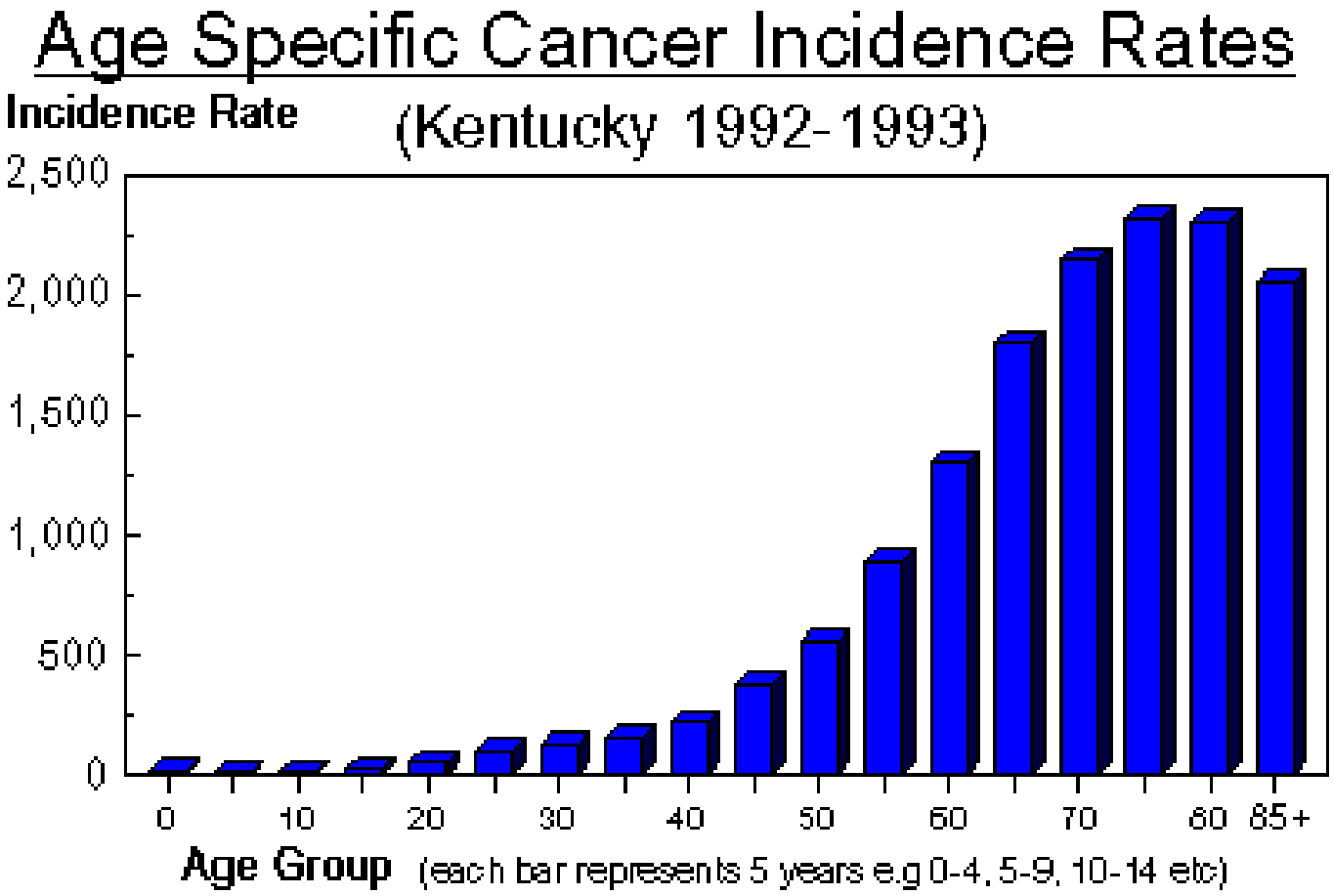
Genetic instability

M. De Palma

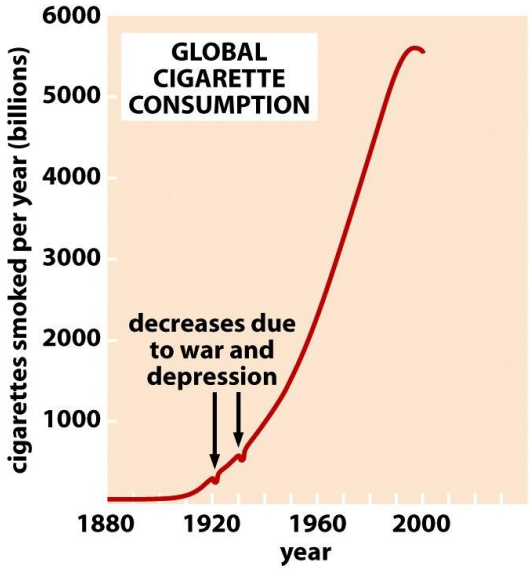
Reminding some concepts…

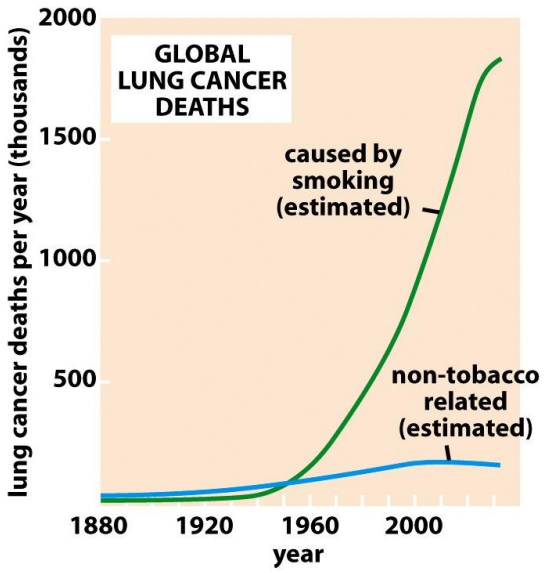
Cancer incidence rates

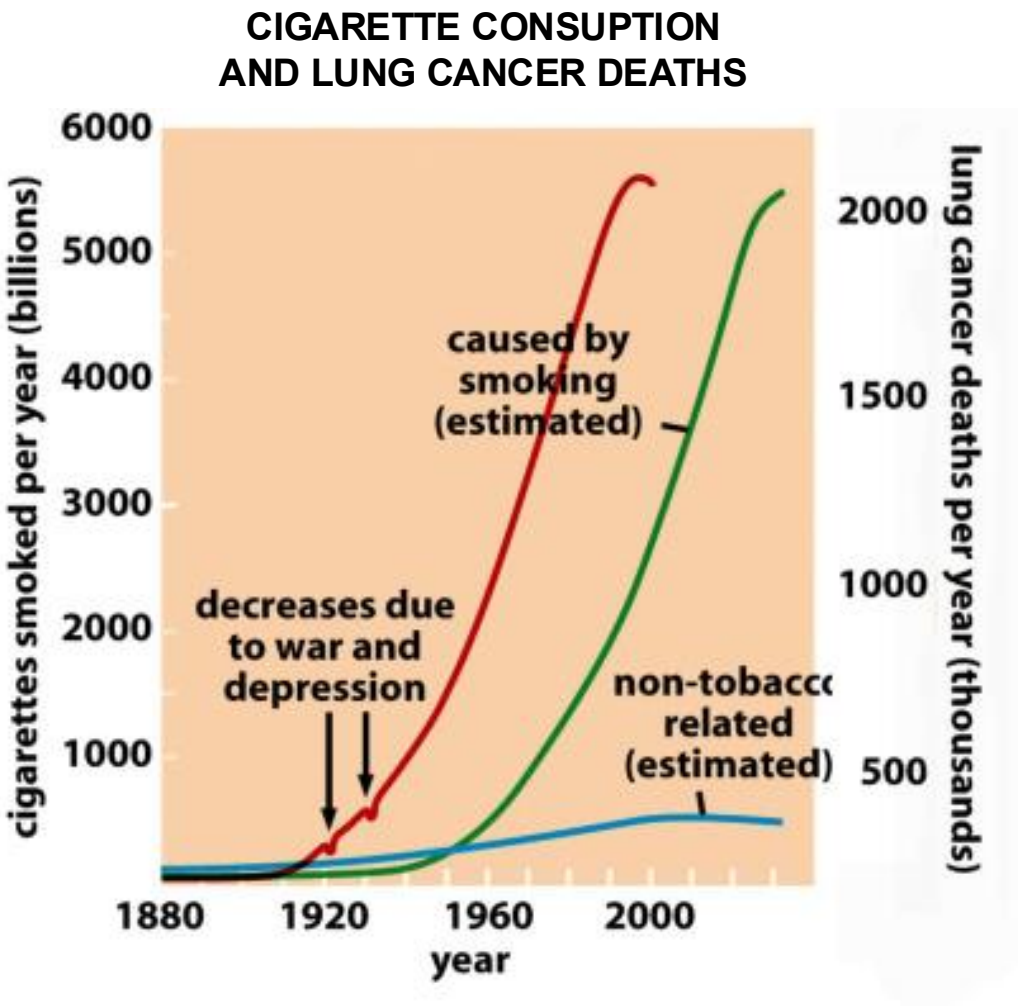


Cancer risk increases with age. This INDIRECTLY suggests that cancer takes many years to develop and become clinically detectable

