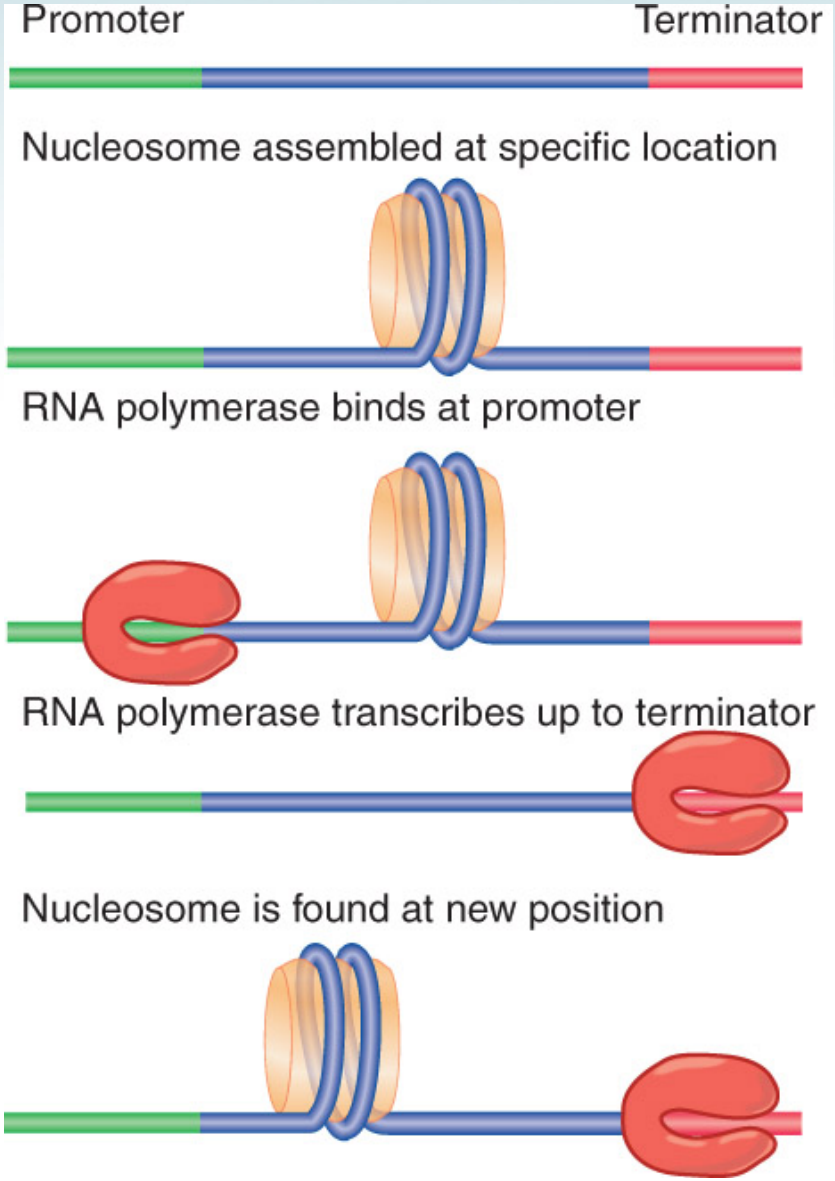
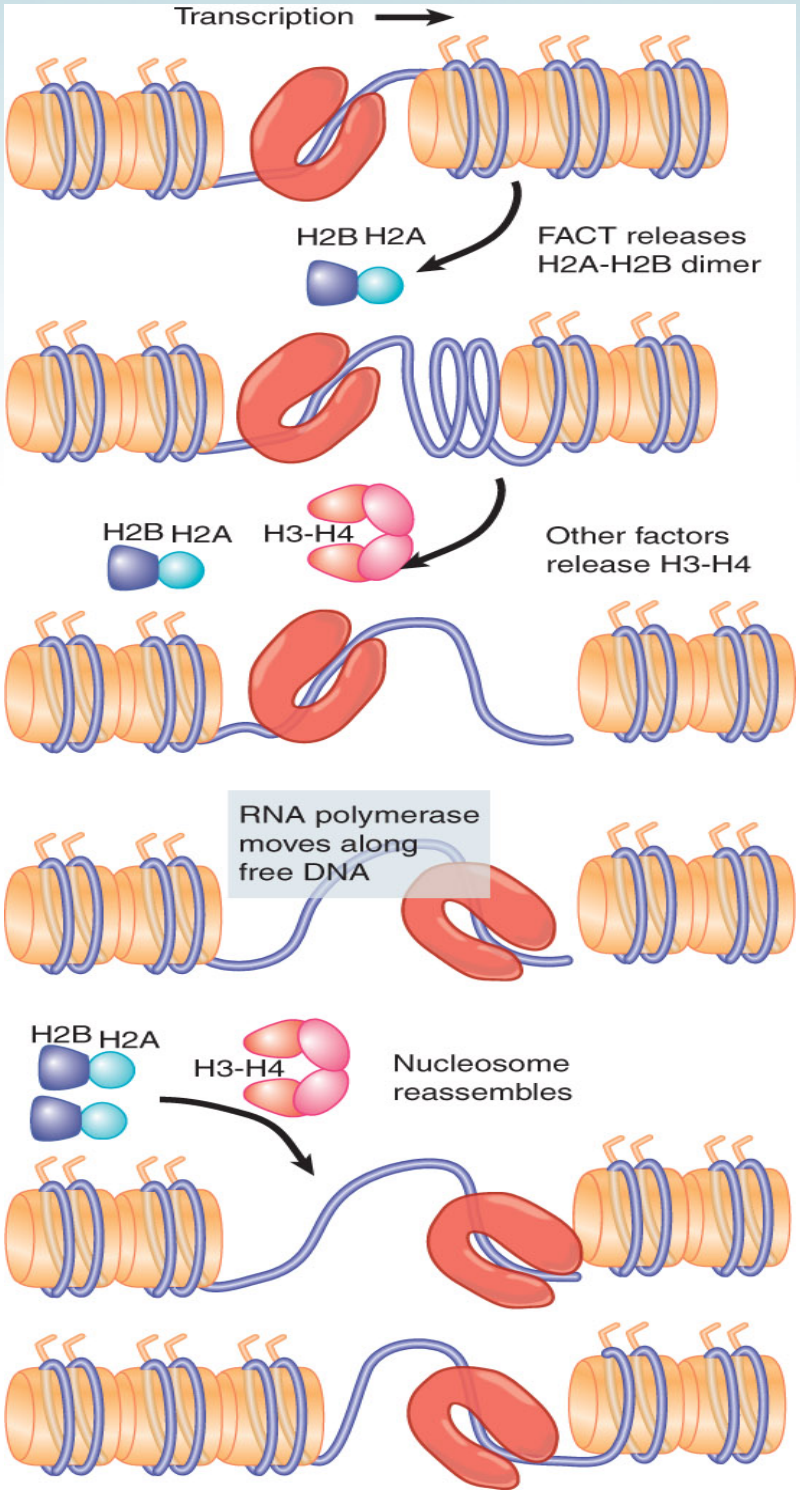
BIOL8620 Eukaryotic Genetics

Mutations, DNA Repair... & Recombination

Chapters 13 &14
& parts of 1 & 2

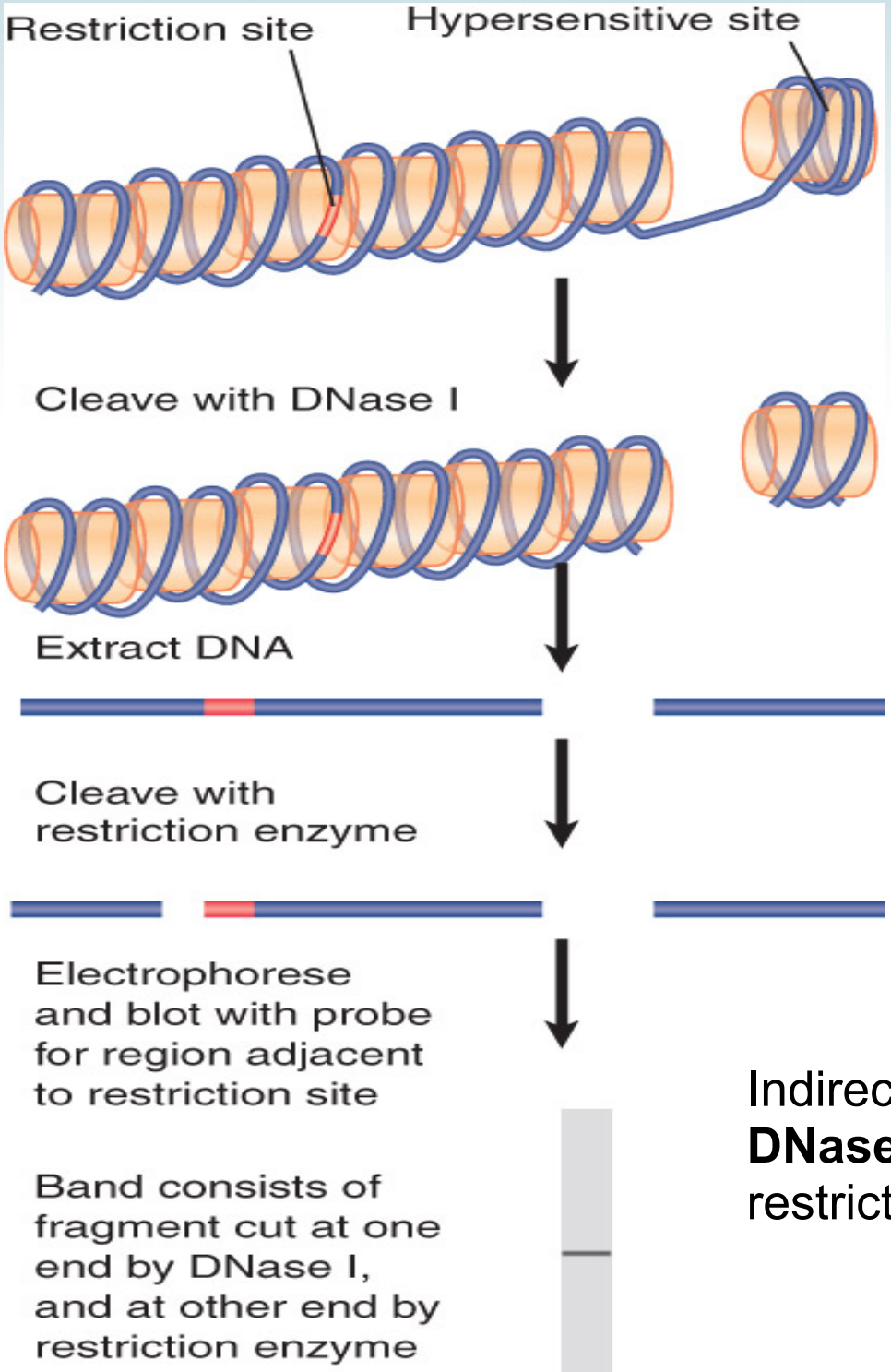


An experiment to test the effect of transcription on nucleosomes shows that the histone octamer is displaced from DNA and rebinds at a new position



- Ancillary factors are required both:
  - for RNA polymerase to displace octamers during transcription
  - for the histones to reassemble into nucleosomes after transcription

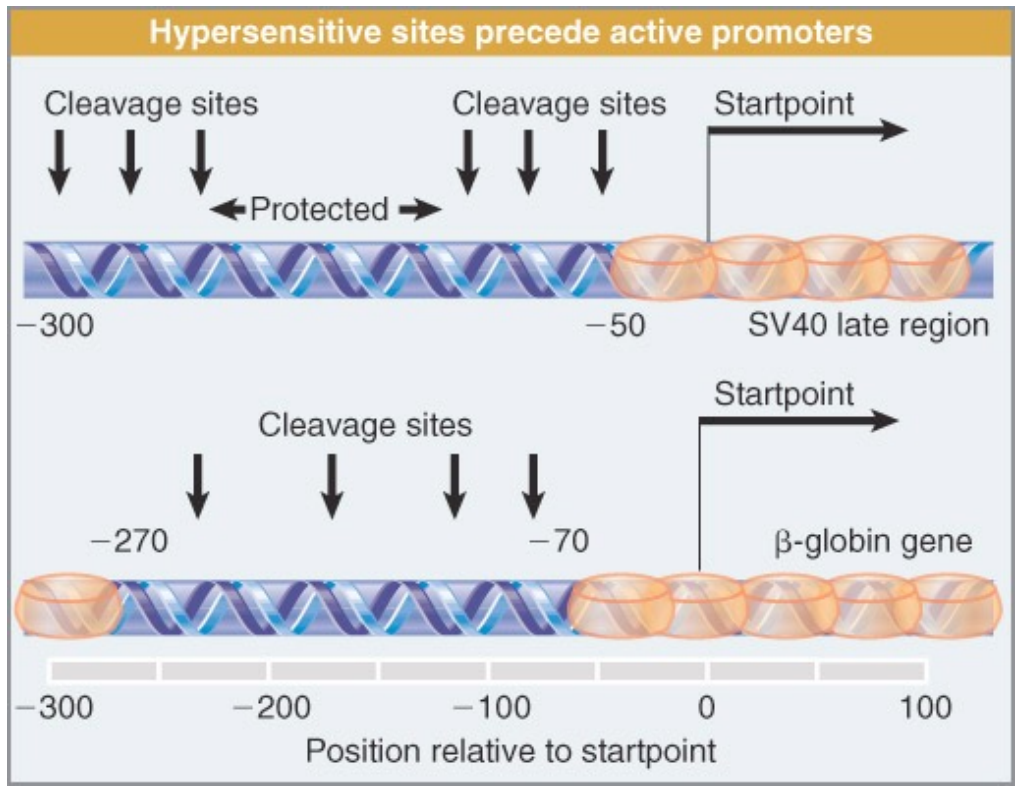
Histone octamers are disassembled ahead of transcription to remove nucleosomes

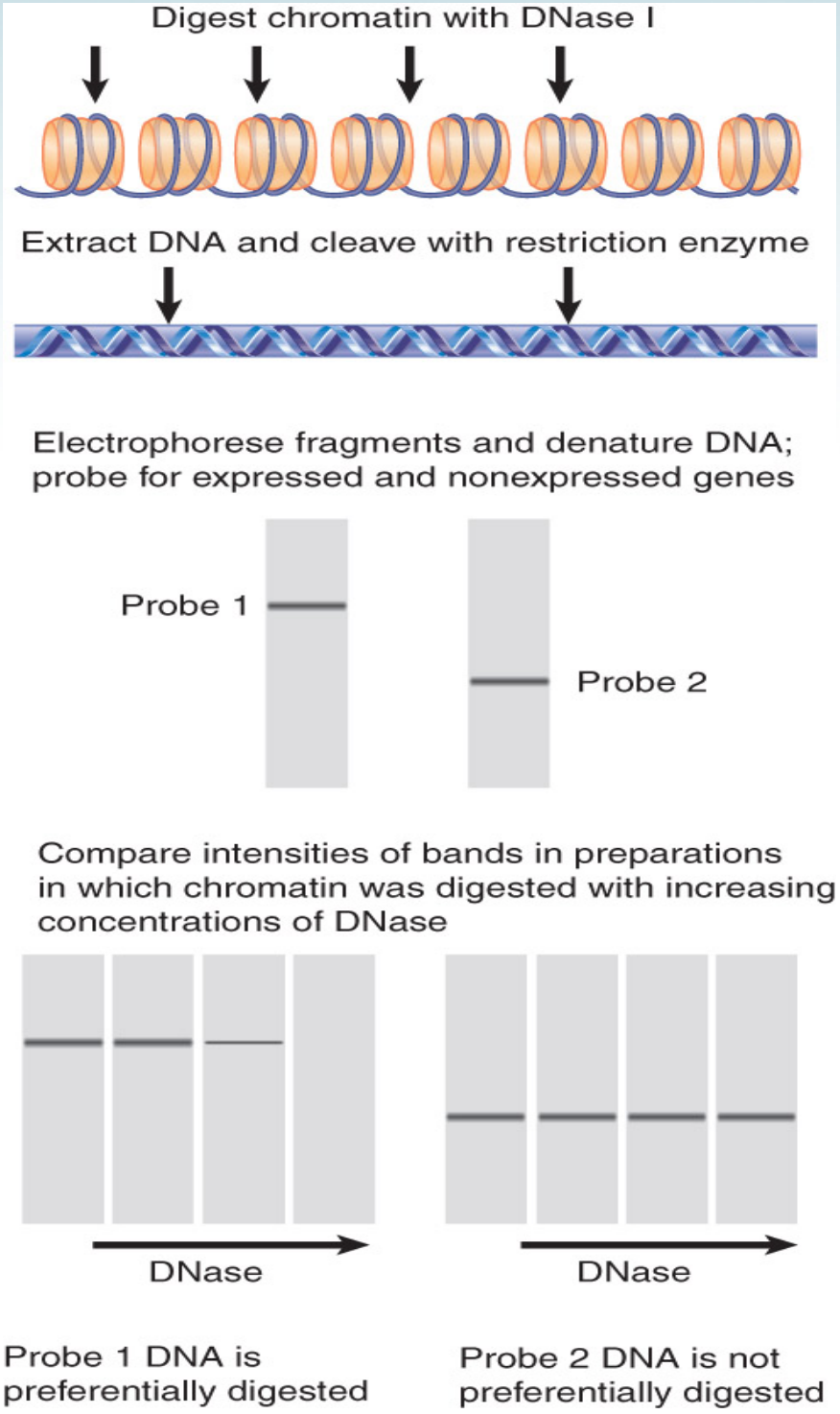


Indirect end-labeling identifies the distance of a **DNase I** hypersensitive site from a “fixed” restriction cleavage site

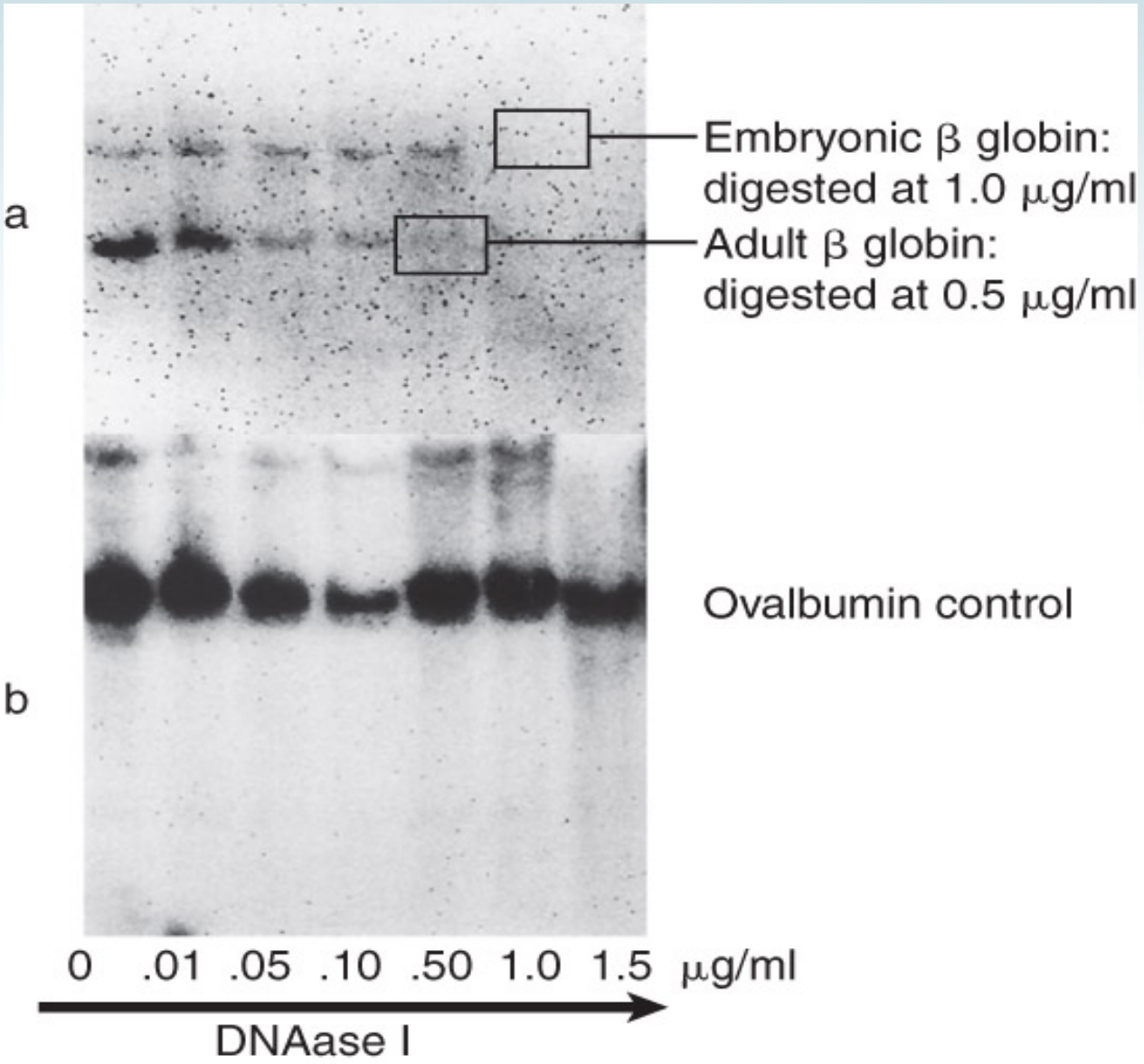
DNAase Hypersensitive Sites Reflect Changes in Chromatin Structure

- Hypersensitive sites are found at the promoters of expressed genes.
- They are generated by the binding of transcription factors that displace histone octamers.



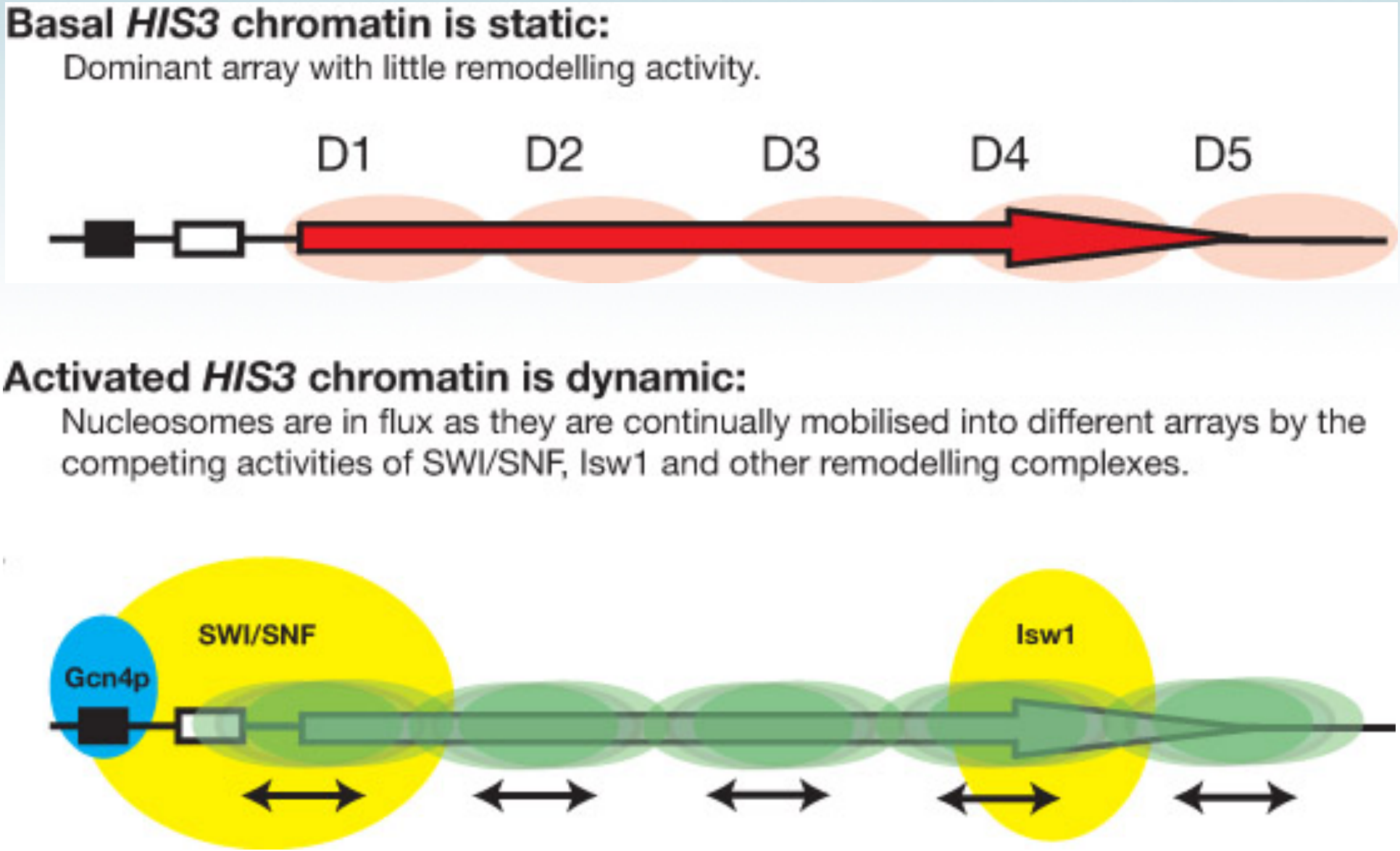


Location of DNase I sensitive sites (at at open promoters for example) can be measured by determining the rate of disappearance of the material hybridizing with discrete probes that and to different fragments of DNA around different DNA sequences.



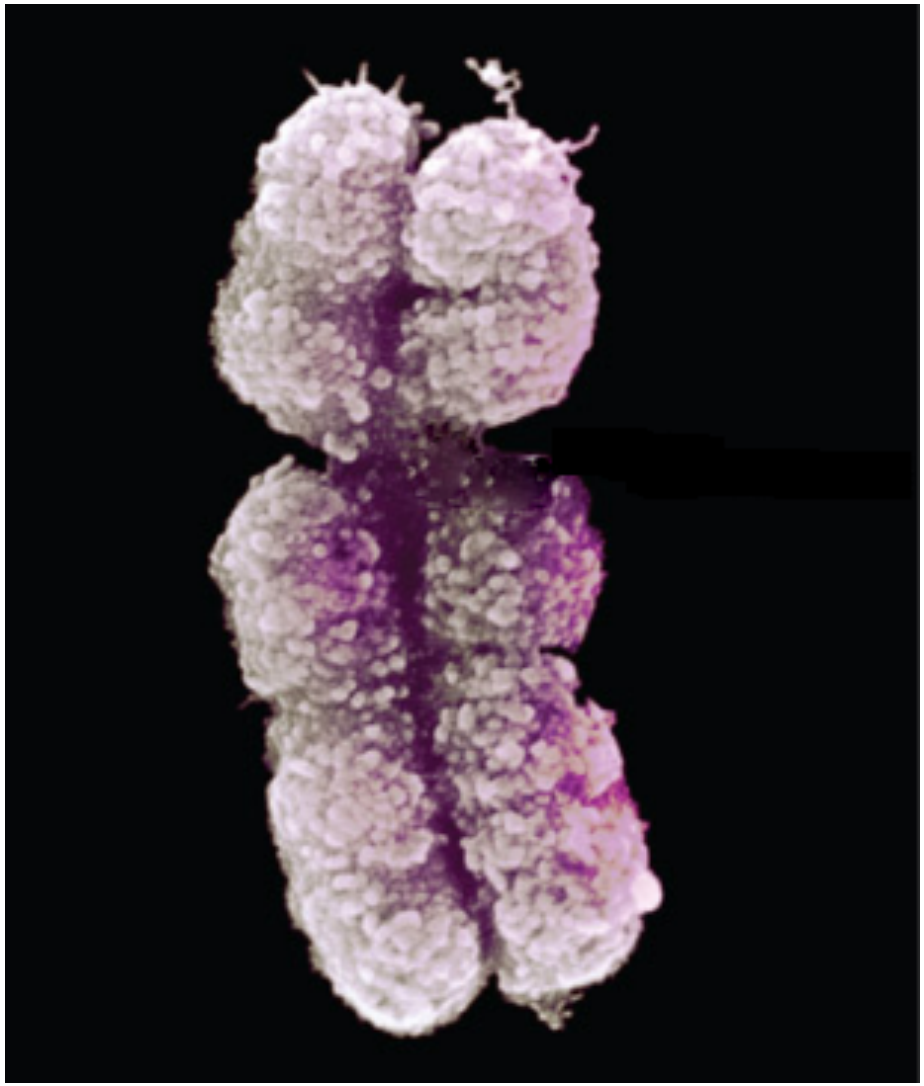
In adult erythroid cells, the **adult ß-globin** gene promoters are more highly sensitive to DNase I digestion than **embryonic ß-globin** gene promoters in adults

Photo courtesy of Harold Weintraub, Fred Hutchinson Cancer Research Center.
Used with permission of Mark Groudine

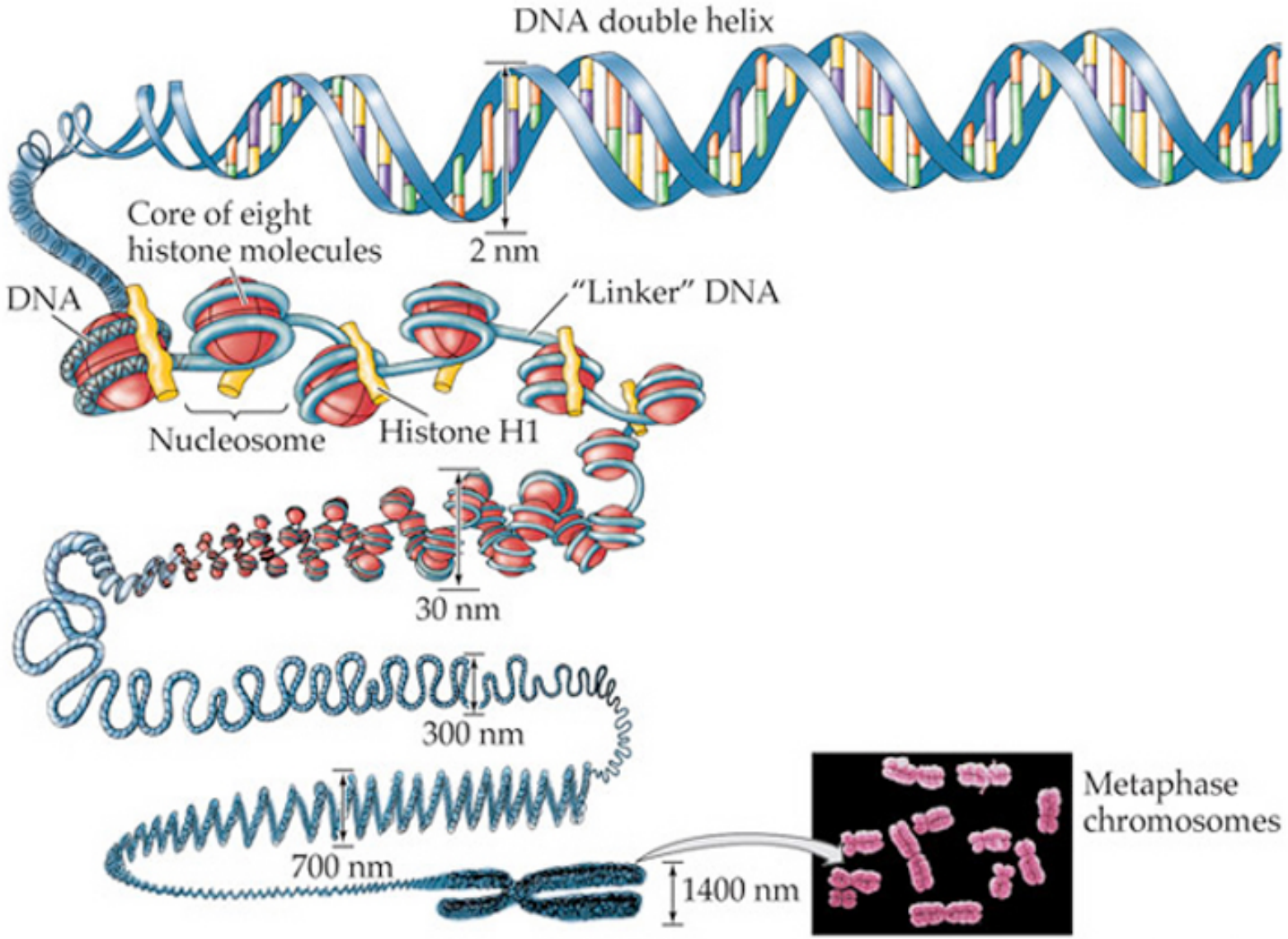


Basal/unactivated HIS3 chromatin is static and shows a single dominant nucleosomal array with little remodeling activity (top panel) Basal/unactivated HIS3 chromatin is static and shows a single dominant nucleosomal array with little remodeling activity (top panel)

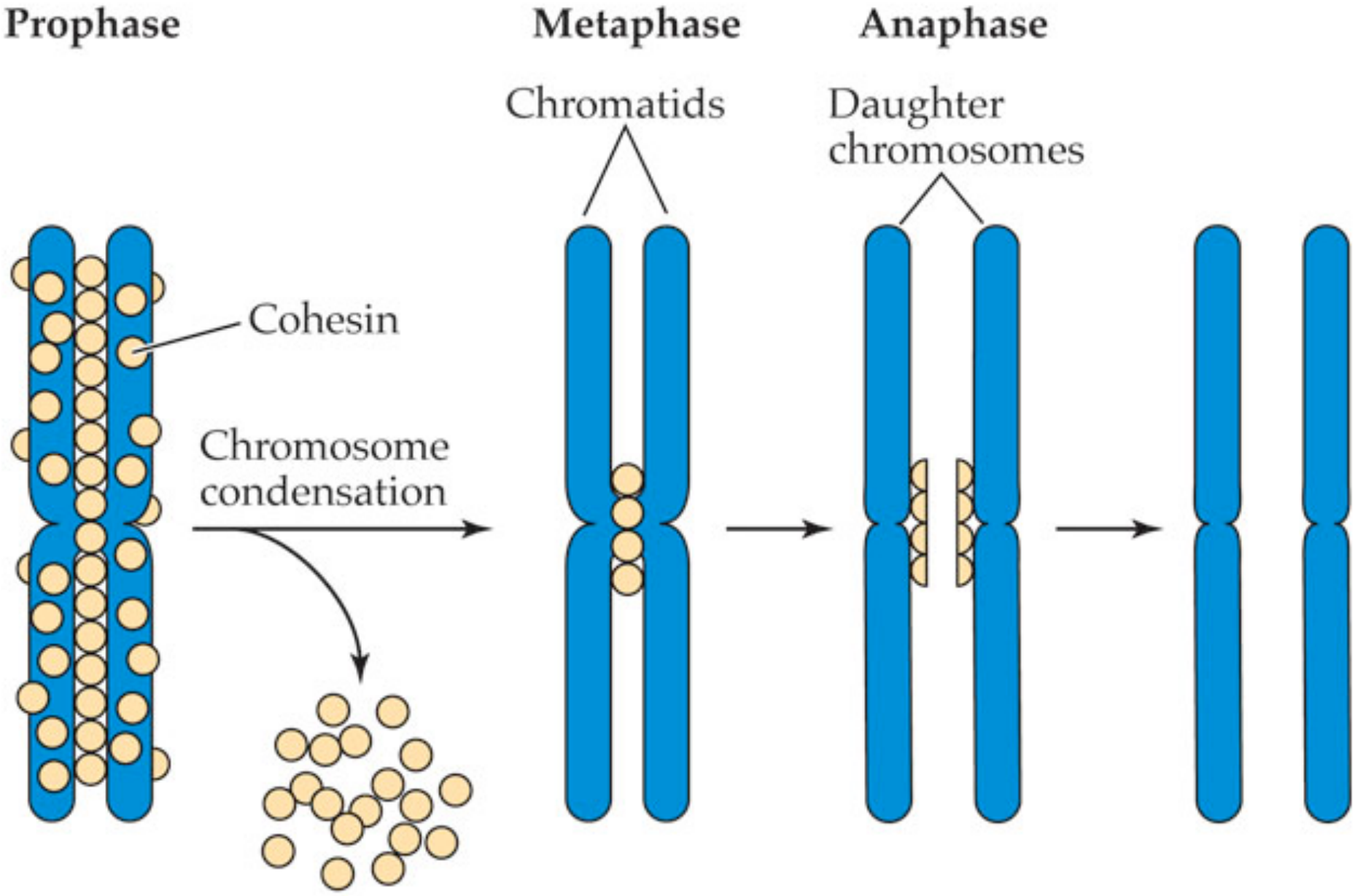
The Eukaryotic chromosome



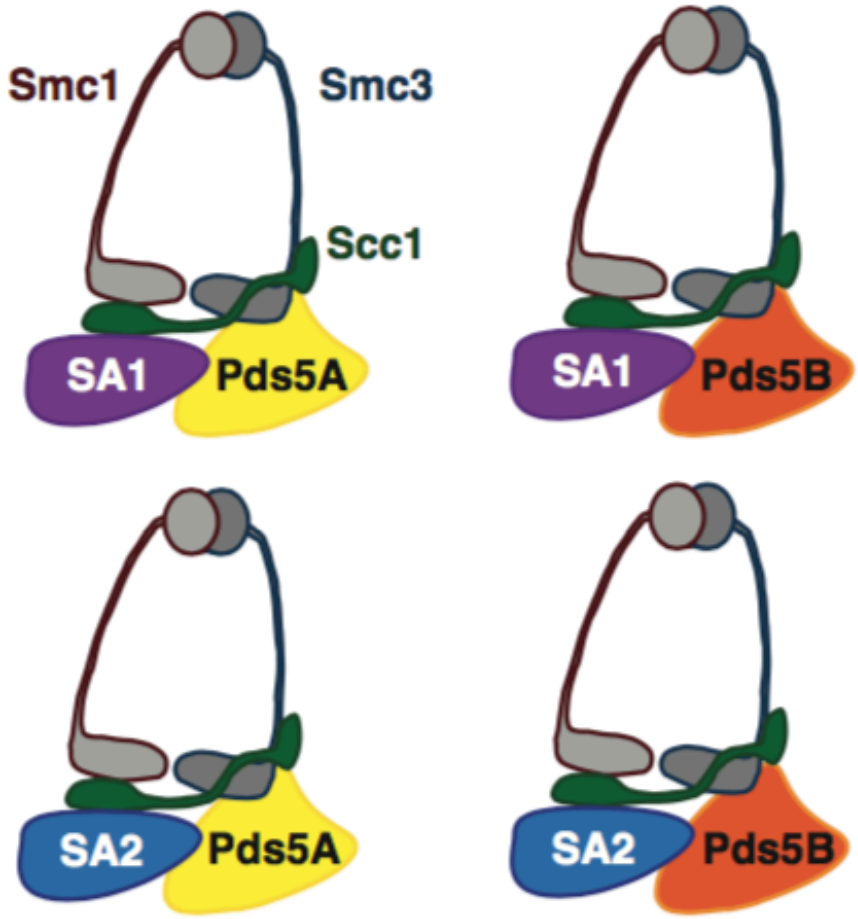
- Organized Structures -banding
- Centromeres
- Telomeres
- Nucleosomes
- **Euchromatin / Heterochromatin**
- **Higher Orders of Chromosomal Structure**



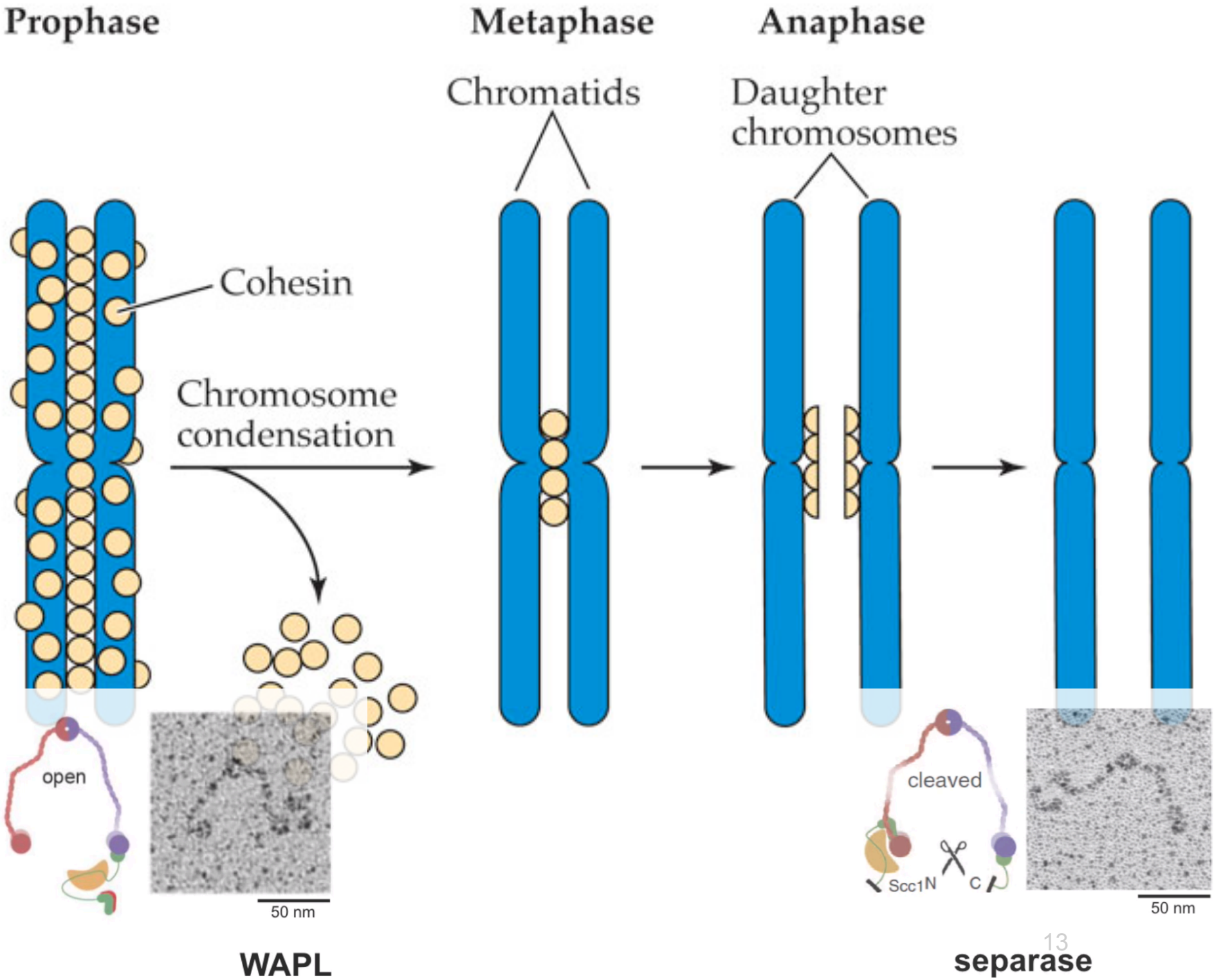
LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 9.6 DNA Packs into a Mitotic Chromosome



LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 9.9 Molecular Biology of Chromatid Attachment and Separation



Cohesins more than just sticky proteins, they are required to snare freshly replicated DNA and have two globular domains separated by a hinge like structure … different variants involved in different cell types that are undergoing different meiosis or mitosis…. Also help to build higher levels of chromatin organization…



WAPL

separase

NUCLEUS, 2018
VOL. 9, NO. 1, 20–32
https://doi.org/10.1080/19491034.2017.1389365

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EXTRA VIEW

Recent evidence that TADs and chromatin loops are dynamic structures

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ABSTRACT

Mammalian genomes are folded into spatial domains, which regulate gene expression by modulating enhancer-promoter contacts. Here, we review recent studies on the structure and function of Topologically Associating Domains (TADs) and chromatin loops. We discuss how loop extrusion models can explain TAD formation and evidence that TADs are formed by the ring-shaped protein complex, cohesin, and that TAD boundaries are established by the DNA-binding protein, CTCF. We discuss our recent genomic, biochemical and single-molecule imaging studies on CTCF and cohesin, which suggest that TADs and chromatin loops are dynamic structures. We highlight complementary polymer simulation studies and Hi-C studies employing acute depletion of CTCF and cohesin, which also support such a dynamic model. We discuss the limitations of each approach and conclude that in aggregate the available evidence argues against stable loops and supports a model where TADs are dynamic structures that continually form and break throughout the cell cycle.

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KEYWORDS
CTCF; cohesin; 3D genome; single-molecule imaging; dynamics; FRAP; topological domains; chromatin loops; loop extrusion; modeling

Figure 1. Chromosome structure and function is organized at multiple scales. At the smallest scale, DNA is folded into a double helix, which gets compacted into »11 nm nucleosomes, whereby **147 bp of DNA wrap around a histone octamer.**

Functionally, nucleosomes regulate access of DNA-binding proteins and serve as modules for epigenetic modifications, which regulate gene expression. At the intermediate scale of tens of kilobases to a few megabases, chromatin is organized into Topologically Associating Domains (TADs) with a median size of a few hundred kilobases.

Functionally, TADs are characterized by preferential contact of loci within them, and critically control enhancer-promoter interactions, and relative insulation from adjacent TADs. At a similar scale of TADs, chromatin is also organized into **epigenomic** A/B "compartments", whereby **active chromatin (A) tends to contact with other segments of active chromatin and localize in proximity of certain nuclear bodies such as nuclear speckles**, while **inactive chromatin (B) tends to contact with inactive chromatin and be associated with the nuclear lamina**.

At the largest scale, particular chromosomes tend to associate with other chromosomes and form stereotyped chromosome territories inside the cell nucleus. The image used to illustrate chromosome territories was generously provided by Stevens et al.[76]

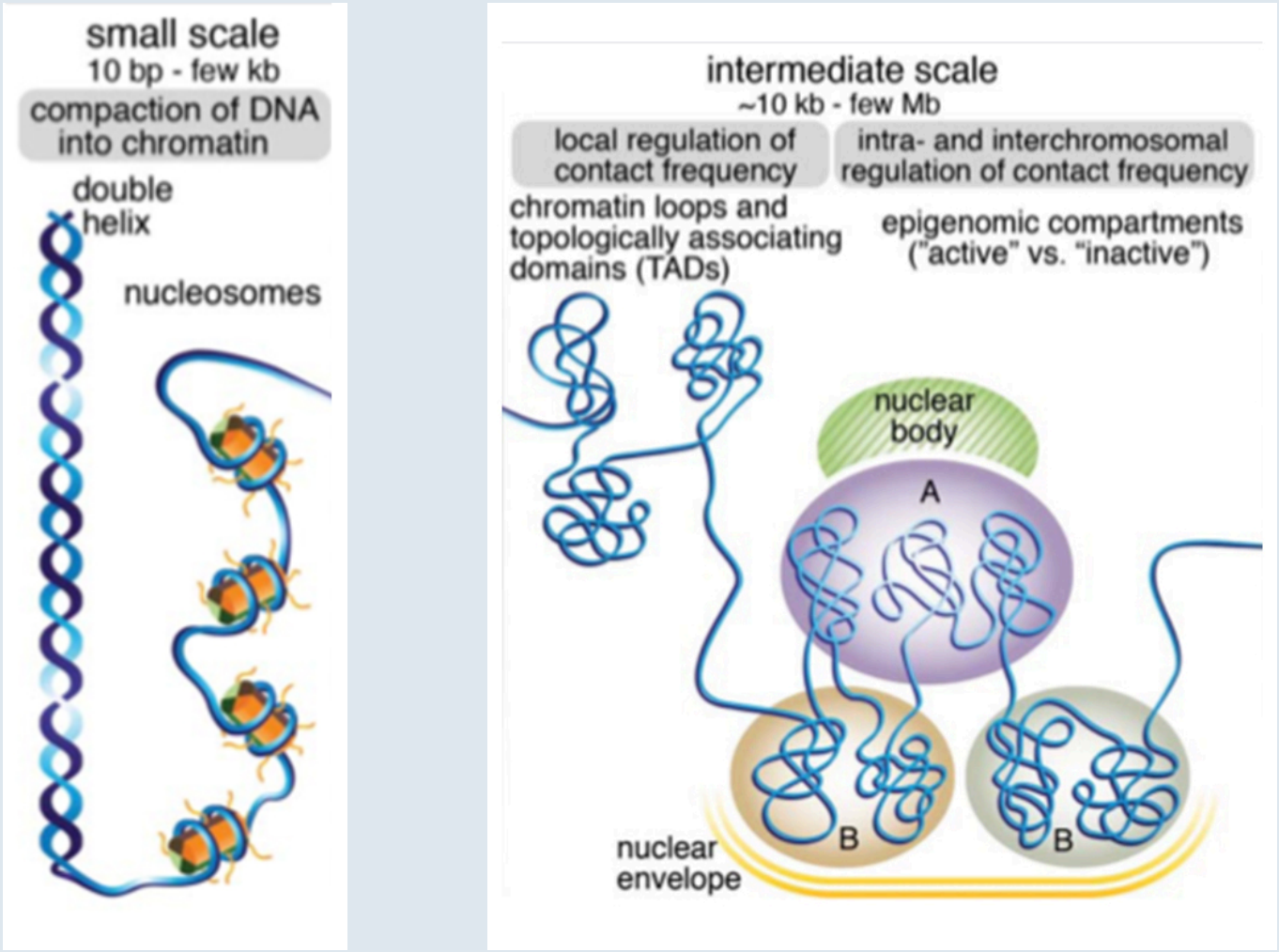


Figure 1. Chromosome structure and function is organized at multiple scales. At the smallest scale, DNA is folded into a double helix, which gets compacted into »11 nm nucleosomes, whereby 147 bp of DNA wrap around a histone octamer. Functionally, nucleosomes regulate access of DNA-binding proteins and serve as modules for epigenetic modifications, which regulate gene expression. At the intermediate scale of tens of kilobases to a few megabases, chromatin is organized into Topologically Associating Domains (TADs) with a median size of a few hundred kilobases. Functionally, TADs are characterized by preferential contact of loci within them, and critically control enhancer-promoter interactions, and relative insulation from adjacent TADs. At a similar scale of TADs, chromatin is also organized into epigenomic A/B "compartments", whereby **active chromatin (A) tends to contact with other segments of active chromatin and localize in proximity of certain nuclear bodies such as nuclear speckles, while inactive chromatin** (B) tends to contact with inactive chromatin and to be associated with the nuclear lamina. At the largest scale, particular chromosomes tend to associate with other chro- mosomes and form stereotyped chromosome territories inside the cell nucleus. The image used to illustrate chromosome territories was generously provided by Stevens et al.[76]

REVIEW

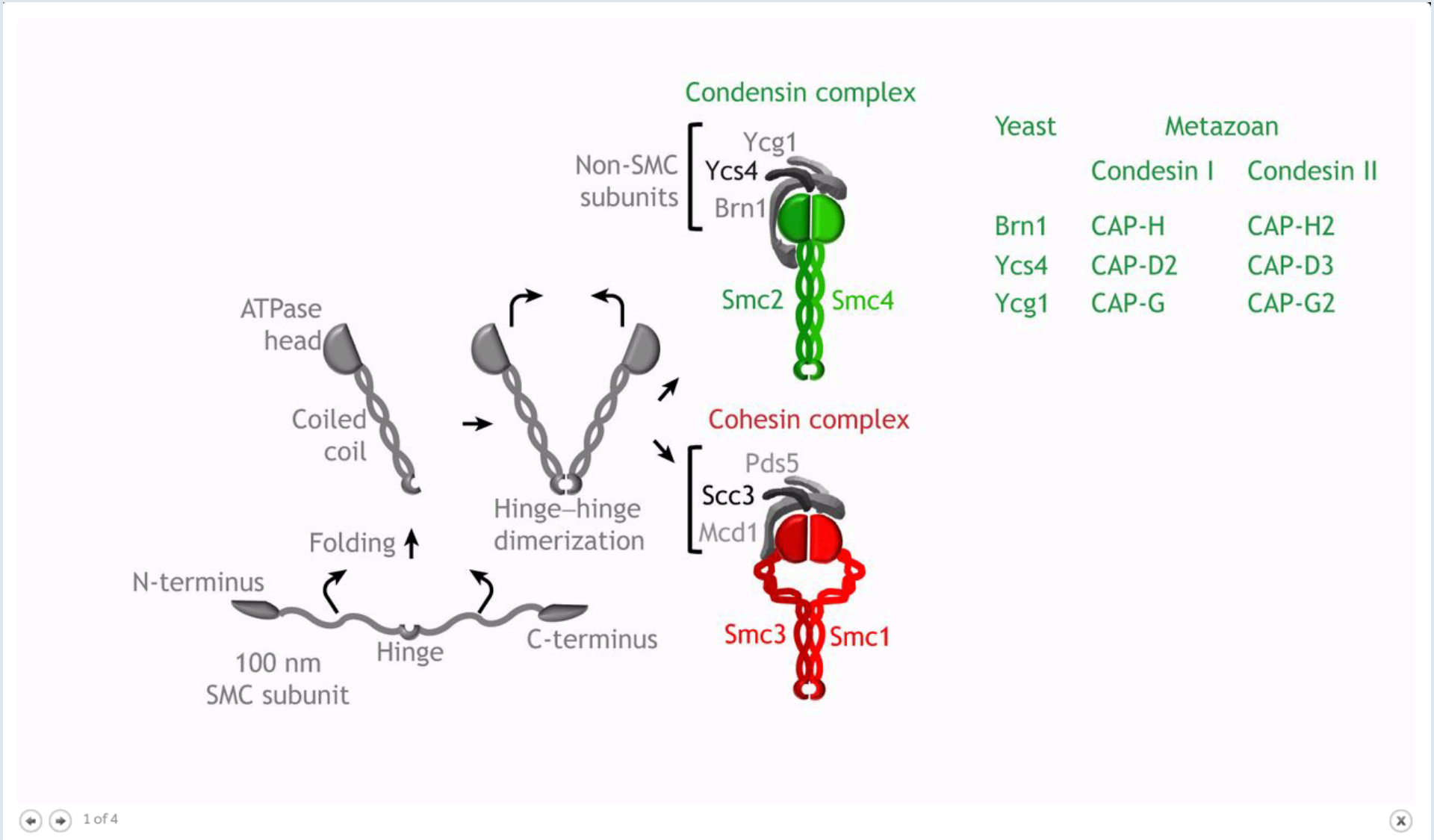
Condensins and cohesins – one of these things is not like the other!

