高中生命科學研究人才培育

植物學特論： 植物的生殖

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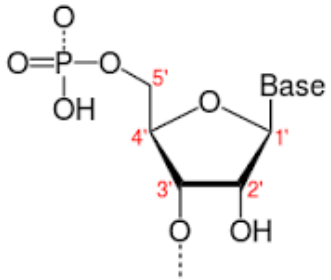
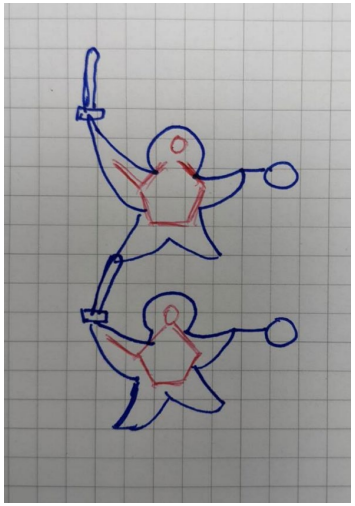
中研院植微所

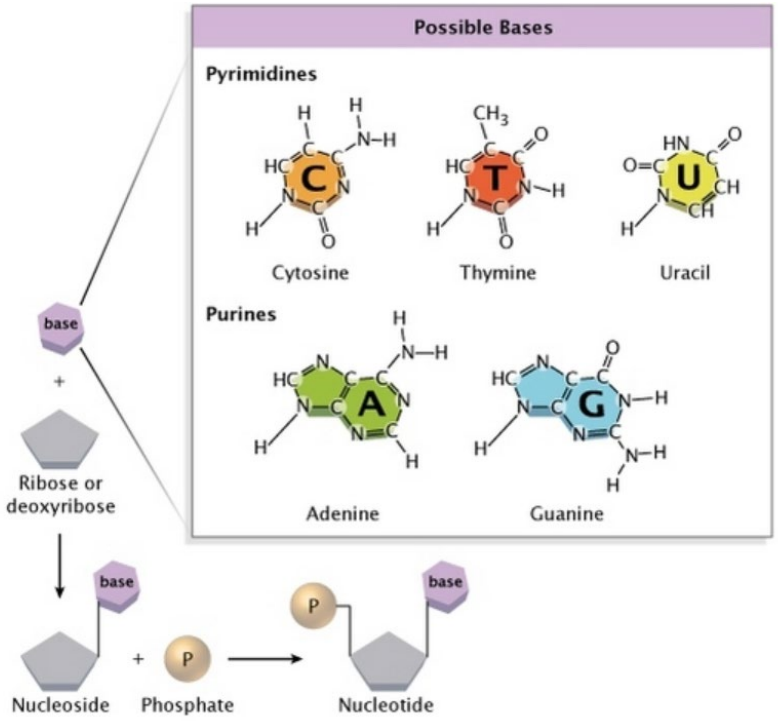
March 30, 2024

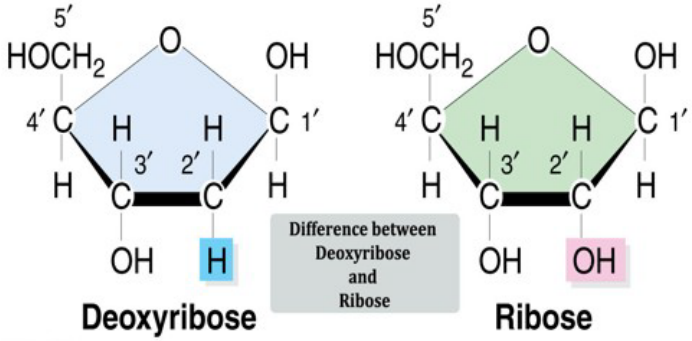
The chemical structure of a nucleotide .





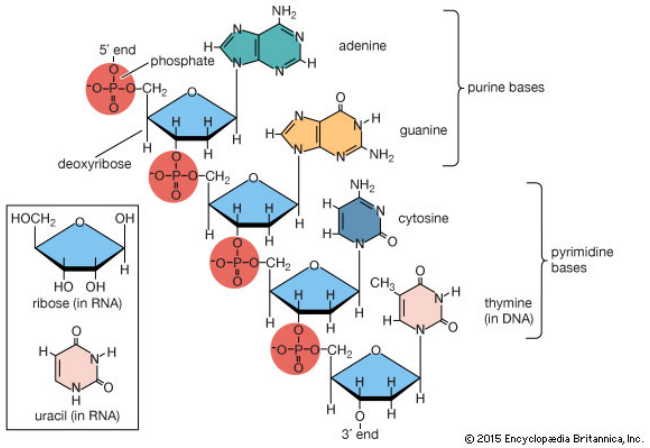
Basic structure: 5 carbon sugar (deoxyribose) is attached one or more phosphate groups and a nitrogen-containing base.



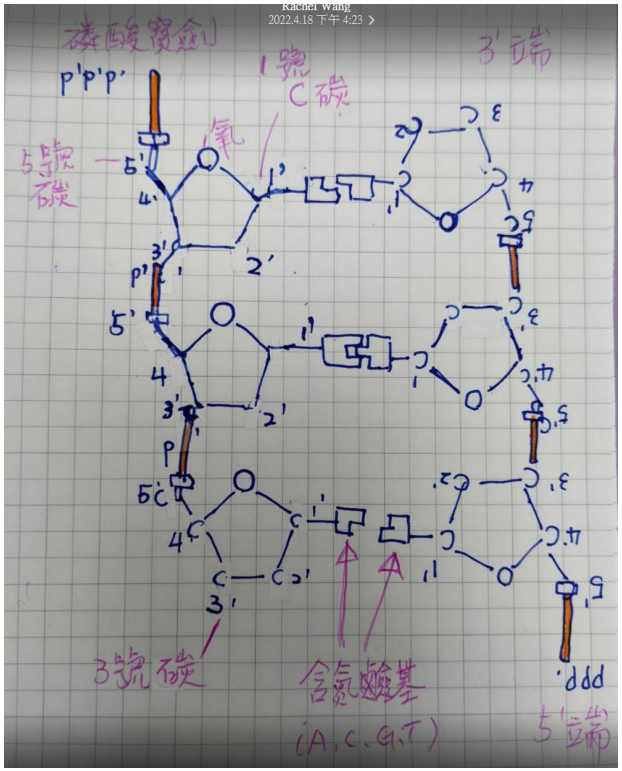


去氧核醣核酸

5’ phosphate



3’ hydroxyl



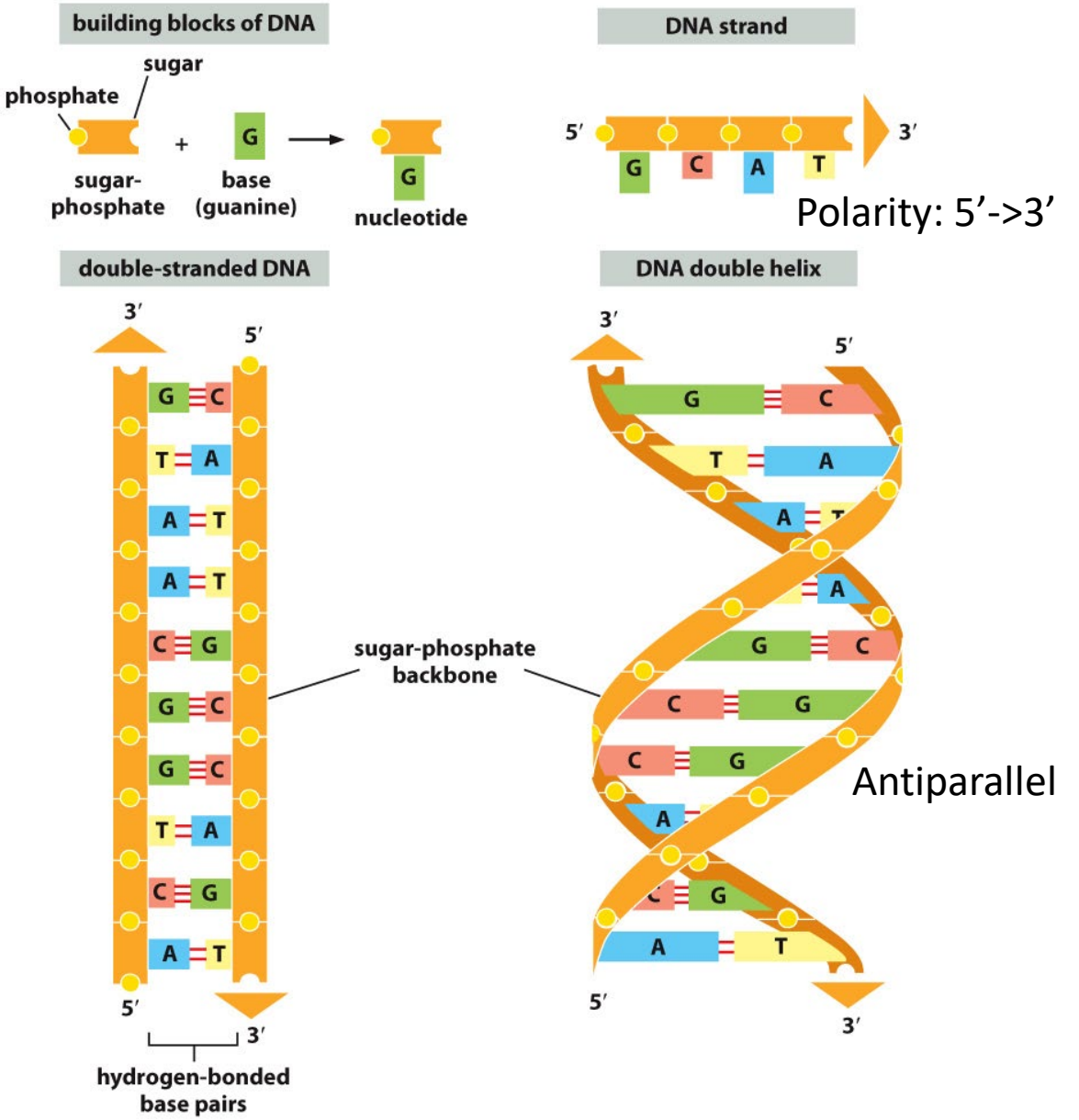
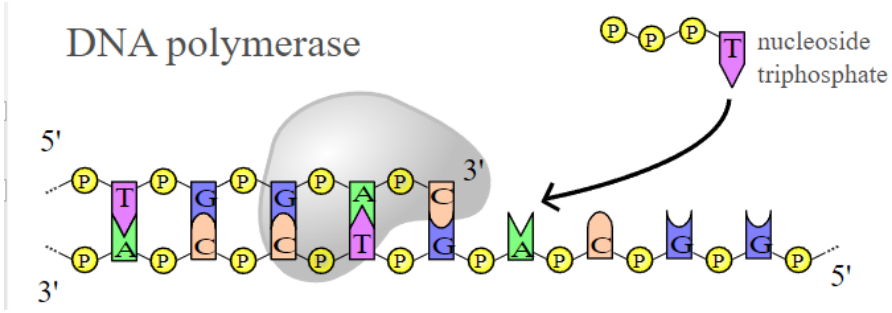
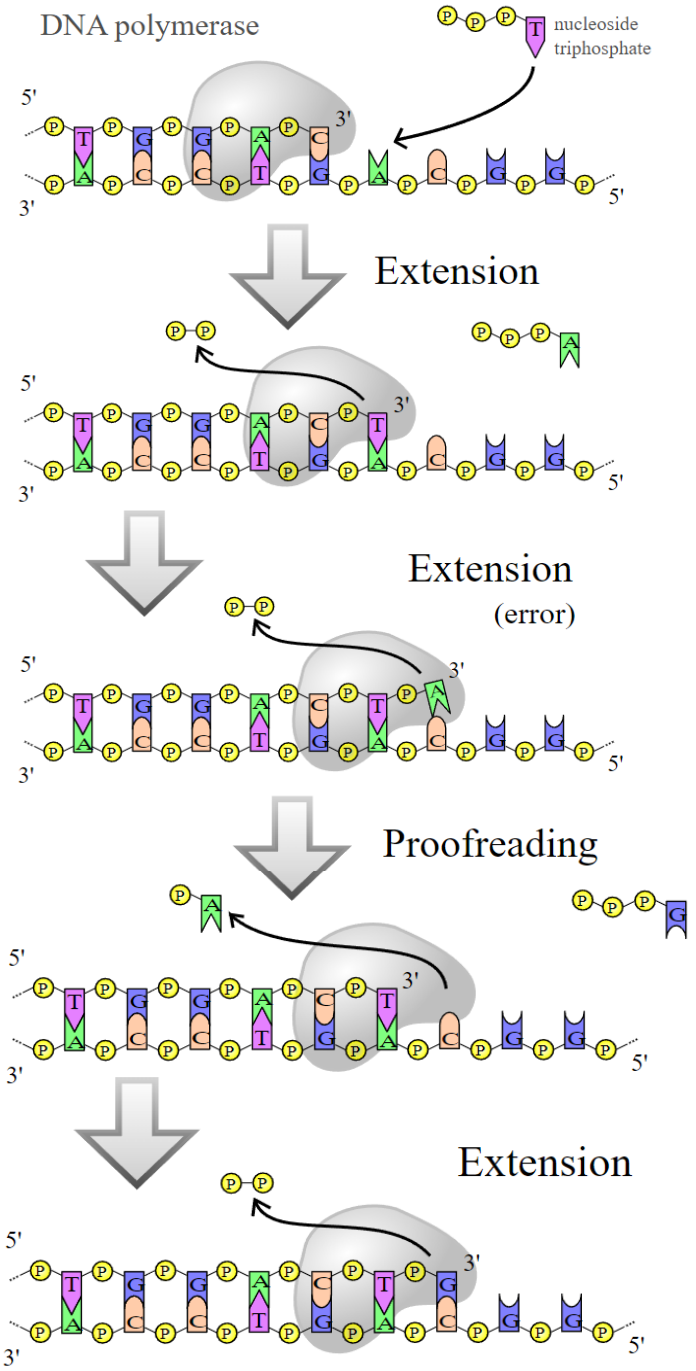


Figure 4-3 Molecular Biology of the Cell 6e (© Garland Science 2015)

DNA 聚合5'→3'?


DNA polymerase
nucleoside
triphosphate
5'
3'
3'
5'

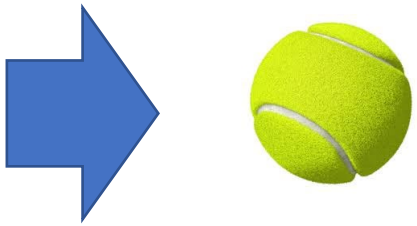

DNA polymerase
nucleoside
triphosphate
Extension
Extension
(error)
Proofreading
Extension

II. CHROMOSOMAL DNA AND ITS PACKAGING IN THE CHROMATIN FIBER

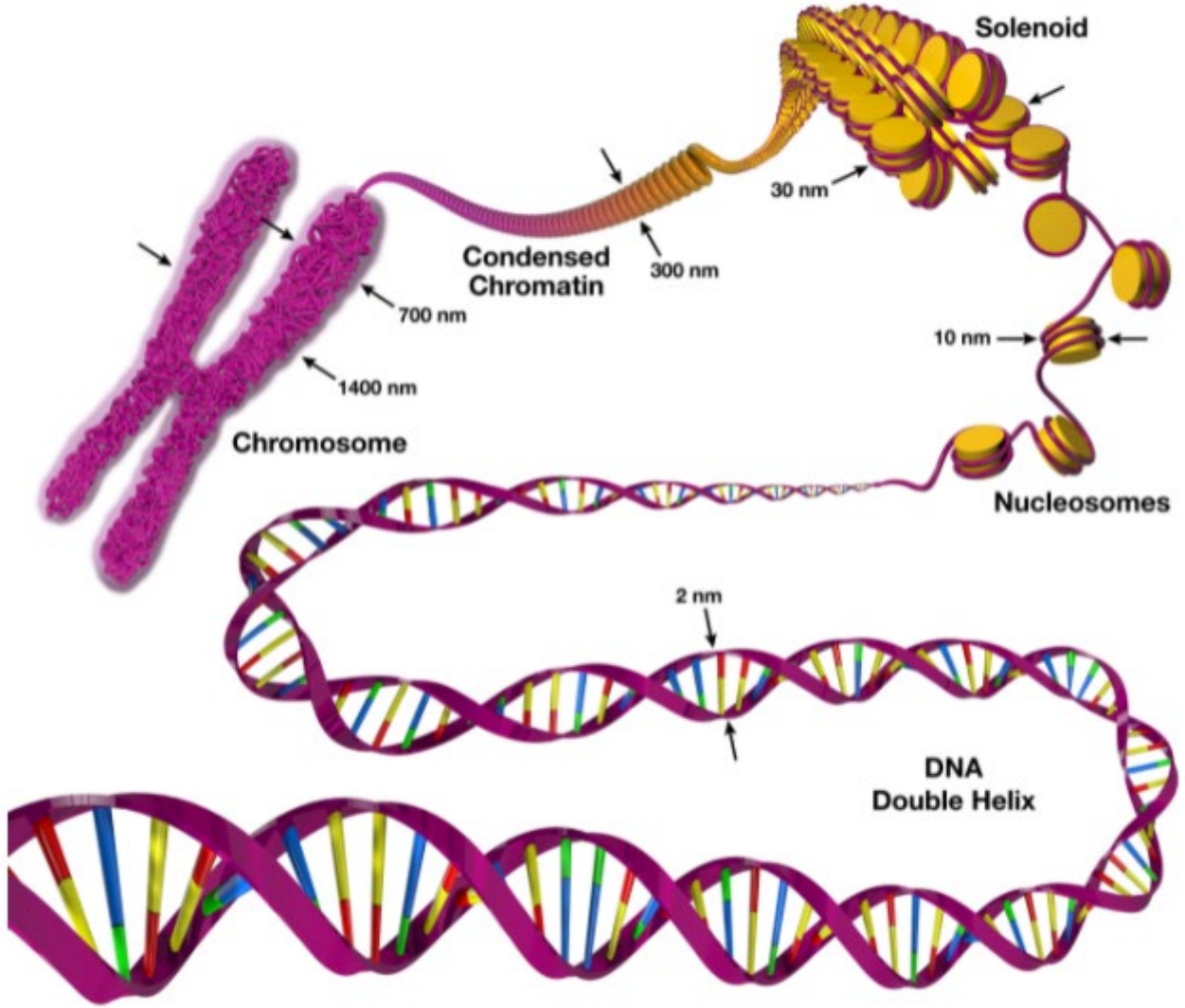
- Eukaryotic DNA Is Packaged into a Set of Chromosomes

Human genome (1C): 3.2 x 10^9 bp
6.4 X 10^9 bp DNA is distributed in 46 different chromosomes.
If DNA in a single human cell can be laid end-to-end, they would reach 2 meters.

How 2 meters of DNA can fit inside a small nucleus which is only 6 μm in diameter (equivalent to pack 40km thread in a tennis ball.)



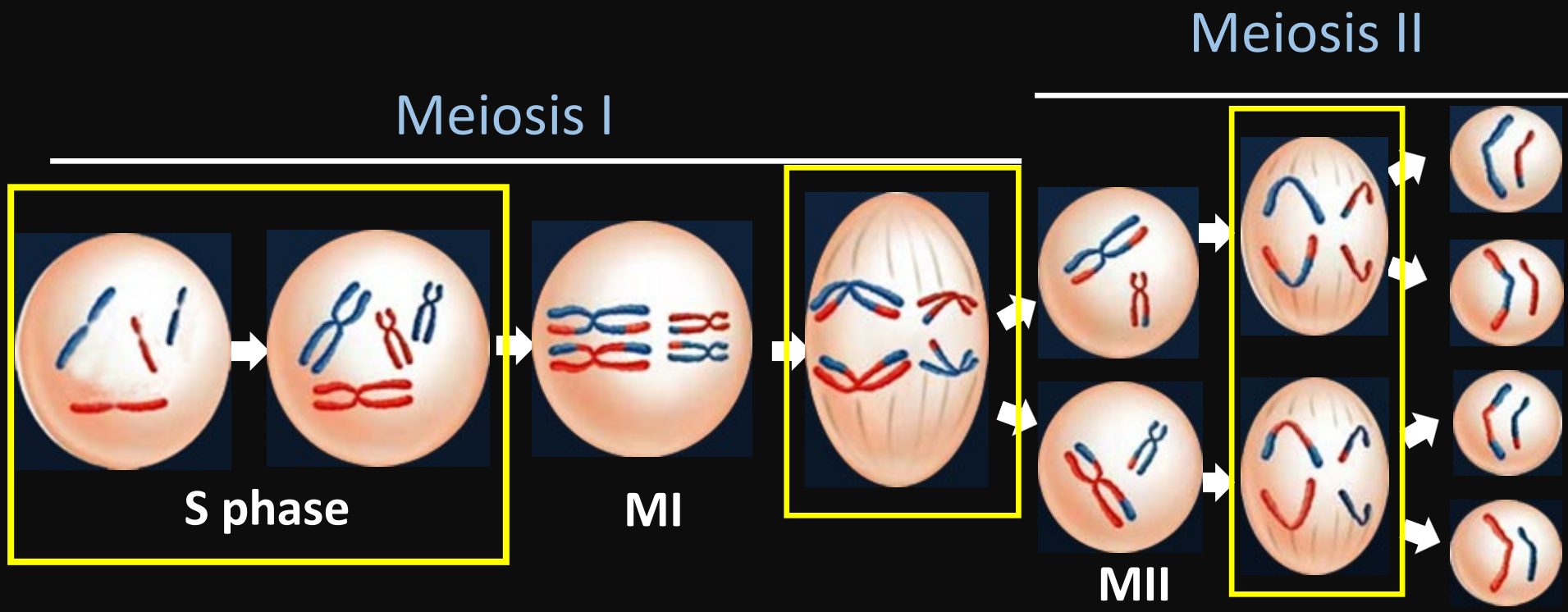
DNA-> 染色質->染色體


Solenoid
30 nm
Condensed
Chromatin
300 nm
700 nm
1400 nm
Chromosome
10 nm
Nucleosomes
2 nm
DNA
Double Helix

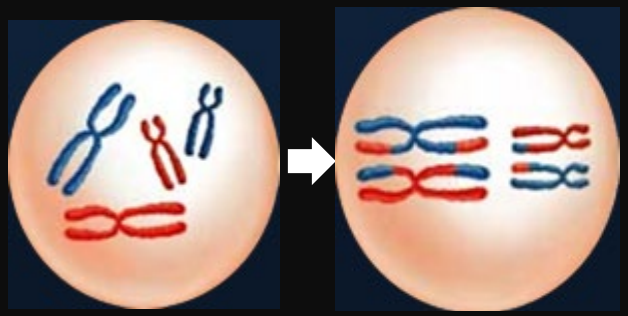
減數分裂

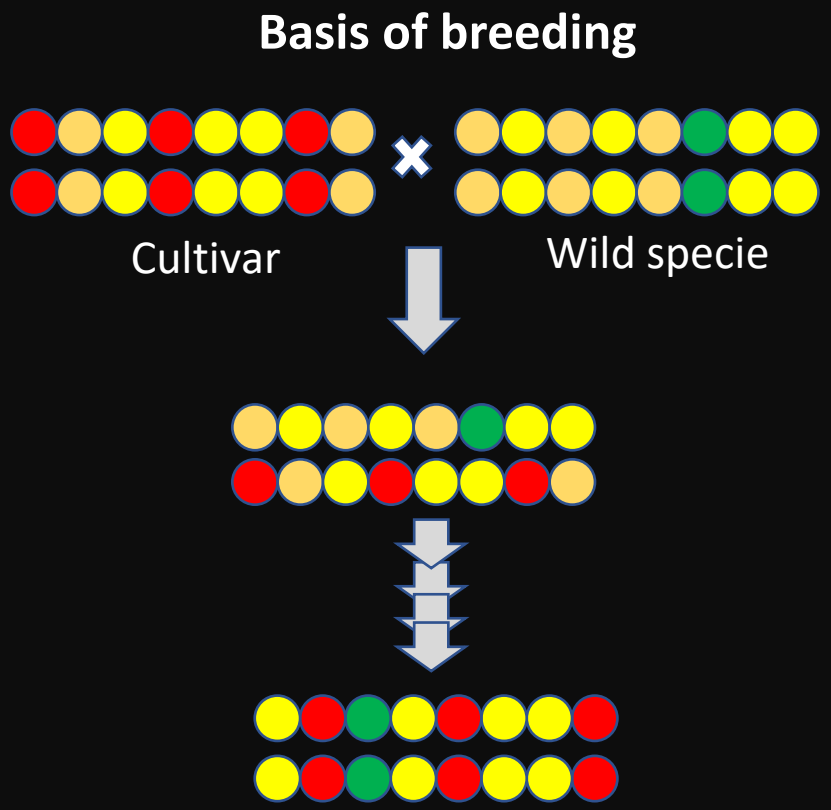
—染色體數目減半並獨立分配
—基因重組(染色體互換)

Meiosis



The mystery:
Homologous pairing and recombination



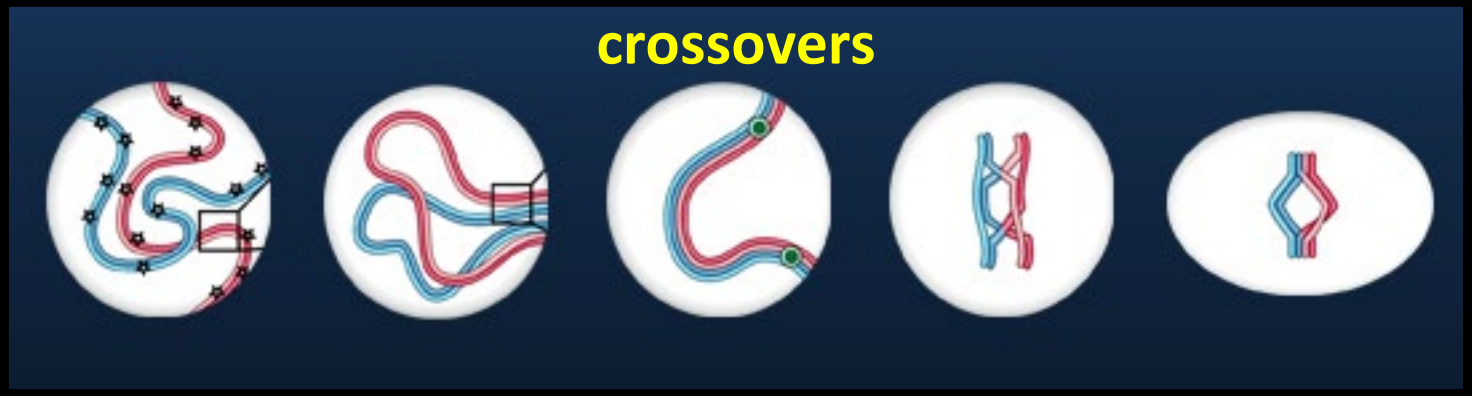


不管是同源染色體正確分離或是互換，

都要先配對

怎麼配????