Mutagens, latency and cancer

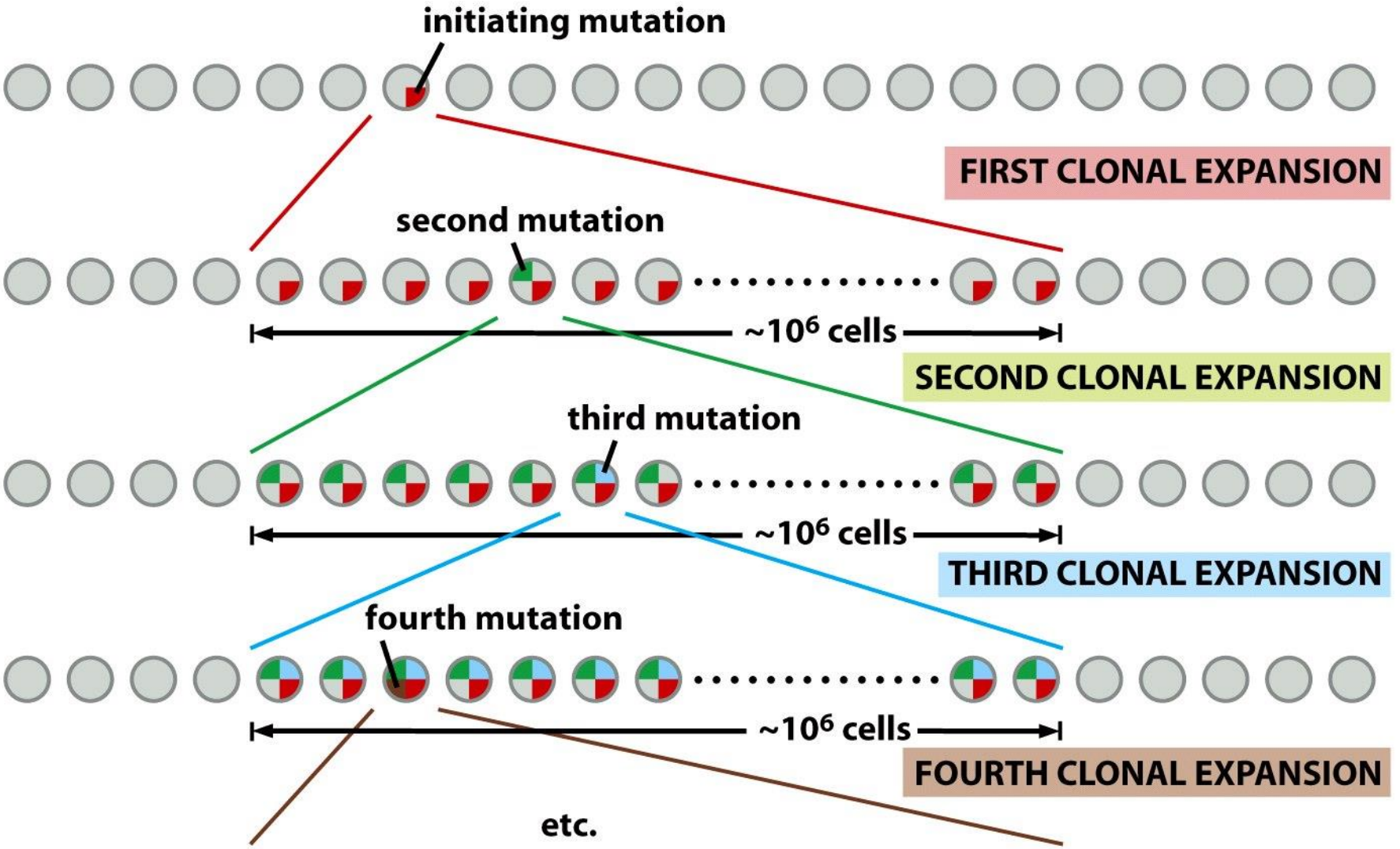




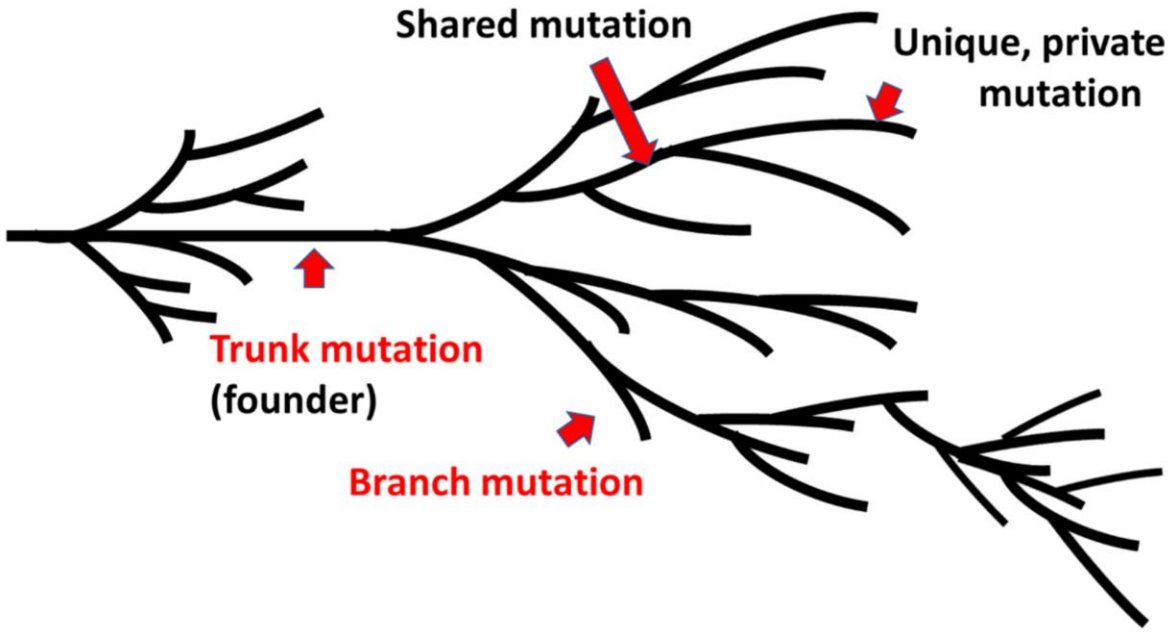


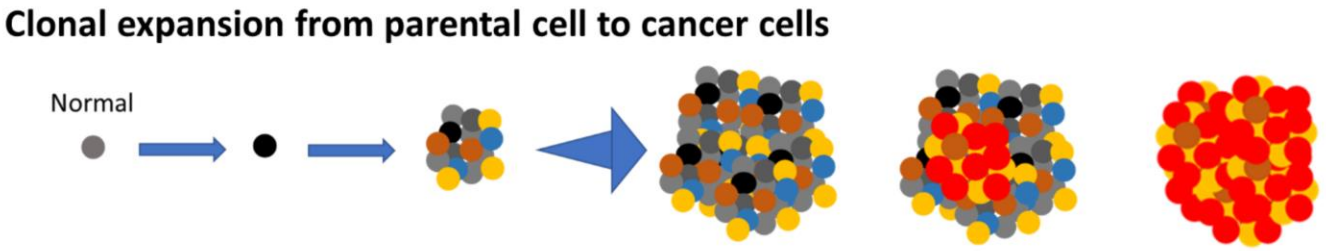
This association DIRECTLY suggests that cancer takes many years to develop and become clinically detectable

Darwinian evolution and clonal expansion during tumorigenesis



Trunk and branching mutations during tumorigenesis





Several mutagenic events are required to transform human cells

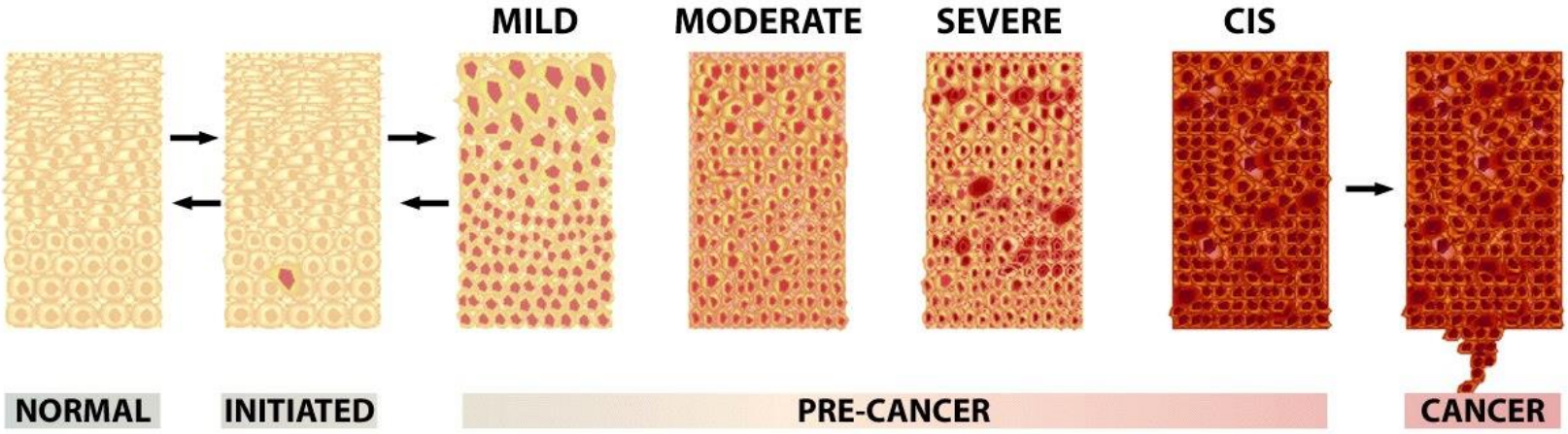
Sufficient to transform mouse cells

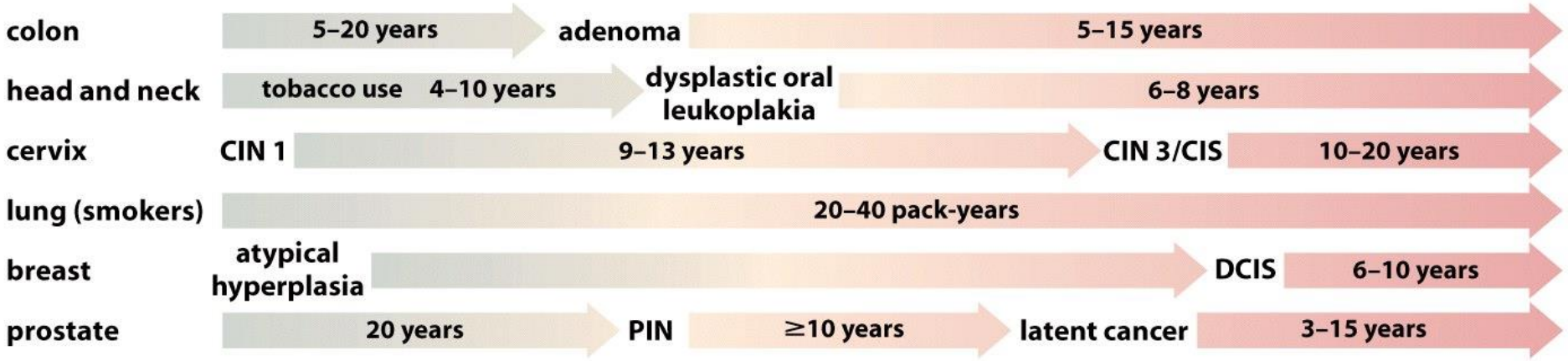
pathway	Ras	pRb	p53	telomeres	PP2A
genes/agents used to deregulate pathway	*ras*	*CDK4 + D1* SV40 *LT* HPV *E7*	DN *p53* SV40 *LT* HPV *E6*	*hTERT* *myc + SV40 LT*	SV40 *sT* sometimes: *myc* *Akt/PKB+Rac1* *PI3K* *B56* shRNA