Robert V. Skibbens

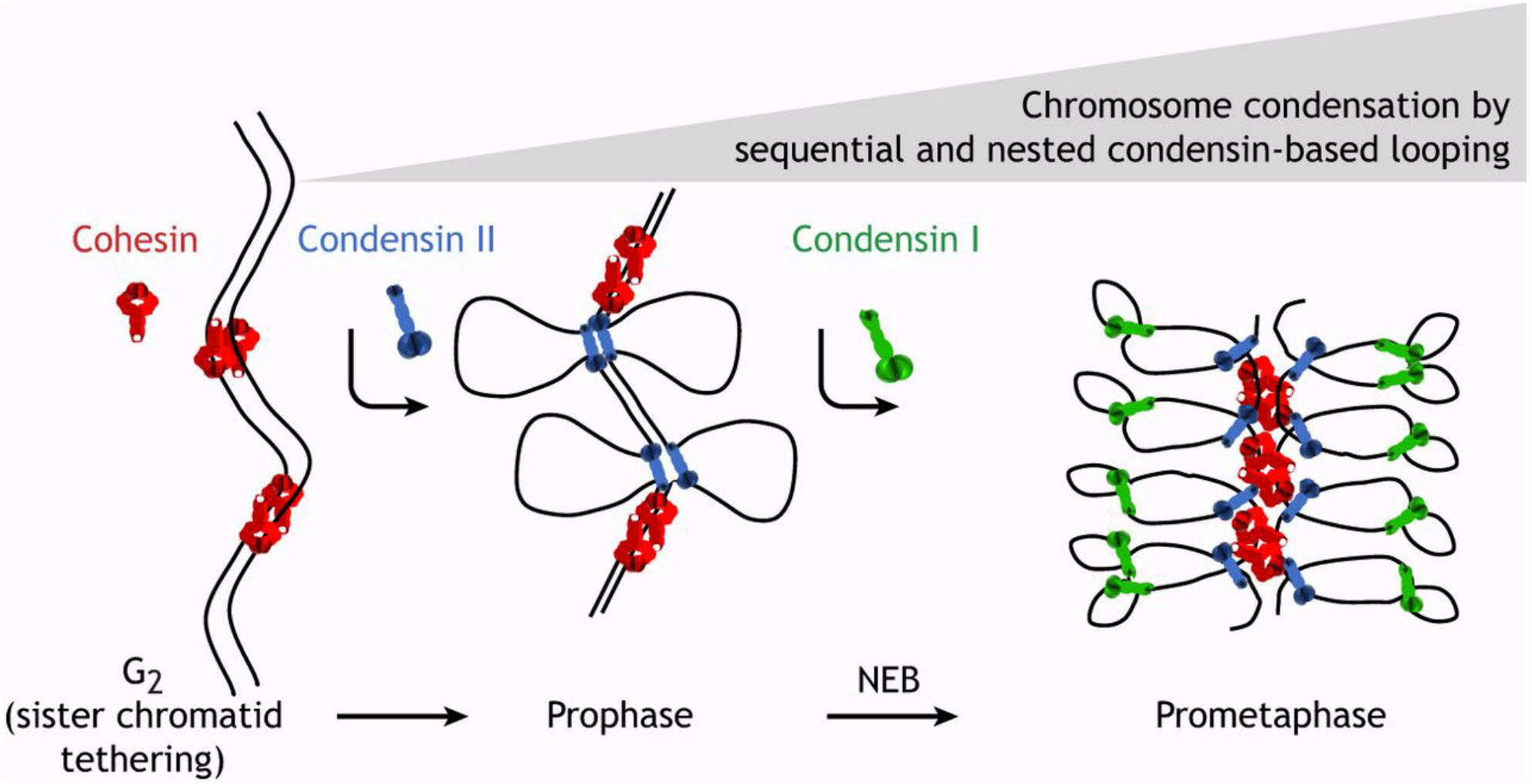
Journal of Cell Science 2019 132: jcs220491 doi: 10.1242/jcs.220491 Published 7 February 2019

ABSTRACT

Condensins and cohesins are highly conserved complexes that tether together DNA loci within a single DNA molecule to produce DNA loops. Condensin and cohesin structures, however, are different, and the DNA loops produced by each underlie distinct cell processes. Condensin rods compact chromosomes during mitosis, with condensin I and II complexes producing spatially defined and nested looping in metazoan cells. Structurally adaptive cohesin rings produce loops, which organize the genome during interphase. Cohesin-mediated loops, termed topologically associating domains or TADs, antagonize the formation of epigenetically defined but untethered DNA volumes, termed compartments. While condensin complexes formed through *cis*-interactions must maintain chromatin compaction throughout mitosis, cohesins remain highly dynamic during interphase to allow for transcription-mediated responses to external cues and the execution of developmental programs. Here, I review differences in condensin and cohesin structures, and highlight recent advances regarding the intramolecular or *cis*-based tetherings through which condensins compact DNA during mitosis and cohesins organize the genome during interphase.



SMC assemblies. The stereotypical folding and dimerization of SMC subunits is shown in gray; 100-nm-long SMC proteins fold at a central hinge and allow for N- and C-terminal binding to form ATPase heads. SMCs dimerize predominantly through hinge-hinge binding but also head–head binding. Condensins (shown in green) are formed from Smc2 and Smc4 heterodimers that recruit the non-SMC subunits Brn1, Ycs4 and Ycg1 to form flexible rods (see Fig. 2). Cohesins (shown in red) are formed from Smc1 (SMC1a or SMC1b in metazoans) and Smc3 heterodimers that recruit the non-SMC subunits Mcd1, Scc3 and Pds5 to form flattened rings (shown) but can adopt other conformations, such as rods, open V-shapes or C-clamps (not shown). Yeast contain single copies of Brn1, Ycs4 and Ycg1, but metazoan cells contain paralogs, which give rise to unique subtypes termed condensin I (CAP-H, CAP-D2 and CAP-G, encoded by NCAPH, NCAPD2 and NCAPG, respectively) and condensin II (CAP-H2, CAP-D3, and CAP-G2, encoded by NCAPH2, NCAPD3 and NCAPG2, respectively). Metazoan cells also contain subunit paralogs (RAD21 or RAD21L for Mcd1, SA1/STAG1, SA2/STAG2 or SA3/STAG3 for Scc3, and PDS5a or PDS5b/APRIN for Pds5), but the assembly of non-SMC subunits into cohesin complexes is less well defined. Note that metazoan cells also contain Sororin, which is absent in yeast

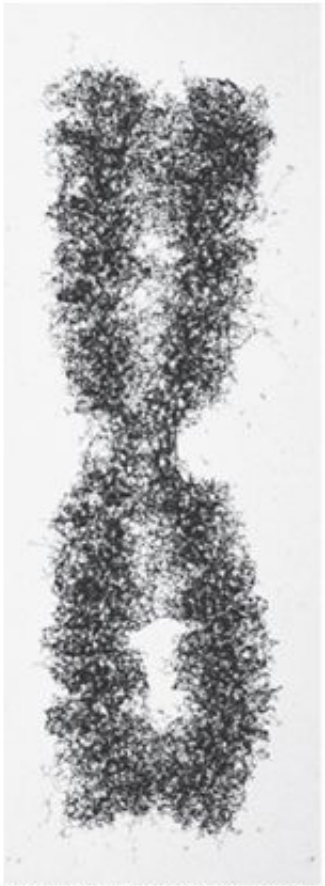


Cell-cycle-regulated chromosome compaction by sequential recruitment of condensins II and I promotes the formation of nested loops. Replicated sister chromatids are tethered together by cohesins (red). During prophase, condensin II (blue) binds DNA and extrudes loops. After nuclear envelope breakdown (NEB), condensin I (green) binds the looped DNA and forms new loops that are nested within the condensin II-generated loops. As cells progress into late prometaphase, DNA loop extrusion and compression of the helical scaffold continues. Not shown is the role for cohesin in generating cis-based compaction, which also promotes chromosome condensation.

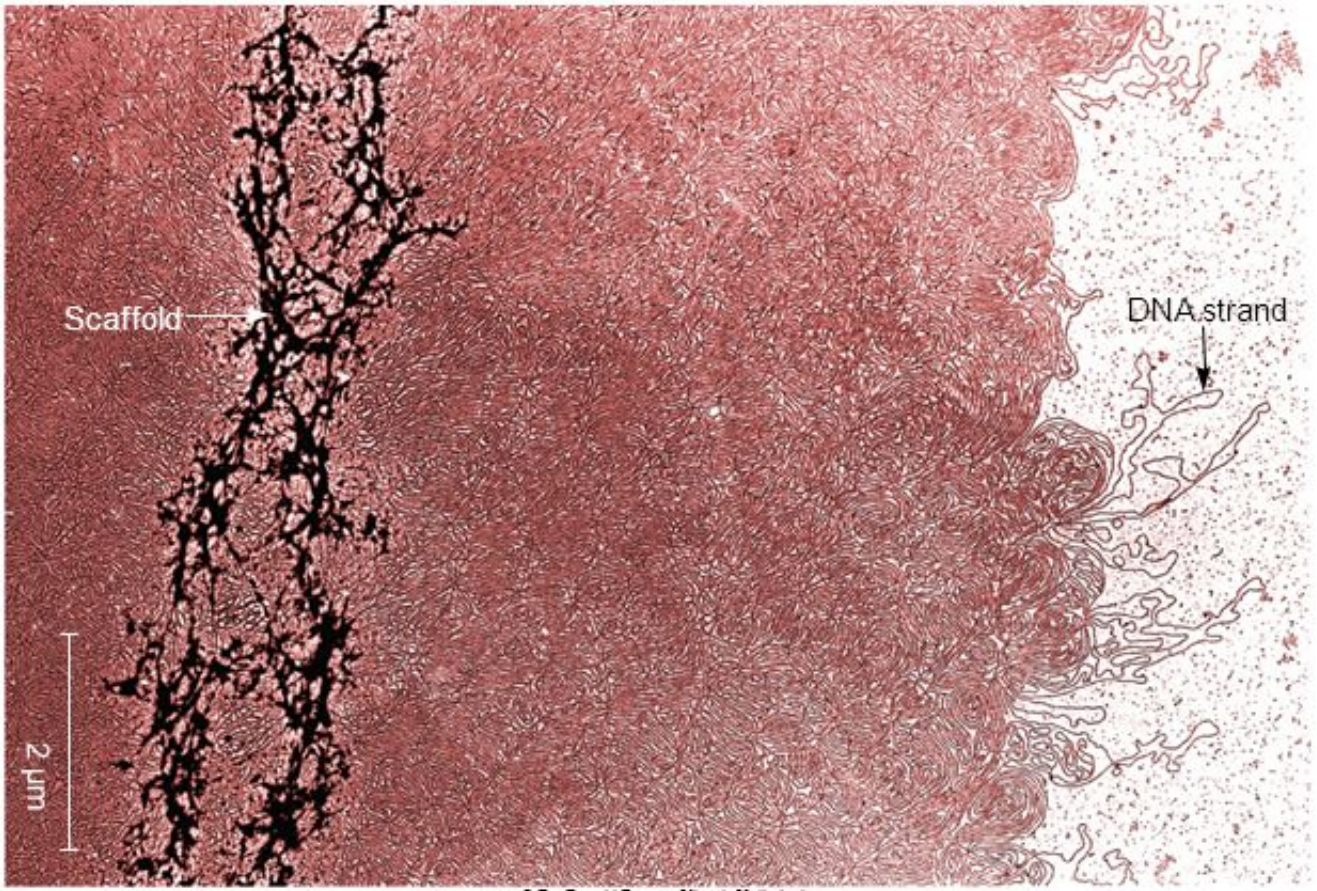
A pathway for mitotic chromosome formation

Gibcus, Samejima, Goloborodko et al., Science (2018)

Metaphase Chromosomes



Metaphase chromosome



Metaphase chromosome treated with high salt to remove histone proteins

Brooker, Fig 12.18

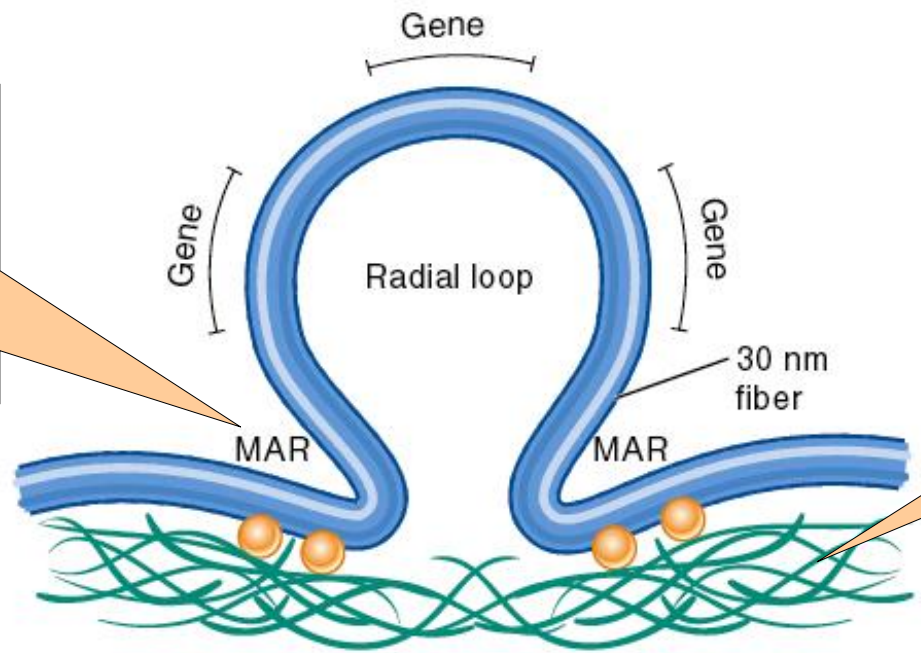
Further Compaction of the Chromosome

- An additional level of compaction involves interaction between the 30 nm fibers and the nuclear matrix

Matrix-attachment regions

or

Scaffold-attachment regions (SARs)



MARs are anchored to the nuclear matrix, thus creating radial loops

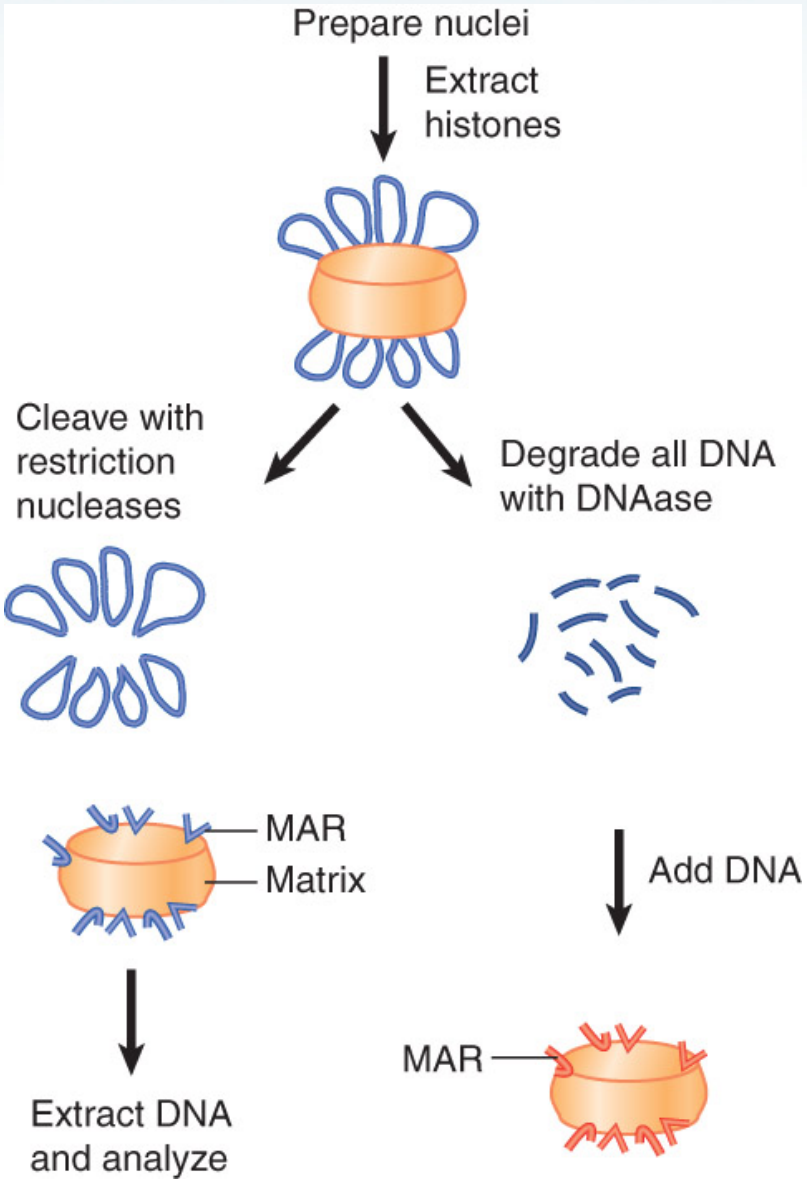
Specific Sequences Attach DNA to an Interphase Matrix

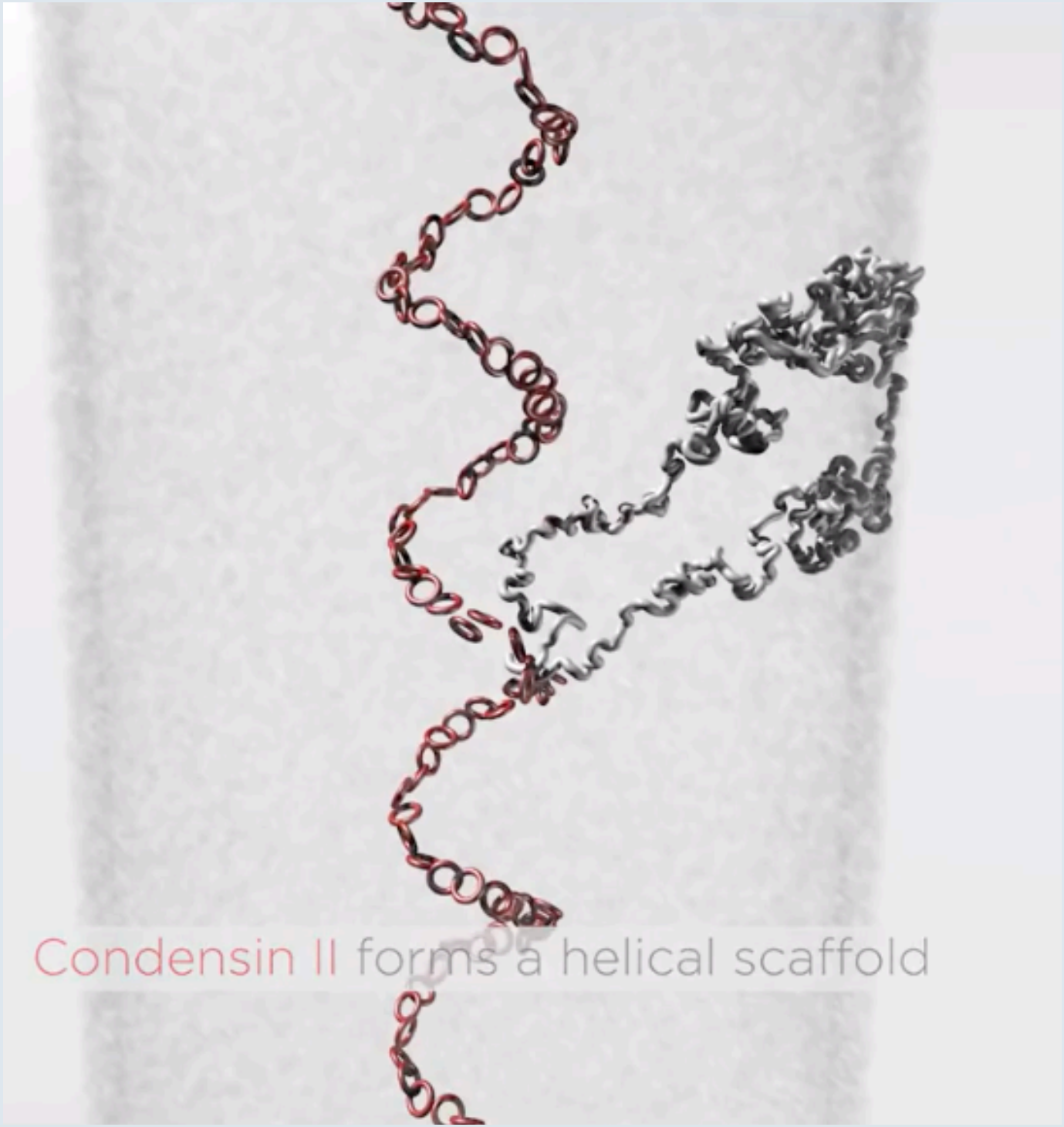
- **S/MAR** (**scaffold/matrix attachment region**), otherwise called **SAR** (**scaffold-attachment region**), or **MAR** (**matrix-associated region**), are sequences in the DNA where it interacts with the chromosomal scaffold as well as the nuclear matrix and/or

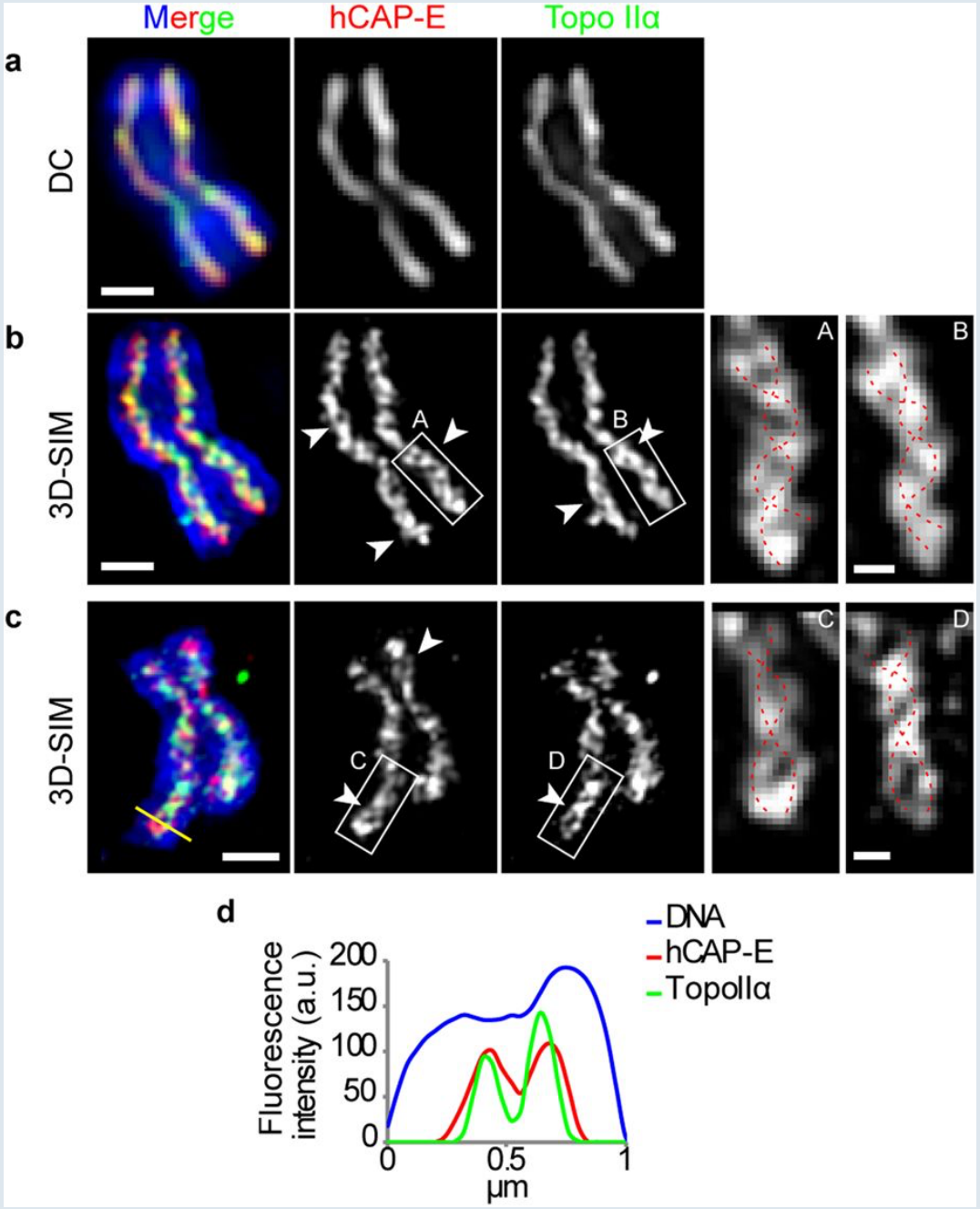
- The MARs are A-T-rich but do not have any specific consensus sequence.

- **Chromosome scaffold –** A proteinaceous structure in the shape of a sister chromatid pair, generated when chromosomes are depleted of histones.

Identifying scaffold-associated regions of DNA




Condensin II forms a helical scaffold



Chromosome Scaffold is a Double-Stranded Assembly of Scaffold Proteins

Rawin Poonperm, Hideaki Takata, Tohru Hamano, Atsushi Matsuda, Susumu Uchiyama, Yasushi Hiraoka & Kiichi Fukui

*Scientific Reports volume**5**, Article number: 11916 (2015)*

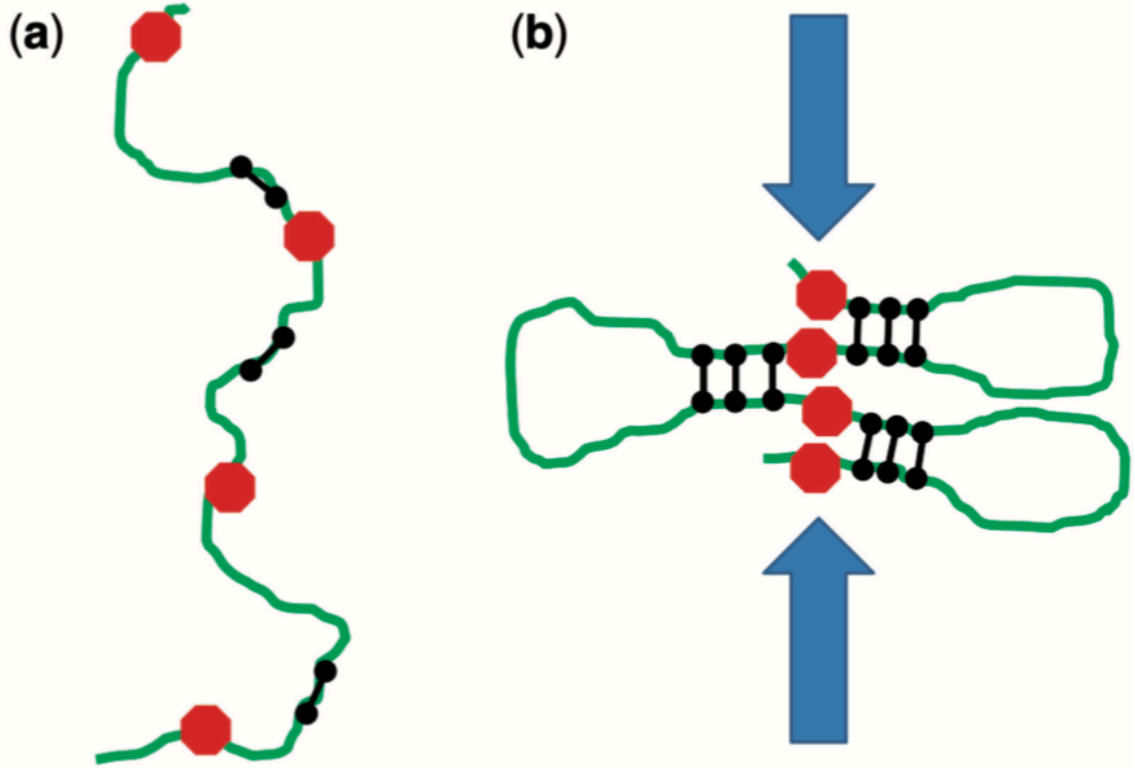
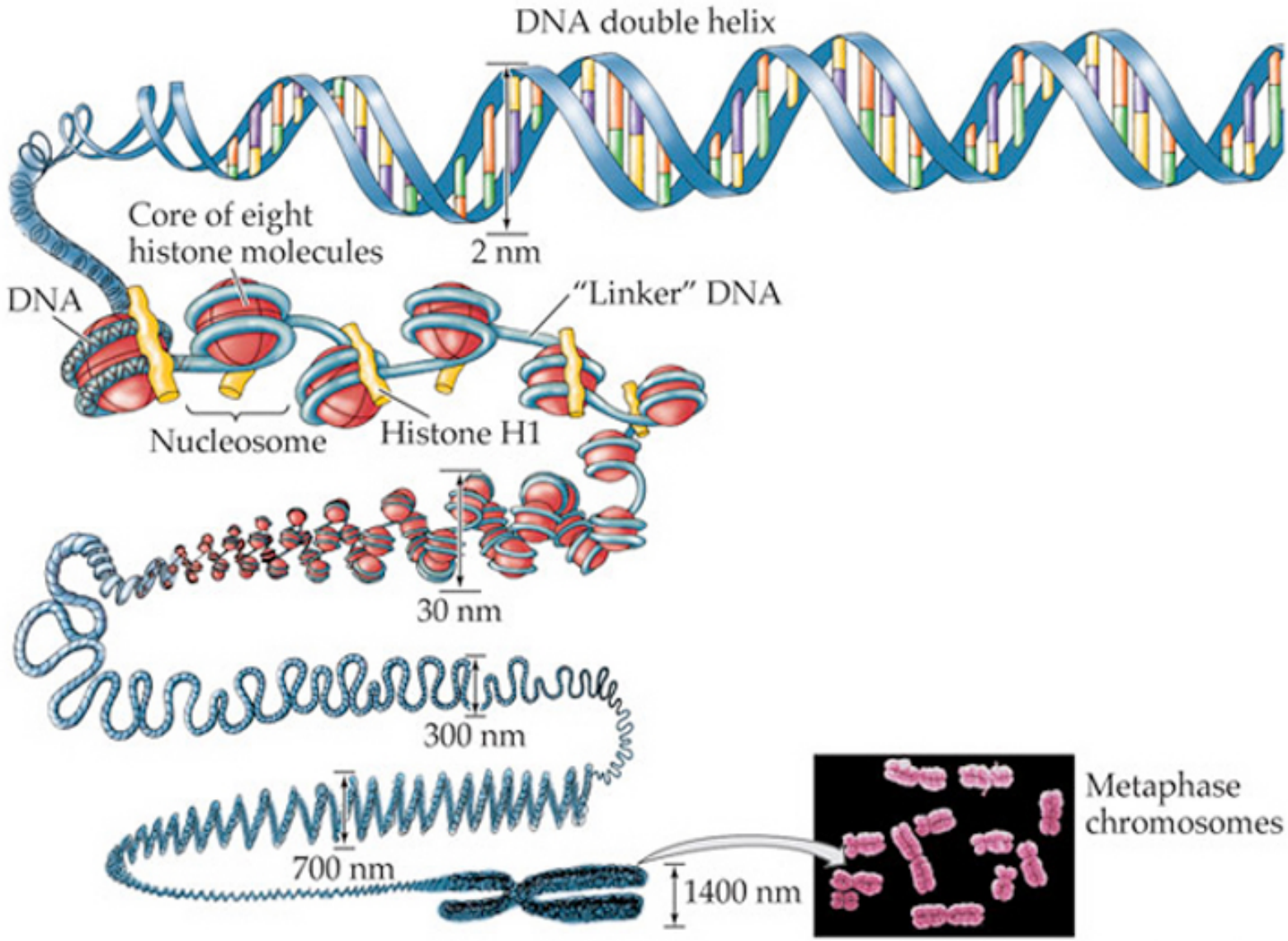
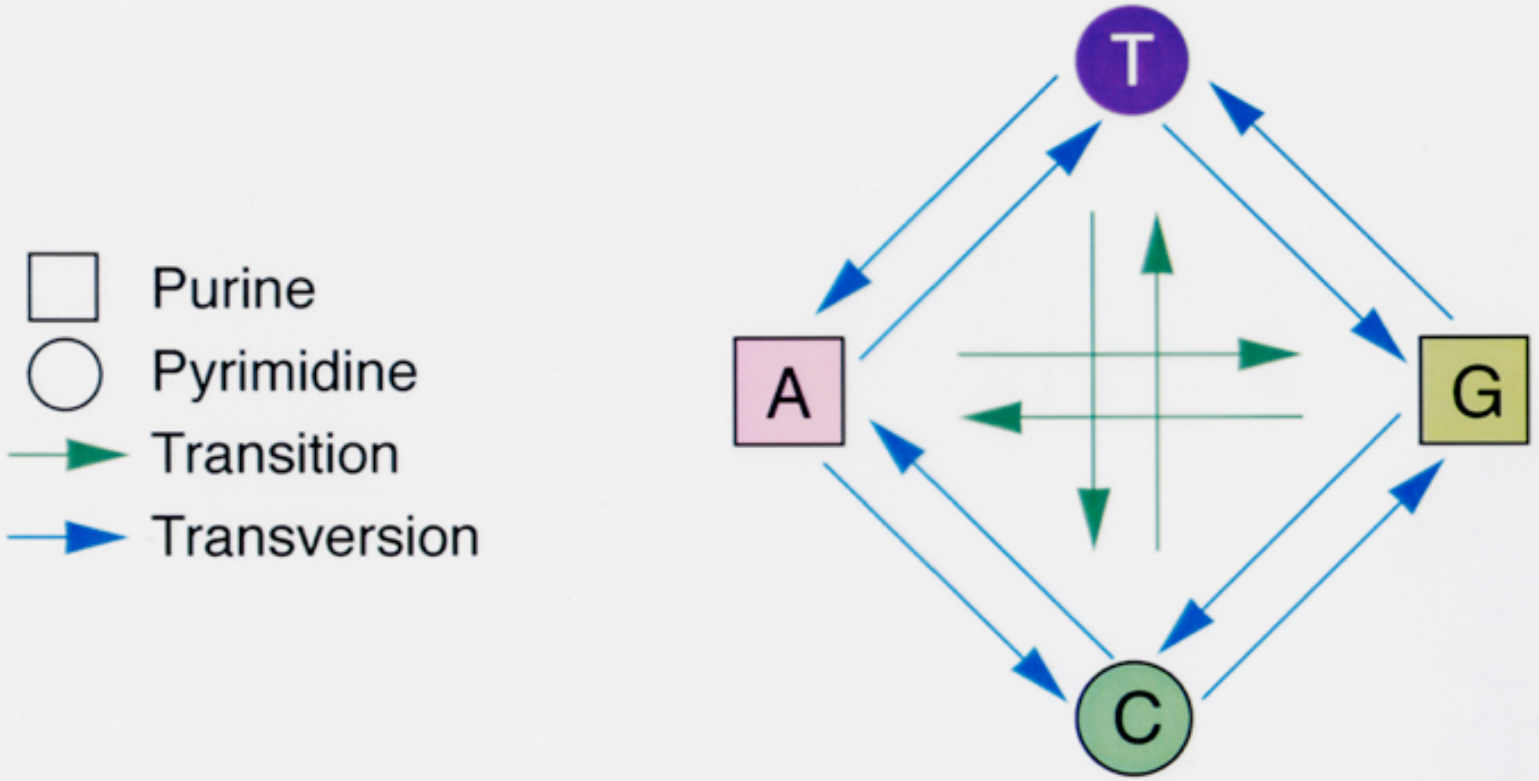


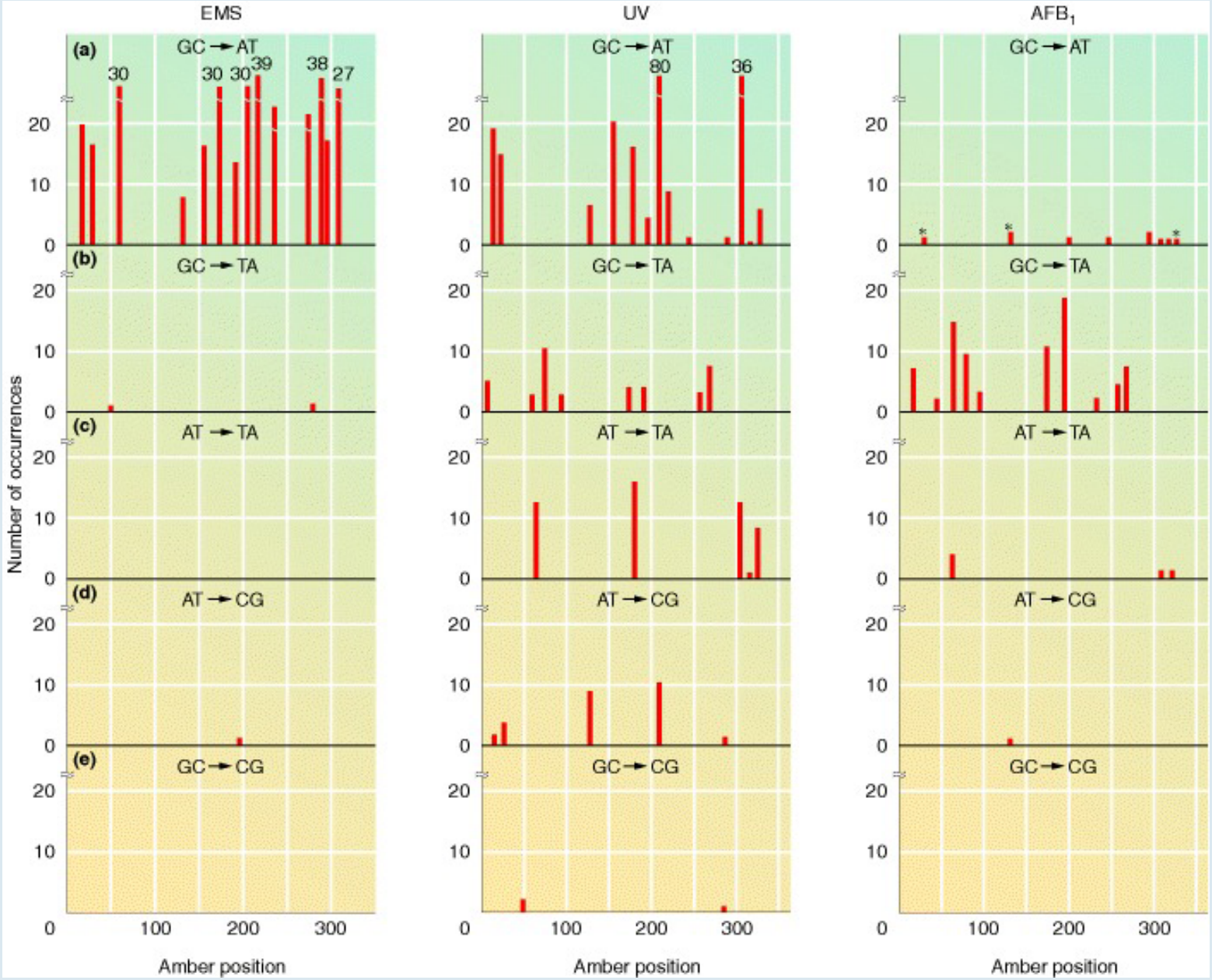
Figure 6. Condensation of multiple loop domains. (**a**) Initially condensins (black dumbbell shapes) bind along a long stretch of chromatin (green), between condensation boundary elements (red octagons). (**b**) As condensation proceeds, condensins organize into a 'stacked' configuration at the bases of chromatin loops defined by the boundary elements. The resultant crowding of chromatin at the bases of the loops generates inter-chromatid tension that will drive topo II to remove inter-chromatid entanglements.



LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 9.6 DNA Packs into a Mitotic Chromosome

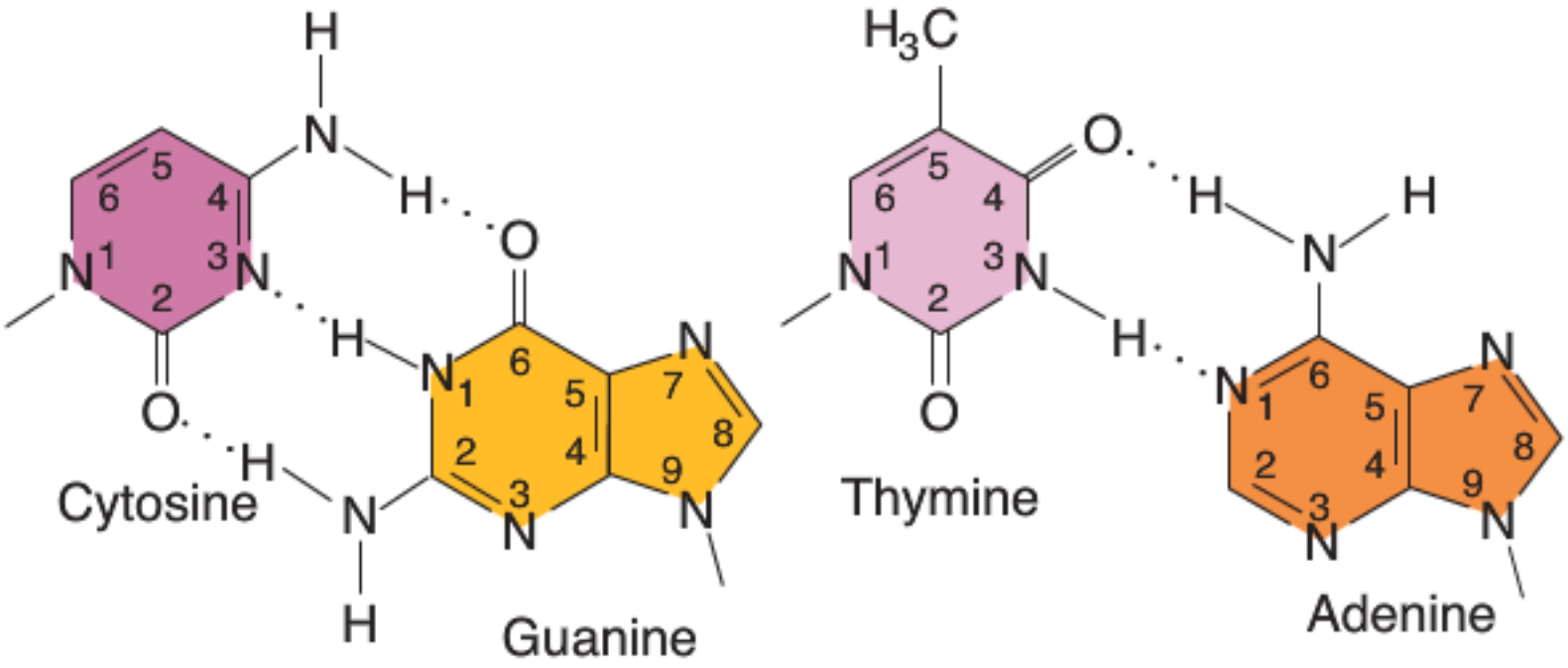


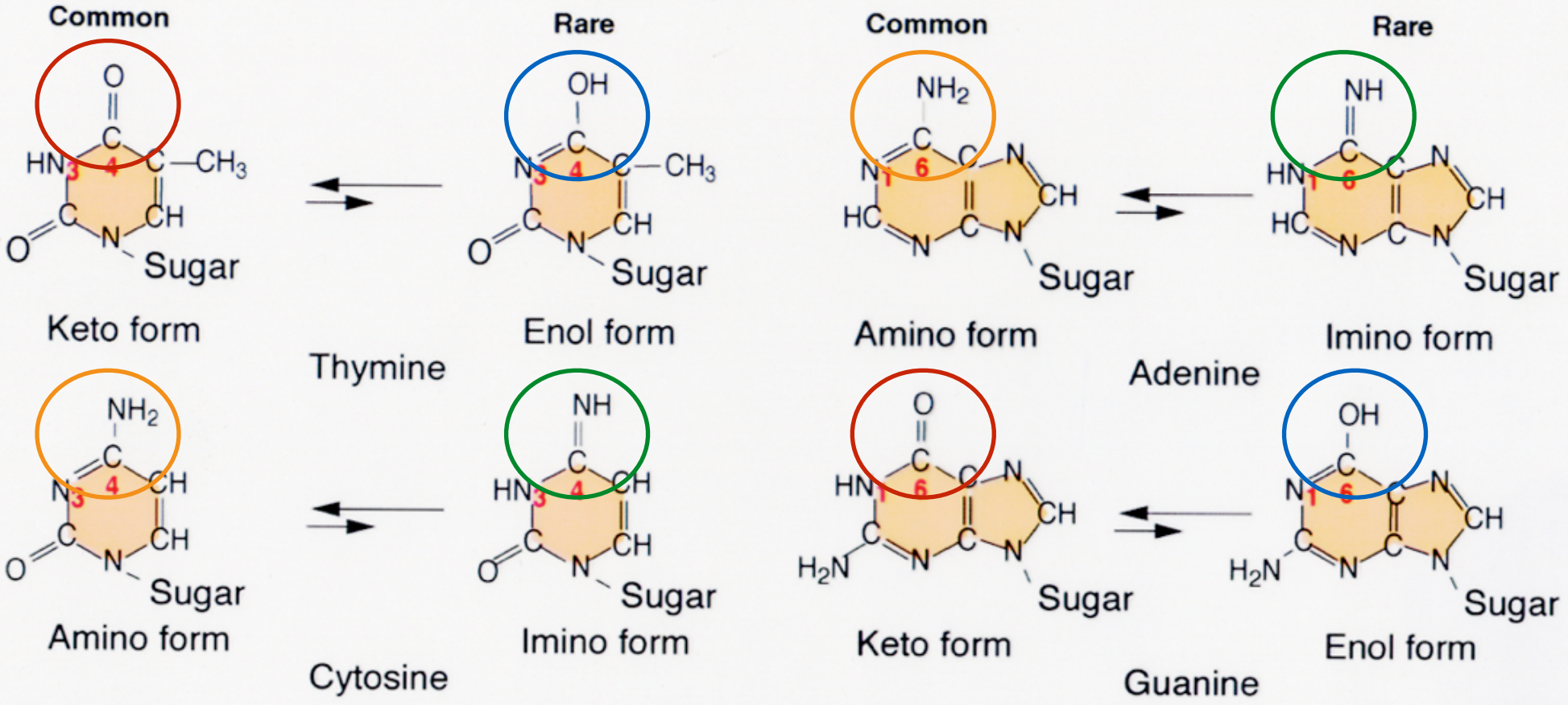
(a) Twelve different base substitutions can occur in DNA.



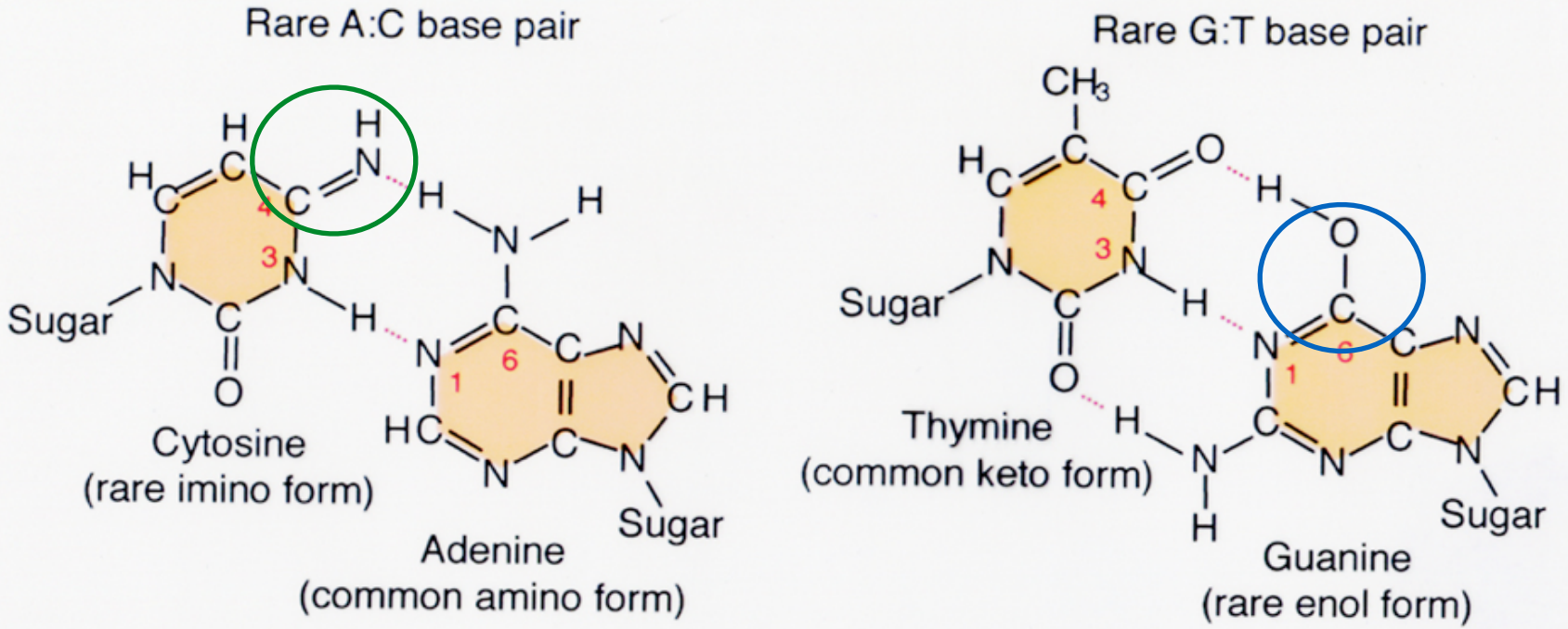
Specificity of mutagens. The distribution of mutations among 36 sites in the lacI gene of E. coli is shown for three mutagens: EMS, UV light, and aflatoxin B1.

Normal base pairing



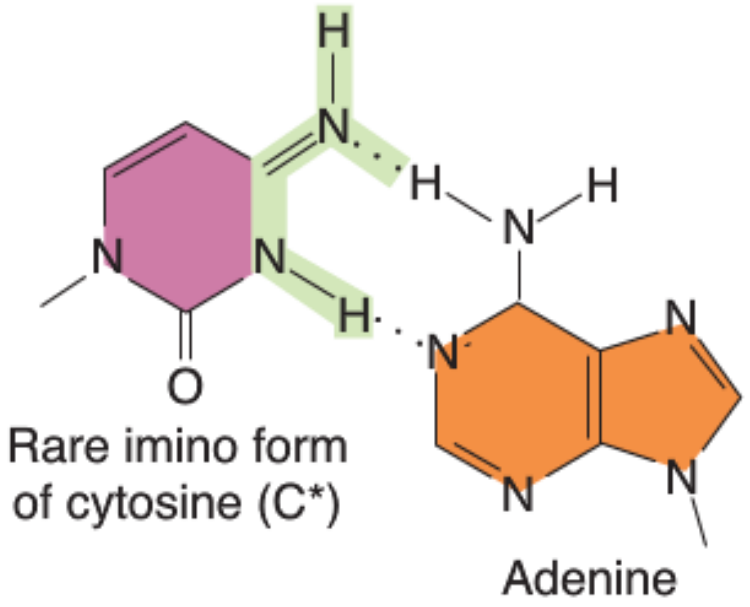


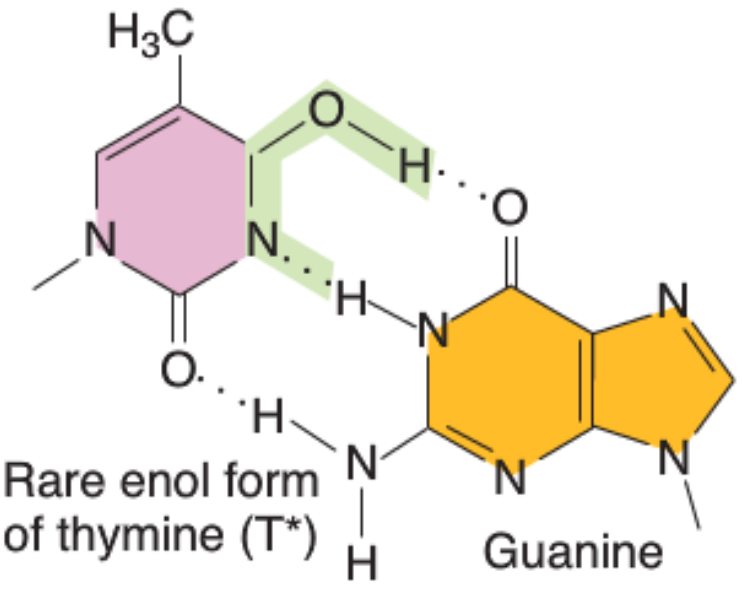
Tautomeric shifts invariably give rise to **transitions**.

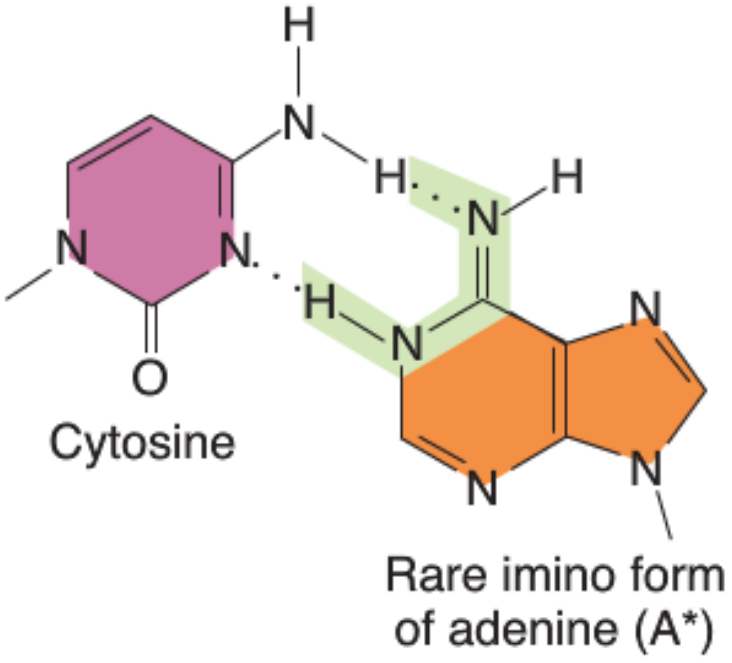


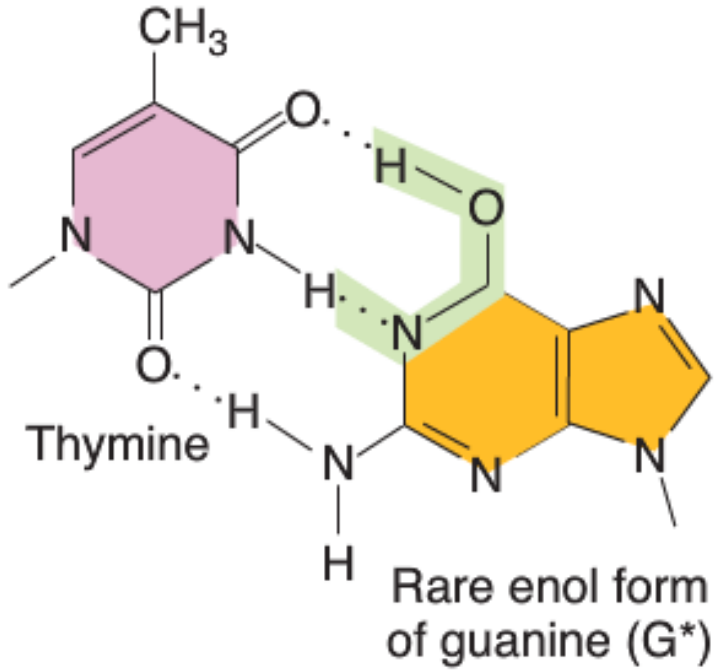
(a) Hydrogen-bonded A:C and G:T base pairs that form when cytosine and guanine are in their rare imino and enol tautomeric forms.