Major processes of meiotic prophase I



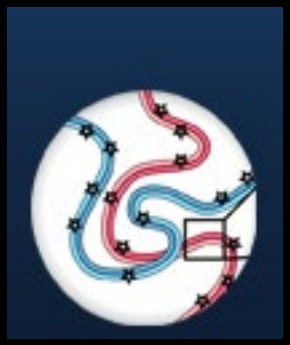
Leptotene Zygotene Pachytene Diplotene Diakinesis

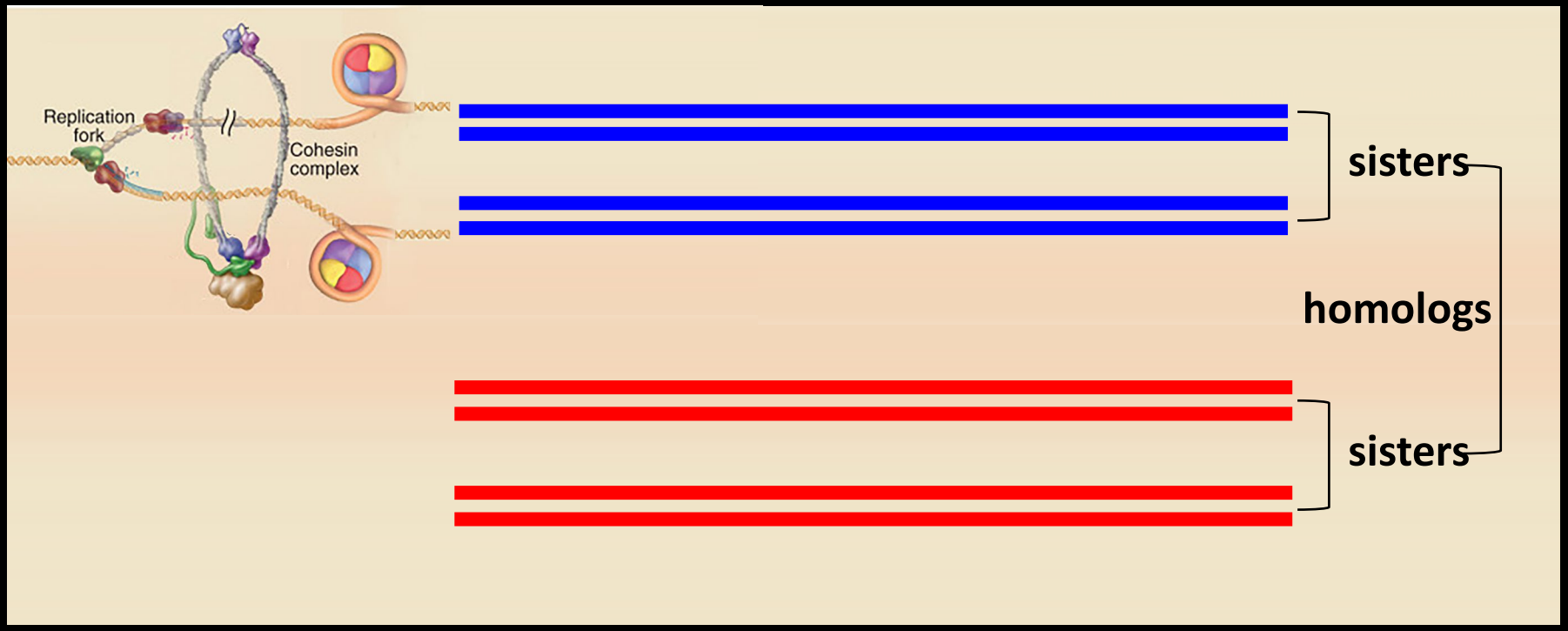
recombination

synapsis

pairing

The DSB repair pathway





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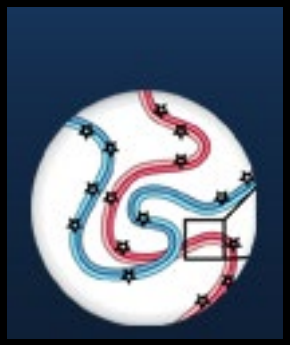

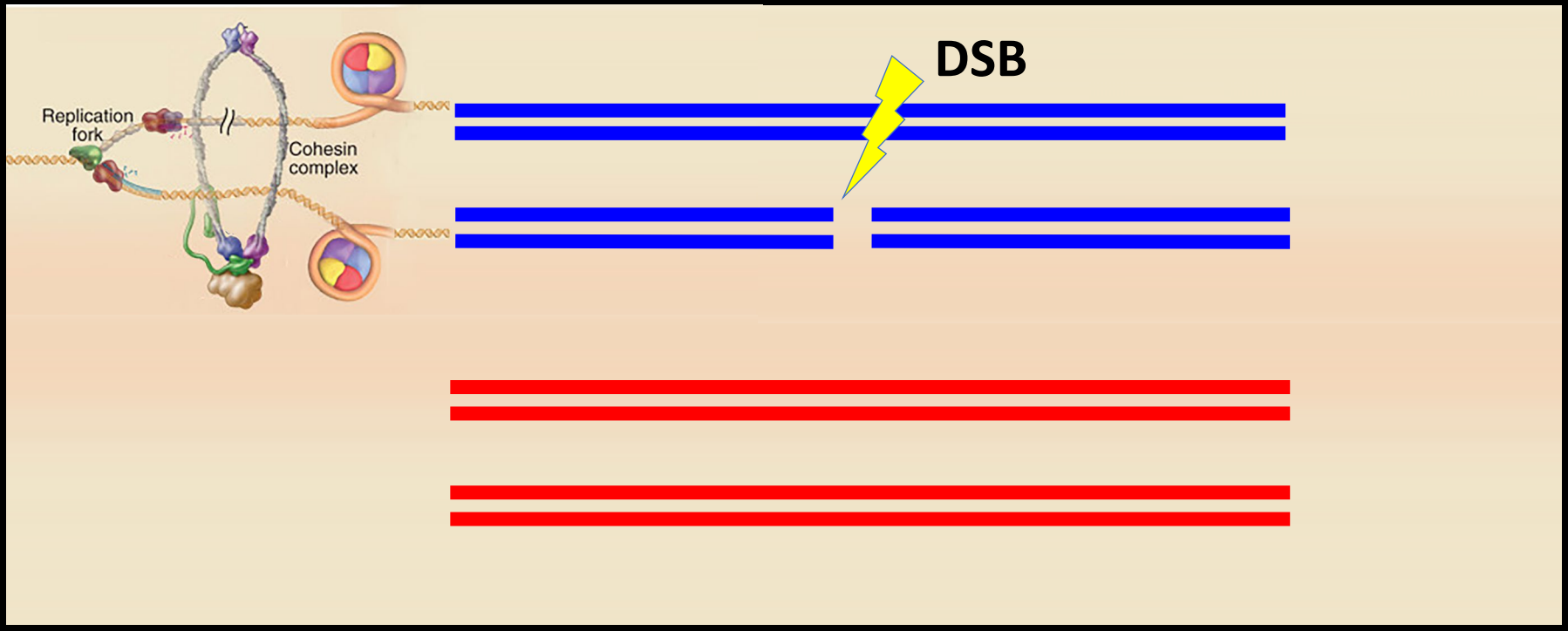

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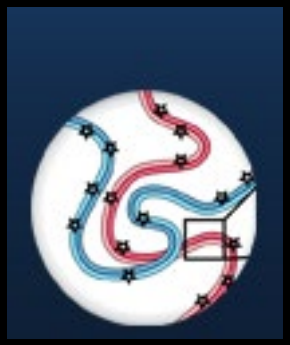

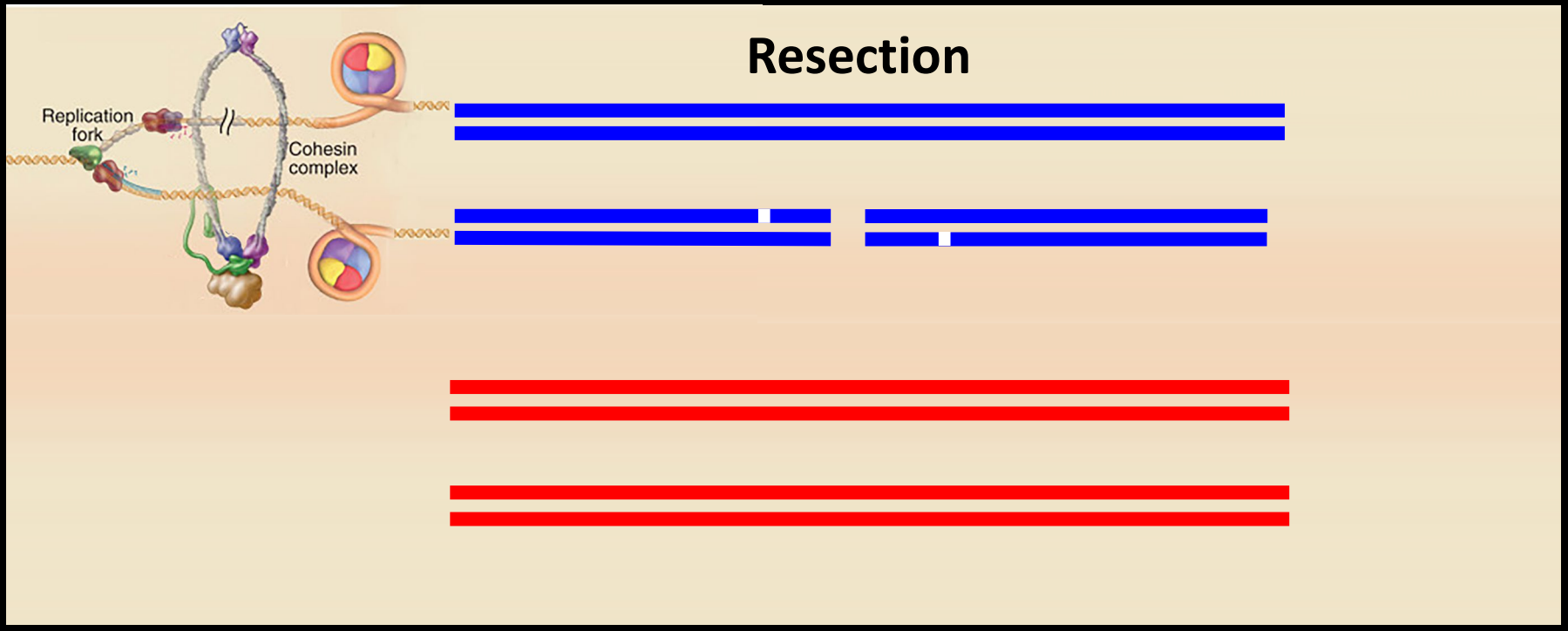

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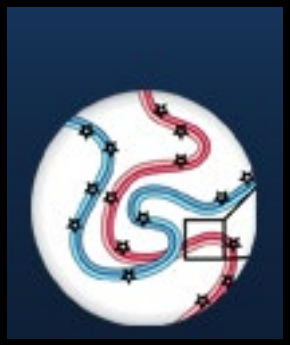

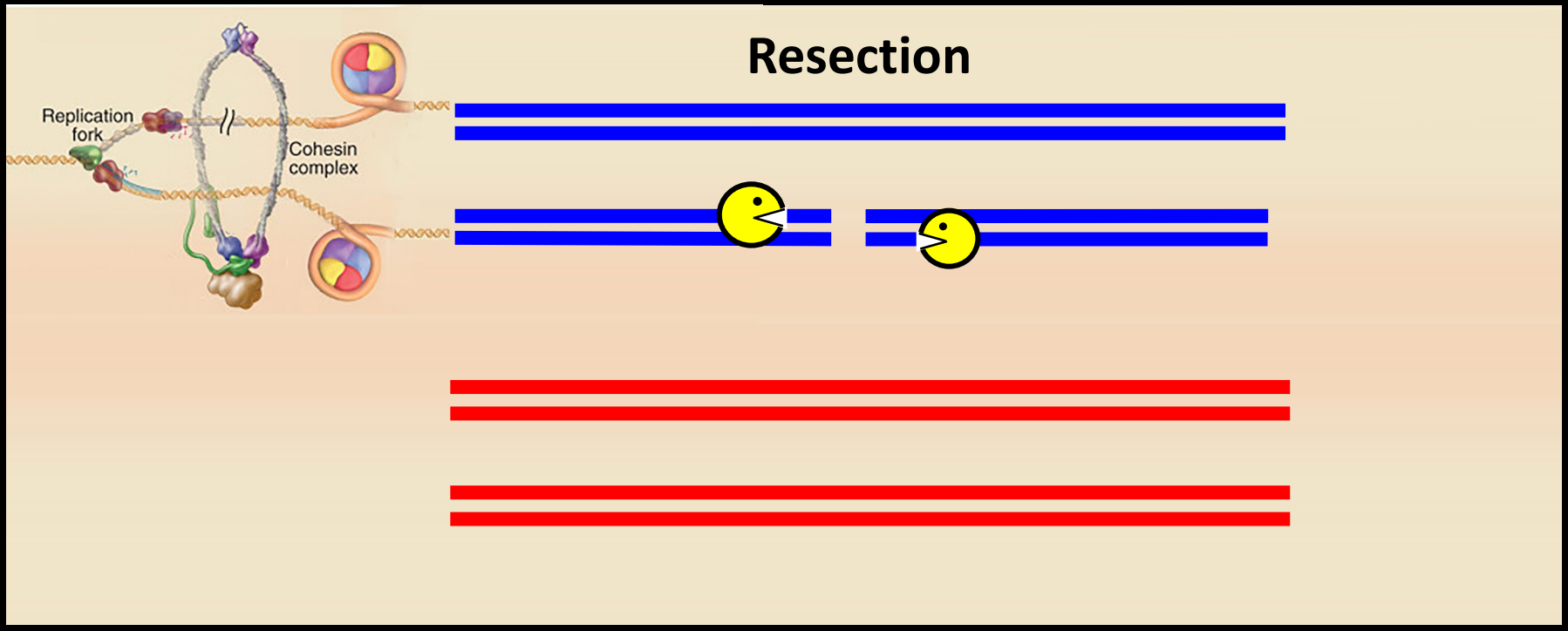

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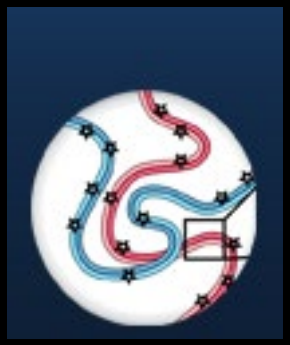

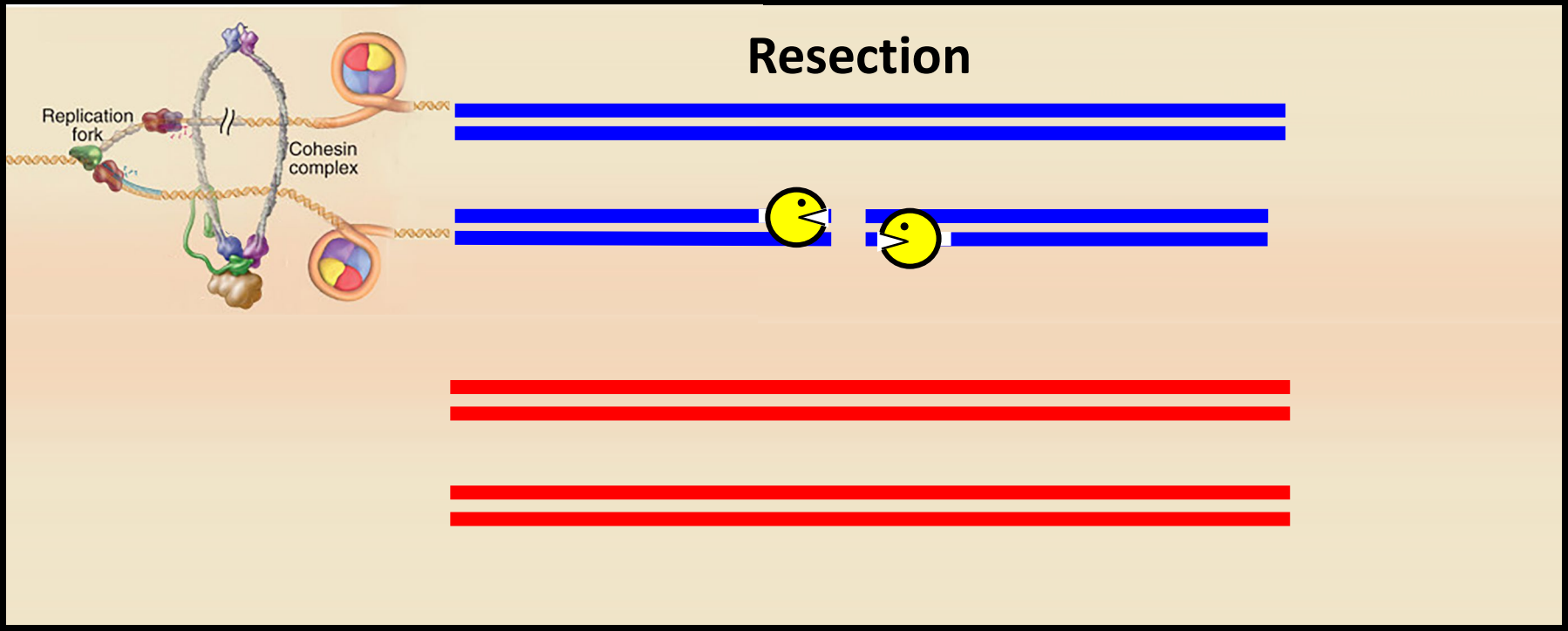

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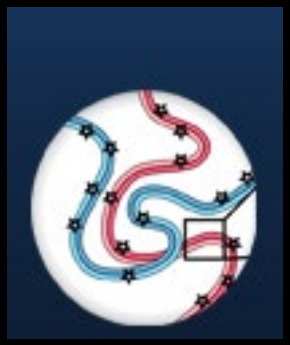

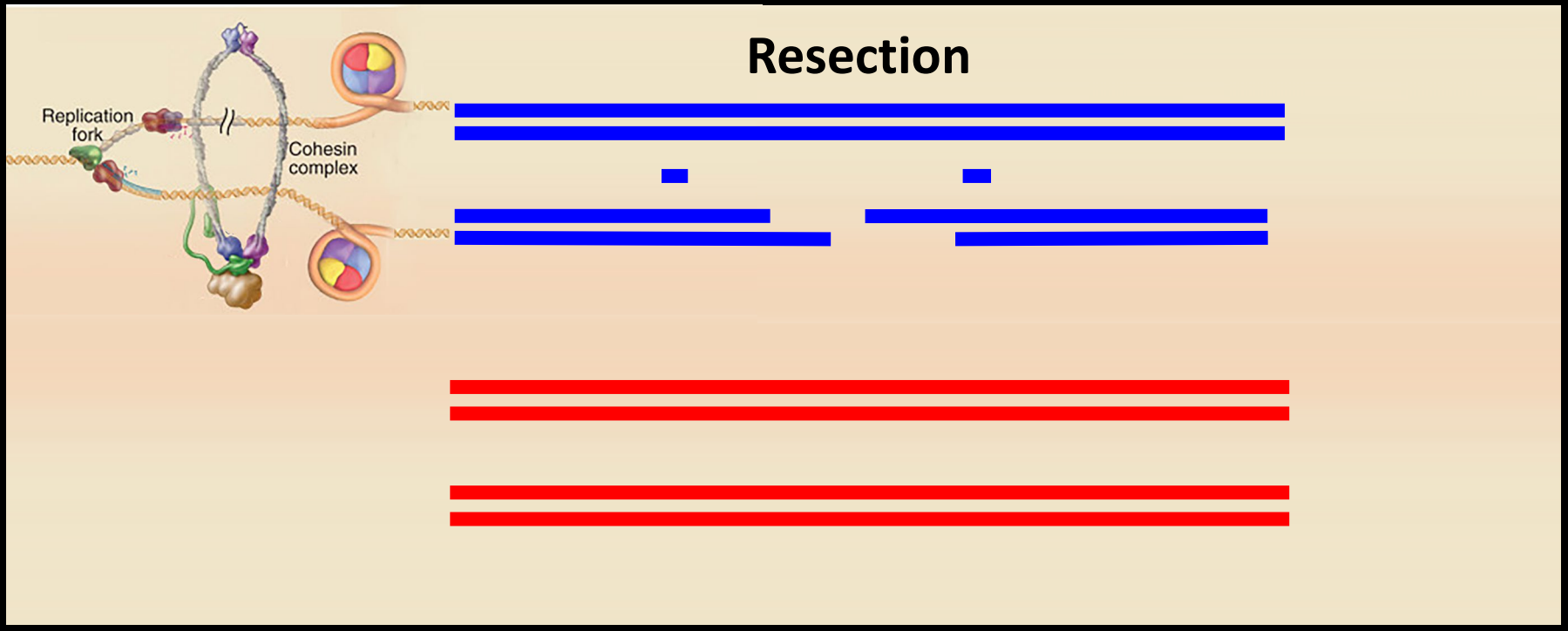

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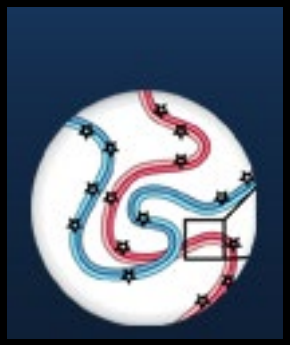

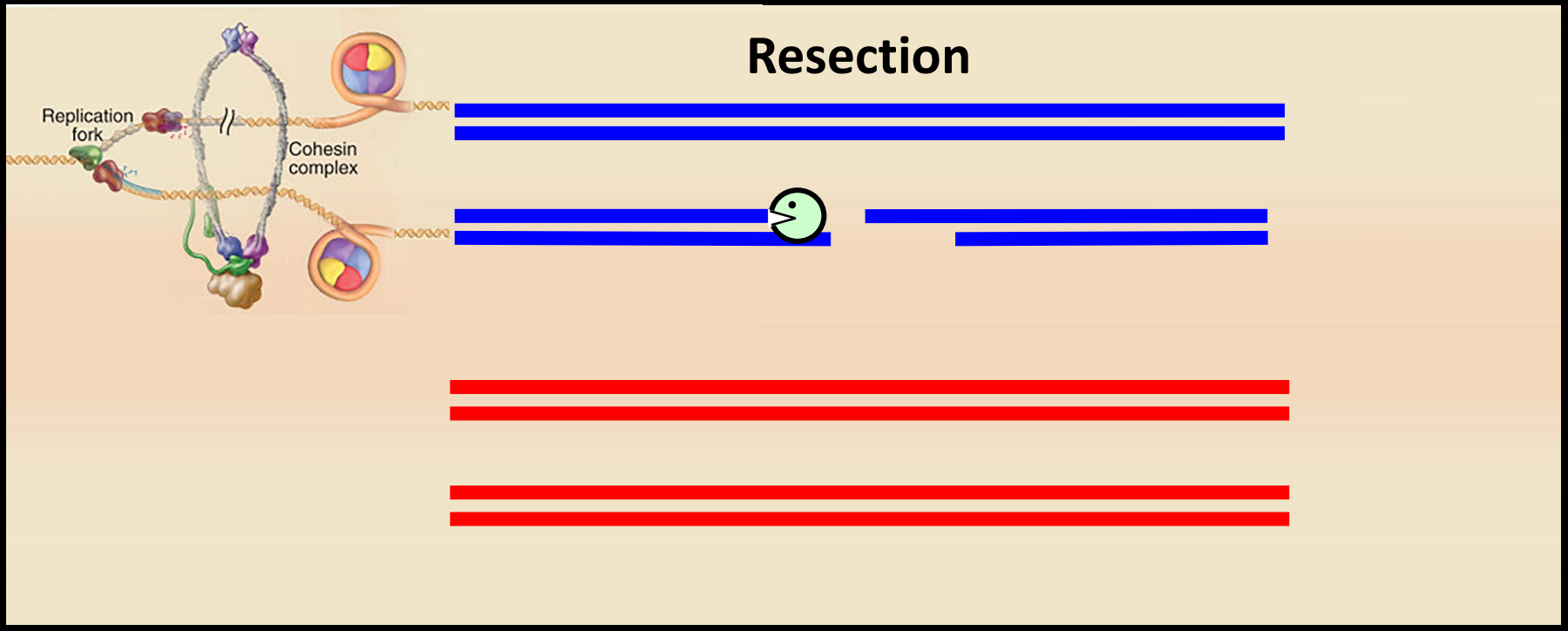

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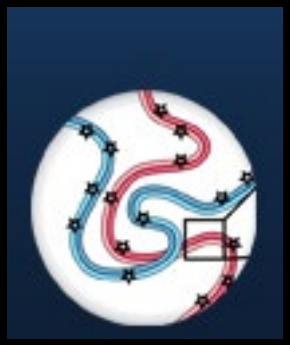