Required to transform human cells

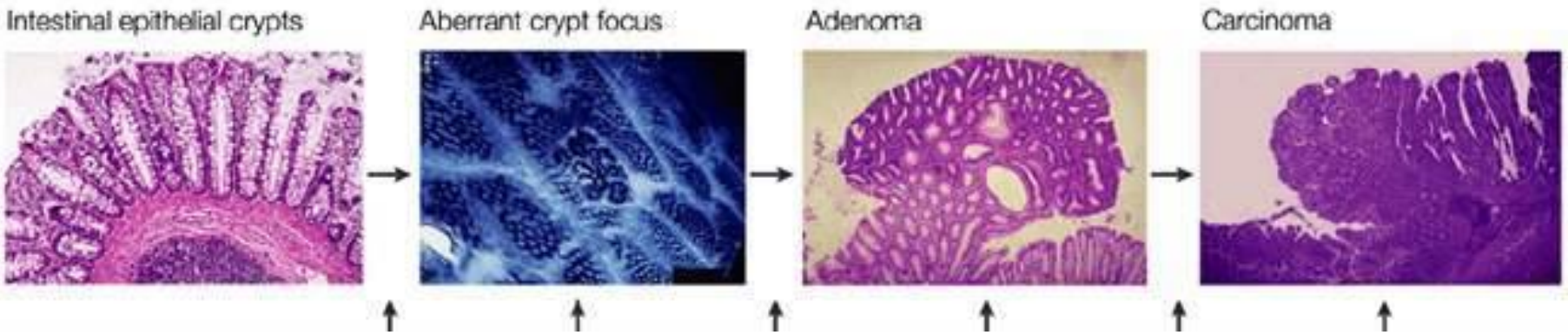
Immortalization: cells proliferate in vitro and do not senescence
Tranformation: cells can form tumors

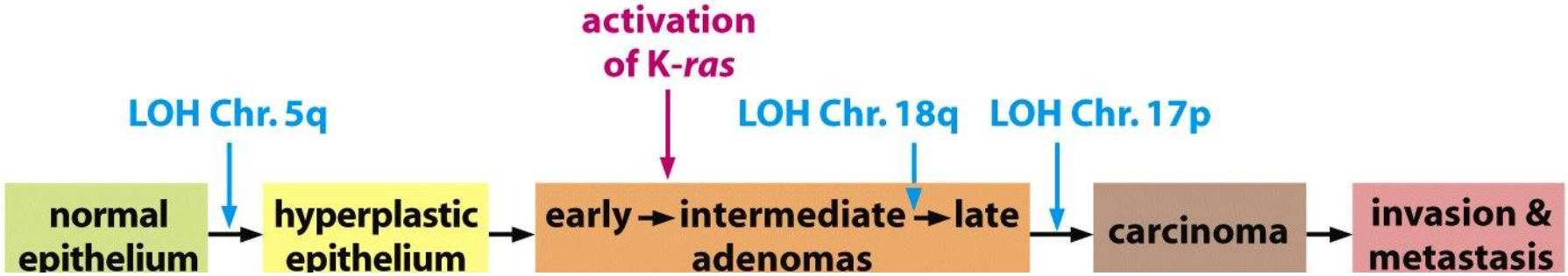
Evidence for multi-step progression in several cancer types

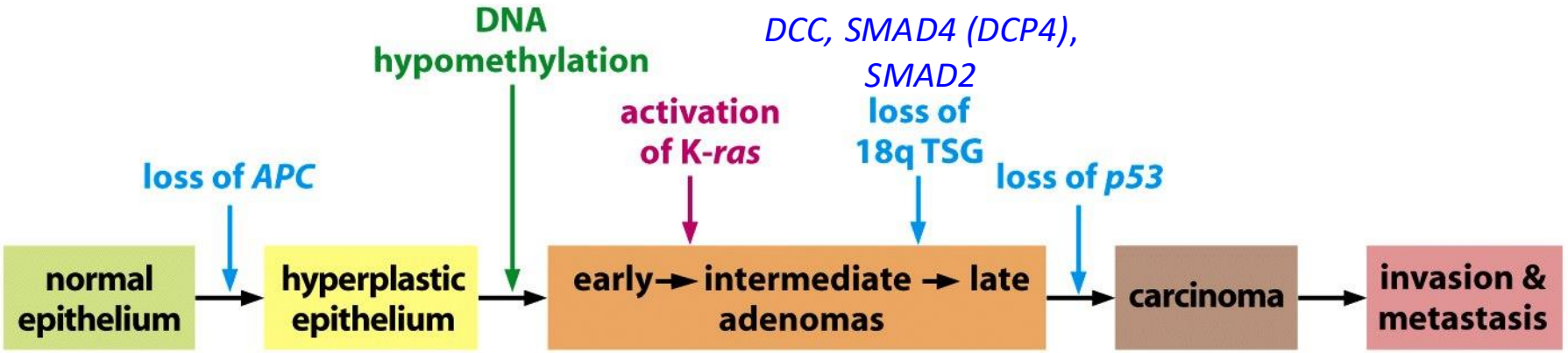




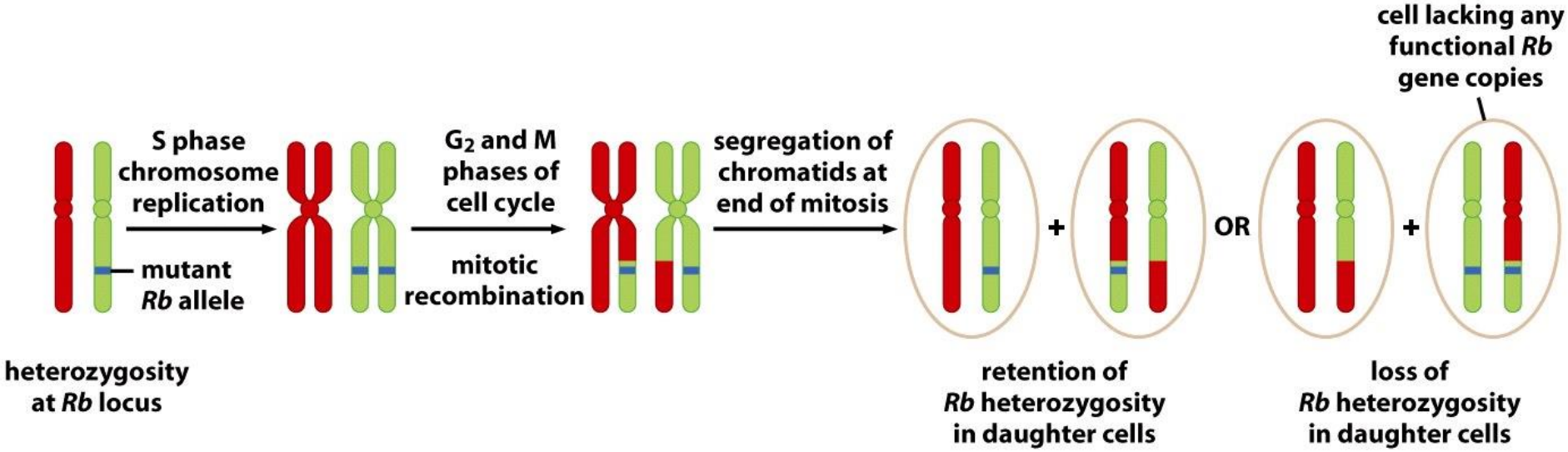
Multi-step progression in colorectal cancer (CRC)







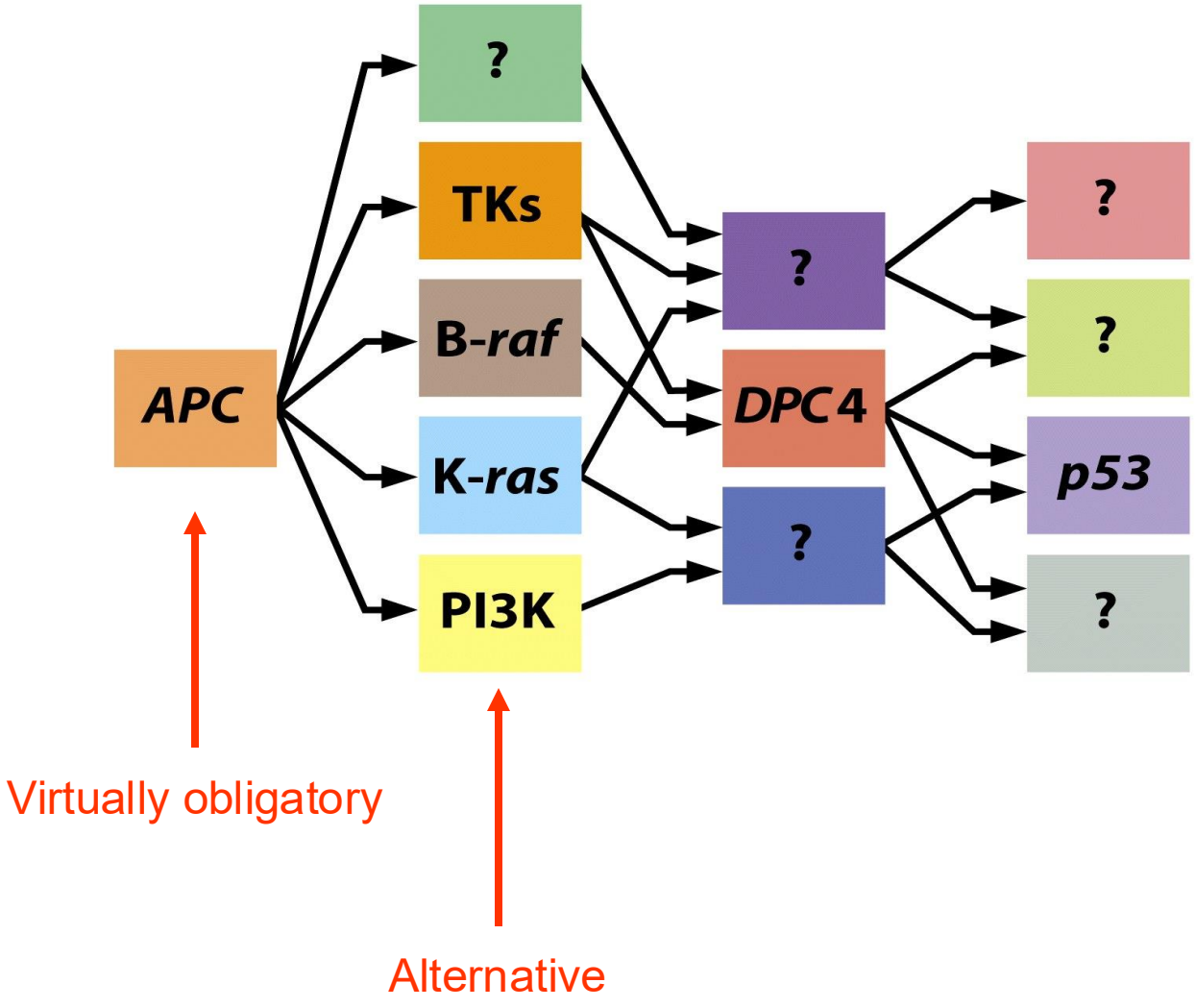
Loss of heterozigosity (LOH)



