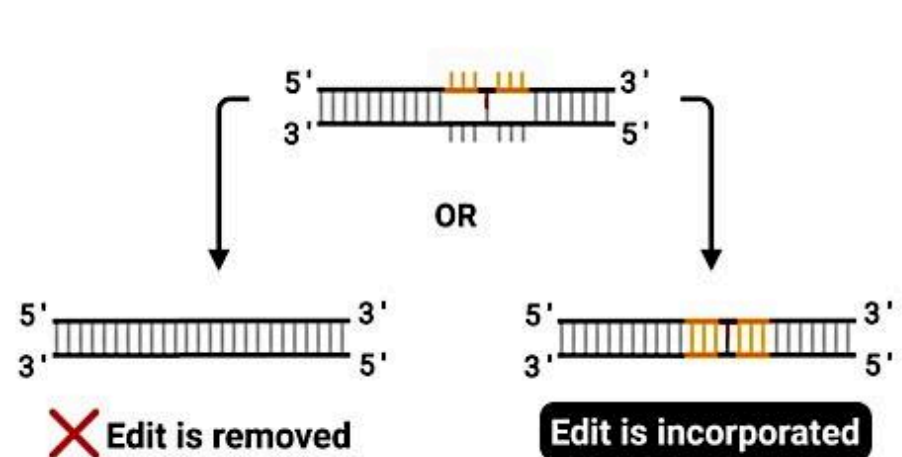
3 Reverse transcription



4 Hybridization of DNA strands and flap cleavage



5 Ligation and mismatch repair



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.

Examples of some types of chromosomal arrangements are :

1. Inversions
2. Deficiencies or deletions
3. Duplications
4. Translocations
5. Changes in number chromosome number, or ploidy.

These changes are important for a number of reasons.

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.

Conception to ongoing pregnancy: the ‘black box’ of early pregnancy loss

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Even when conditions are optimal, the maximum chance of a clinically recognized pregnancy occurring in a given menstrual cycle is 30–40%. Increasing evidence points to preclinical pregnancy loss rather than failure of conception as the principal cause for the relatively low fecundity observed in humans. While sensitive assays for hCG have provided a glimpse of the events occurring between implantation and the missed menstrual period, new cytogenetic techniques have further opened this ‘black box’, providing novel insights into the causes of early pregnancy wastage. In this article, the evidence and causes of preclinical or ‘occult’ pregnancy are reviewed, and the implications for the infertile patient are addressed.

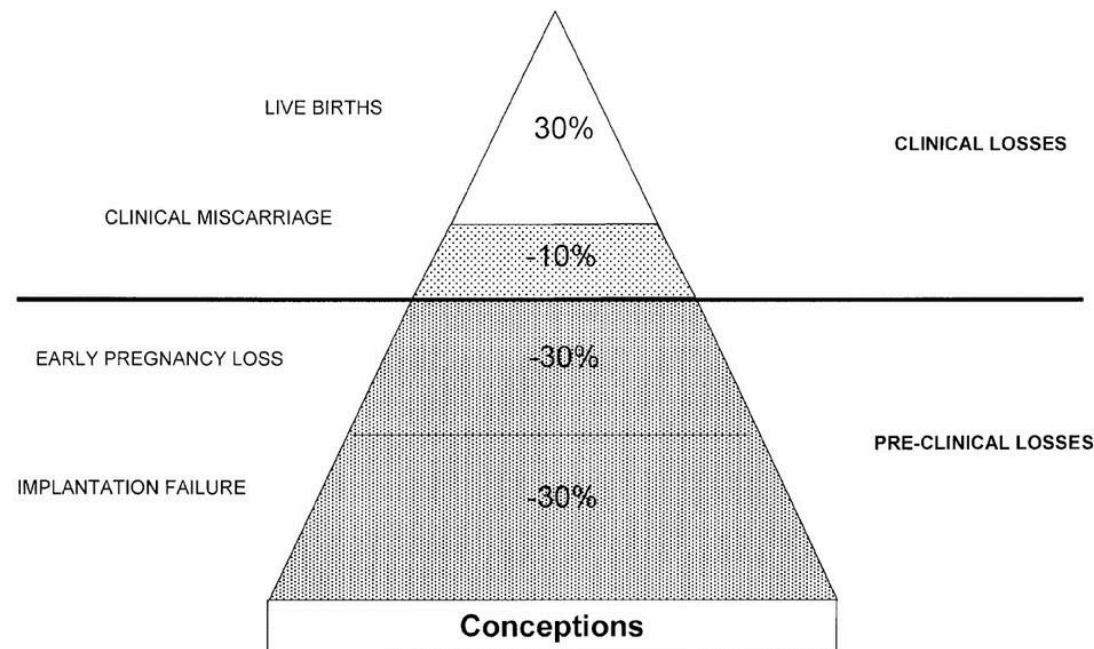


Figure 1. The Pregnancy Loss Iceberg: an overview of the outcome of spontaneous human pregnancy. A total of 70% of conceptions are lost prior to live birth. The majority of these losses occur prior to the time of the missed menstrual period, and are not revealed. (Adapted from Chard, 1991.)



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



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

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.

Aneuploidy and Confined Chromosomal Mosaicism in the Developing Human Brain

Yuri B. Yurov^{1,2,3*}, Ivan Y. Iourov^{1,2,3}, Svetlana G. Vorsanova^{1,2,3}, Thomas Liehr³, Alexei D. Kolotii², Sergei I. Kutsev⁴, Franck Pellestor⁵, Alfia K. Beresheva^{1,2}, Irina A. Demidova^{1,2}, Viktor S. Kravets², Viktor V. Monakhov¹, Ilia V. Soloviev¹

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, intercellular/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

NEUROSCIENCE

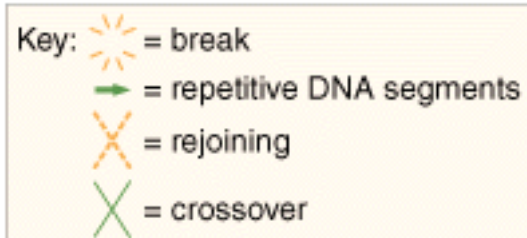
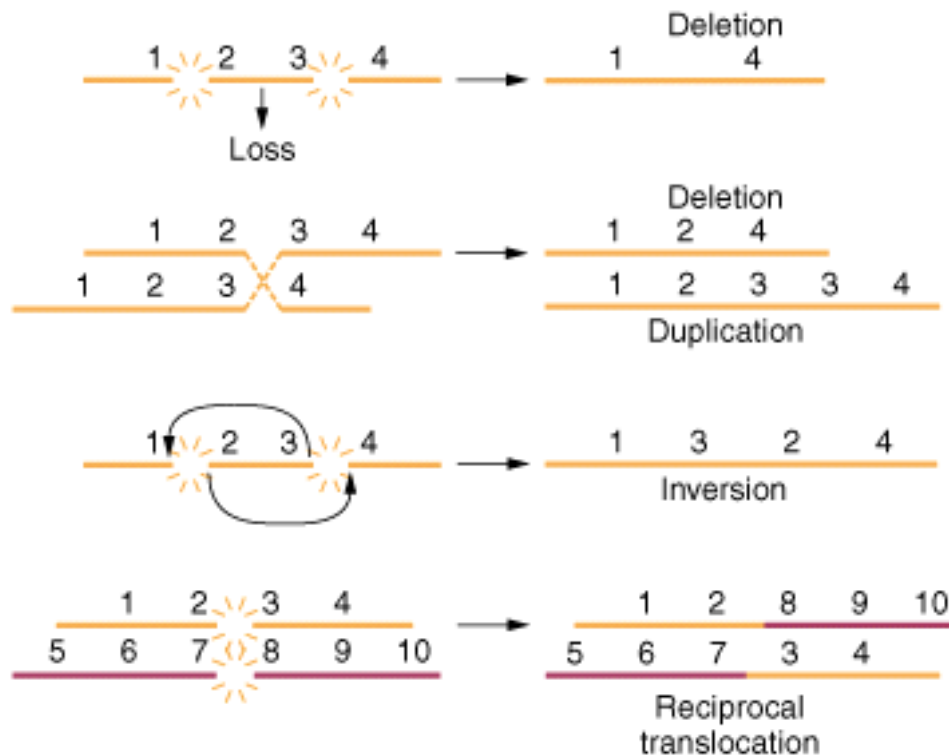
Scientists Surprised to Find No Two Neurons Are Genetically Alike

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

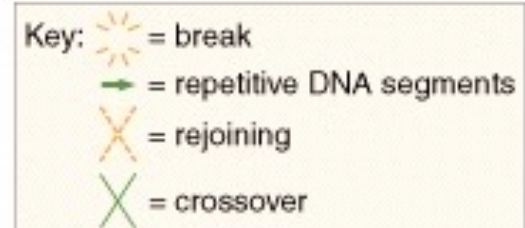
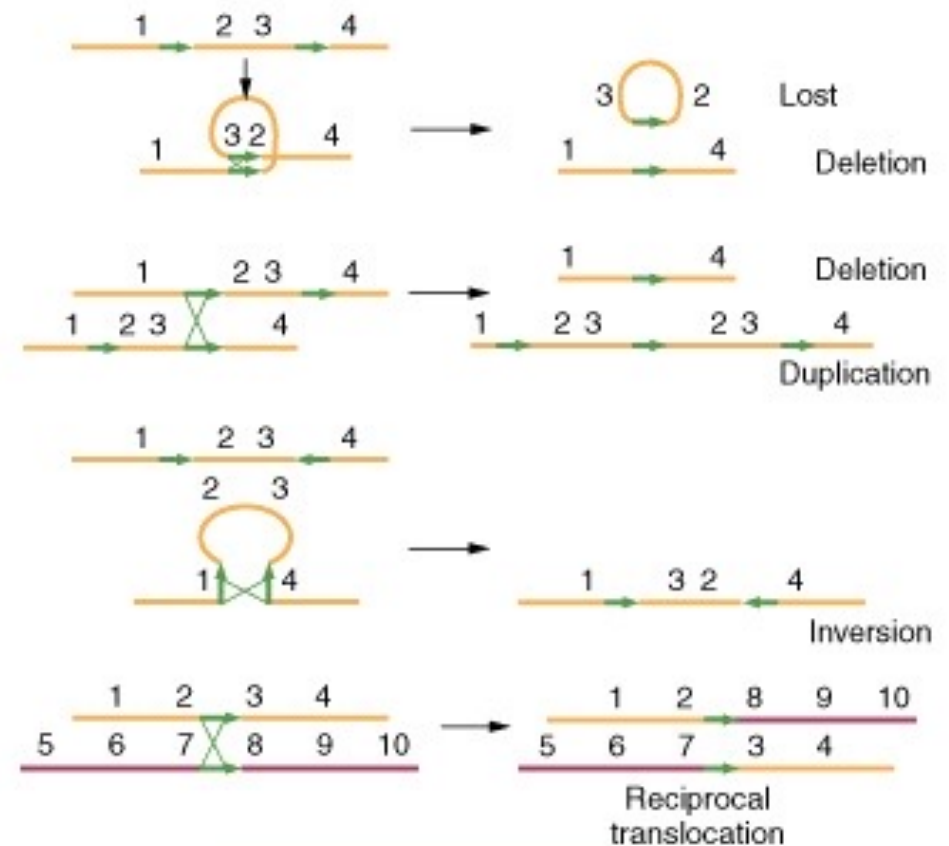
By Simon Makin on May 3, 2017

Chromosome rearrangements: how do they happen?

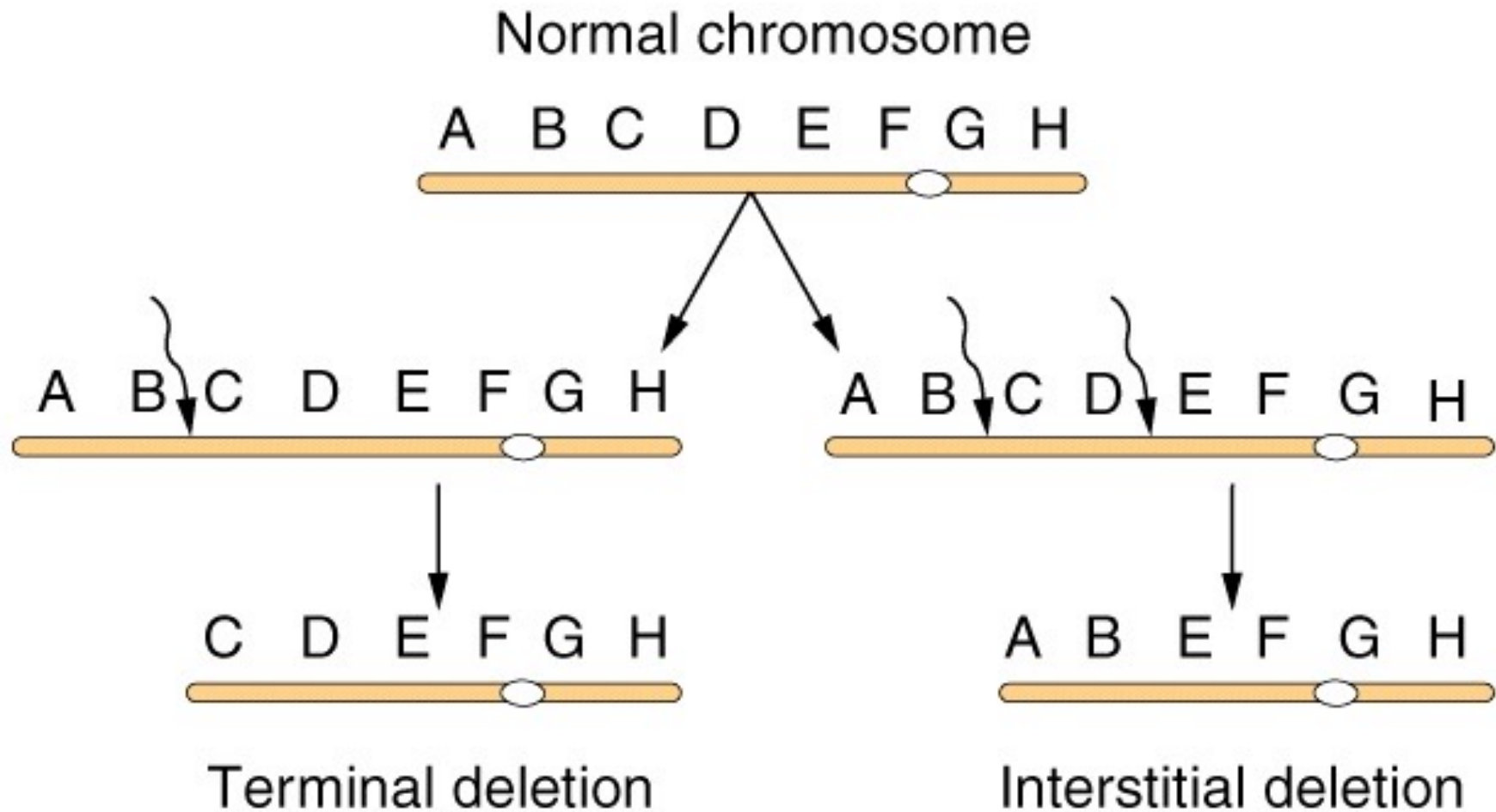
(a) By breakage and rejoining

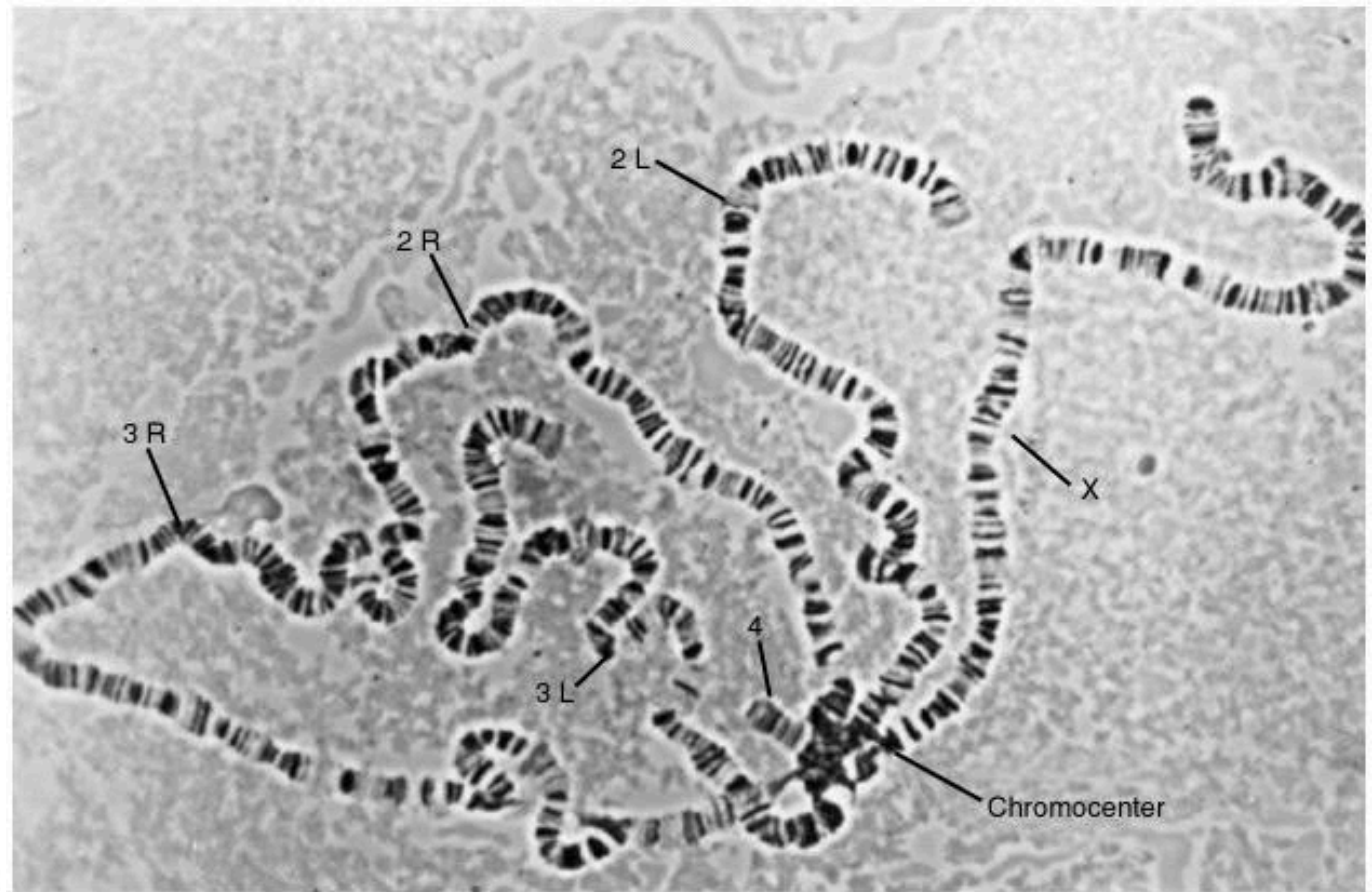
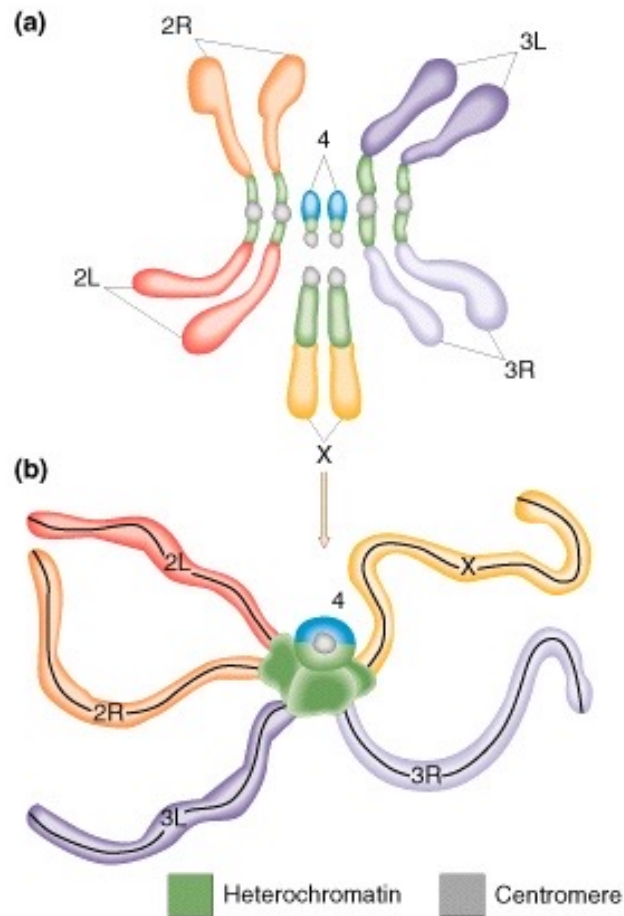


(b) By crossing-over between repetitive DNA



Deletions





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.