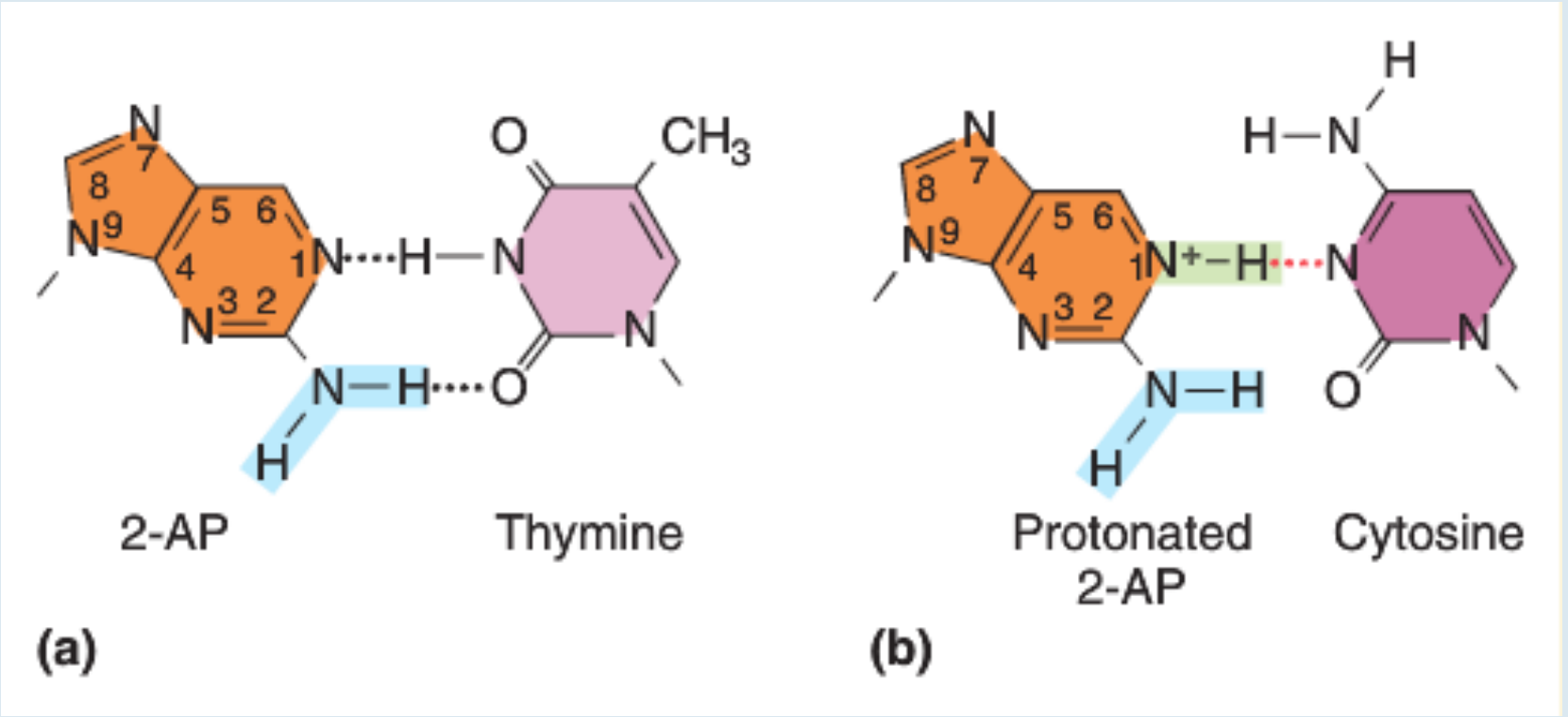
Mismatched bases



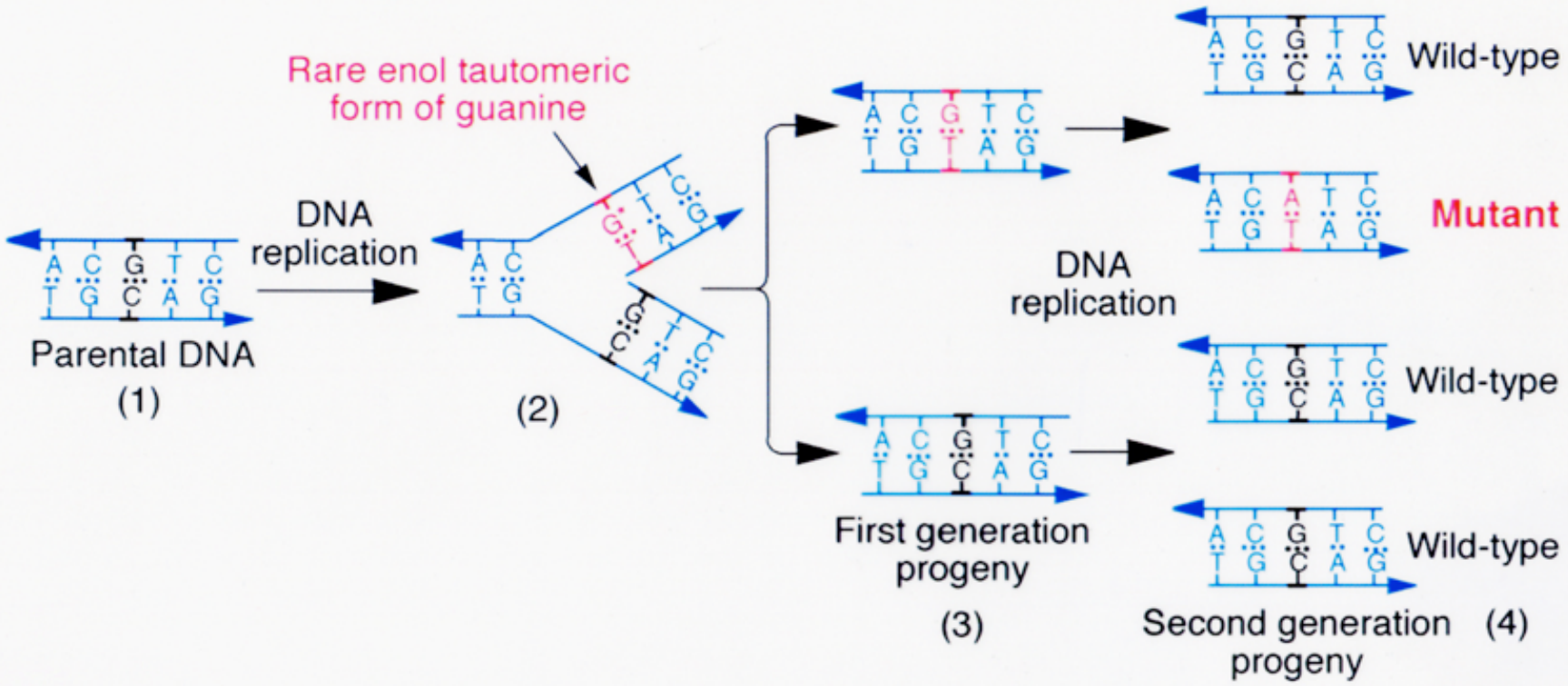






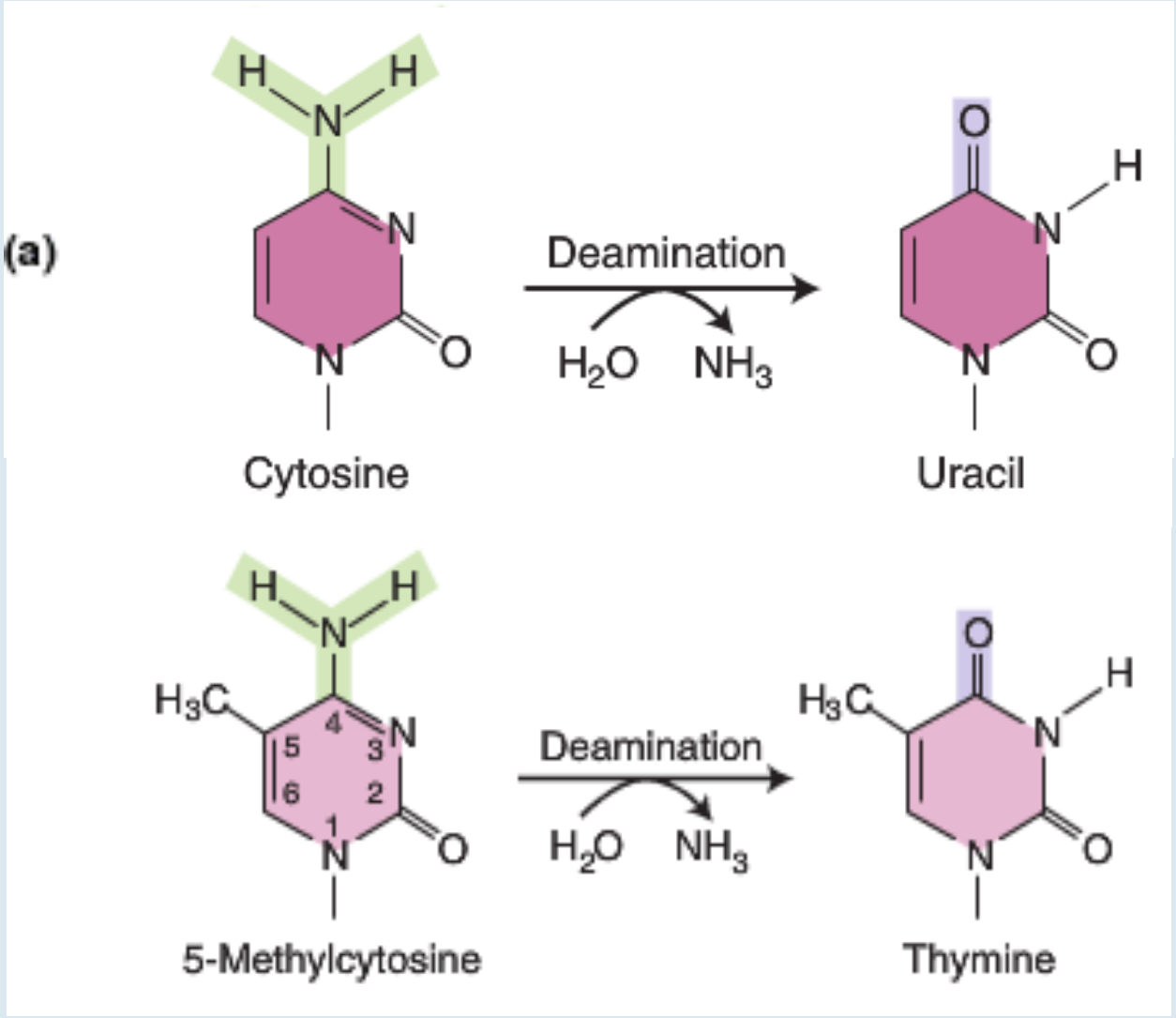


The incorporation of an adenine analogue, 2-amino purine provides a base that can pair with (a) adenine or (b) with cytossine -in its protonated form.

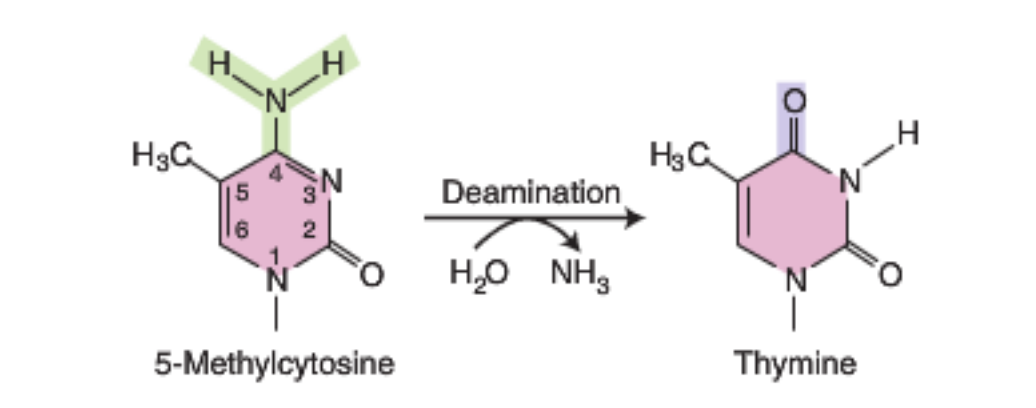


(b) Mechanism by which tautomeric shifts in the bases in DNA cause mutations.

Since corrections of these changes can occur at either of these steps, the observed frequency of transitions is about **10^{-8} - 10^{-10} /base/replication cycle**.



Deamination of (a) cytosine and 5-methylcytosine.



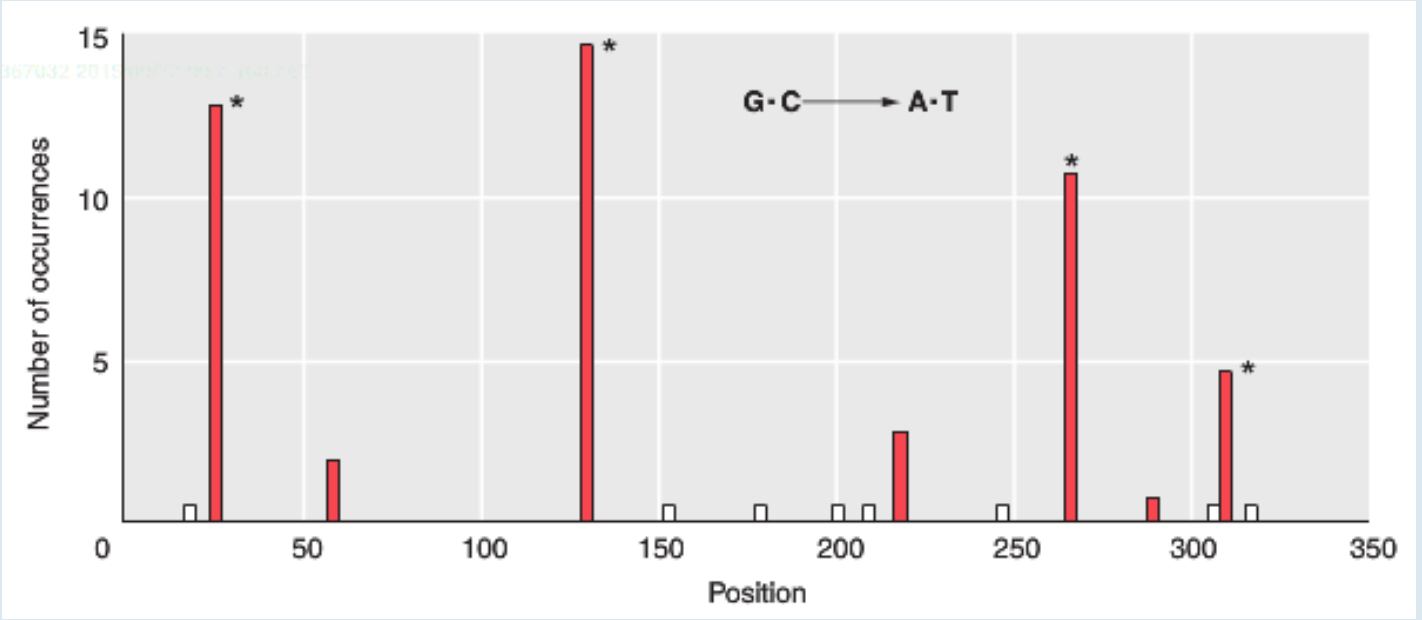
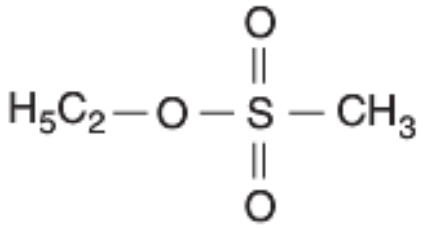
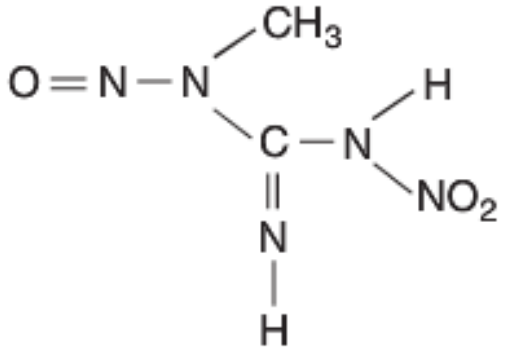


FIGURE 16-21 Methylcytosine hotspots in *E. coli*. Nonsense mutations at 15 different sites in *lacI* were scored. All resulted in G • C → A • T transitions. The asterisk (*) marks the positions of 5-methylcytosines, and the white bars mark sites where transitions known to occur were not isolated in this group. [*Data from C. Coulondre, J. H. Miller, P. J. Farabaugh, and W. Gilbert, "Molecular Basis of Base Substitution Hotspots in Escherichia coli,"* Nature *274, 1978, 775.*]

Specific mispairing Some mutagens are not incorporated into the DNA but instead alter a base in such a way that it will form a specific mispair. Certain alkylating agents, such as ethylmethanesulfonate (EMS) and the widely used nitrosoguanidine (NG), operate by this pathway.

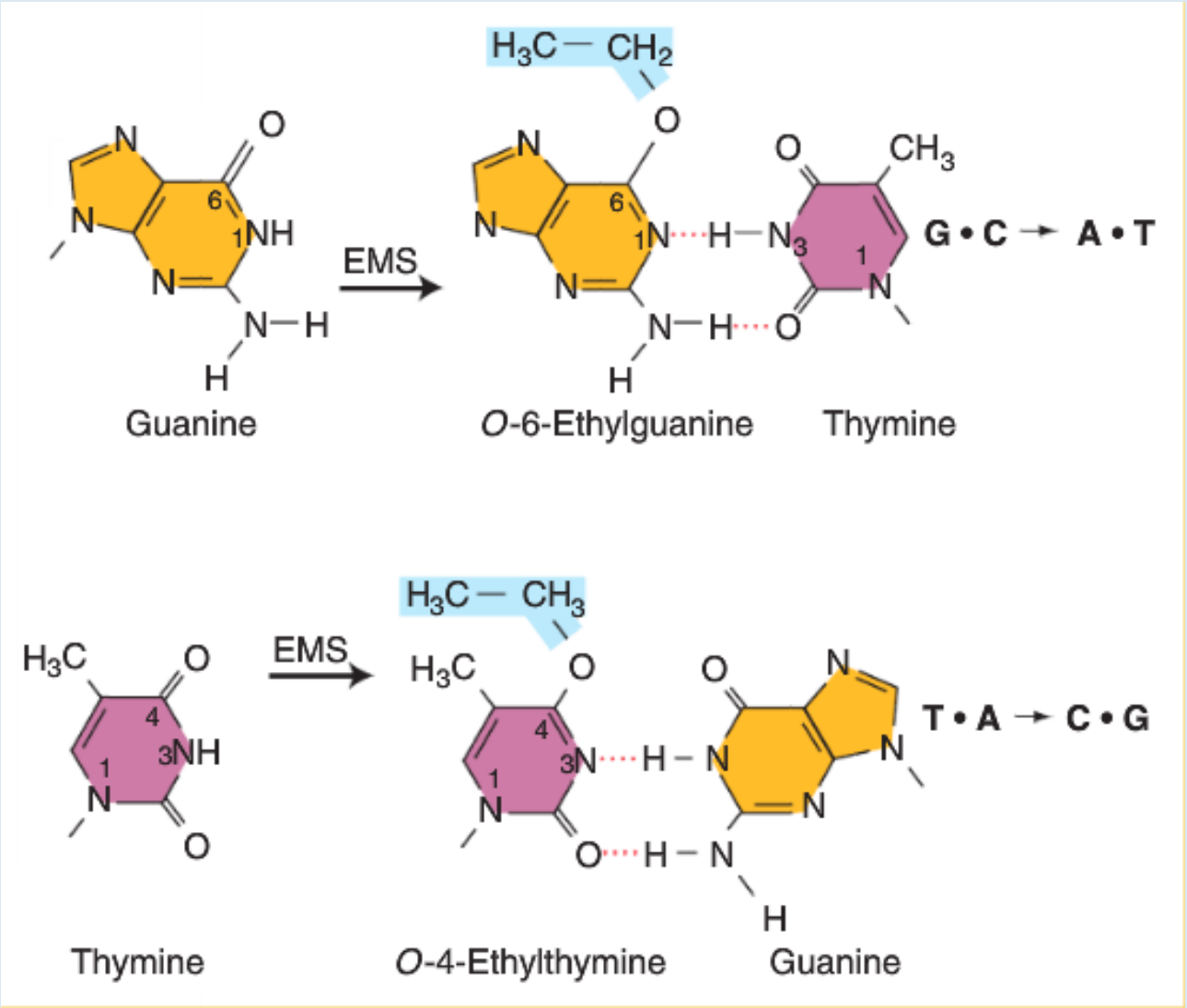


EMS



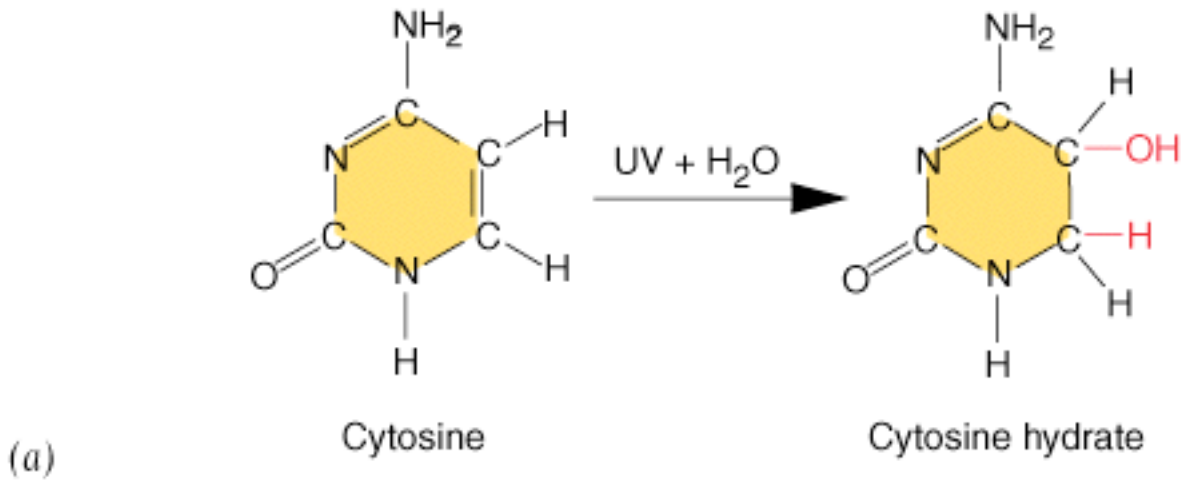
NG

Such agents add alkyl groups (an ethyl group in EMS and a methyl group in NG) to many positions on all four bases. However, the formation of a mutation is best correlated with an addition to the oxygen at position 6 of guanine to create an *O*-6-alkylguanine. This addition leads to direct mispairing with thymine, as shown in Figure 16-13, and would result in G · C → A · T transitions at the next round of replication.

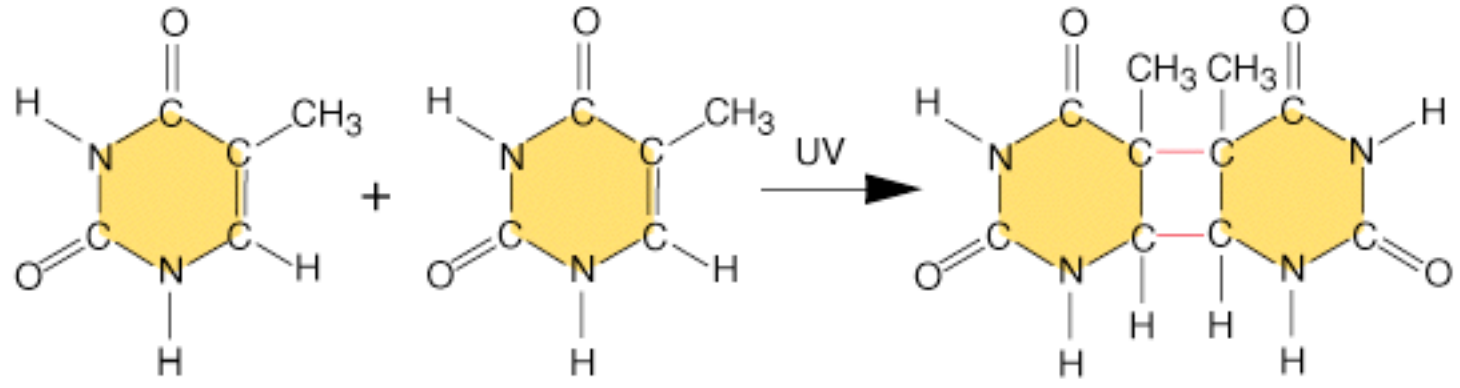


Alkylation-induced specific mispairing.

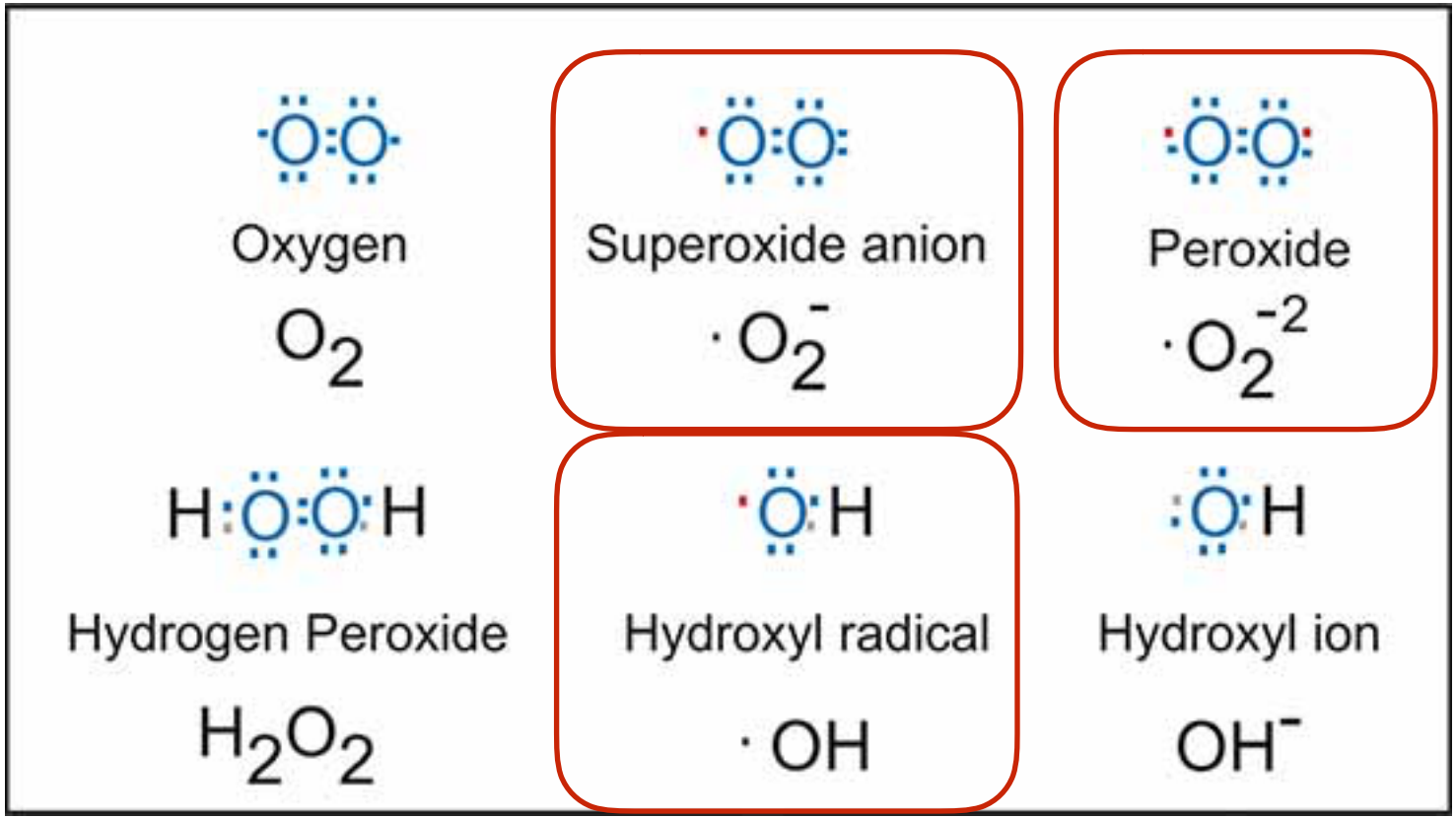
The alkylation (in this case, **EMS (e**thane methyl sulfonate)-generated ethylation) of the O-6 position of guanine and the O-4 position of thymine can lead to direct mispairing with thymine and guanine, respectively, as shown here.

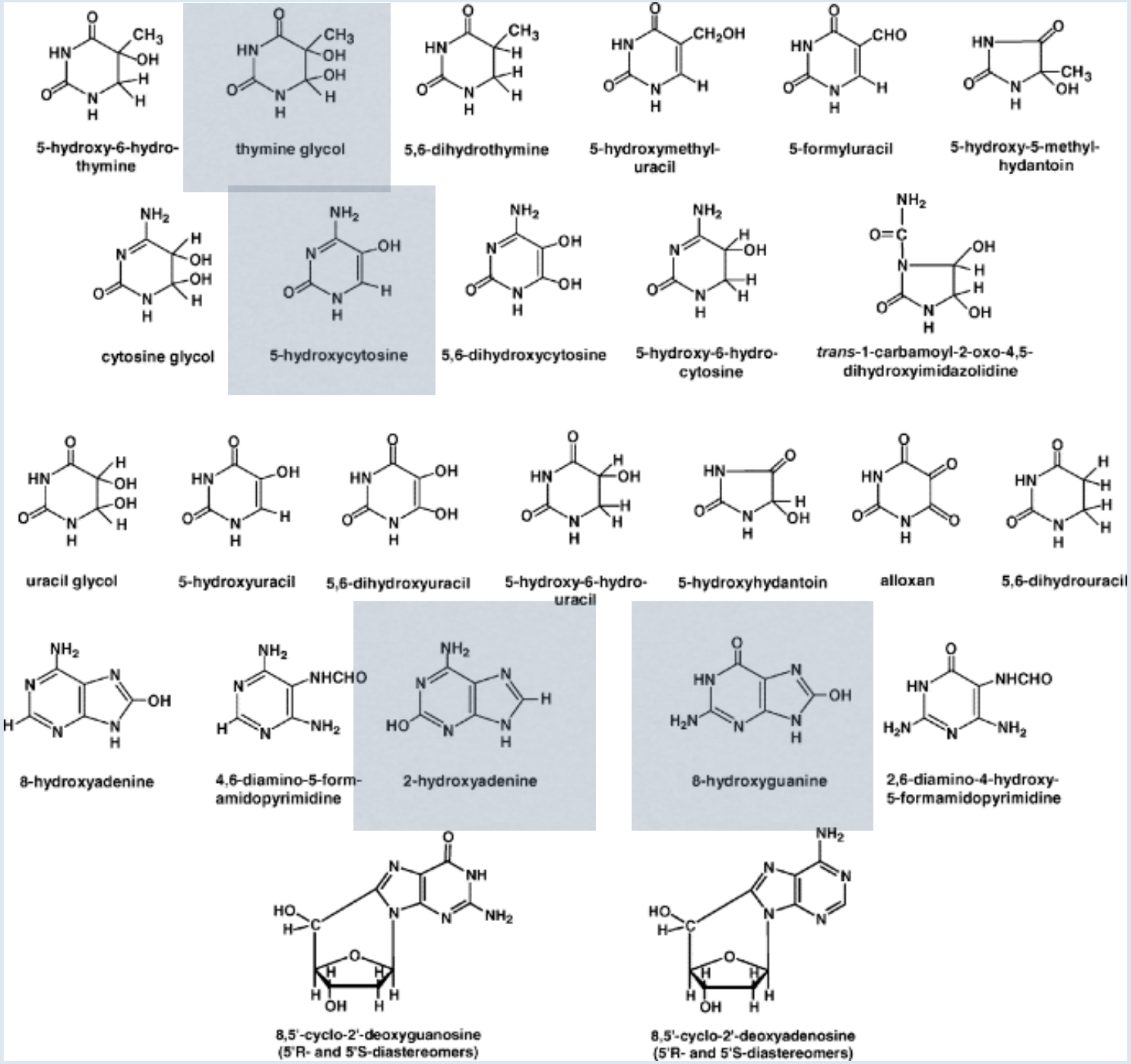


(a)

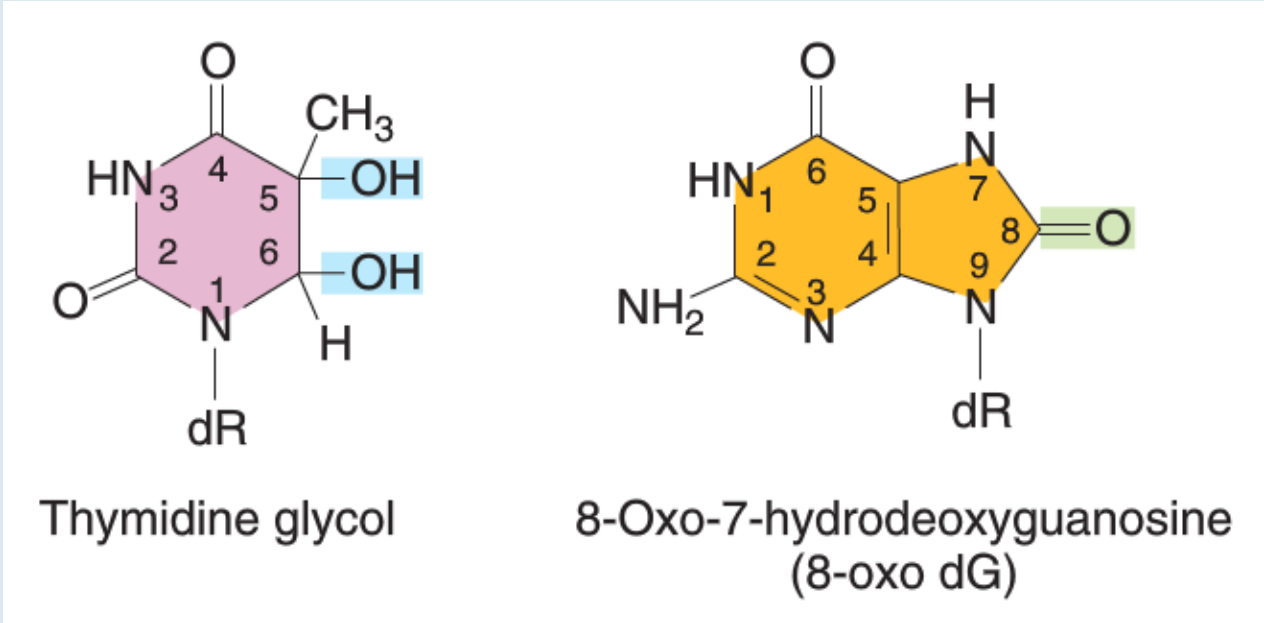


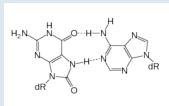
(b)


Oxygen
O_2
Superoxide anion
$\cdot O_2^-$
Peroxide
$\cdot O_2^{-2}$
Hydrogen Peroxide
H_2O_2
Hydroxyl radical
$\cdot OH$
Hydroxyl ion
OH^-



Additional modified bases that can result from oxidative damage.



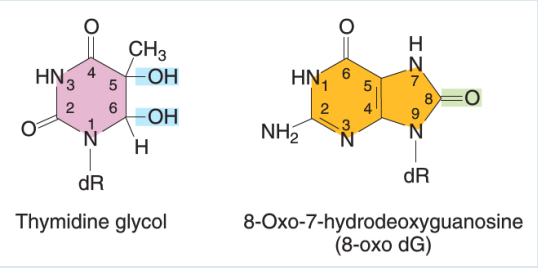


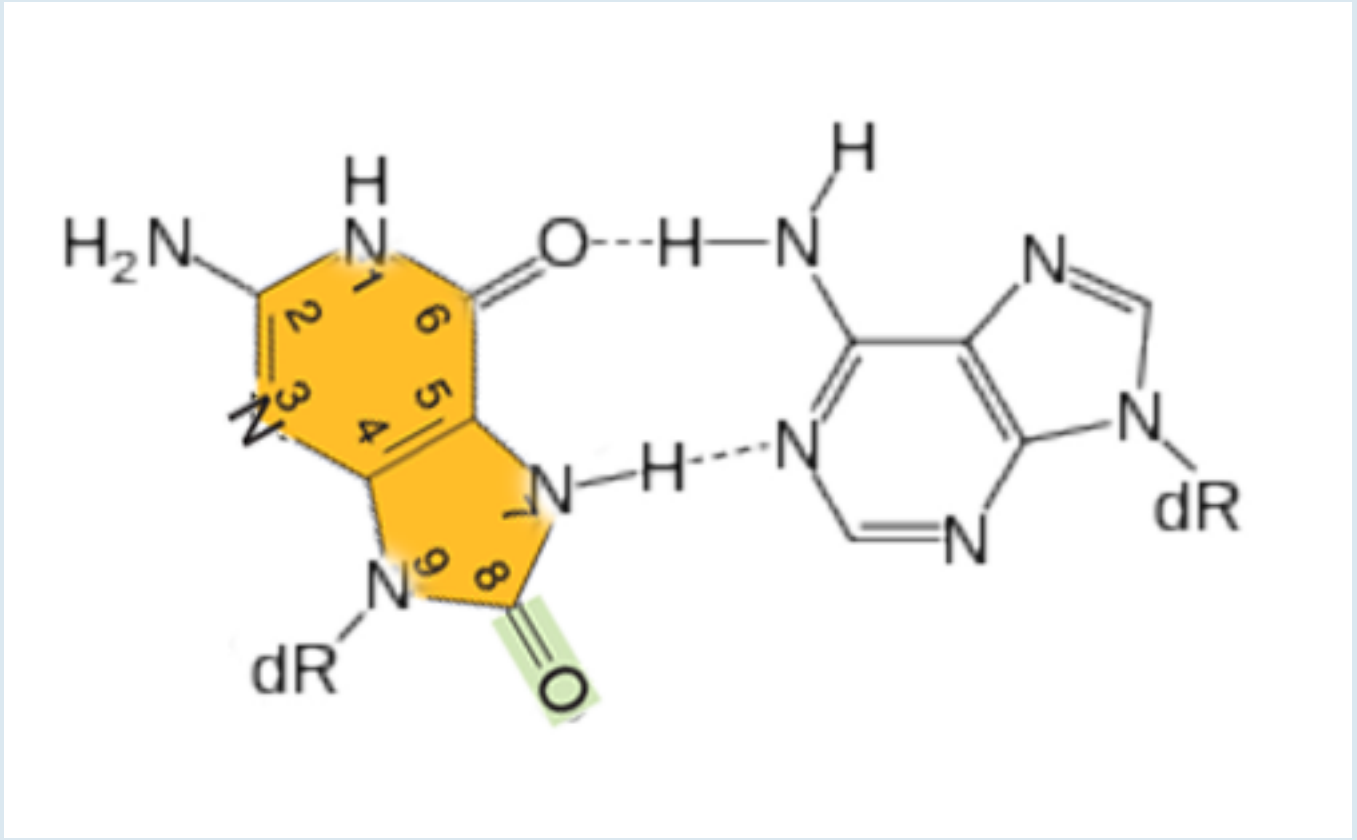
DNA damage products formed after attack by oxygen radicals. dR = deoxyribose.

Oxidatively damaged bases represent a third type of spontaneous lesion implicated in mutagenesis. Active oxygen species, such as superoxide radicals ($O_2\cdot$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH\cdot$), are produced as by-products of normal aerobic metabolism.

Thymidine glycol, blocks DNA replication -if unrepaired, but has not yet been implicated in mutagenesis.

8-oxo-7-hydrodeoxyguanosine (8-oxodG, or GO) product (which frequently mispairs with **A**, results in a high level of **G → T transversions**).





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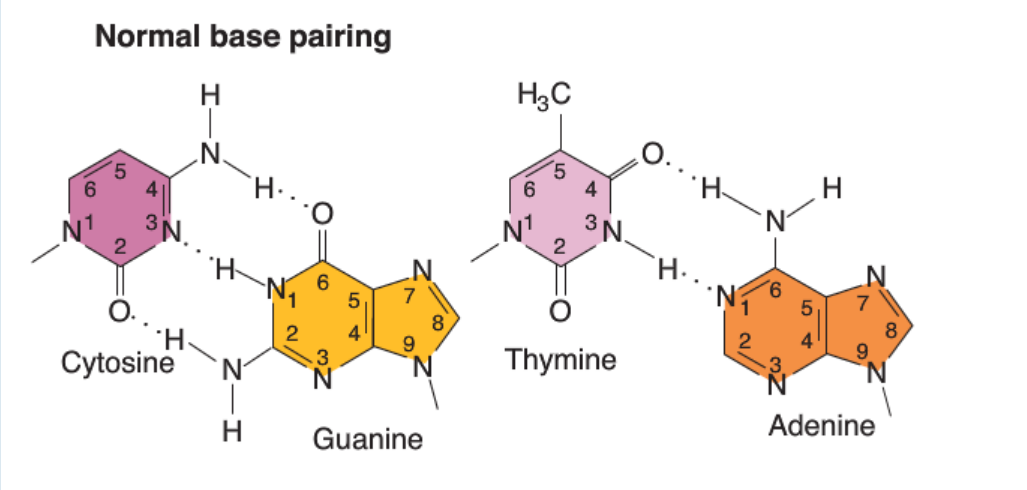
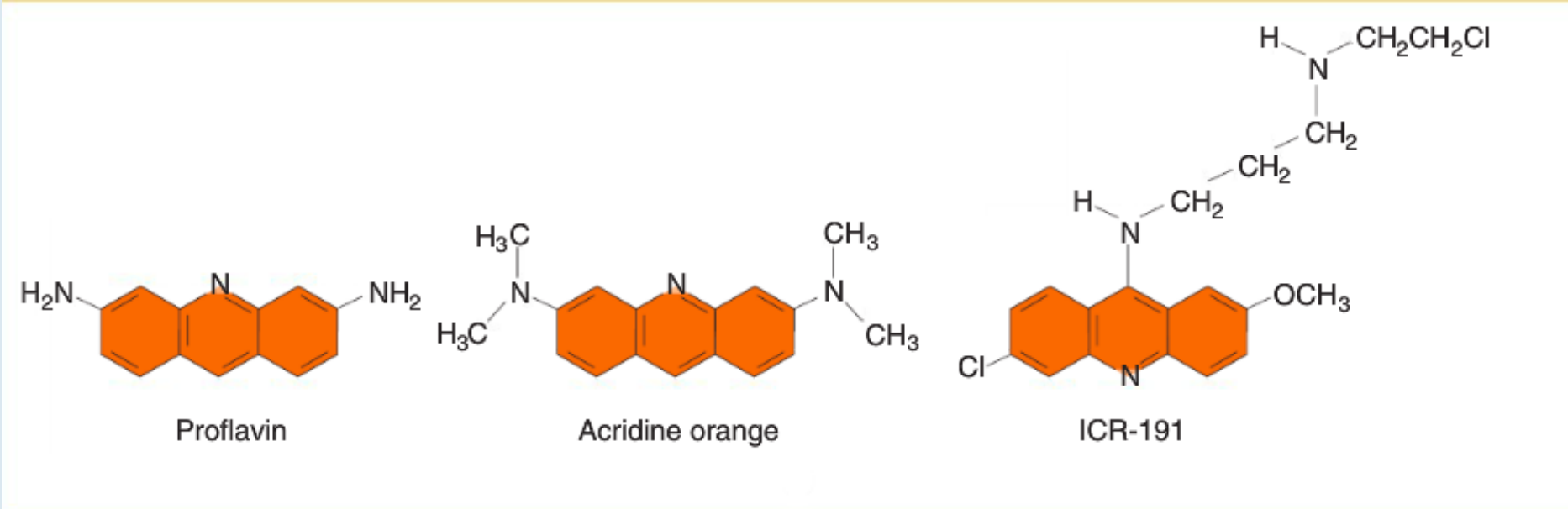
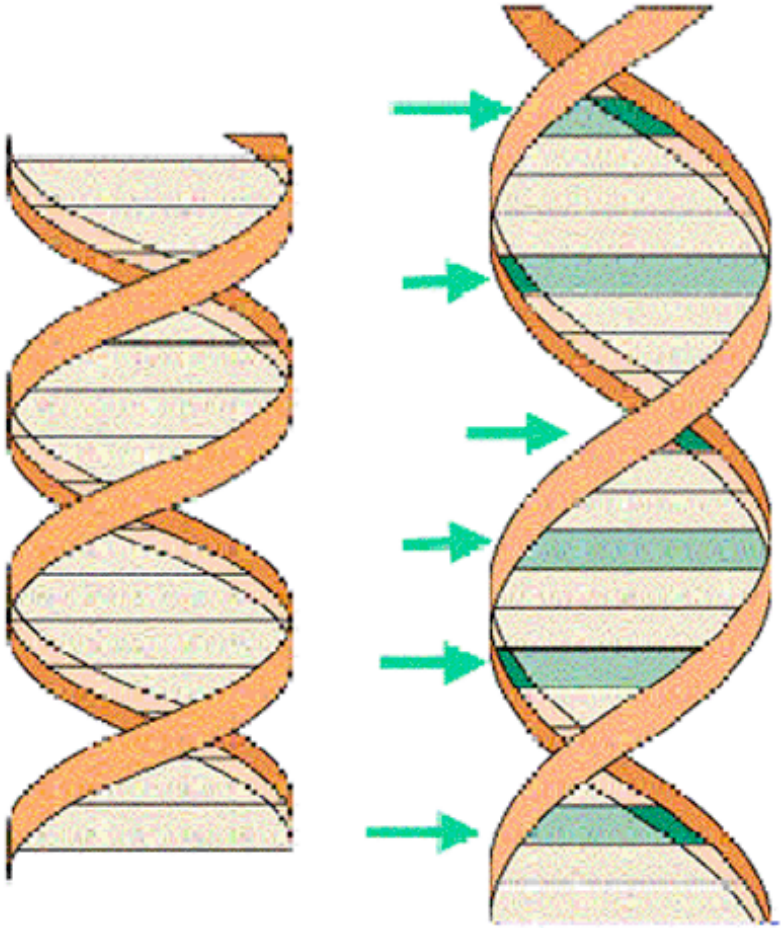




Intercalating agents
Structures of the common agents **proflavin**, **acridine orange**, and **ICR-191**.

Intercalating agents slip between the nitrogenous bases stacked at the center of the DNA molecule. This occurrence can lead to single-nucleotide-pair insertions and deletions. (From L. S. Lerman, *Proceedings of the National Academy of Sciences USA* 49, 1963, 94.)



Original DNA

DNA stretched by intercalated ligands

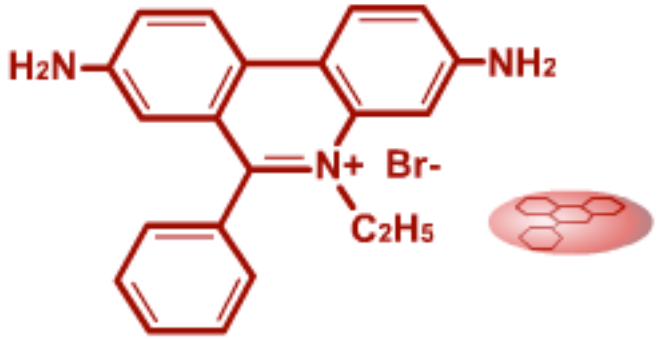
Stretching changes the frame needed by DNA Polymerase during replication.

Extra nucleotides are added during replication

The DNA reading frame for RNA synthesis is changed:

- amino acid changes
- altered protein

Ethidium Bromide



Ethidium Bromide (EtBr) is a planar compound which intercalates between base pairs in the DNA double helix. Each molecule of EtBr which intercalates causes the double helix to unwind, decreasing Tw and increasing Wr.

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http://sites.fas.harvard.edu/~biotext/animations/etbr.html

Figure 16-4. A simplified version of the **Streisinger model for frameshift formation. (a–c)** In DNA synthesis, the newly synthesized strand slips, looping out one or several bases. This loop is stabilized by the pairing afforded by the repetitive-sequence unit (the A bases in this case).
An addition of one base pair, A–T, will result at the next round of replication in this example. (d–f) If, instead of the newly synthesized strand, the template strand slips, then a deletion results.

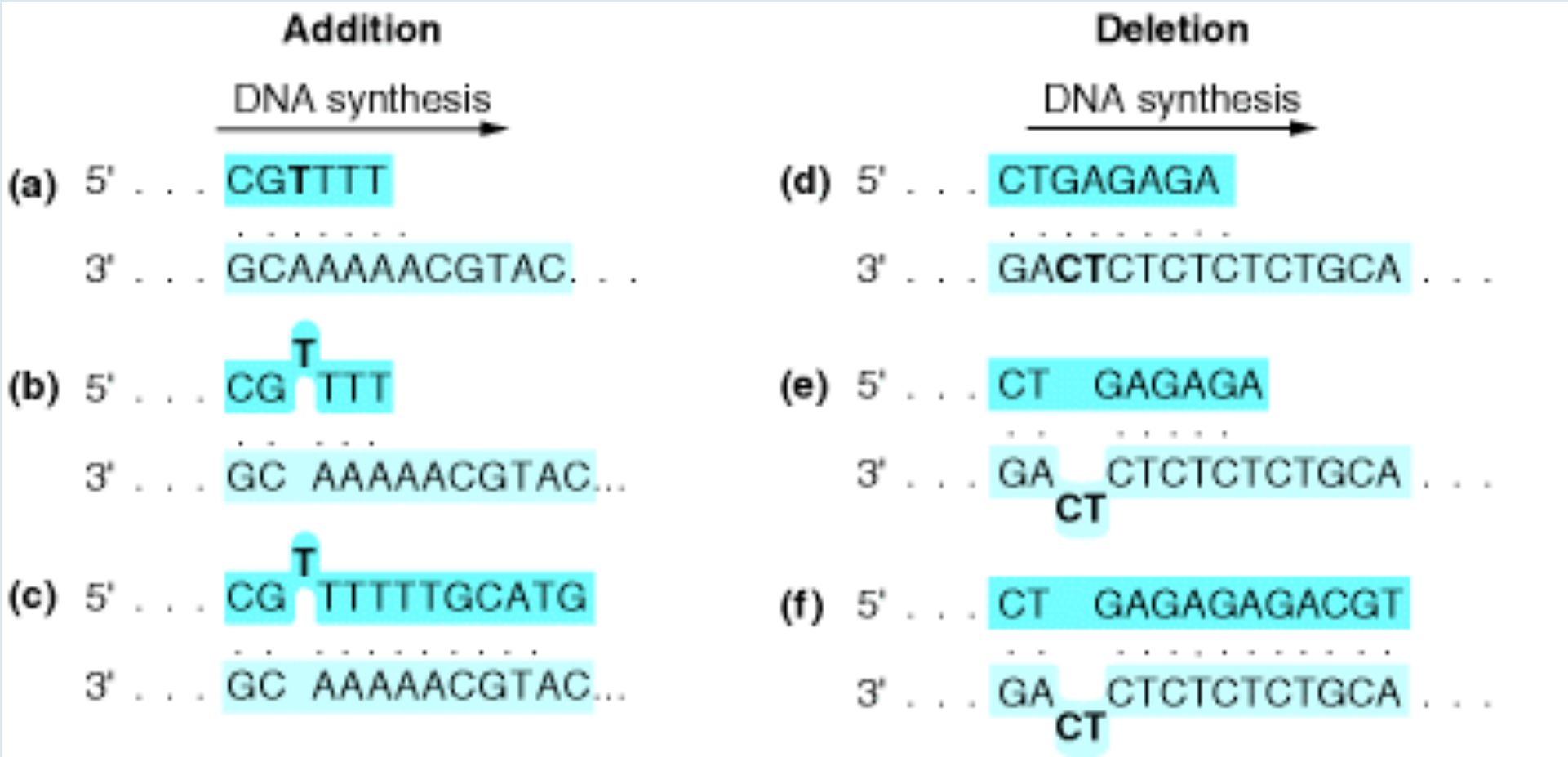

Figure 16-4. A simplified version of the Streisinger model for frameshift formation. (a–c) In DNA synthesis, the newly synthesized strand slips, looping out one or several bases. This loop is stabilized by the pairing afforded by the repetitive-sequence unit (the A bases in this case). An addition of one base pair, A–T, will result at the next round of replication in this example. (d–f) If, instead of the newly synthesized strand, the template strand slips, then a deletion results. Here the repeating unit is a CT dinucleotide. After slippage, a deletion of two base pairs (C–G and T–A) would result at the next round of replication.

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Estimate of the Mutation Rate per Nucleotide in Humans

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Communicating editor: A. G. CLARK

Many previous estimates of the mutation rate in humans have relied on screens of visible mutants. We investigated the rate and pattern of mutations at the nucleotide level by comparing pseudogenes in humans and chimpanzees to (i) provide an estimate of the average mutation rate per nucleotide, (ii) assess heterogeneity of mutation rate at different sites and for different types of mutations, (iii) test the hypothesis that the X chromosome has a lower mutation rate than autosomes, and (iv) estimate the deleterious mutation rate. Eighteen processed pseudogenes were sequenced, including 12 on autosomes and 6 on the X chromosome. The average mutation rate was estimated to be ~2.5×10^{-8} mutations per nucleotide site or 175 mutations per diploid genome per generation. Rates of mutation for both transitions and transversions at CpG dinucleotides are one order of magnitude higher than mutation rates at other sites. Single nucleotide substitutions are 10 times more frequent than length mutations. Comparison of rates of evolution for X-linked and autosomal pseudogenes suggests that the male mutation rate is 4 times the female mutation rate, but provides no evidence for a reduction in mutation rate that is specific to the X chromosome. Using conservative calculations of the proportion of the genome subject to purifying selection, we estimate that the genomic deleterious mutation rate (U) is at least 3. This high rate is difficult to reconcile with multiplicative fitness effects of individual mutations and suggests that synergistic epistasis among harmful mutations may be common.

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Mutation Rate in Human Microsatellites: Influence of the Structure and Length of the Tandem Repeat

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2 Gerichtlich-Medizinisches Institut, Graz, Austria

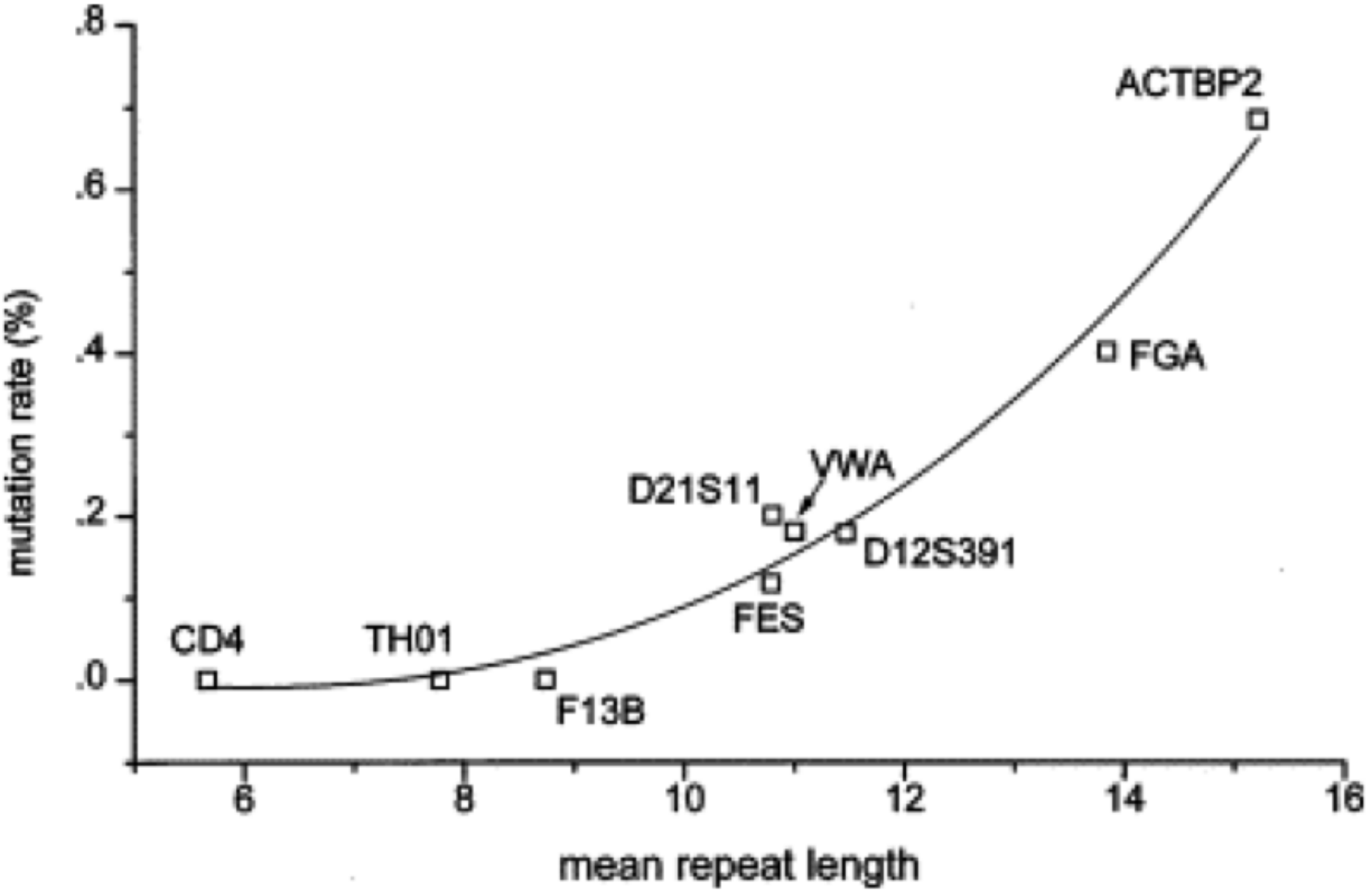
3 Institut für Gerichtliche Medizin, Salzburg

Received 10 December 1997; Accepted 15 April 1998. Available online 21 December 2007.