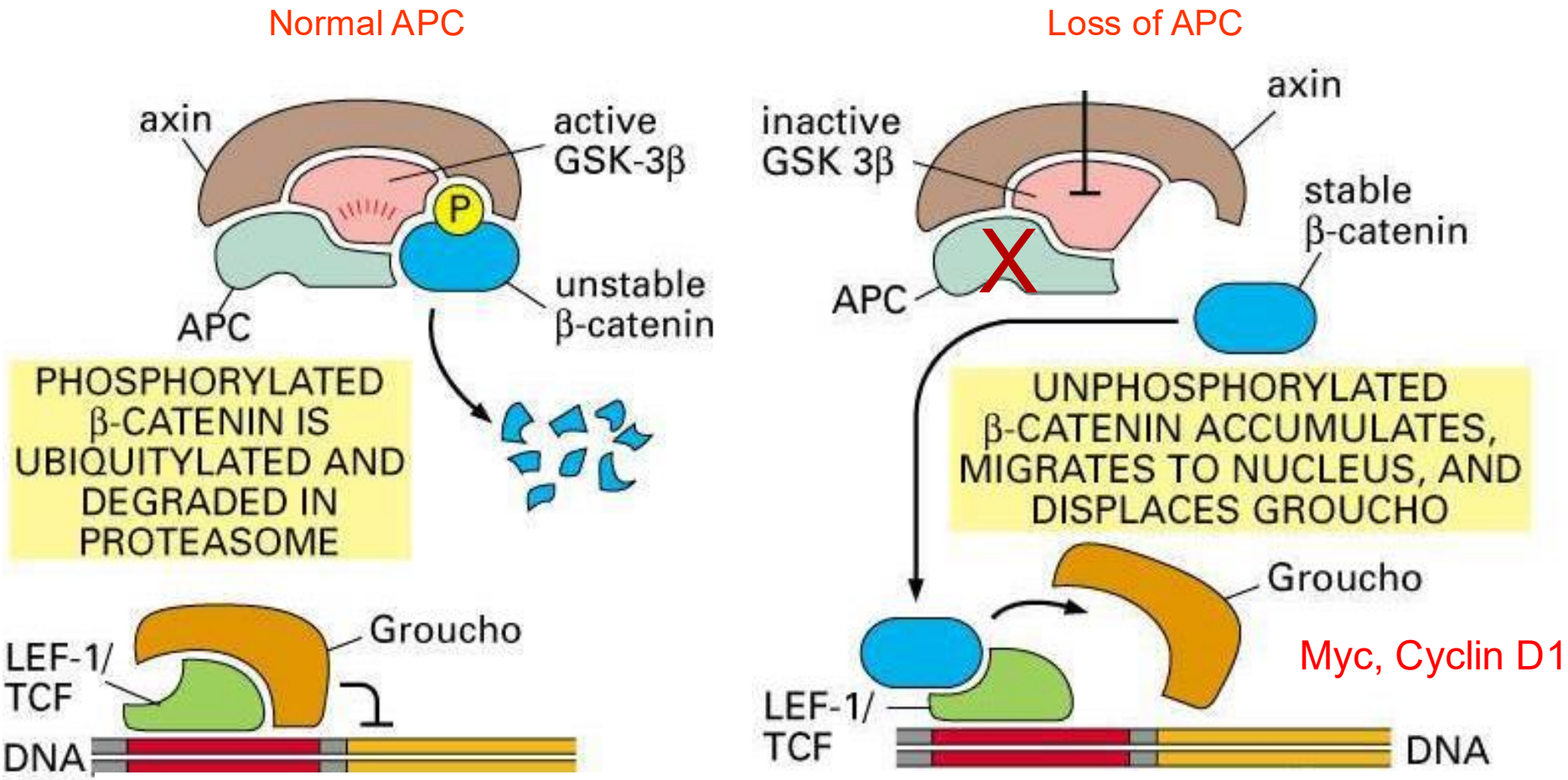
(pRb illustrated as example for mitotic recombination. Note that LOH may equally happen through other mechanisms, e.g., epigenetic silencing of the second allele)

Figure 7.8 *The Biology of Cancer* (© Garland Science 2007)

Alternative genetic alterations may underlie multi-step tumor progression in CRC



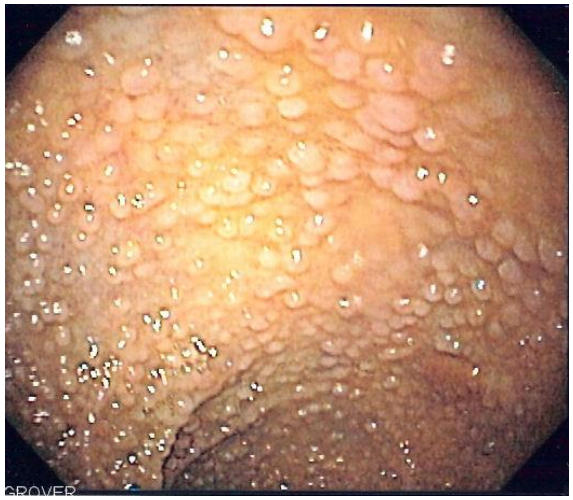
Role of APC in multi-step progression of CRC



APC, adenomatous polyposis coli

Familial adenomatous polyposis (FAP): germ-line inactivation of a single APC allele

- Rare disease; germ-line (monoallelic) mutation in APC is inherited
- One APC allele is mutated in each of the billions of epithelial cells that line the colonic mucosa
- LOH occurs quite frequently (1 in 10,000 cell generations)…
- … so dozens to thousands of small polyps (early adenomas) develop in the colon that lack both APC alleles
- One or several polyps inevitably progress to adenocarcinoma within 20-40 years (through the acquisition of additional, sporadic driver mutations) ⟶ long latency



HNPCC syndrome (hereditary nonpolyposis colorectal cancer)

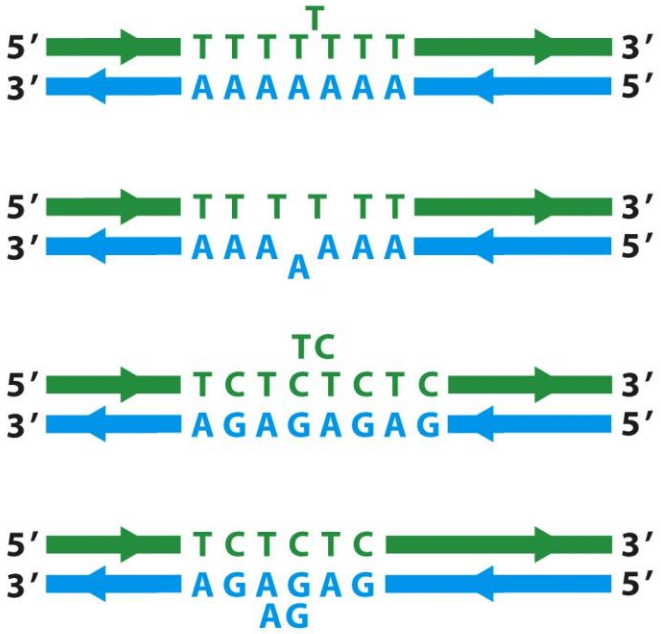
- More commonly known as **Lynch syndrome**
- Rare, germ-line (autosomal dominant) disease
- Benign lesions form at the same frequency/timing as in the normal population
- Once formed, benign lesions (adenomas) may progress rapidly into aggressive invasive adenocarcinomas.
- Responsible of 2-3% of colon cancers (hereditary form), but the same mechanism can occur sporadically and account for another 10% of CRC cases.
- Germ-line mutations affect the DNA repair system: **mismatch (MMR) repair enzymes**