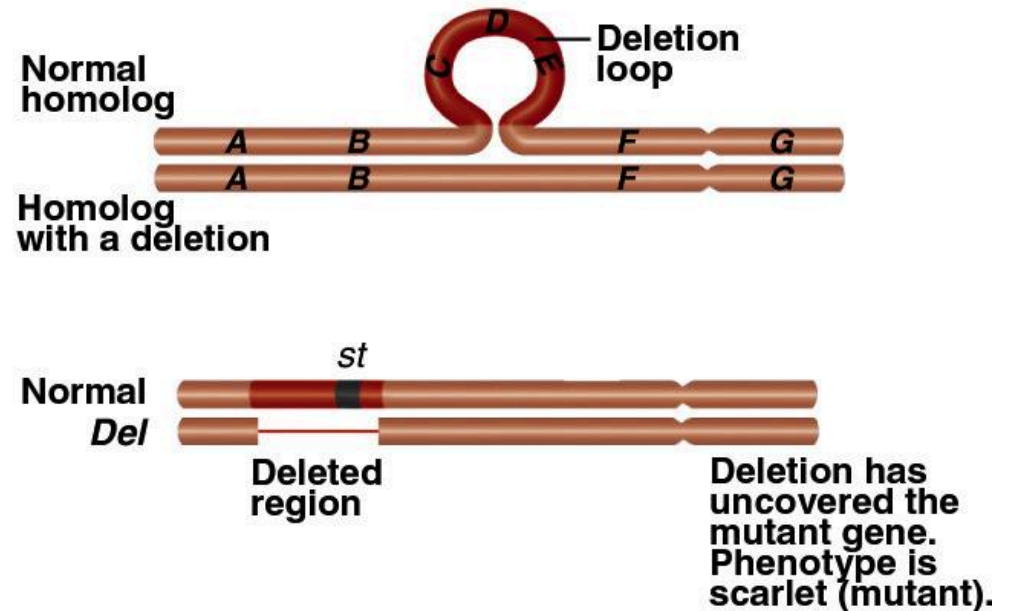
Deficiencies are most useful for gene mapping. By recording which bands are missing, and which genes are removed by making Df/mutant heterozygotes one can generate a map of the relative order, and rough position of genes on a chromosome.

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.

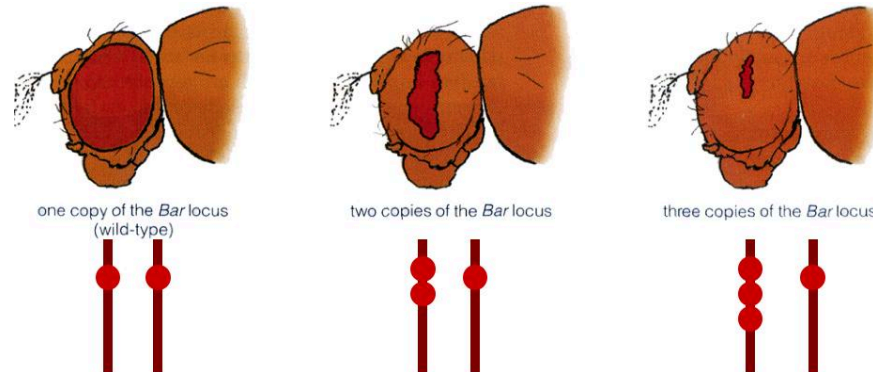
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Behavior of deletions



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 $\frac{B}{B} \times \frac{+}{Y}$

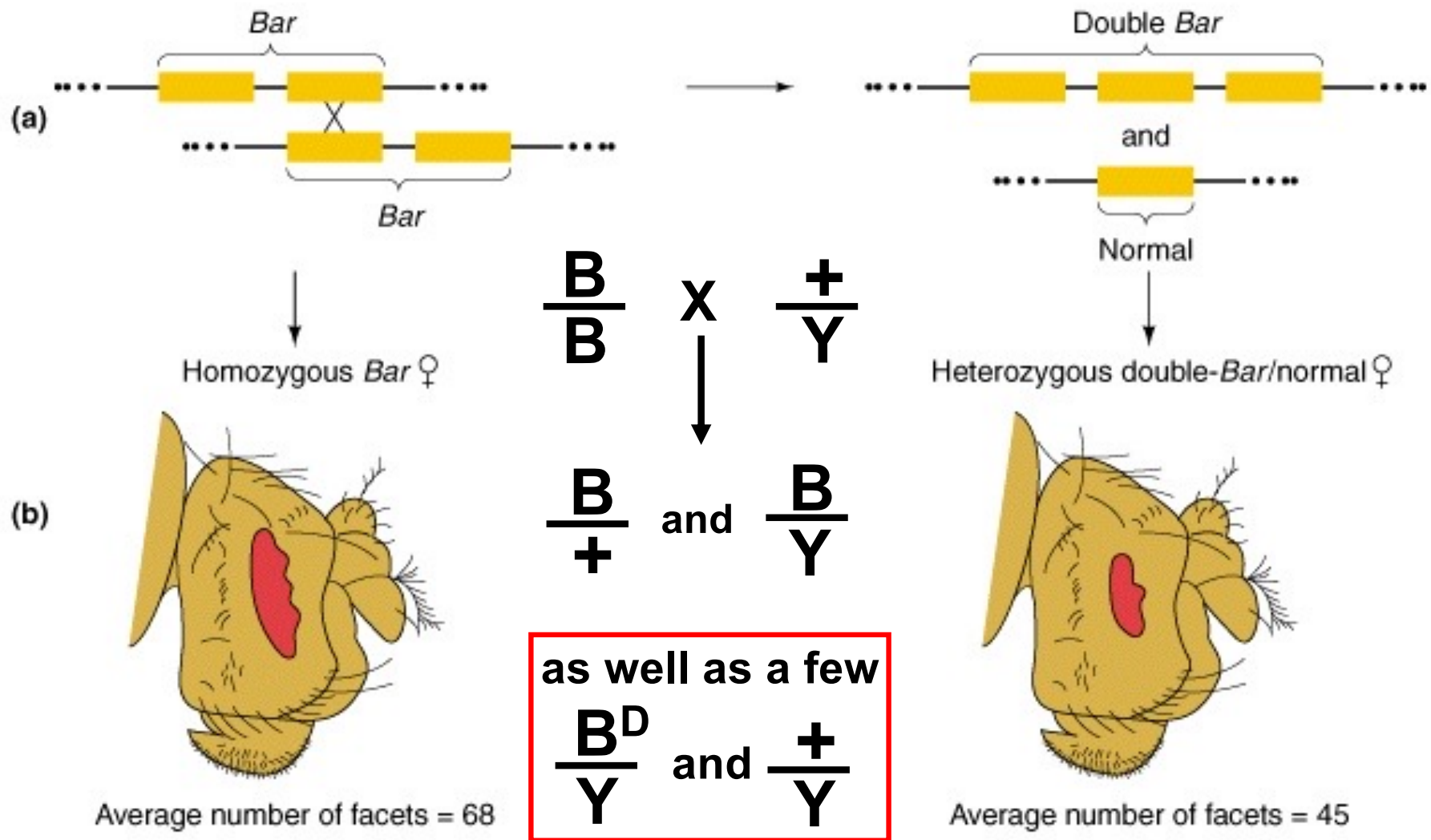
Among the $\frac{B}{+}$ and $\frac{B}{Y}$ progeny he observed

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.



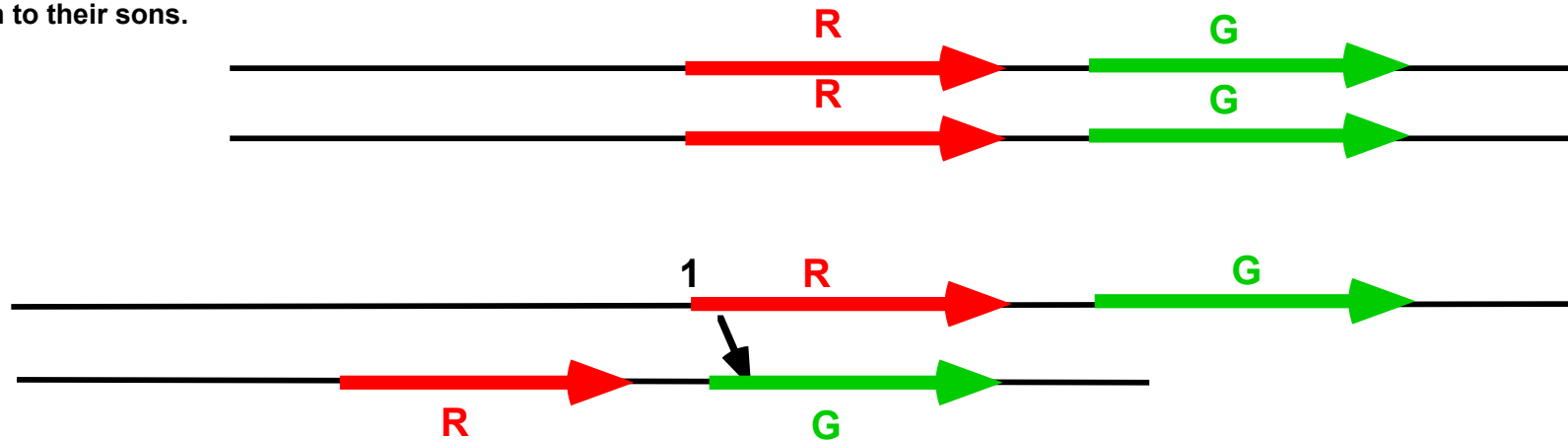
Red-green colorblindness

5% in males

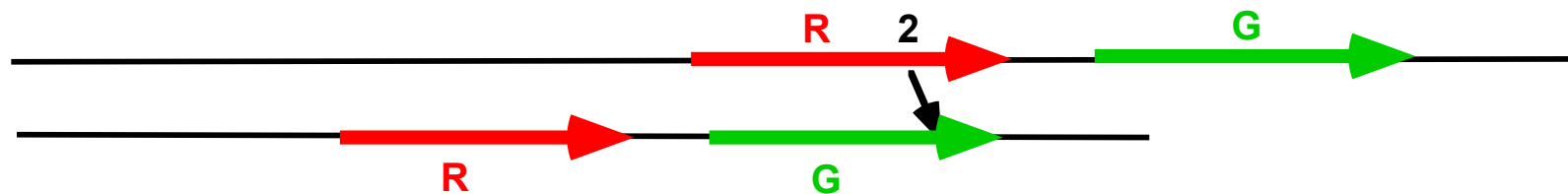
Red-green colorblindness offers an example of the role recombination between duplicated segments of DNA plays in shaping the human genome. Light is received in the retina by rhodopsins, light sensitive proteins. One class of rhodopsin is red sensitive, while a second is green sensitive. These genes are 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.



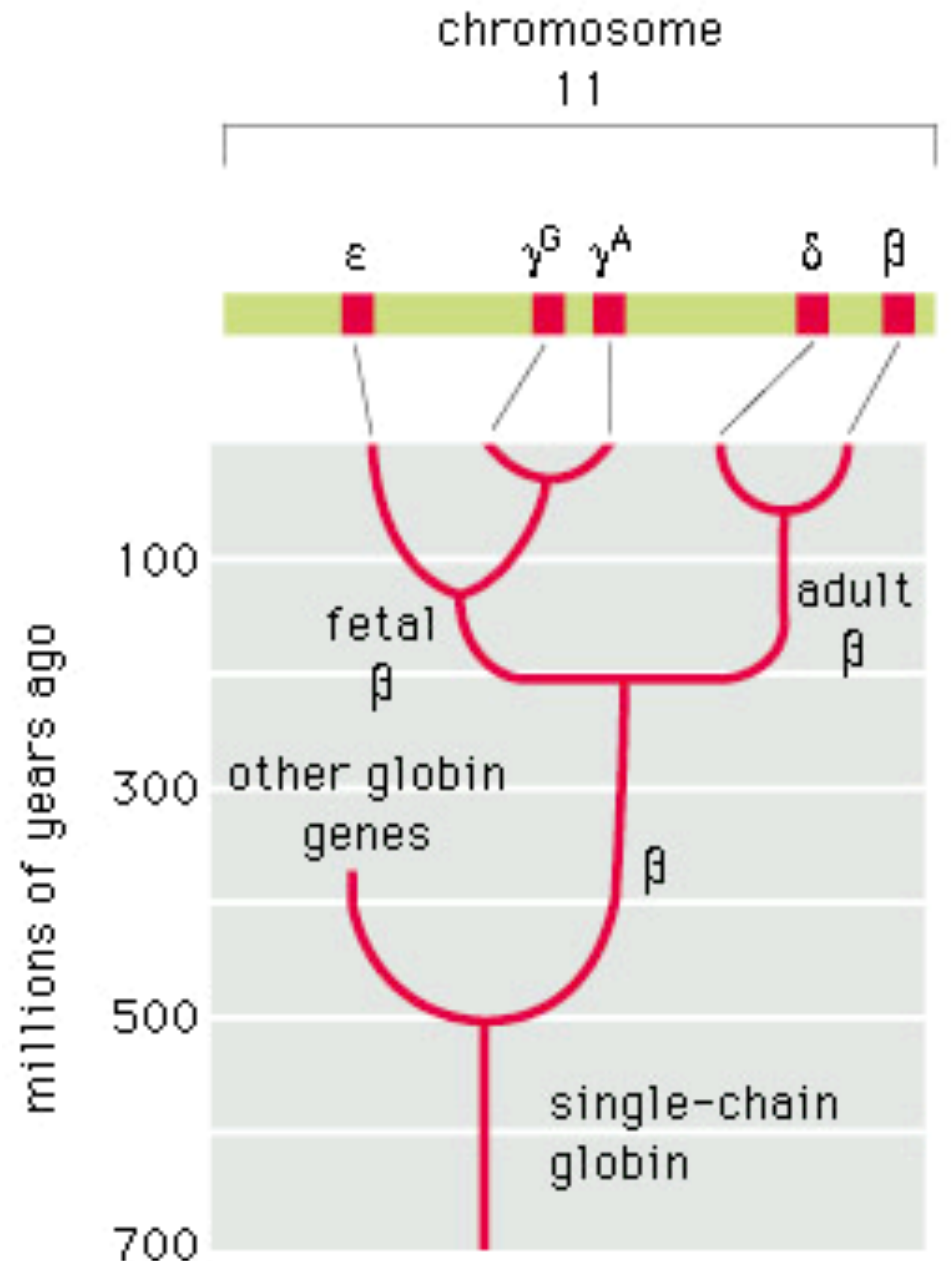
2. The rhodopsin pigment is most like the intact red-sensitive rhodopsin. Recombinant individuals cannot see green.

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.

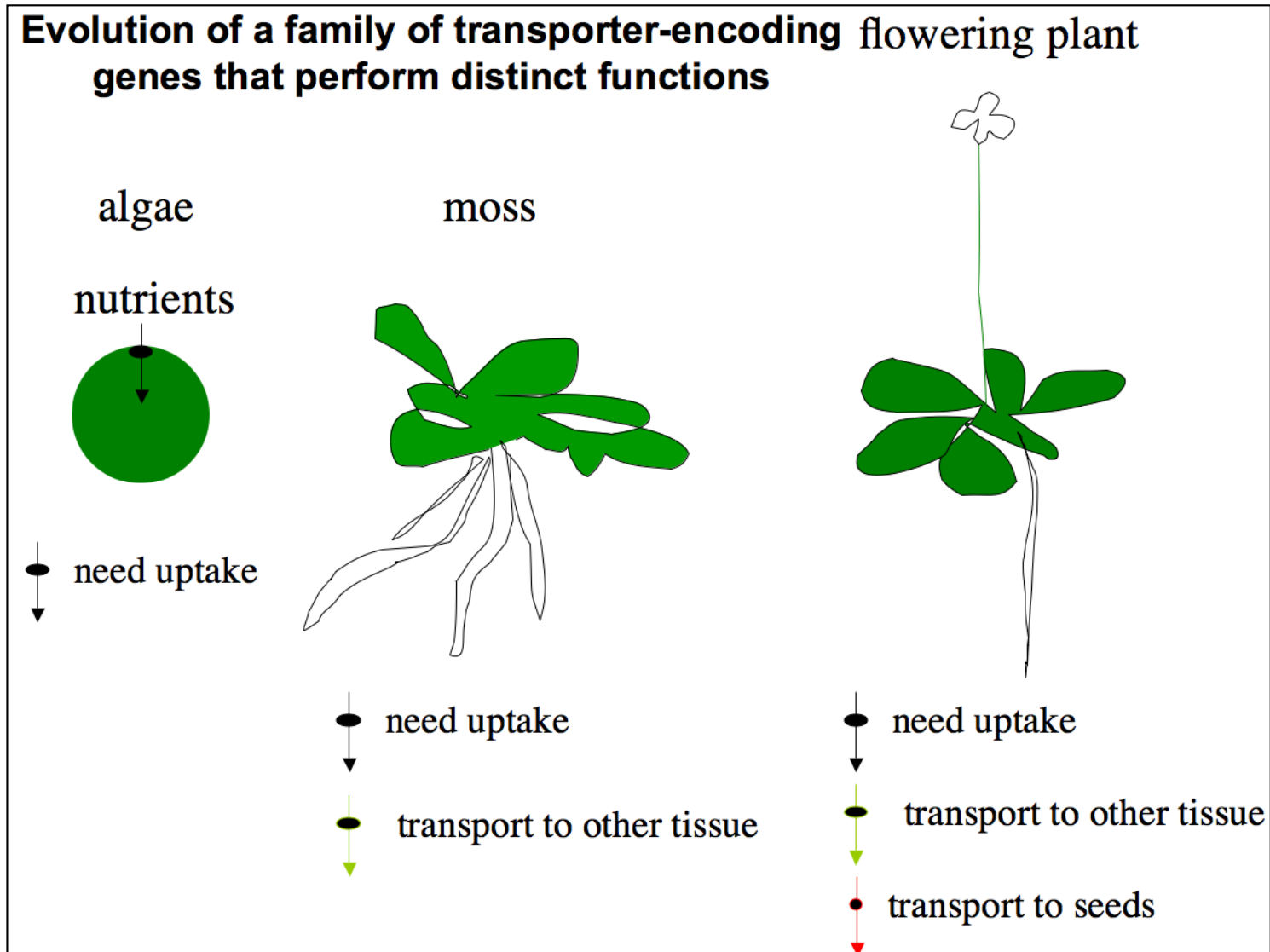
There are 5 beta-globin genes, each produced at a different time during embryonic, fetal, and adult development. Each with a different oxygen-binding and -releasing characteristic appropriate for the time it is expressed. And each under independent regulation.