Summary

In 10,844 parent/child allelic transfers at nine short-tandem-repeat (STR) loci, 23 isolated STR mismatches were observed. The parenthood in each of these cases was highly validated (probability >99.97%). The event was always repeat related, owing to either a single-step mutation (n=22) or a double-step mutation (n=1). The mutation rate was between 0 and 7×10^{-3} per locus per gamete per generation. No mutations were observed in three of the nine loci. Mutation events in the male germ line were five to six times more frequent than in the female germ line. A positive exponential correlation between the geometric mean of the number of uninterrupted repeats and the mutation rate was observed. Our data demonstrate that mutation rates of different loci can differ by several orders of magnitude and that different alleles at one locus exhibit different mutation rates.

Author Keywords: Microsatellites; Mutation; Short tandem repeat (STR); Slippage rate; Single step



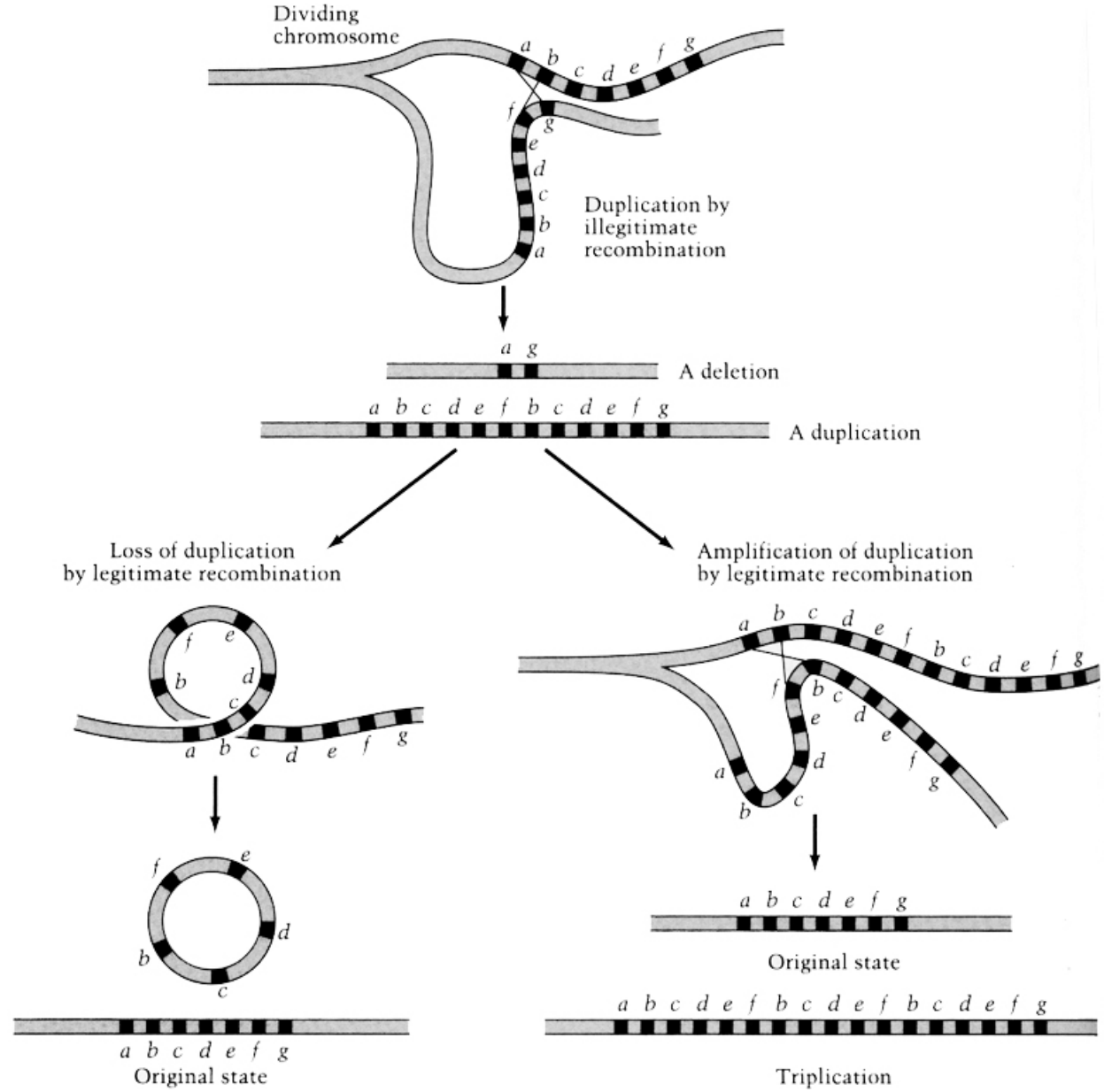
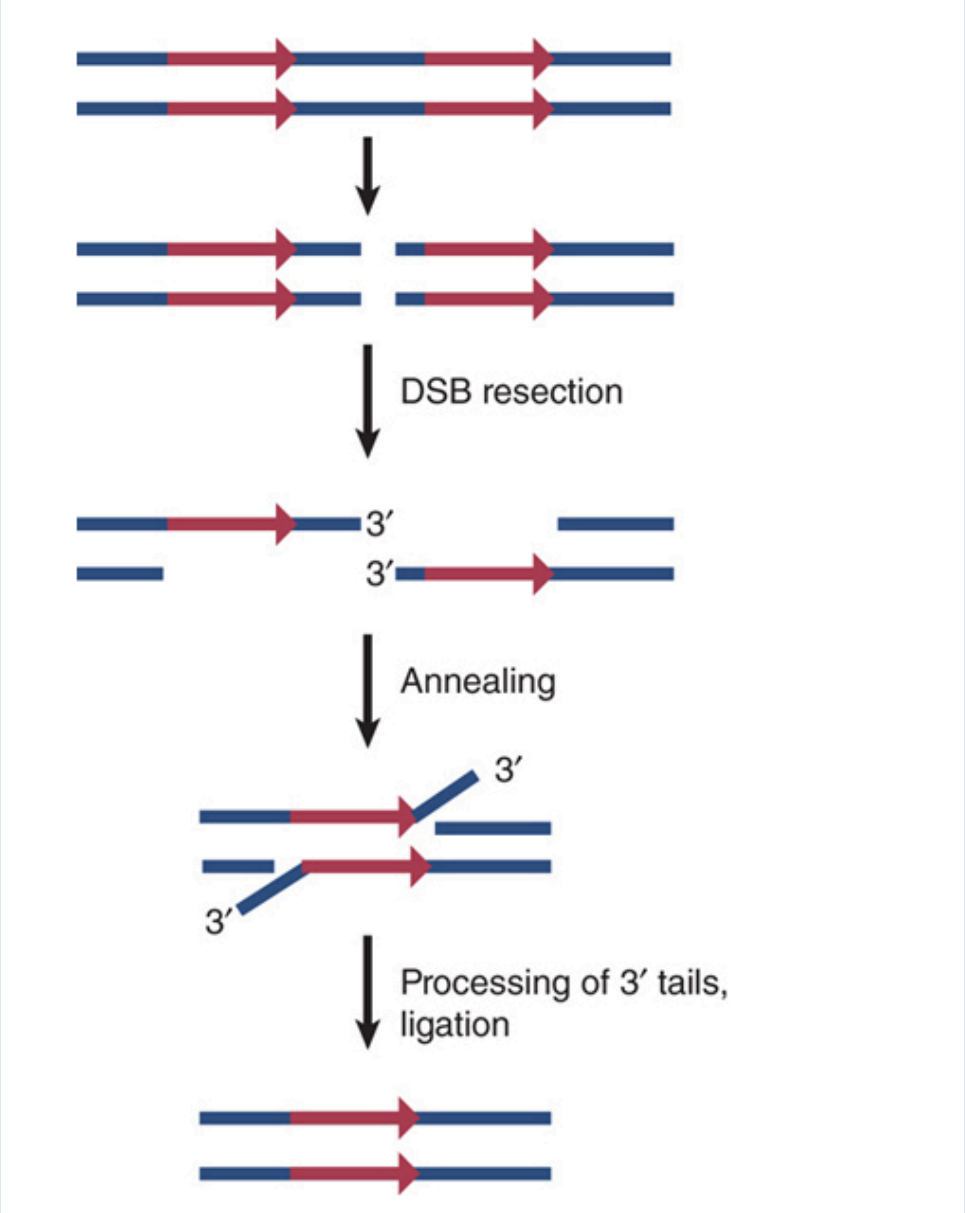


Figure 6

Probable mechanisms for the generation, loss, and amplification of duplication mutations. (After Anderson and Roth, 1977.)



A double-strand break occurring between direct repeats, depicted as red arrows. Following end processing to form single-strand tails with 3′–OH ends, the single strands anneal by homology at the red arrows. The single-strand tails are removed by endonucleases that recognize branch structures. The end product is double-strand break repair with a deletion of the sequences between the repeats and loss of one repeat sequence.

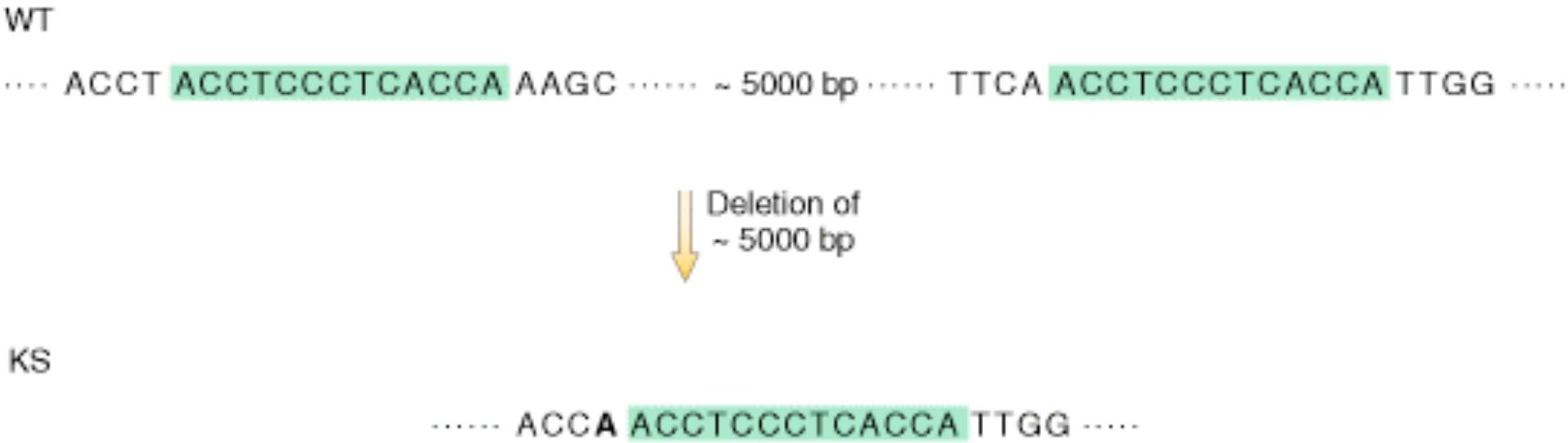


Figure 16-11. Sequences of wild-type (WT) mitochondrial DNA and deleted DNA (KS) from a patient with **Kearns-Sayre syndrome.**

The 13-base boxed sequence is identical in both WT and KS and serves as a breakpoint for the DNA deletion. A single base (boldface type) is altered in KS, aside from the deleted segment.

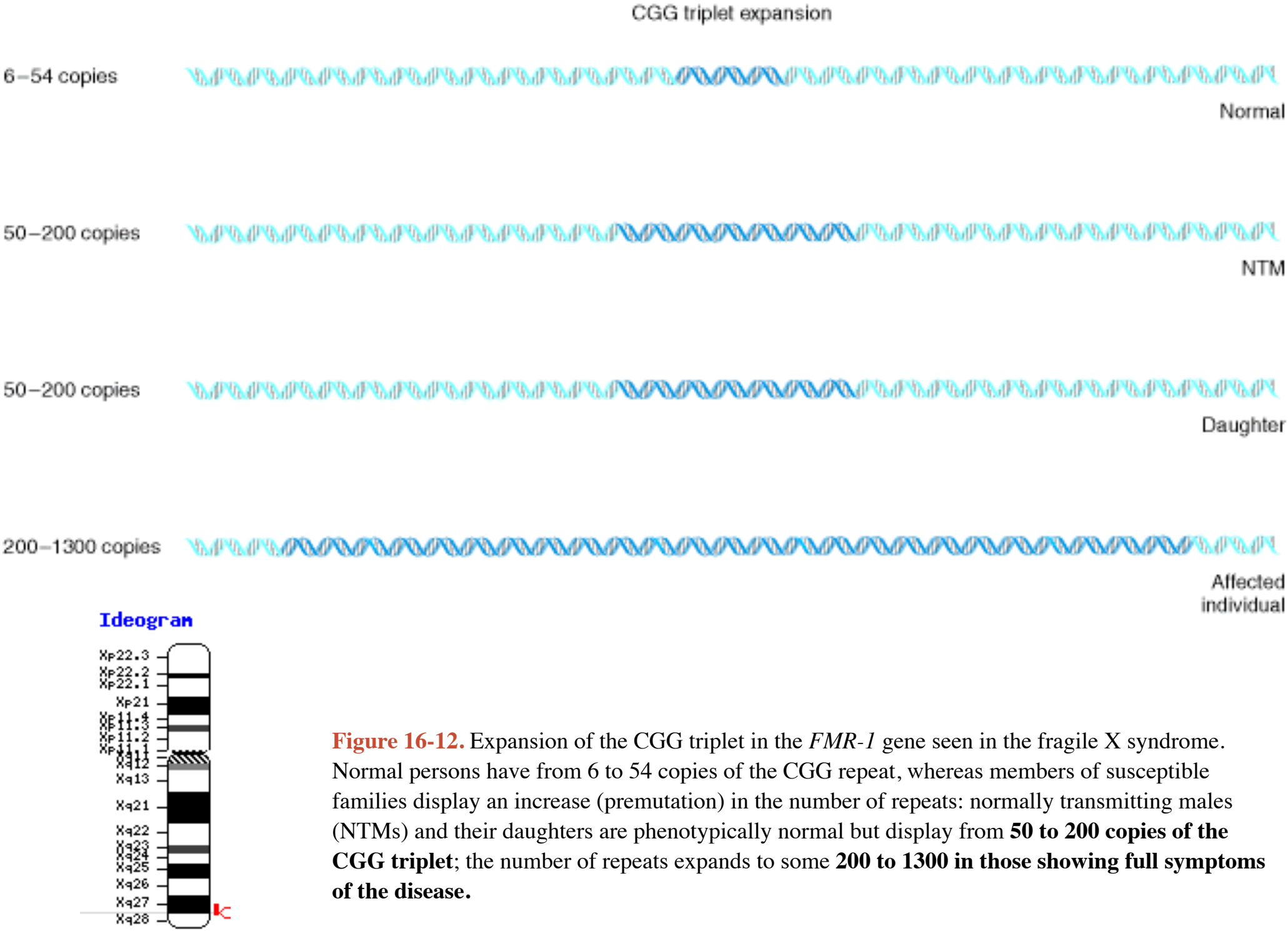
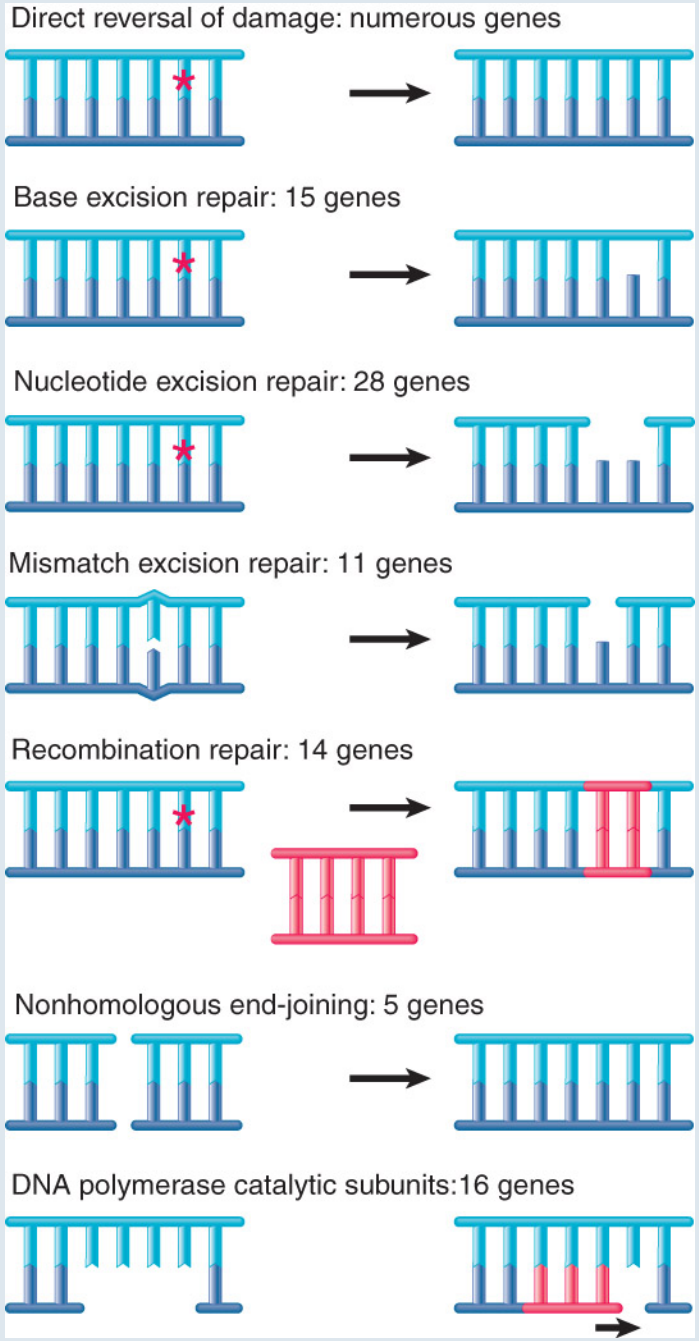


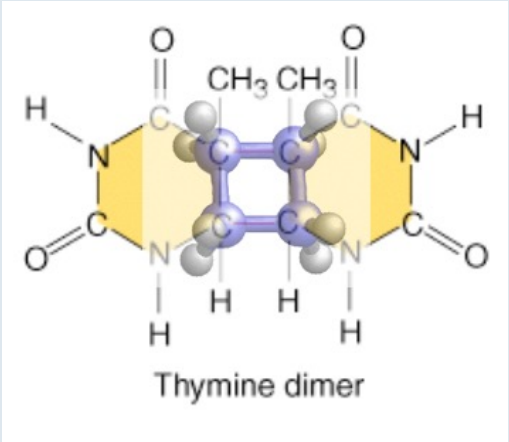
Figure 16-12. Expansion of the CGG triplet in the *FMR-1* gene seen in the fragile X syndrome. Normal persons have from 6 to 54 copies of the CGG repeat, whereas members of susceptible families display an increase (premutation) in the number of repeats: normally transmitting males (NTMs) and their daughters are phenotypically normal but display from **50 to 200 copies of the CGG triplet**; the number of repeats expands to some **200 to 1300 in those showing full symptoms of the disease.**



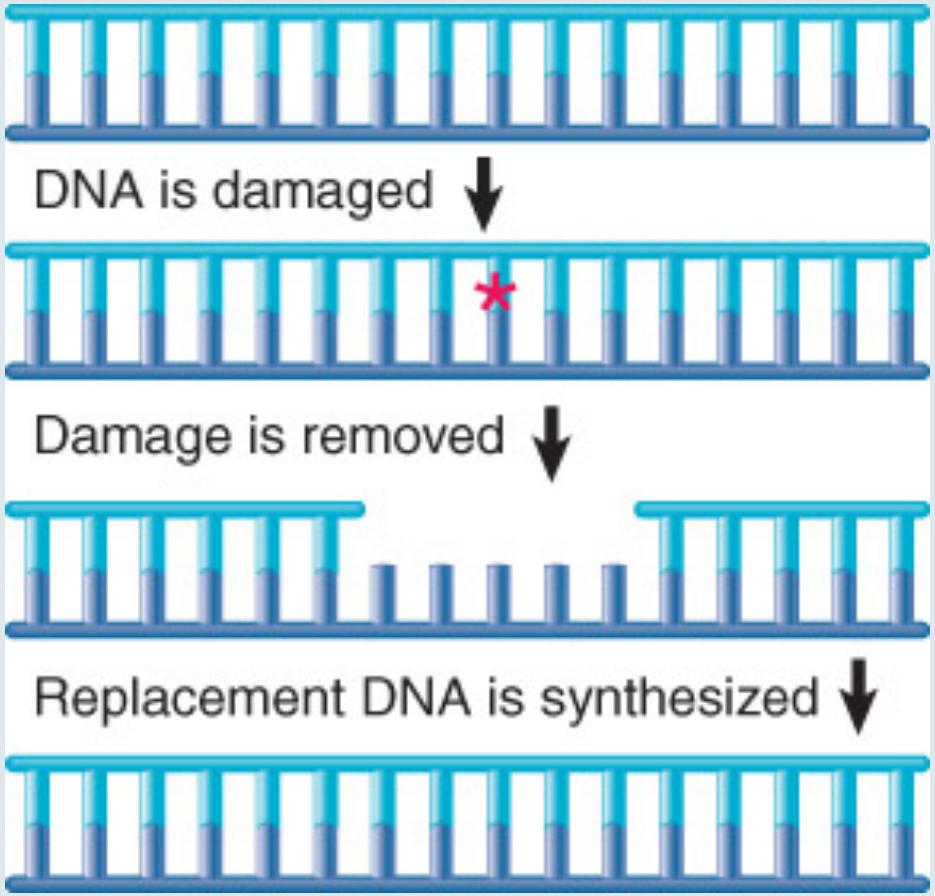
- **mismatch repair (MMR) –** A type of repair that corrects mispaired bases, typically immediately following replication.

  – The process preferentially corrects the sequence of the daughter strand by distinguishing the daughter strand and parental strand, sometimes on the basis of their states of methylation.

- **photoreactivation –** A repair mechanism that uses a white light-dependent enzyme to split cyclobutane pyrimidine dimers formed by ultraviolet light.



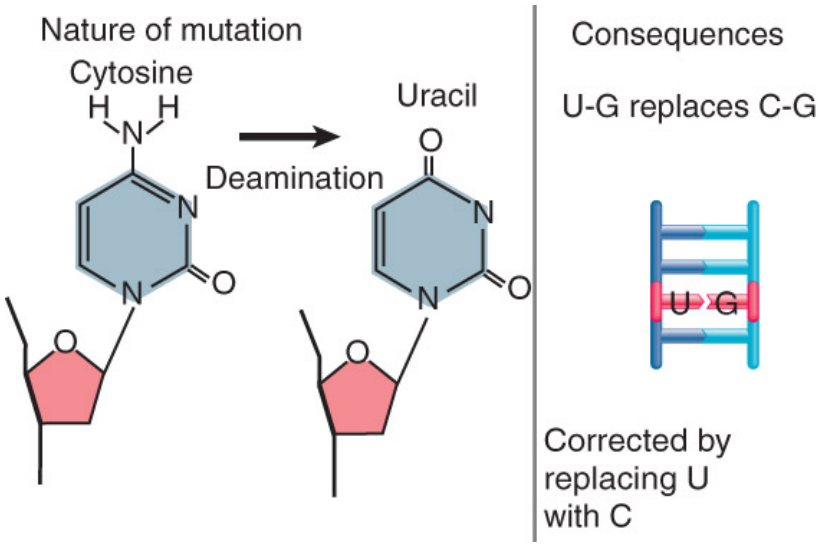
- **excision repair –** A type of repair system in which one strand of DNA is directly excised and then replaced by re-synthesis using the complementary strand as template.



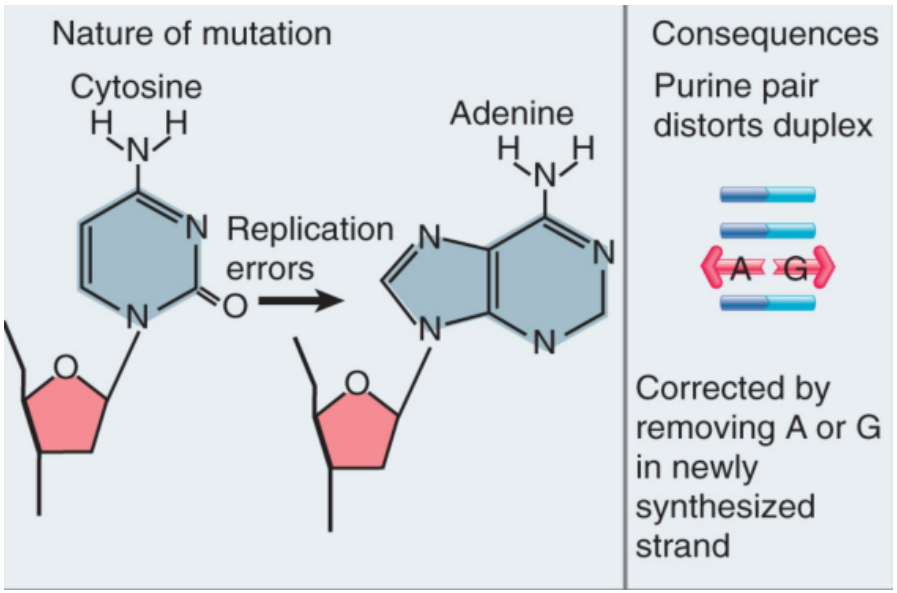
Excision repair directly replaces damaged DNA by resynthesizing a replacement stretch for the damaged strand.

Repair Systems Correct Damage to DNA

- Repair systems recognize DNA sequences that do not conform to standard base pairs.
- Excision systems remove one strand of DNA at the site of damage and then replace it.

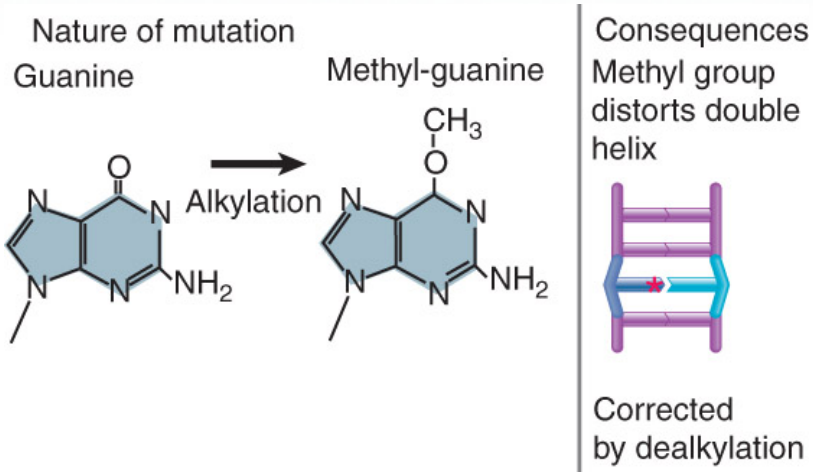


Deamination is reversed by replacing U with C

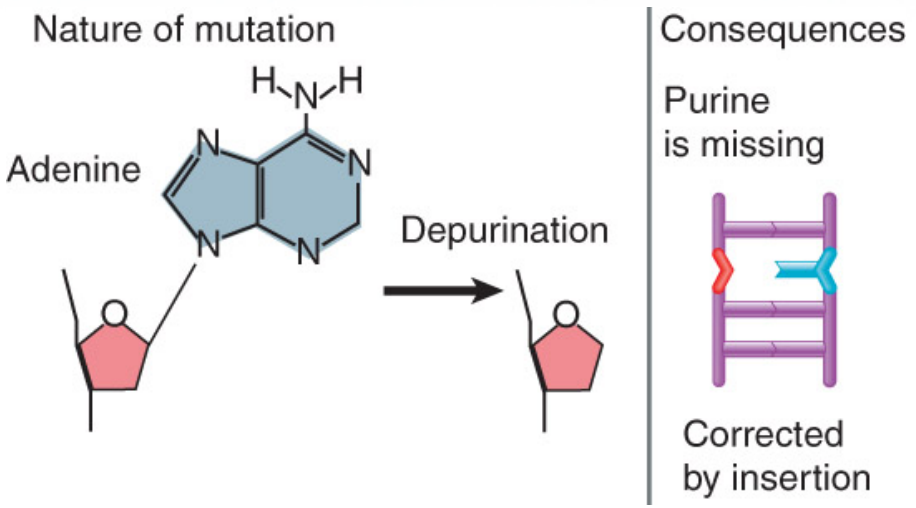


Replication errors introduce mismatched base pairs

Repair Systems Correct Damage to DNA

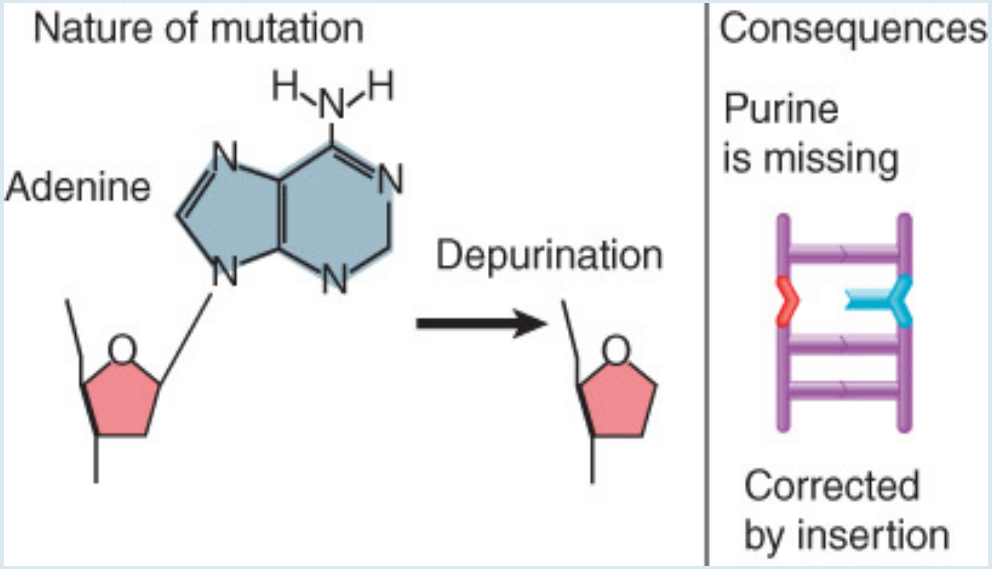


Methylation can distort the structure of DNA



Depurination requires base replacement

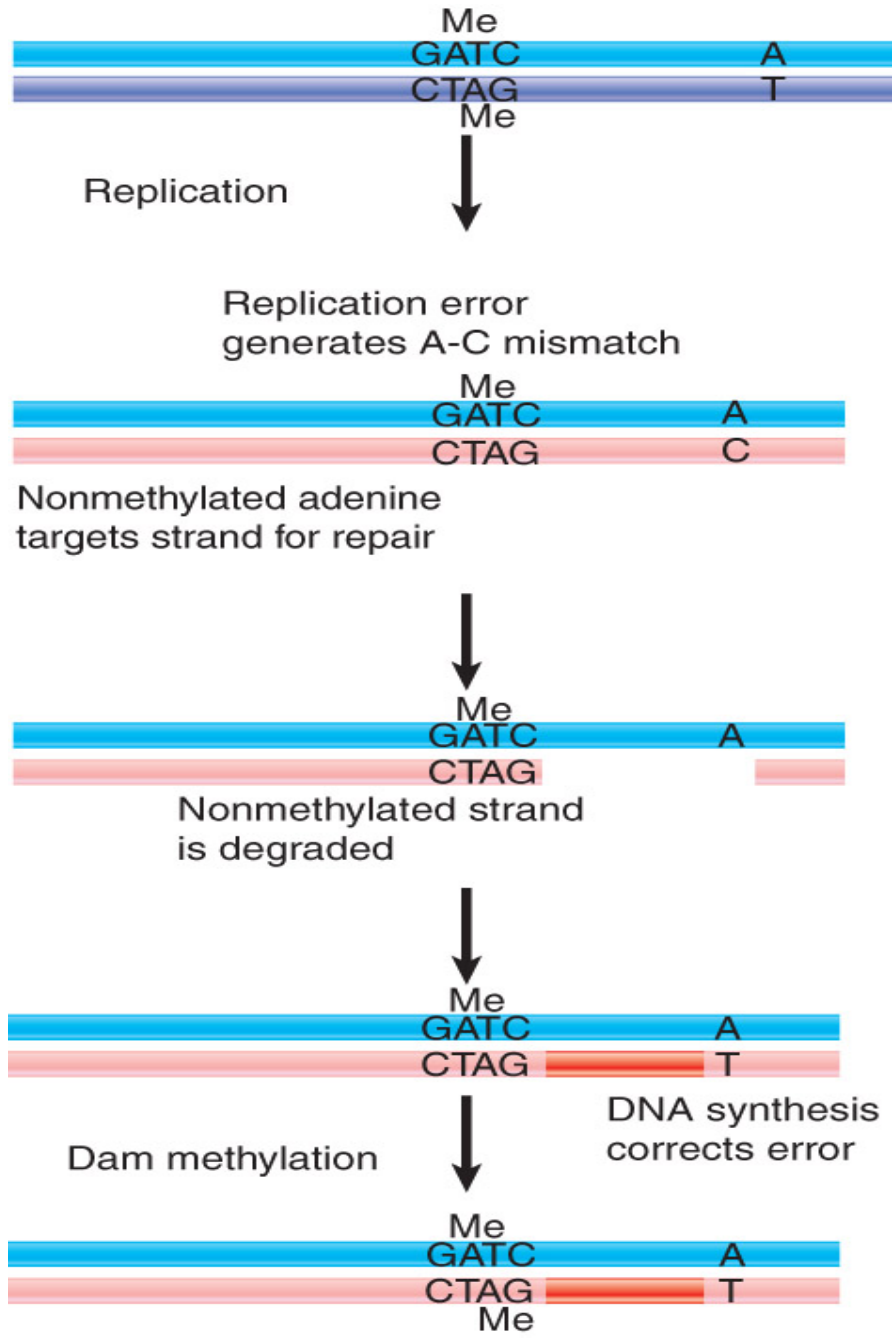
Controlling the Direction of Mismatch Repair

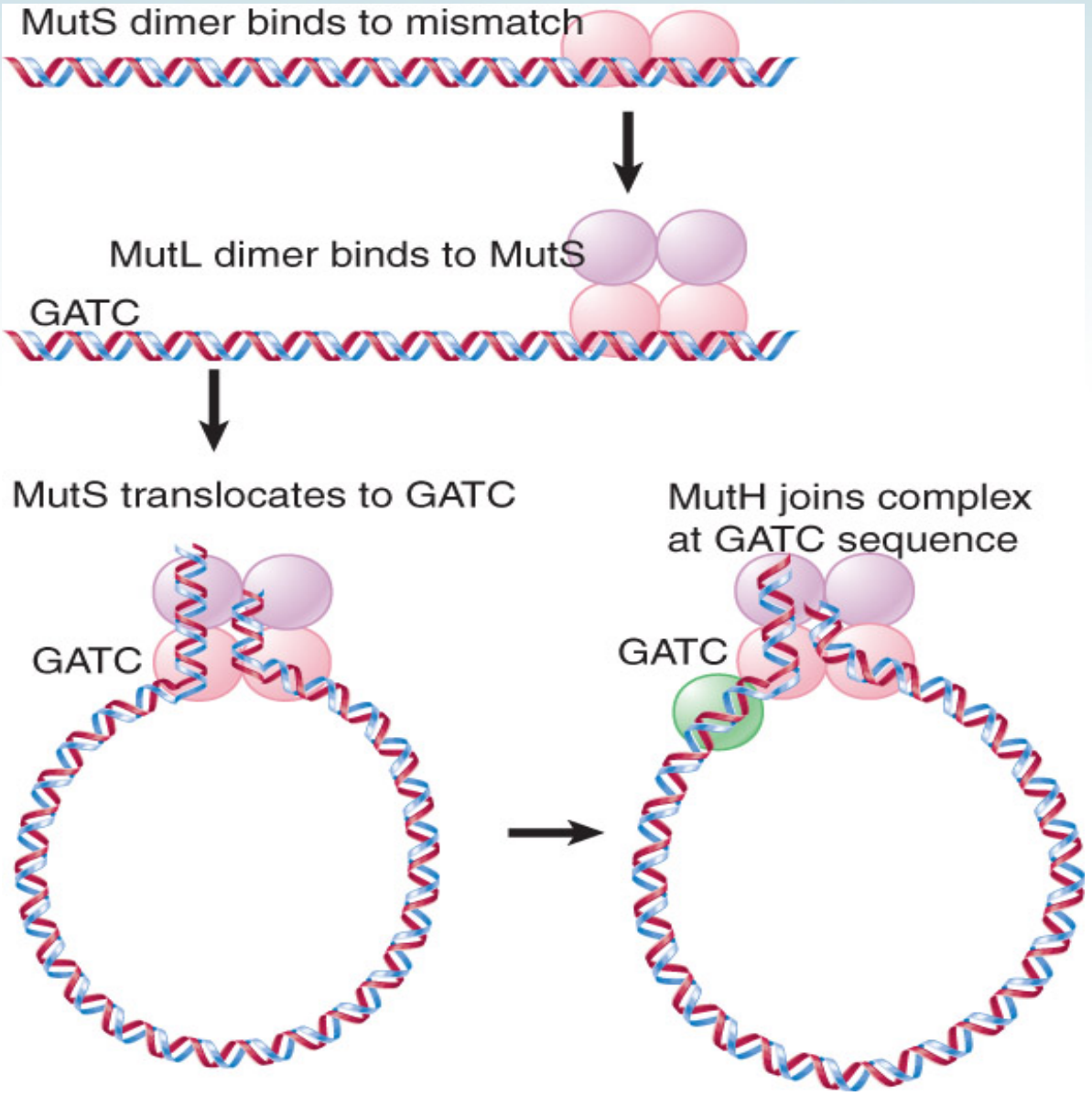


GATC sequences are targets for the **Dam methylase** (in *E. coli*)

CpG repeats are often targets for **DNA methyl transferases (DNMTs)** in eukaryotes

- There is a bias in the selection of which strand to replace at mismatches.
- The strand lacking methylation at a hemimethylated $^{GATC}_{CTAG}$ is usually replaced.
- The mismatch repair system is used to remove errors in a newly synthesized strand of DNA. At G-T and C-T mismatches, the T is preferentially removed.


Me
GATC
A
CTAG
T
Me
Replication
Replication error
generates A-C mismatch
Me
GATC
A
CTAG
C
Nonmethylated adenine
targets strand for repair
Me
GATC
A
CTAG
Nonmethylated strand
is degraded
Me
GATC
A
CTAG
T
DNA synthesis
corrects error
Dam methylation
Me
GATC
A
CTAG
T
Me



MutSL binds mismatches on unmethylated DNA strands

Eukaryotes have **MutL** homologues designated **Mlh1** and **Pms1**. They form **heterodimers** which the mimic function of the homodimeric **MutL** in *E. coli*.

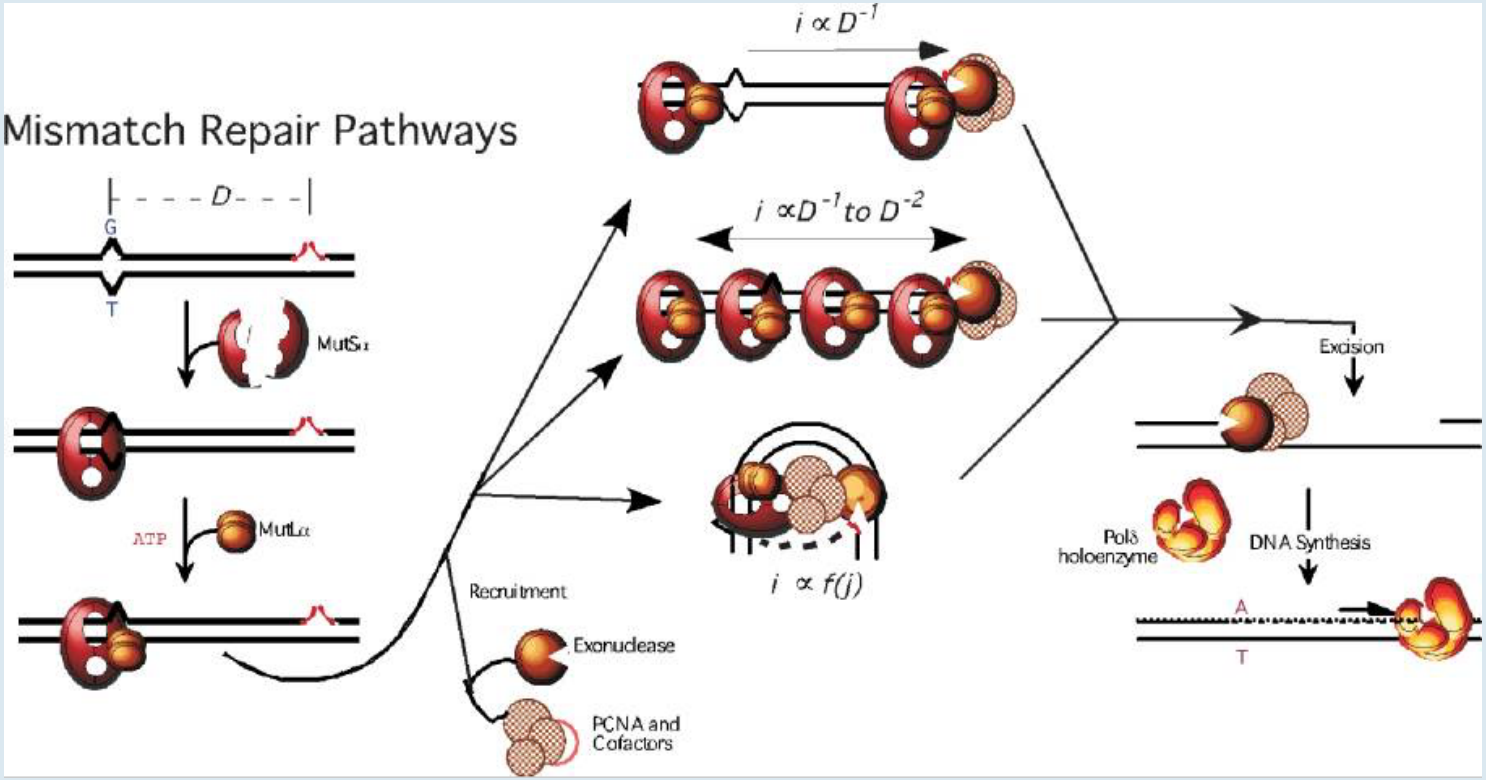
The human homologue of prokaryotic MutL has three forms designated as **MutLα**, **MutLβ** and **MutLγ** -and there is a noticeable absence of a **MutH homologue.**

They effectively lack the equivalent of a MutH p

The **MutLα** complex is made of two subunits **MLH1 and PMS2,** the **MutLβ** heterodimer is made of **MLH1 and PMS1**, while **MutLγ** is made of **MLH1 and MLH3**.

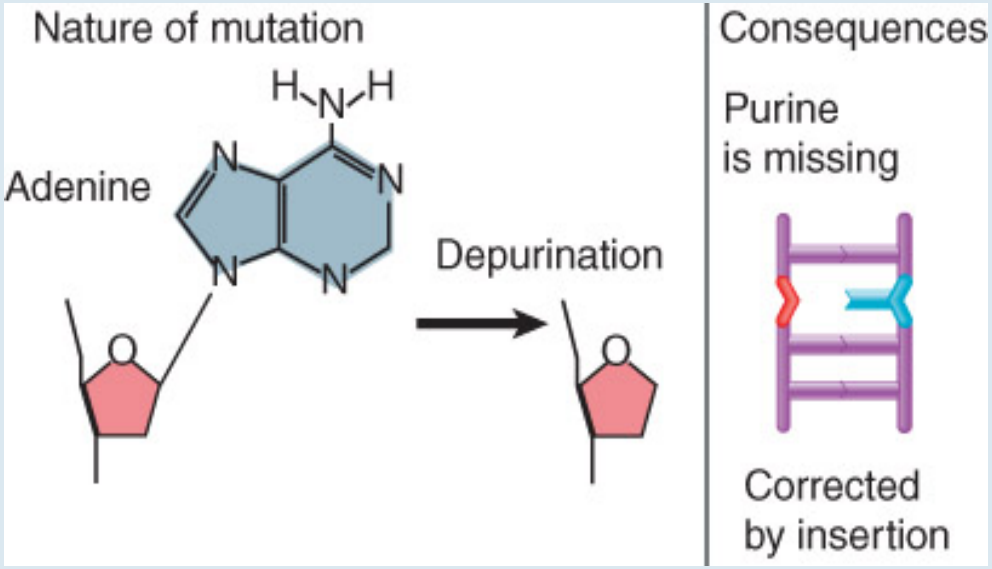
MutLα acts as the matchmaker or facilitator. **MutS** homologues form two major heterodimers: Msh2/Msh6 (**MutSα**) and Msh2/Msh3 (**MutSβ**).

The **MutSα pathway** is also involved -primarily in **base substitution** and **small loop mismatch repair**, where the **MutSa** can interact with associated proteins, such as with the **PCNA (the Sliding clamp of the DNA replicase)**. These strand interruptions serve as entry points for an exonuclease activity that removes mismatched DNA.



Nucleic Acids Res. 2007 Nov; 35(20): 6727–6739.

Controlling the Direction of Mismatch Repair

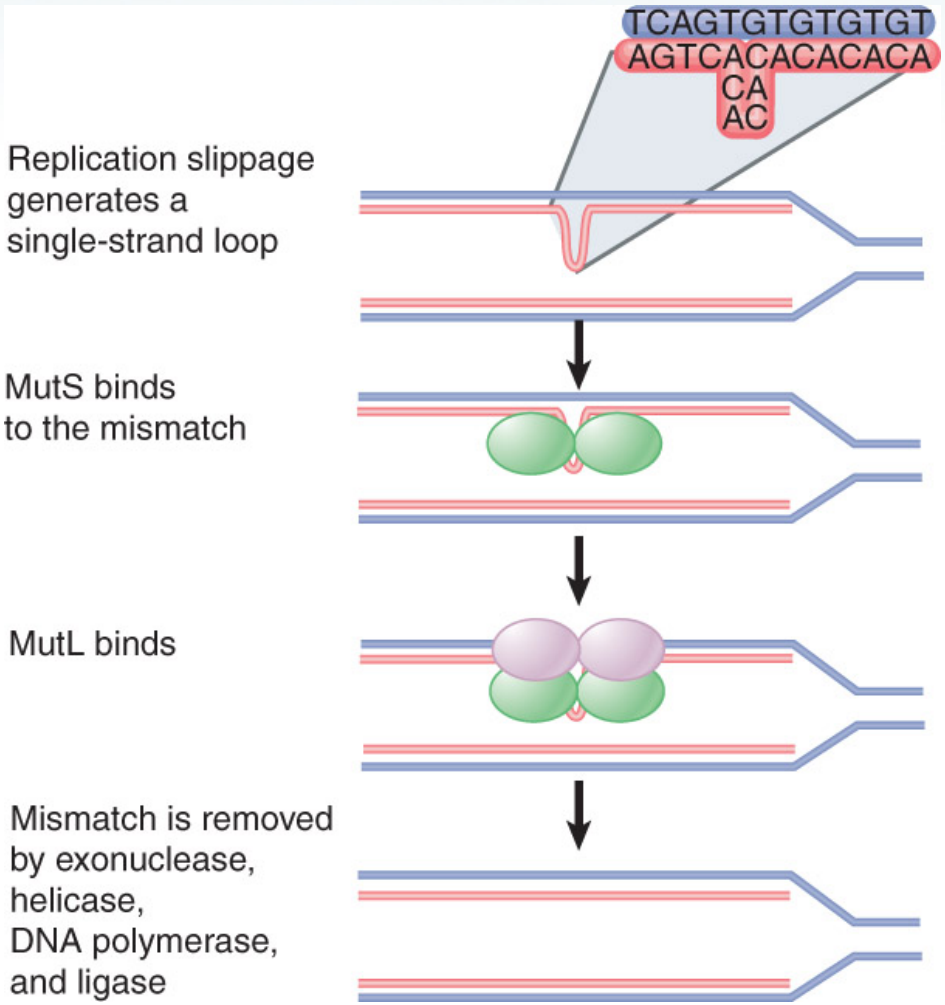


GATC sequences are targets for the **Dam methylase** (in *E. coli*)

CpG repeats are often targets for **DNA methyl transferases (DNMTs)** in eukaryotes

- There is a bias in the selection of which strand to replace at mismatches.
- The strand lacking methylation at a hemimethylated $^{GATC}_{CTAG}$ is usually replaced.
- The mismatch repair system is used to remove errors in a newly synthesized strand of DNA. At G-T and C-T mismatches, the T is preferentially removed.

Controlling the Direction of Mismatch Repair

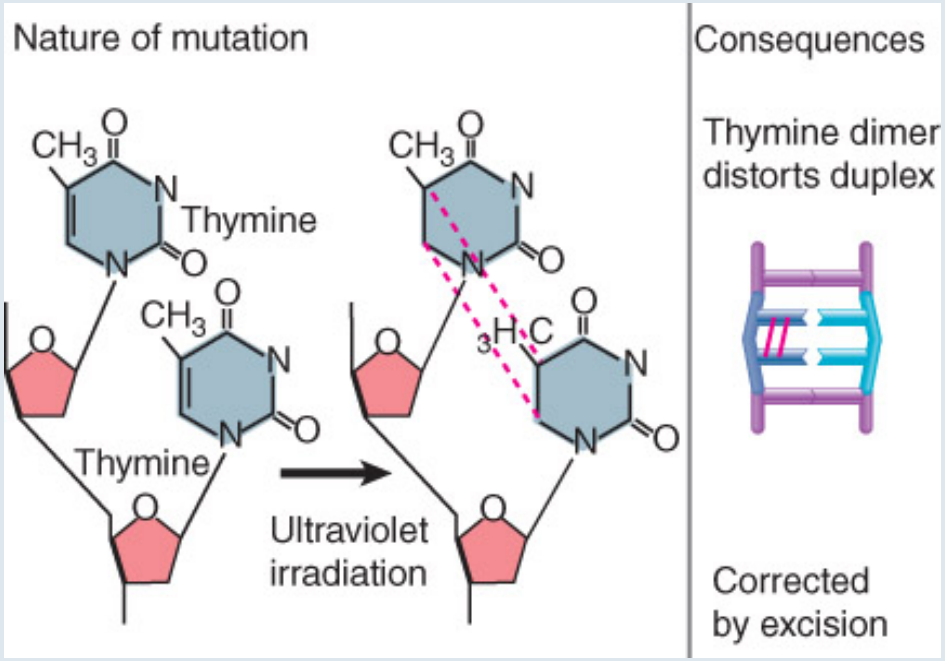


- Eukaryotic MutS/L homologous systems repair mismatches and insertion/ deletion loops.

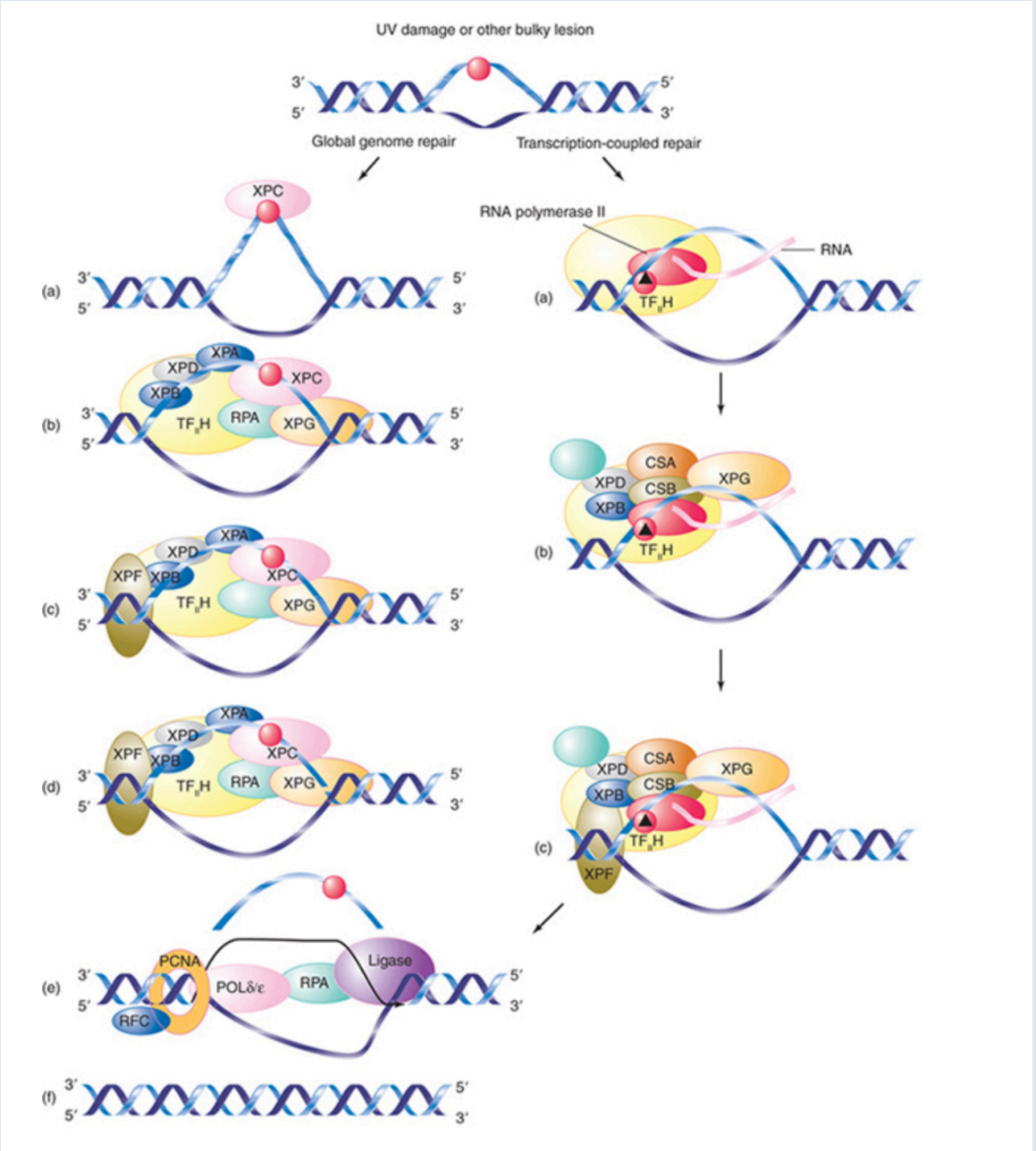
MutSα/MutL repair replication slippages
MutSα and MutL form heterodimers in eukaryotes

Repair Systems Correct Damage to DNA

- Recombination-repair systems use recombination to replace the double-stranded region that has been damaged.
- All these systems are prone to introducing errors during the repair process.
- Photoreactivation is a nonmutagenic repair system that acts specifically on **pyrimidine dimers**.



Ultraviolet irradiation causes dimer formation between adjacent thymines. The dimer blocks replication and transcription.



Nucleotide excision repair occurs via two major pathways: **global genome repair,** in which **XPC** recognizes damage anywhere in the genome, and **transcription-coupled repair**, in which the transcribed strand of active genes is preferentially repaired and the damage is recognized by an elongating RNA polymerase.