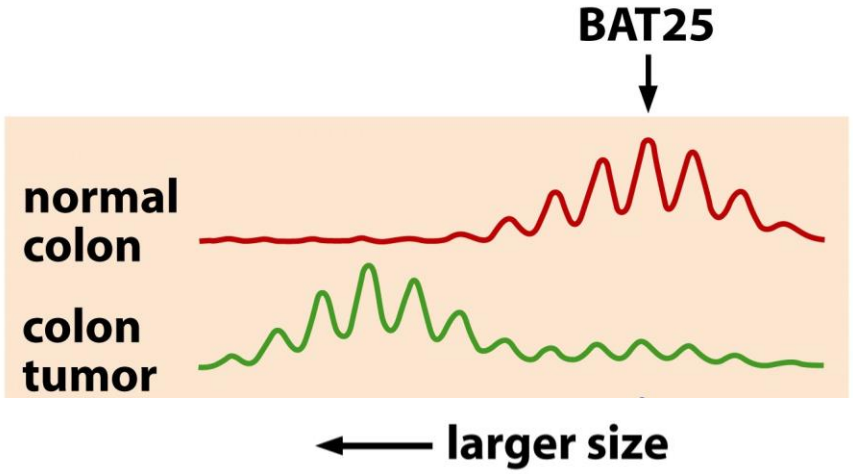
Microsatellites and DNA instability

Errors during DNA polymerization (there are ca 15 DNA polymerases in the human genome, 3 involved in DNA replication)

Errors mainly occur at repetitive regions, **microsatellites**, which are simple sequence repeats of 6-7 bps present in both coding and noncoding sequences
-> **microsatellite instability**

Mismatches in microsatellites

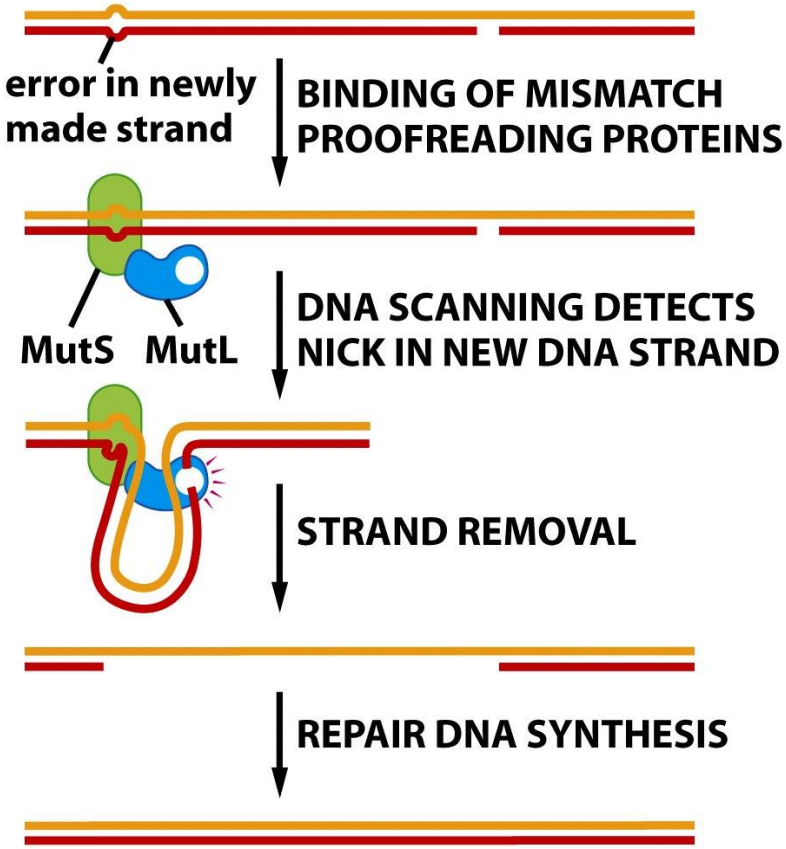




Size of the microsatellite BAT25 is increased in cancer tissue (expansion of dinucleotide repeats CACACA…).

Microsatellite instability is present in many cancer types. It suggests defects in the repair of such mutations in the cancer cells....

Mismatch repair (MMR) enzymes



These enzymes correct mis-matches (wrong pairing) introduced by DNA polymerases

Significance of MMR system (mutation rate):

DNA polymerase mismatches: 10^{-5}

+ repair: 3' -> 5' exonucl. activity: 10^{-7}

+ repair: MMR enzymes: 10^{-9}

MutS homologs (eg. *MSH2/MSH6*)
MutL homolog (eg. *MLH1/PMS1*)

Mutations in the MMR enzymes can be recognized by increased microsatellite instability

HNPCC syndrome (hereditary nonpolyposis colorectal cancer)

- Rare, germ-line (autosomal dominant) disease (Lynch syndrome)
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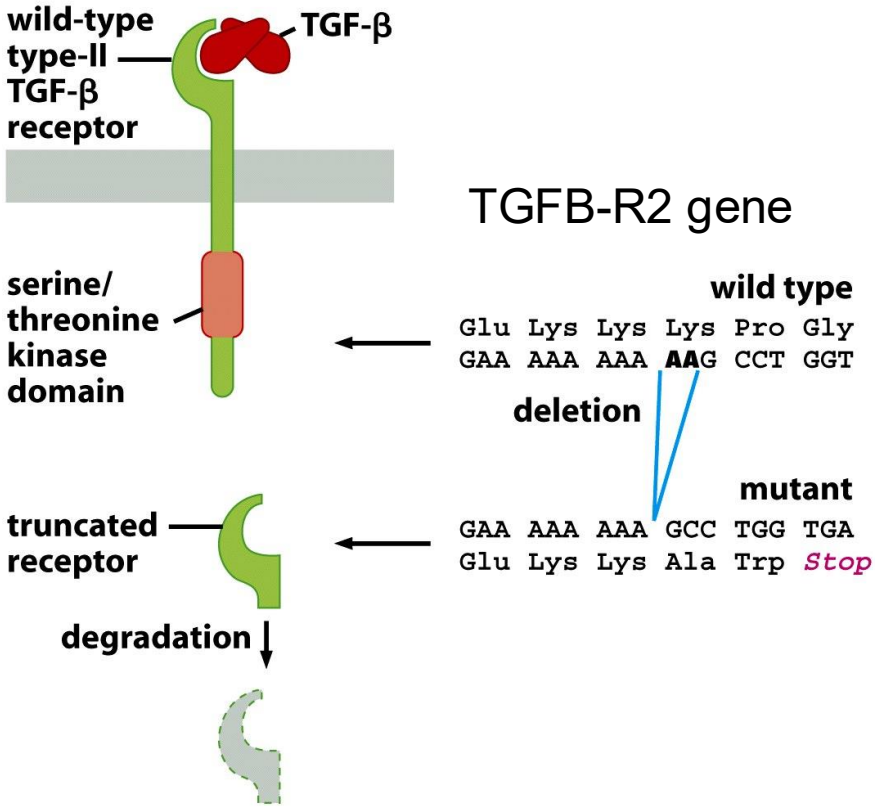
- Germ-line mutations affect the DNA repair system: **mismatch (MMR) repair enzymes**

- One allele is mutated in each of the billions of epithelial cells that line the colonic mucosa; second allele lost by LOH (occasionally in MLH1) or other events, like promoter hypermethylation (MSH2).
- Mutations in MMR enzymes increase the rate of mutations in **microsatellites**: short DNA repeats like AAAAAAA (A7) contained in some genes (in addition to non-coding regions).
- Such repeats cause DNA polymerases to occasionally skip or duplicate one A, leading to, e.g., TTTTTT (T6) or T8 in the copied strand.
- In the absence of MMR enzymes, these errors are not corrected and mutations occur rapidly ⟶ short latency

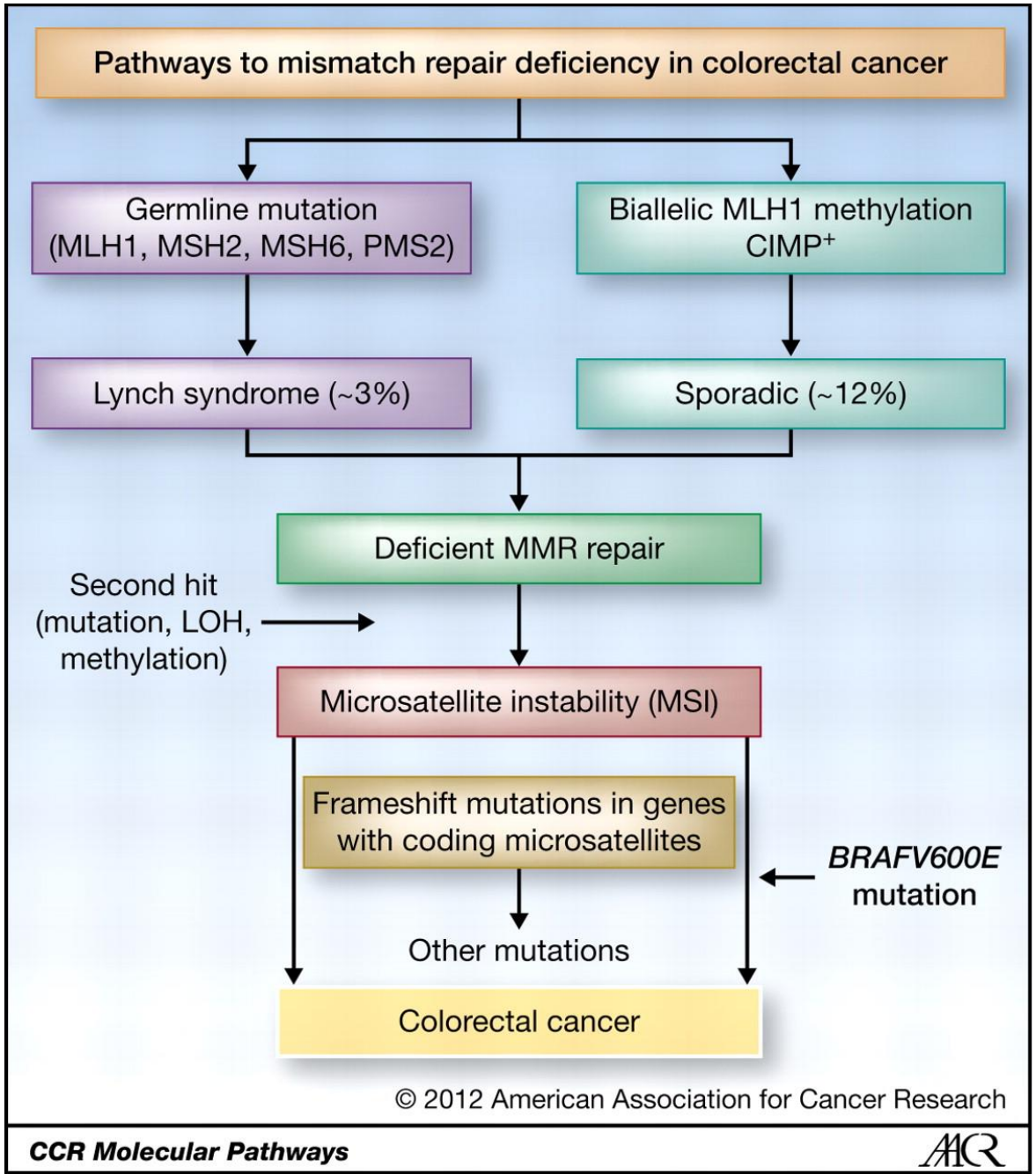
HNPCC syndrome (hereditary nonpolyposis colorectal cancer)