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.

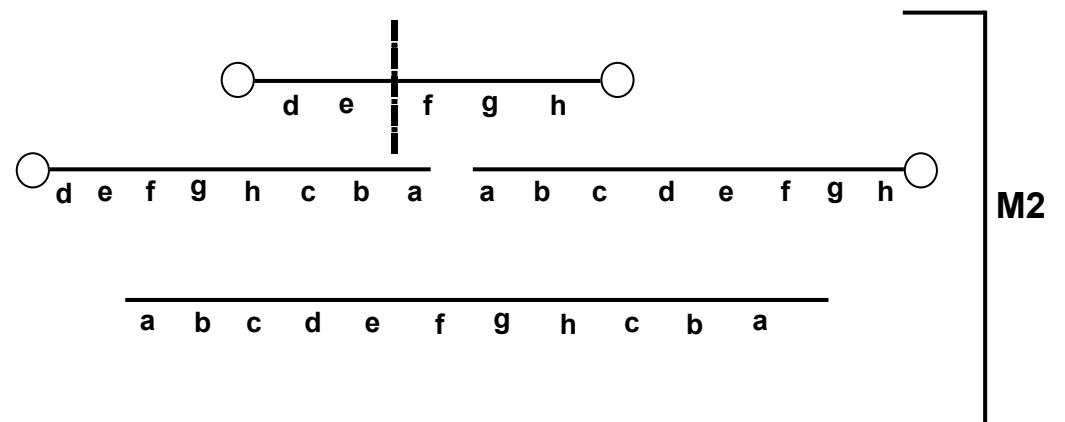
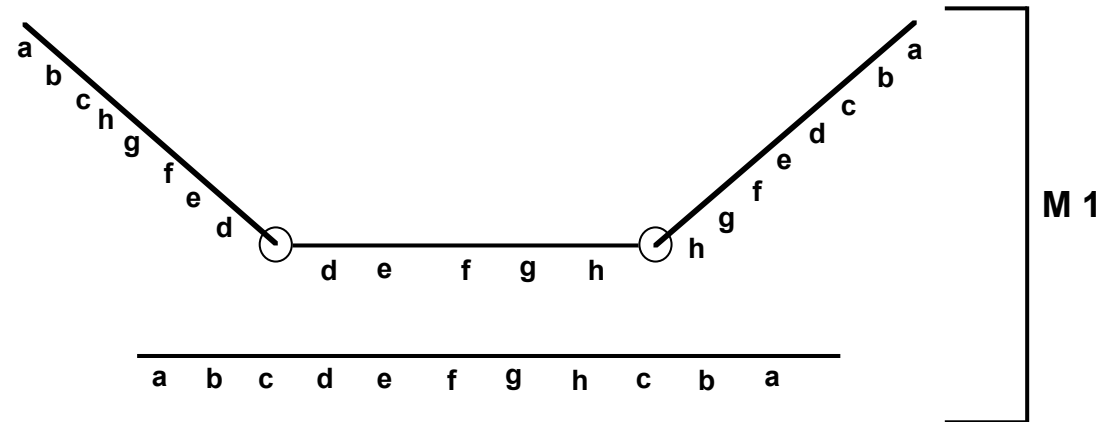
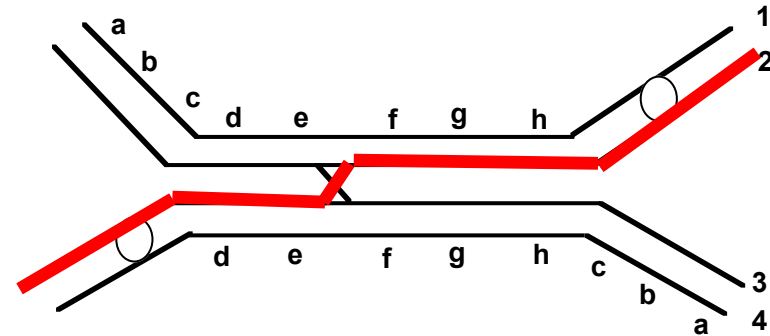
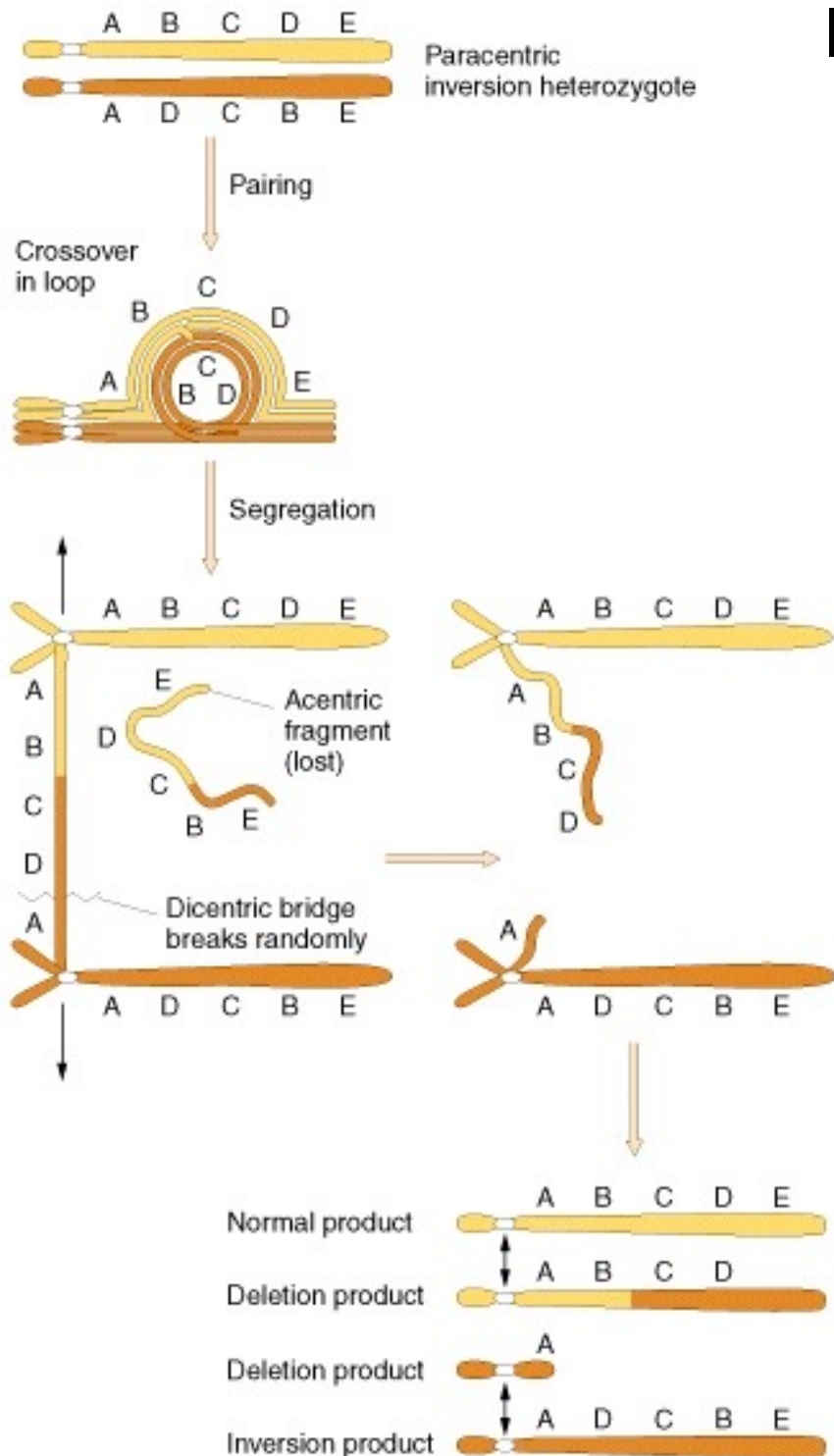


Duplication in Evolution

- ...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 an 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 contribute 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 kernels 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

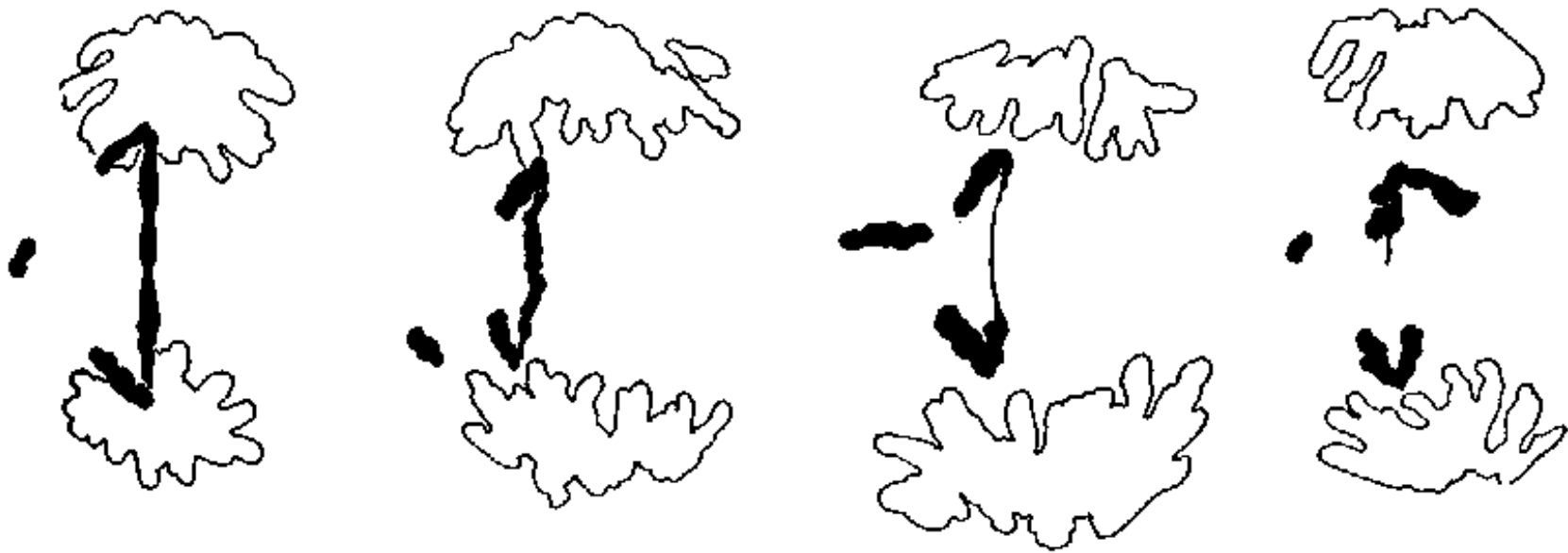
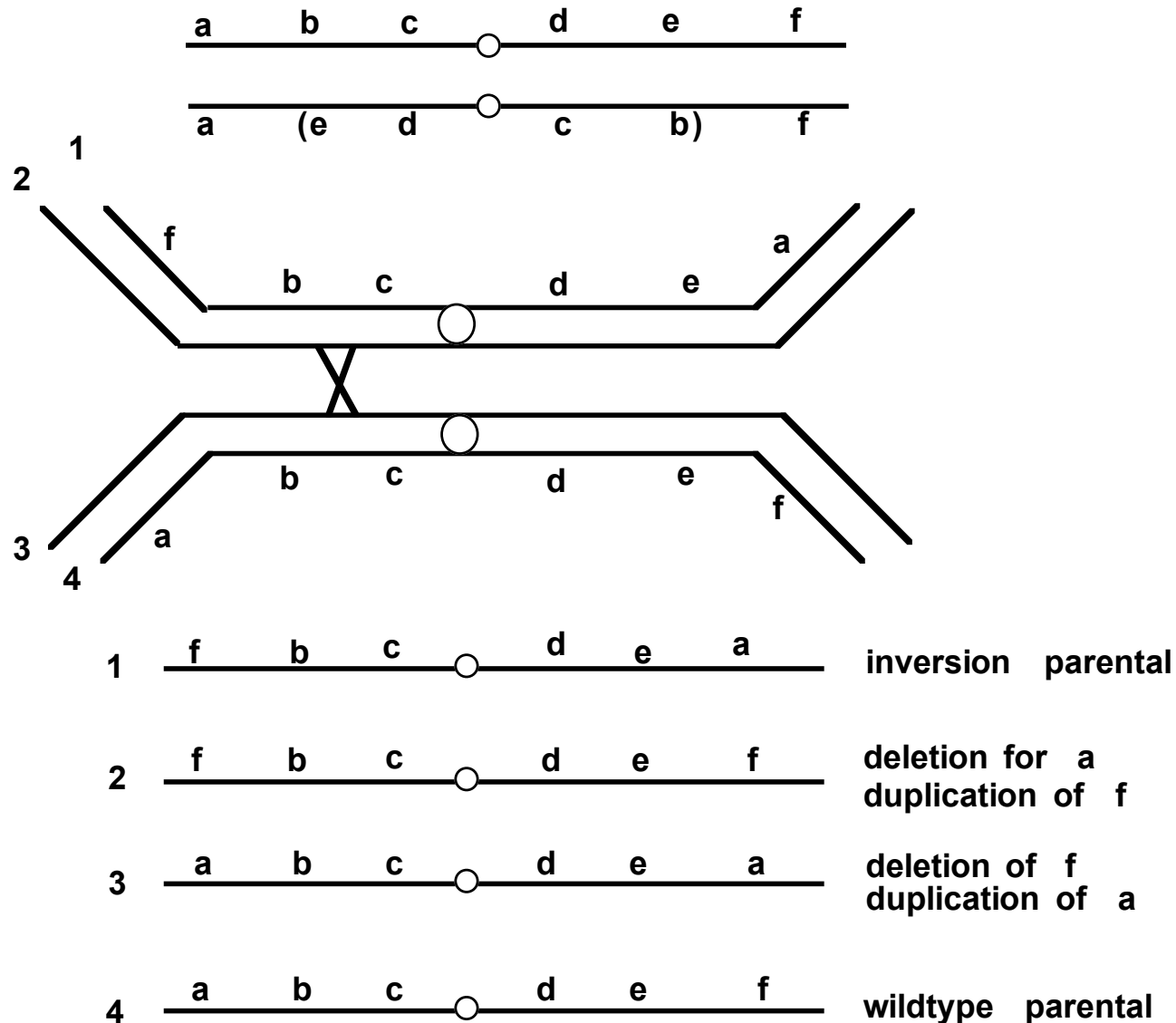


Fig. 51.—Telophase of first meiotic division in *Fritillaria* showing chromatin bridges and acentric fragments resulting from crossing over between segments inverted with respect to each other. The difference in the size of the fragments results from differences in positions of the different inversions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



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.

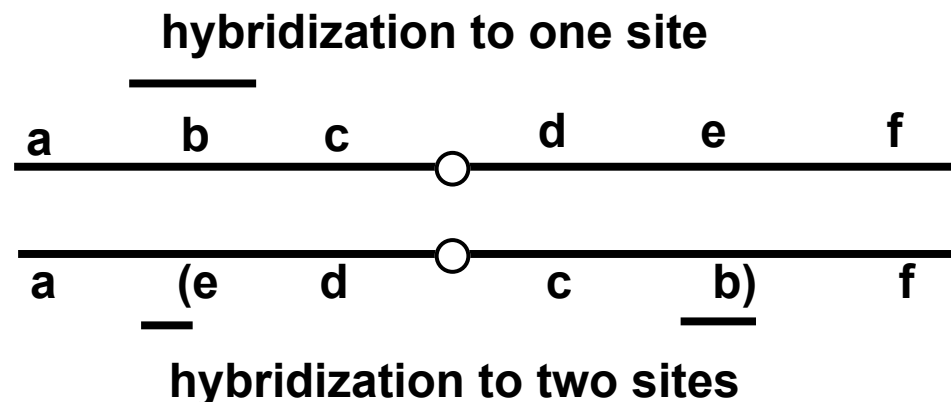
Band order and the presence of loops in polytene chromosomes.

Chromatin bridge fragments at anaphase of meiosis.

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

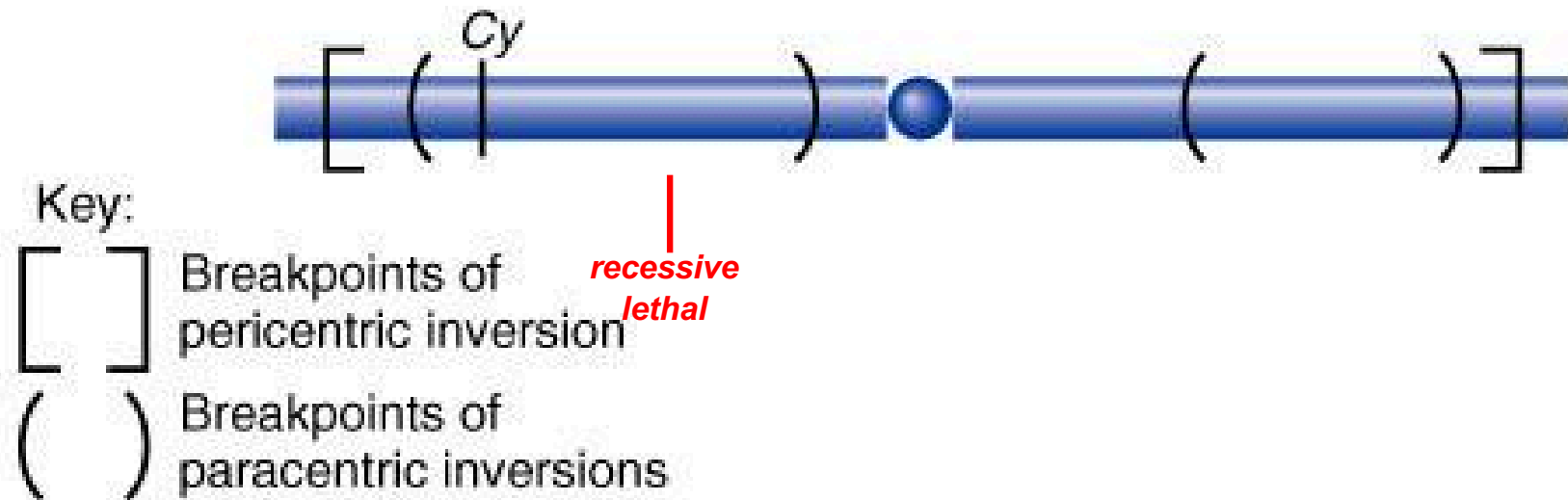
- *kni* is embryonic lethal
- What will happen to this cross:
 - *kni*-/+ X *kni*-/+
 - 1 *kni*-/*kni*- (all die)
 - 2 *kni*-/+ (viable)
 - 1 +/+ (viable)
 - *kni*-/+ X +/+
 - 1 *kni*-/+ (viable)
 - 1 +/+