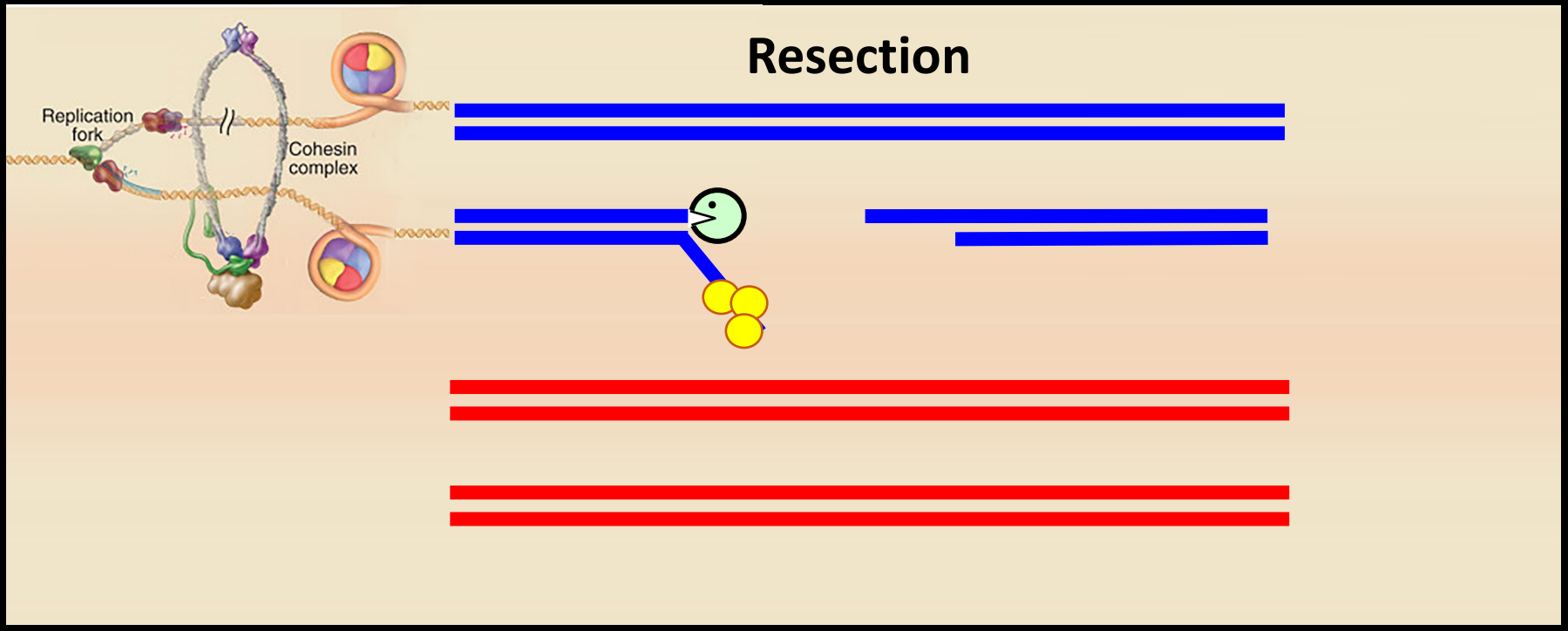

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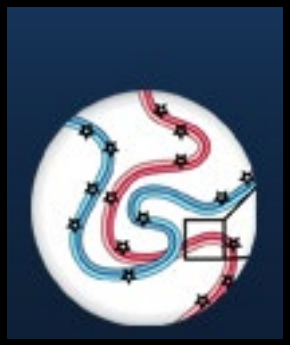

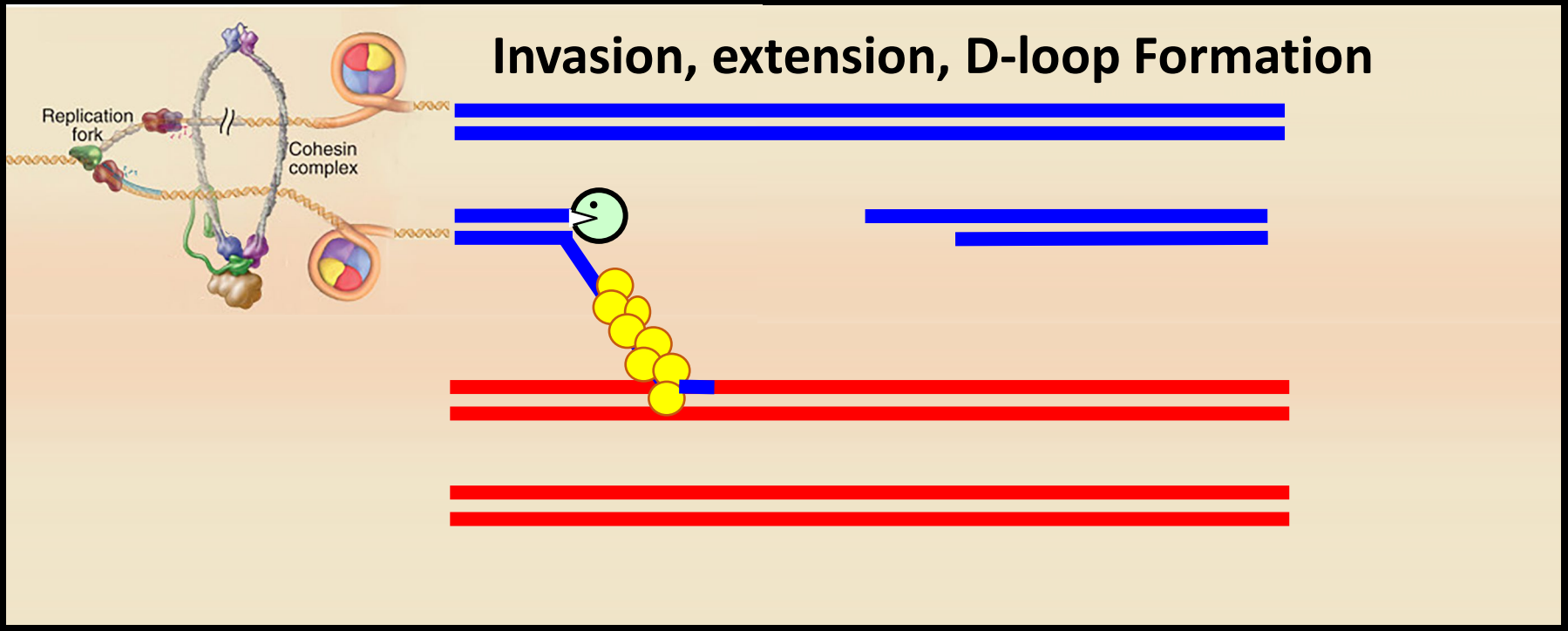

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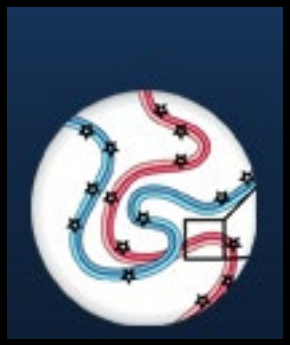

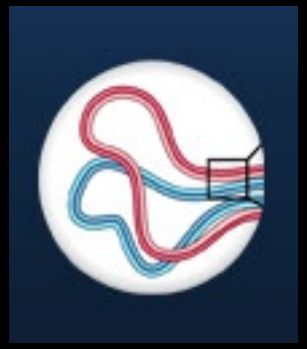

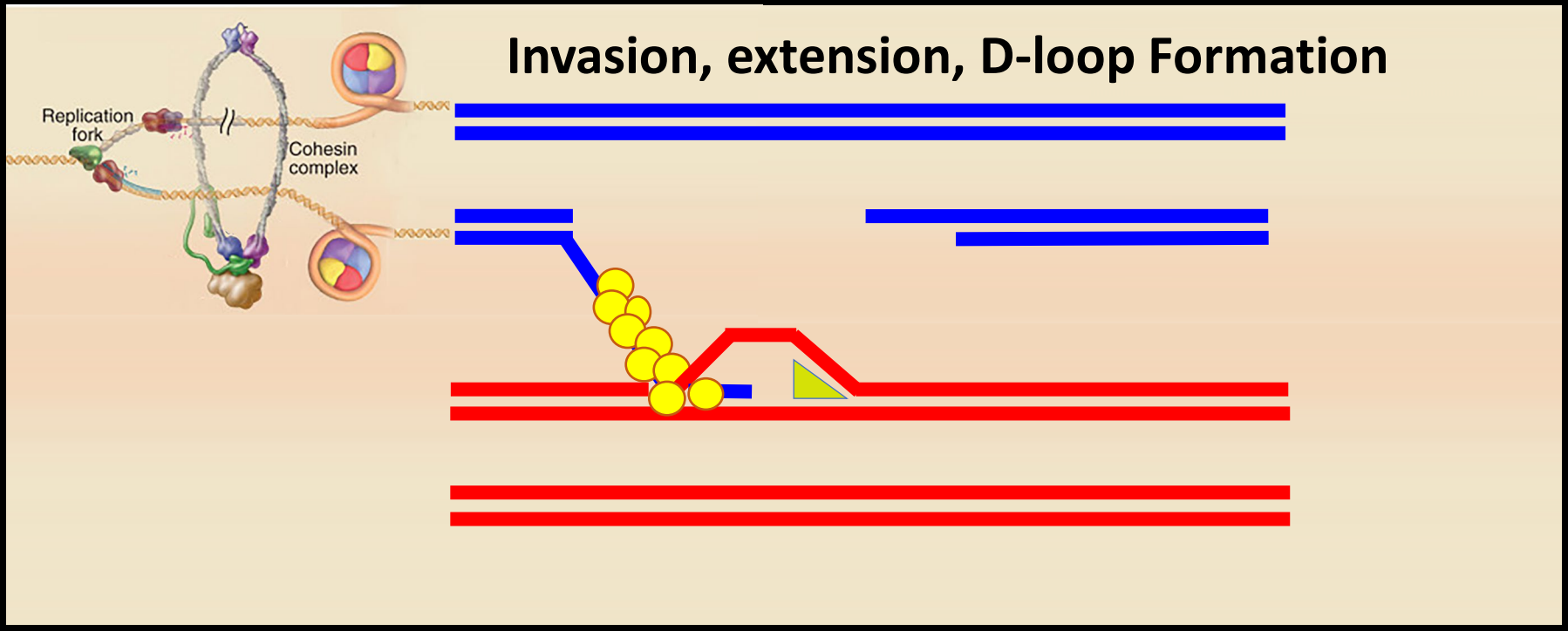

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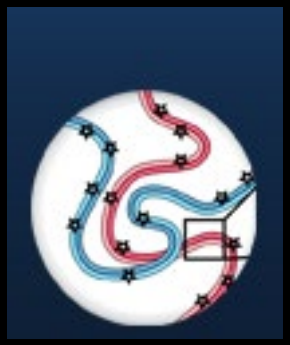

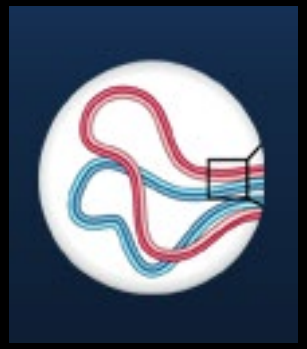

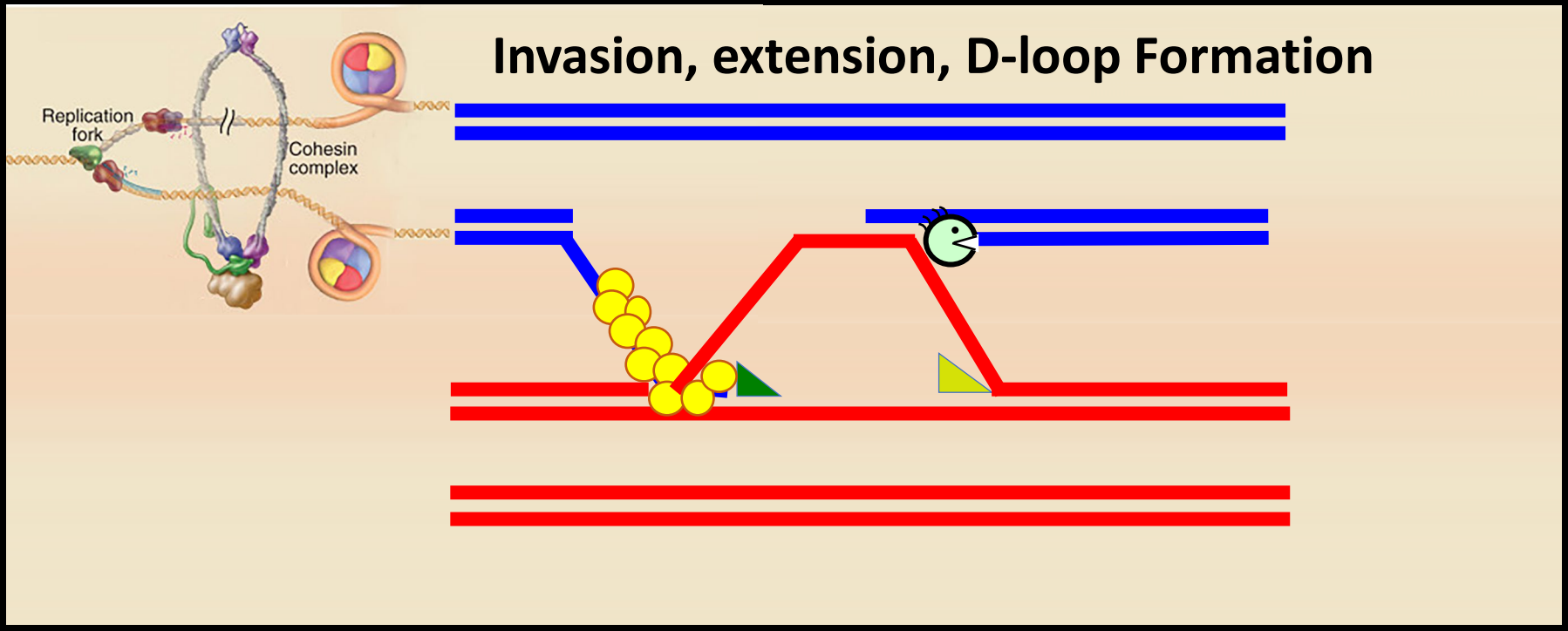

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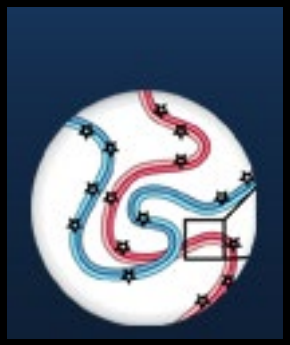

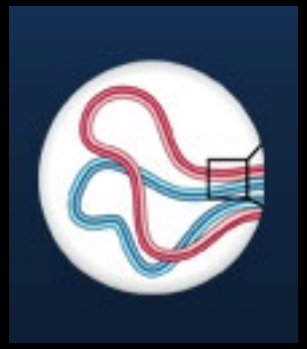

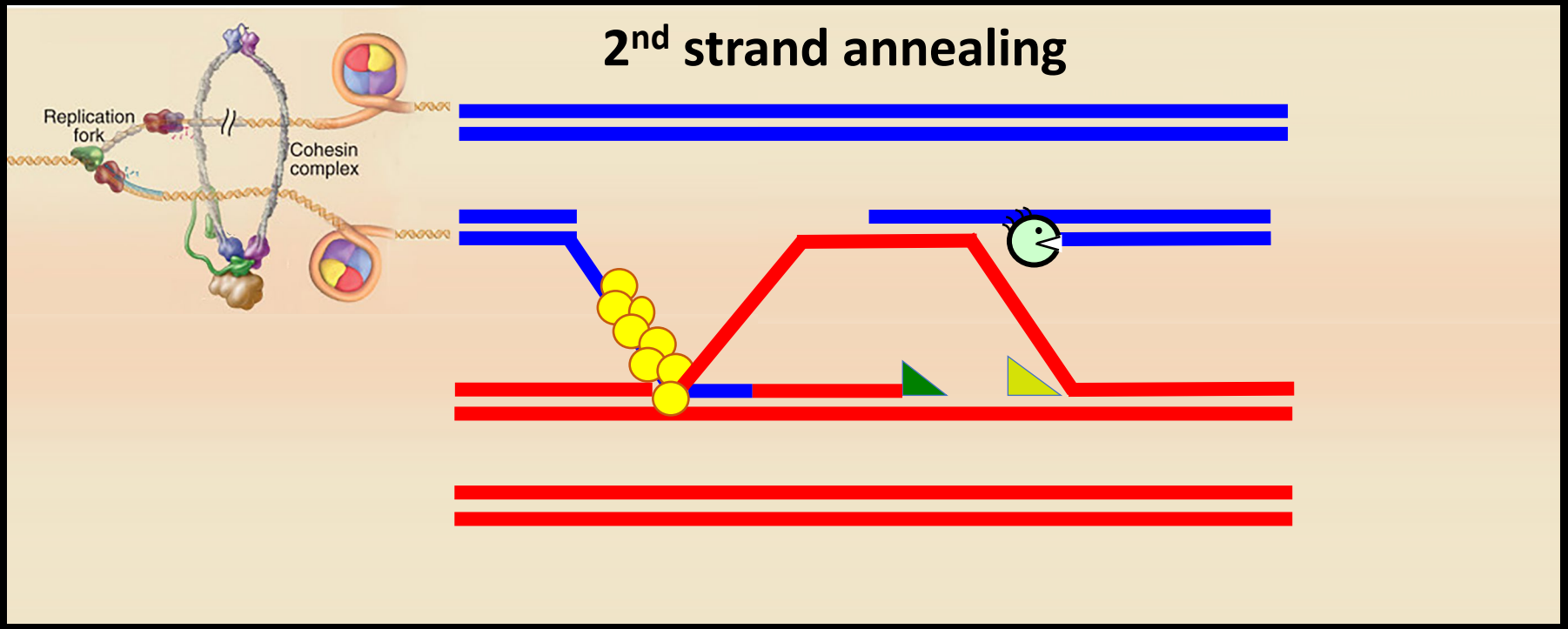

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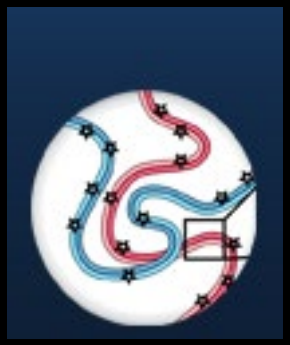

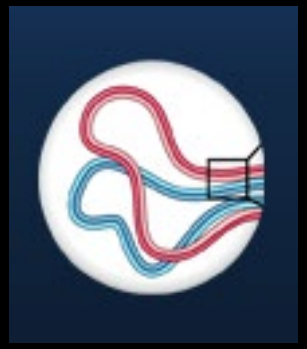

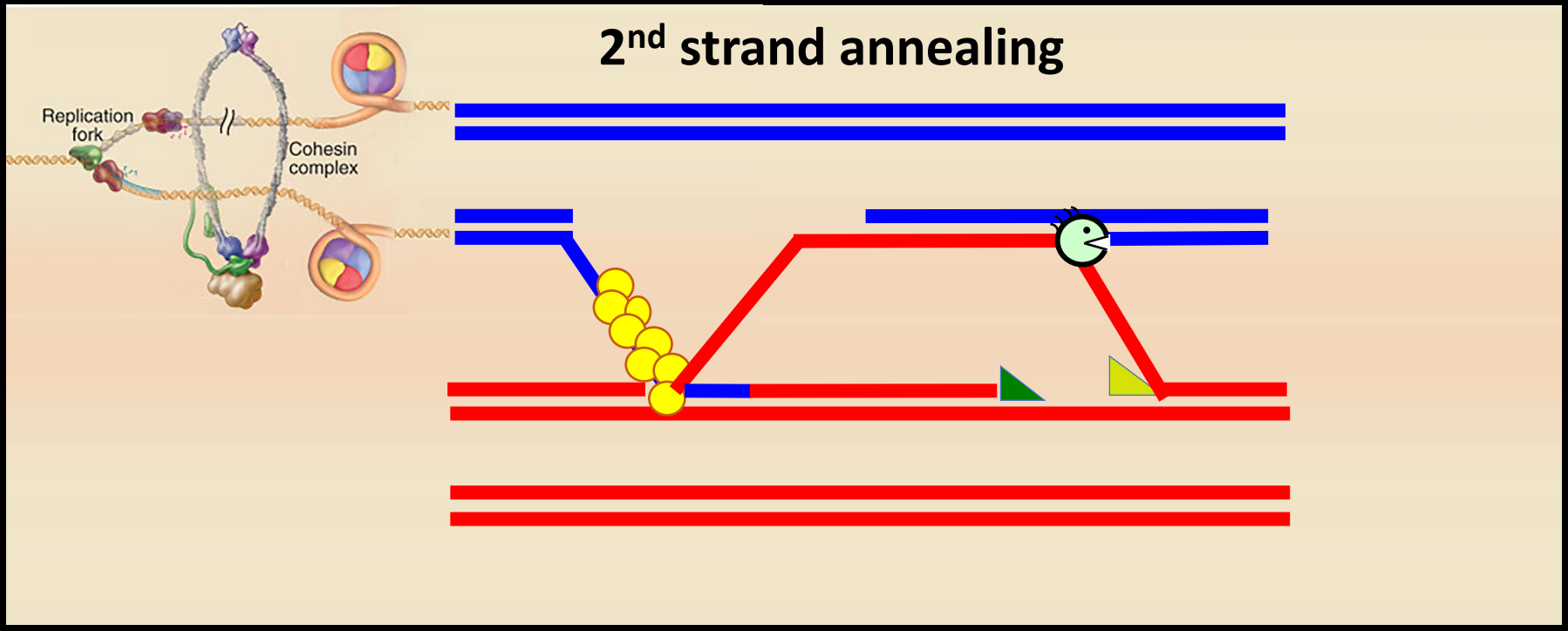

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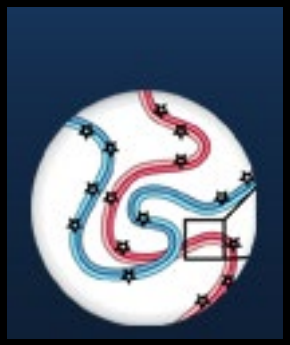
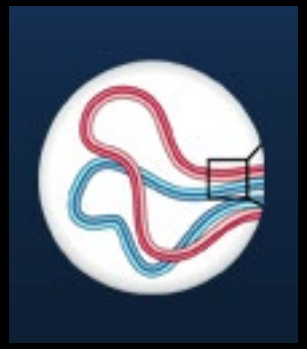
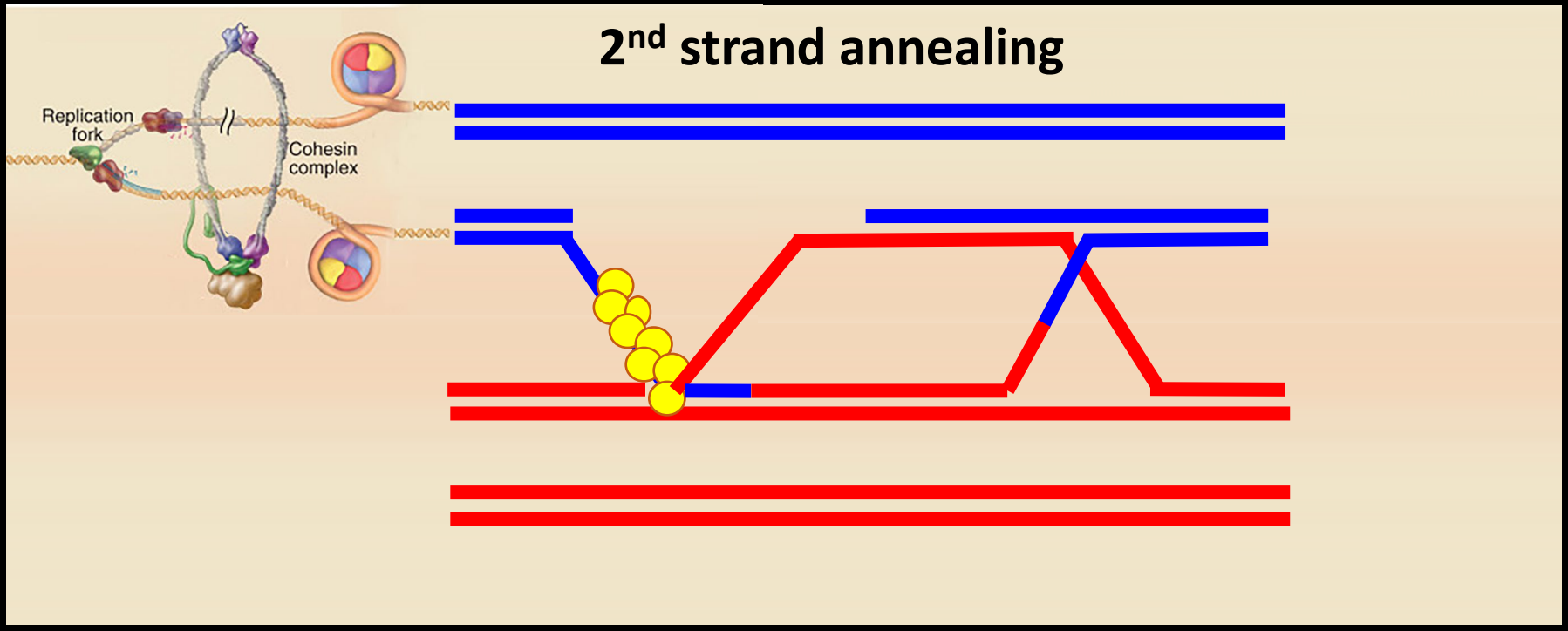
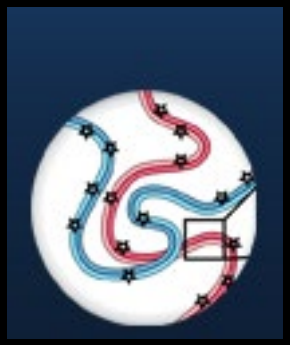
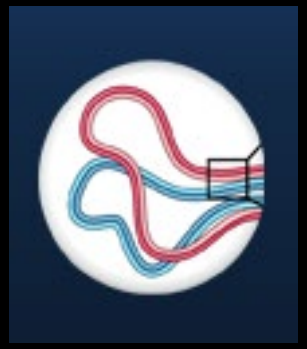