Base Excision Repair Systems Invariably Require **Glycosylases**

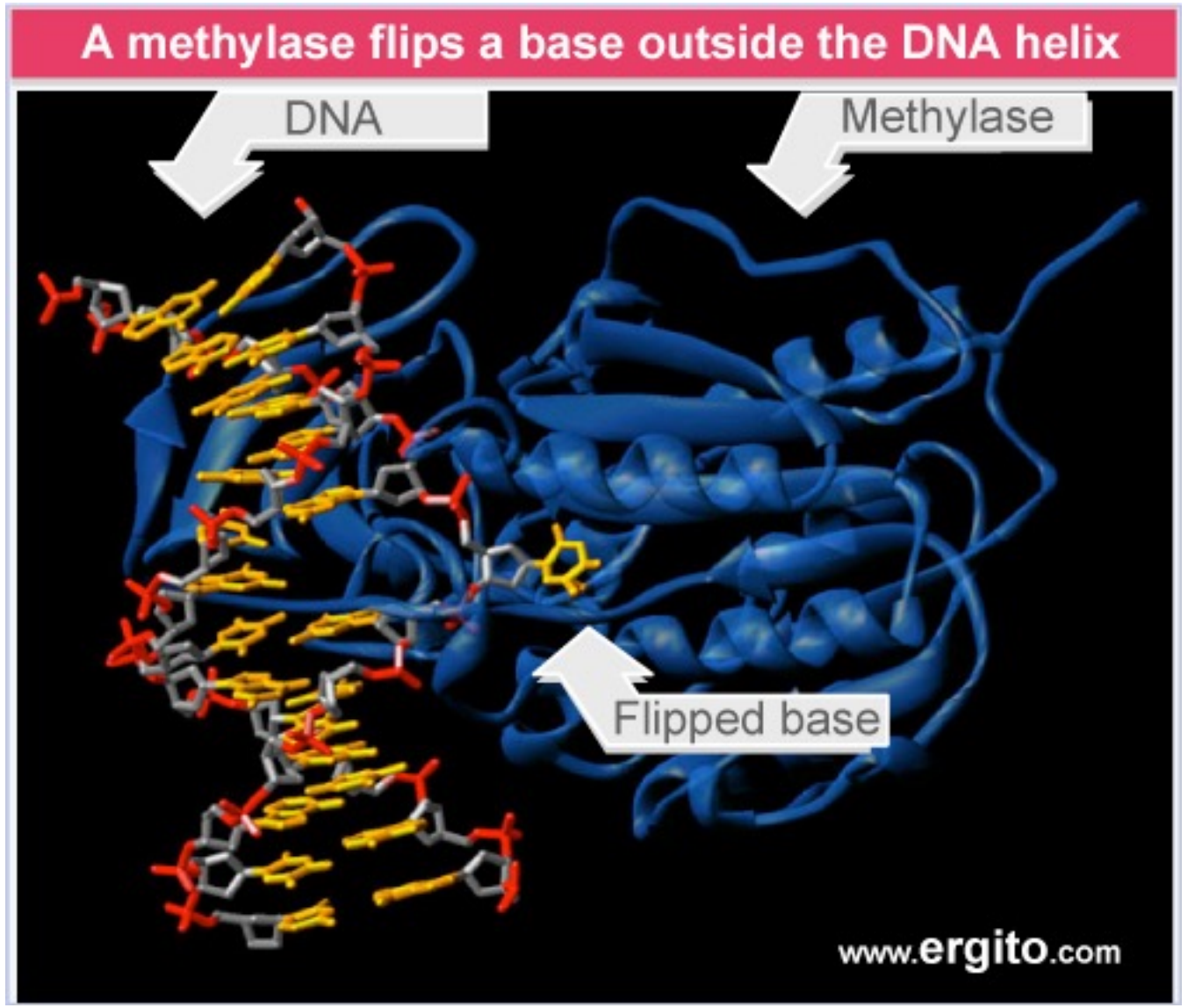
- **Uracils** and **alkylated bases** are recognized by **glycosylases** and removed directly from DNA.

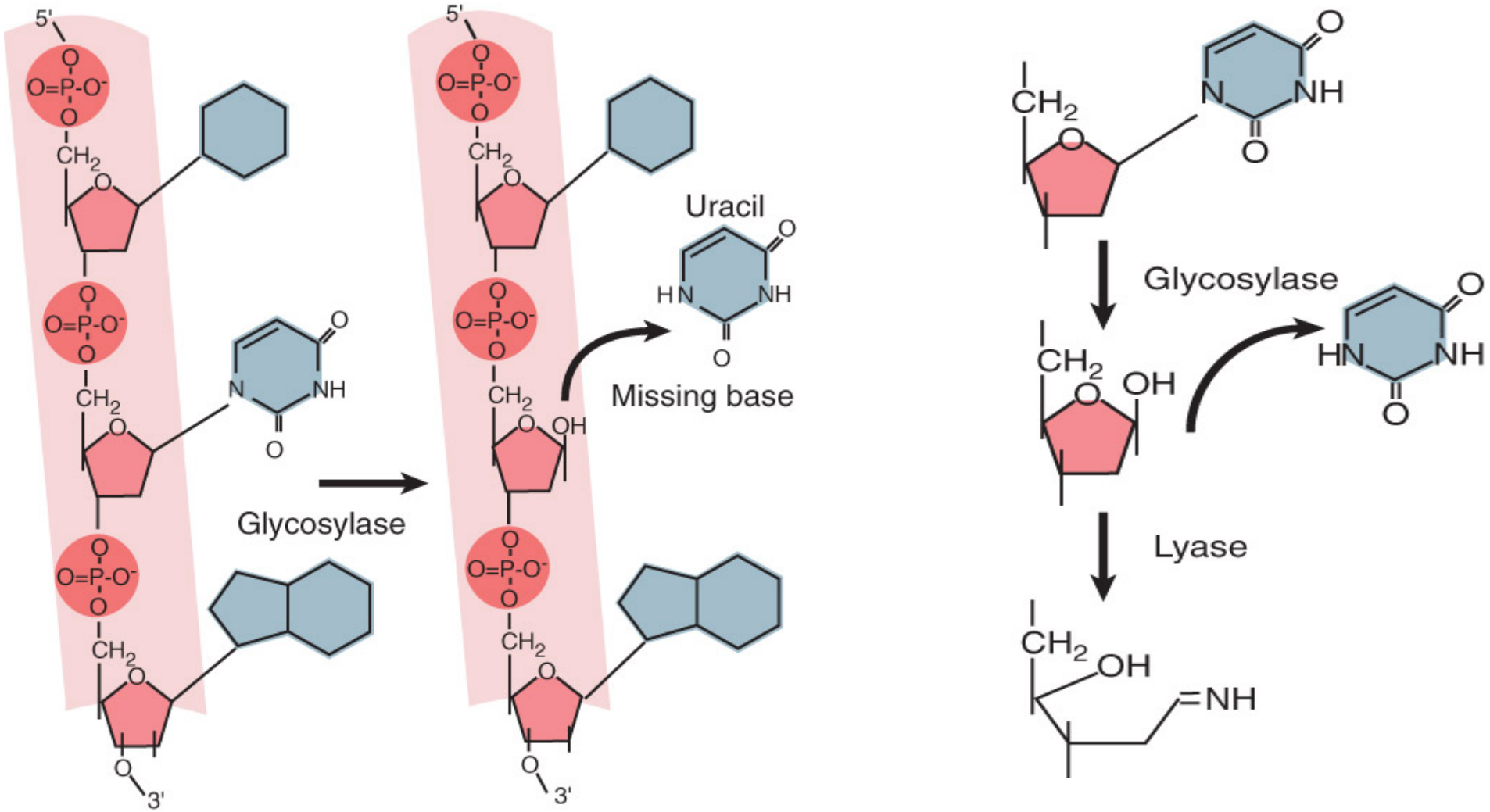
- Glycosylases and photolyase (a **lyase**) act by flipping the base out of the double helix, where, depending on the reaction, it is either removed or modified and returned to the helix.

Glycosylases in bacteria, yeast and humans[5][6]

E. coli	Yeast (S. cerevisiae)	Human	Type	Substrates
AlkA	Mag1	MPG	monofunctional	3-meA, hypoxanthine
UDG	Ung1	UNG	monofunctional	uracil
Fpg	Ogg1	hOGG1	bifunctional	8-oxoG, FapyG
Nth	Ntg1	hNTH1	bifunctional	Tg, hoU, hoC, urea, FapyG
	Ntg2			
Nei	Not present	hNEIL1	bifunctional	Tg, hoU, hoC, urea, FapyG, FapyA
		hNEIL2		AP site, hoU
		hNEIL3		unknown
MutY	Not present	hMYH	monofunctional	A:8-oxoG
Not present	Not present	hSMUG1	monofunctional	U, hoU, hmU, fU
Not present	Not present	TDG	monofunctional	T:G mispair
Not present	Not present	MBD4	monofunctional	T:G mispair

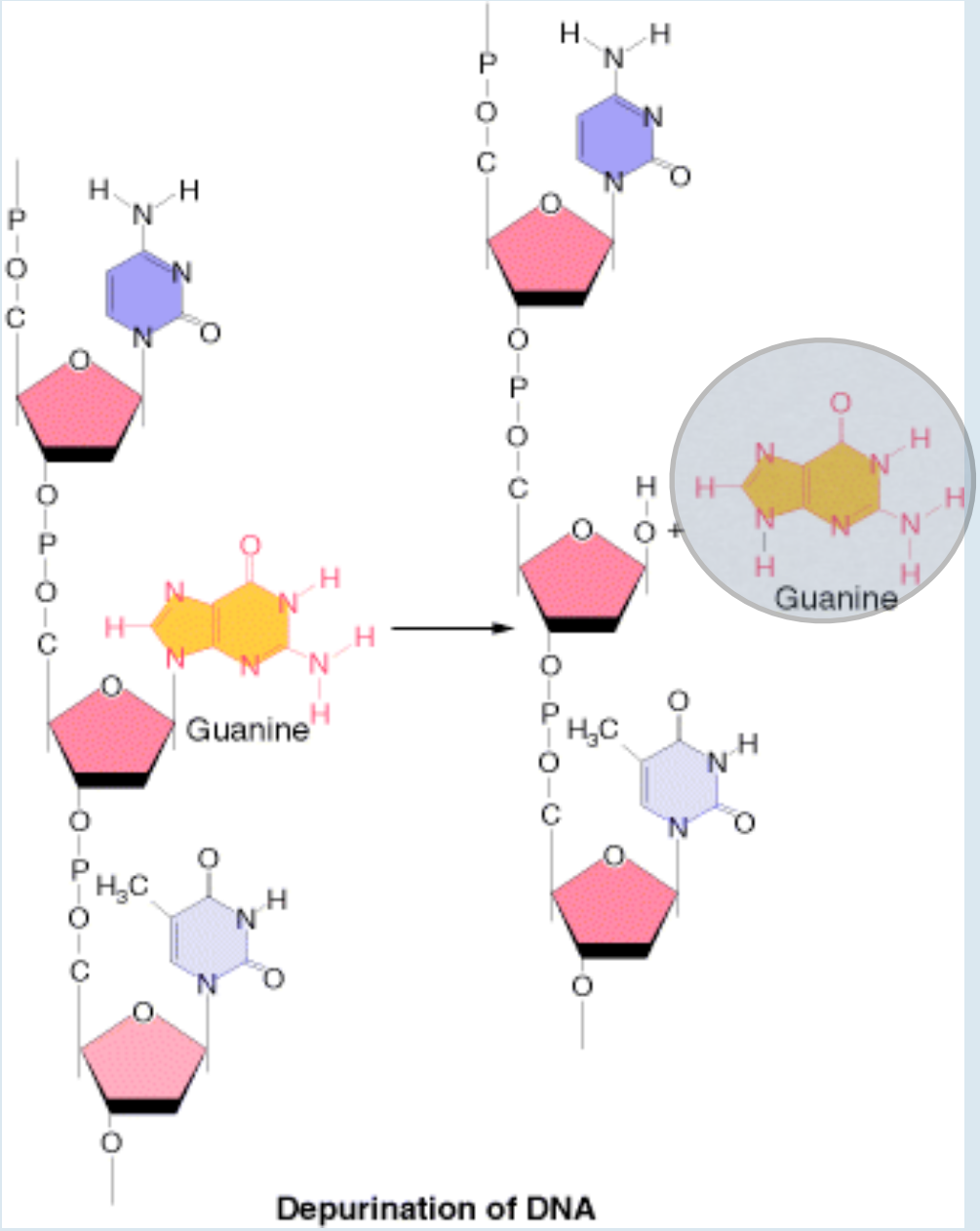
DNA glycosylases can be grouped into the following categories based on their substrate(s):


A methylase flips a base outside the DNA helix
DNA
Methylase
Flipped base
www.ergito.com



Glycosylase activity is followed by the endonuclease **APE1,** which cleaves the polynucleotide chain on the 5’ side.

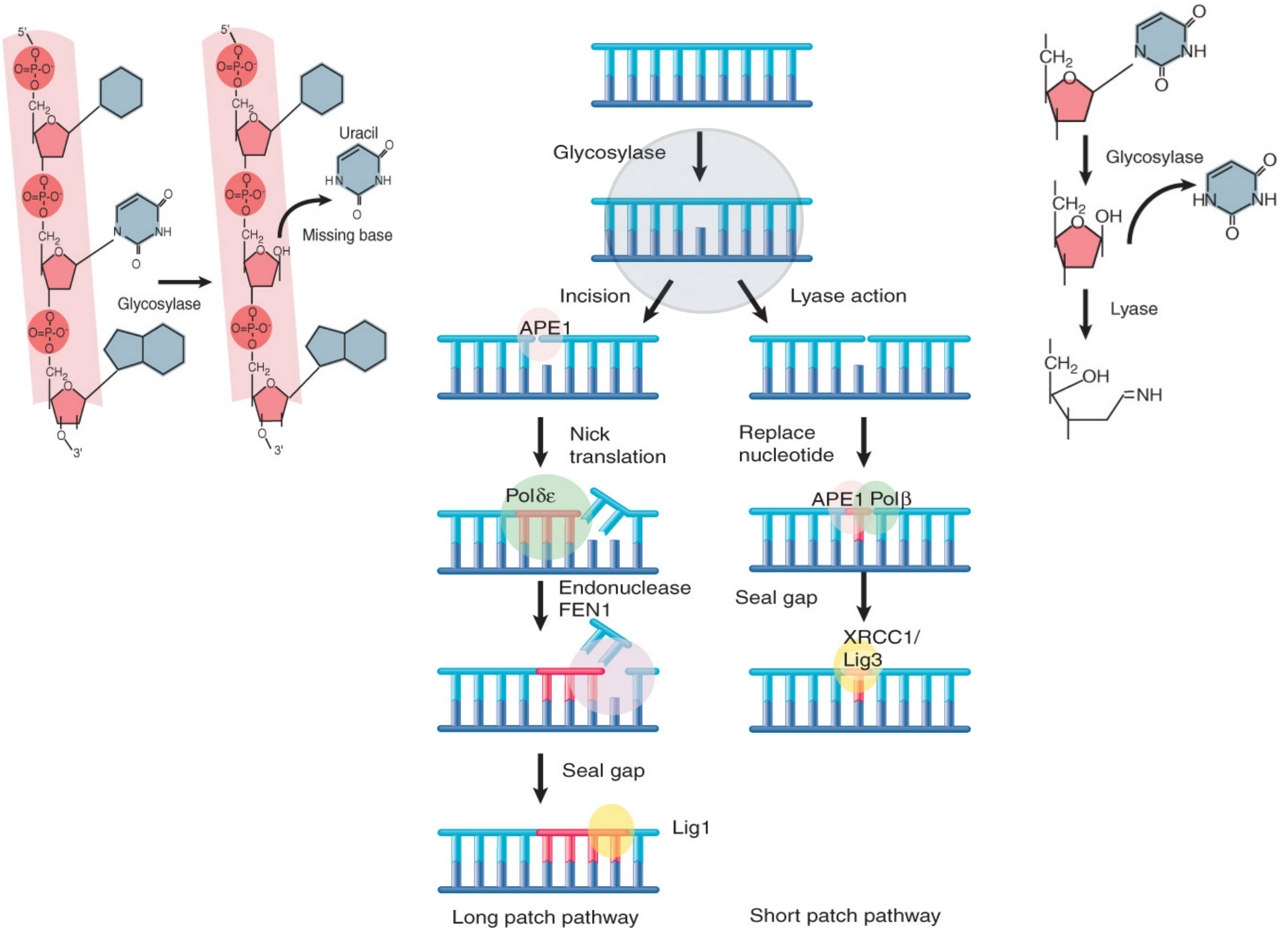
This in turn attracts a replication complex including the **DNA polymerase δ/ε and ancillary components,** which undertakes a process of **nick translation extending for 2-10 nucleotides**. The displaced material is removed by the **endonuclease FEN1.** The enzyme ligase-1 seals the chain. This is called the long patch pathway.

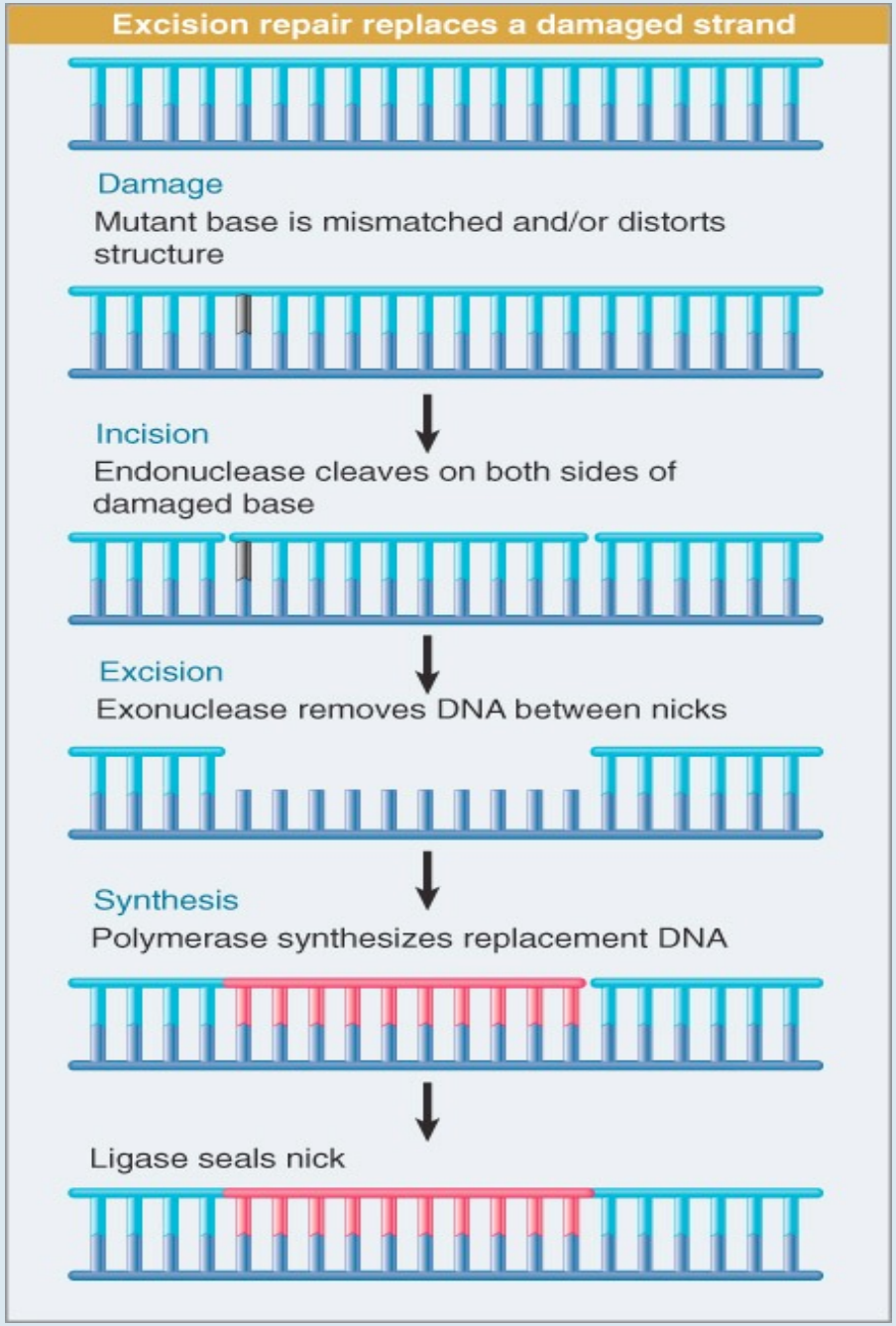


The loss of a purine residue (guanine) from a single strand of DNA, but the sugar-phosphate backbone is left intact resulting in an "**apurinic**" site.

Base Excision Repair Systems Require Glycosylases

- Base excision repair is triggered by directly removing a damaged base from DNA.
- Base removal triggers the removal and replacement of a stretch of polynucleotides.
- The nature of the base removal reaction determines which of two pathways for excision repair is activated.
- The **polδ/ε pathway replaces a long polynucleotide** stretch; the **polβ pathway replaces a short stretch (sometime "single"bp).**


Glycosylase
Uracil
Missing base
Glycosylase
Lyase
Incision
Lyase action
APE1
Nick translation
Replace nucleotide
Polδε
APE1 Polβ
Endonuclease FEN1
Seal gap
XRCC1/ Lig3
Seal gap
Lig1
Long patch pathway
Short patch pathway


Excision repair replaces a damaged strand
Damage
Mutant base is mismatched and/or distorts structure
Incision
Endonuclease cleaves on both sides of damaged base
Excision
Exonuclease removes DNA between nicks
Synthesis
Polymerase synthesizes replacement DNA
Ligase seals nick

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Estimate of the Mutation Rate per Nucleotide in Humans

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Communicating editor: A. G. CLARK

Many previous estimates of the mutation rate in humans have relied on screens of visible mutants. We investigated the rate and pattern of mutations at the nucleotide level by comparing pseudogenes in humans and chimpanzees to (i) provide an estimate of the average mutation rate per nucleotide, (ii) assess heterogeneity of mutation rate at different sites and for different types of mutations, (iii) test the hypothesis that the X chromosome has a lower mutation rate than autosomes, and (iv) estimate the deleterious mutation rate. Eighteen processed pseudogenes were sequenced, including 12 on autosomes and 6 on the X chromosome. The average mutation rate was estimated to be $\sim 2.5 \times 10^{-8}$ mutations per nucleotide site or 175 mutations per diploid genome per generation. Rates of mutation for both transitions and transversions at CpG dinucleotides are one order of magnitude higher than mutation rates at other sites. Single nucleotide substitutions are 10 times more frequent than length mutations. Comparison of rates of evolution for X-linked and autosomal pseudogenes suggests that the male mutation rate is 4 times the female mutation rate, but provides no evidence for a reduction in mutation rate that is specific to the X chromosome. Using conservative calculations of the proportion of the genome subject to purifying selection, we estimate that the genomic deleterious mutation rate (U) is at least 3. This high rate is difficult to reconcile with multiplicative fitness effects of individual mutations and suggests that synergistic epistasis among harmful mutations may be common.

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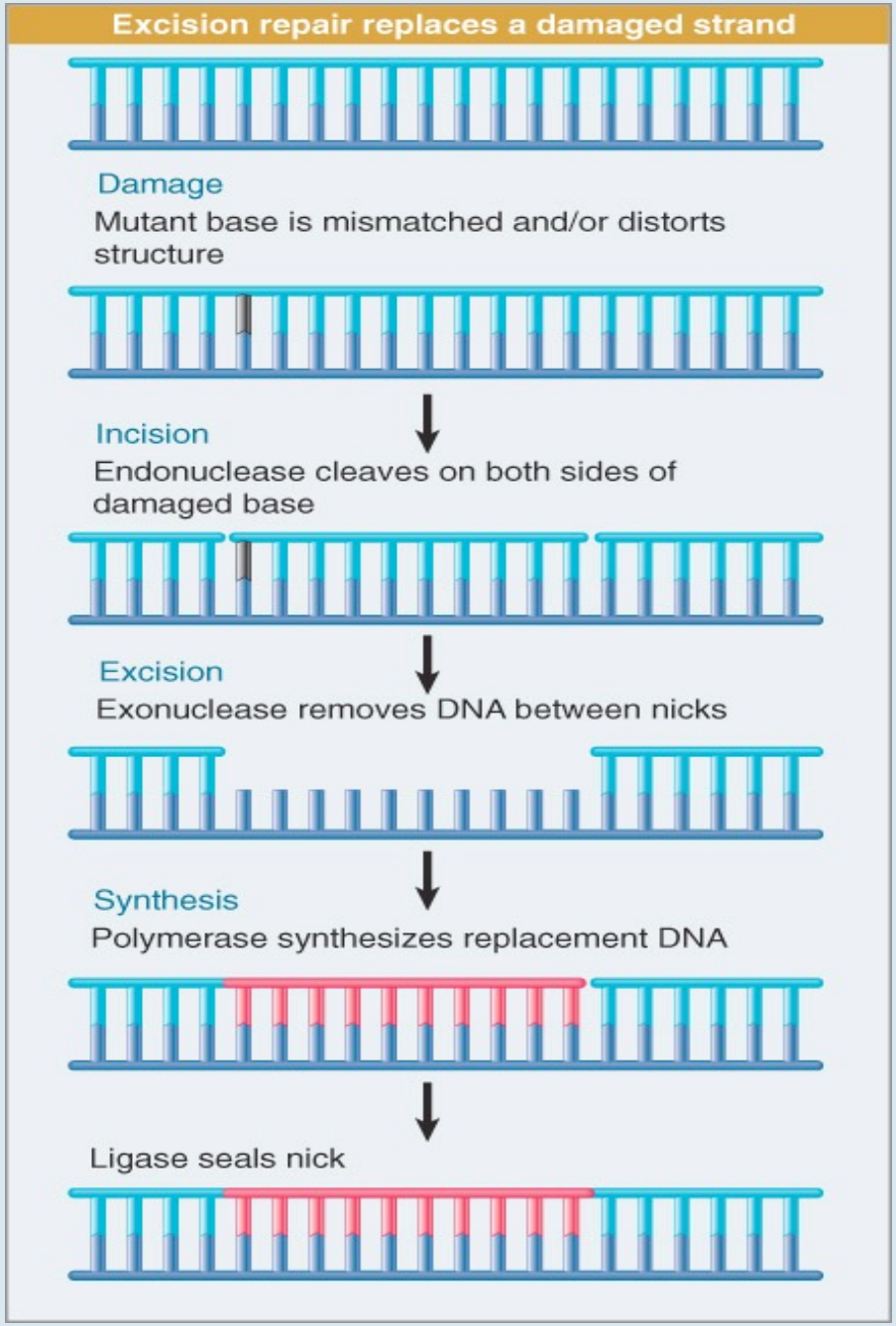
Excision repair replaces a damaged strand
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Polymerase synthesizes replacement DNA
Ligase seals nick

Molecular Cell 23, 155–160, July 21, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.molcel.2006.05.034

DNA Replication: Keep Moving and Don't Mind the Gap

Short Review

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1230 York Avenue
New York City, New York 10021

As the replication fork progresses, synthesis of the discontinuous lagging strand requires frequent priming and cycling of the lagging strand polymerase to the new primers. It appears that this mechanism also permits bypass of template lesions on both strands, leaving the damage behind in a single-strand gap and precluding fork stalling or collapse.

Introduction

The standard view of DNA replication is that it is semi-discontinuous, with the leading strand synthesized as a single uninterrupted chain and the lagging strand as a series of short (<2 kb) Okazaki fragments. Despite the prevalence of this view and the support it has gained from in vitro studies, it is not in agreement with cytological evidence in *E. coli*, which routinely shows discontinuities in both strands during DNA replication (reviewed in Wang [2005]). This does not necessarily imply that there is a regular cycle of reinitiation on the leading strand, but it suggests that priming can and does recur on the leading strand as the replication fork moves away from the origin, most likely in response to damage on the

tural bridge between the replicative helicase and the leading and lagging strand polymerases at the prow of the replication fork.

Cellular DNA polymerases cannot initiate synthesis in the absence of a nucleic acid primer, so the first step in DNA synthesis is the formation of a short RNA primer (~10 nt) by specialized RNA polymerases known as primases (Kornberg and Baker, 1992). In principle, leading strand synthesis requires only a single priming event, whereas frequent repriming is the hallmark of discontinuous lagging strand synthesis. The distribution of primers, ~1–2 kb apart on the lagging strand, is governed by dynamic interactions between DnaB and the DnaG primase (Tougu and Marians, 1996) and possibly, according to a recent report, by interactions between separate primase molecules bound to a single DnaB on the lagging strand (Corn et al., 2005).

Primase remains bound to the 3′ terminus of the RNA primer through contact with SSB, the single-strand DNA (ssDNA) binding protein, which binds and protects ssDNA ahead of the primer (Figure 1A). The Pol III core polymerase then replaces primase at the primer terminus in a three-part switch activated by the χ subunit of the γ complex clamp loader (Yuzhakov et al., 1999). χ displaces primase by competitive binding to SSB, whereas the clamp-loading subunits of γ complex (the AAA+ proteins γ, τ_2, δ, and δ') form a helical structure that completely encases the newly cleared primer-template junction (for a detailed review of clamp loaders and how their structures confer specificity for the primed

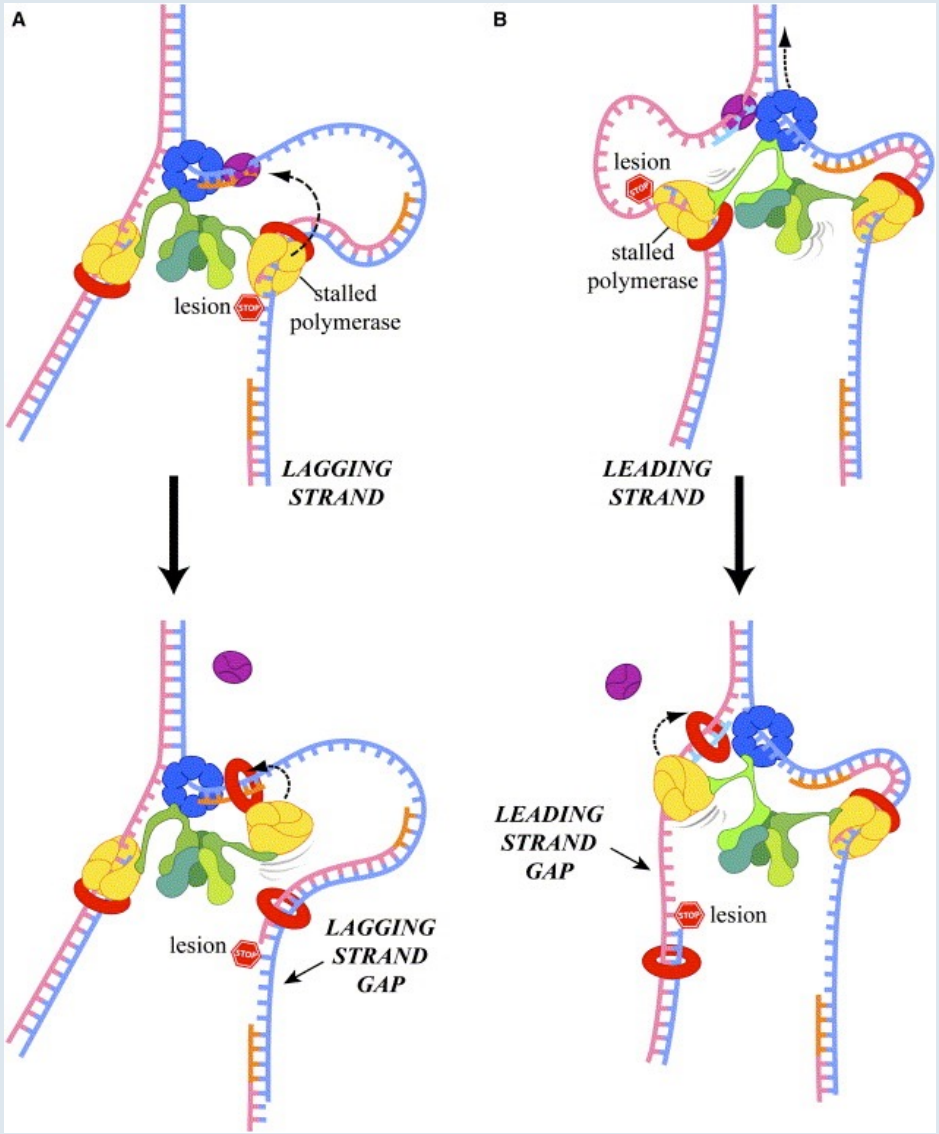


Figure 2. Replisomes Bypass Template Damage

(A) Upon encountering a lesion on the lagging strand template (stop sign), leading strand synthesis continues and the stalled lagging strand polymerase recycles (dotted arrow) to a new primer/template junction, leaving a single-strand gap with a template lesion (bottom).

(B) The leading strand polymerase stalls upon encountering a lesion (top). The helicase recruits primase to reinitiate leading strand synthesis ahead of the lesion, leaving a single-strand gap (bottom). If stalling causes the replication fork to collapse, additional factors (e.g., PriA or PriC) are required to reload the helicase at the collapsed fork. For (A) and (B), gaps that are left behind in either strand can be repaired with high fidelity by recombination processes using the new sister chromatid as a template. Artwork by Dr. Nina Yao.

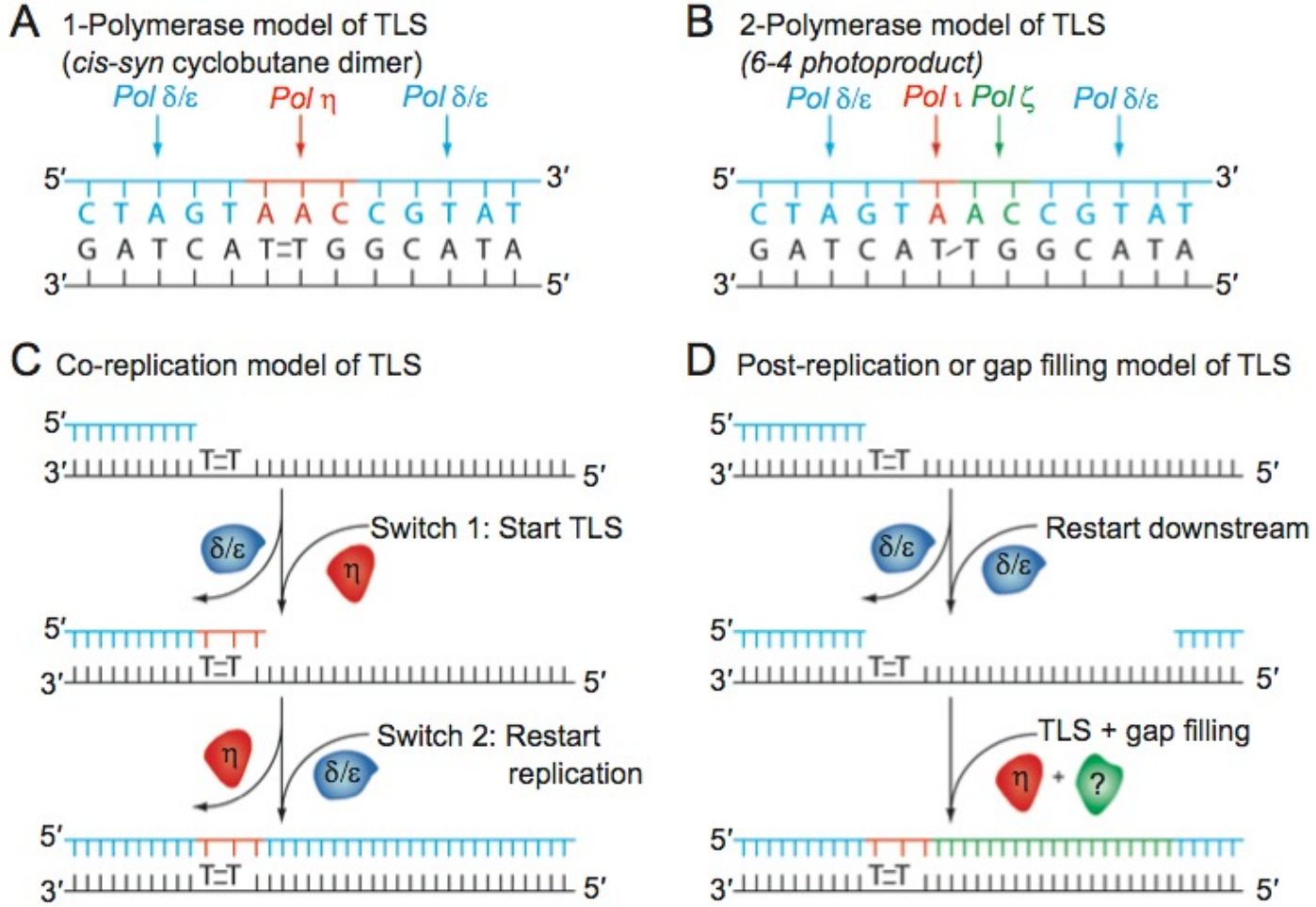
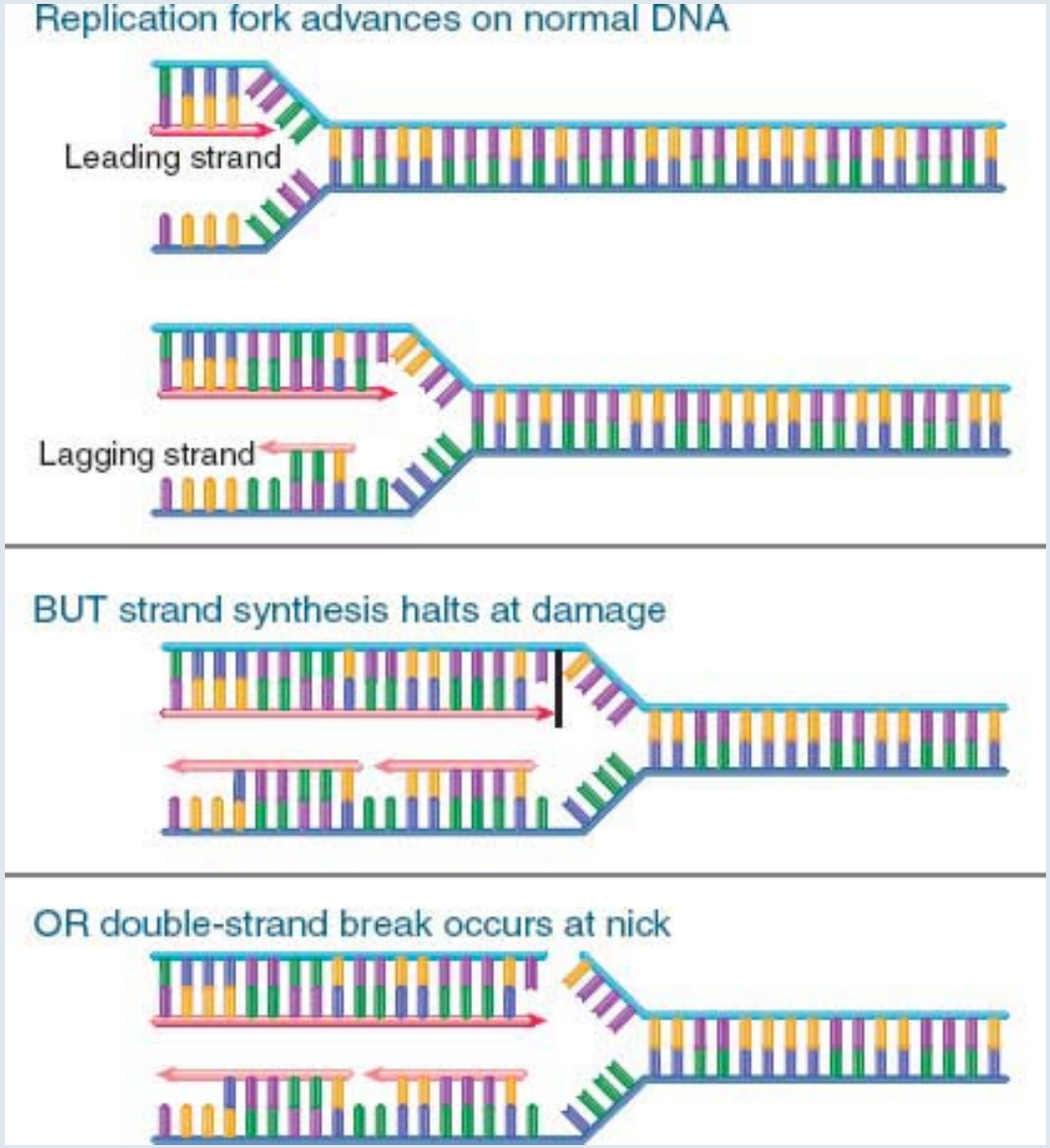


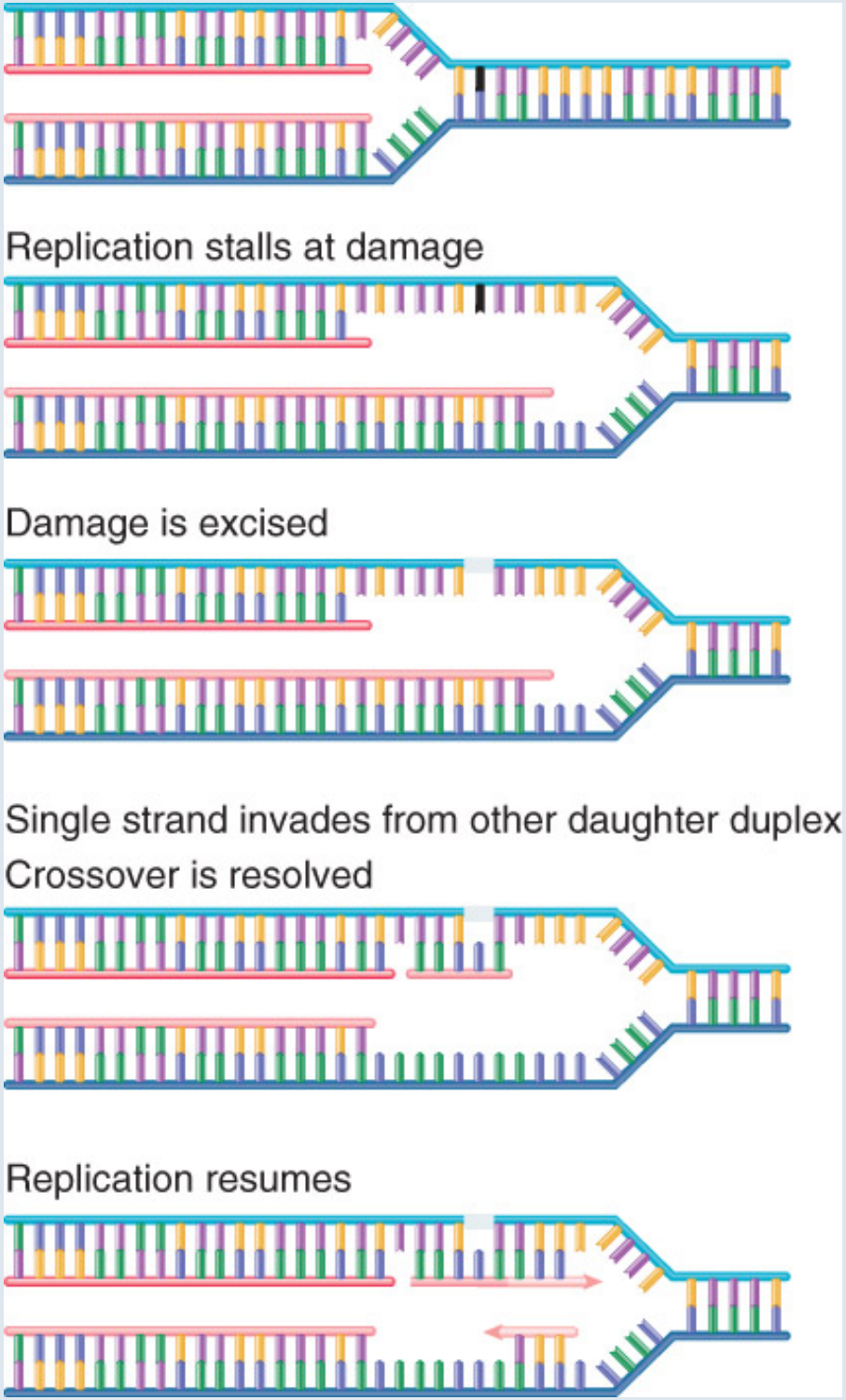
Figure 3 Models of translesion synthesis. **(A)** The 1-polymerase model of TLS, shown here for a thymine-thymine dimer, states that a single polymerase is responsible for the complete bypass of a lesion, including insertion opposite all lesion bases and extension from the primer terminus opposite a damaged template base. **(B)** The 2-polymerase model of TLS, shown here for a thymine-thymine 6-4 photoproduct, states that different polymerases are responsible for the insertion steps at the various lesion positions. In the example given, note that while pol ζ is responsible for extension from the template-3′ T primer terminus, it also carries out an insertion at the 5′ T position of the lesion. For a single base lesion, the insertion step would be opposite undamaged DNA. A more comprehensive listing of 2-polymerase/lesion combinations is given elsewhere [11]. Note that for both examples given, the actual TLS reaction is flanked relatively closely both upstream (1-2 bases) and downstream (1-5 bases) of the lesion by replicative polymerase synthesis. **(C)** Model for TLS that occurs at a replication fork during the process of ongoing synthesis. **(D)** Model for TLS that takes place as a "gap-filling" reaction, away from the main replication machinery. Note that both of these models are consistent with either the 1- or 2-polymerase model of TLS given in panels **A** and **B**. In both cases, post-translational modification of PCNA and possible other proteins is critical for the polymerase switch. Note that panels A and B are models of the actual TLS process while panels **C** and **D** depict models for the timing of TLS. As such (and as noted in the text), there is overlap between the panels.

Lesion Bypass Requires Polymerase Replacement



- A replication fork stalls when it arrives at damaged DNA.
- The replication complex must be replaced by a specialized DNA polymerase for **lesion bypass**.

The replication fork stalls and may collapse when it reaches a damaged base or a nick in DNA. Arrowheads indicate 3′ ends.

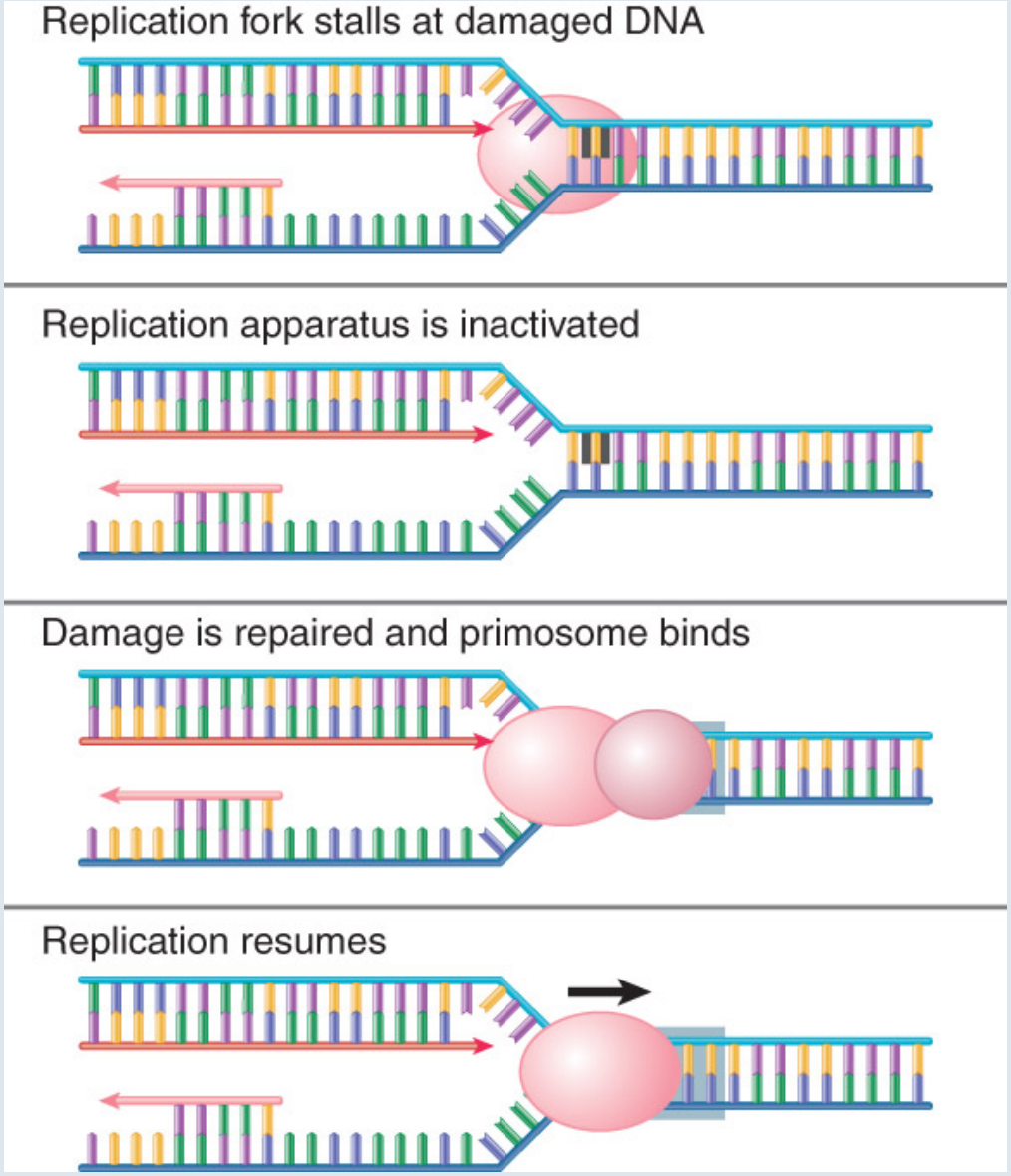


Lesion Bypass Requires Polymerase Replacement

- A replication fork stalls when it arrives at damaged DNA.
- The replication complex must be replaced by a specialized DNA polymerase for **lesion bypass**.

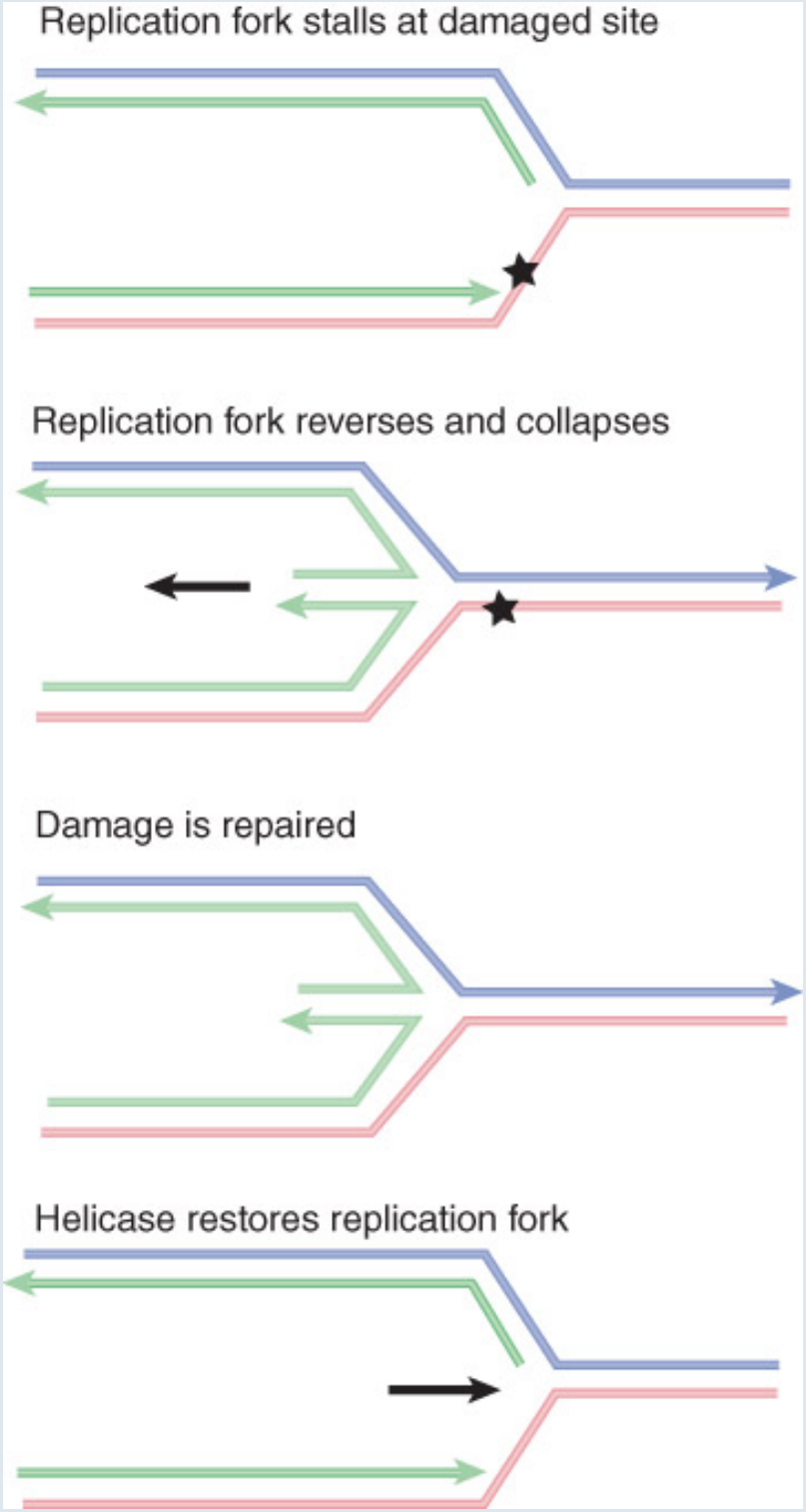
When replication halts at damaged DNA, the damaged sequence is excised and the complementary (newly synthesized) strand of the other daughter duplex crosses over to repair the gap. Replication can now resume, and the gaps are filled in

Lesion Bypass Requires Polymerase Replacement



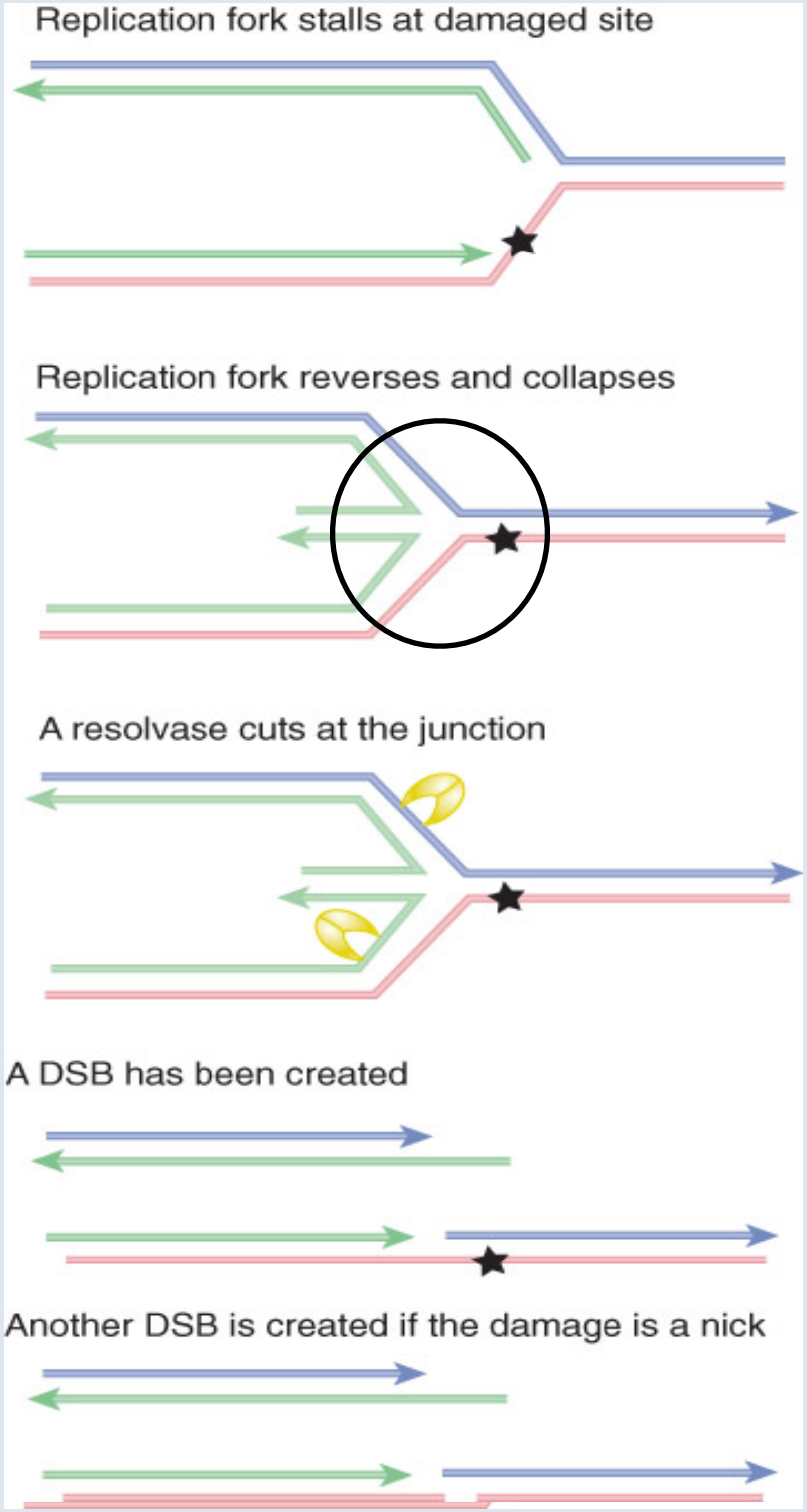
- After the damage has been repaired, the **primosome** is required to reinitiate replication by reinserting the replication complex.