Inversions, a genetic tool for preserving embryonic lethal mutations

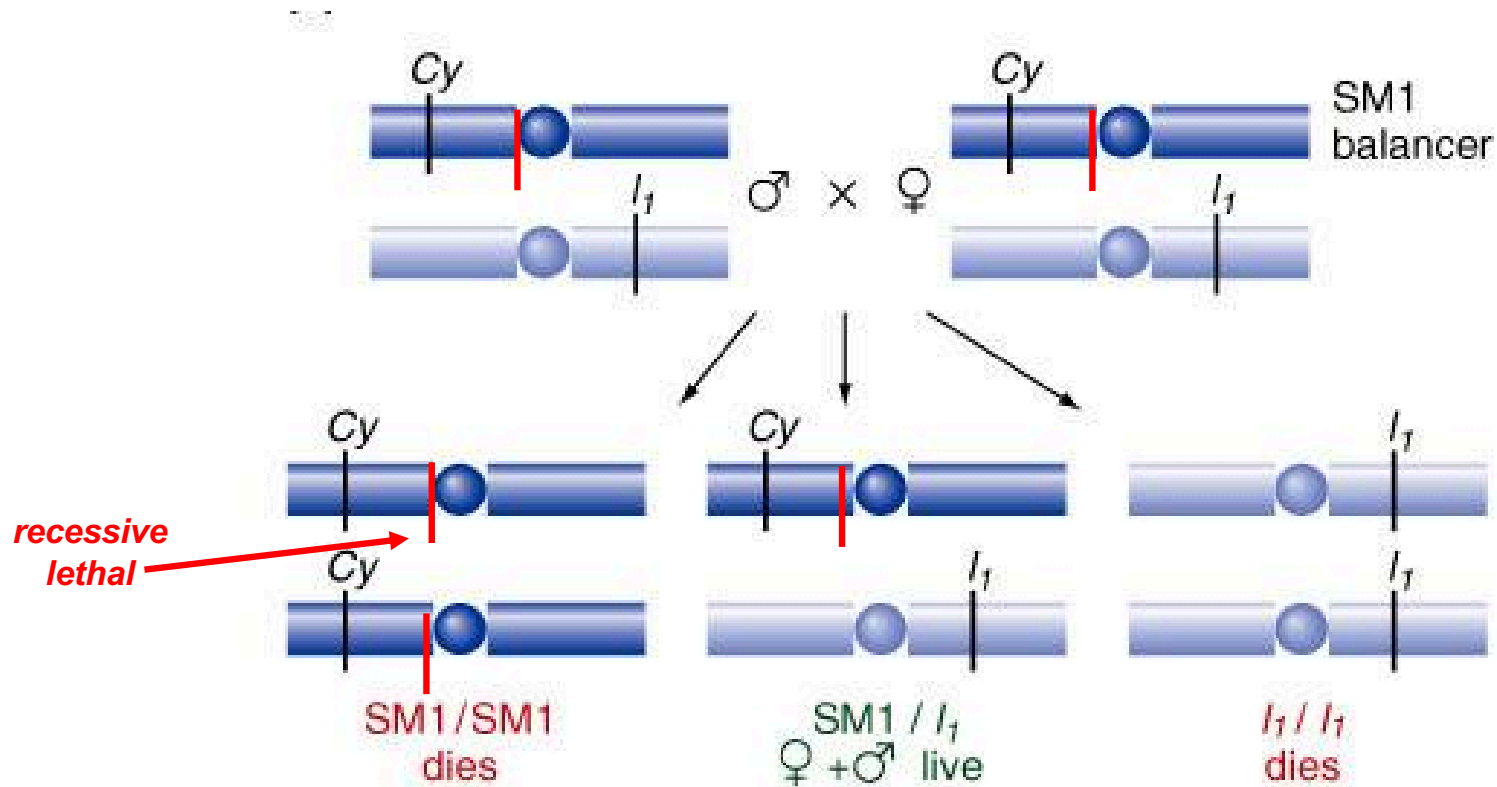
- **Balancer chromosomes allow the maintenance of alleles that are lethal when homozygous**
 - **Carries multiple, overlapping inversions to prevent recombination**
 - **Carries recessive lethal mutation to prevent survival of homozygotes**
 - **Carries dominant marker (e.g. *Curly*) to allow research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

SM1 balancer chromosome

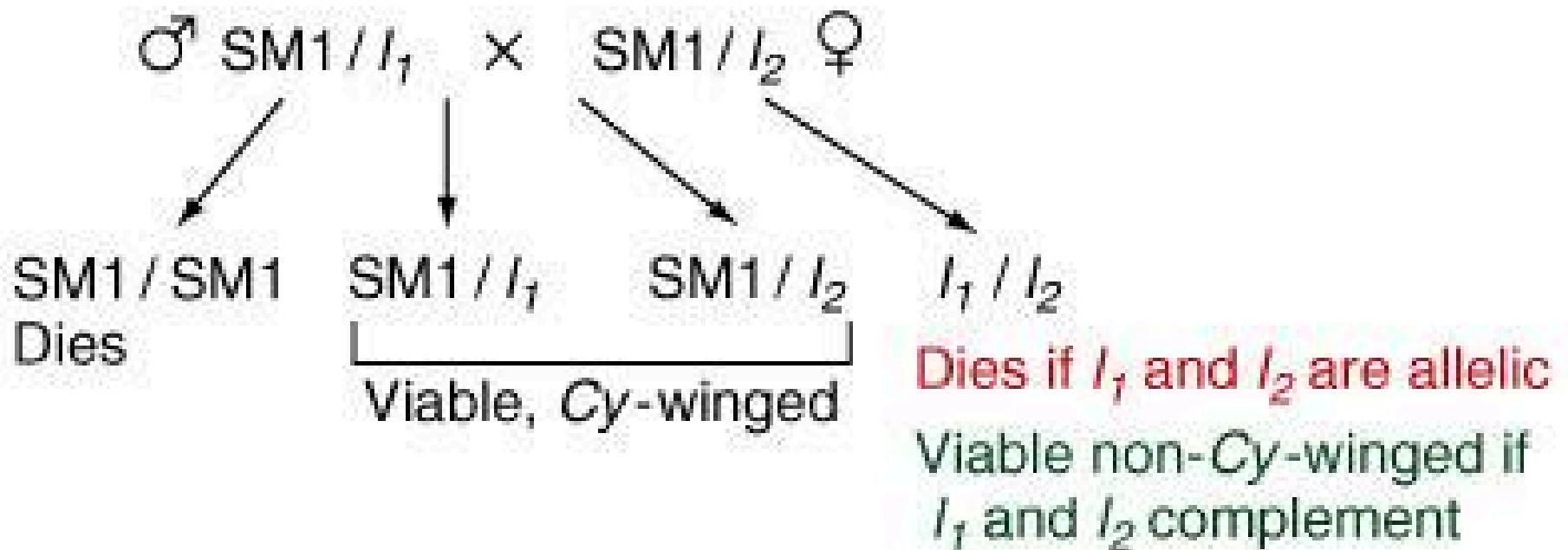
(a) SM1 balancer chromosome



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.

$$\begin{array}{l}
 \frac{sn^*}{Y} \times \frac{sn}{Bal} \longrightarrow \frac{sn^*}{sn} ; \frac{sn^*}{Bal} ; \frac{Y}{Bal} ; \frac{sn}{Y} \\
 \frac{sn^*}{Bal} \times \frac{Y}{Bal} \longrightarrow \frac{sn^*}{Bal} ; \boxed{\frac{sn^*}{Y}} ; \frac{Y}{Bal} ; \frac{Bal}{Bal}
 \end{array}$$

hemizygous male

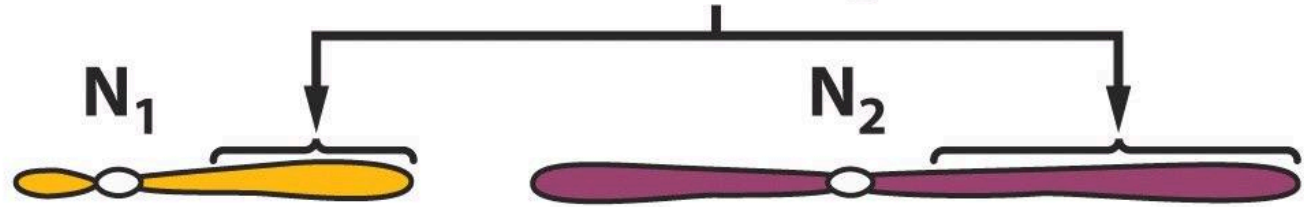
very sickly

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

Translocation heterozygote

Original position of
translocated segments

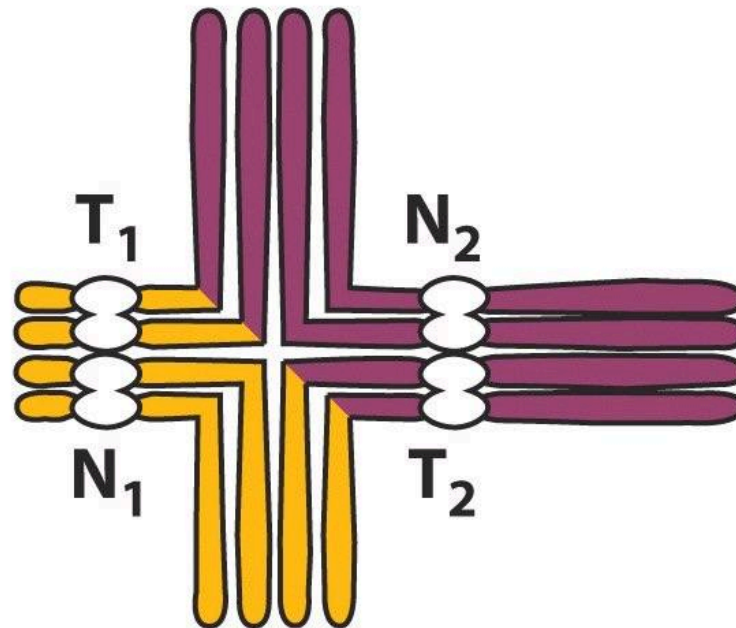
Normal



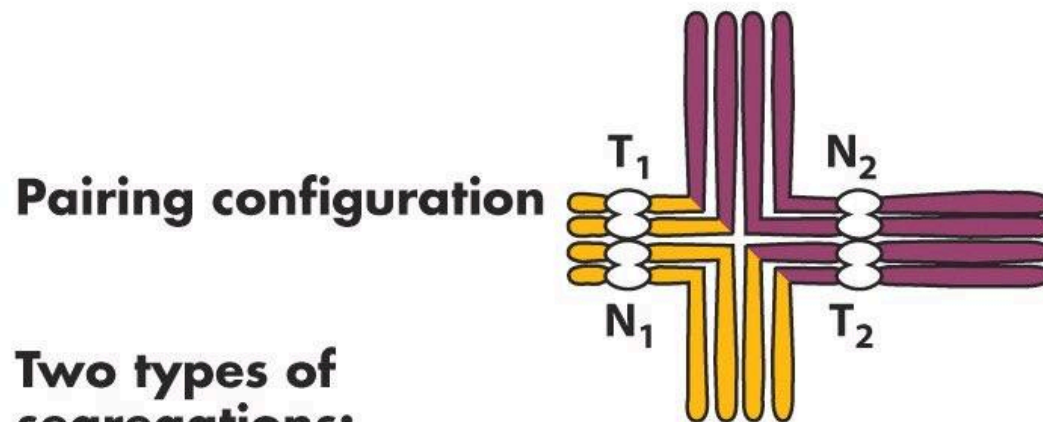
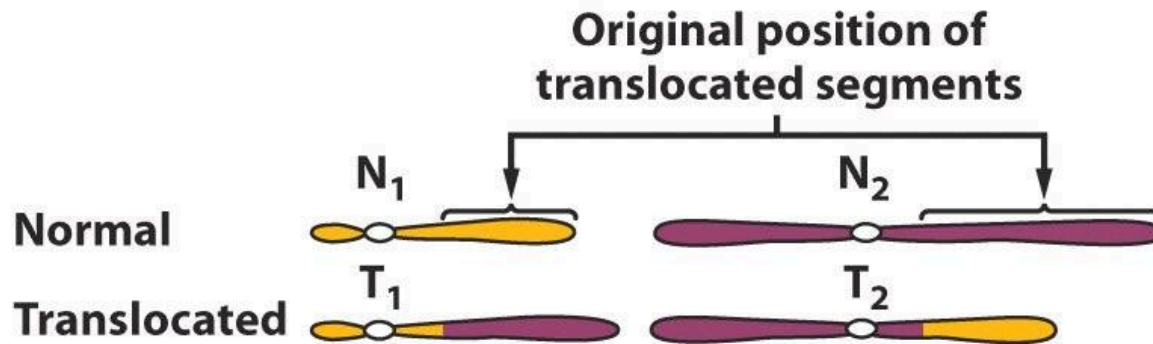
Translocated



Pairing configuration



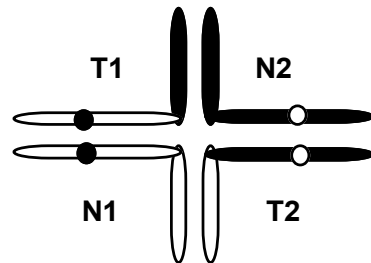
Translocation heterozygote



Two types of
segregations:

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

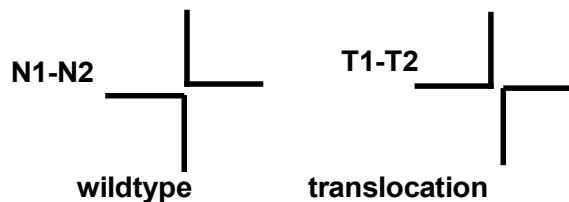
In a cross between translocation heterozygotes, only a small fraction of the zygotes are viable. In plants most translocation heterozygotes are viable, while in *Drosophila* they are lethal. There is a simple explanation for this. In plants translocations must be transmitted through a haploid multicellular 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.



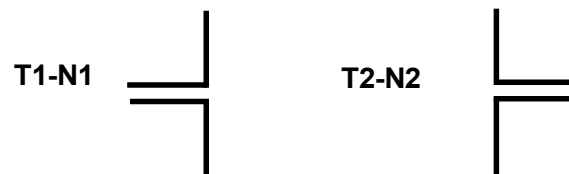
Adjacent-1 segregation
Duplication + deficiency



Alternate segregation
Viable



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



A cross between translocation heterozygotes, in animals, = 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

In plants the pattern simplifies because unbalanced translocation gametes are inviable in the gametophyte stage. Thus certain zygote 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



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

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.

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

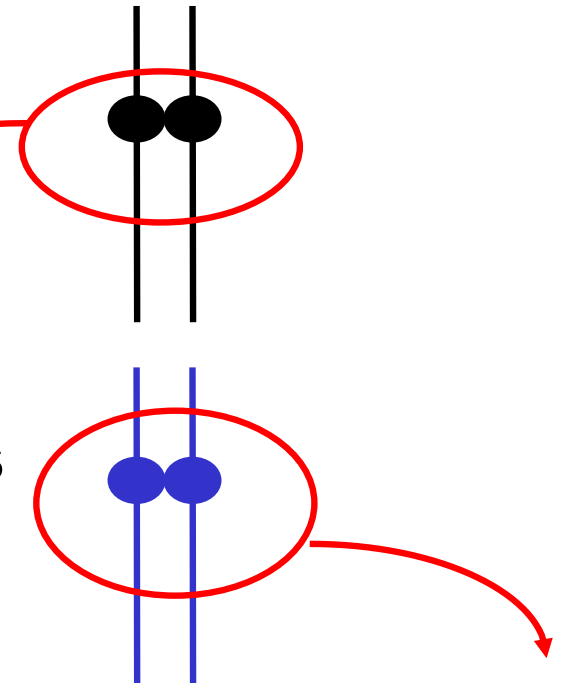
X = the number of chromosomes (in monoploids), or X, the basic chromosome set number, in polyploids such as triploids.

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.

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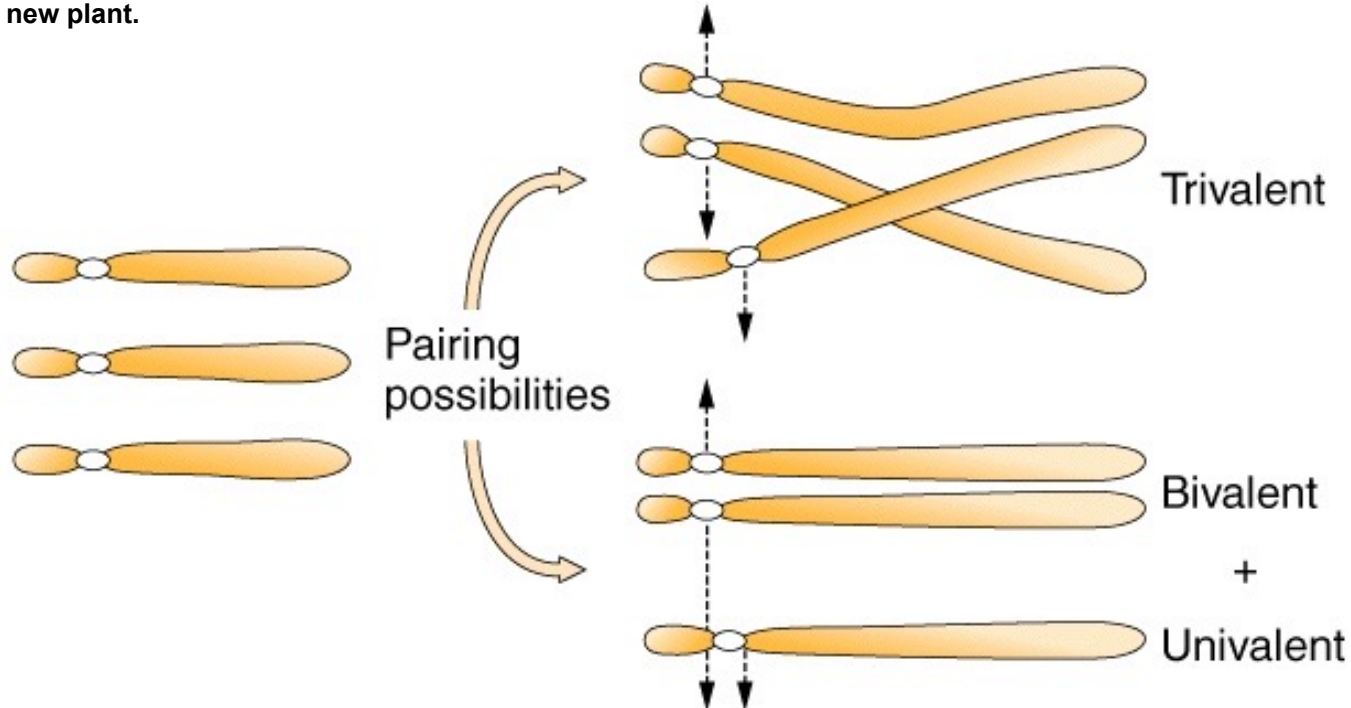
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Triploids are sterile.