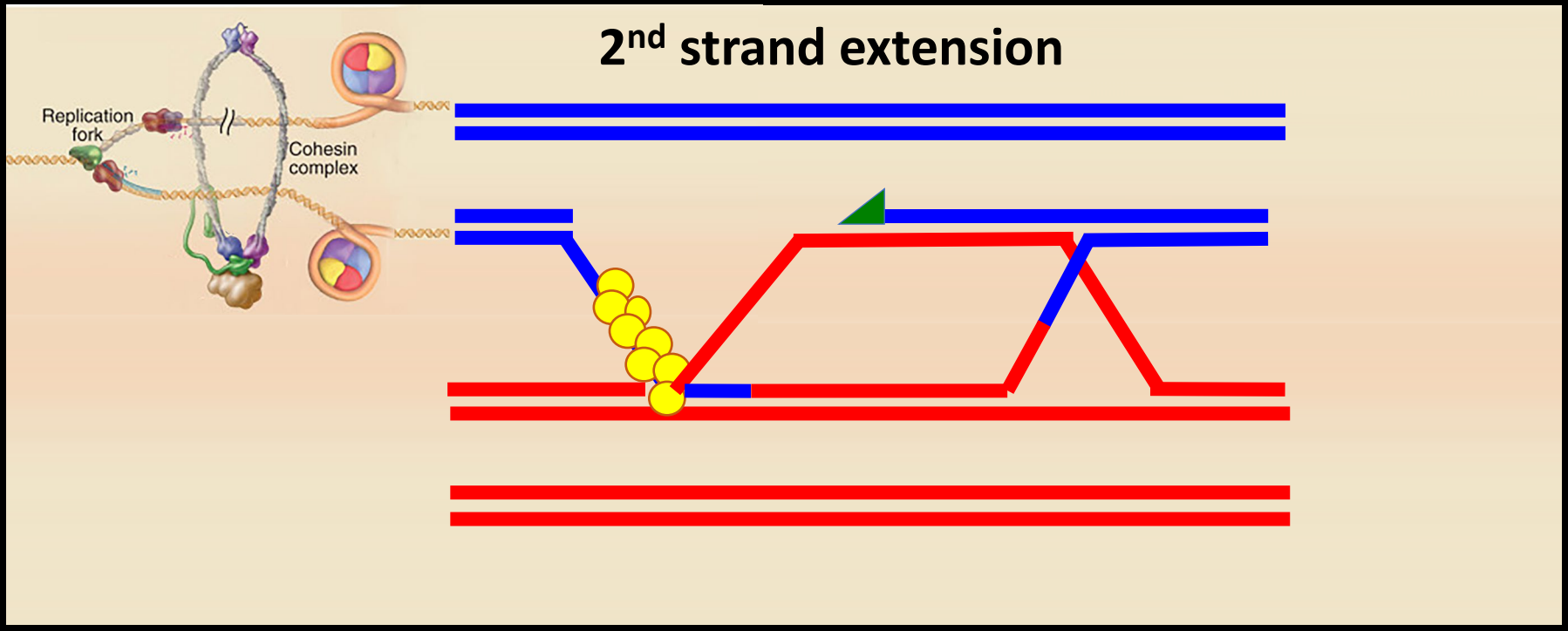




The DSB repair pathway







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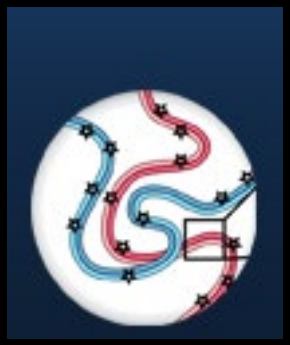

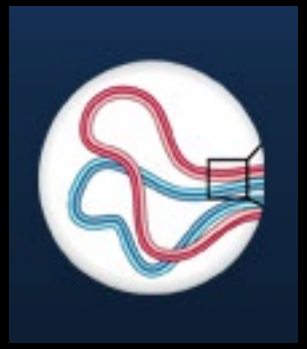

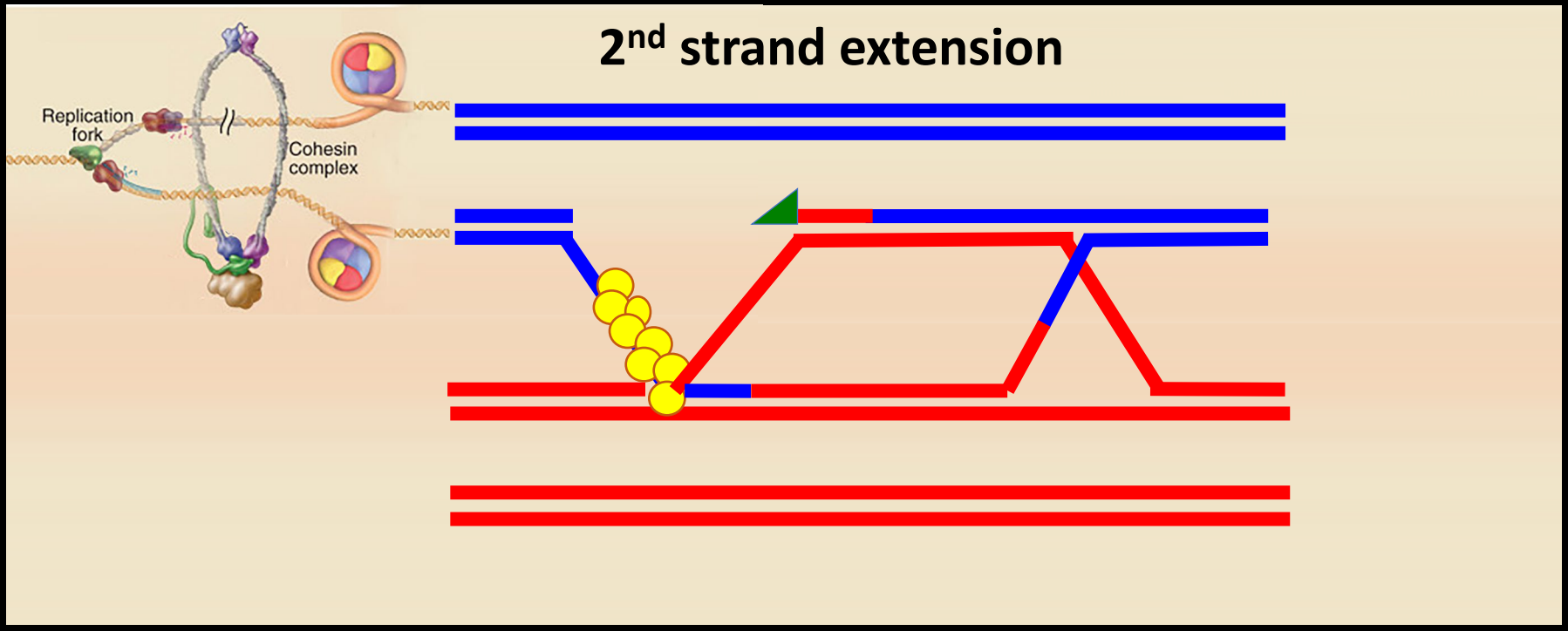

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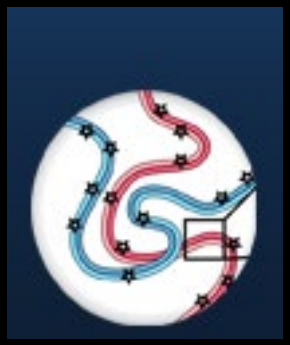
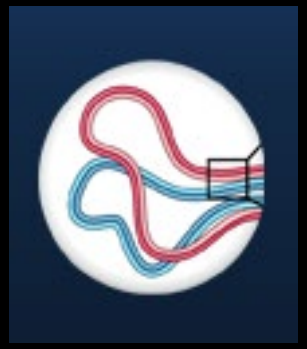
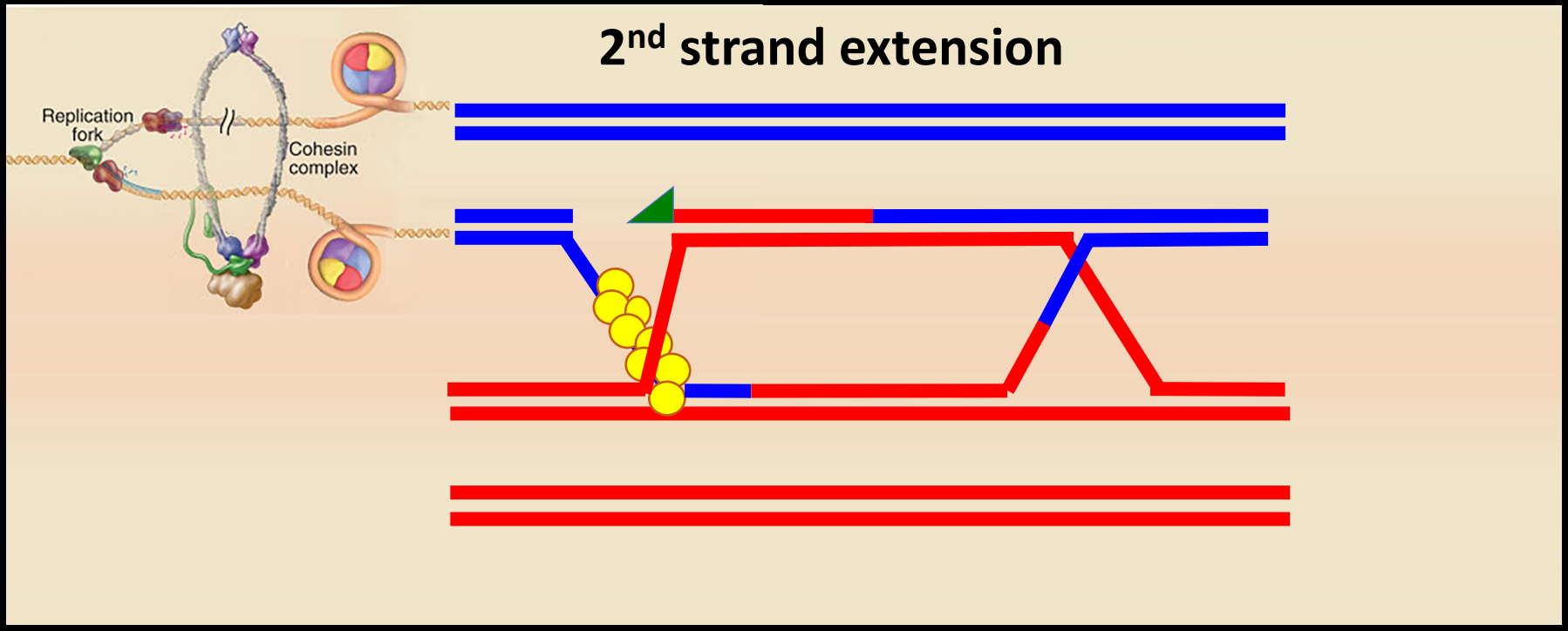
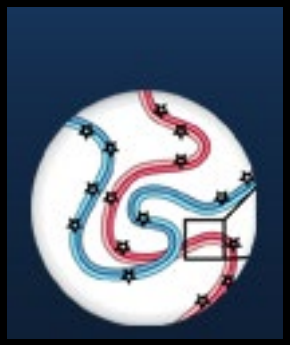
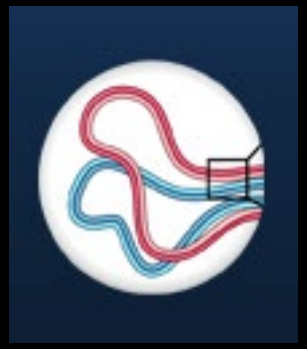


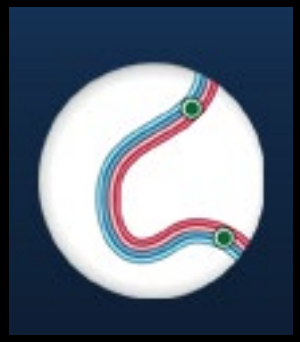


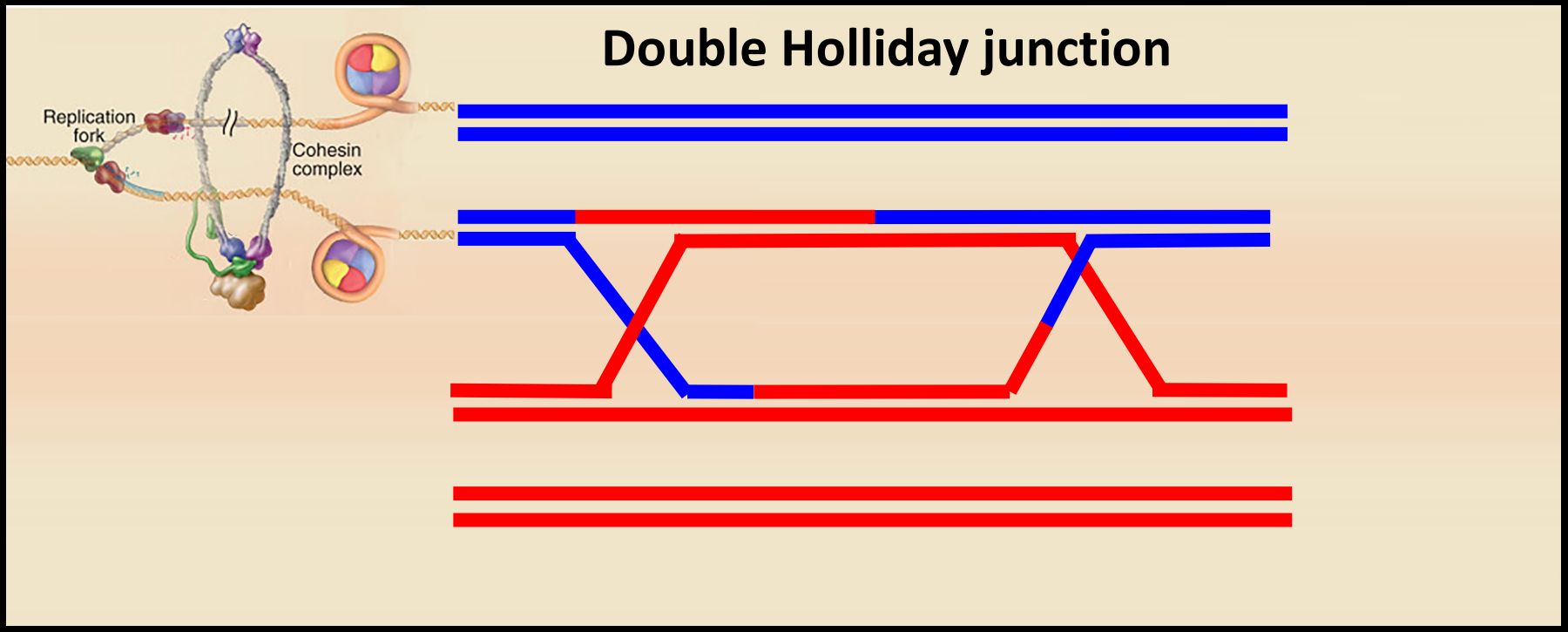


The DSB repair pathway

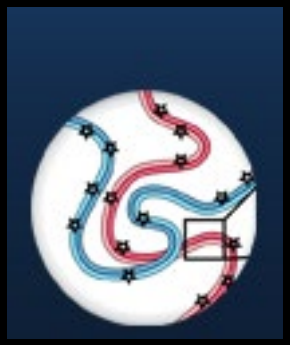


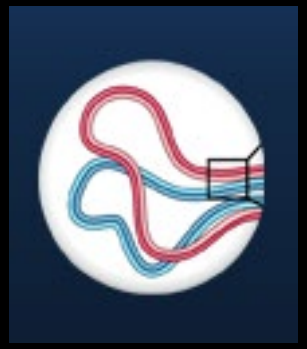


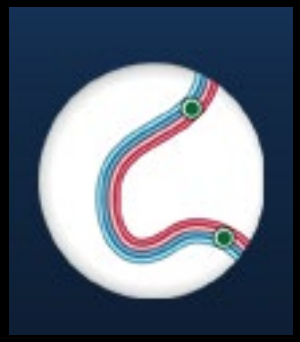


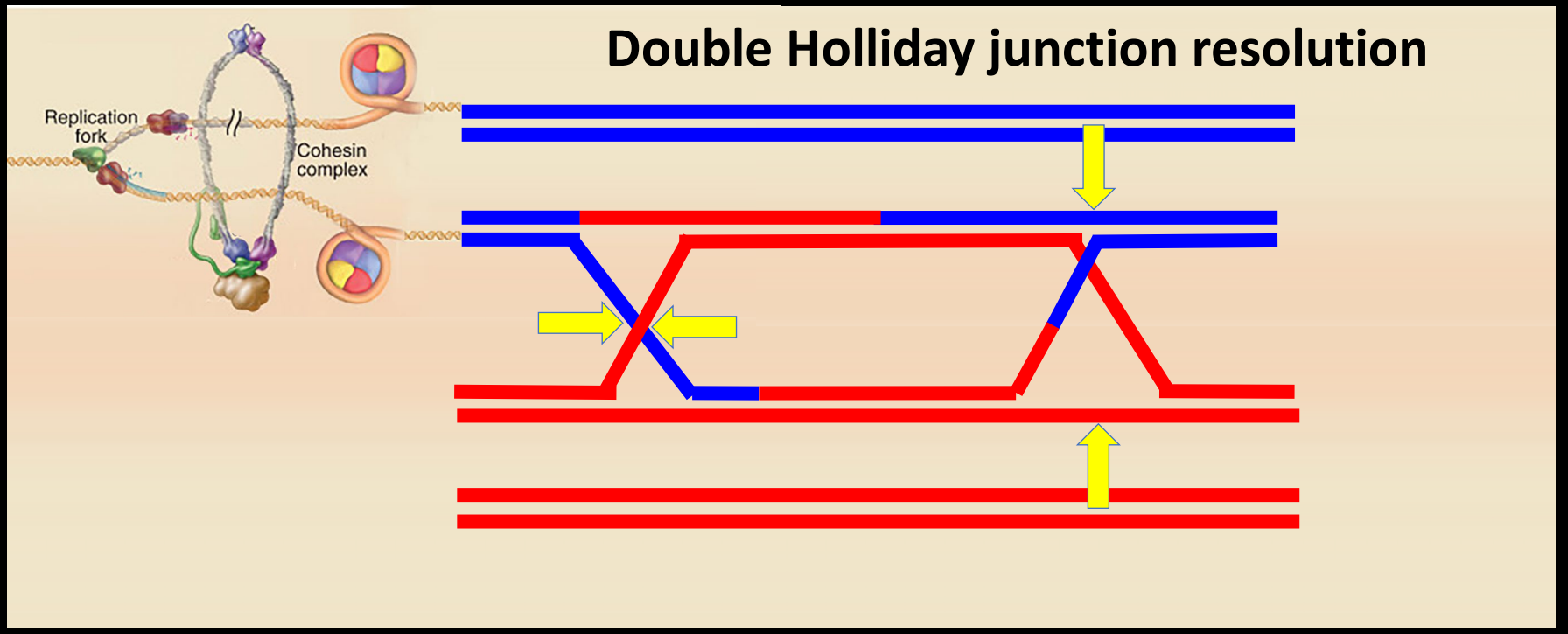


The DSB repair pathway

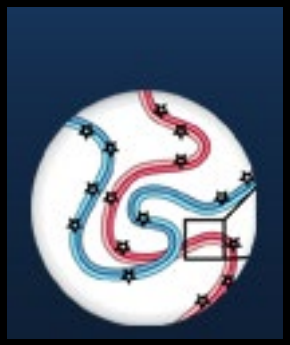


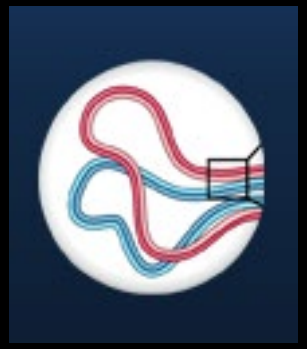


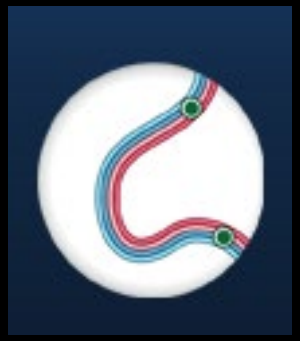


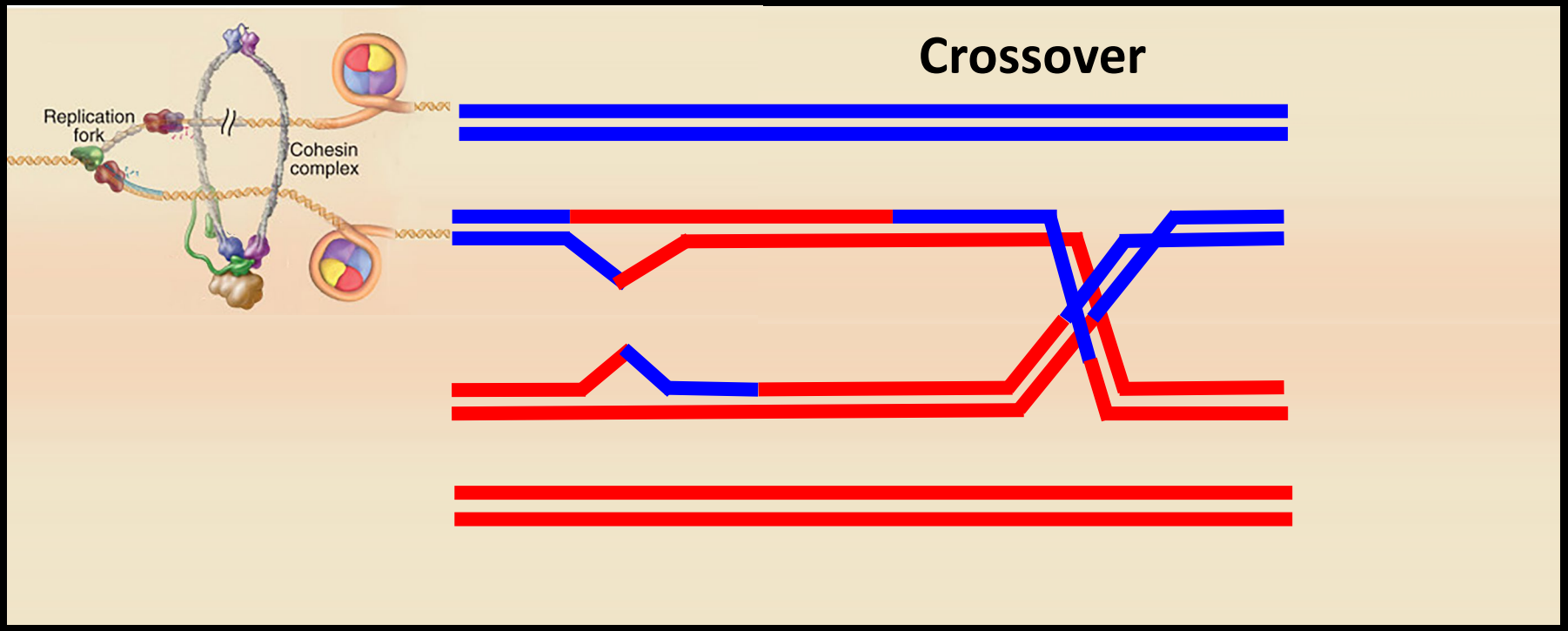


The DSB repair pathway

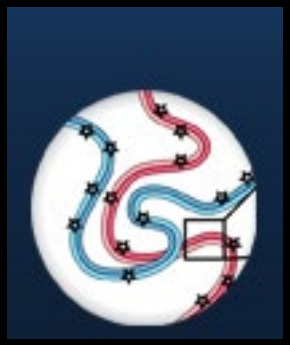
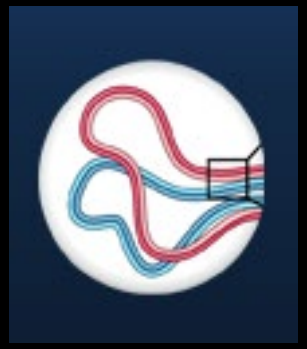
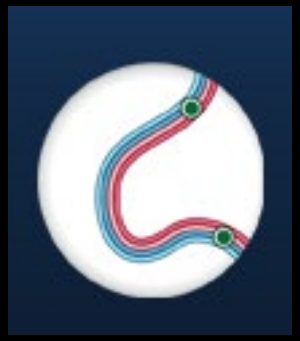