OMIM name	Genes implicated in HNPCC	Frequency of mutations in HNPCC families
HNPCC1	**MSH2**	approximately 60%
HNPCC2	**MLH1**	approximately 30%
HNPCC5	MSH6	7-10%
HNPCC4	PMS2	relatively infrequent, <5%
HNPCC3	PMS1	case report
HNPCC6	TGFBR2	case report
HNPCC7	MLH3	disputed



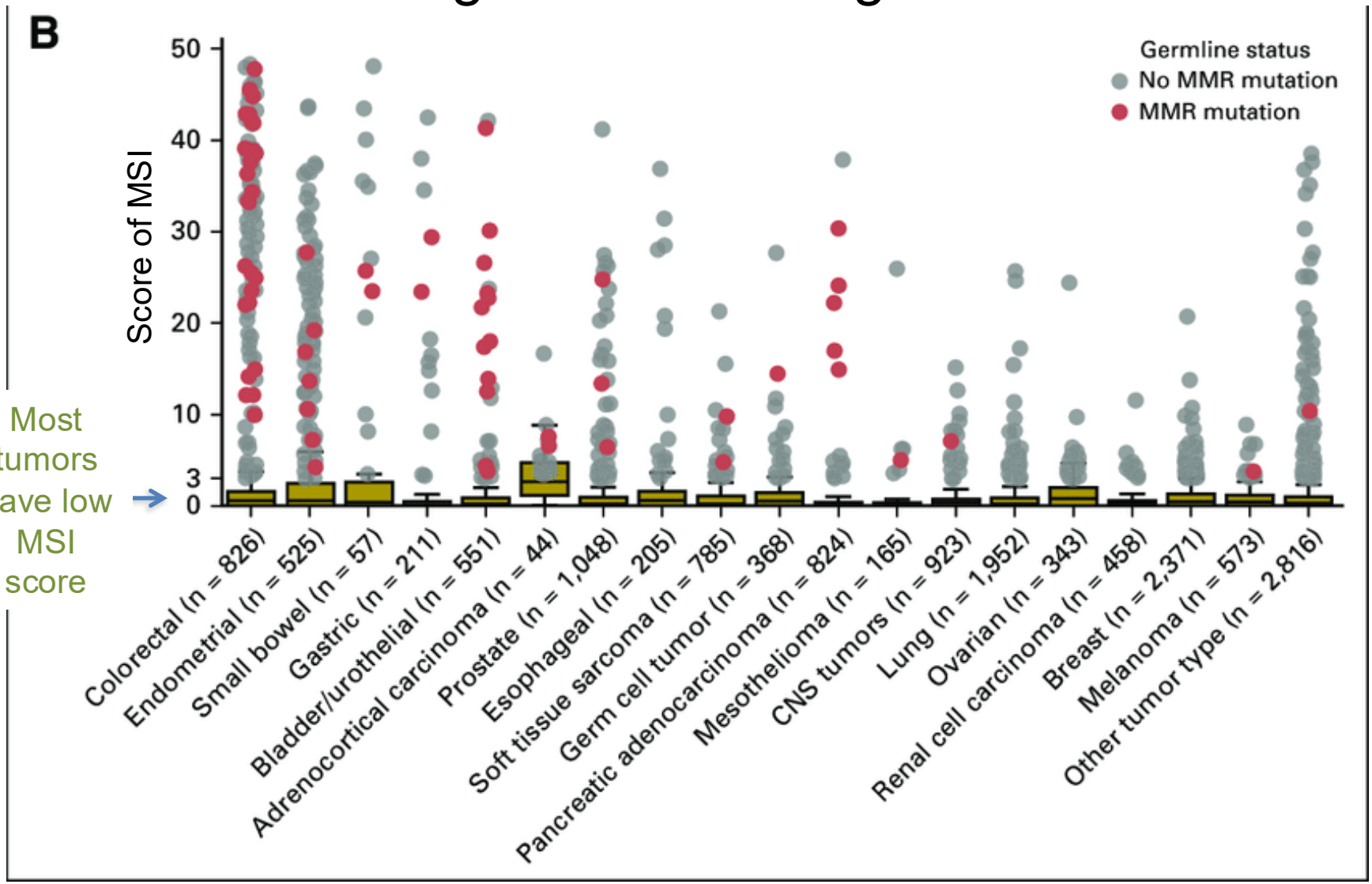
A microsatellite is present in the TGFB-R2 gene. Loss of TGFB-R2 leads to loss of this important anti-mitogenic signal. This homozygous mutation is present in 90% of HNPCC colon cancers

Mismatch repair (MMR) deficiency in colorectal cancer



About 15% of all CRCs are due to microsatellate instabilty (MSI)

Microsatellite instability (MSI) is variably frequent (1-12%) across cancer types but is not always associated with *known* germline MMR gene mutations



Latham et al., Journal of Clinical Oncology 37(4):JCO.18.00283

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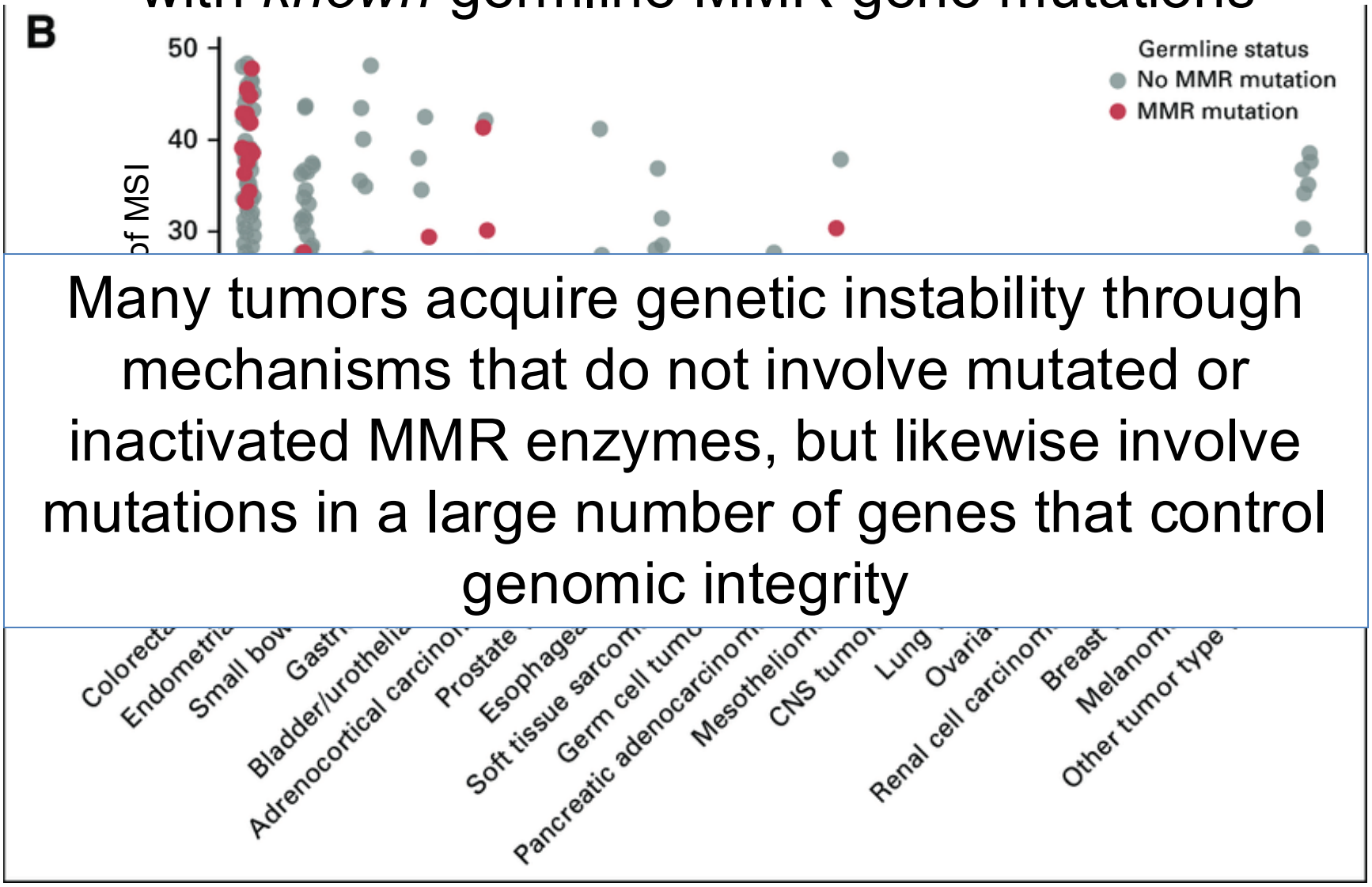
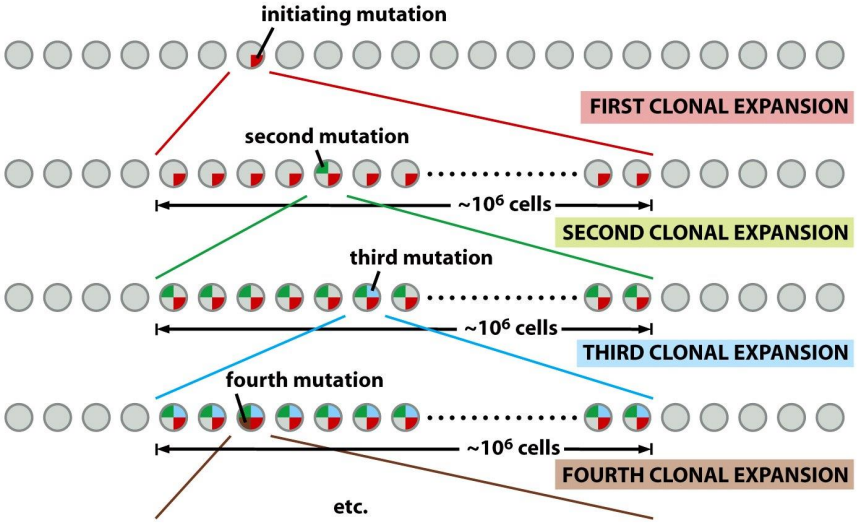