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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 dem-

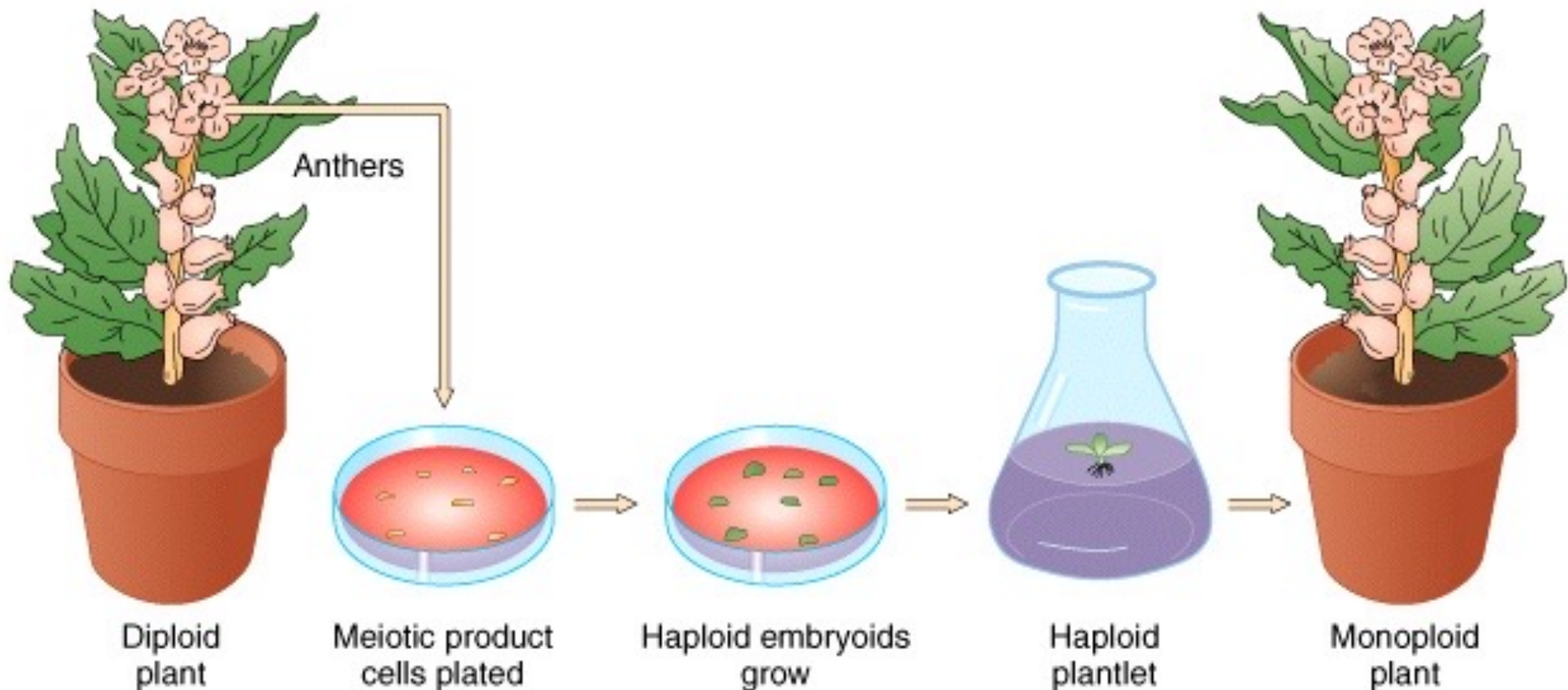
planting spermatogonia into sterile xenogeneic recipients.

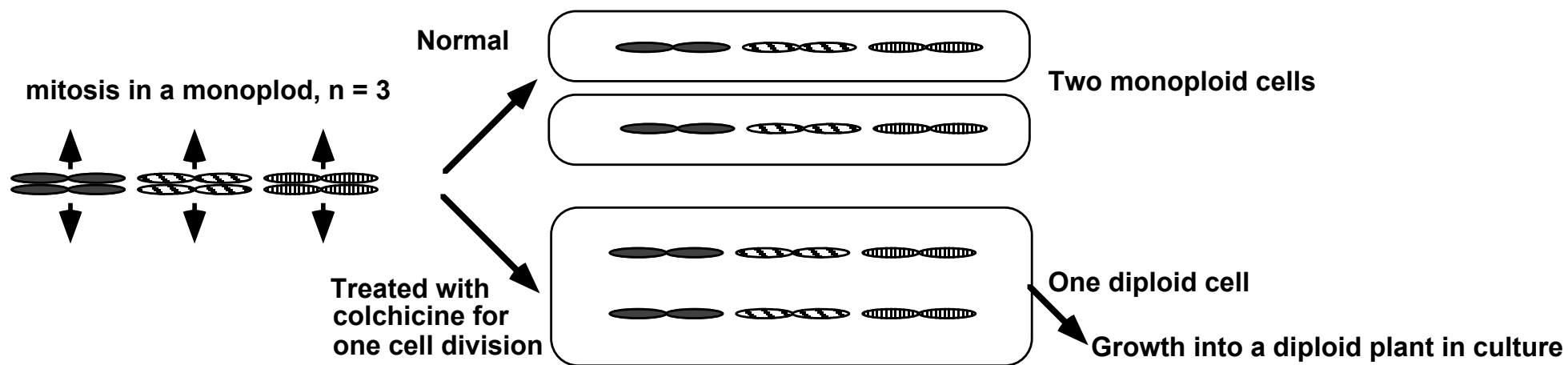
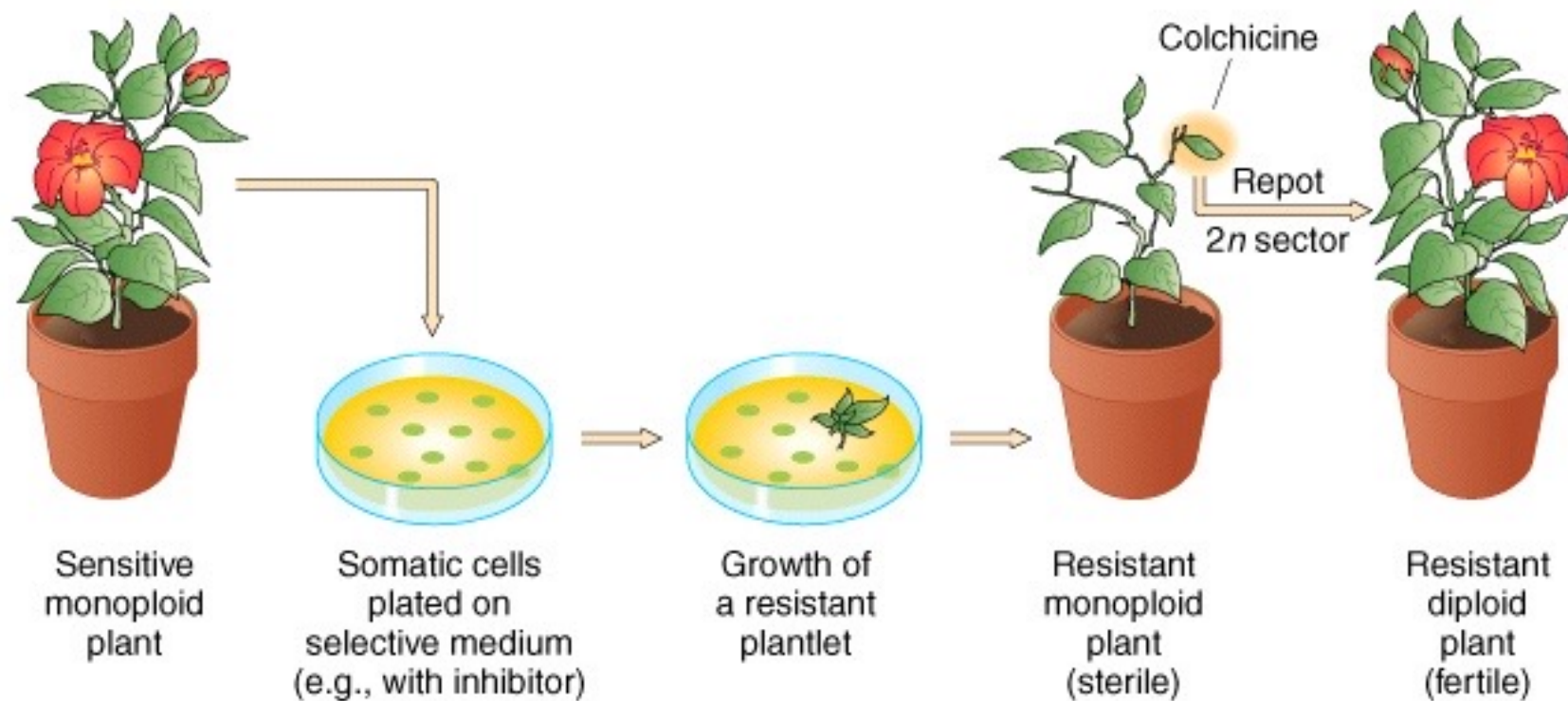
In this study, spermatogonia of *pvasa-Gfp* (where Gfp represents green fluorescent protein) hemizygous (*pvasa-Gfp*/–) and dominant orange-colored mutant heterozygous [*OR*/wild type (*WT*)] adult rainbow trout (*Oncorhynchus mykiss*) were intraperitoneally microinjected into newly hatched embryos of 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 F₁ 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.



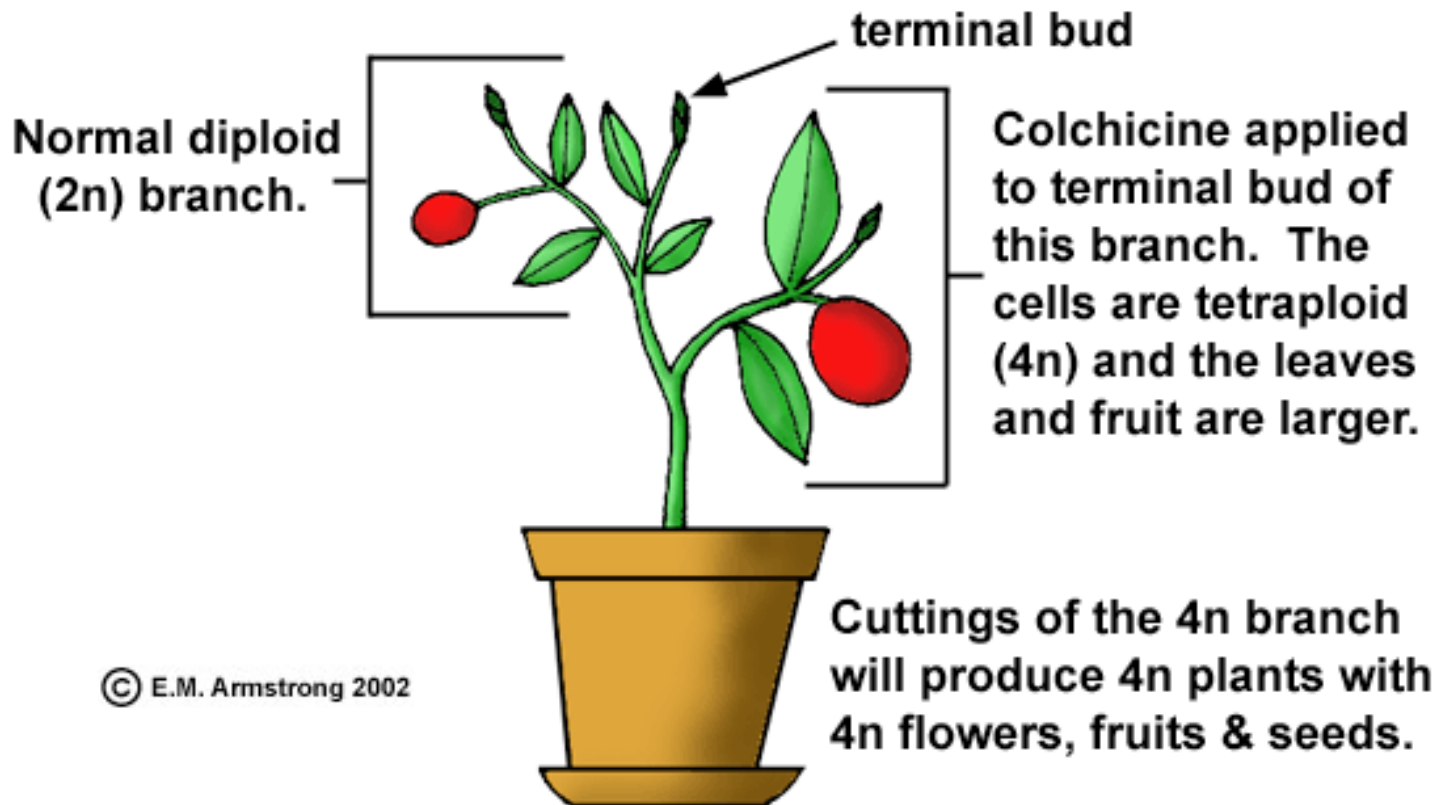


Autopolyploidy

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

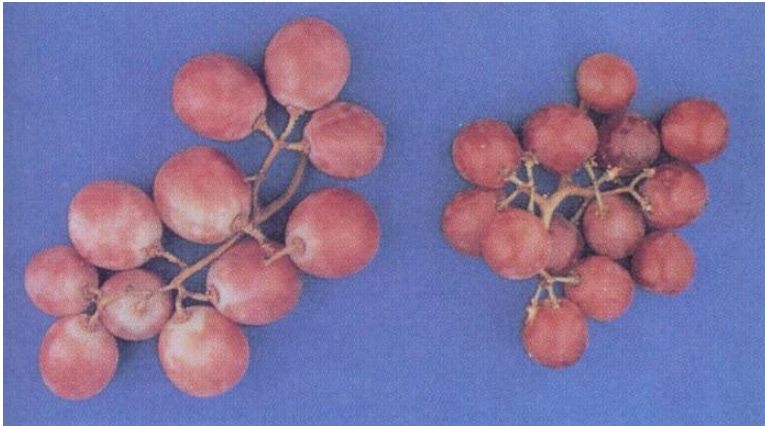
...the additional set of chromosomes is identical to the normal haploid complement of that species.

Can artificially use colchicine to increase ploidy.



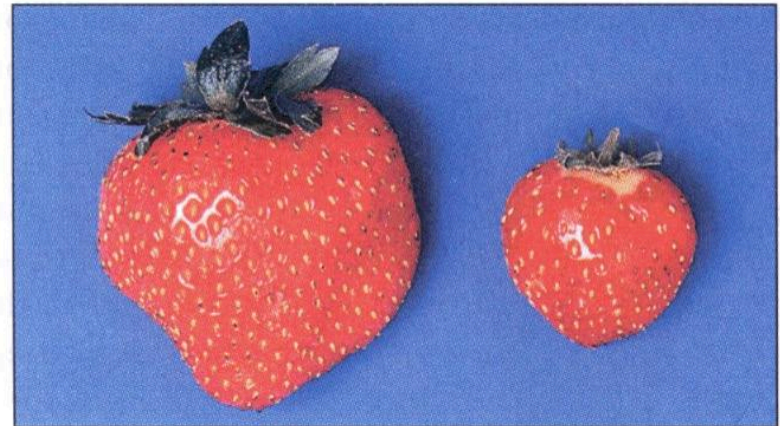
Autopolyploidy Applications

- 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*

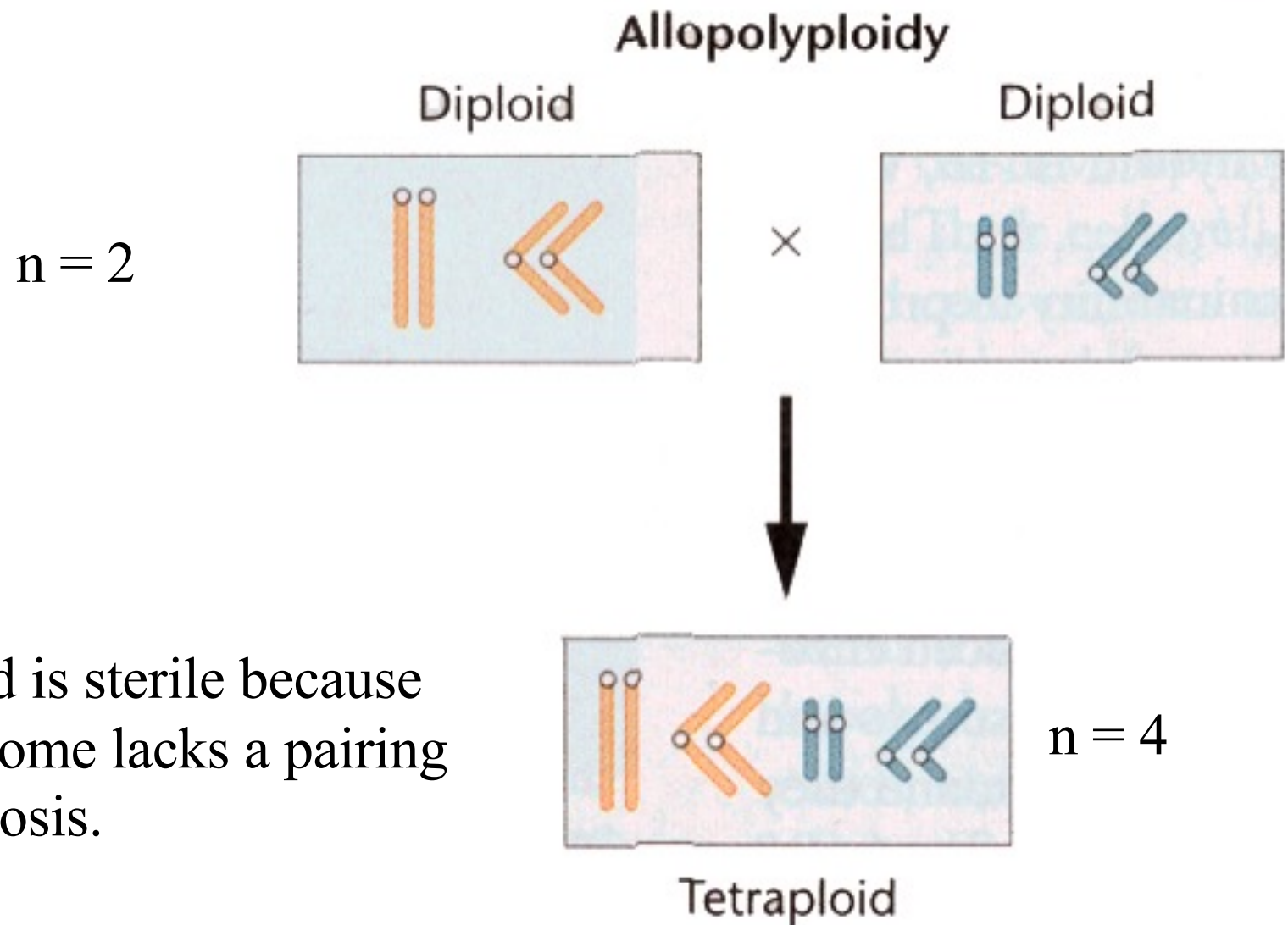
These are essentially the same frog - it is the *balance* between the number of gene copies that is important for proper development, not necessarily the *total number* of gene copies

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 homologous, but are referred to as homeologous.

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

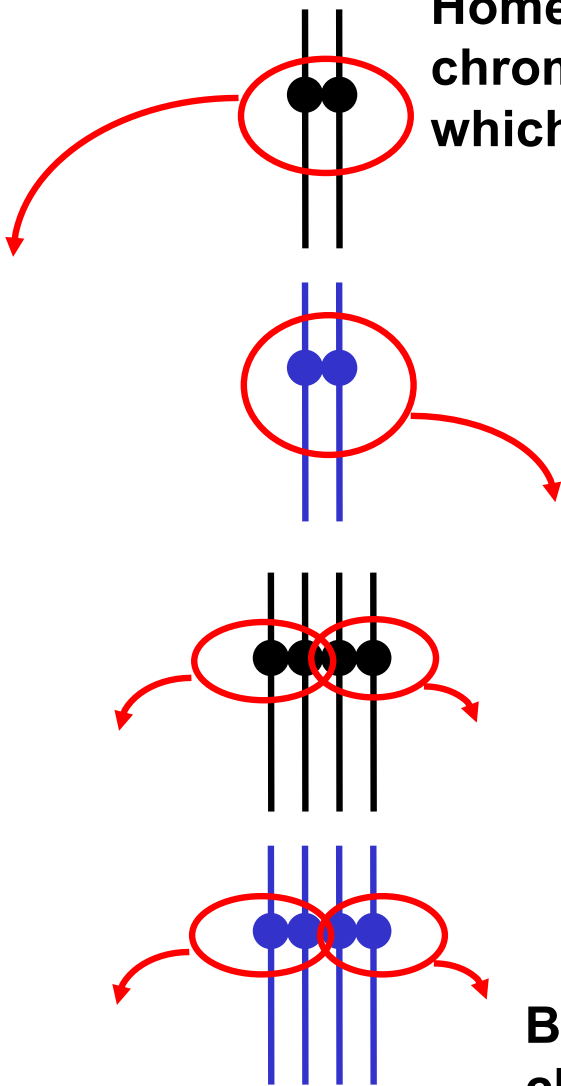


This tetraploid is sterile because each chromosome lacks a pairing partner at meiosis.