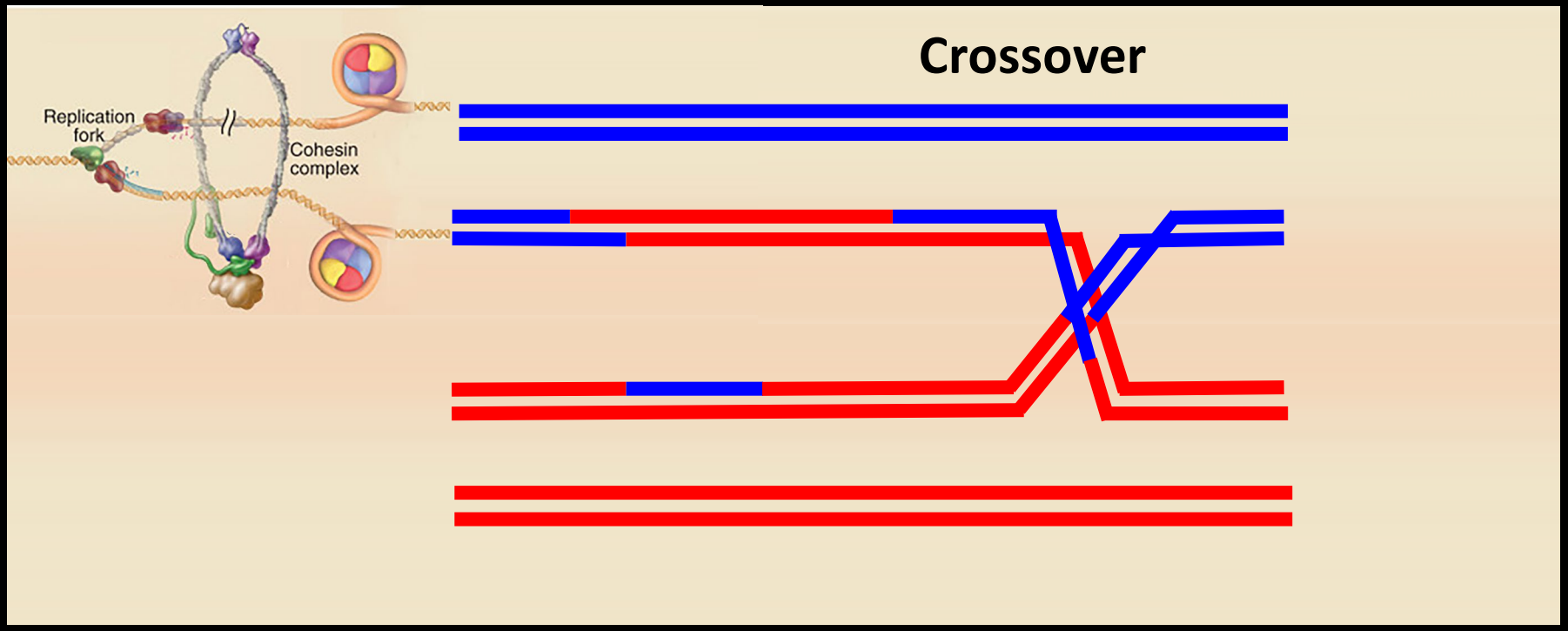






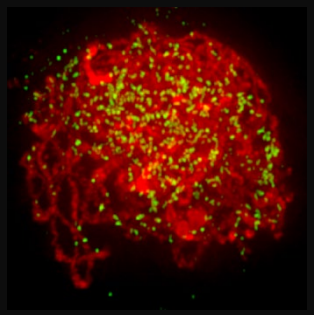
The DSB repair pathway

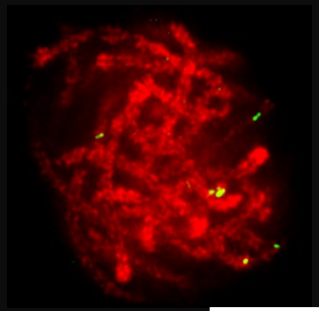



From DSBs to COs with interference

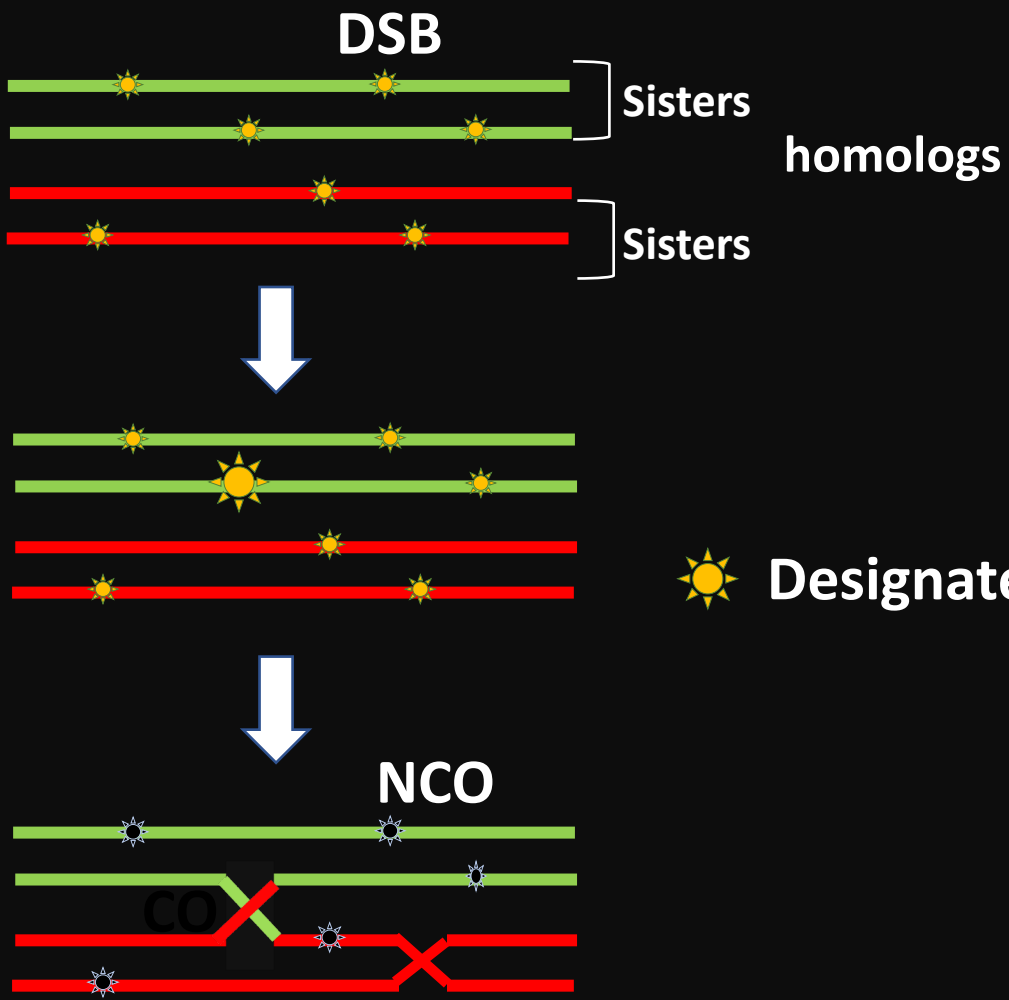
500 RAD51 foci



20 RAD51 foci



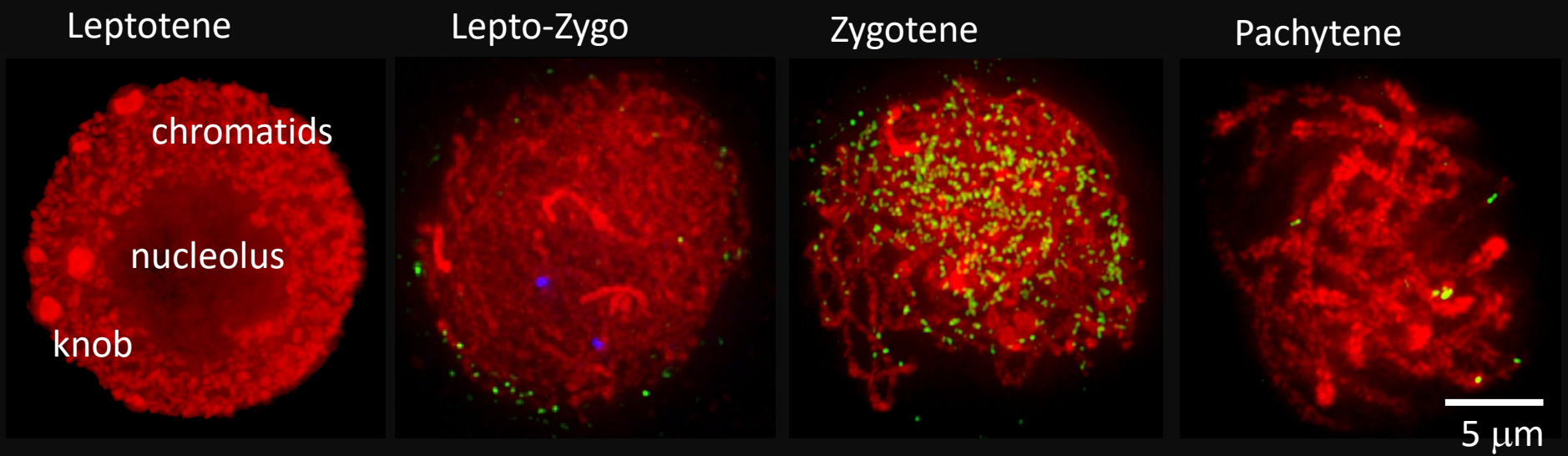
5 µm



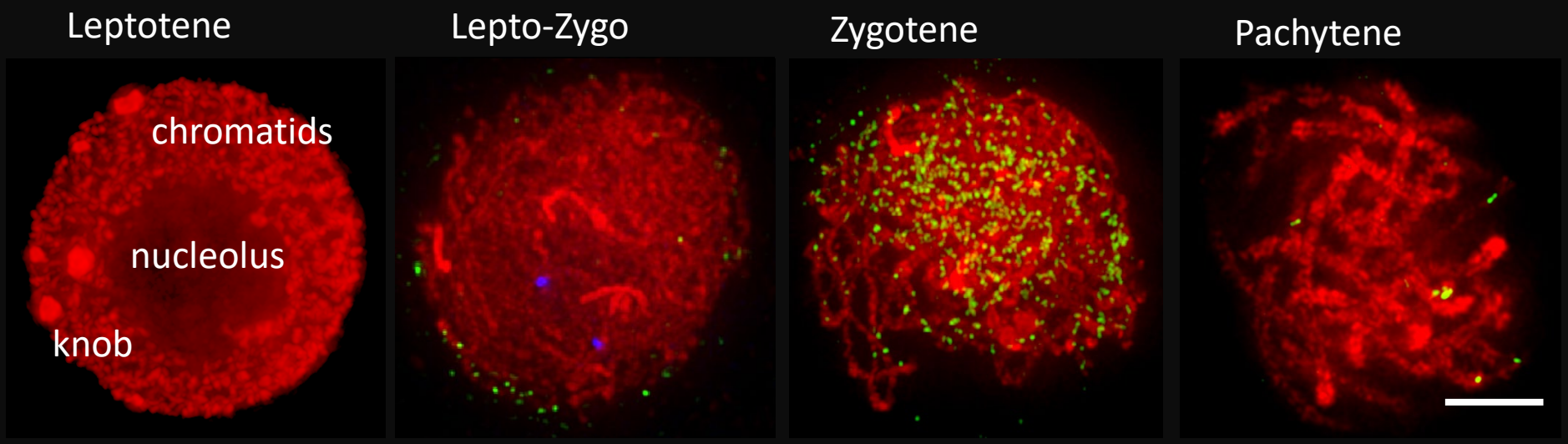
Designated DSB

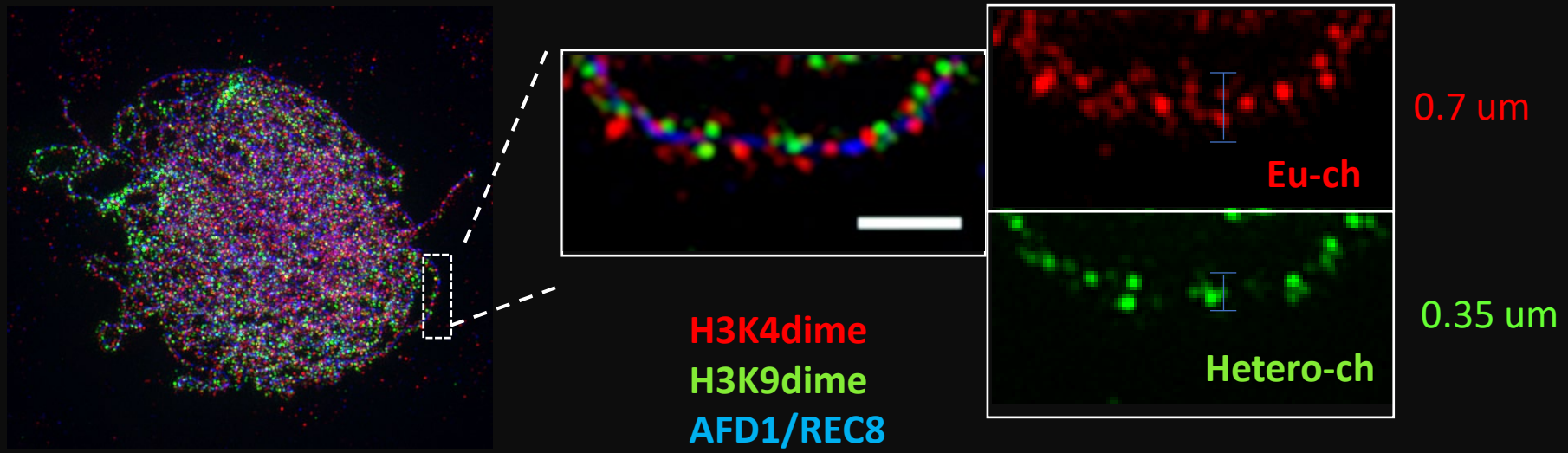
SCE (sister chromatid exchange)

Male Meiosis in Maize



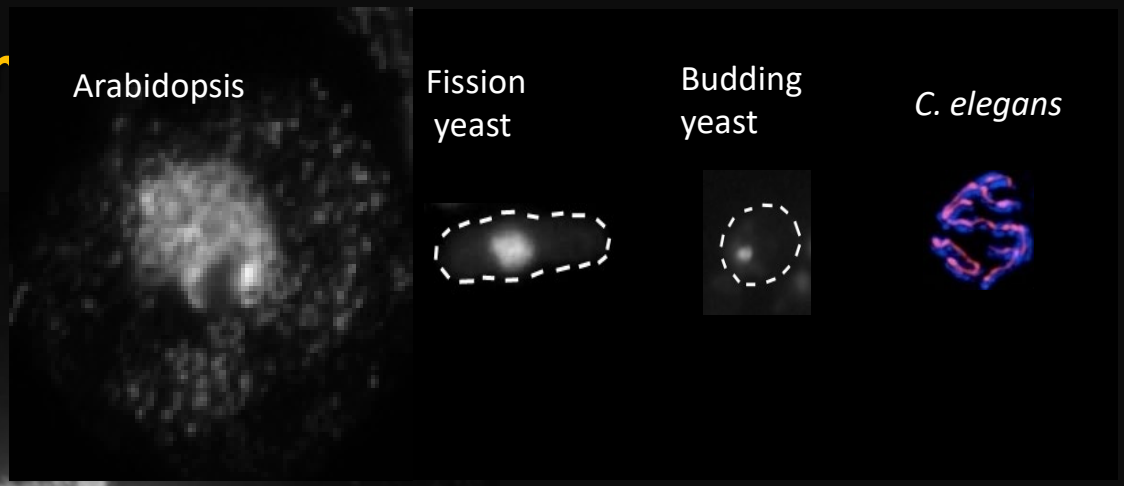
Male Meiosis in Maize

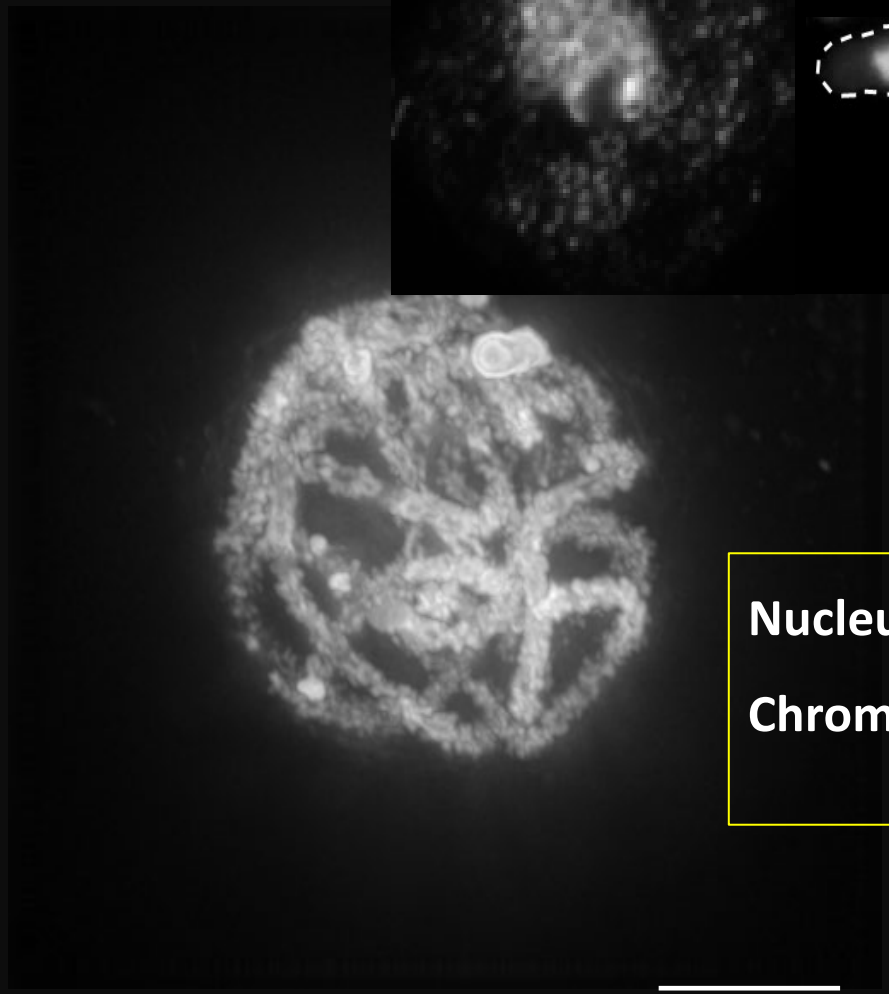




Super-resolution microscopy

Maize meiotic chrom





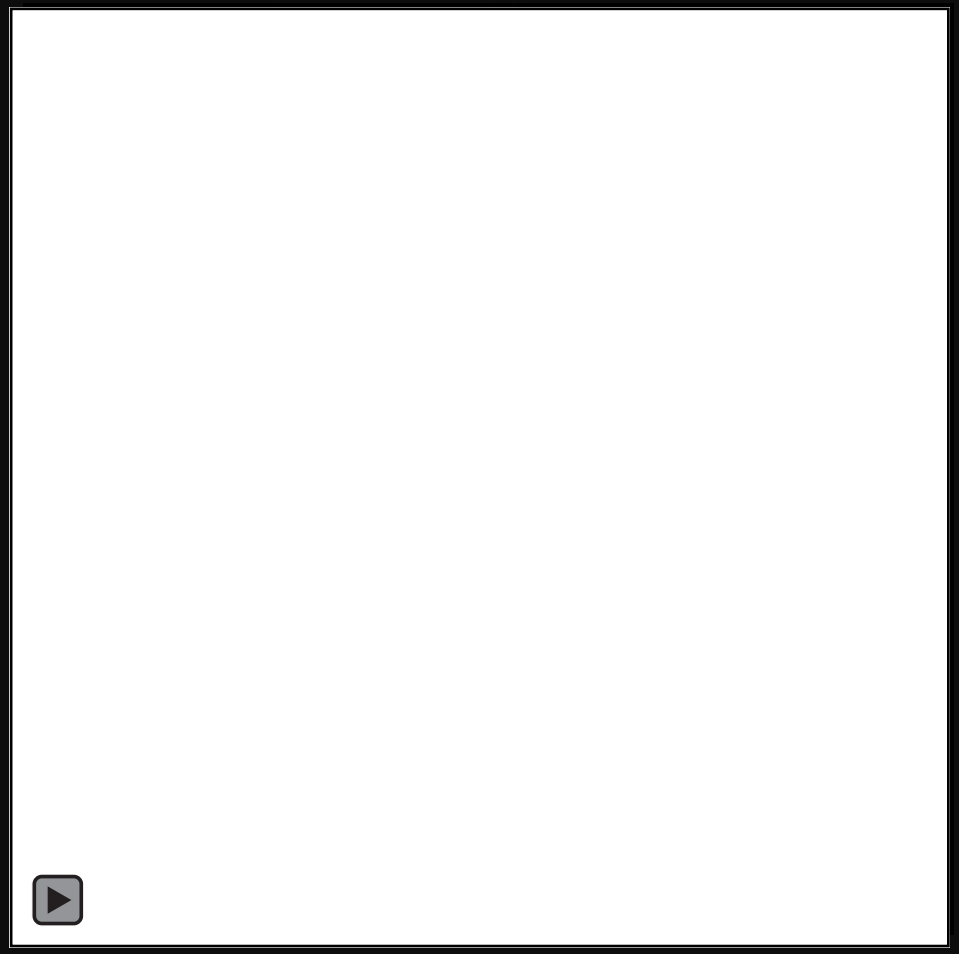
Nucleus diameter: 25 μm

Chromosome width:1 μm

10 um

Taken by Deconvolution Microscope

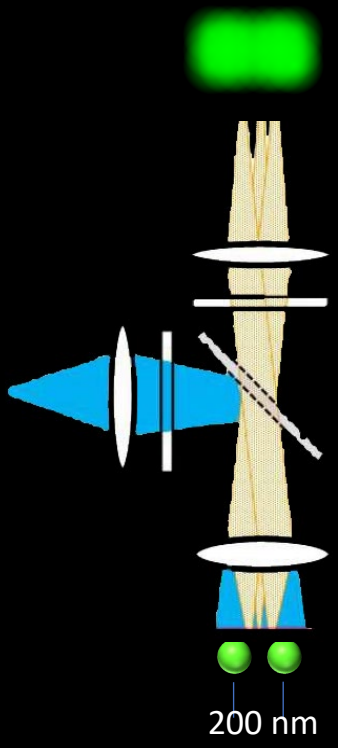
Maize meiocytes are amenable to super-resolution microscopy



AFD1/REC8 antibody

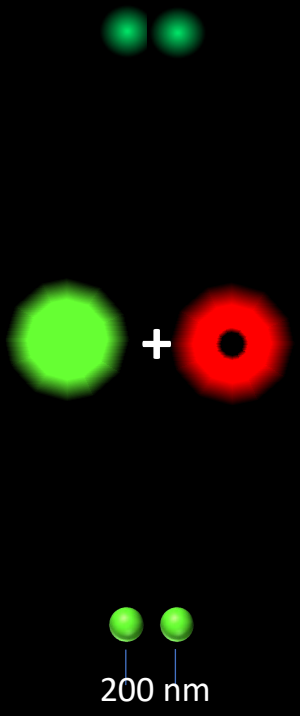
Included in the textbook *"Molecular Biology of the Cell",* 6th edition by Alberts et al. 2015

Resolution limit and super-resolution microscopy



200 nm

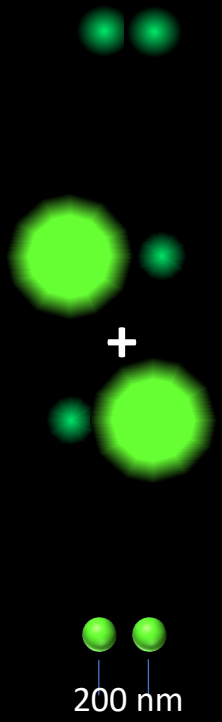
STED



200 nm

Klar and Hell (1999)

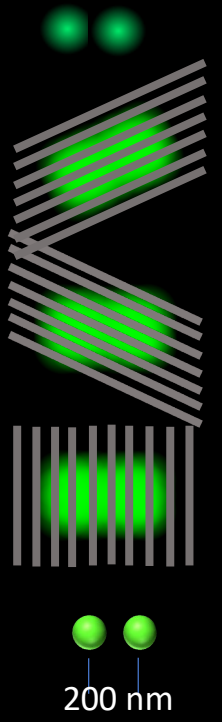
PALM/STORM



200 nm

Rust et al (2006)
Betzig et al. (2006)

SIM

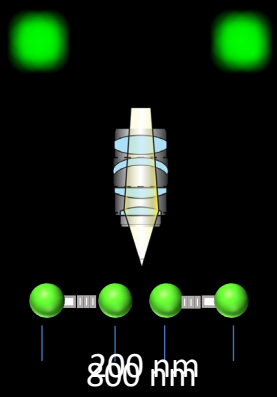


200 nm

Gustafsson (2000)

Expansion Microscopy (ExM)

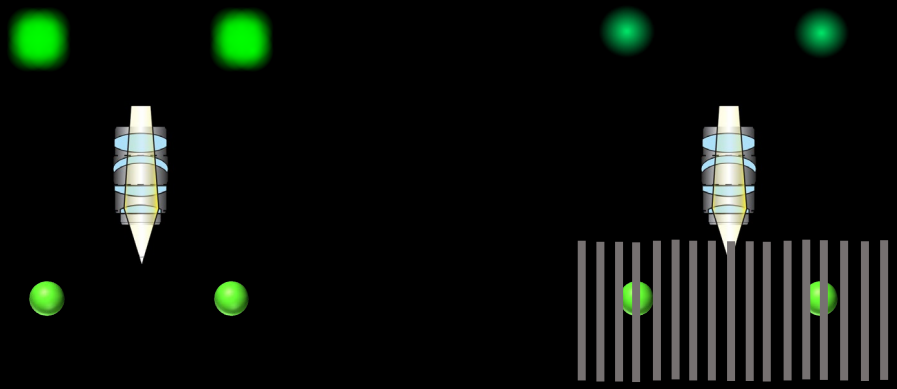
Make samples physically BIGGER



Ed Boyden
Chen et al (2015)

Expansion Microscopy (ExM)

By 3D-SIM



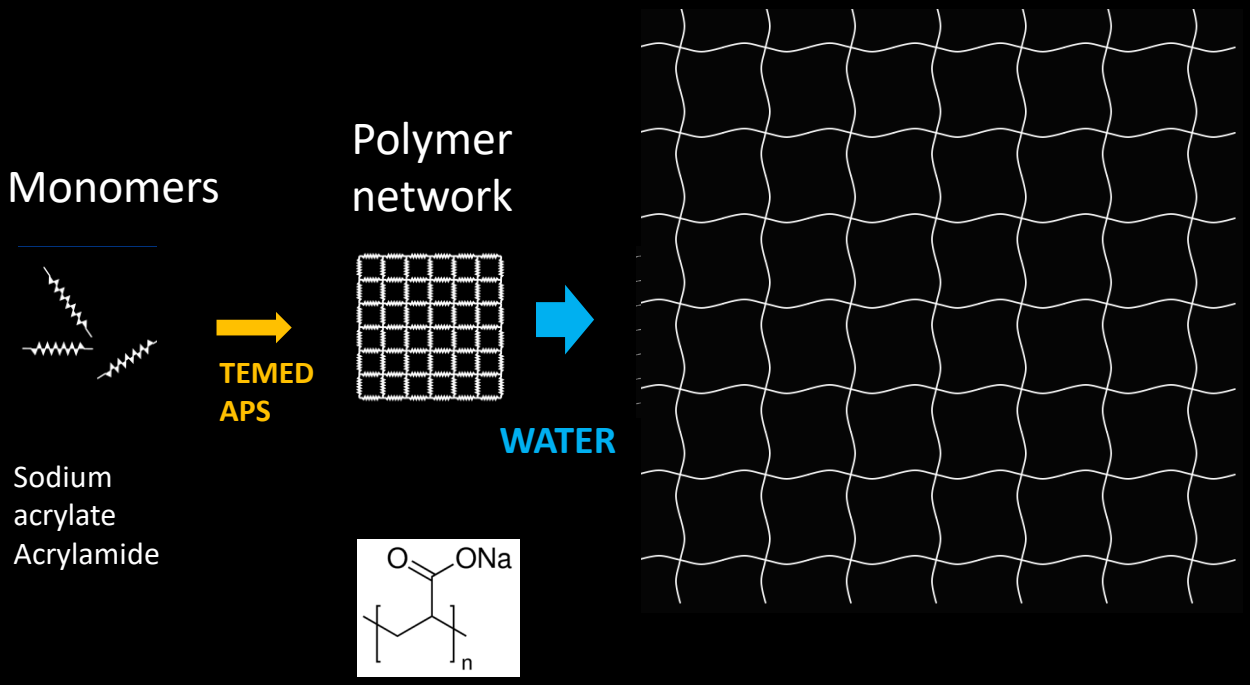
Resolution improves ~ 8 folds.