The **primosome** is required to restart a stalled replication fork after the DNA has been repaired



Recombination Is an Important Mechanism to Recover from Replication Errors

- A replication fork may stall when it encounters a damaged site or a nick in DNA.
- A stalled fork may restart after repairing the damage and use a helicase to move the fork forward.
-



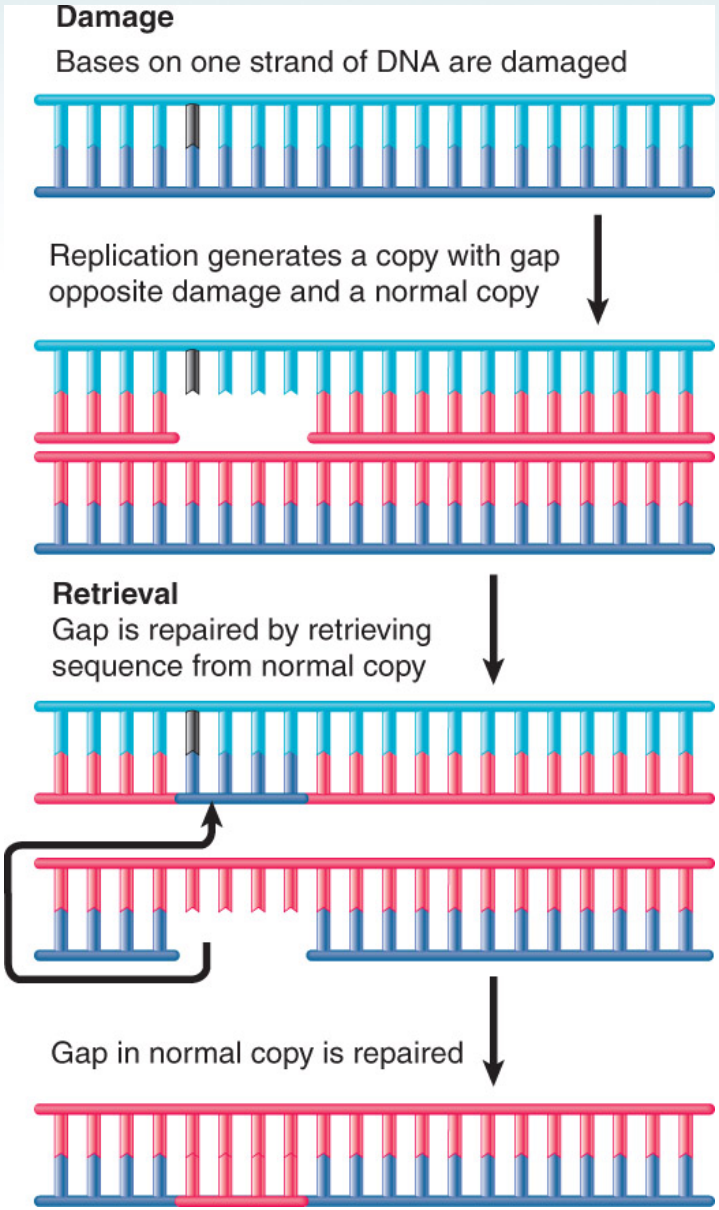
Recombination Is an Important Mechanism to Recover from Replication Errors

- A replication fork may stall when it encounters a damaged site or a nick in DNA.
- A stalled fork may restart after repairing the damage and use a helicase to move the fork forward.

or

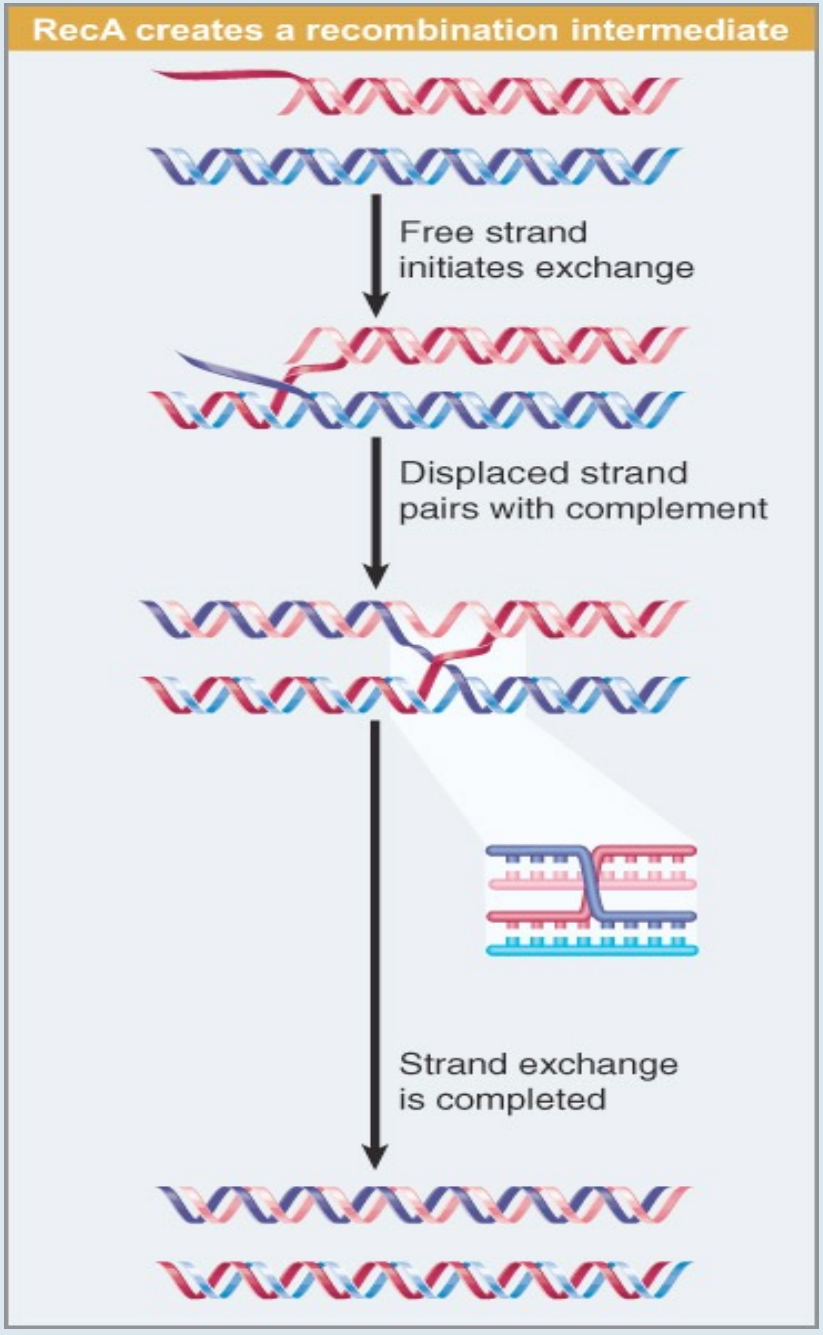
- a stalled fork may reverse by pairing between the two newly synthesized strands.
- The structure of the stalled fork is the same as a **Holliday junction** and may be converted to a duplex and **DSB** using DNA resolvases…..

Recombination-Repair Systems



- The single strand of another duplex is used to replace the gap (**single-strand exchange**).
- The damaged sequence is then removed and resynthesized.

Recombination-repair uses two duplexes



RecA creates a recombination intermediate

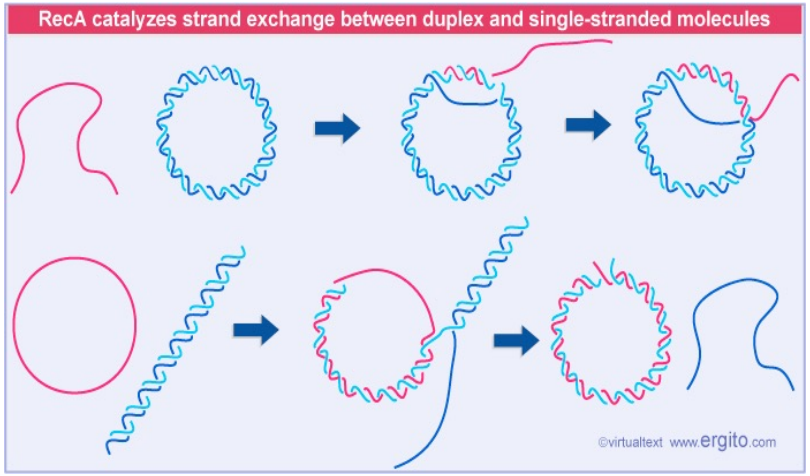
RecA

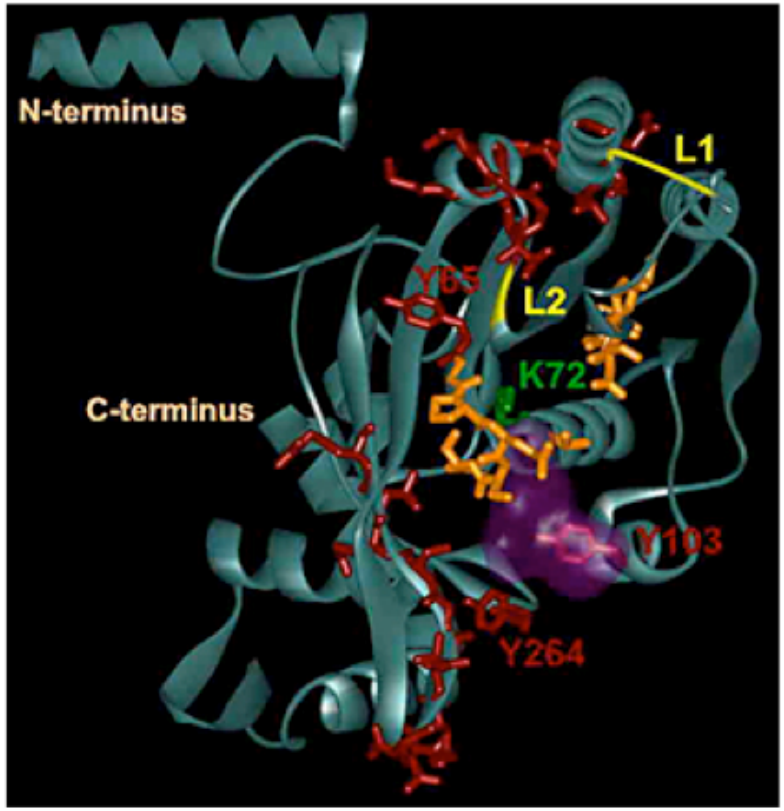
38 kDa protein that polymerizes onto SS DNA 5'-3'

Catalyzes strand exchange

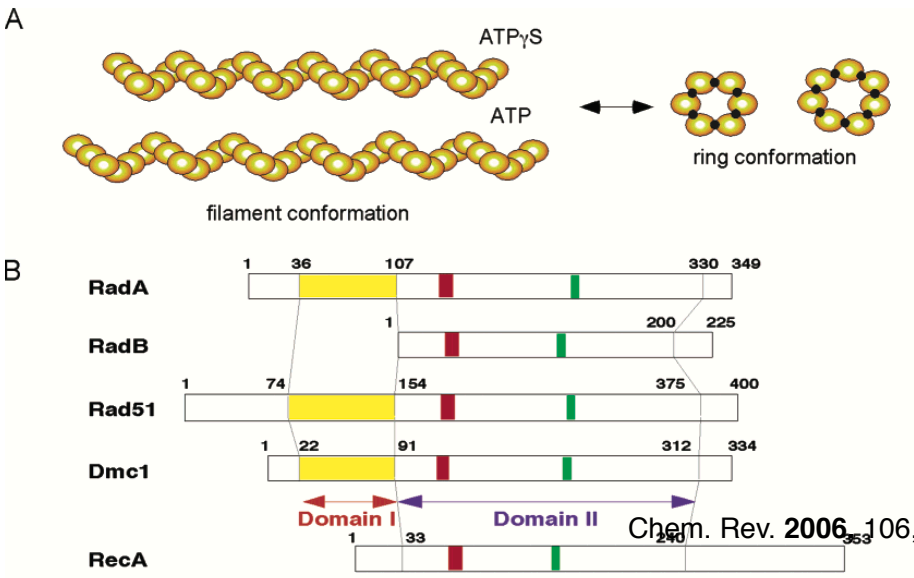
ATPase activity

Works as hexamer





RecA monomer



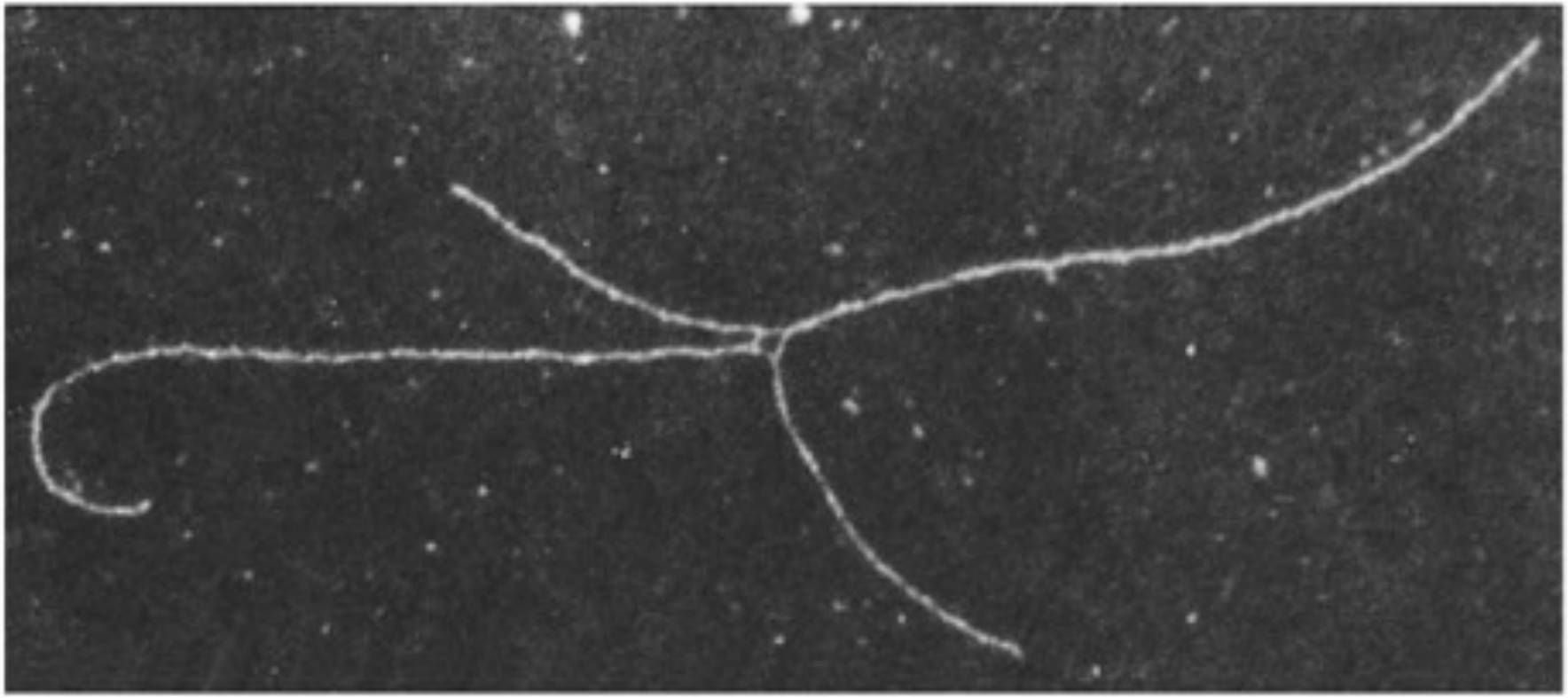
Chem. Rev. **2006**, 106, 324–339

Figure 2. Structural view of RecA/RadA/Rad51 recombinase. (A) Two different structural states of the RecA proteins. The structural states are affected by the protein concentration, the bound nucleotide state (ATP, ADP, or no nucleotide), and the presence/absence of DNA. (B) Schematic drawings of RecA, RadA, and Rad51. Conserved domains among the three protein family members are designated as domain II. This region contains the ATP binding/hydrolysis site as well as the oligomerization domain. The red and green boxes indicate the Walker A and B motifs, respectively. Domain I (yellow box) is conserved only in Eukaryotes and Archaea. The amino acid residue numbers of each protein are derived from *P. furiosus* RadA and RadB, *S. cerevisiae* Rad51 and Dmc1, and *E. coli* RecA. (C) Crystal structures of the core domains of RecA (*Mycobacterium tuberculosis*; left), RadA (*P. furiosus*; middle), and Rad51 (human; right). Notice that all three proteins share a similar architecture. ADP/AlF_4, an ATP analogue, shown in a ball-and-stick model, is bound to the RecA nucleotide binding pocket.

Mechanisms of Maintaining Genetic Stability by Homologous Recombination

Yoshizumi Ishino,*,†,‡ Tatsuya Nishino,§,| and Kosuke Morikawa*,§

Chem. Rev. **2006,** 106, 324–339



Electron micrograph of a Holliday junction that was detected during recombination of plasmid DNAs in *E. coli*. The molecule illustrates a Holliday junction in the open configuration resulting from rotation of the crossed-strand intermediate. [Courtesy of Huntington Potter, University of South Florida, and David Dressler, University of Oxford.

Nucleic Acids Research

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The role of Holliday junction resolvases in the repair of spontaneous and induced DNA damage

Neta Agmon, Moran Yovel, Yaniv Harari, Batia Liefshitz and Martin Kupiec*

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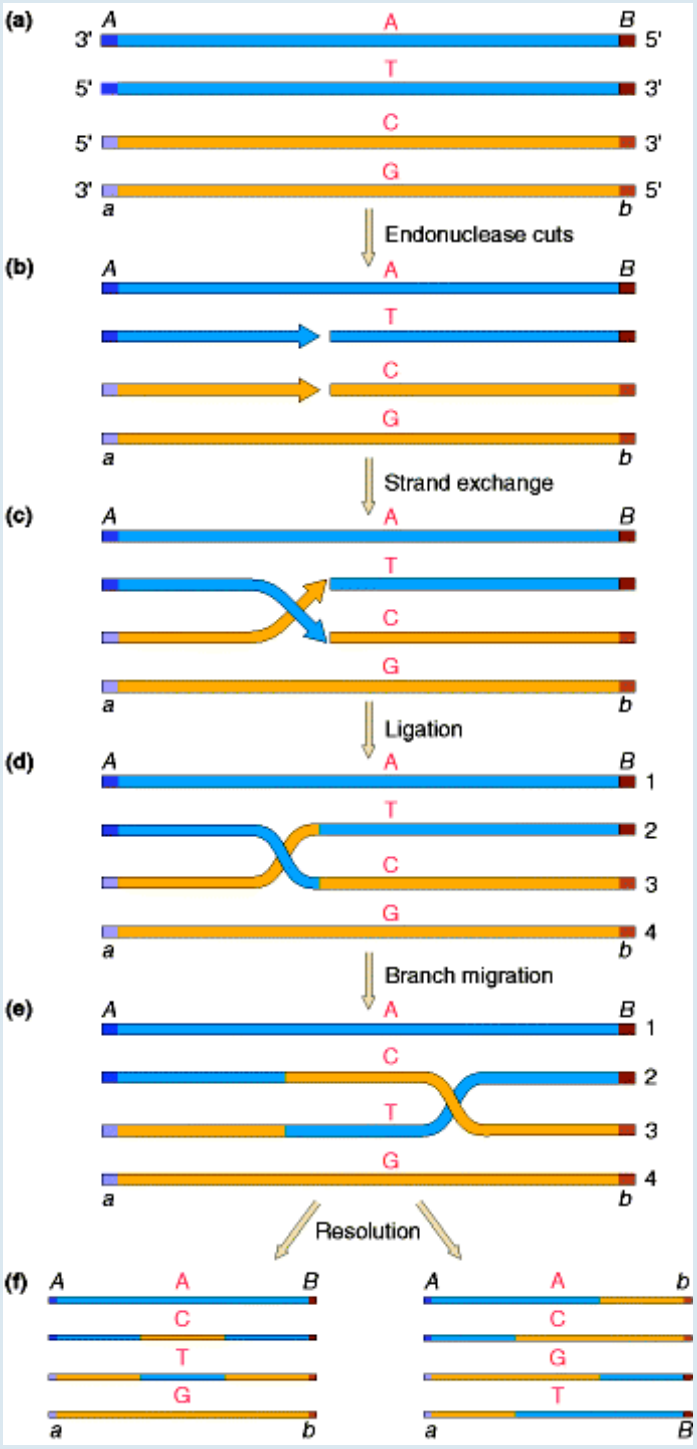
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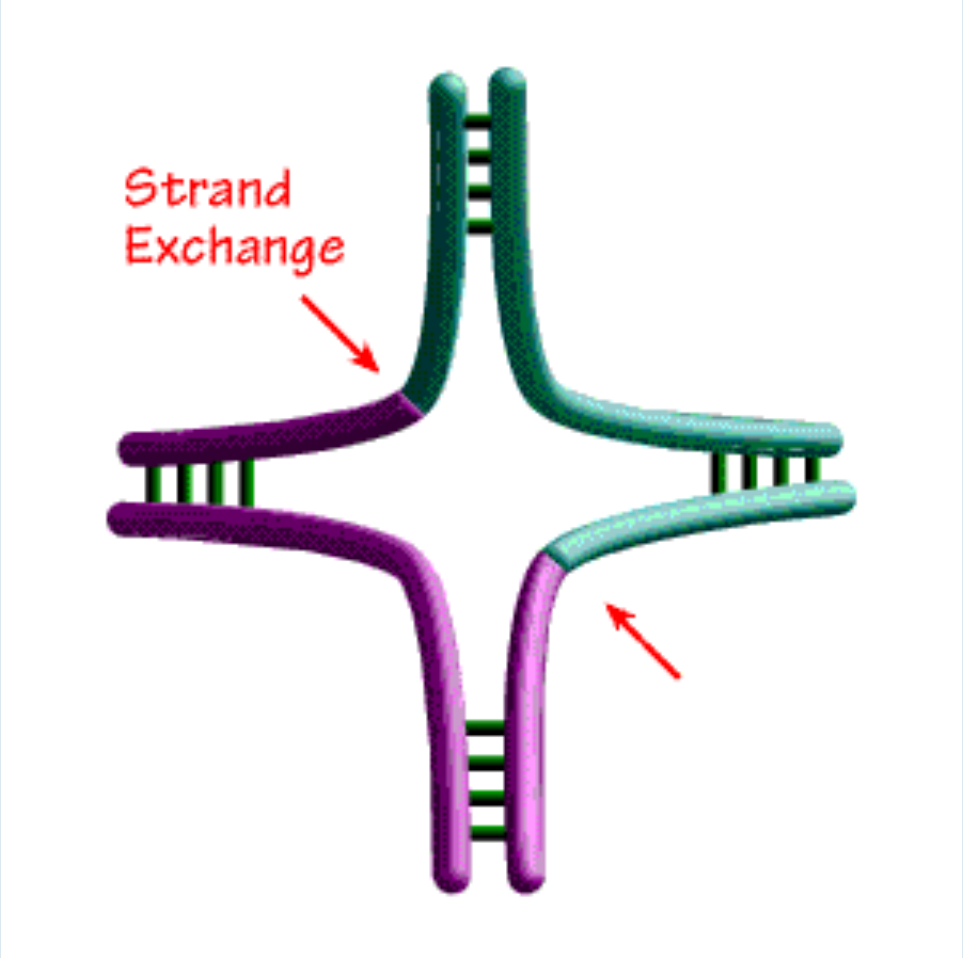
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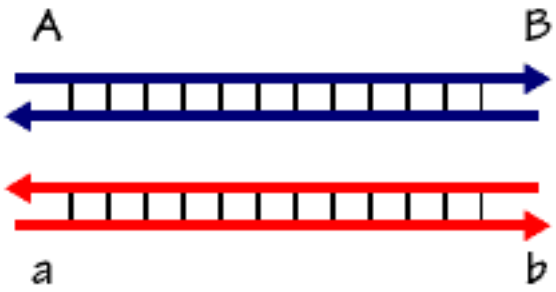
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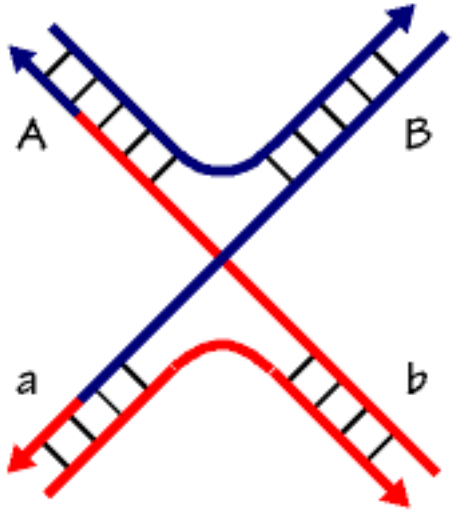
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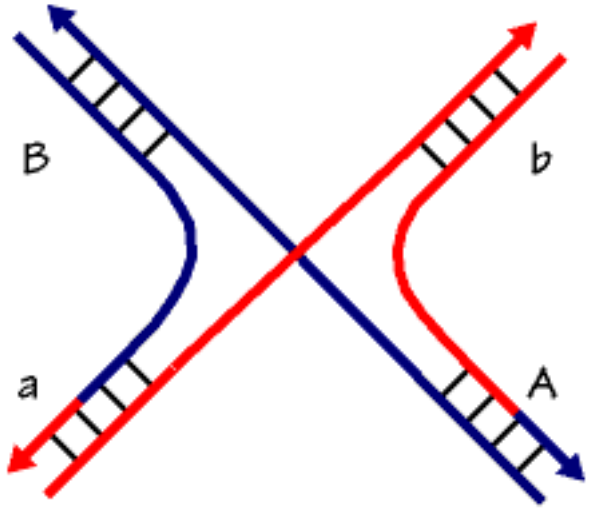
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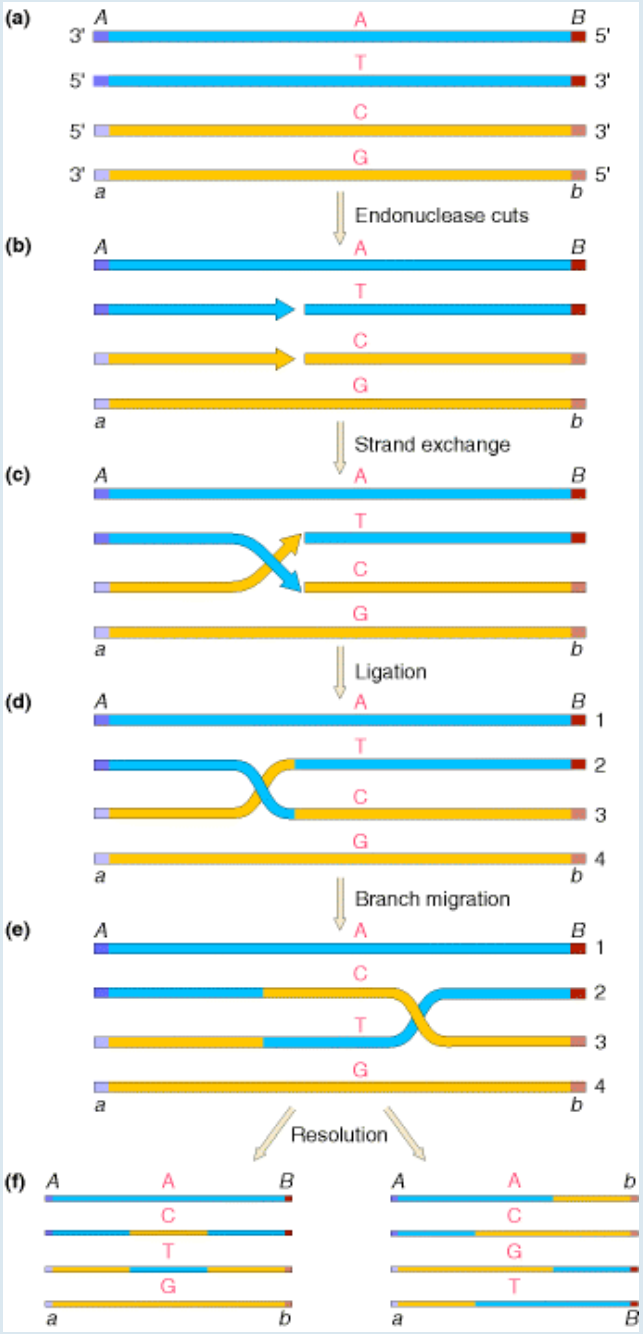
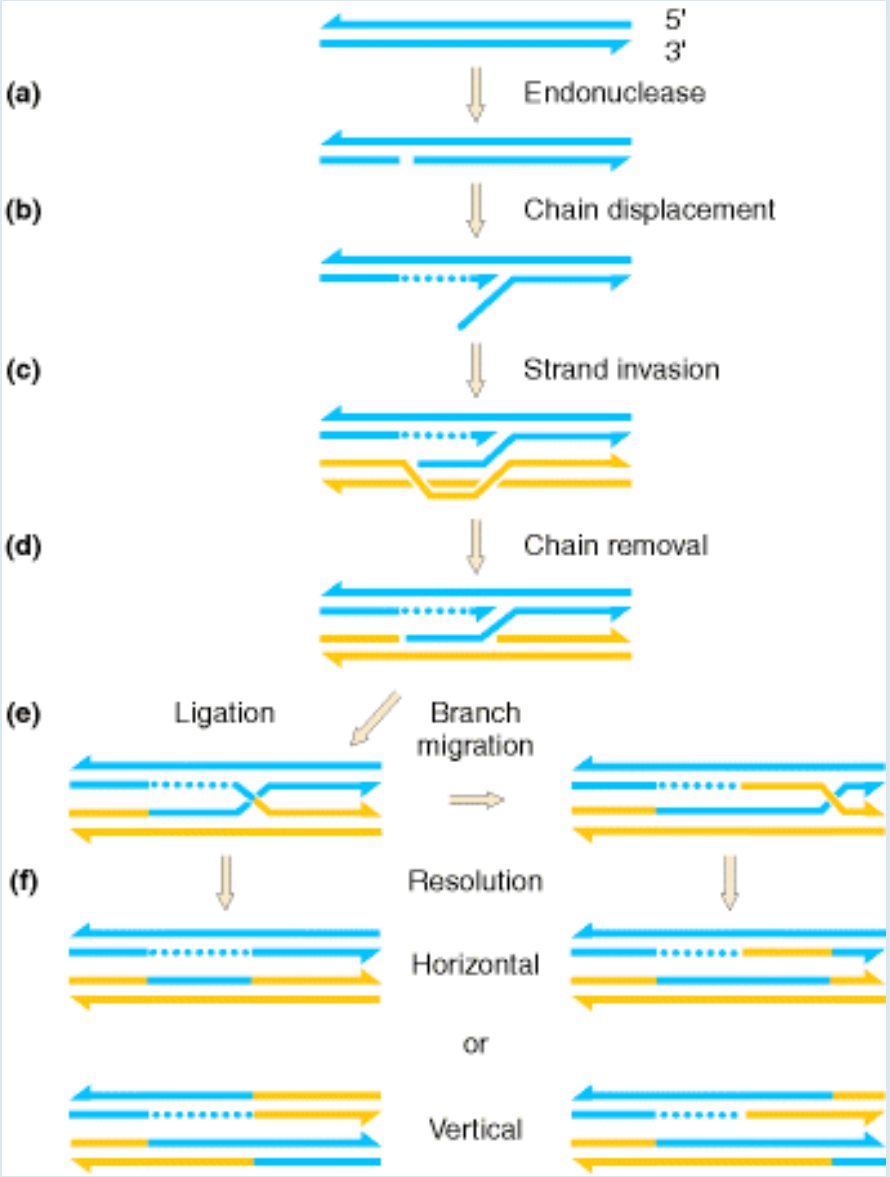
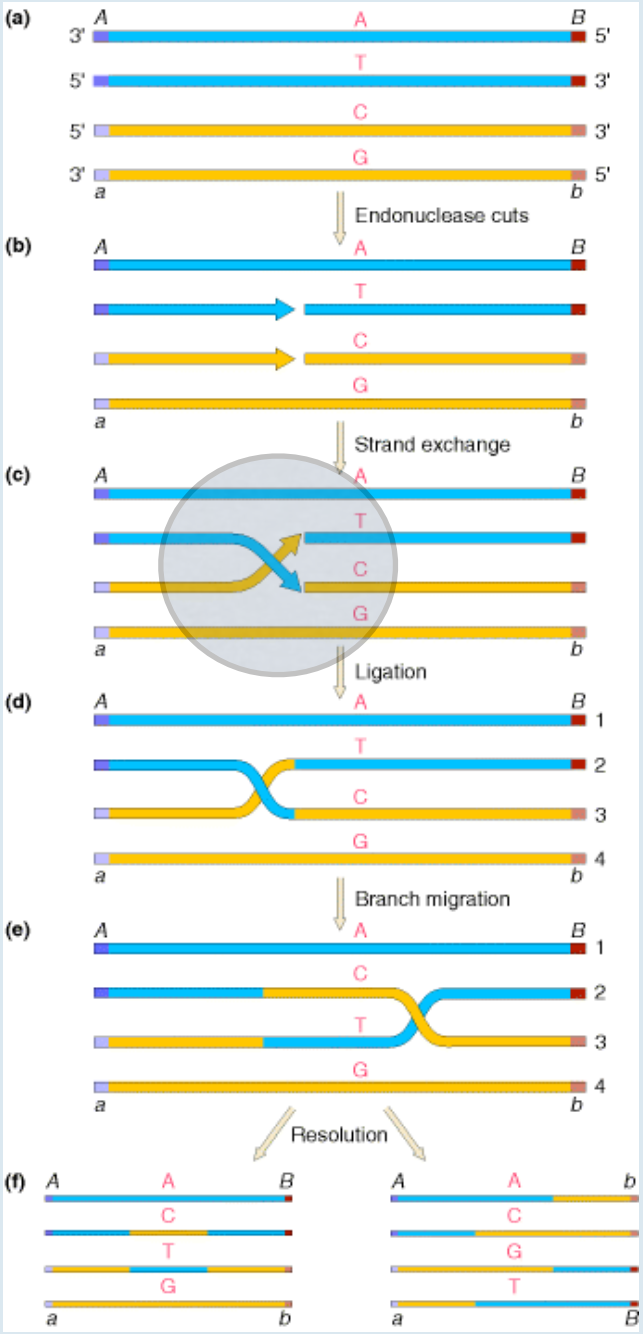


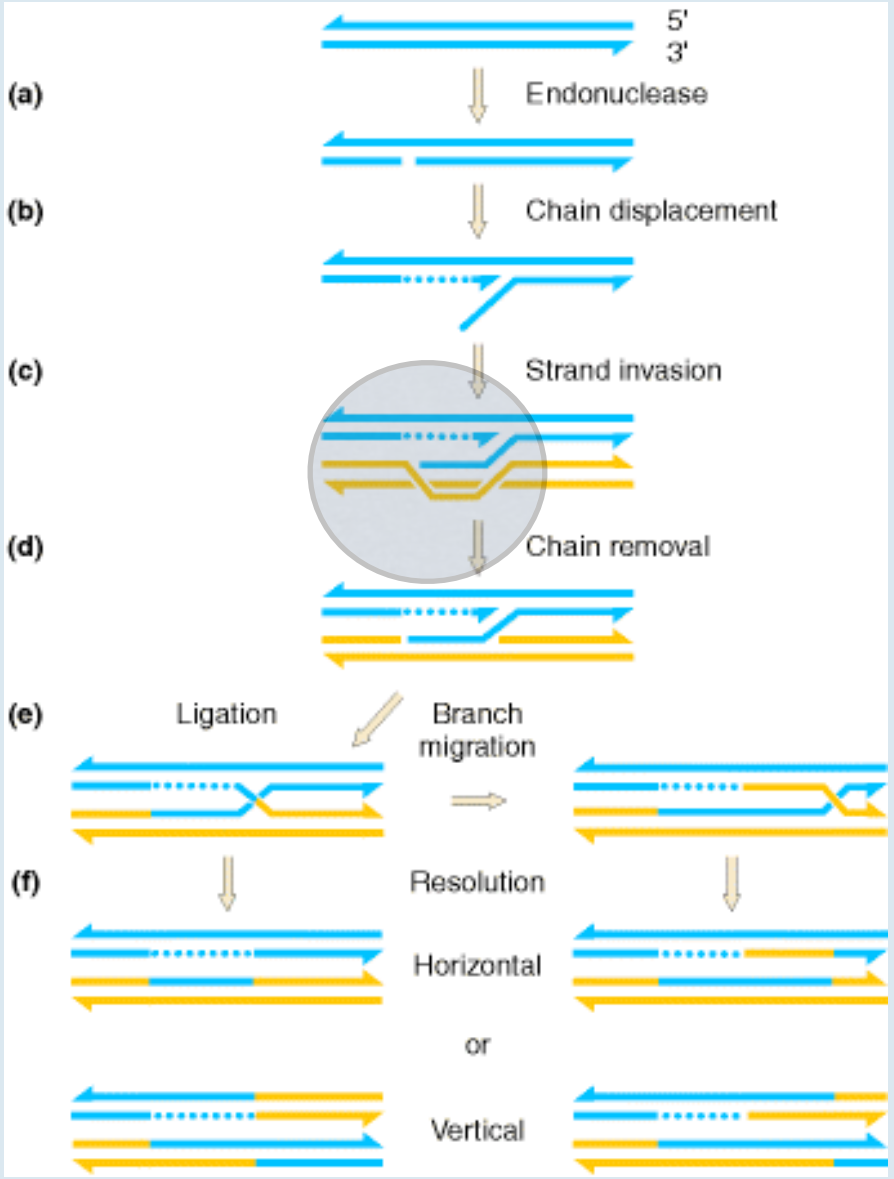
Holliday ("double nick")



Meselson-Radding ("single nick")



Holliday ("double nick")



Meselson-Radding ("single nick")

In both the original **Holliday** and **Meselson-Radding** models for genetic recombination, the initiation events for recombination are **single-strand nicks** that result in the generation of **heteroduplex DNA**.

However, the finding in yeast was that transformation is stimulated **1,000-fold** when a **double-strand break** is introduced into a circular donor plasmid

.... giving rise to an additional model, the **double-strand-break DSB model**, originally formulated by **Jack Szostak, Terry Orr-Weaver, and Rodney Rothstein.**

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Review

The double-strand-break repair model for recombination

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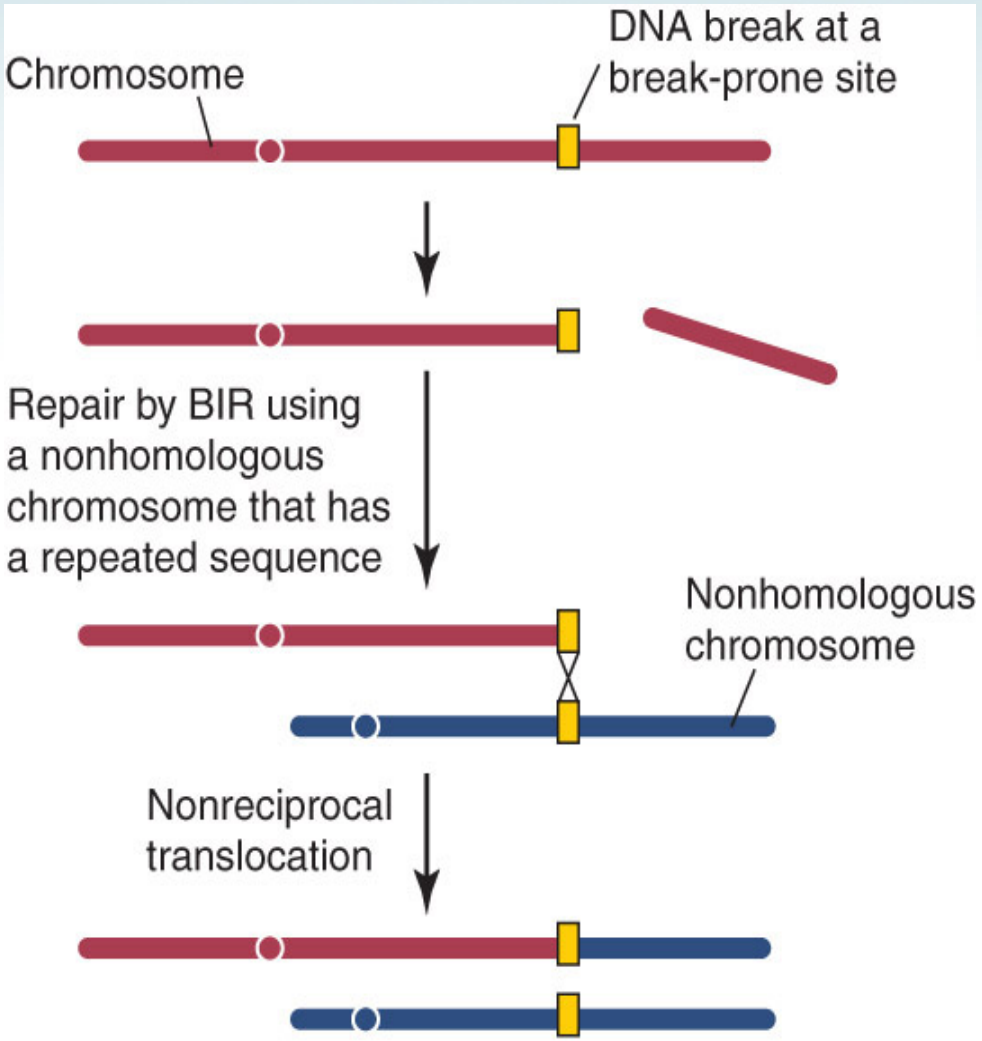
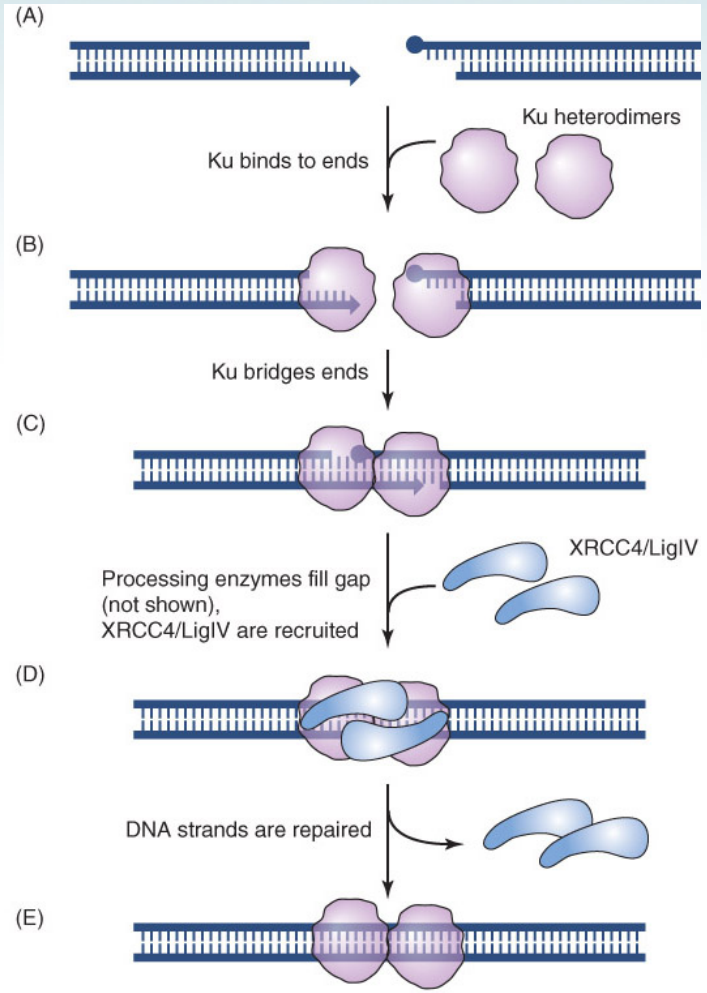
Abstract

Gene conversion is the nonreciprocal transfer of information from one DNA duplex to another; in meiosis, it is frequently associated with crossing-over. We review the genetic properties of meiotic recombination and previous models of conversion and crossing-over. In these models, recombination is initiated by single-strand nicks, and heteroduplex DNA is generated. Gene conversion is explained by the repair of mismatches present in heteroduplex DNA. We propose a new mechanism for meiotic recombination, in which events are initiated by double-strand breaks that are enlarged to double-strand gaps. Gene conversion can then occur by the repair of a double-strand gap, and postmeiotic segregation can result from heteroduplex DNA formed at the boundaries of the gap-repair region. The repair of double-strand gaps is an efficient process in yeast, and is known to be associated with crossing-over. The genetic implications of the double-strand-break repair model are explored.



Break Induced Replication (BIR) initiates translocations



Break Induced Replication (BIR) promotes Non-Homologous “end-joining” involves Ku proteins… which force interactions

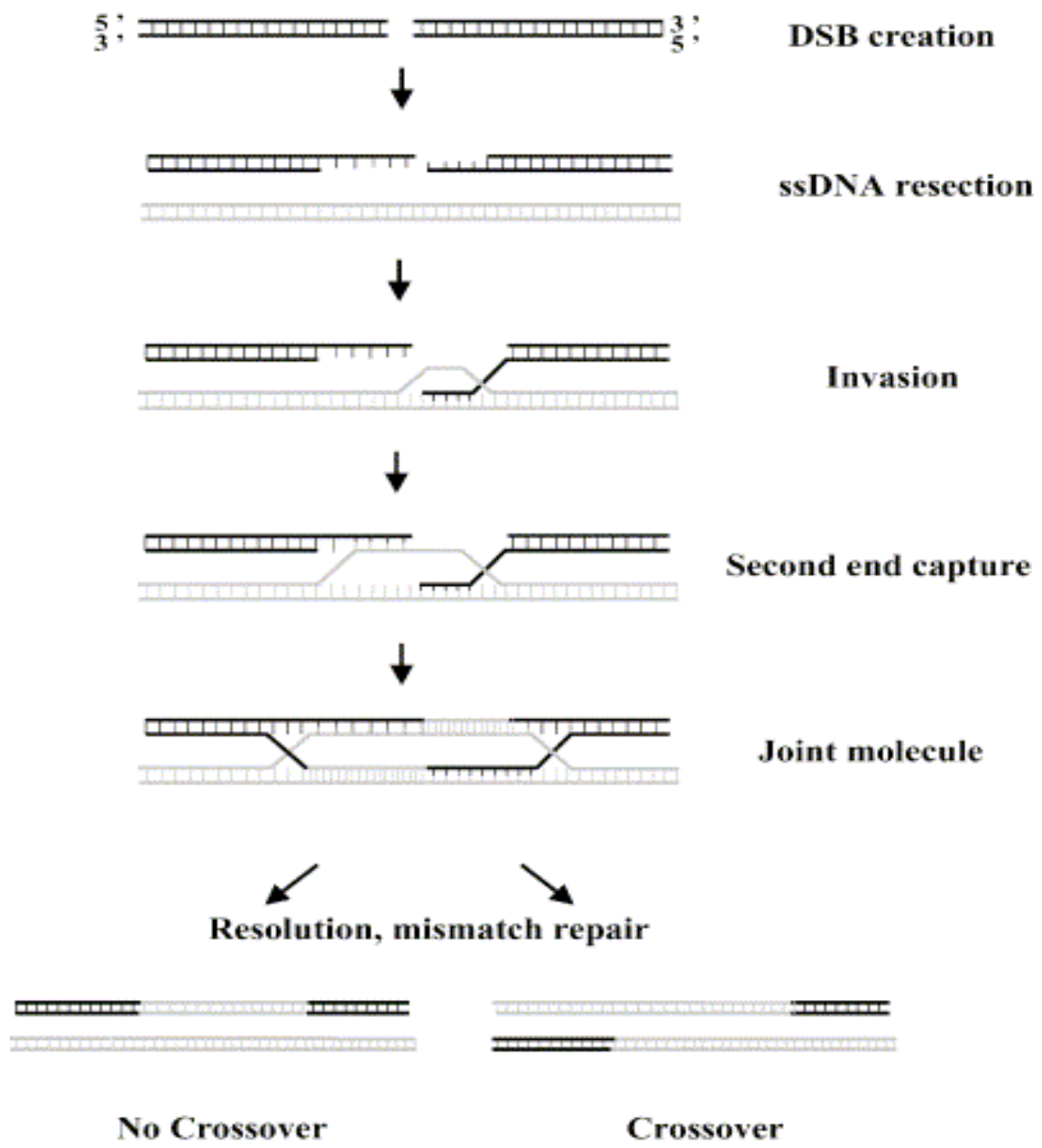
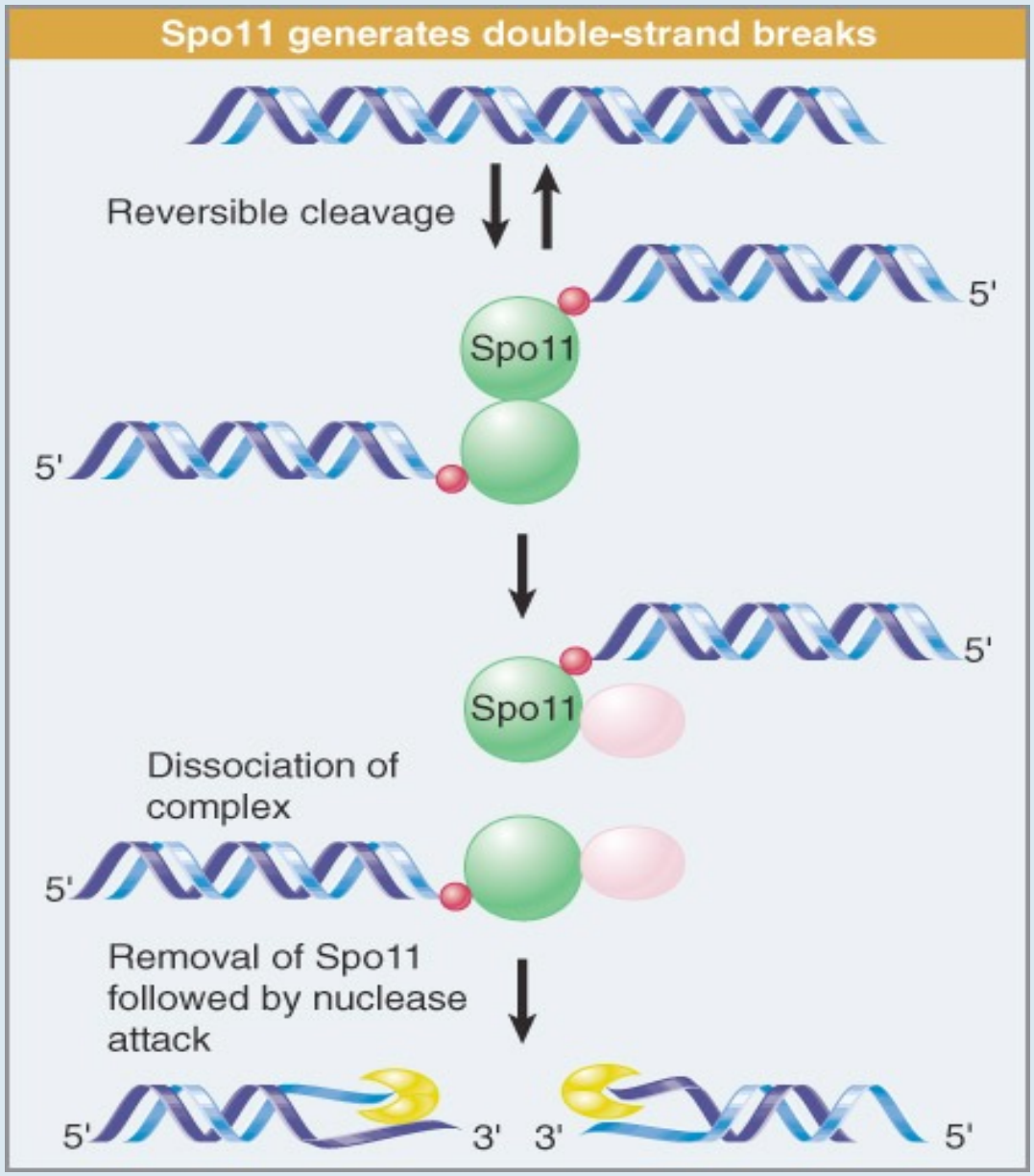
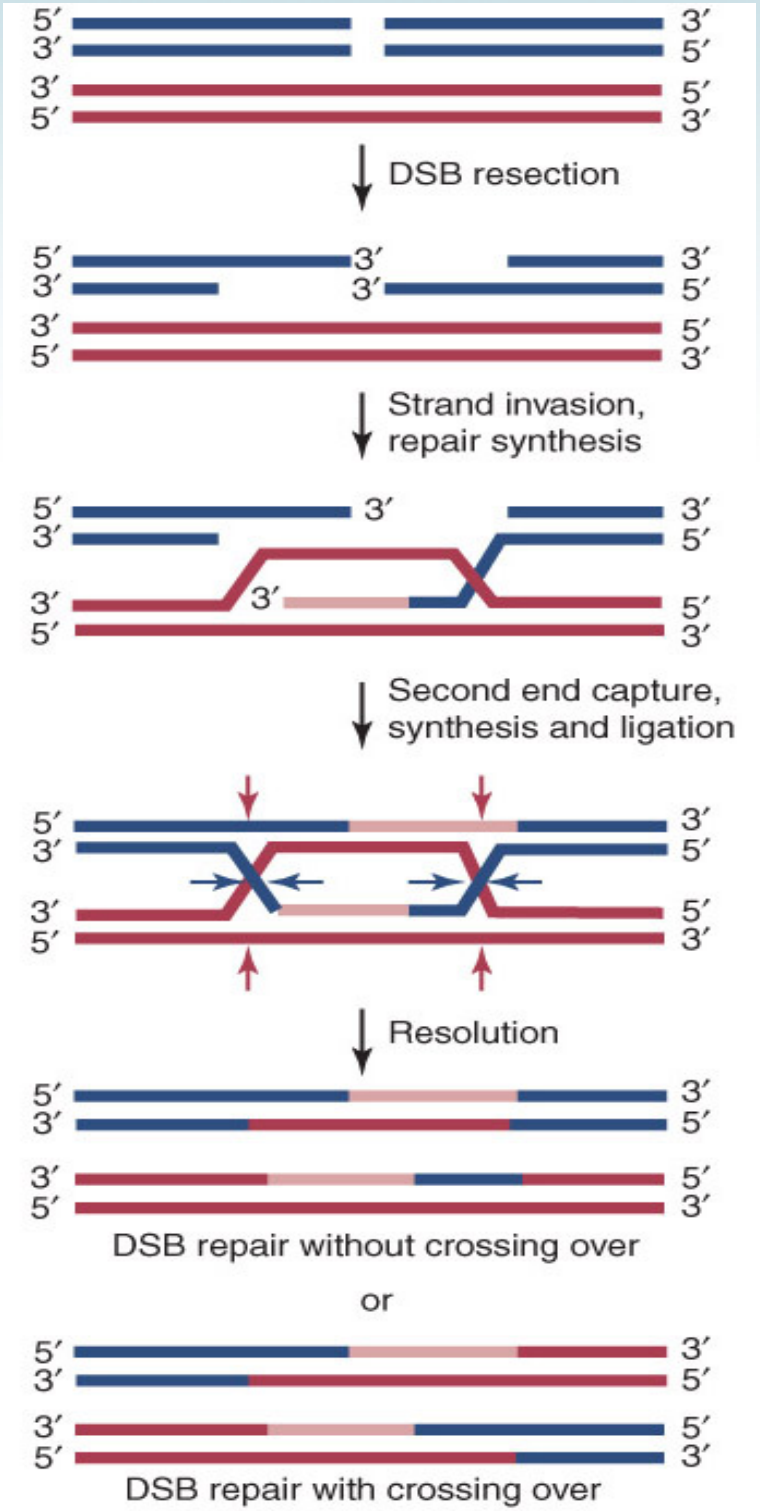


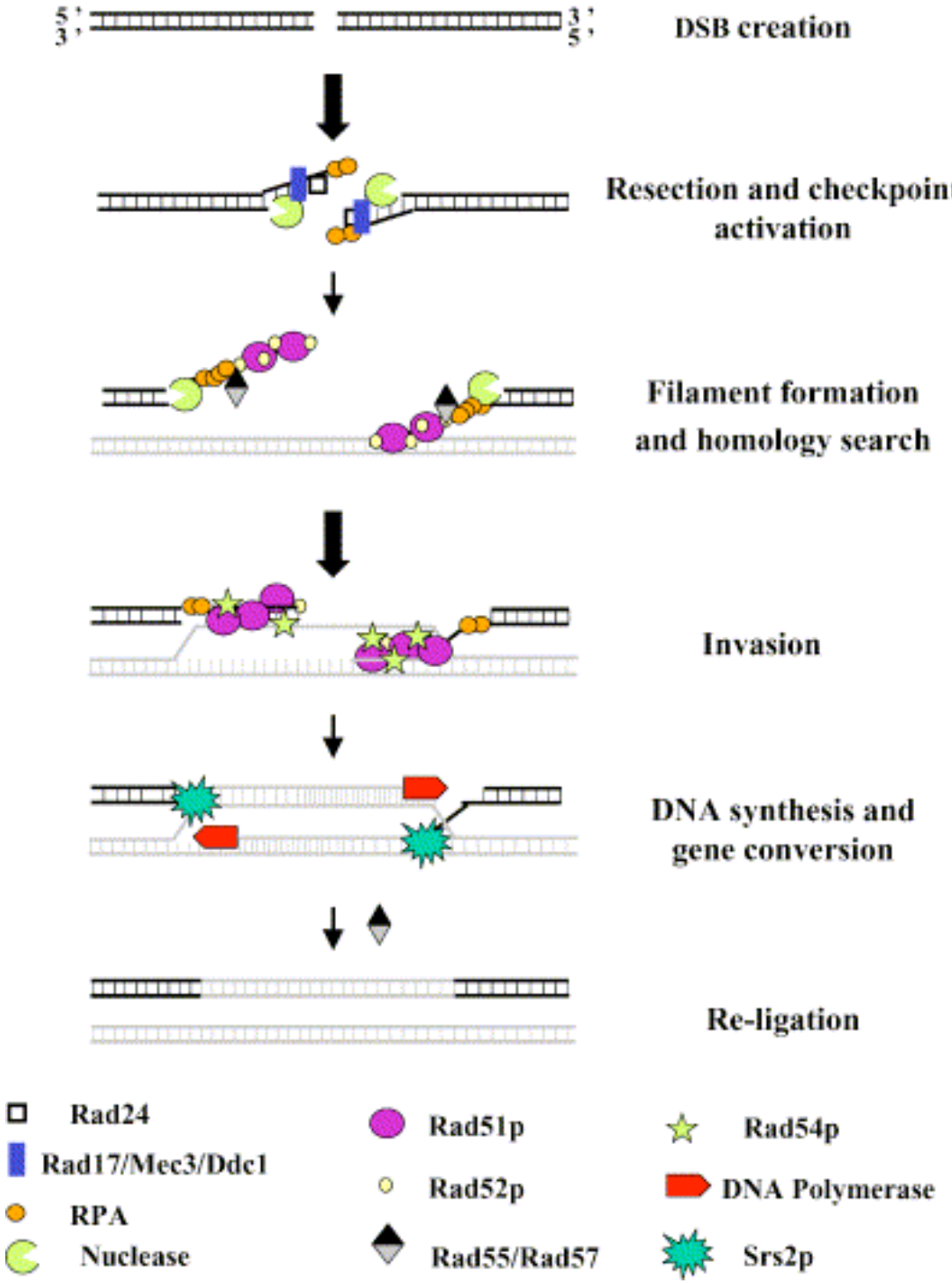
Fig. 1. Current DSB-initiated model of homologous recombination (modified from [9]).