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

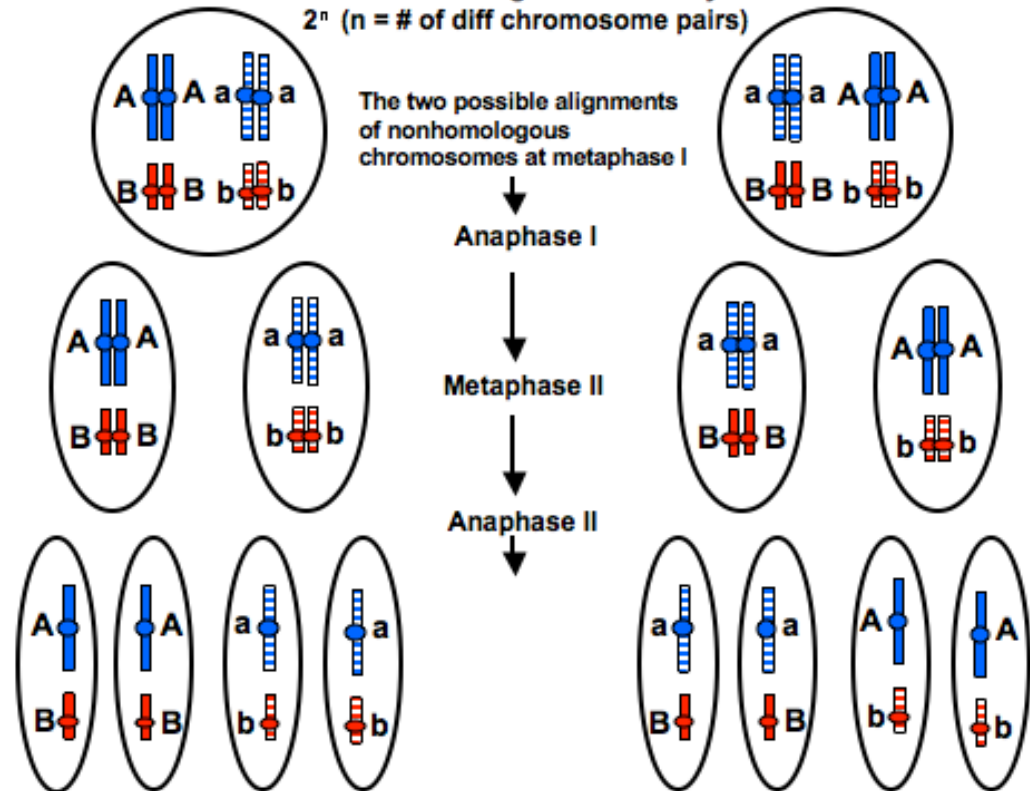
If cross two related species, ok until meiosis.

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



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

Meiosis without recombination. Independent assortment of homologs to different Poles creates gamete diversity 2^n (n = # of diff chromosome pairs)



The Sad Tail of *Raphanobrassica*

Cast

Dr. G. Karpechenko, Russian Plant breeder,

Brassica oleracea ($2n = 18$),

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

Raphanus sativus ($2n = 18$),

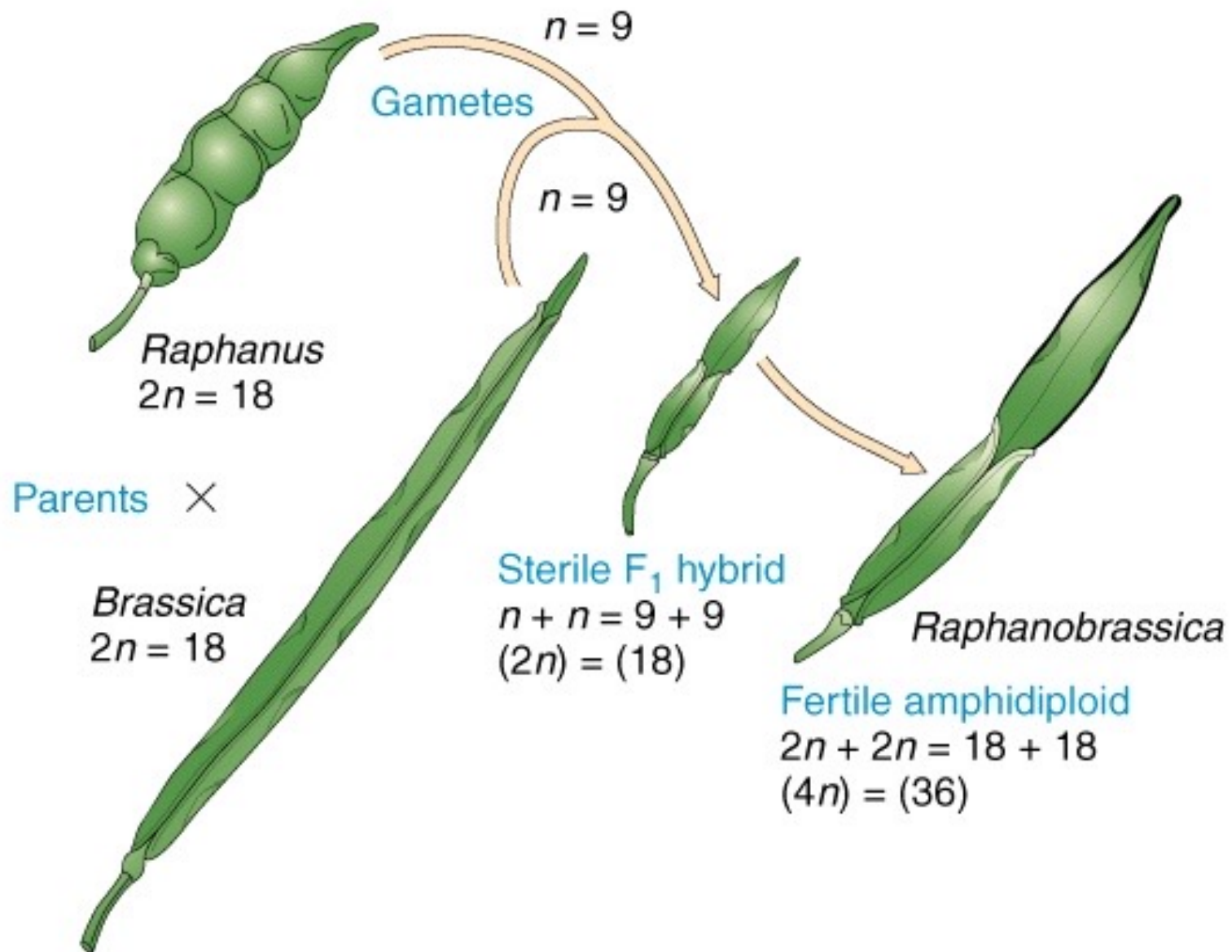
- the common radish, large root food stock, worthless leaves.

Georgi Karpechenko

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

cabbage + radish = cabrad?





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

Triticum (wheat)

$$6n = 42$$

Gametes: $3n = 21$

Secale (Rye)

$$2n = 14$$

gametes: $n = 7$

Sterile hybrid

$$4n = 28$$

Fertile 'Triticale'

$$8n = 56$$

**Chromosome
doubling with
colchicine.**

**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.

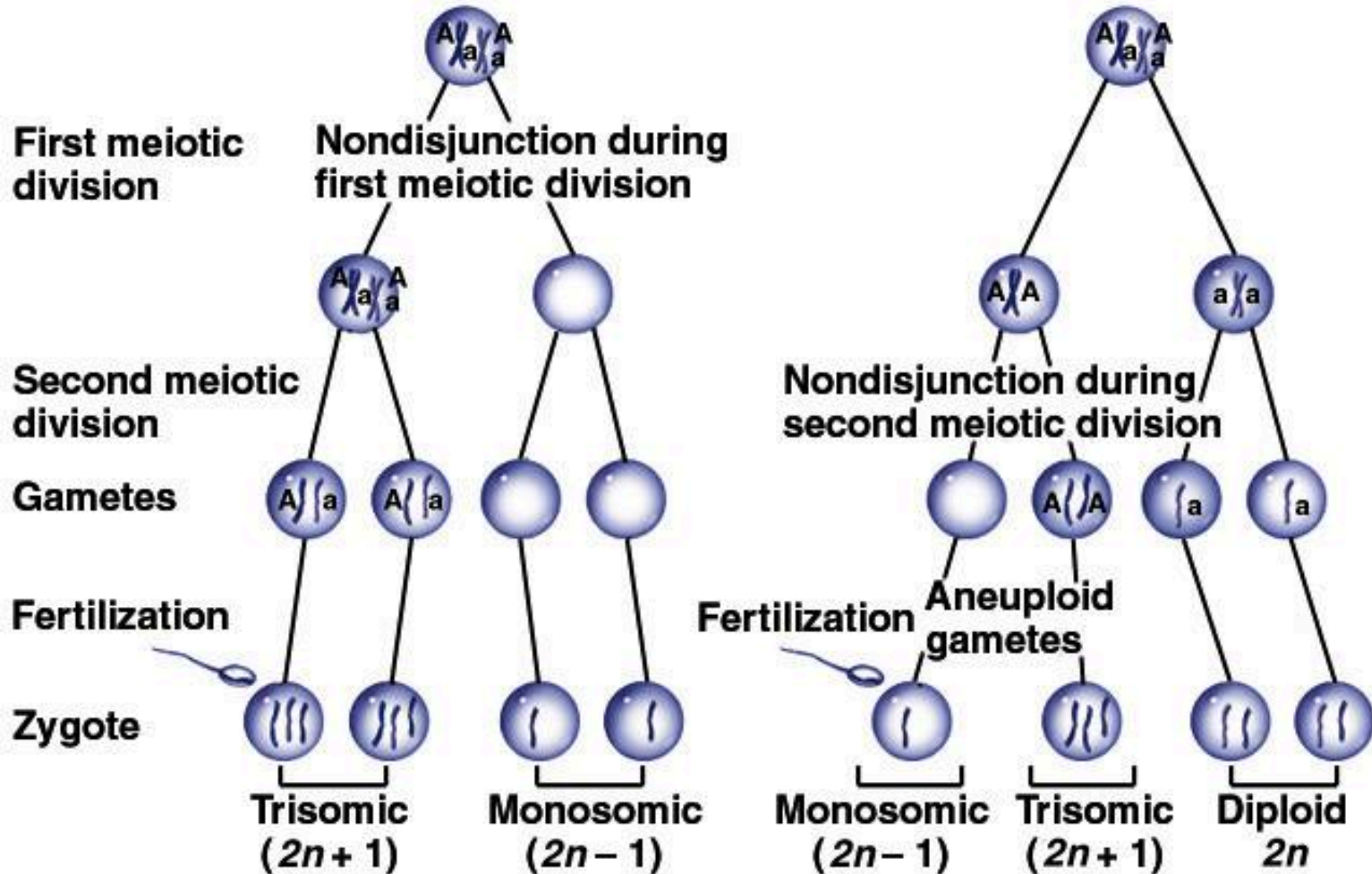
Aneuploidy

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!

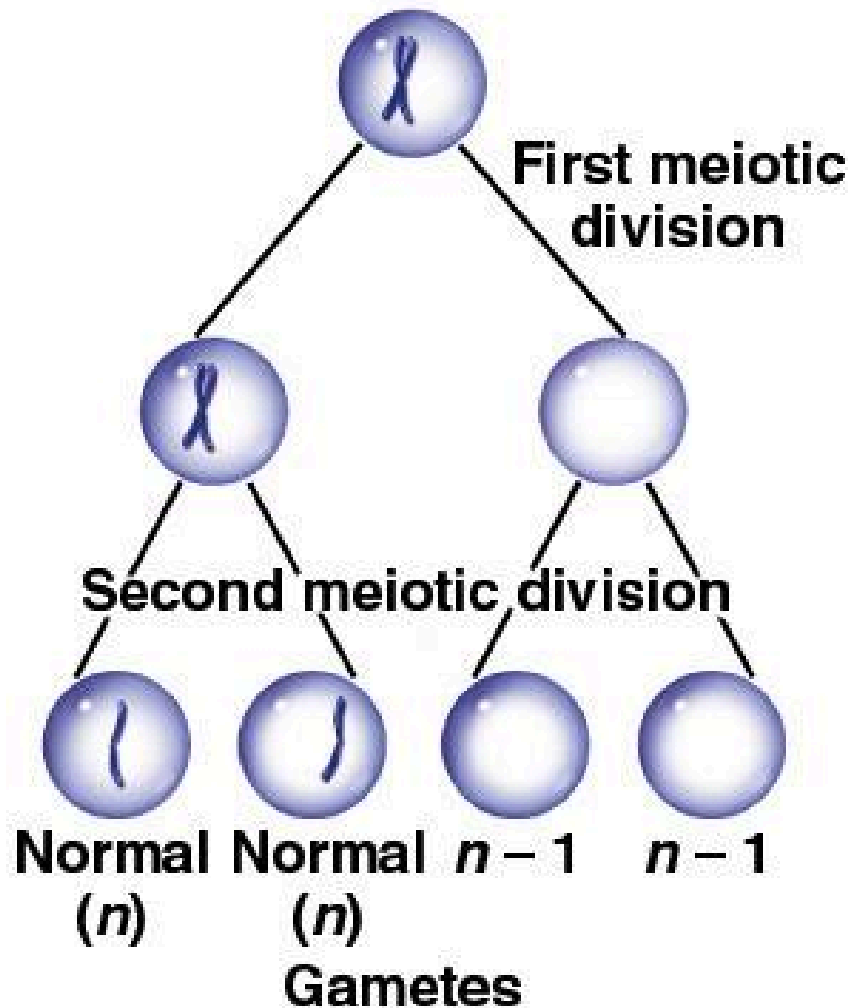
What are the consequences of aneuploidy?

Aneuploidy

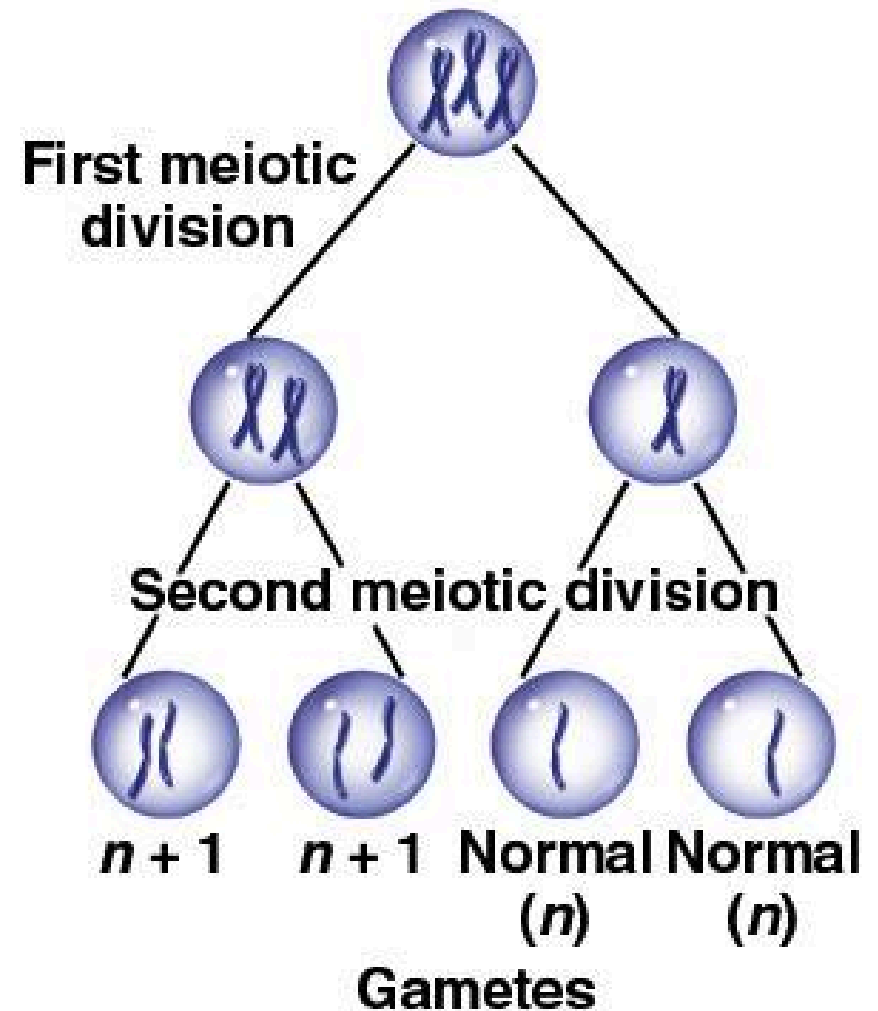


Aneuploidy

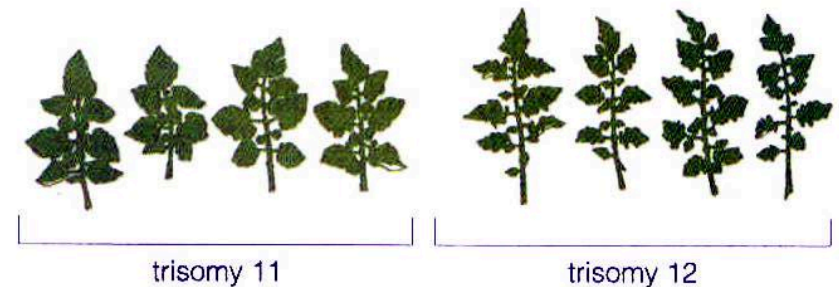
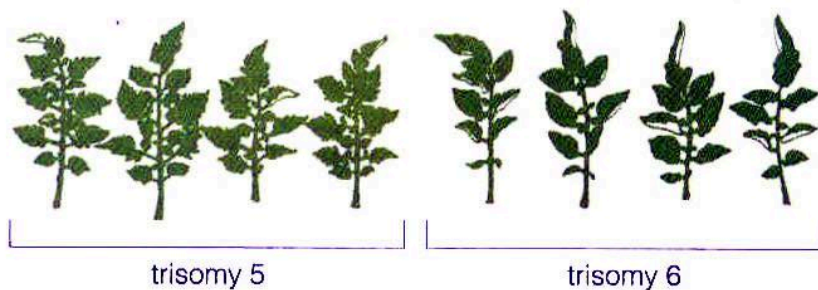
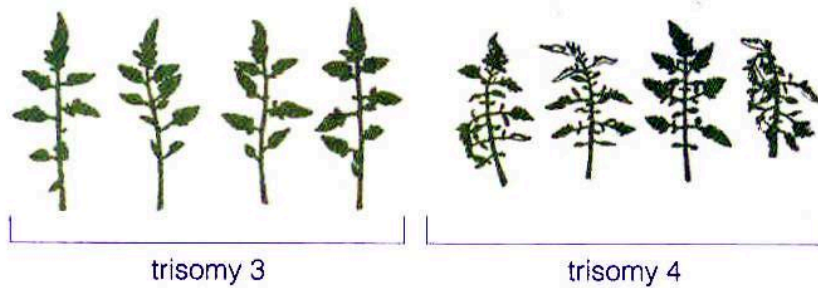
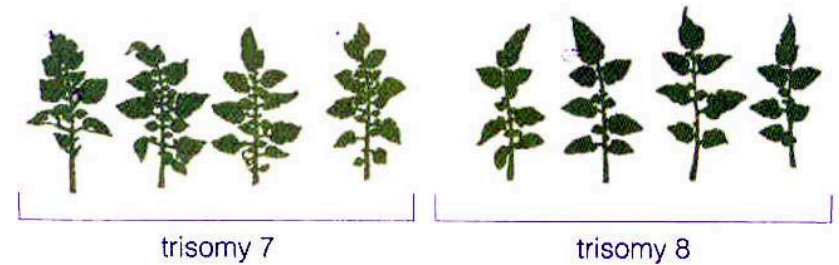
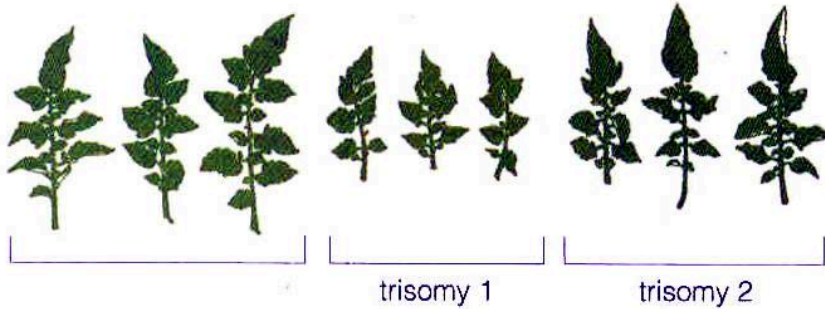
**Germ cell in a
monosomic individual**