Many tumors acquire genetic instability through mechanisms that do not involve mutated or inactivated MMR enzymes, but likewise involve mutations in a large number of genes that control genomic integrity

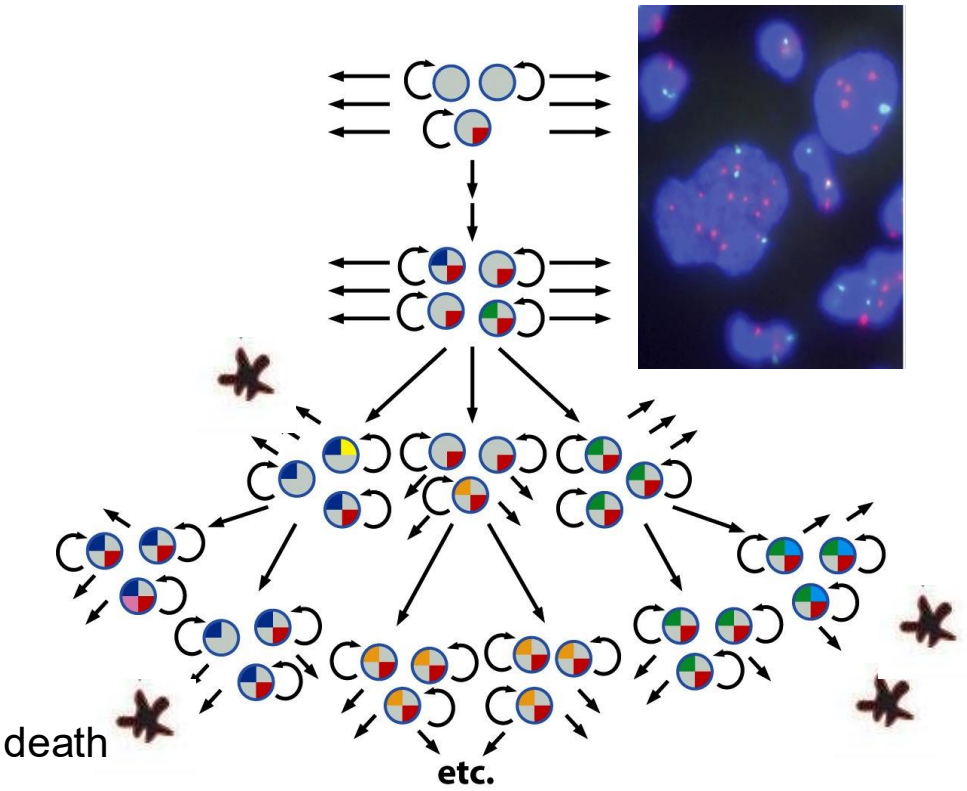
Latham et al., Journal of Clinical Oncology 37(4):JCO.18.00283

Because of genetic instability, the rate of mutations exceeds the rate at which Darwinian selection positively selects fit clones

Theoretical



Reality



Sequencing of cancer genomes reveals thousands of mutations in each tumor, which reflect both the presence of multiple clonal populations and the occurrence of hundreds of (neutral) mutations in each clone

Genetic instability and Darwinian selection

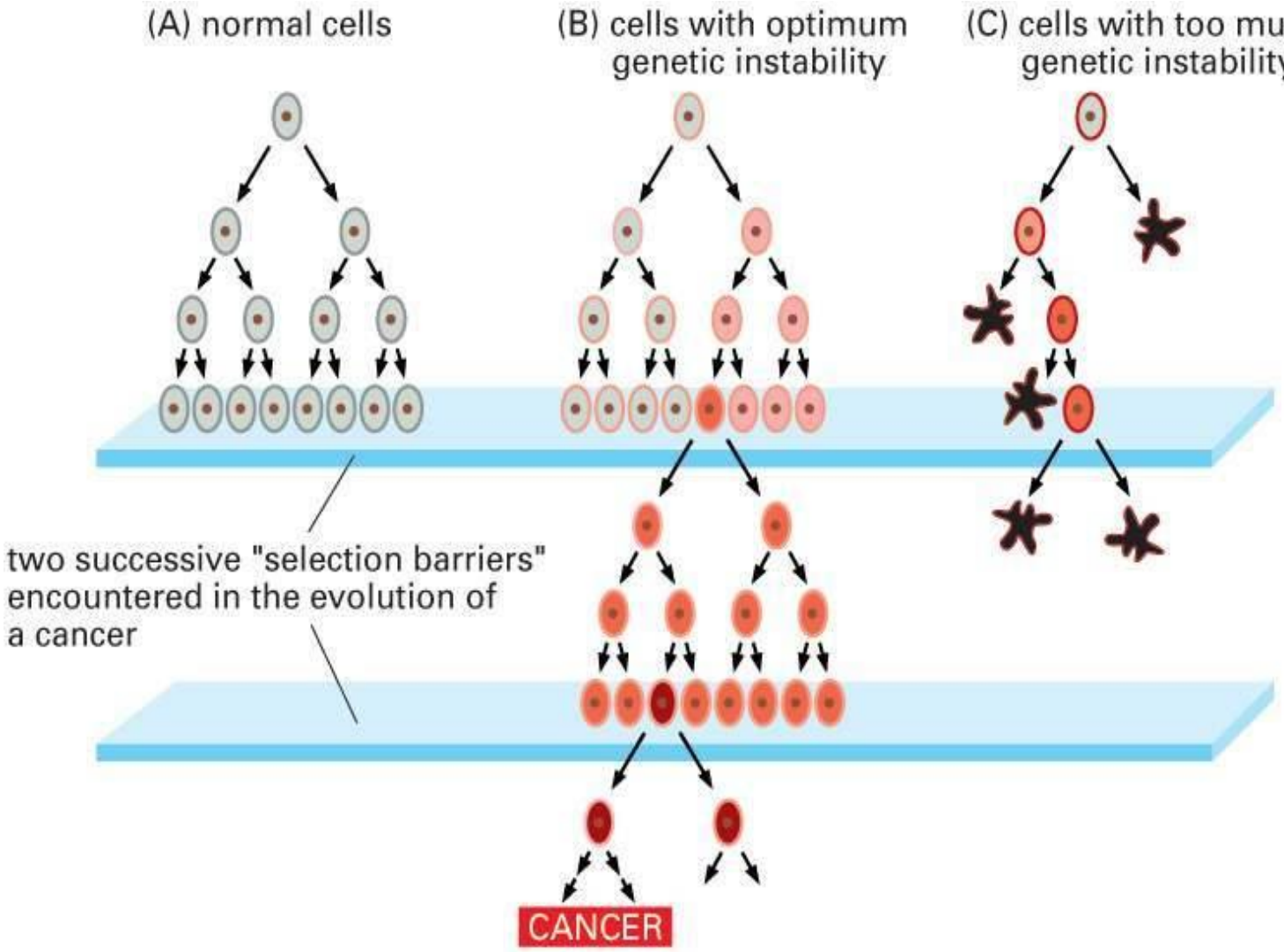


Figure 23–13. Molecular Biology of the Cell, 4th Edition.

Inherited (rare) mutations, sporadic mutations, LOH, and genetic instability, all contribute to the onset of cancer

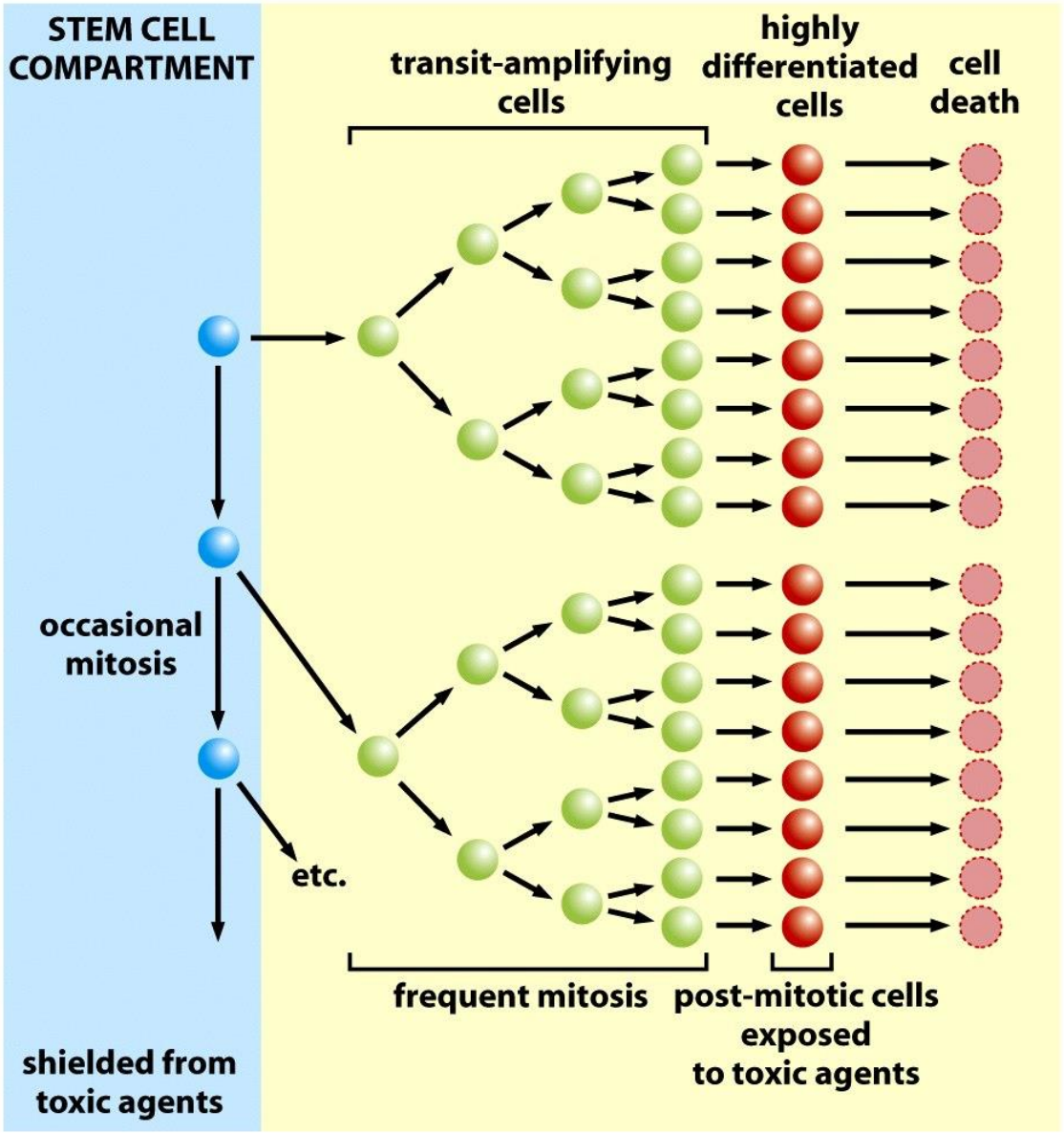
How do our cells cope with this?