Spo11 and other endonucleases generates double-strand breaks.



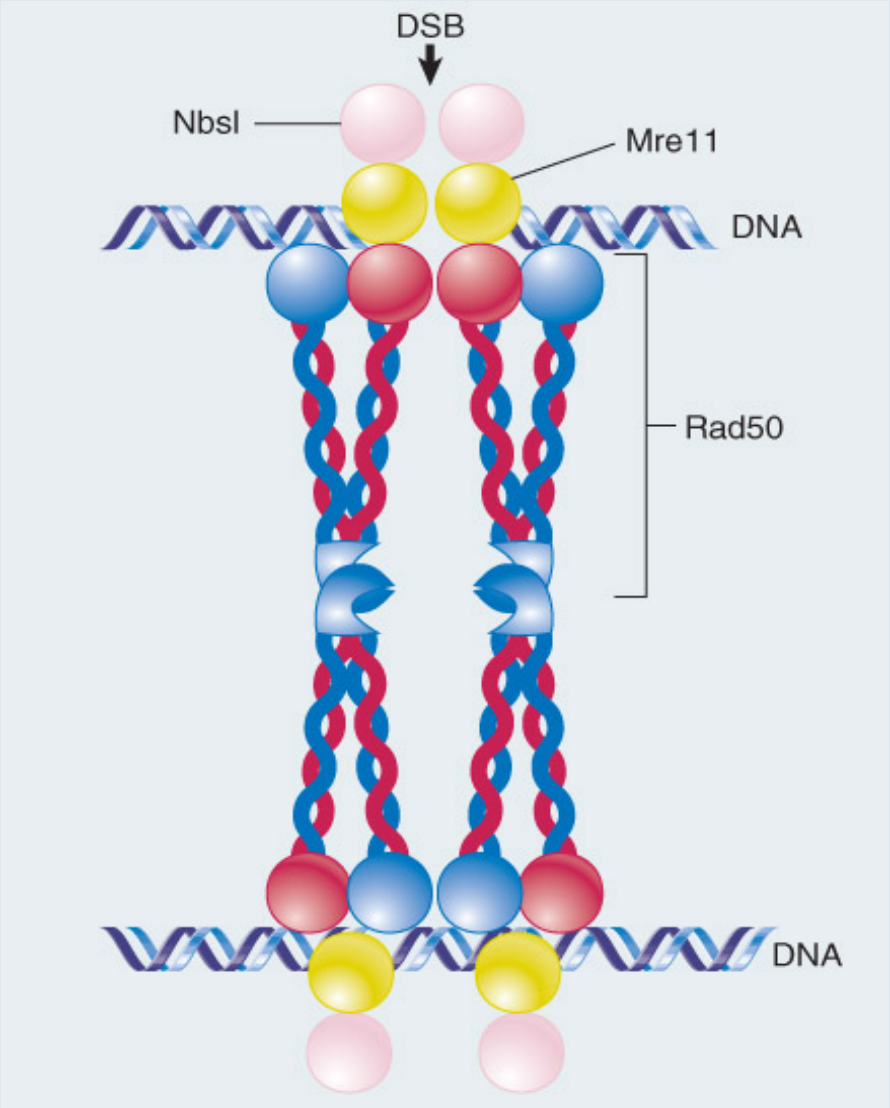
Double-strand break-repair model of homologous recombination.



- The RecA homologue, **Rad51,** forms a nucleoprotein filament on the single-stranded regions, assisted by **Rad52** and **Rad55/57.**

- **Rad54** and **Rdh54/Rad54B** are involved in homology search and strand invasion.

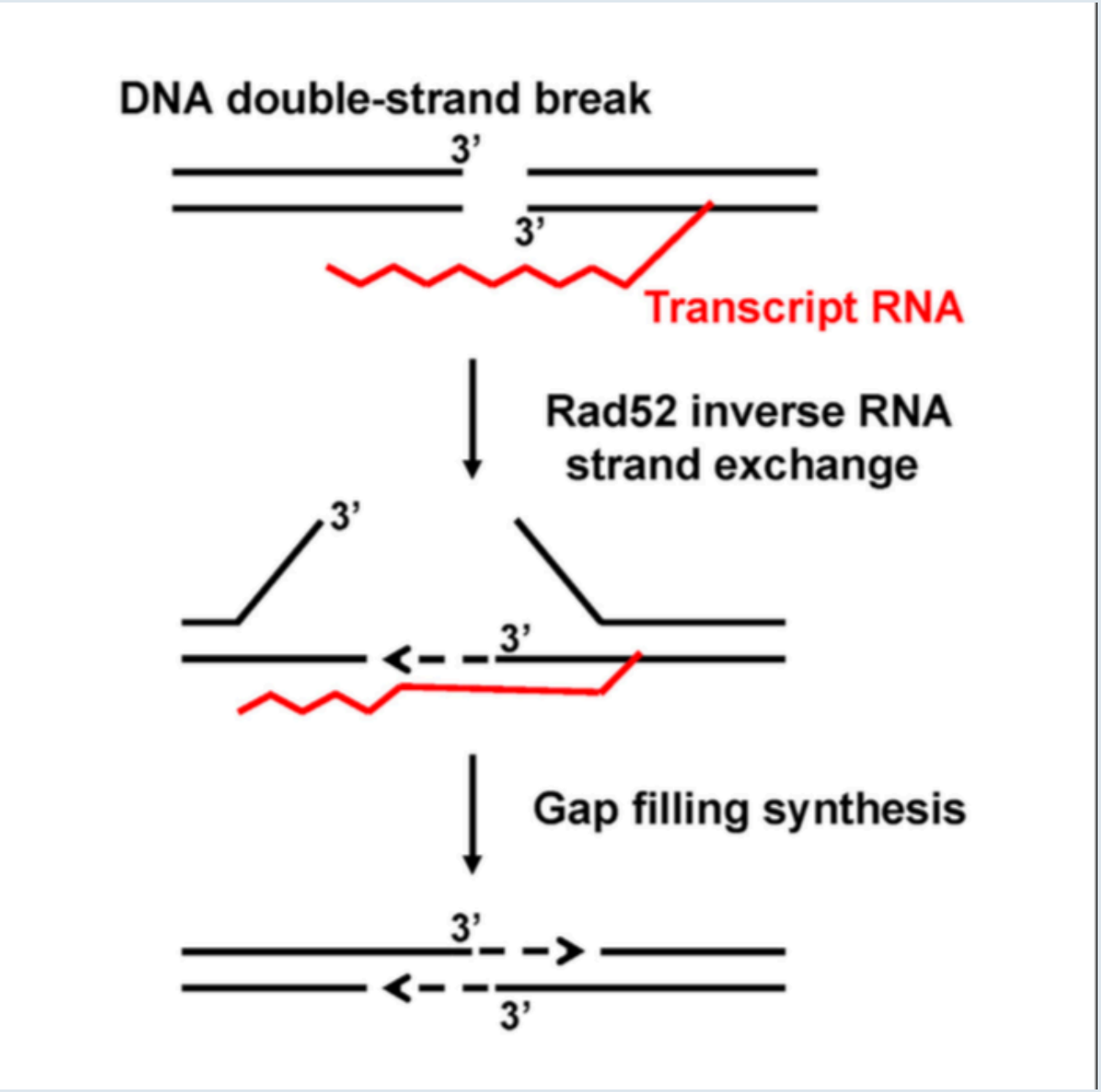
- The yeast *RAD* mutations were identified by radiation-sensitive phenotypes and are in genes that code for repair systems.



The MRN complex, required for 5' end resection, also serves as a DNA bridge to prevent broken ends from separating.

- The **RecA homologue Rad51** forms a nucleoprotein filament on the single-stranded regions, assisted by **Rad52** and **Rad55/57.**
- The ***RAD52*** assembly of genes (which includes **Rad50)** is required for recombination repair, and is intergral to the formation of synaptonemal complexes in yeast
- The **MRX** (yeast) or **MRN** (mammals) complex is required to form a single-stranded region at each DNA end.

Rad52 Inverse Strand Exchange Drives RNA-Templated DNA Double-Strand Break Repair



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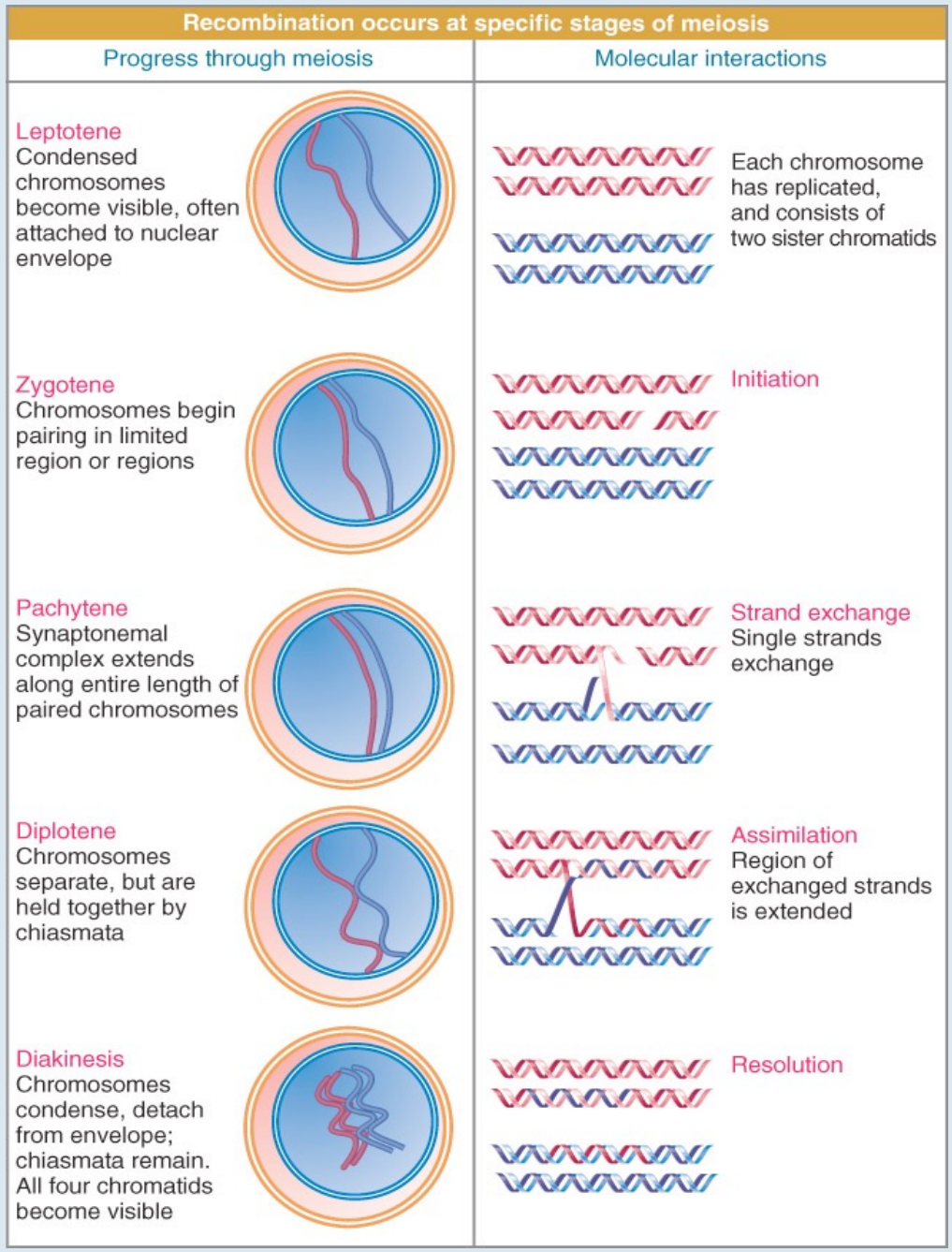
Mitotic vs. Meiotic recombination?



Homologous recombination is a reaction between two duplexes of DNA. Its critical feature is that the enzymes responsible can use any pair of homologous sequences as substrates (although some types of sequences may be favored over others). The frequency of recombination is not constant throughout the genome, but is influenced by both global and local effects.

MOLECULAR AND CELLULAR BIOLOGY, Mar. 2007, p. 1868–1880 Vol. 27, No. 5
0270-7306/07/$08.00+0 doi:10.1128/MCB.02063-06

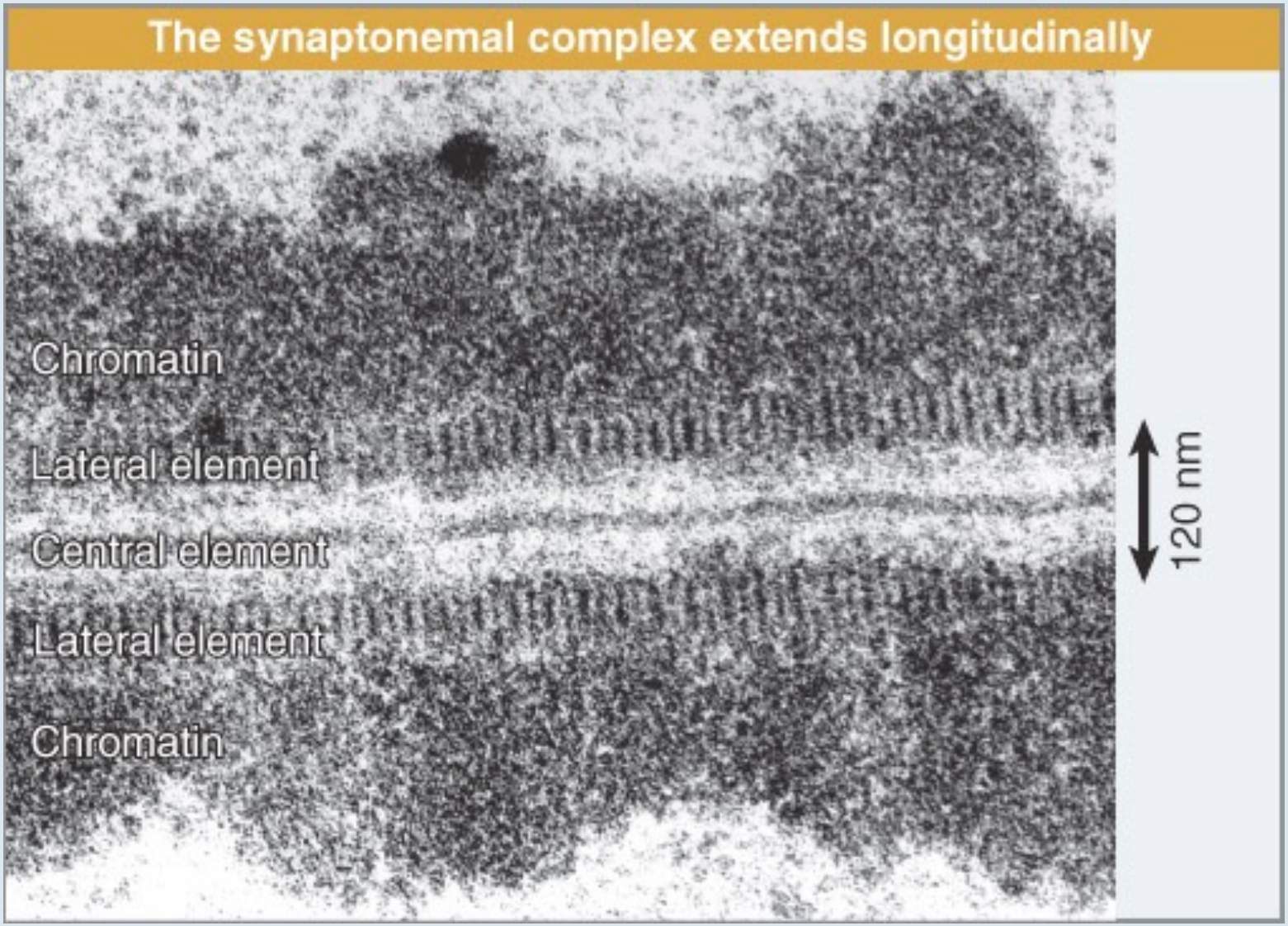
Genome-Wide Redistribution of Meiotic Double-Strand Breaks in *Saccharomyces cerevisiae*▽†

Nicolas Robine,[1,2] Norio Uematsu,[1]‡ Franck Amiot,[3] Xavier Gidrol,[3] Emmanuel Barillot,[2] Alain Nicolas,[1] and Valérie Borde[1]*

Institut Curie, Recombinaison et Instabilité Génétique, Centre de Recherche, UMR7147 CNRS-Institut Curie-Université P. et M. Curie, 26 rue d'Ulm, 75248 Paris Cedex 05, France[1]; Institut Curie, Service de Bioinformatique, 26 rue d'Ulm, 75248 Paris Cedex 05, France[2]; and Service de Génomique Fonctionnelle, CEA, 2 rue Gaston Crémieux, CP5722, 91057 Evry Cedex, France[3]

Received 3 November 2006/Returned for modification 30 November 2006/Accepted 12 December 2006

Meiotic recombination is initiated by the formation of programmed DNA double-strand breaks (DSBs) catalyzed by the Spo11 protein. DSBs are not randomly distributed along chromosomes. To better understand factors that control the distribution of DSBs in budding yeast, we have examined the genome-wide binding and cleavage properties of the Gal4 DNA binding domain (Gal4BD)-Spo11 fusion protein. We found that Gal4BD-Spo11 cleaves only a subset of its binding sites, indicating that the association of Spo11 with chromatin is not sufficient for DSB formation. In centromere-associated regions, the centromere itself prevents DSB cleavage by tethered Gal4BD-Spo11 since its displacement restores targeted DSB formation. In addition, we observed that new DSBs introduced by Gal4BD-Spo11 inhibit surrounding DSB formation over long distances (up to 60 kb), keeping constant the number of DSBs per chromosomal region. Together, these results demonstrate that the targeting of Spo11 to new chromosomal locations leads to both local stimulation and genome-wide redistribution of recombination initiation and that some chromosomal regions are inherently cold regardless of the presence of Spo11.



Reproduced from D. von Wettstein. 1971. Proc. Natl. Acad. Sci. USA. 68: 851-855.
Photo courtesy of D. von Wettstein, Washington State University

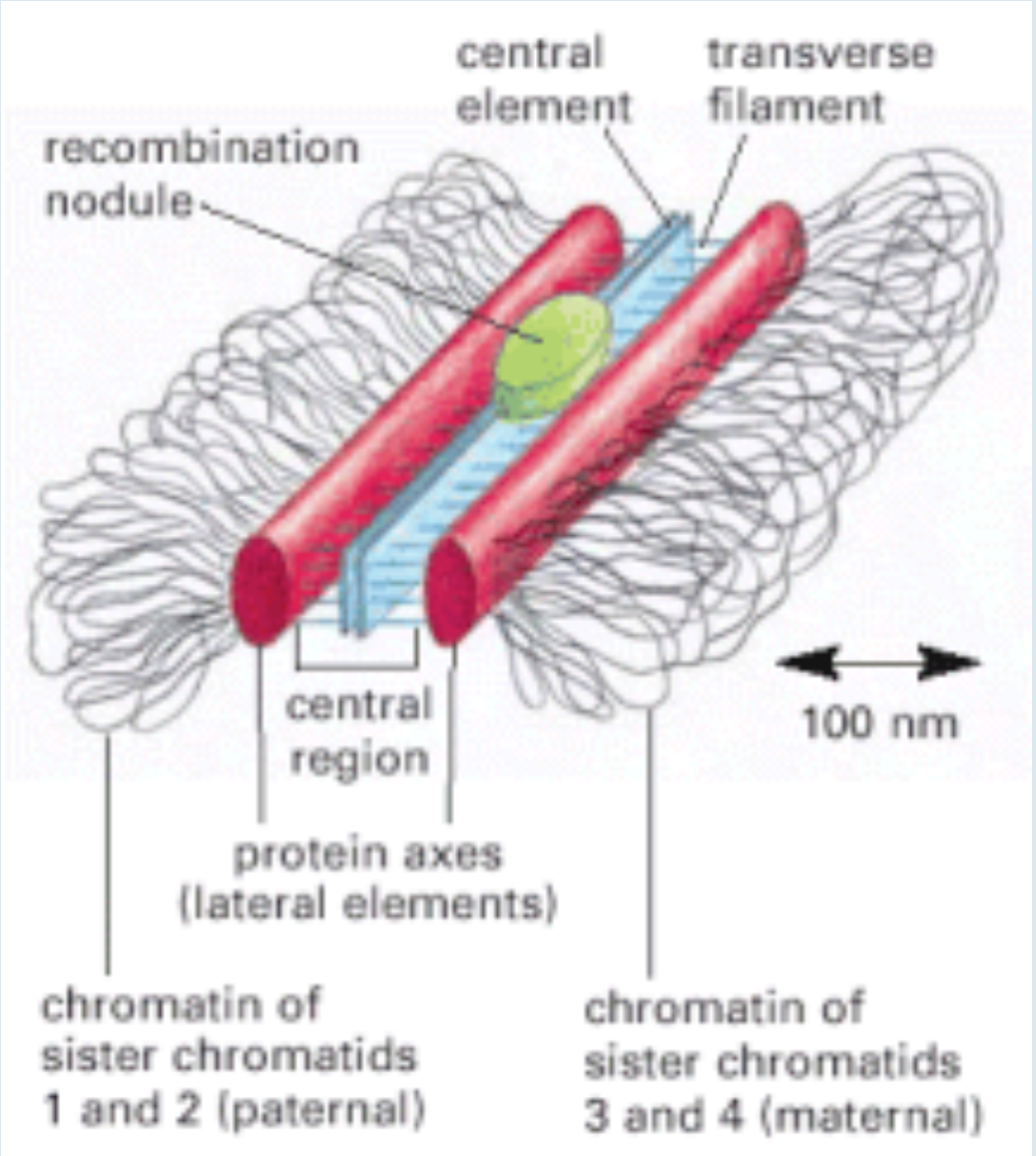


Figure 20–13. A mature synaptonemal complex. Only a short section of the long ladderlike complex is shown. A similar synaptonemal complex is present in organisms as diverse as yeasts and humans

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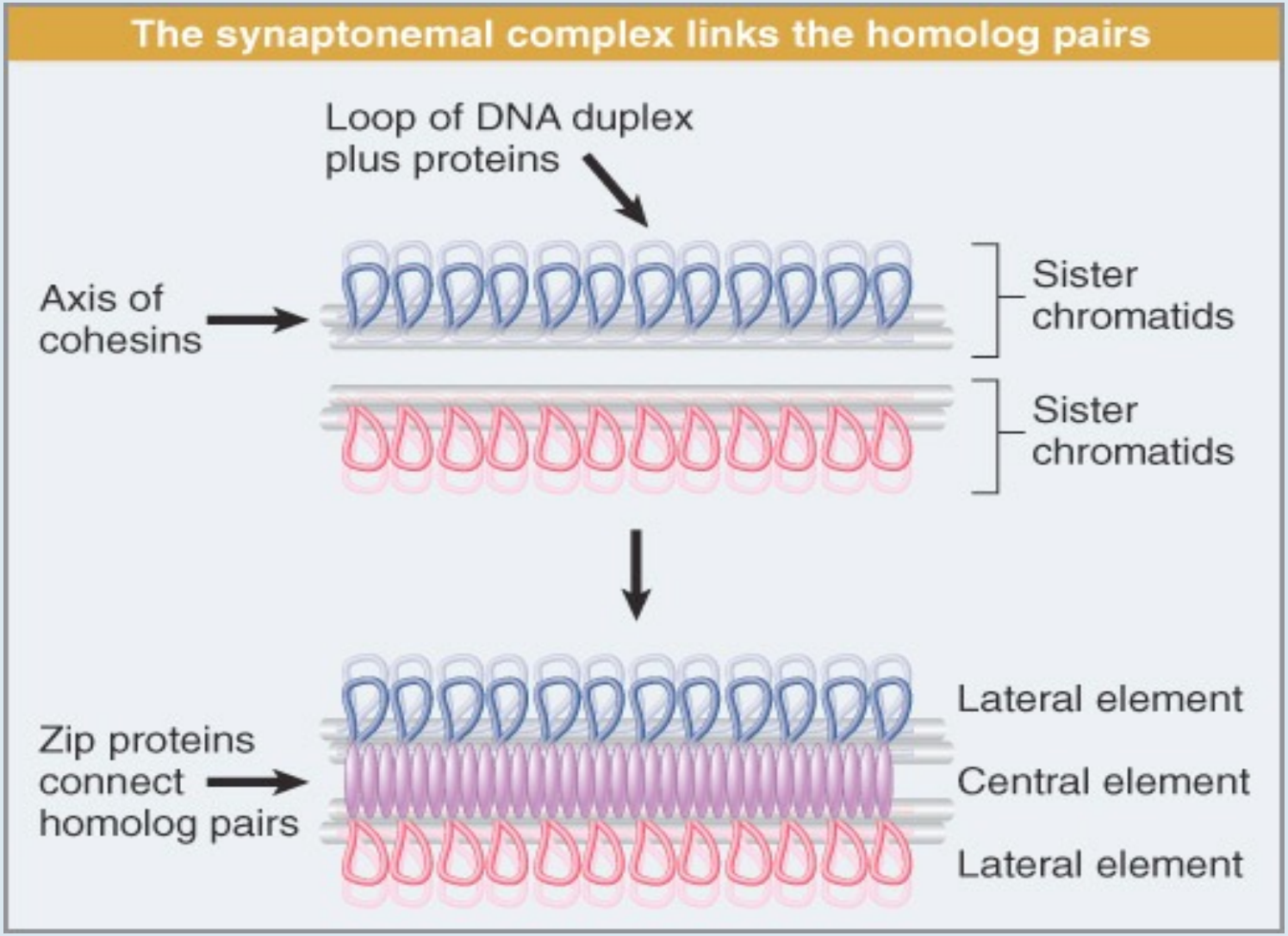

Synapsis begins when each chromosome (sister chromatid pair) condenses around a structure called the **axial element**, which is apparently proteinaceous. Then the axial elements of corresponding chromosomes become aligned, and the synaptonemal complex forms as a tripartite structure, in which the axial elements, now called **lateral elements**, are separated from each other by a **central element**. Figure 15.9 shows an example.

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doi: 10.1007/978-1-4939-0888-2_11

The roles of cohesins in mitosis, meiosis, and human health and disease

Amanda S. Brooker and Karen M. Berkowitz

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Summary

Go to:

Mitosis and meiosis are essential processes that occur during development. Throughout these processes, cohesion is required to keep the sister chromatids together until their separation at anaphase. Cohesion is created by multi-protein subunit complexes called cohesins. Although the subunits differ slightly in mitosis and meiosis, the canonical cohesin complex is composed of four subunits that are quite diverse. The cohesin complexes are also important for DNA repair, gene expression, development, and genome integrity. Here we provide an overview of the roles of cohesins during these different events, as well as their roles in human health and disease, including the cohesinopathies. Although the exact roles and mechanisms of these proteins are still being elucidated, this review will serve as a guide for the current knowledge of cohesins.

Keywords: cohesin, mitosis, meiosis, sister chromatid cohesion, cell cycle, chromosome segregation, aneuploidy, human health, cohesinopathies, maternal age effect

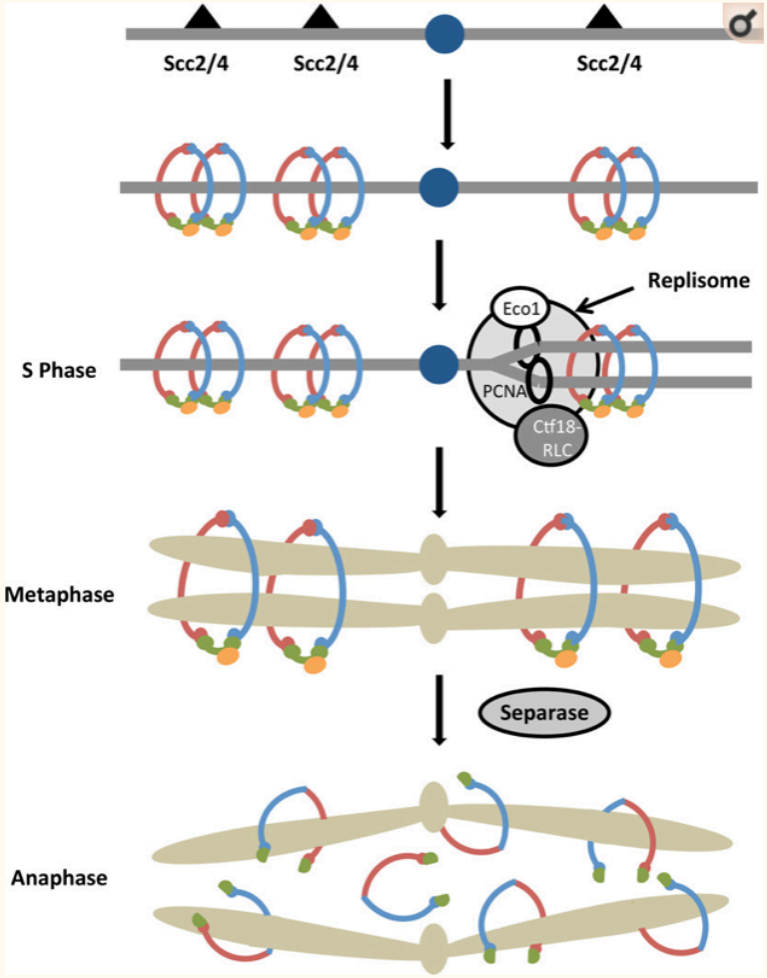

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Figure 2

Cohesion in yeast mitosis. Cohesin complexes require the Scc2/Scc4 protein complex in order to be loaded on chromosomes. Several proteins act together to establish cohesion during DNA replication. These proteins include Eco1 acetyltransferase, the CTF18-RLC complex, and the polymerase-associated protein Ctf4. Tension at centromeres is generated by the bipolar attachment of kinetochores to the mitotic spindle. Following biorientation of sister chromatids, separase is activated to cleave the Scc1 subunit resulting in removal of cohesin complexes, loss of cohesion, and separation of sister chromatids.

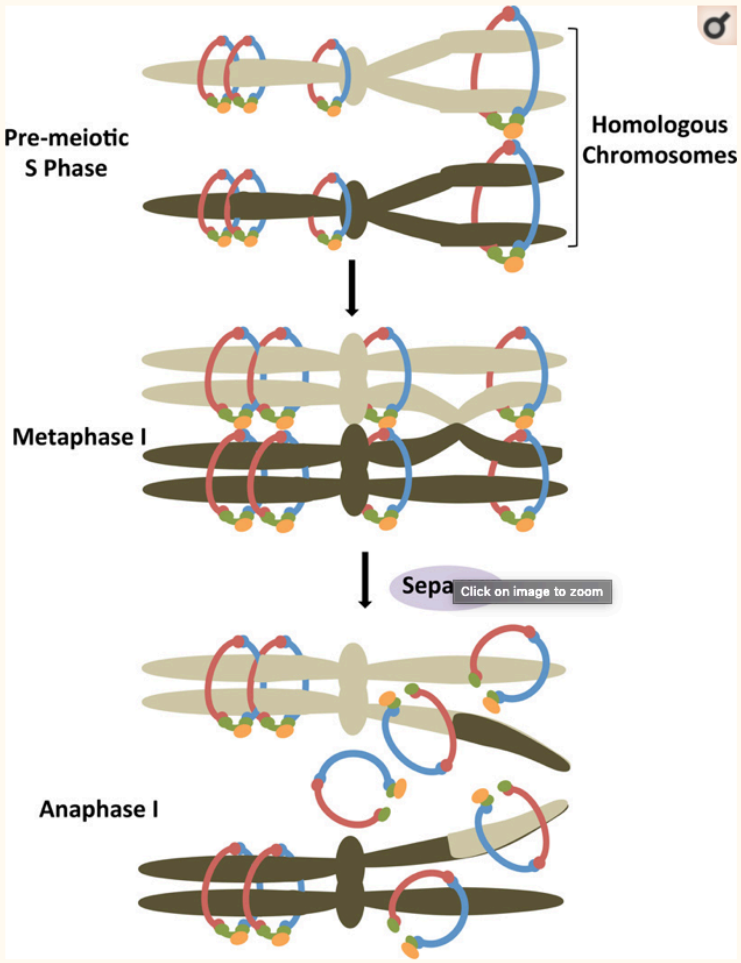

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Figure 4

Cohesion in yeast meiosis I. Rec8 replaces Scc1 of the cohesin complex in S phase. During prophase I homologous chromosomes pair and meiotic recombination leads to DNA crossovers between non-sister chromatids. In order for homologous chromosomes to segregate, kinetochores of sister chromatid pairs must each be mono-oriented to opposite poles during metaphase I. Separase cleavage of Rec8 during anaphase I, much like that during mitosis, resolves the cohesion distal to crossovers to allow segregation of homologues. In order to allow for the proper biorientation and segregation of sister chromatids during meiosis II, cohesion proximal to centromeres is preserved.

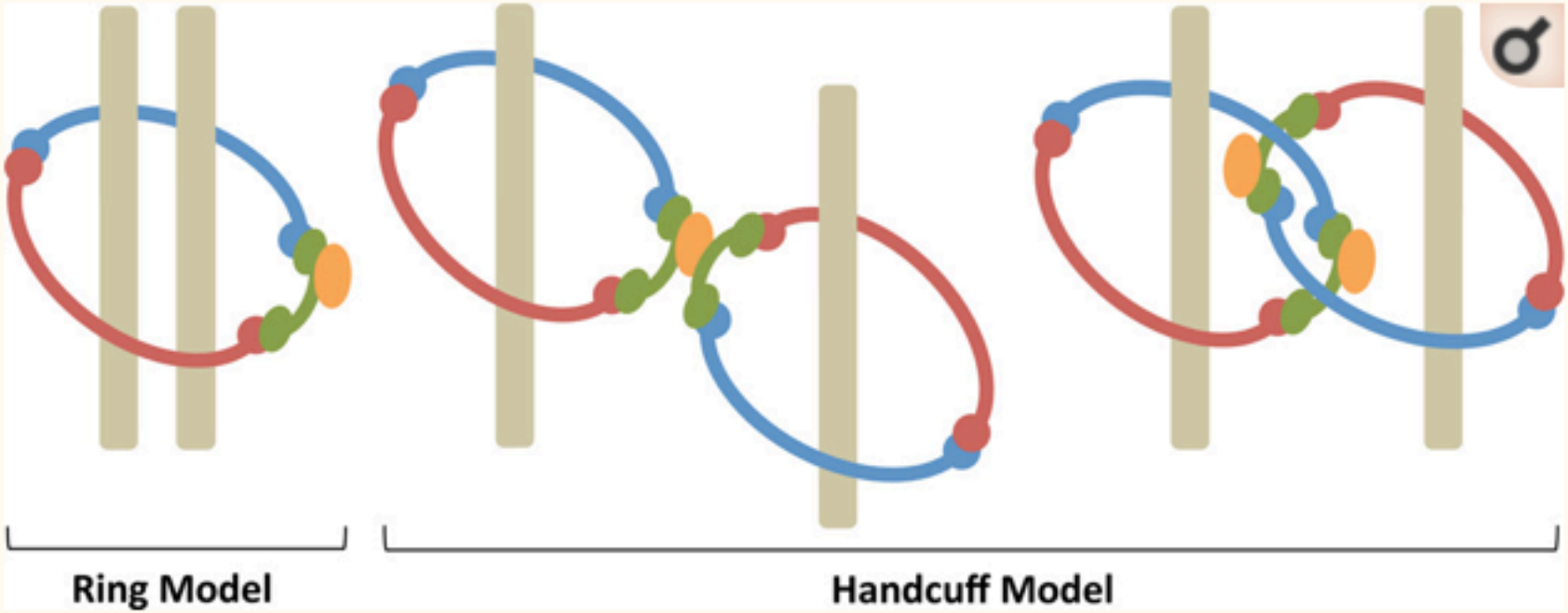

Figure 3

Models of cohesin rings. **(A)** One ring model predicts that both sister chromatids are entrapped within a single cohesin ring. **(B)** Another type of ring model, the "handcuff" model, proposes that each of two cohesin rings entraps one sister chromatid, either by binding a single Scc3 subunit or topological interconnection between rings.

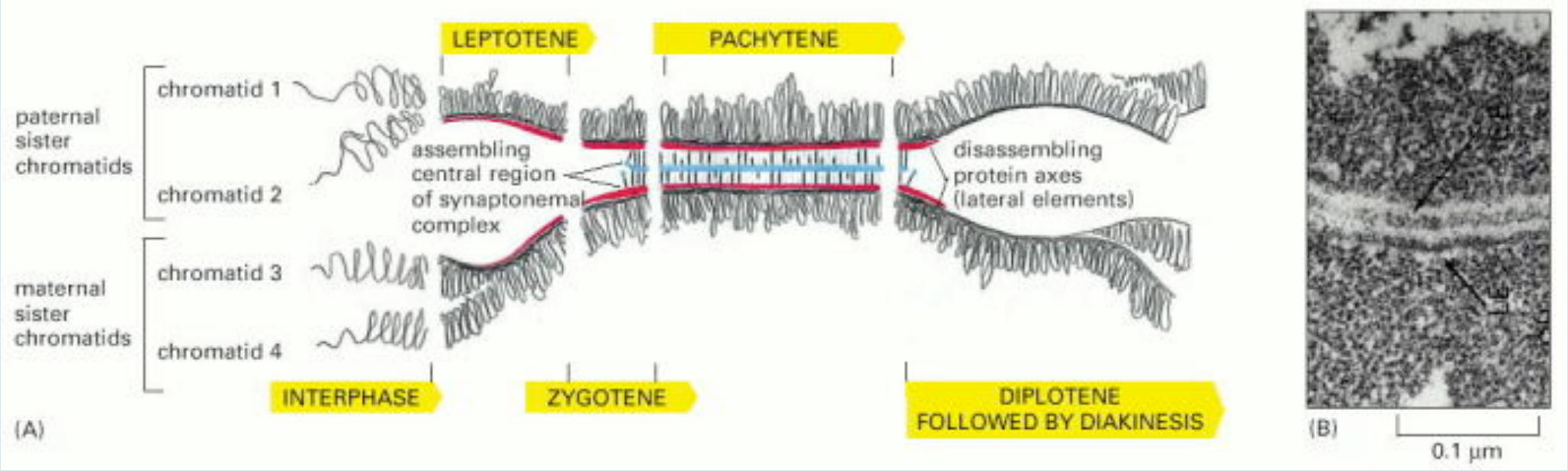

Figure 20–12. Chromosome synapsis and desynapsis during the different stages of meiotic prophase I. (A) A single bivalent is shown. The pachytene stage is defined as the period during which a fully formed synaptonemal complex exists. At leptotene, the two sister chromatids condense, and their chromatin loops each extend from a common protein axis (red). As meiosis progresses, the two homologs become tightly connected by proteins that form the central region of the synaptonemal complex, composed of a central element (blue), transverse filaments (thin black lines), and the lateral elements (red) that anchor the chromatin loops. In the gametes of many female animals, but not those of mammals, the subsequent diplotene stage is an enormously prolonged period of cell growth, during which the chromosomes are decondensed and very active in transcription. Diplotene ends with diakinesis—the stage of transition to metaphase—in which the chromosomes recondense and transcription halts. In male gametes, diplotene and diakinesis are briefer and less distinct. (B) An electron micrograph of a synaptonemal complex from a meiotic cell at pachytene in a lily flower. (B, courtesy of Brian Wells.)

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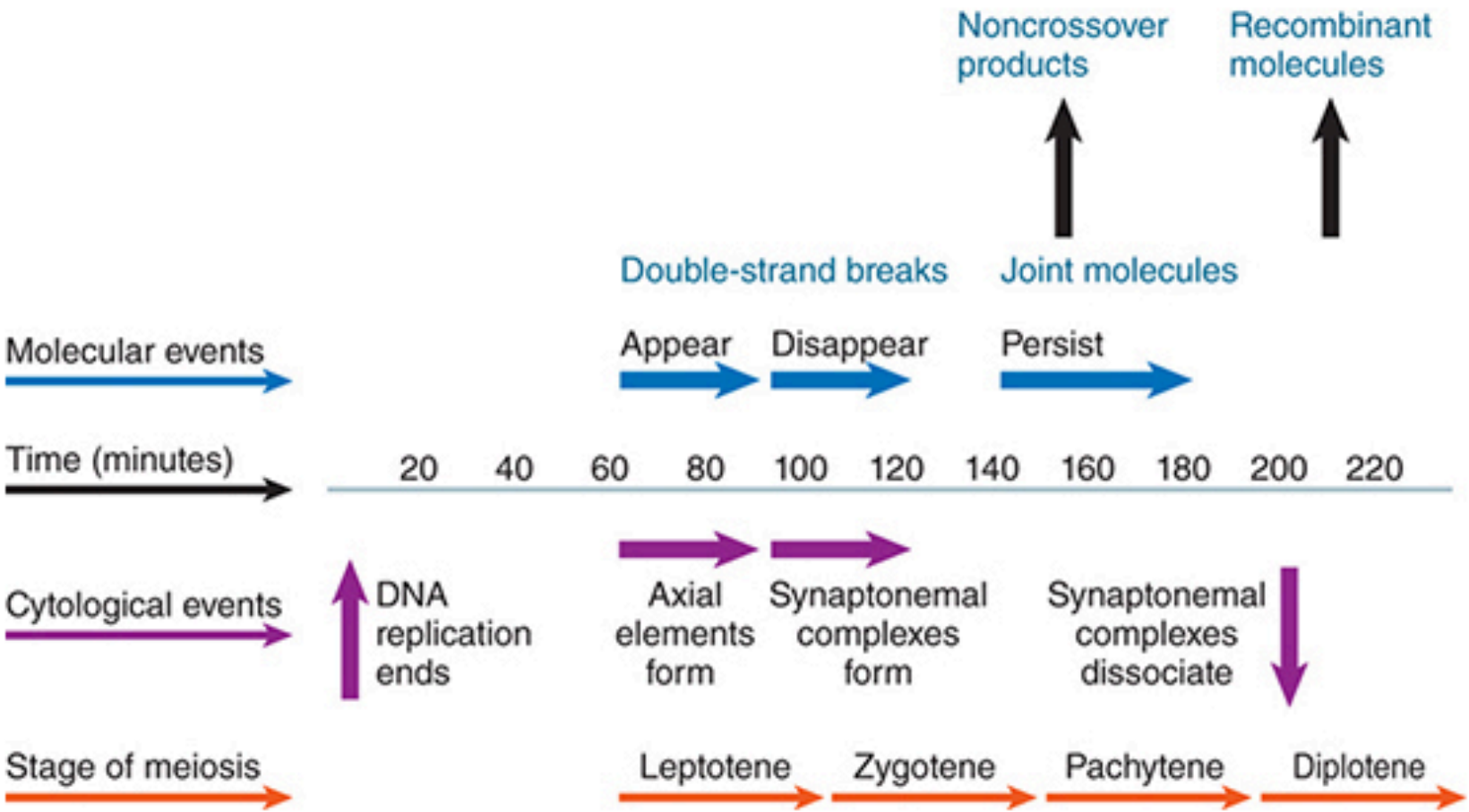
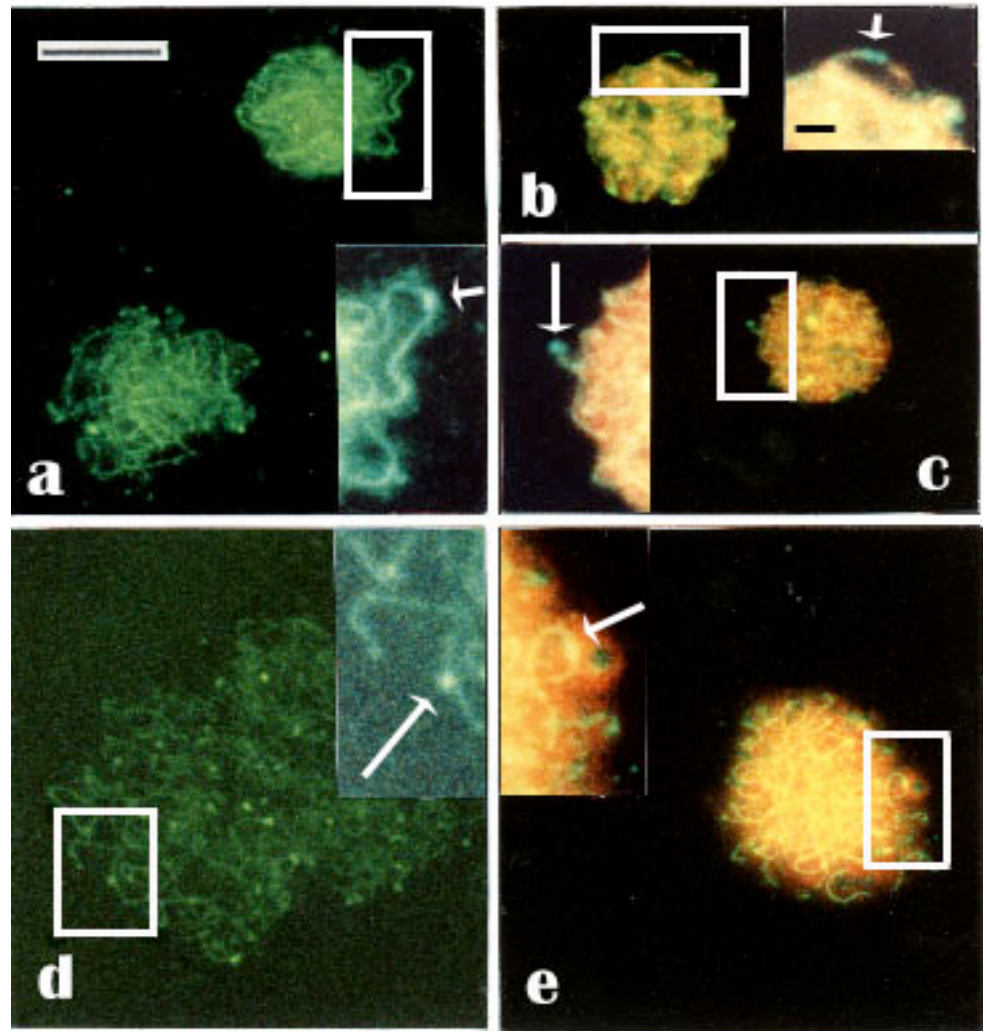
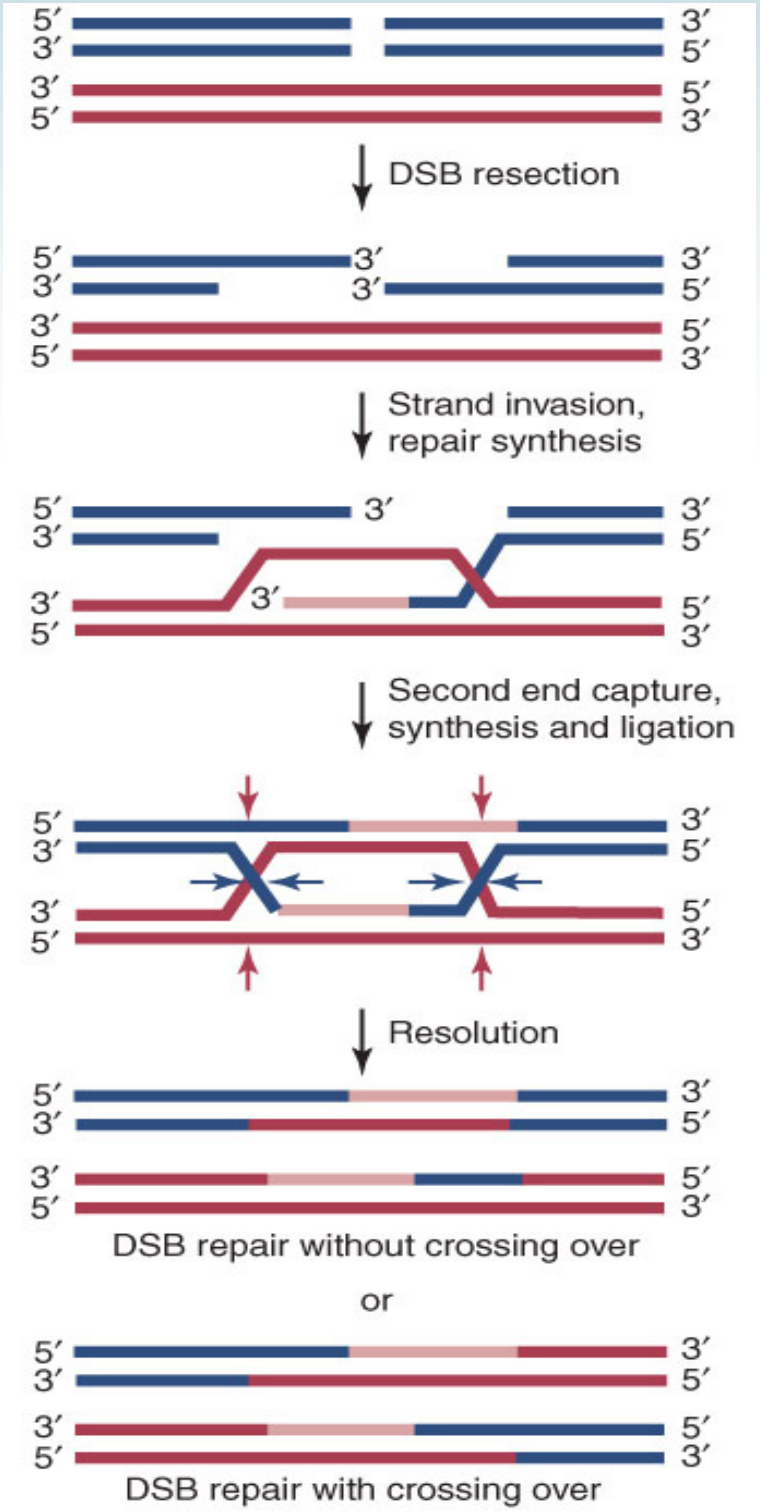


Double-strand breaks appear when axial elements form and disappear during the extension of synaptonemal complexes. Joint molecules appear and persist until DNA recombinants are detected at the end of pachytene.