Ed Boyden
Chen et al (2015)

Expansion Microscopy (ExM)

Use of polyelectrolyte hydrogel

4X expansion in X, Y and Z = 64X in volume

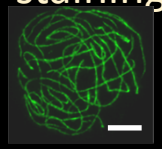


negatively charged carboxylic groups (羧酸)

Expansion Microscopy (ExM)

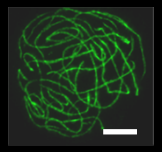
ProExM (Nature Biotech, 2016)

Ab staining



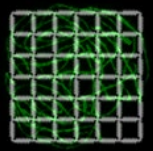
DSY2/RED1

Anchoring



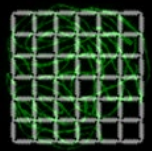
acryloyl-X SE (AcX)

Gelation vs Cross-linking

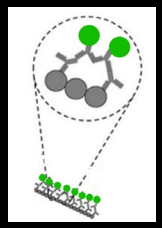


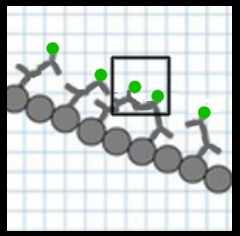
Hydrogel matrix

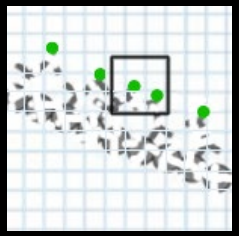
Mechanical homogenization

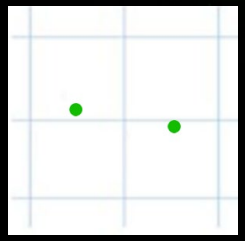


Proteinase K

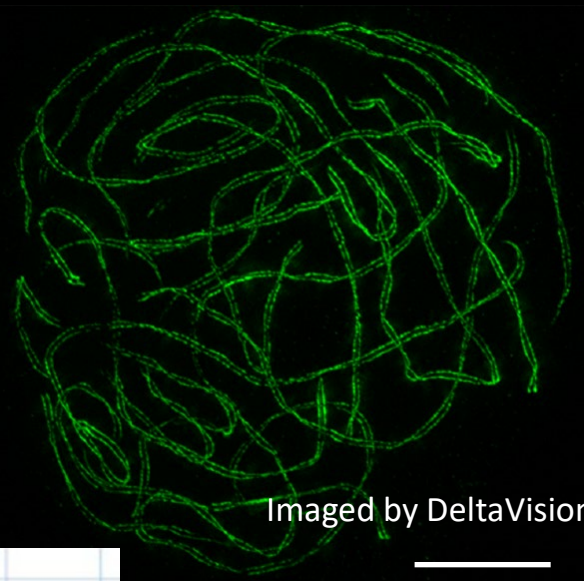








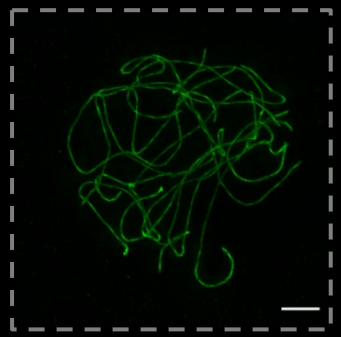
Expansion



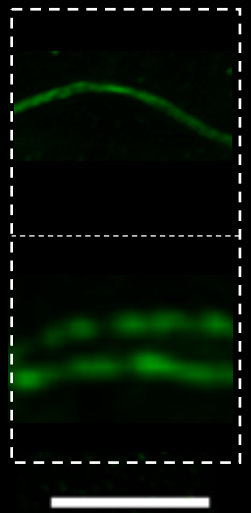
Imaged by DeltaVision

ExM of maize meiocytes

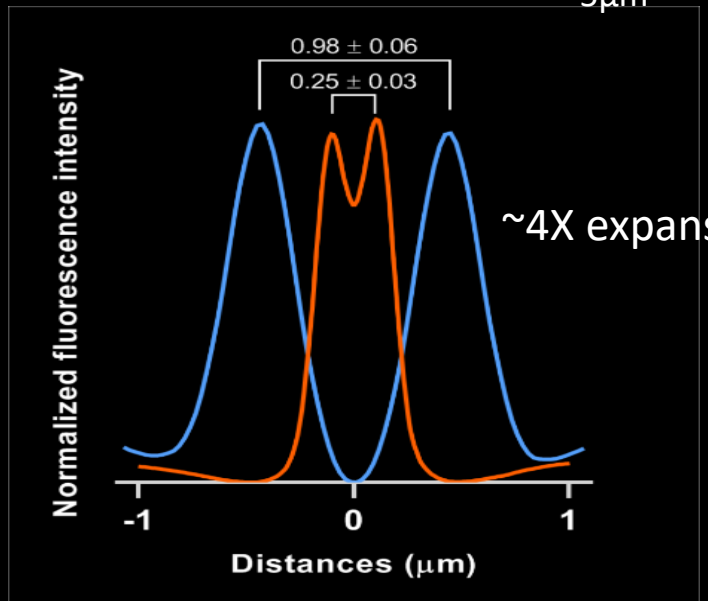
Deltavision



DSY2

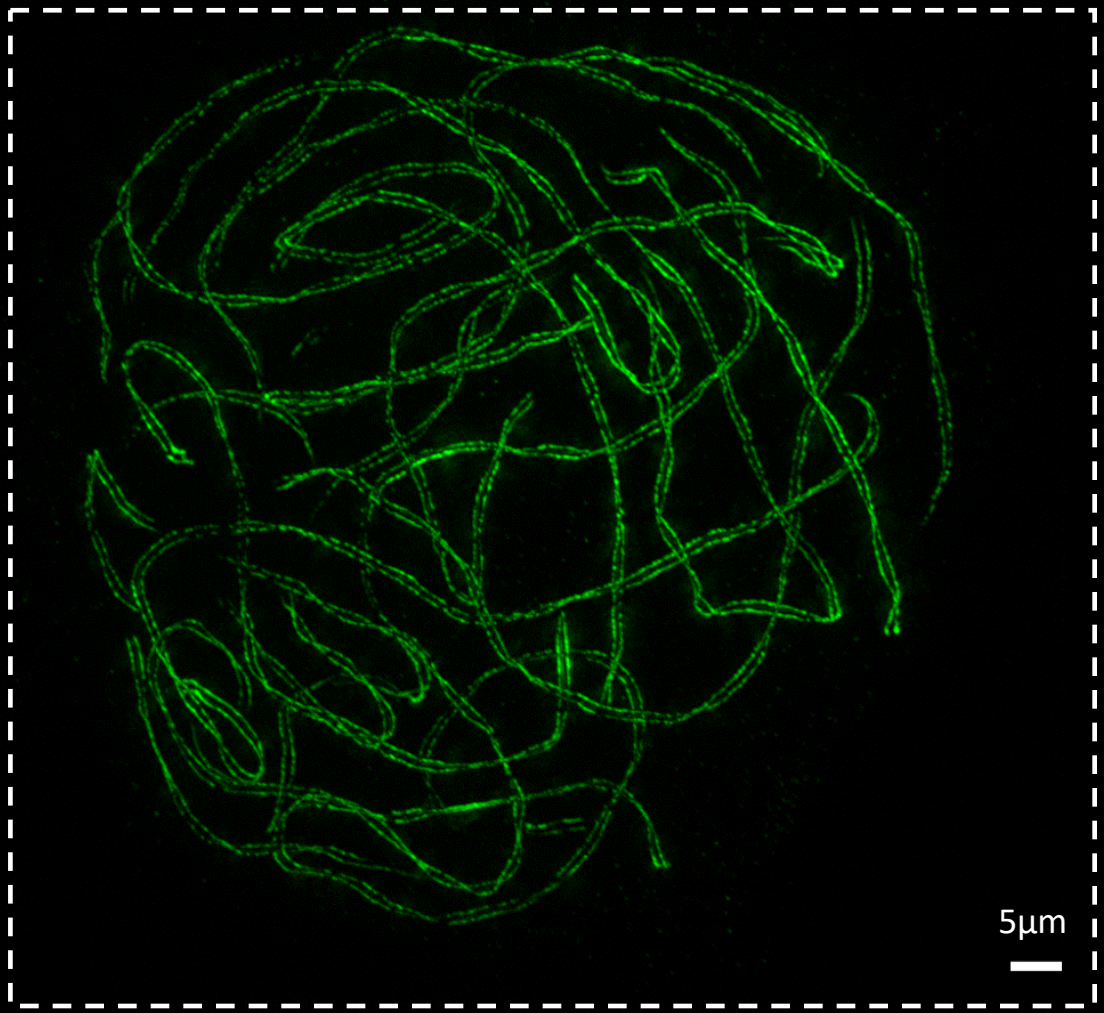


5µm

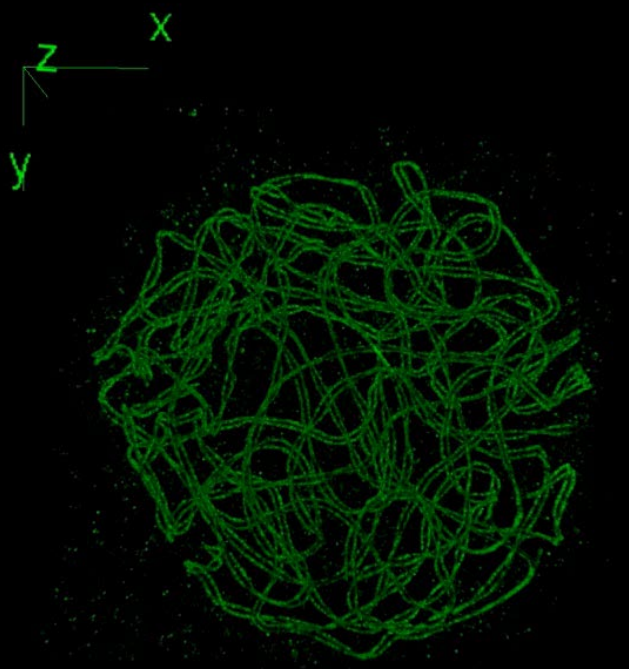


~4X expansion

ExM + Deltavision

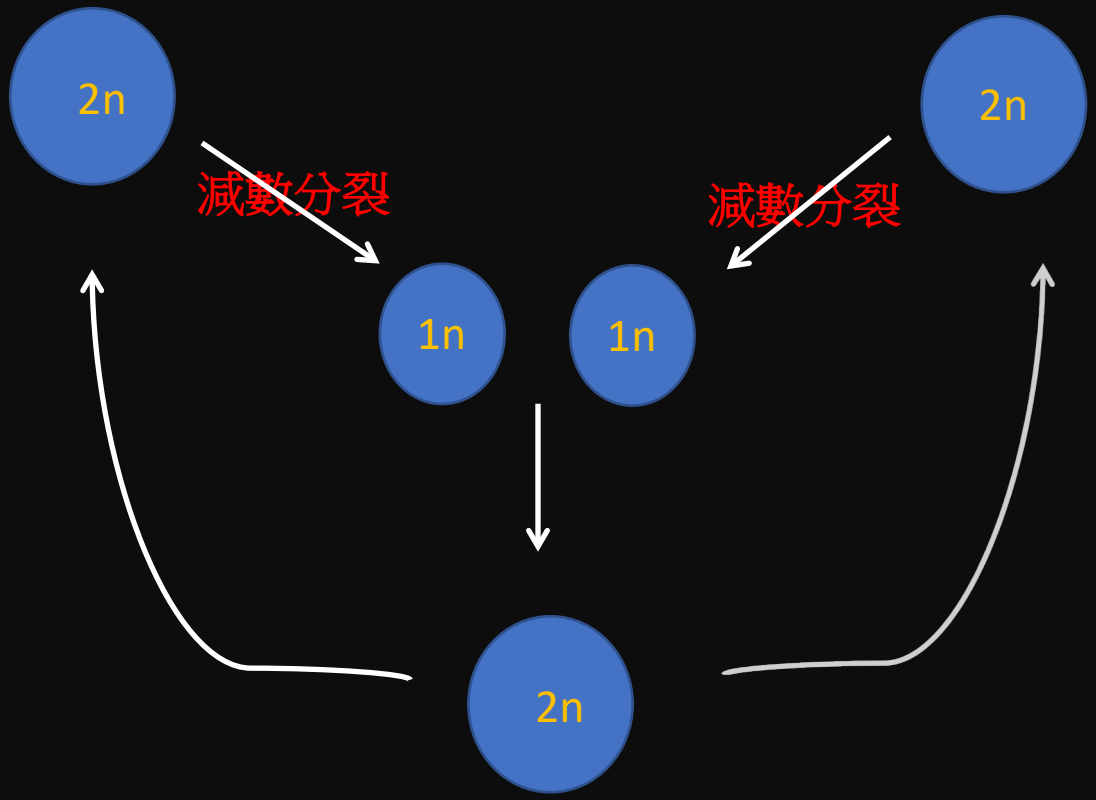


Expansion Microscopy (ExM)

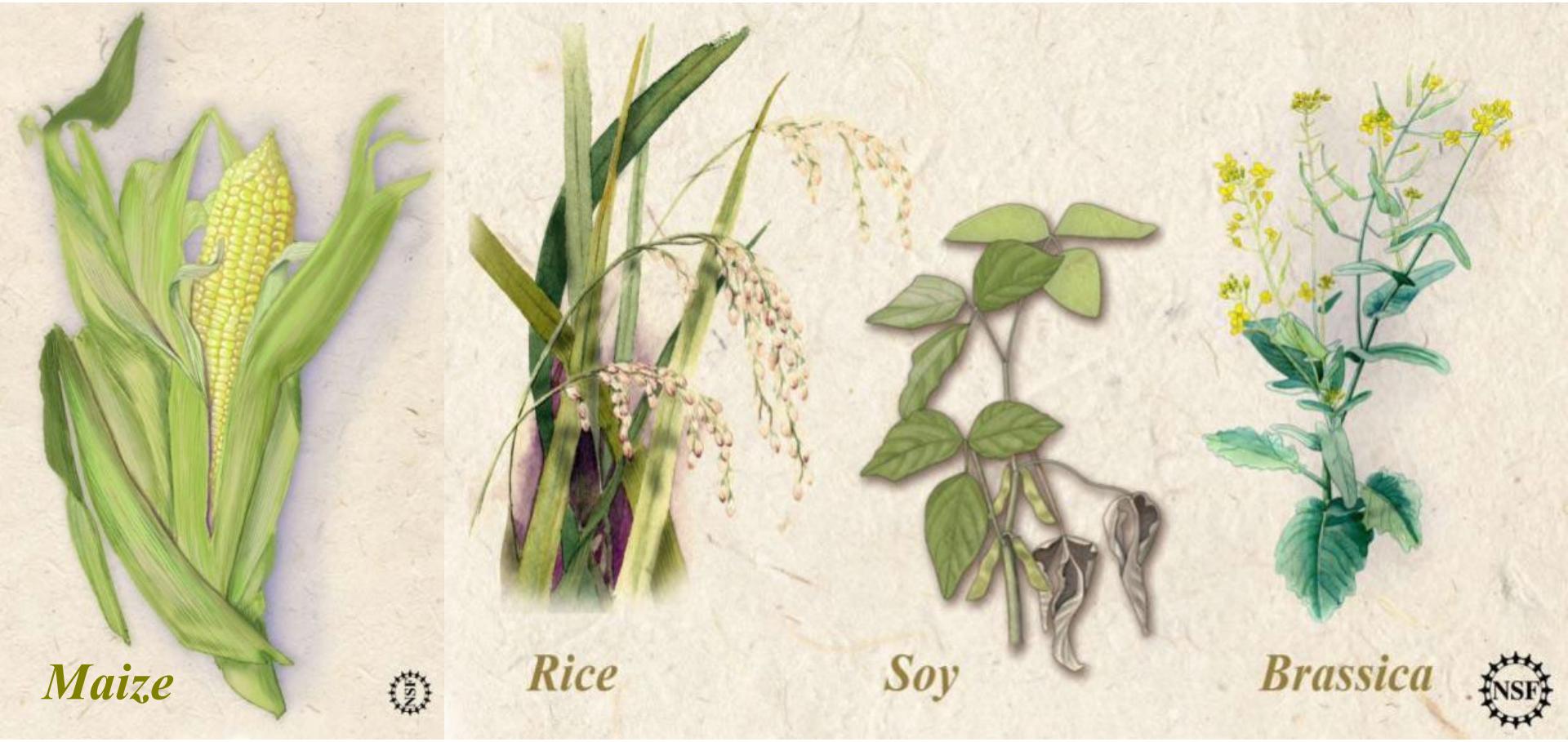


生命起源為
無性生殖
單倍體（1n）

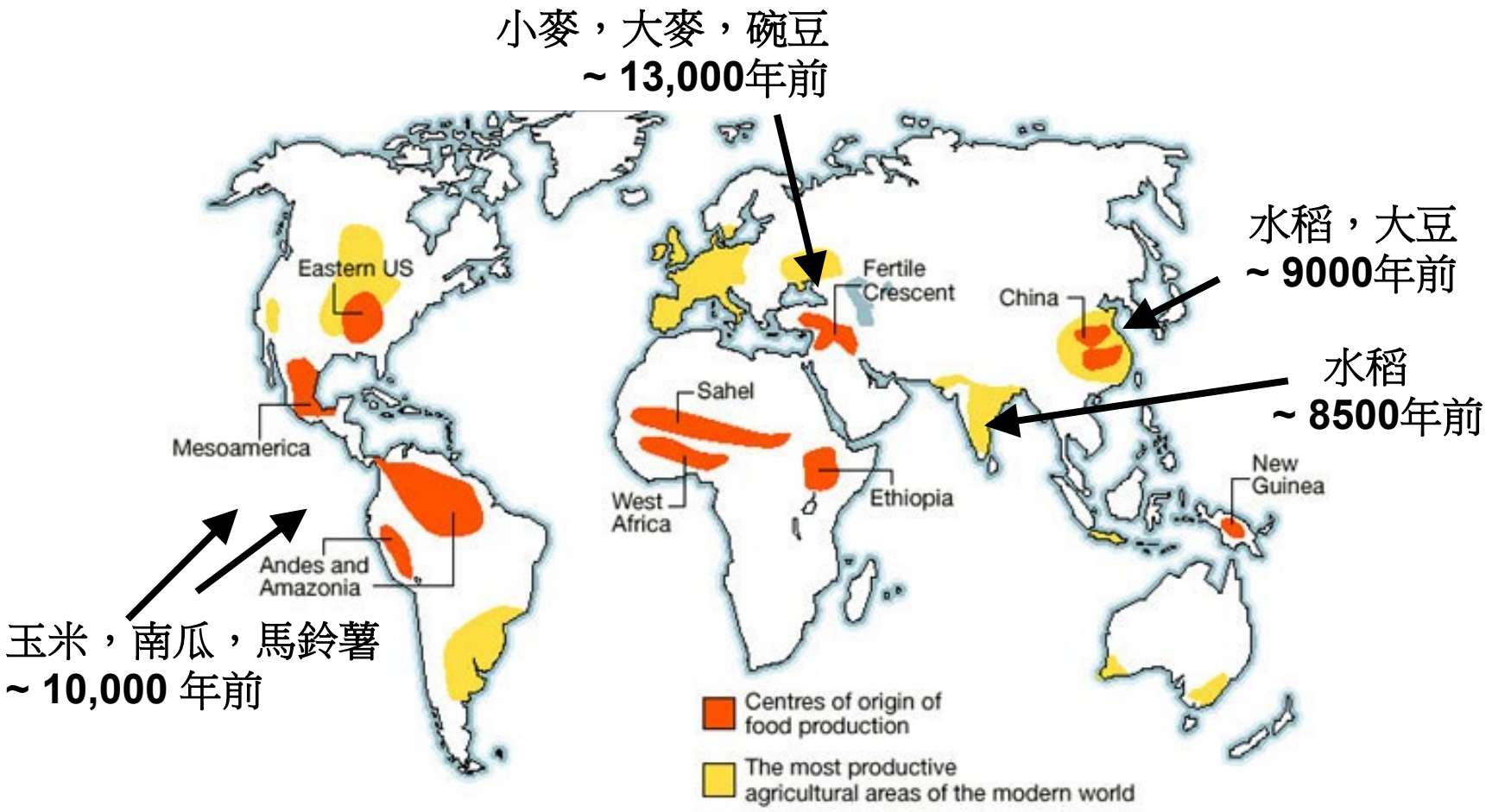
有性生殖



作物育種在面對挑戰中的角色與準備

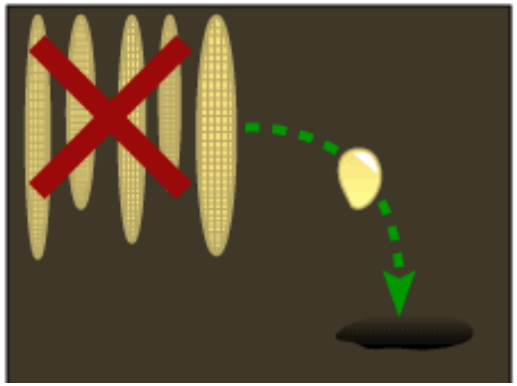


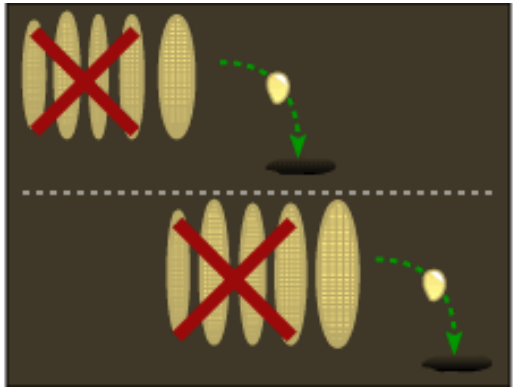
人類已經開始育種/馴化工程數千年



育種/馴化:透過突變，篩選特定性狀

自然突變(多樣性)





種植”好”的植物產生的種子增加”好的基因”在後代中的代表性

玉米的馴化

大芻草

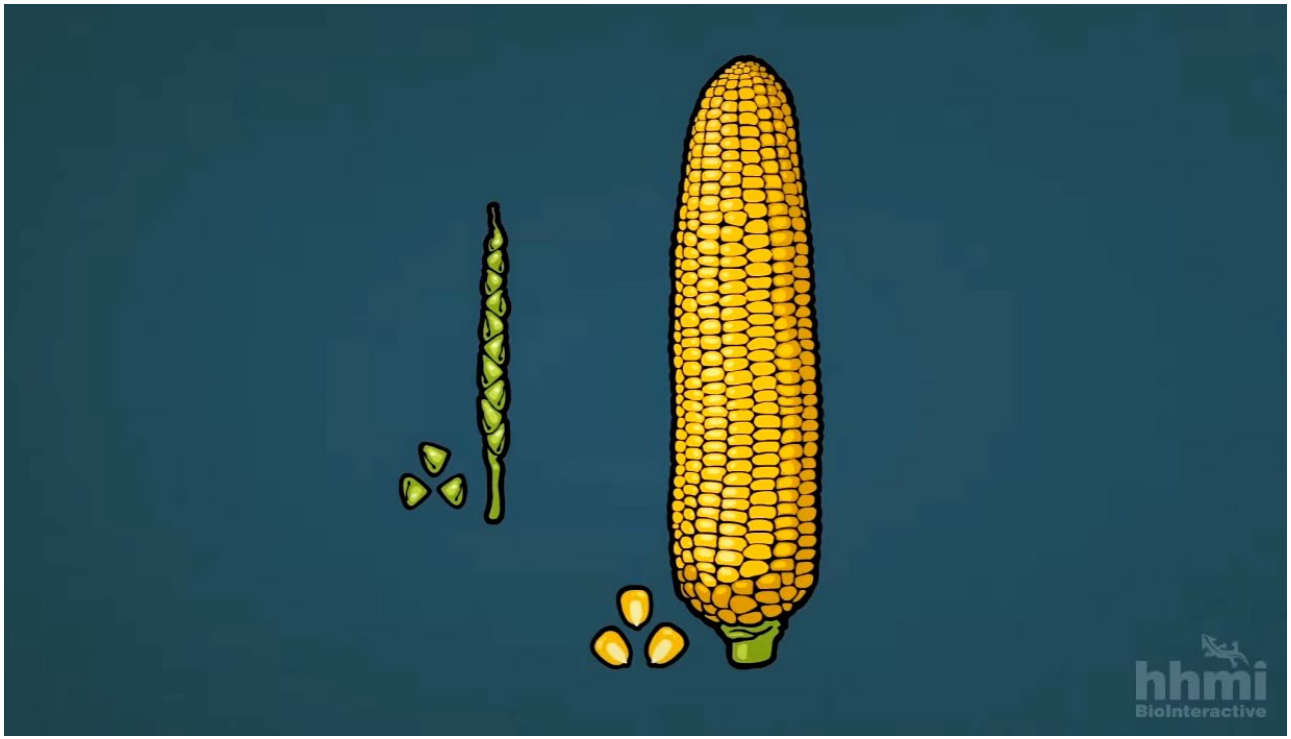
現代玉米

馴化的力量

Map of the Tehuacán Valley matorral

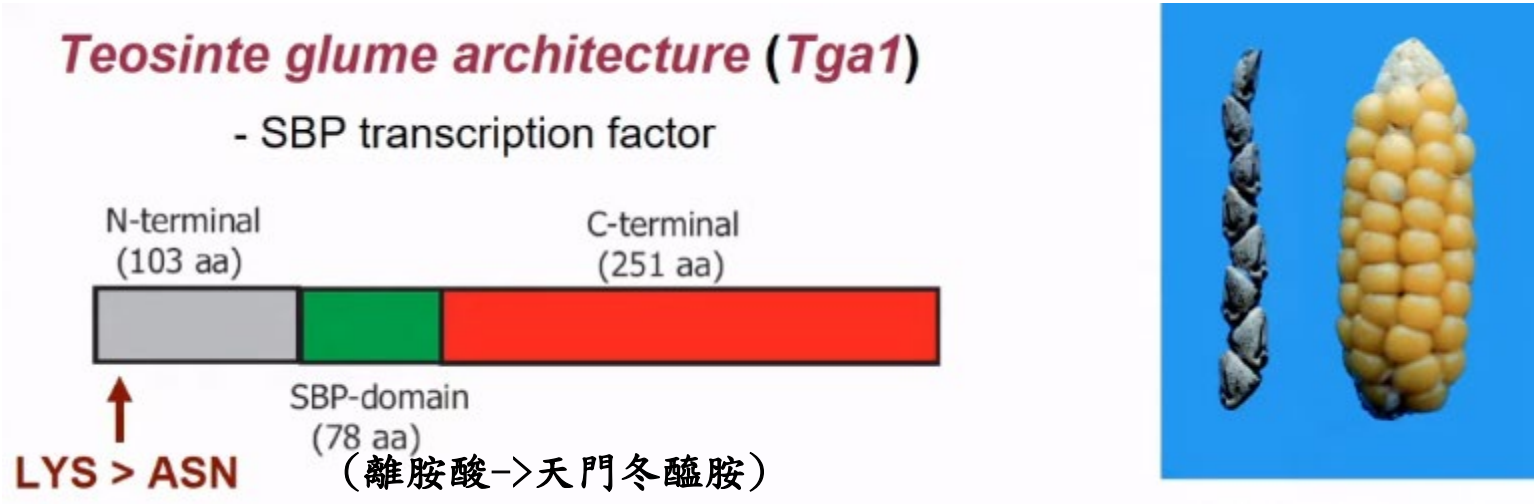
從大芻草到玉米：基因改變了多少

George Beadle (1903-1989)