**Germ cell in a
trisomic individual**



Plants just don't care that much: the tomato



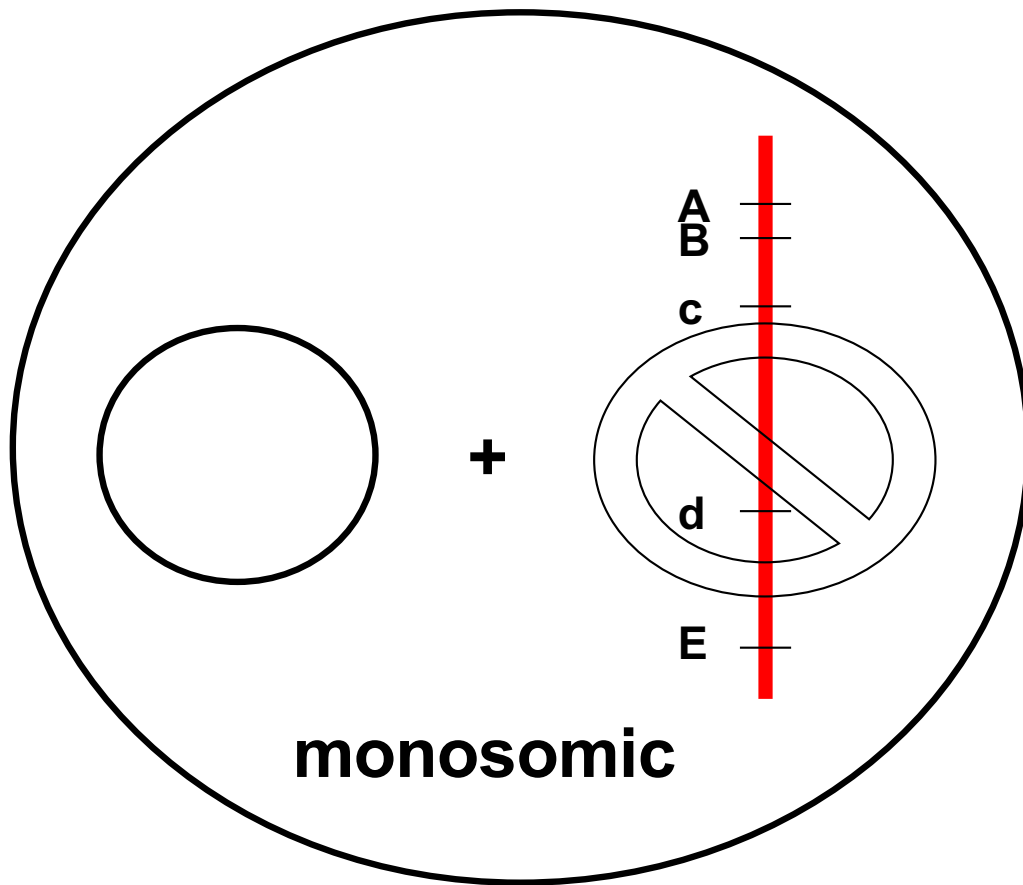
Human Autosomes first!

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

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

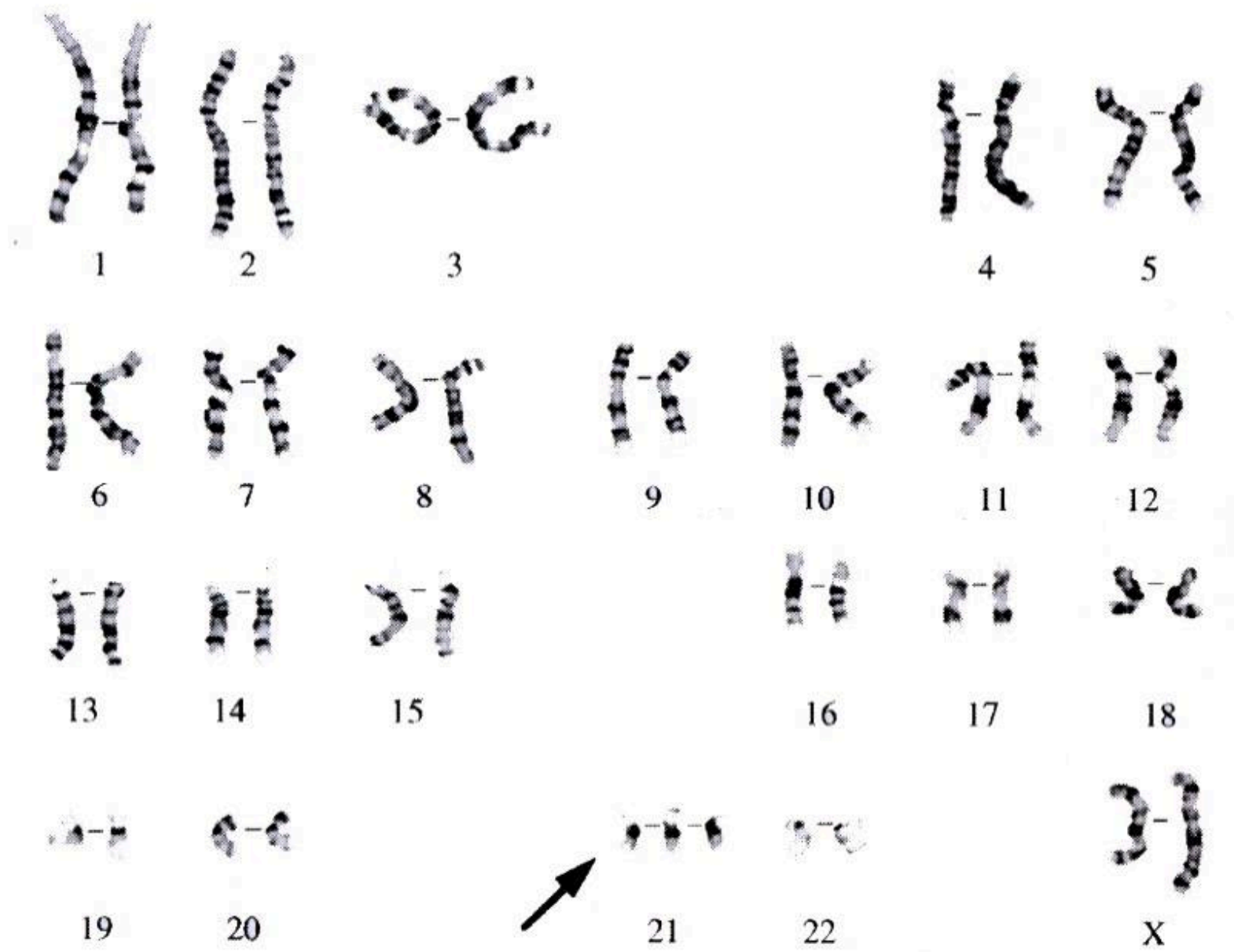
Autosomal Monosomy

- 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)



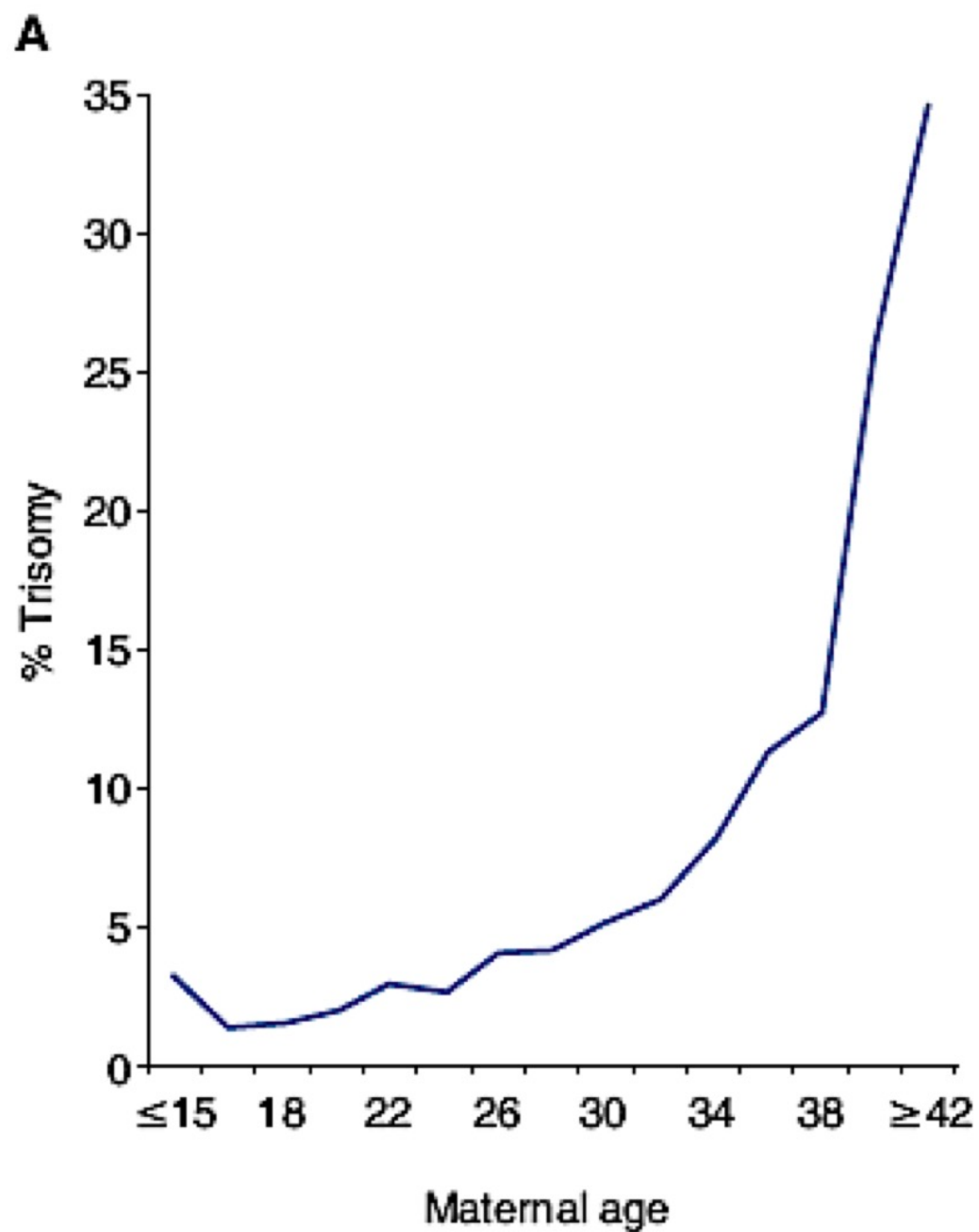
Trisomy in Humans

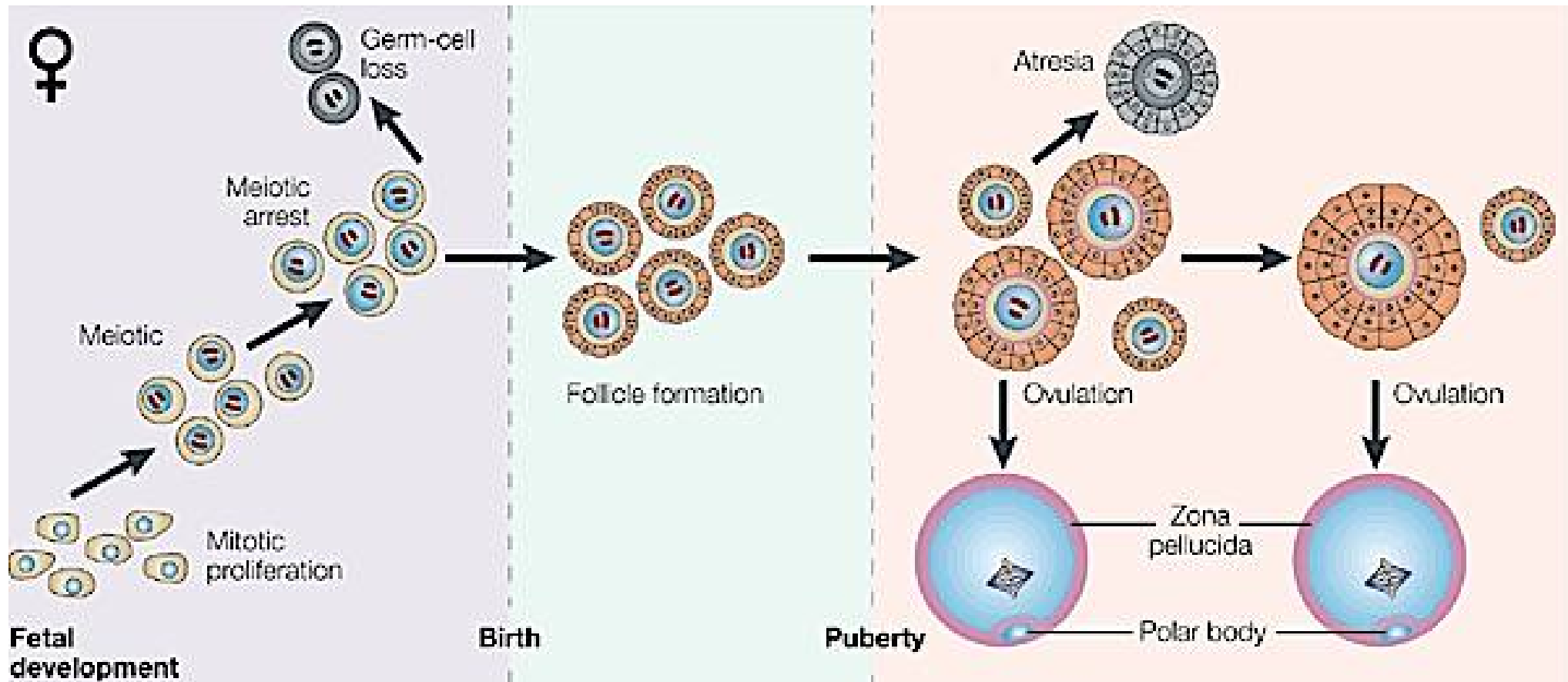
Down Syndrome

(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.

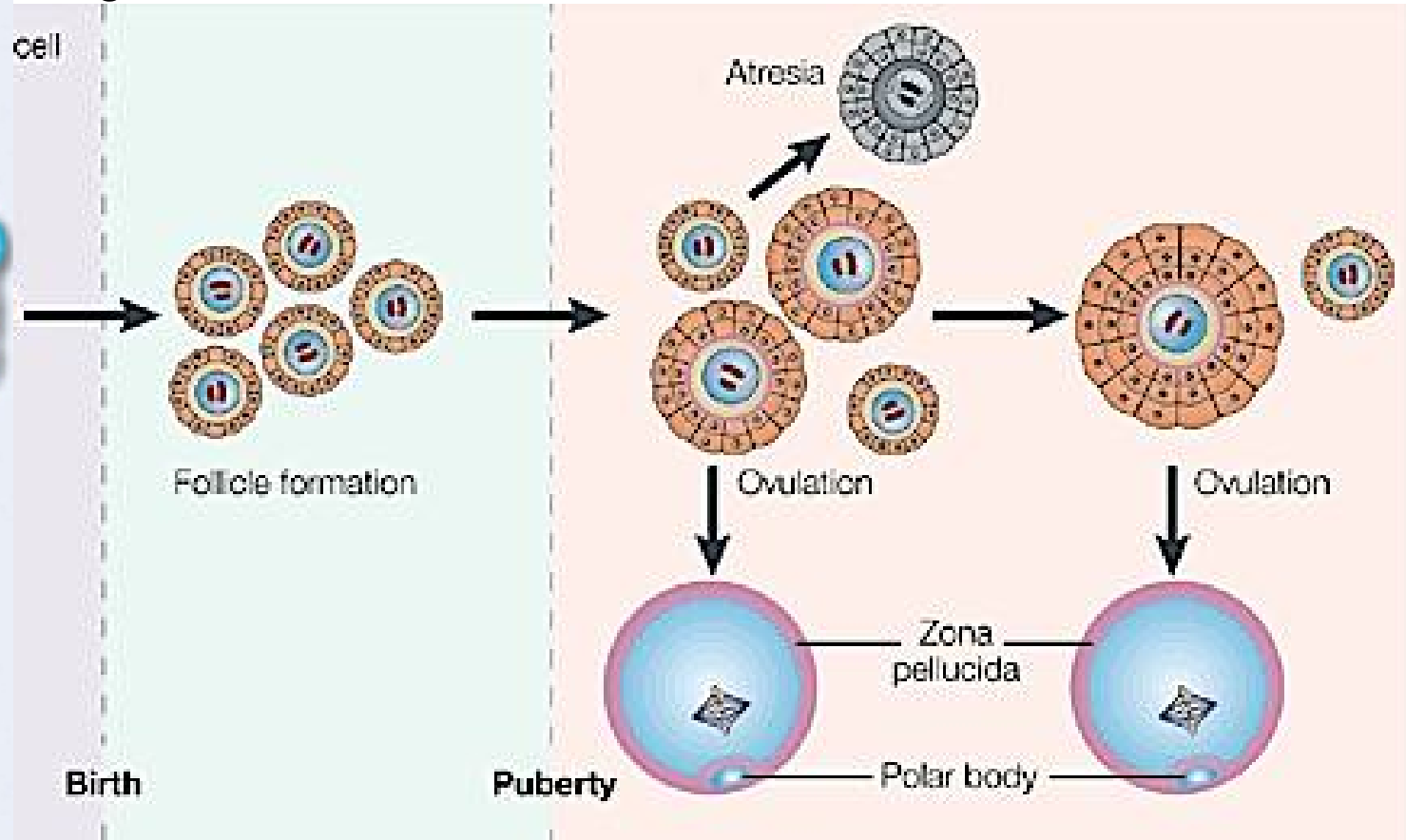
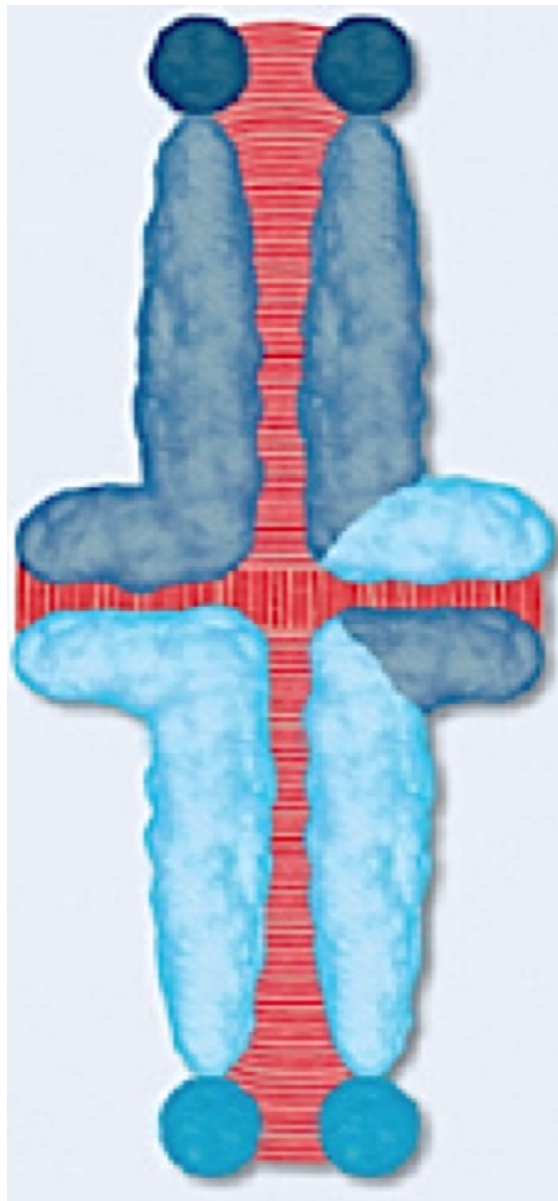
Maternal Age and trisomy