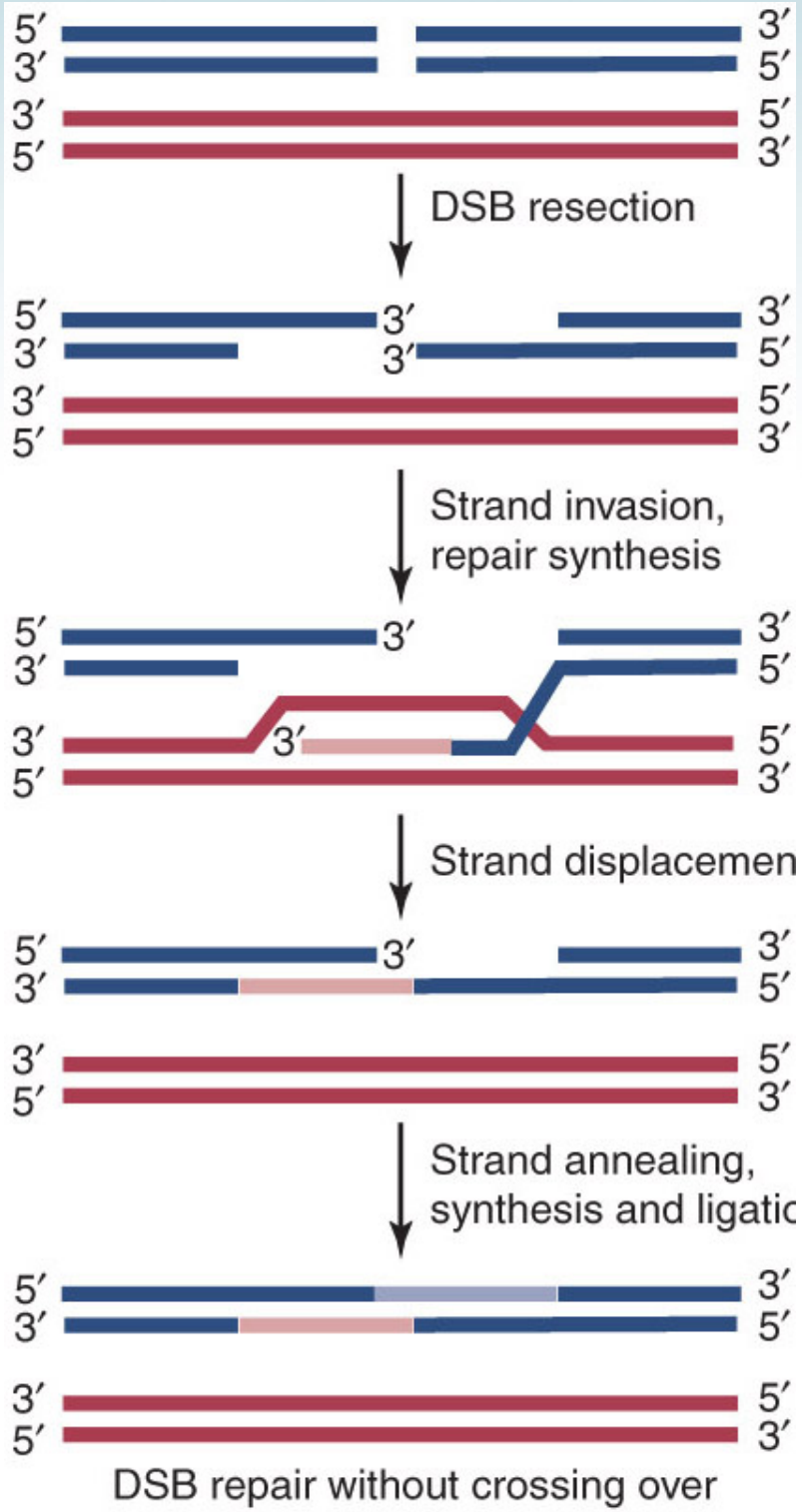


Indirect immunofluorescence using anti-RecA and FITC-conjugated secondary antibodies of meiotic nuclei with suggested recombination nodules. The nuclei represent early to late pachytene estimated by the presence of the SCs and the comparative size of the nuclei.
- a-c. Early to middle pachytene nuclei.
- d, e. Middle to late pachytene nuclei.
- a, d. PI omitted.
- b, c and -e. Counterstaining with PI.
- Insets: Details of the nodules (arrows) on the SCs indicate
- a, e. Ellipsoidal nodules.
- b, c and -d. Spherical nodules
- c. A terminal spherical nodule.
- b.Two spherical tandem nodules. Note, that many of the nuclei contain several nodules. Some chromosome cores with nodules (framed) are seen at higher magnification in the insets. - Bar 10 µm - In insets: -Bar 1 µm.

Immunological detection of RecA-related proteins in bull meiotic nucle. http://www.csc.fi/jpr/emt/engelhar/ASCB.html



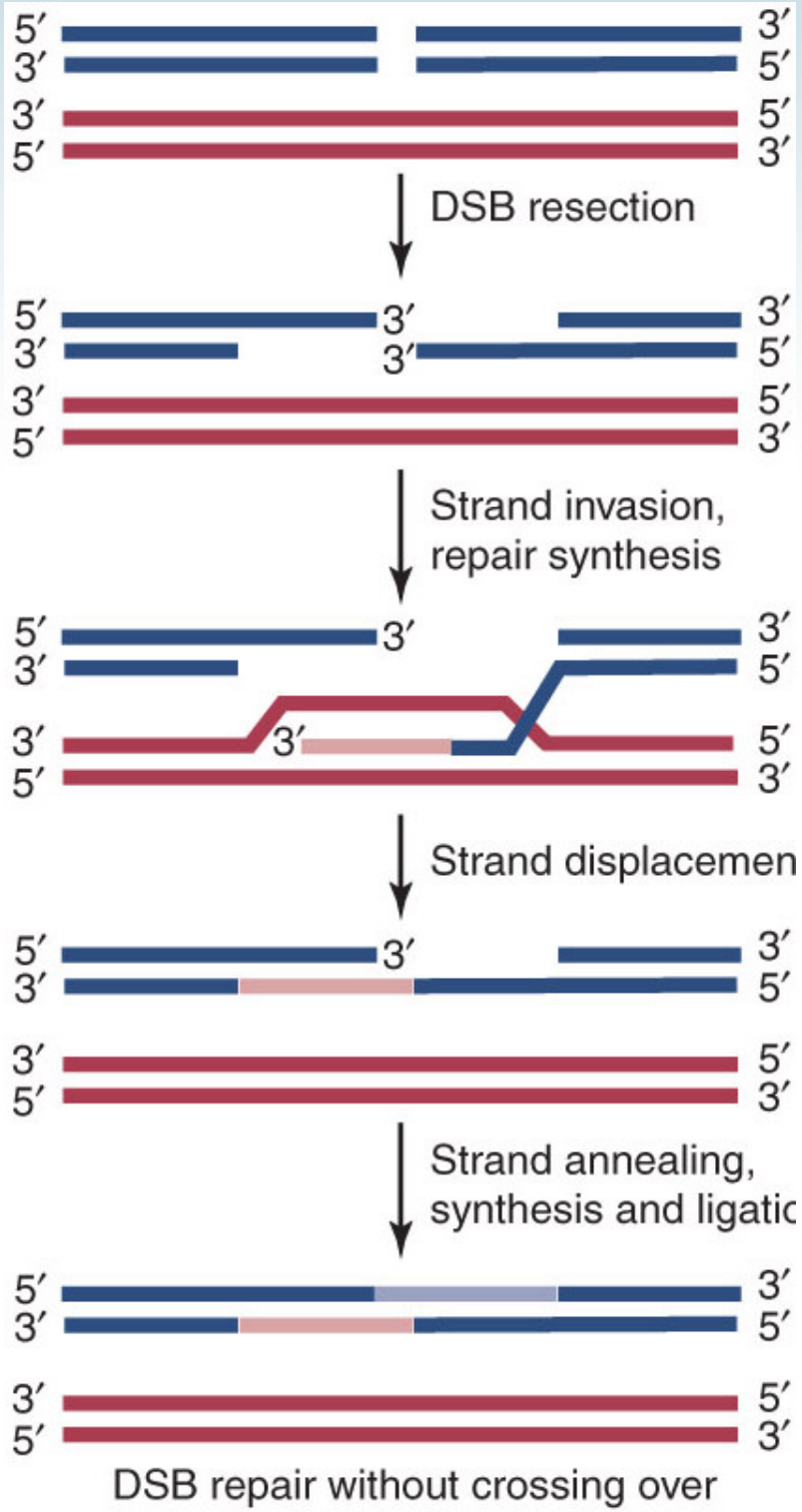
Double-strand break-repair model of homologous recombination.



The Synthesis-Dependent Strand-Annealing Model

ALTERNATIVE

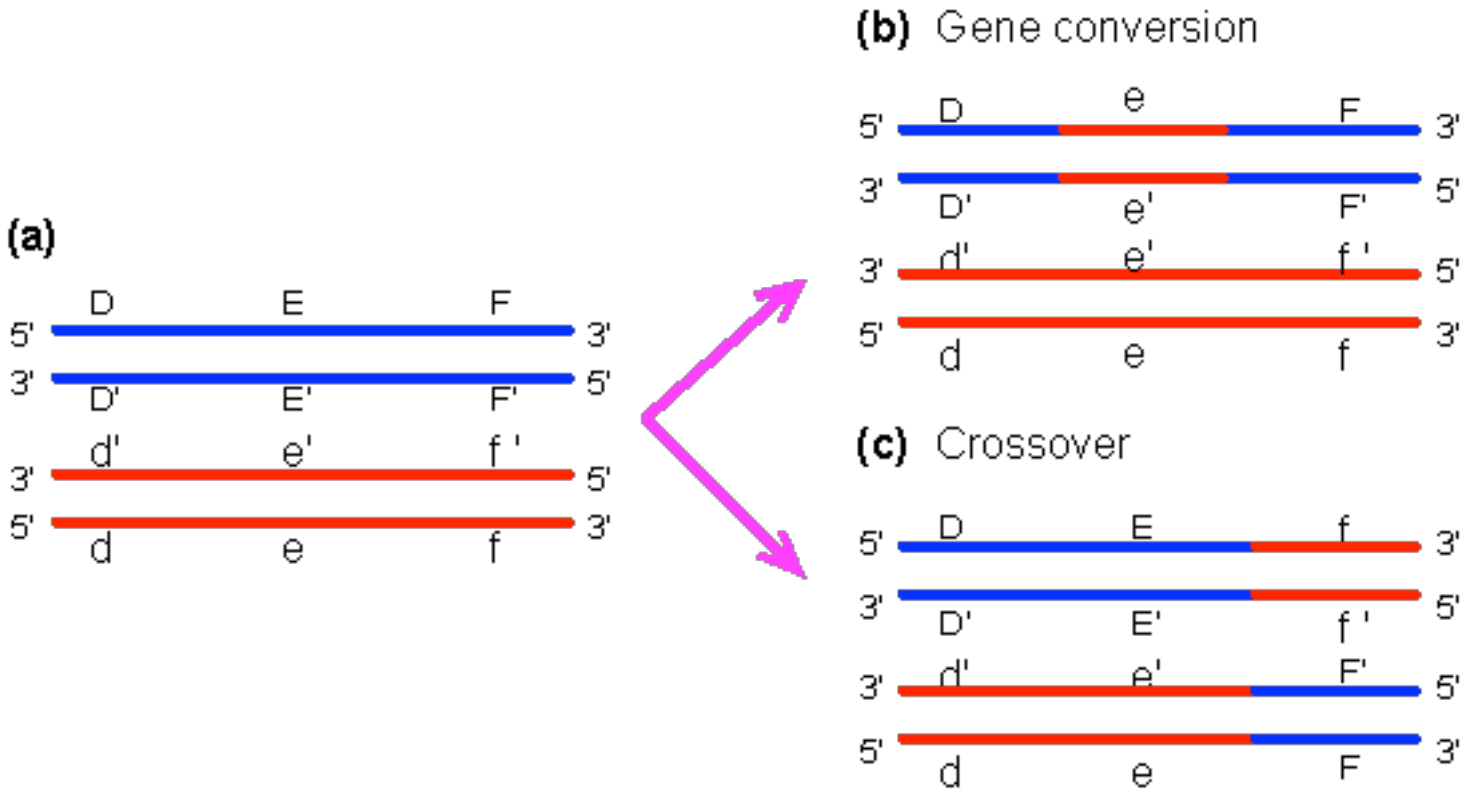
- The synthesis-dependent strand-annealing model (SDSA) is relevant for **mitotic recombination**.



The Synthesis-Dependent Strand-Annealing Model

ALTERNATIVE

- The synthesis-dependent strand-annealing model (SDSA) is relevant for **mitotic recombination**, as it also produces **gene conversions** from double-strand breaks without having associated crossovers.



(a) Two DNA molecules. **(b)** Gene conversion - the red DNA **donates** part of its genetic information (e-e' region) to the blue DNA. **(c)** DNA crossover - the two DNAs **exchange** part of their genetic information (f-f' and F-F').

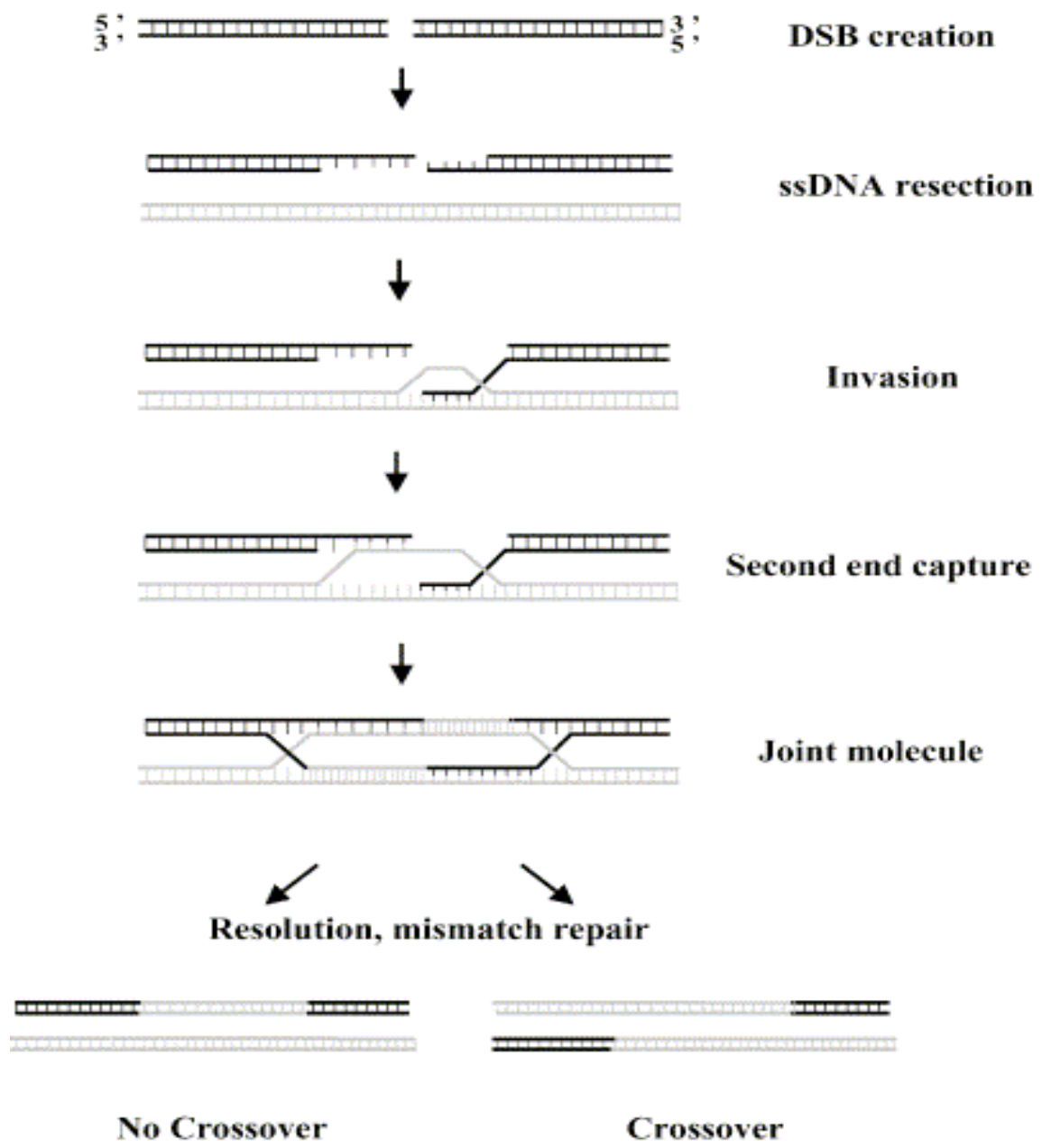
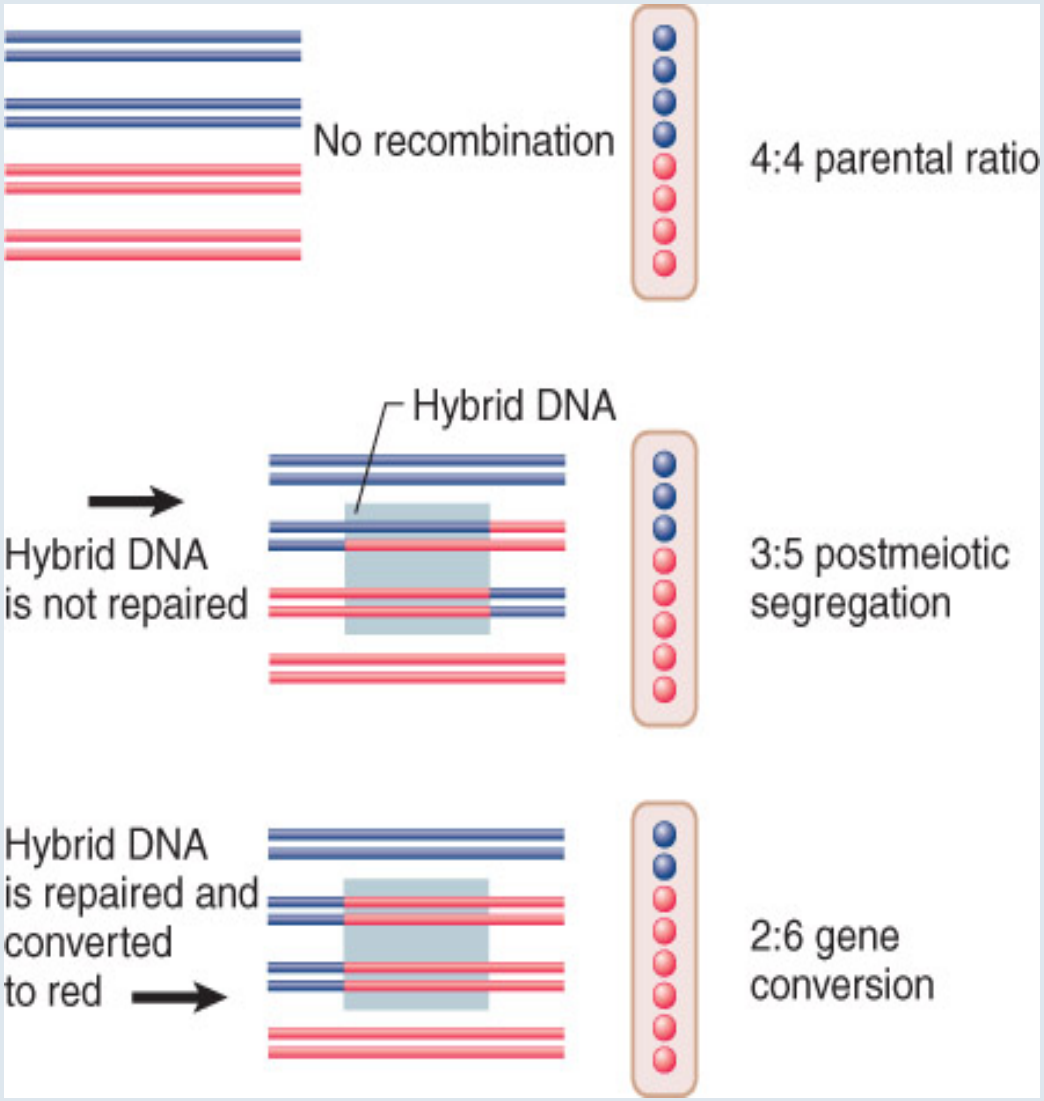


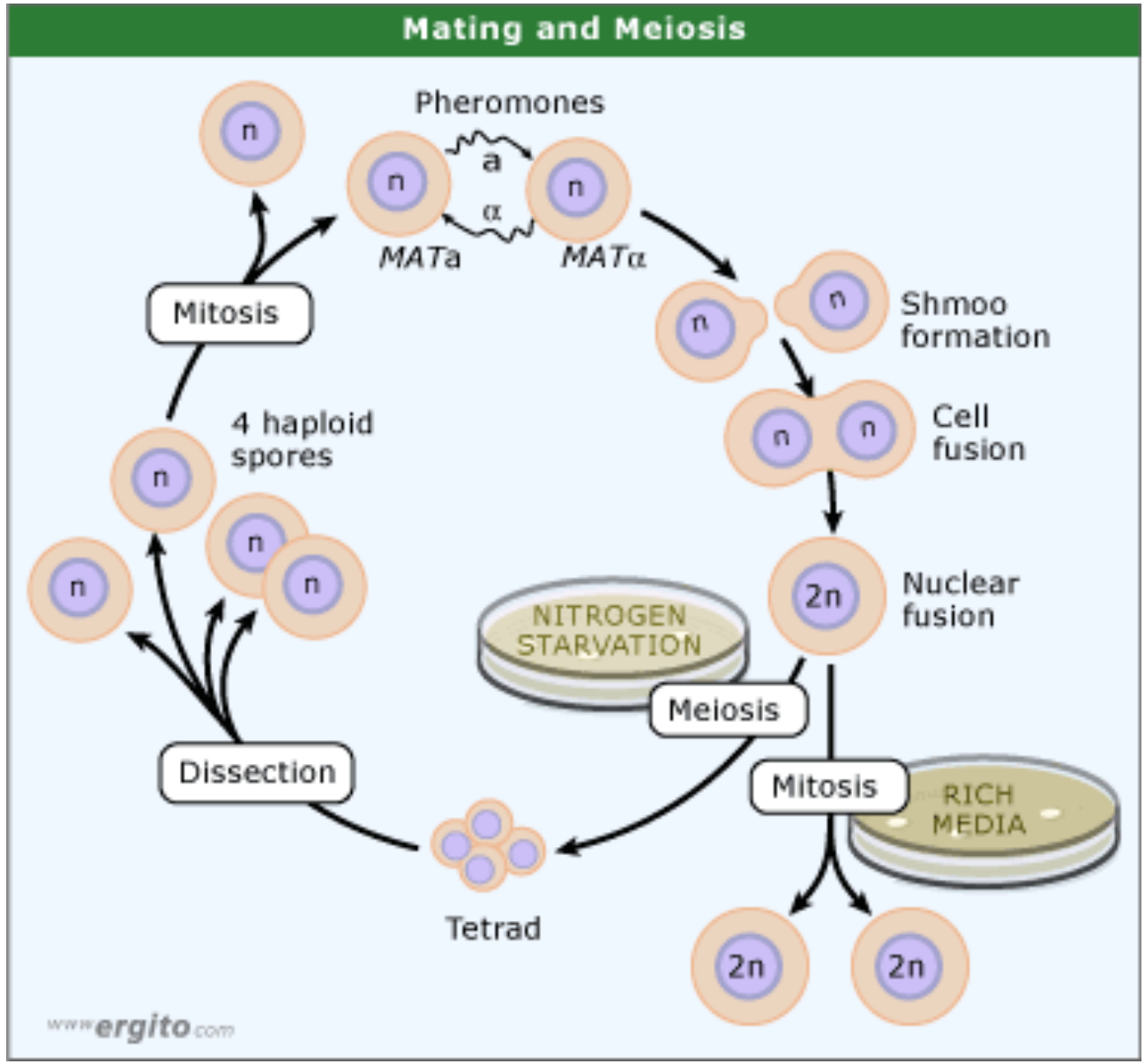
Fig. 1. Current DSB-initiated model of homologous recombination (modified from [9]).

Gene Conversion Accounts for Interallelic Recombination

- **Mismatch (gap) repair** of heteroduplex DNA generates nonreciprocal recombinant products called **gene conversions**.

Spore formation in the ascomycetes allows determination of the genetic constitution of each of the DNA strands involved in meiosis.




Mating and Meiosis
Pheromones
a
α
n
MATa
MATα
Shmoo formation
Cell fusion
2n
Nuclear fusion
NITROGEN STARVATION
Meiosis
Mitosis
RICH MEDIA
Tetrad
Dissection
4 haploid spores
Mitosis
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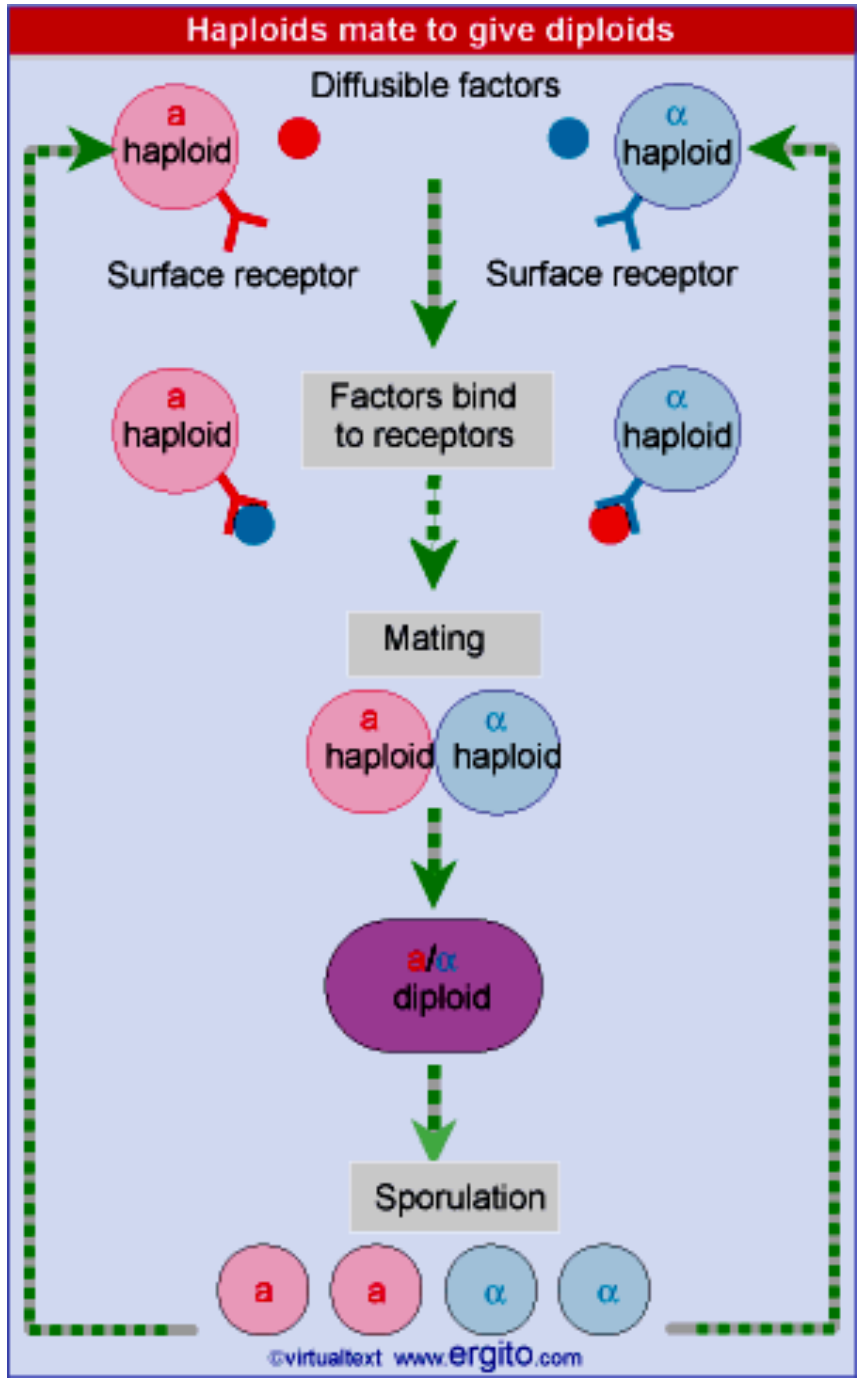
Haploids mate to give diploids
Diffusible factors
a haploid
α haploid
Surface receptor
Surface receptor
a haploid
Factors bind to receptors
α haploid
Mating
a haploid
α haploid
a/α diploid
Sporulation
a
a
α
α
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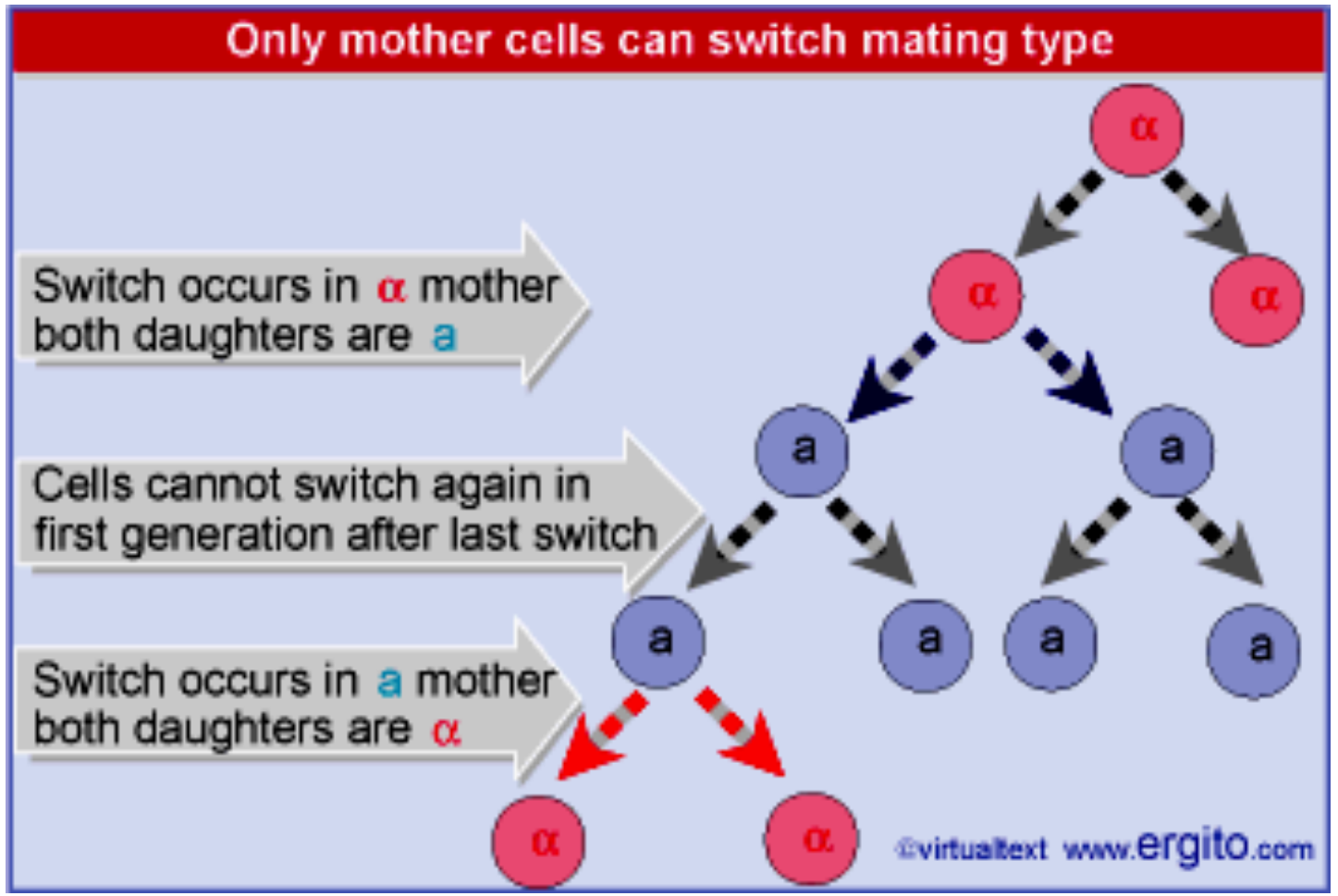
Only mother cells can switch mating type
Switch occurs in α mother both daughters are a
Cells cannot switch again in first generation after last switch
Switch occurs in a mother both daughters are α
α
α
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α
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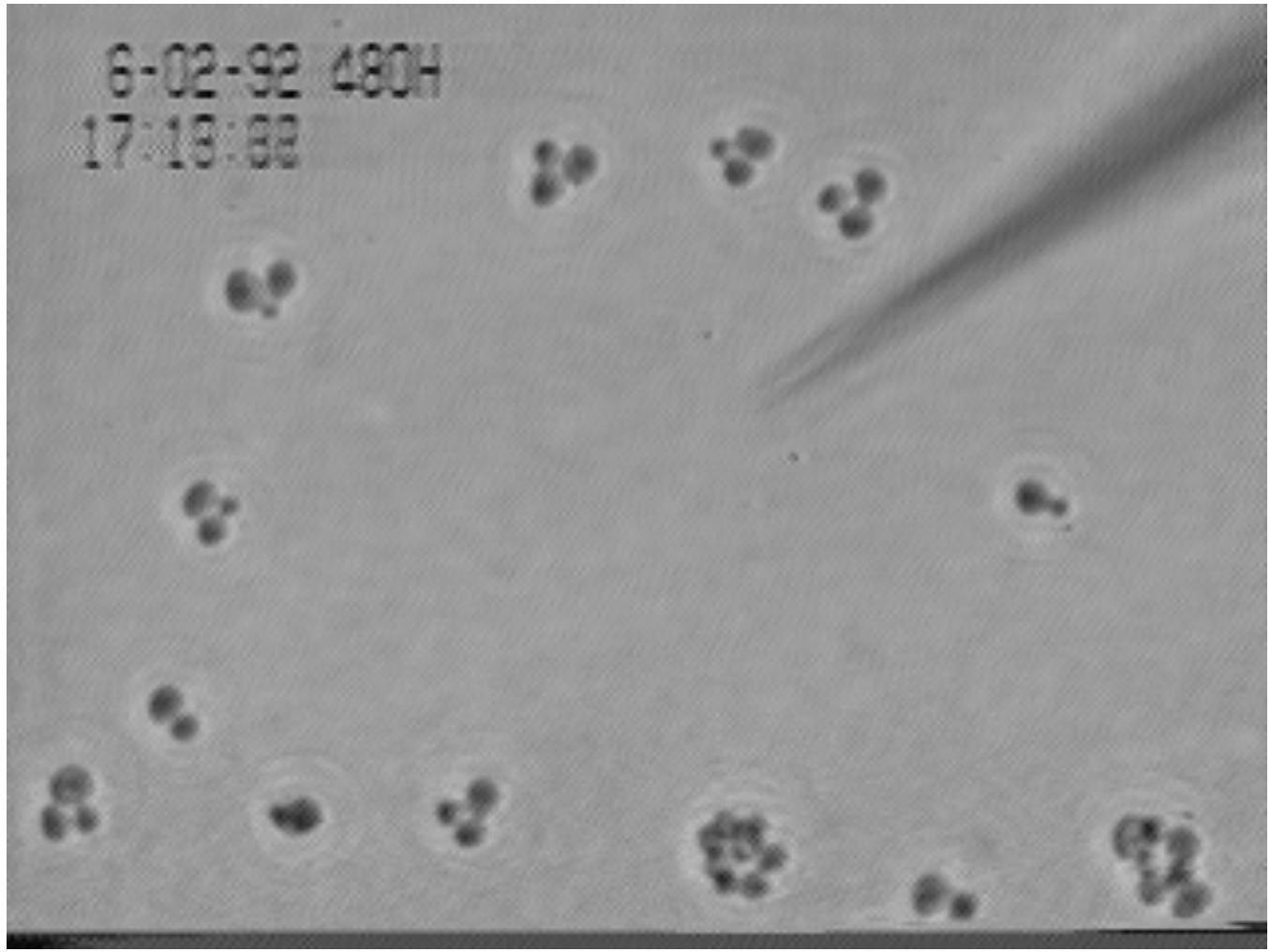
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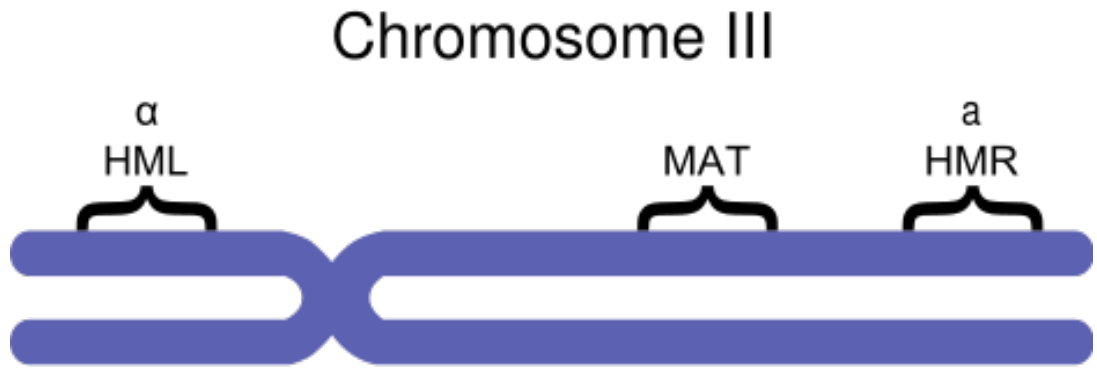


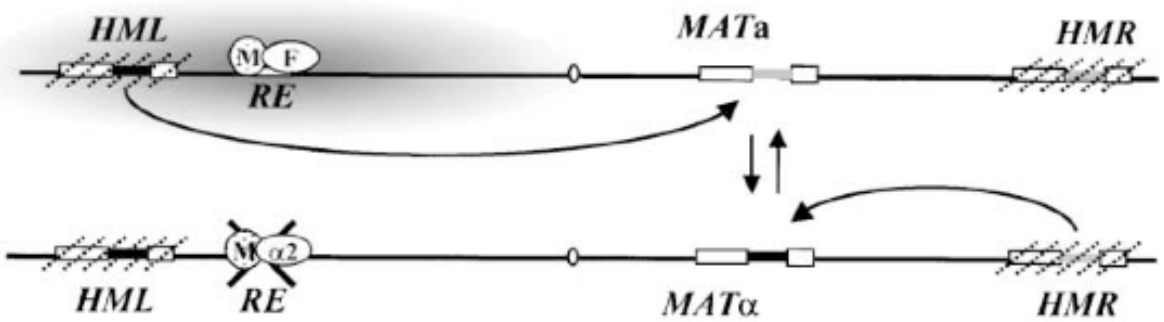
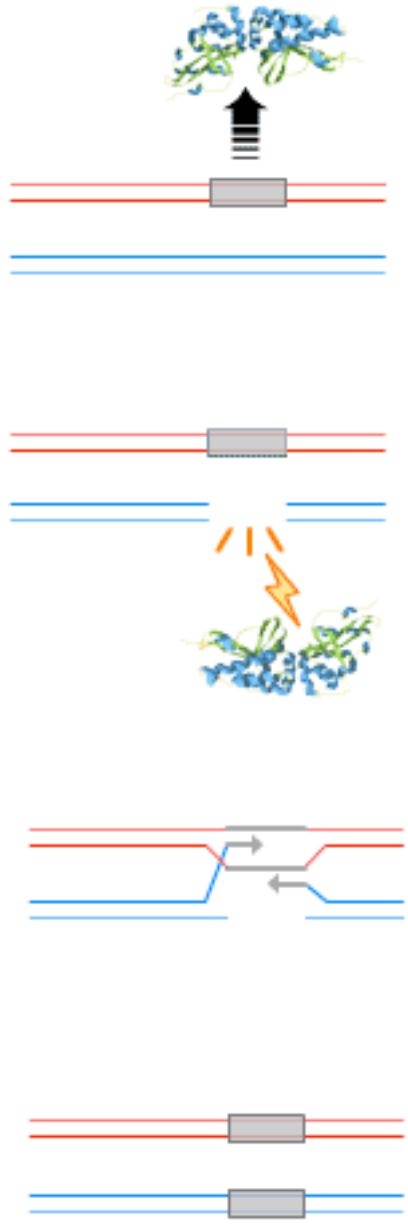


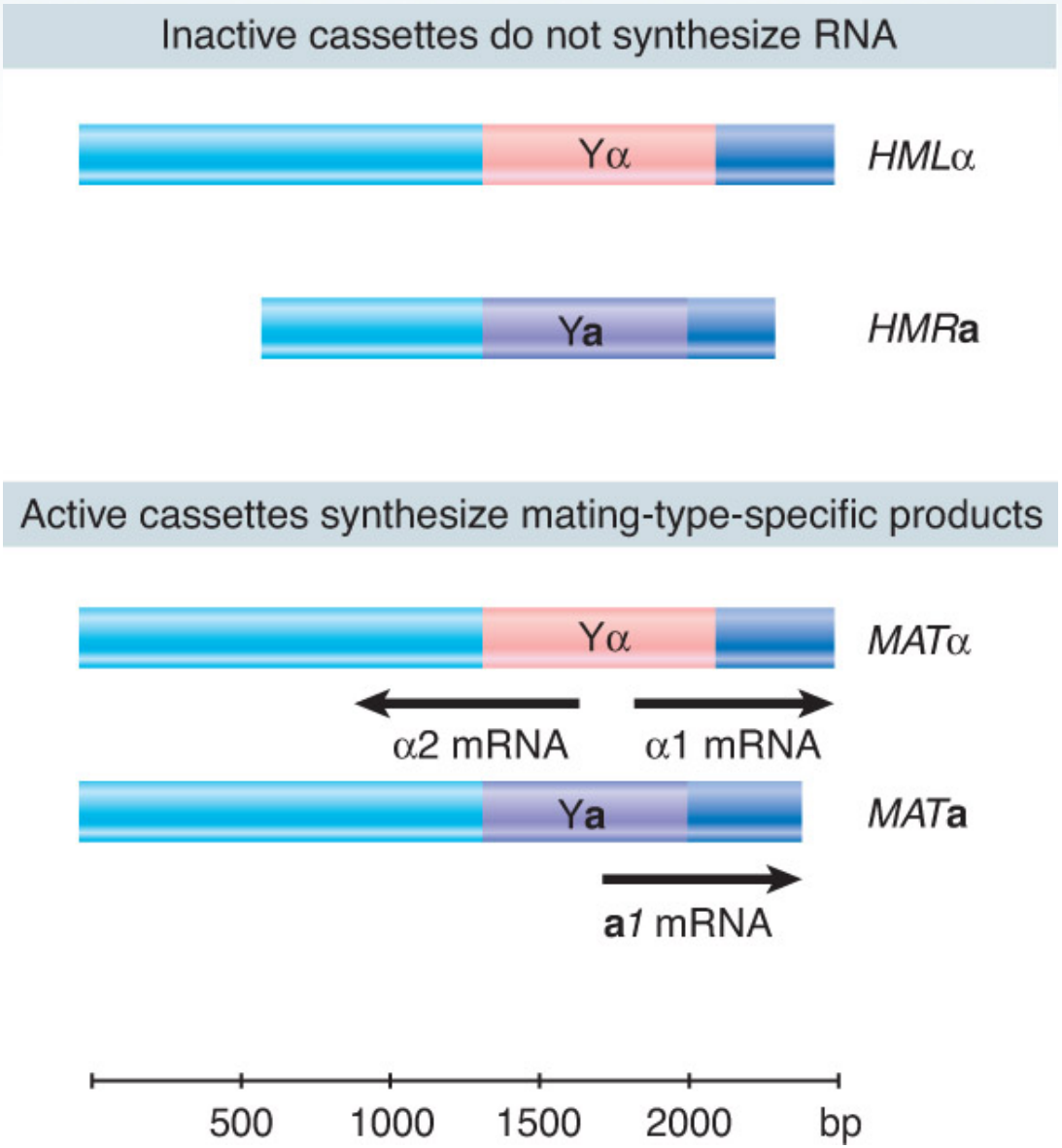
FIGURE 1.—Mating-type switching exhibits cell type-dependent donor preference. A diagram of chromosome III indicating the relative positions of the donor loci, *HML* and *HMR*, the *MAT* locus, and the recombination enhancer, *RE*, is shown. *HML* and *HMR* are transcriptionally silent, indicated by the hatched lines, while *MAT* is transcriptionally active, giving rise to the mating type of the cell. Open rectangles at the three mating loci indicate blocks of homology while the thick line indicates the allele-specific region (α allele is black, **a** allele is gray). In **a** cells (top), Mcm1 (M) and Fkh1 (F), a forkhead transcription factor, occupy *RE* and promote enhanced recombination potential (shaded area) extending over *HML*, rendering it the preferred donor during mating-type switching and resulting in conversion from *MAT***a** to *MAT*α. In α cells (bottom), α2 binds to *RE*, precluding occupation by Fkh1, to suppress enhanced recombination potential, rendering *HMR* the preferred donor through *RE*-independent mechanisms and resulting in conversion from *MAT*α to *MAT***a**.

Matthew P Scott, Paul Matsudaira, Harvey Lodish, James Darnell, Lawrence Zipursky, Chris A Kaiser, Arnold Berk, Monty Krieger (2004). *Molecular Cell Biology, Fifth Edition*. WH Freeman and Col, NY



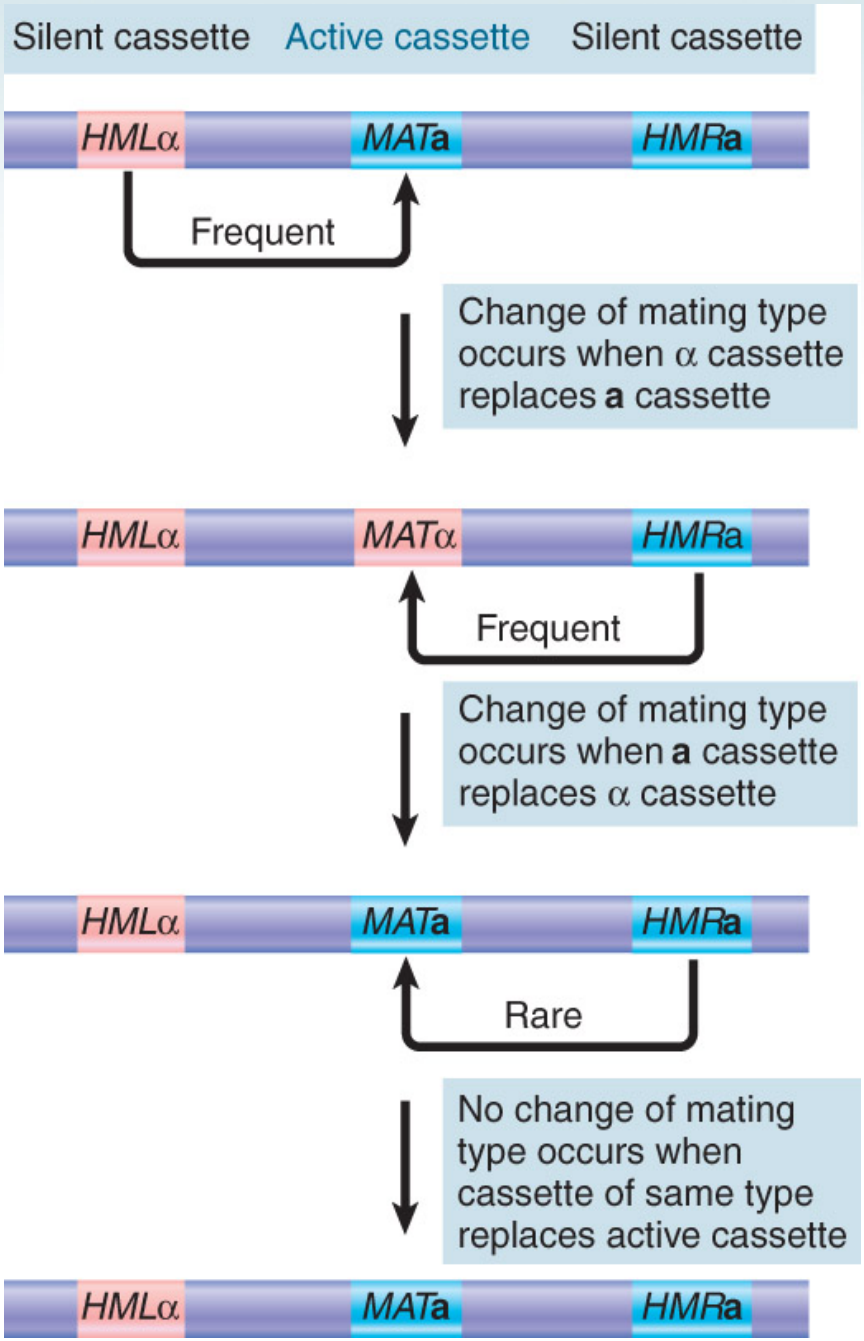
a unidirectional genetic exchange.....

Yeast Can Switch Silent and Active Loci for Mating Type



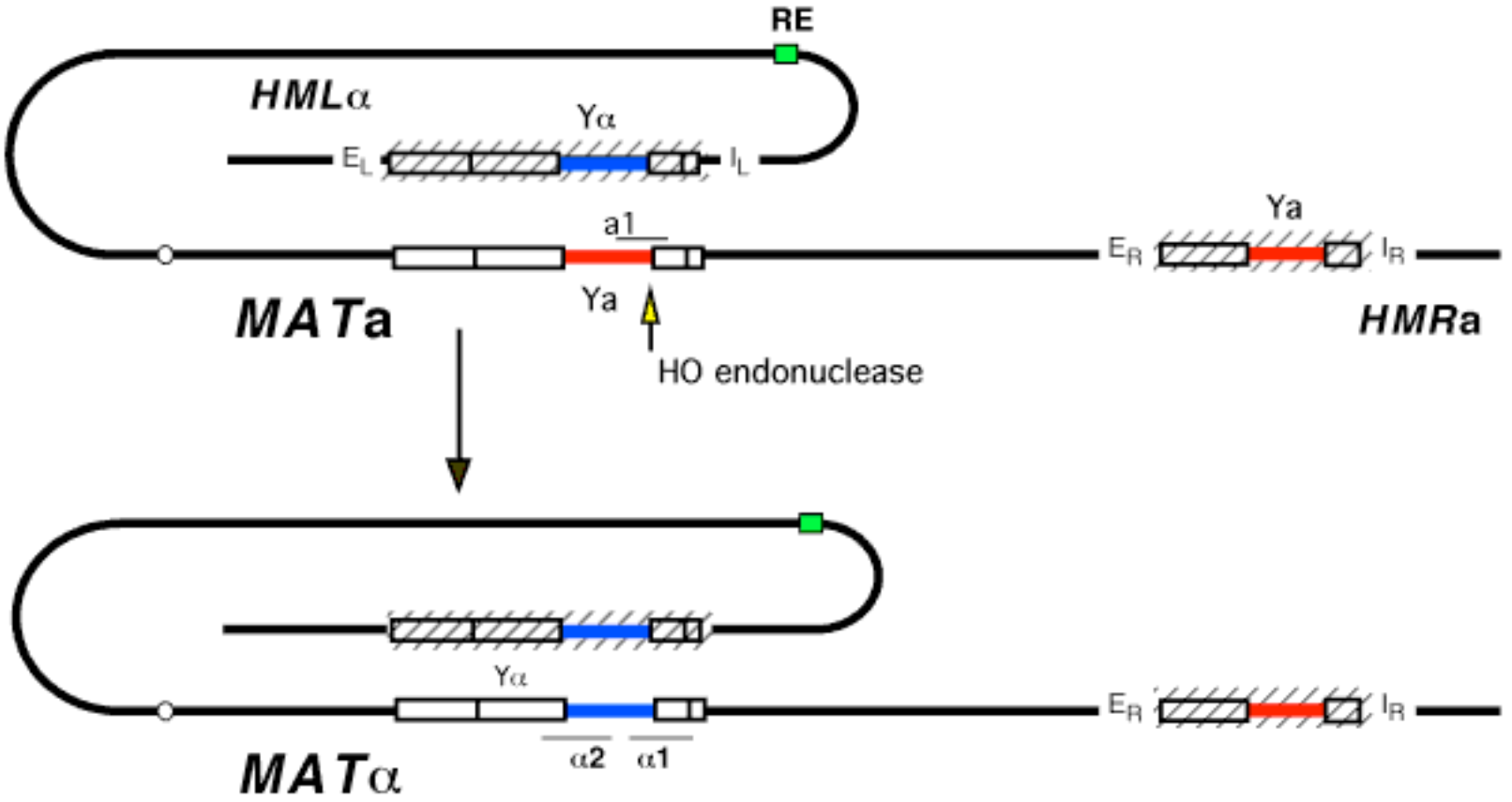
- Switching occurs if *MAT***a** is replaced by *HMR*α or *MAT*α is replaced by *HMR***a**.

FIGURE 34: Mating type loci organization



- The yeast mating type locus *MAT*, a **mating type cassette**, has either the *MAT***a** or *MAT*α genotype.
- Yeast with the dominant allele *HO* switch their mating type at a frequency ~10^{-6}.
- The allele at *MAT* is called the active cassette.
- There are also two silent cassettes, *HML*α and *HMR***a**.

Cassette model for mating type


RE
HMLα
Yα
EL
IL
a1
Ya
ER
IR
MATa
Ya
HMRa
HO endonuclease
Yα
ER
IR
MATα
α2
α1

Research Highlight

Nature Reviews Genetics **11**, 592 (September 2010) | doi:10.1038/nrg2848

Corrected online: 19 August 2010

Mutation rate: DNA repair and indels boost errors

Mar

Mutation rates vary with genomic locus and cellular context, but there are many unanswered questions regarding when, where and how elevated mutation rates occur. Two papers now implicate DNA repair in increased local mutation rates, and the results may influence future studies of genetic variation and tumorigenesis.

Mitotic gene conversion is a mechanism for repairing DNA double-strand breaks (DSBs) in which a homologous sequence (donor) is used as a template for repair. Hicks and colleagues took advantage of the gene conversion that is a normal part of mating-type (*MAT*) switching in *Saccharomyces cerevisiae* to screen for errors generated by this repair process. **They inserted a gene (*URA3*) into the normal donor sequence that enabled them to chemically select cells in which mutations had arisen during gene conversion. Strikingly, they found that the mutation rate associated with gene conversion was 1,400 times higher than the rate of spontaneous mutation in the same genomic region.**

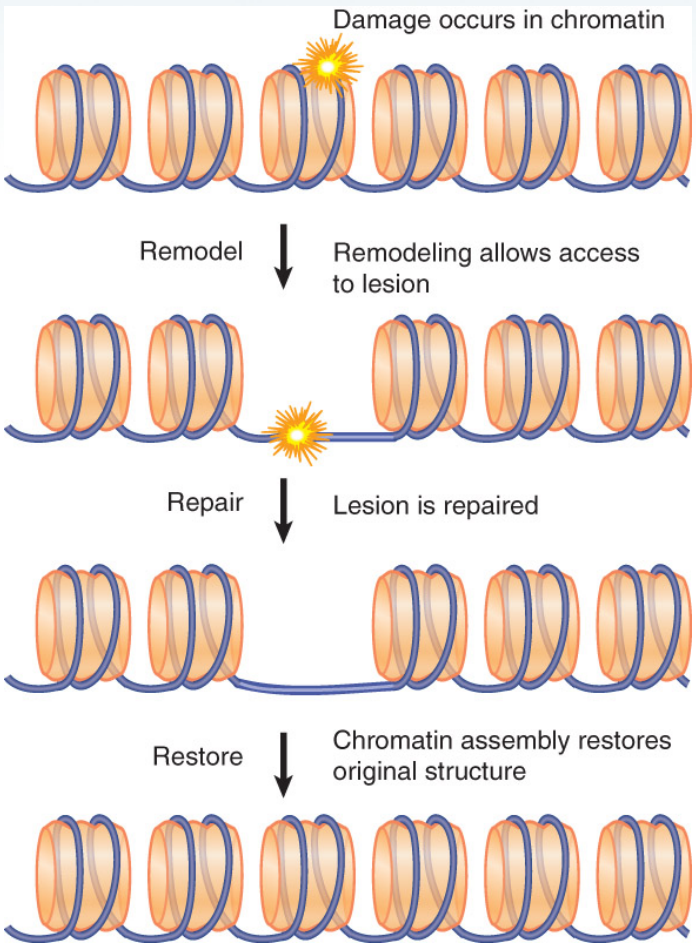
Hicks *et al*. showed that the majority of mutations were single-base-pair substitutions, but a high proportion were one-base-pair deletions and complex mutations that are probably caused by template-switching during gene conversion. Surprisingly, the normally high-fidelity DNA polymerase-δ seems to cause these template switches. This finding suggests that gene conversion is less processive than the DNA synthesis that takes place during S phase. Furthermore, the authors suggest that some mutations required for carcinogenesis could result from gene conversion being used to repair the increased numbers of DSBs triggered by activated oncogenes.

* DNA Repair in Eukaryotes Occurs in the Context of Chromatin

- Different patterns of histone modifications may distinguish stages of repair or different pathways of repair.

- Chromatin “Remodelers” and “chaperones” are required to reset chromatin structure before and after completion of repair.

* DNA Repair in Eukaryotes Occurs in the Context of Chromatin



DNA damage in chromatin requires chromatin remodeling and histone modification

- Both histone modification and chromatin remodeling are essential for repair of DNA damage in chromatin.
- H2A phosphorylation (γ**-H2AX**) is a conserved double-strand break-dependent modification that actively recruits chromatin modifying activities and facilitates assembly of repair factors.