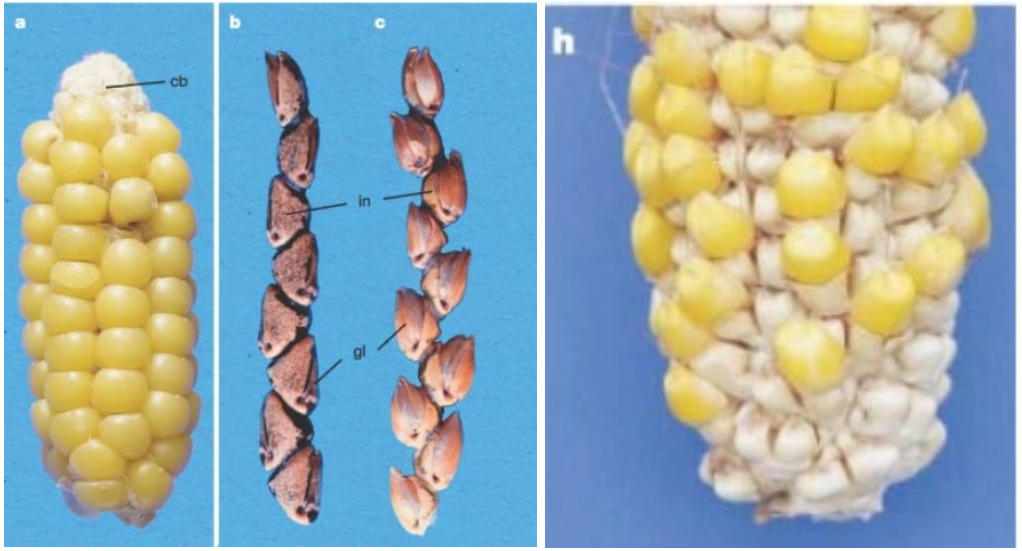


HHMI BioInteractive Video

基因 *Tga1* 控制部分種子硬殼的性狀



tga1 *Tga1*

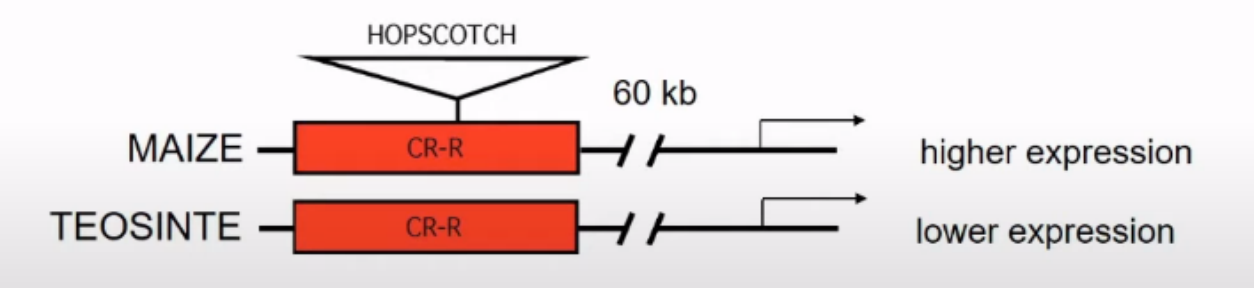


(Doebley's group, 2005, Nature)

基因 *Tb1* 控制植株分蘖

Teosinte branched1 (tb1)

TCP transcription factor

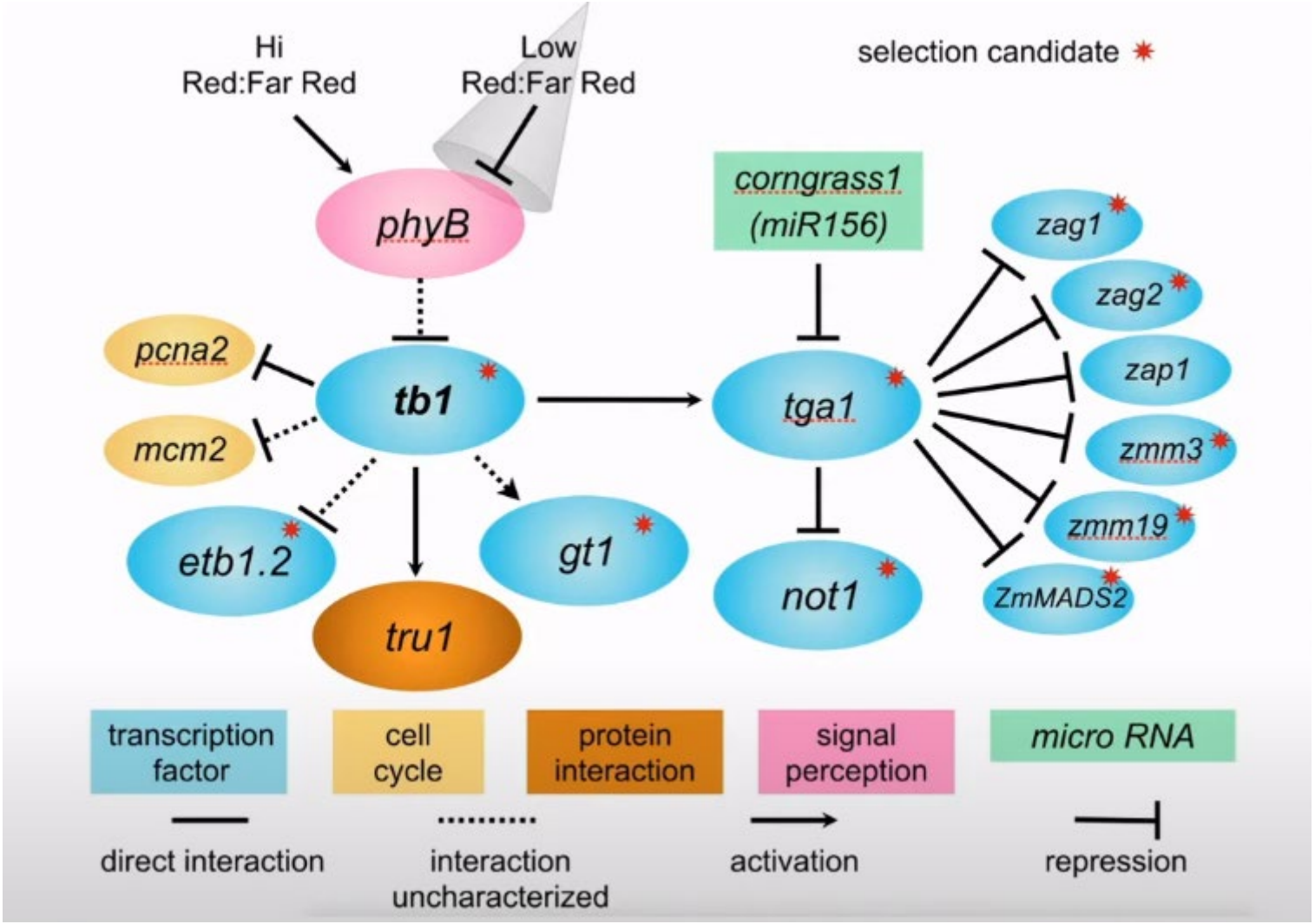


大芻草(*tb1*)　　玉米 (*Tb1*)　　*teosinte branched1*

玉米带有大芻草
tb1 基因版本

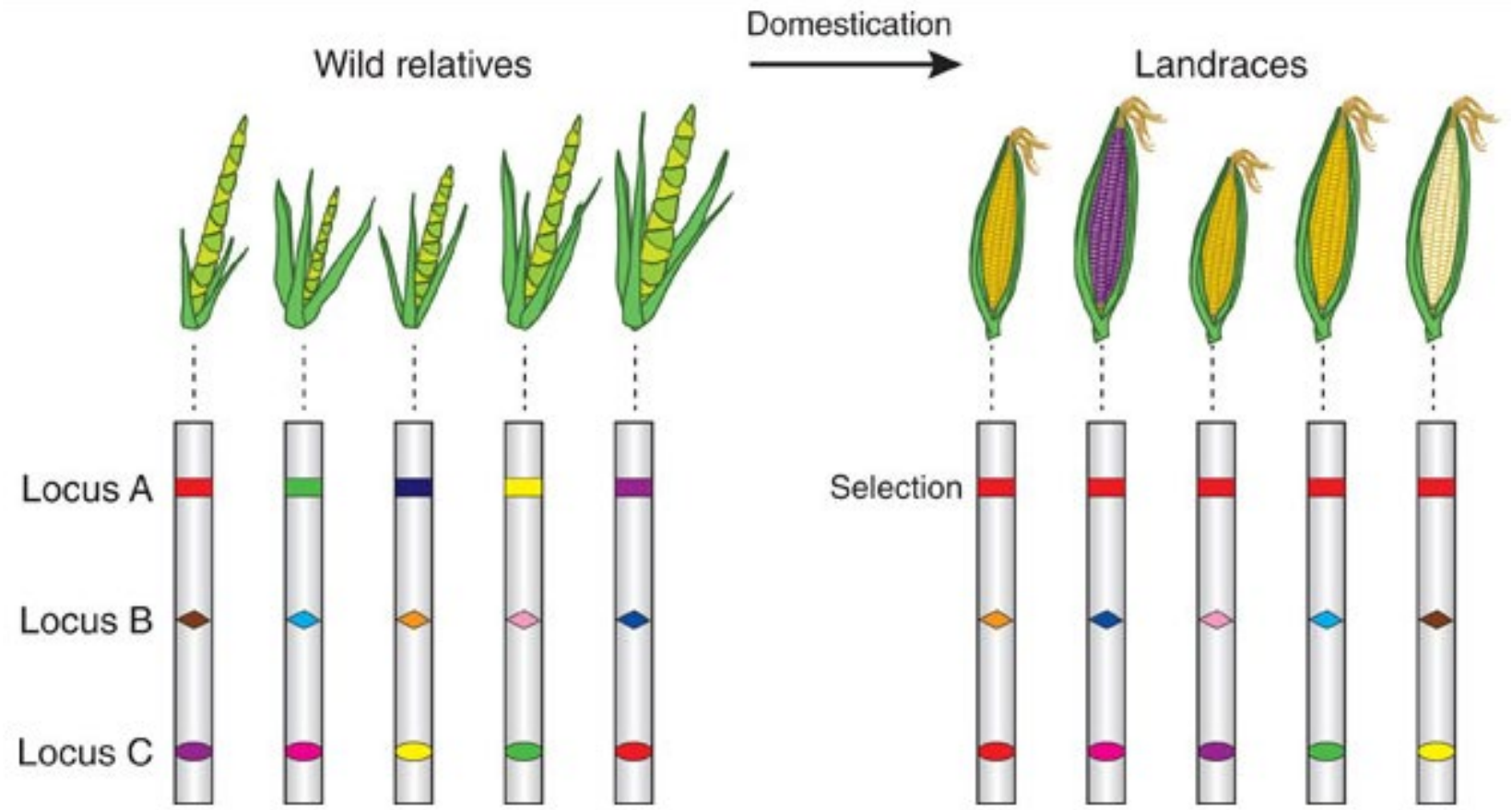
(Doebley et al., 1995. Genetics)

玉米馴化基因調控網路

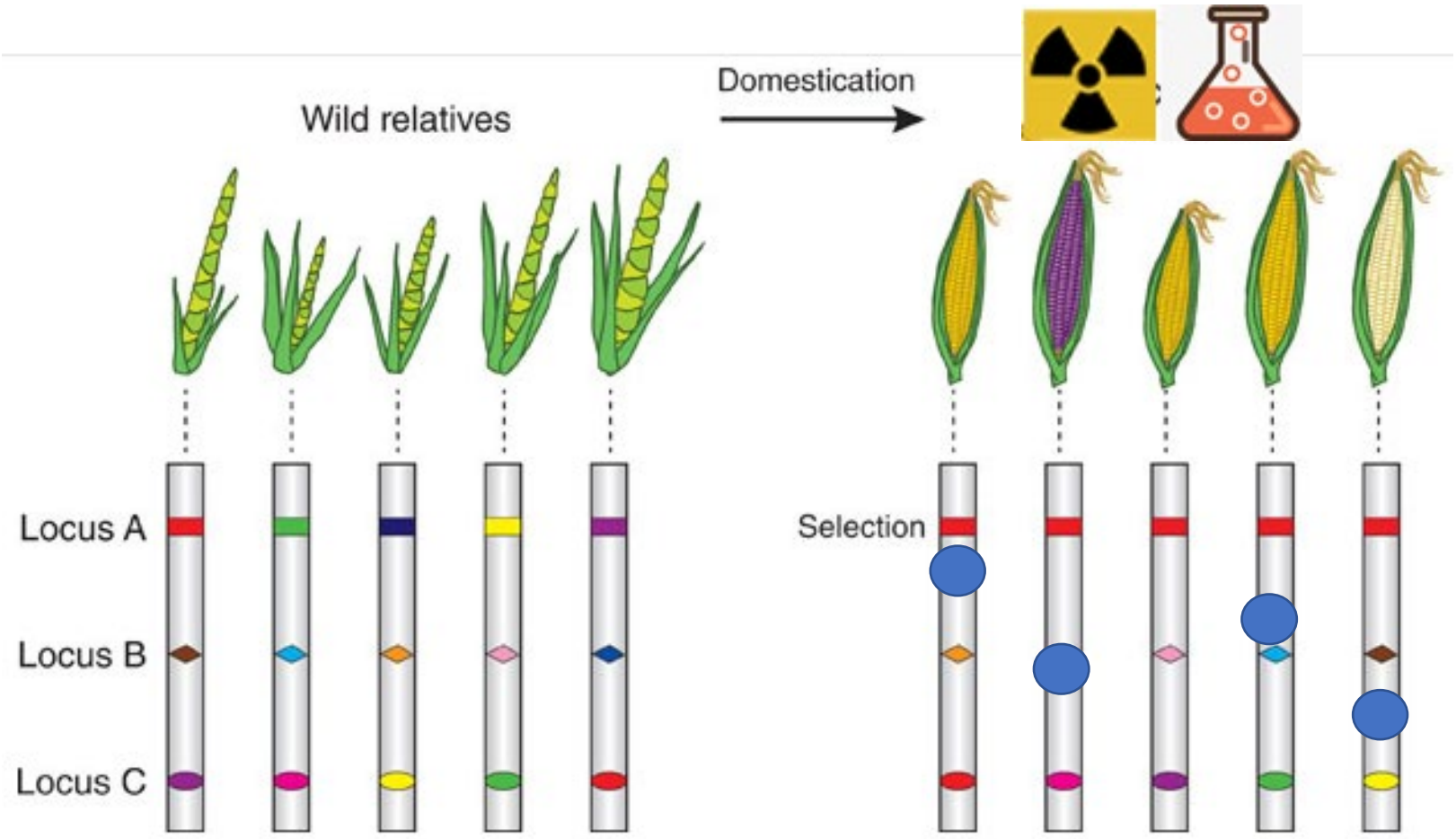


(Trends in Genetics, 2019)

利用天然突變，再人為選拔



人為增加突變機會，產生基因型多樣性



分子標記輔助育種

Poor tomato
but disease
resistant

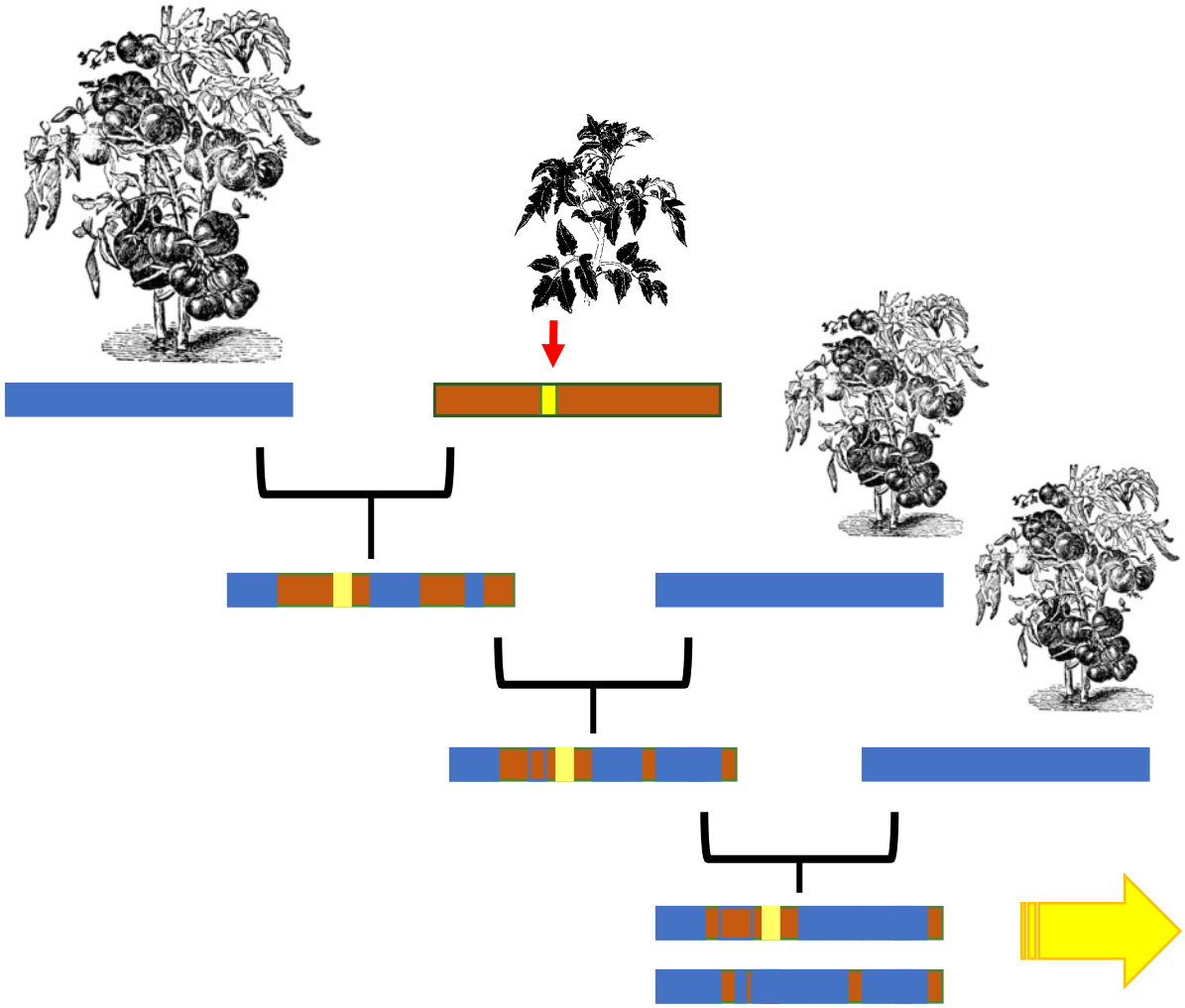
Elite tomato

F1

F2

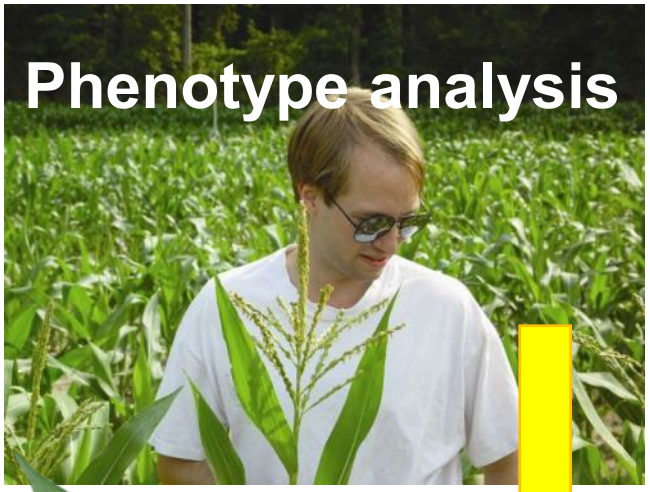
Photo by Stephen Ausmus USDA

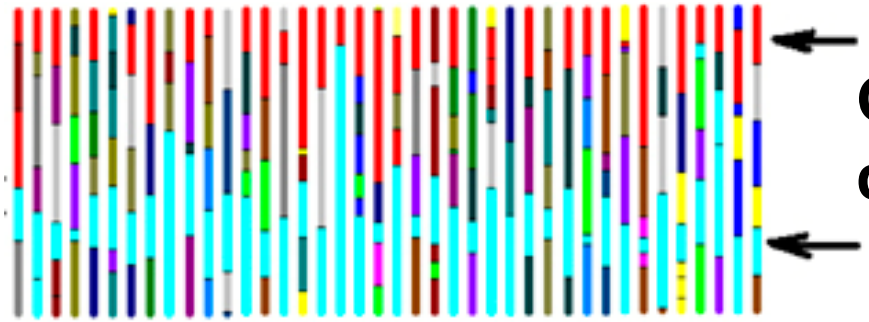
分子標記輔助育種



After several generations, **elite**, **disease resistant** tomato

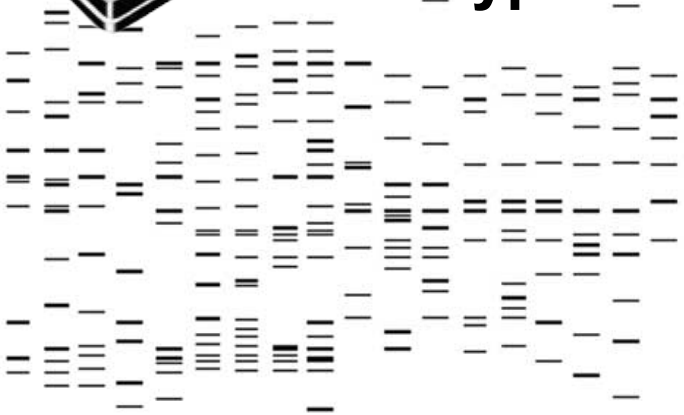
全基因組關聯分析加快育種效率





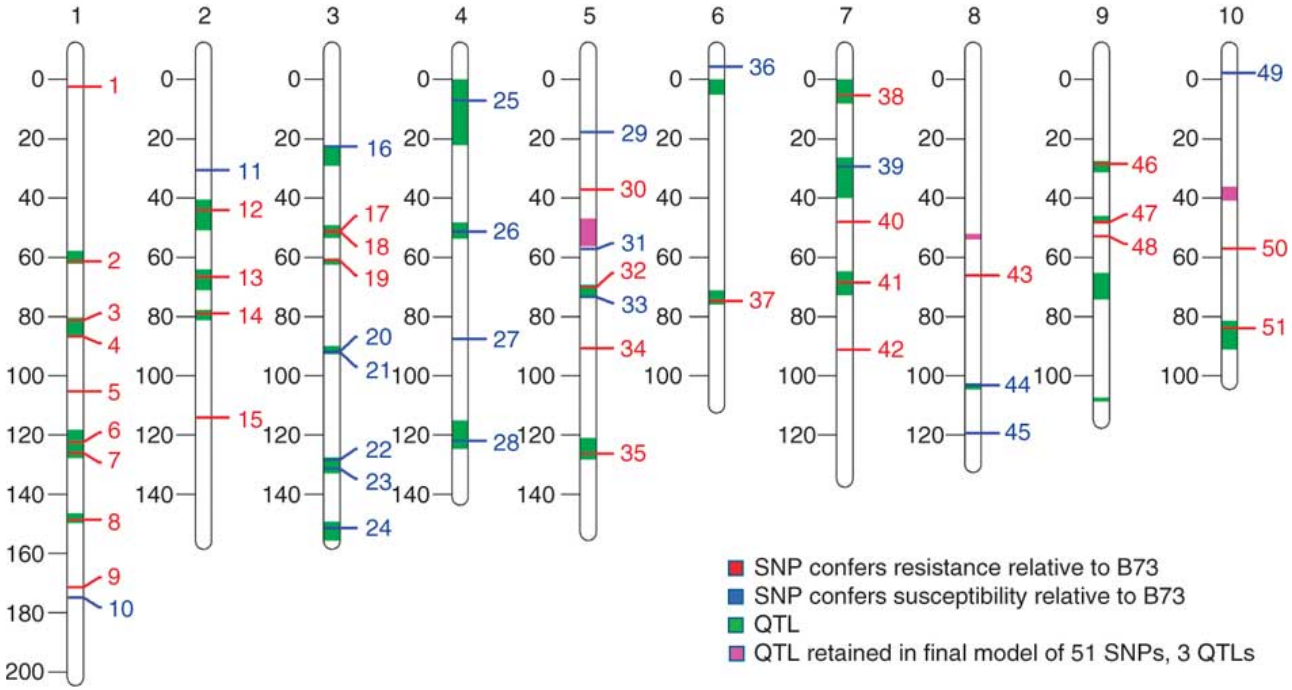
Gene discovery

Genotype analysis



Association analysis

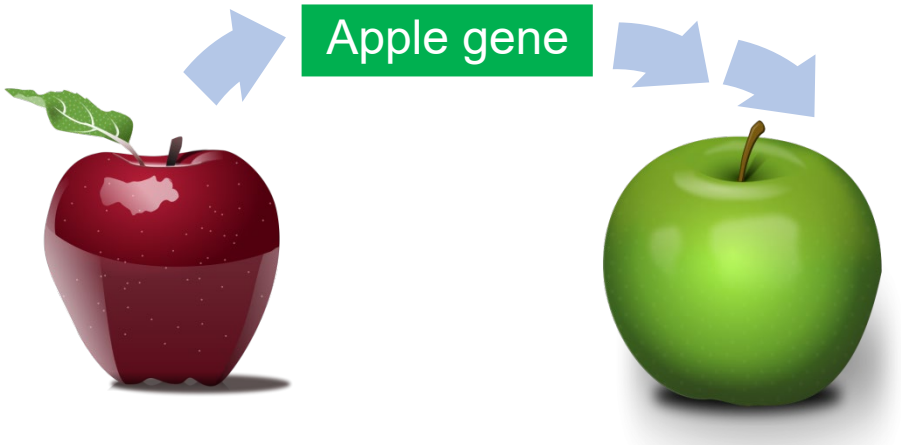
全基因組關聯分析快速找到抗病基因



Similar studies have led to the identification of genes contributing to other agronomically important traits including drought tolerance

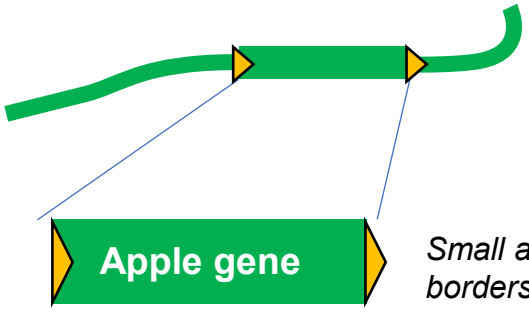
Reprinted by permission from Macmillan Publishers Ltd Kump, K.L., Bradbury, P.J., Wisser, R.J., Buckler, E.S., Belcher, A.R., Oropeza-Rosas, M.A., Zwonitzer, J.C., Kresovich, S., McMullen, M.D., Ware, D., Balint-Kurti, P.J., and Holland, J.B. (2011). Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. Nat Genet 43: 163-168.