- 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 Trisomies

- **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

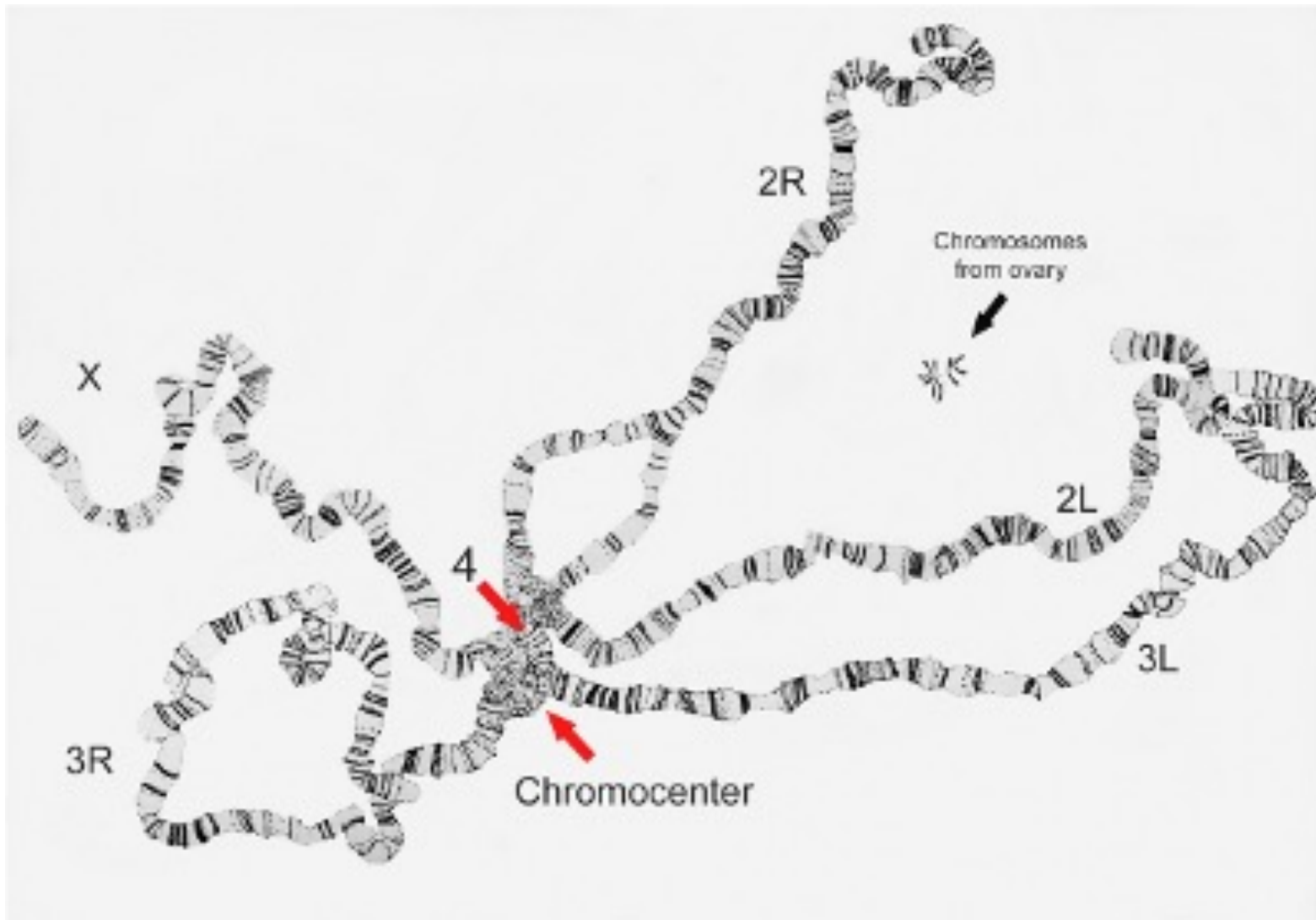


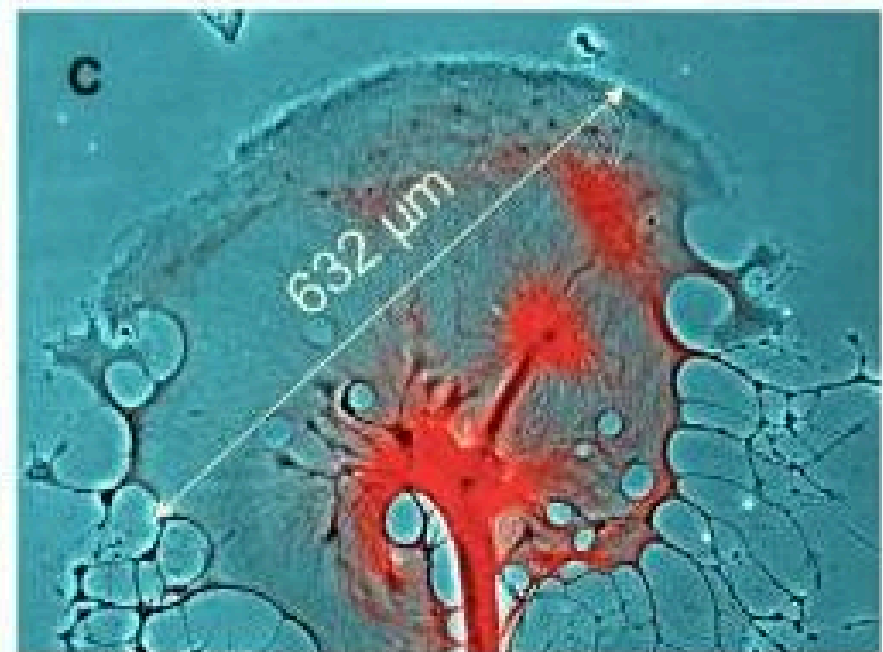
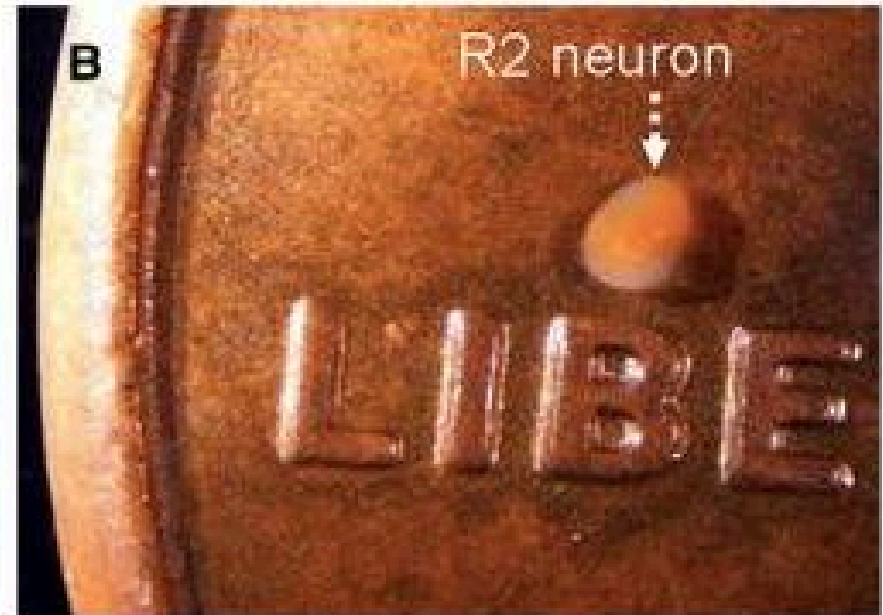
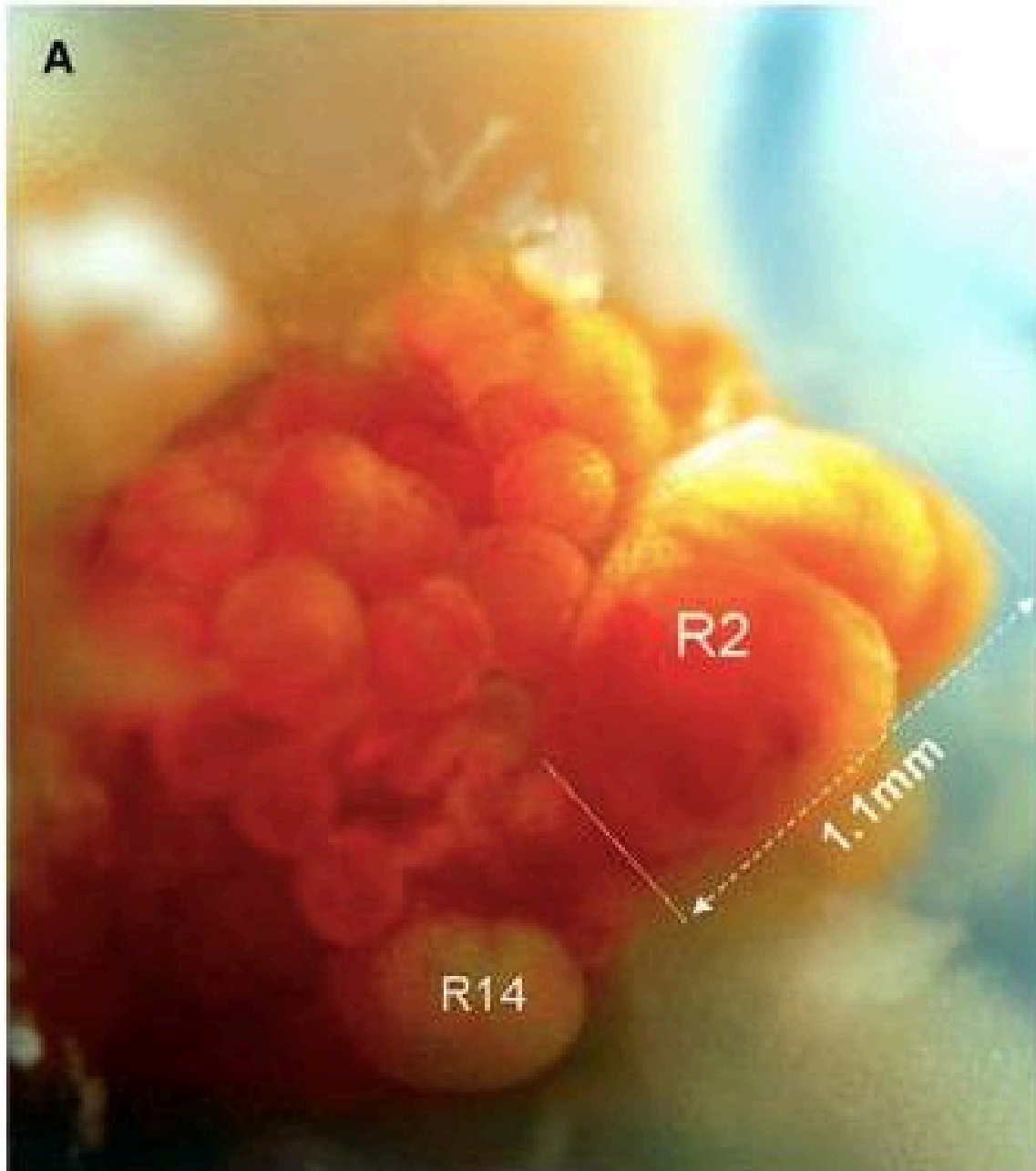
TABLE 1 - High-level polyteny in animals and plants. C values are based on DNA measurements, n values on nuclear volumetry.

Species	Tissue or cell	Maximum level of polyteny	Reference
ANIMALS			
<i>Aplysia californica</i>	Giant neurons	200,000 C	LASEK and DOWER 1971 ¹⁰¹
<i>Ascaris suum</i>	Esophageal gland	262,144 C	ANISIMOV 1976 ¹⁰²
<i>Bombyx mori</i>	Silk gland	524,288 C	GAGE 1974 ¹⁰³
	id, posterior zone	1,048,576 C	PERDRIX-GILOT 1978 ¹⁰⁴
<i>Chironomus tentans</i>	Salivary gland	32,768 n	BEERMANN 1952 ¹⁰⁵
<i>Chironomus thummi</i>	Salivary gland	16,384 C	RASCH 1970 ¹⁰⁶
<i>Drosophila melanogaster</i>	Salivary gland	2,048 C	RASCH 1970 ¹⁰⁶
<i>Microtus arvalis</i>	Trophoblast	2,048 C	ZYBINA <i>et al.</i> 1975 ¹⁰⁷
<i>Mus musculus</i>	Trophoblast	1,024 C	BARLOW and SHERMAN 1972 ¹⁰⁸
<i>Rattus rattus</i>	Trophoblast	4,096 C	NAGL 1972 ¹⁰⁹
<i>Sciara coprophila</i>	Salivary gland	8,192 C	RASCH 1970 ¹⁰⁶
PLANTS			
<i>Arum maculatum</i>	Endosperm haustorium *	24,576 n	ERBRICH 1965 ¹¹⁰
<i>Echinocystis lobata</i>	Endosperm, central	3,072 n	TURALA 1966 ¹¹¹
<i>Melampyrum nemorosum</i>	Endosperm haustrium	1,536 n	ERBRICH 1965 ¹¹⁰
<i>Phaseolus coccineus</i>	Suspensor	8,192 C	BRADY 1973 ¹¹²
<i>Phaseolus vulgaris</i>	Suspensor	4,096 C	NAGL 1974 ¹¹³
<i>Scilla bifolia</i>	Elaosome	4,096 n	SPETA 1972 ¹¹⁴
<i>Scilla decidua</i>	Antipodal **	1,024 C	FRISCH and NAGL 1979 ¹¹⁵

* Endosperm cells are initially triploid (3n).

** Antipodal cells are initially haploid (n).

Aplysia R2 neuron



Region-specific amplification/underreplication

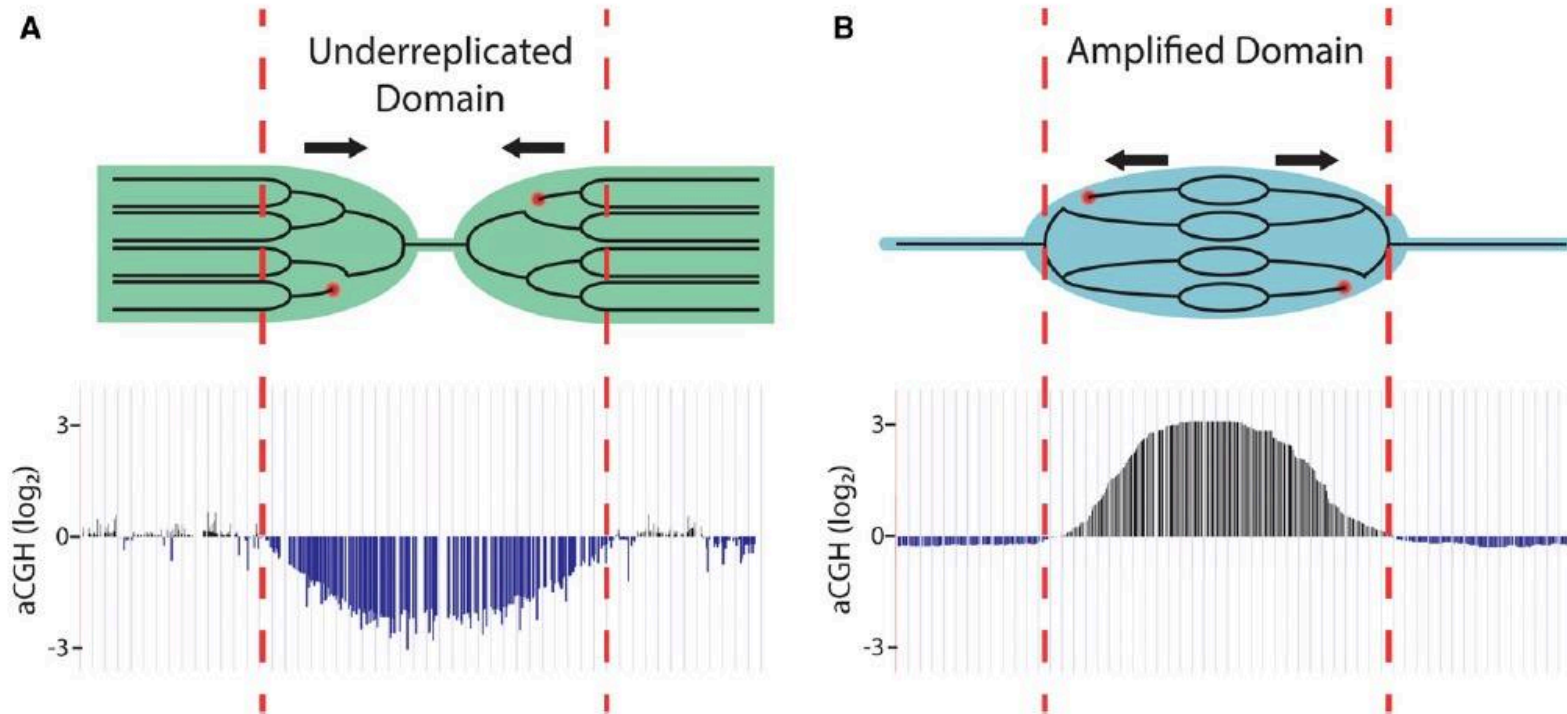


Figure 3 Differential DNA replication. (A) Underreplication results from two effects: absence of replication origins and initiation within a domain coupled with impaired progression of replication forks initiating from flanking origins (arrows indicate direction of fork progression). Replication forks 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	<i>Cp7Fa</i> , <i>Cp7Fb</i> , <i>Cp7Fc</i> , <i>Cp36</i> , <i>Cp38</i>
22B	4	10B–13	None
30B	4	10B	<i>CG11381</i> , <i>CG13113</i> , <i>CG13114</i> ^a
34B	6	10B, 13	<i>Vm34Ca</i>
62D	4	10B, 13	<i>yellow-g</i> , <i>yellow-g2</i>
66D	60–80	10B–11	<i>Cp18</i> , <i>Cp15</i> , <i>Cp19</i> , <i>Cp16</i>

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