同源基改 vs 異種基改



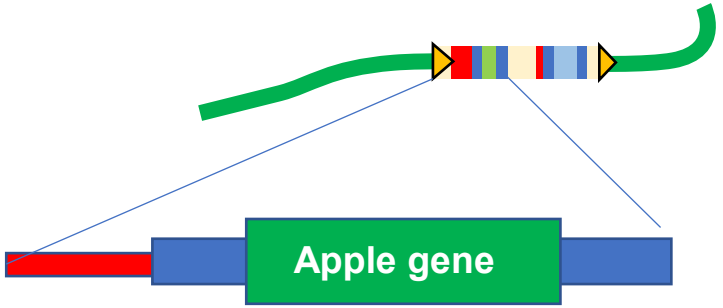
Some traits can be modified by the introduction of a cisgene – a gene from the same or closely-related species

That might mean that little foreign DNA is introduced (“cisgenic”)



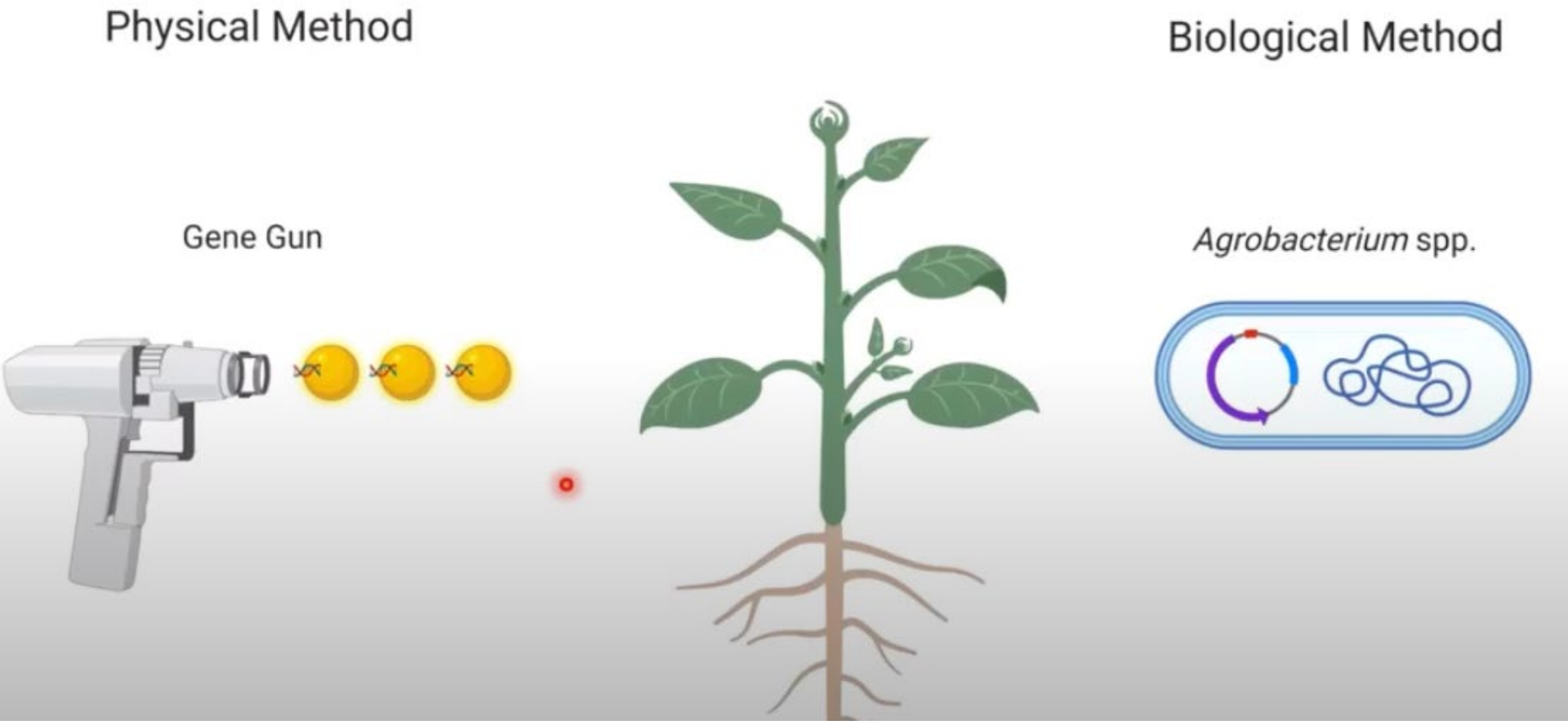
Small amounts of DNA from T-DNA borders may be incorporated

Or, bacterial and viral DNA may be included, but no protein-coding regions from other organisms (“intragenic”)

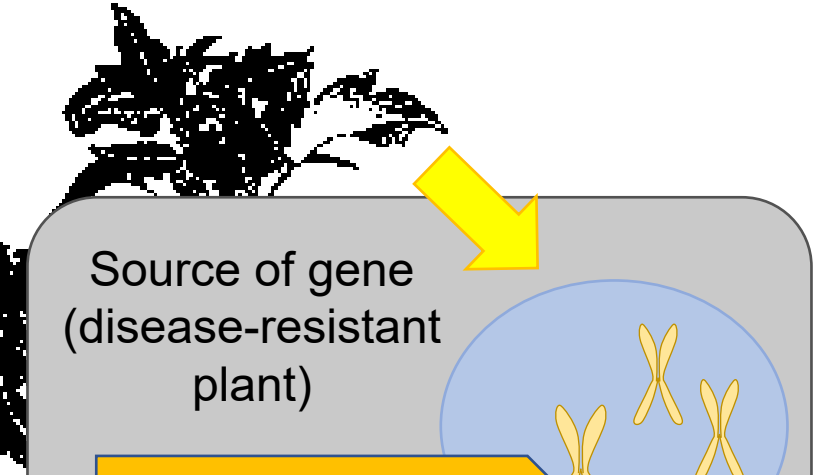


Schouten, H.J., Krens, F.A. and Jacobsen, E. (2006). Cisgenic plants are similar to traditionally bred plants. EMBO Rep. 7: 750-753.

基因轉殖方法


Physical Method
Biological Method
Gene Gun
Agrobacterium spp.

基因轉殖（基改）



Source of gene (disease-resistant plant)

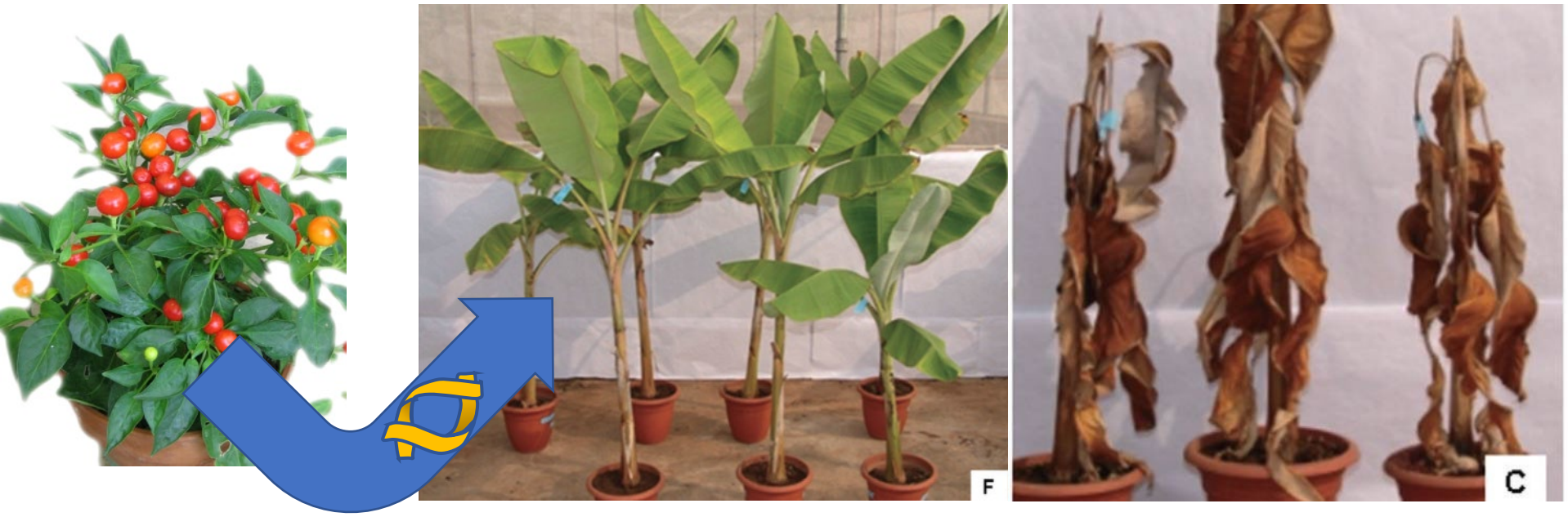
Gene of interest

Isolate gene of interest using molecular biology methods

Recombine into recipient plant DNA

Once a gene is introduced into the plant genome it functions like any other gene

基因轉殖(基改)香蕉



Resistant **Susceptible**

Banana bacterial wilt (*Xanthomonas campestris* pv. *musacearum*) is destroying plants in eastern Africa. Transgenic plants carrying a resistance gene from pepper are resistant to the disease

Tripathi, L., Mwaka, H., Tripathi, J.N., and Tushemereirwe, W.K. (2010). Expression of sweet pepper Hrap gene in banana enhances resistance to Xanthomonas campestris pv. musacearum. Molecular Plant Pathology 11: 721-731.

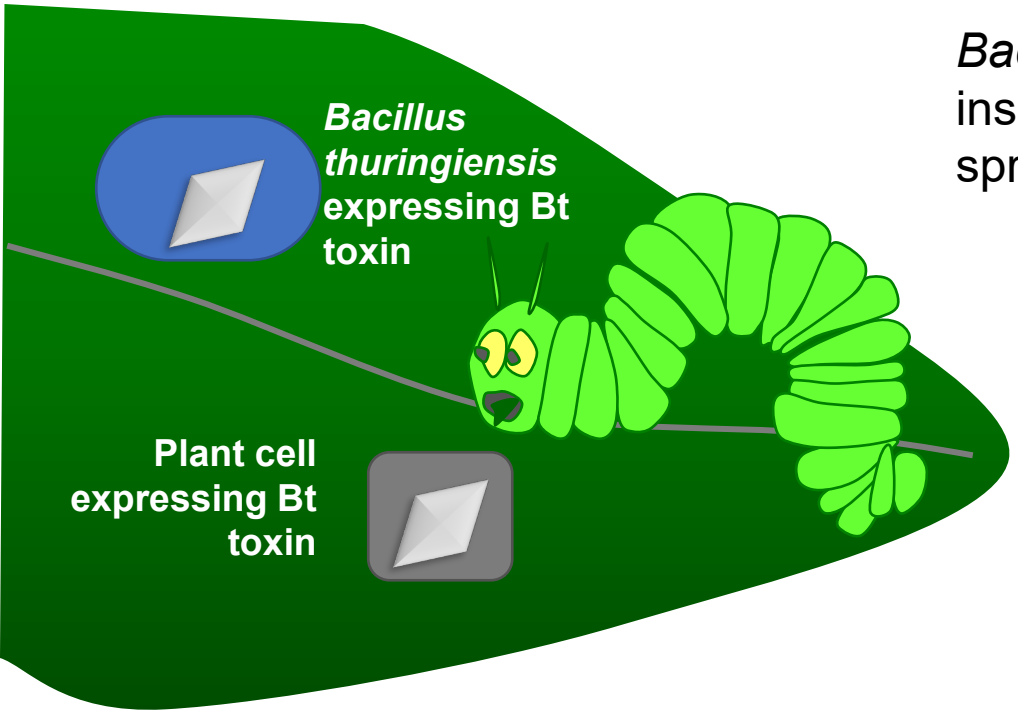
基因轉殖(基改)花生

Wild-type peanut plant

Peanut plant expressing the *Bt* gene

Photo by Herb Pilcher USDA

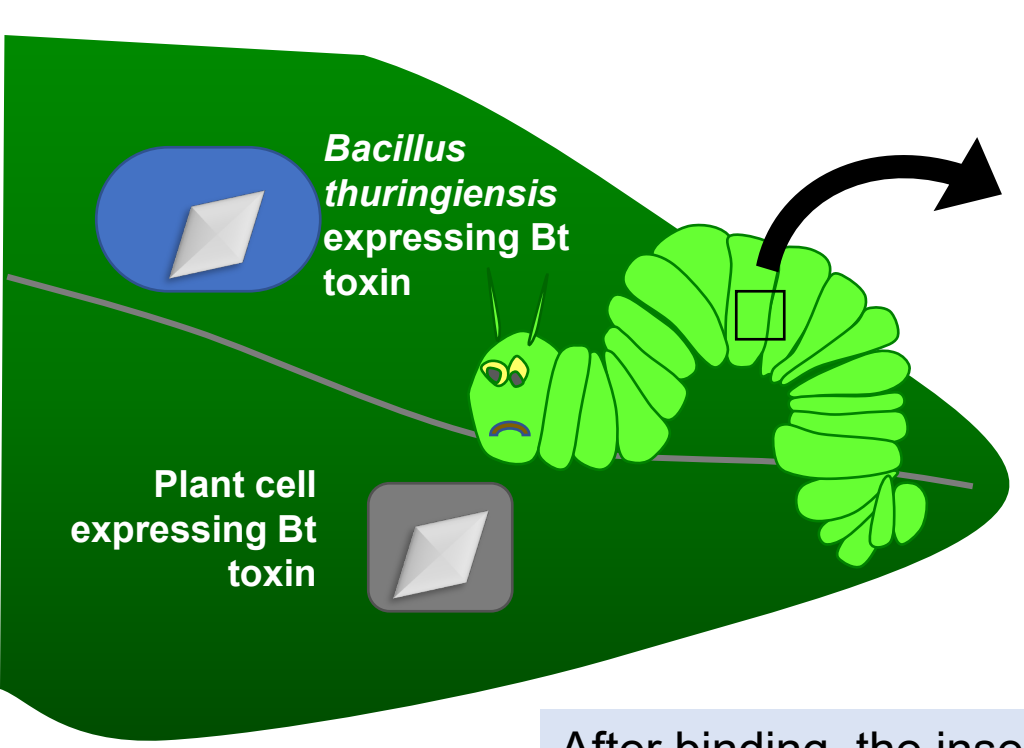
Bacillus thuringiensis (Bt) 細菌的基因對抗毛毛蟲

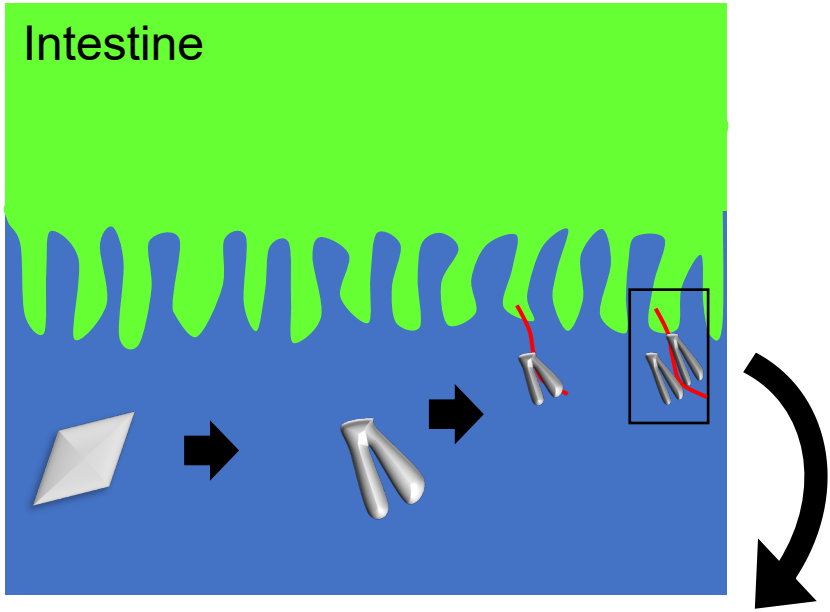


Bacillus thuringiensis expressing insecticidal Bt toxin can be sprayed onto plants

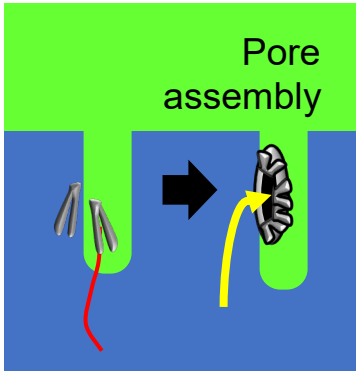
Or the plants can be engineered to express the *Bt* gene coding for Bt toxin

Bacillus thuringiensis (Bt) 細菌的基因對抗毛毛蟲



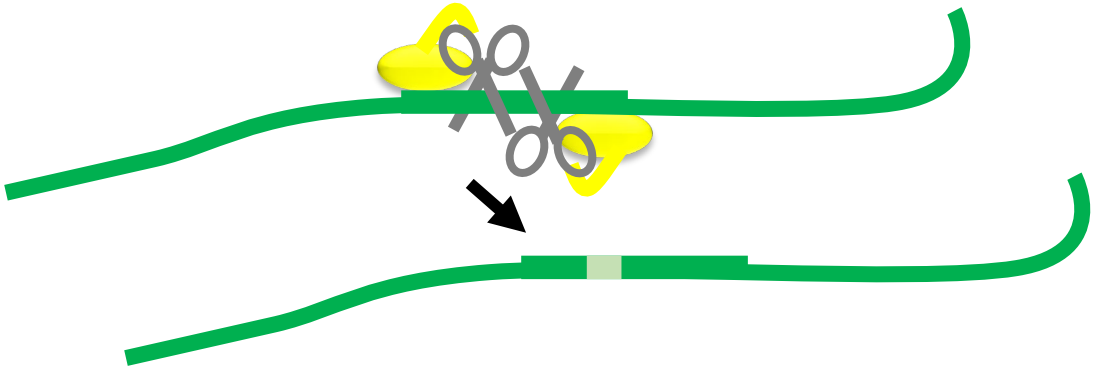


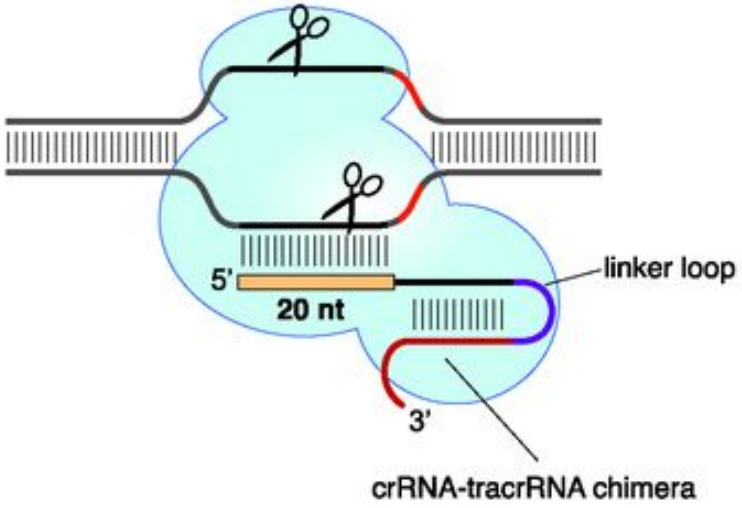
After binding, the insecticidal proteins assemble to form a pore in the lining of the insect intestine which kills the insect



基因編輯

Zinc-finger nucleases (ZFNs) and **transcription activator-like effector nucleases (TALENs)** are proteins that can produce double-strand DNA breaks that when repaired introduce site-specific mutations or insertions

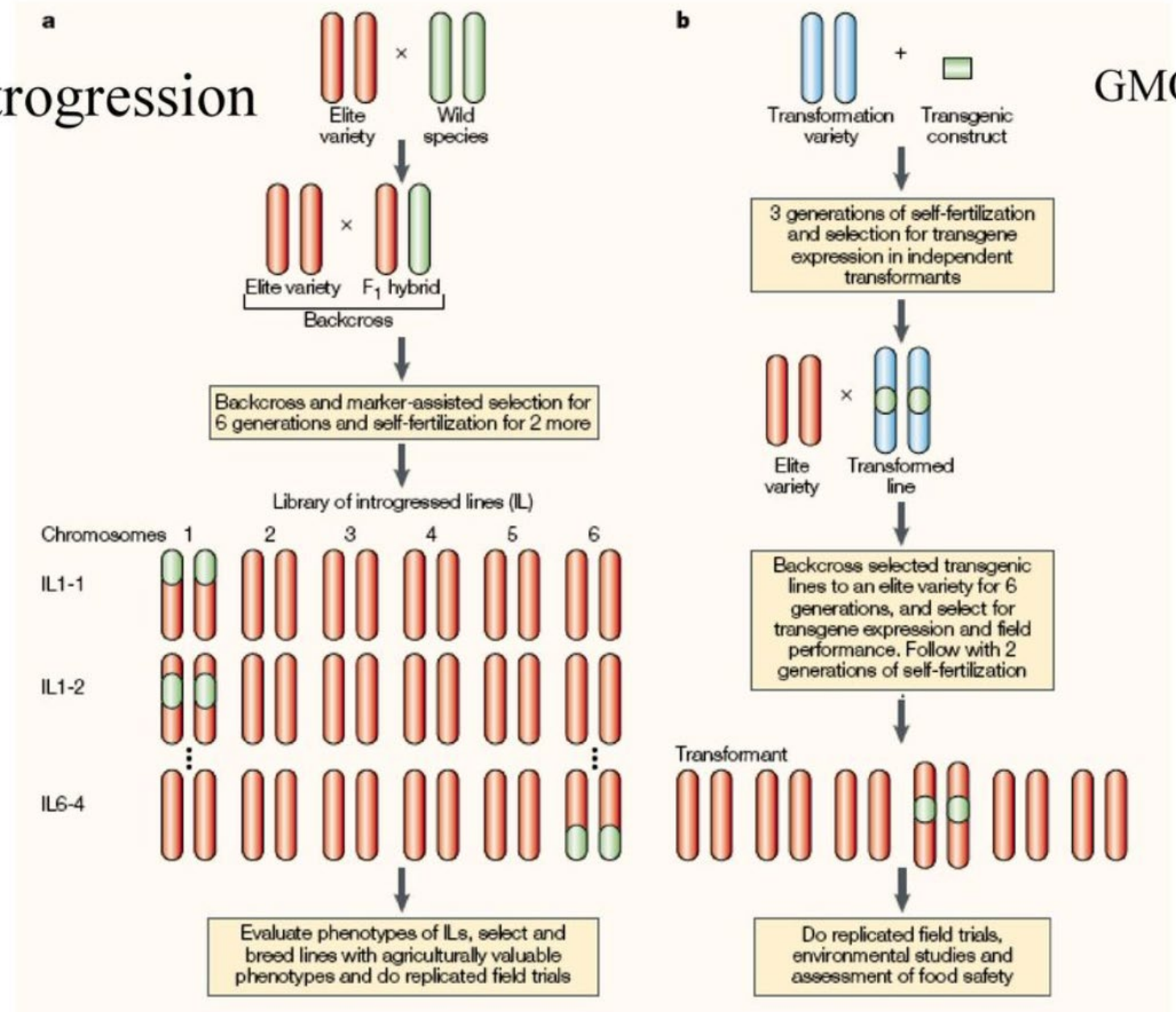




The **clustered regularly interspaced short palindromic repeats (CRISPR) / CRISPR-associated (Cas)** system uses RNAs to target nucleases to specific sites; when repaired, site-specific mutations or insertions are introduced

From Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A. and Charpentier, E. (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science. 337: 816-821 with permission from AAAS; see also Bhaya, D., Davison, M. and Barrangou, R. (2011). CRISPR-Cas Systems in Bacteria and Archaea: Versatile small RNAs for adaptive defense and regulation. Annu. Rev. Genet. 45: 273-297.

遺傳導入還是基因轉殖或是基因編輯



育種過程都會經過有性生殖