Low rates of stem cell divisions

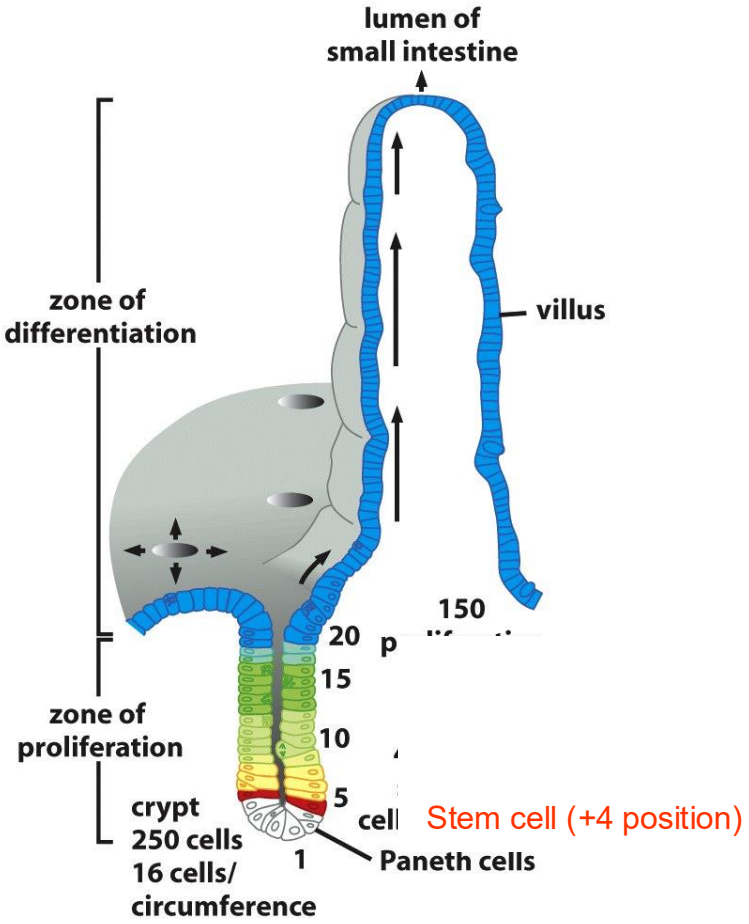
Physical and biochemical protection from mutagenic events

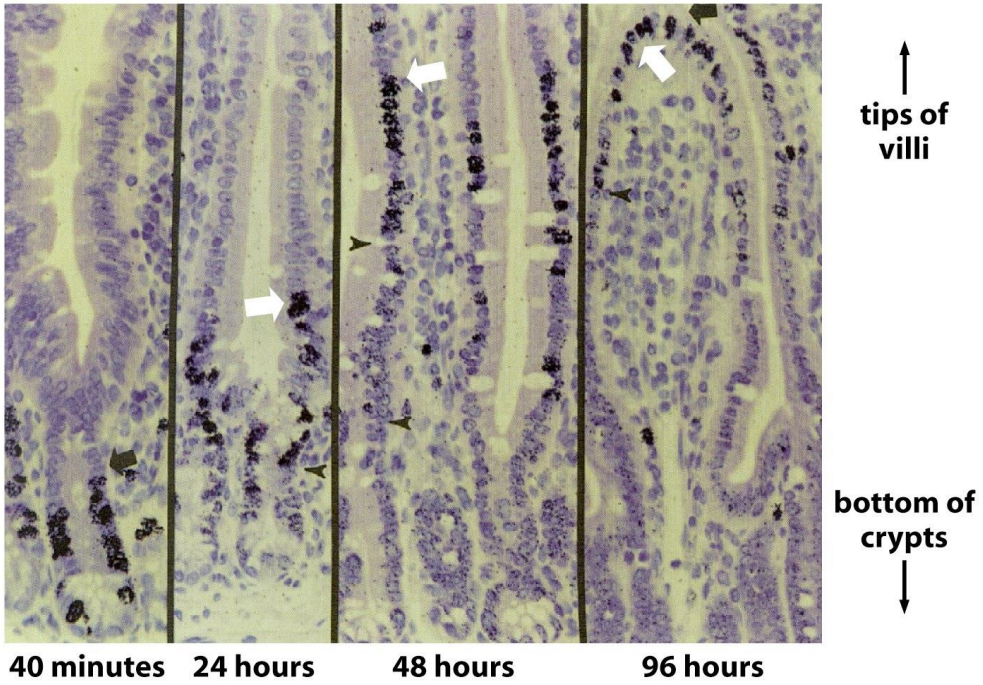
DNA repair enzymes

Stem cells


STEM CELL COMPARTMENT
transit-amplifying cells
highly differentiated cells
cell death
occasional mitosis
etc.
frequent mitosis
post-mitotic cells exposed to toxic agents
shielded from toxic agents

Intestinal stem cells





Labeling of the DNA of rapidly proliferating cells (pulse) and analysis after xx hours (chase). The marker traces the fate of the proliferating cells

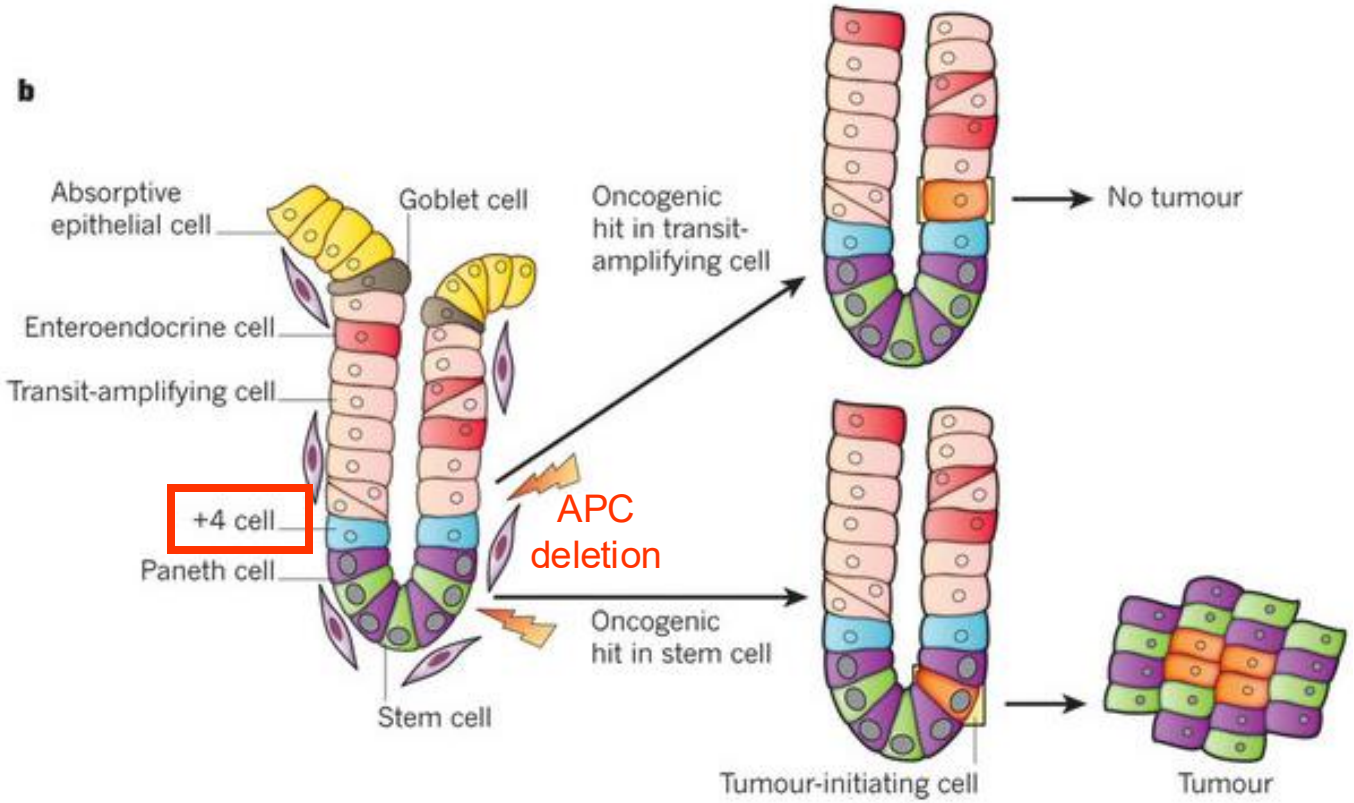
+4 stem cells have low proliferation rates, but they generate rapidly proliferating progenitors (+5-10 position) that – while migrating toward the tip of the villus – differentiate into mature enterocytes and goblet cells, which are short lived. The whole program takes only 3-5 days!

Normal stem cells may be the cells of origin of cancer

LETTERS

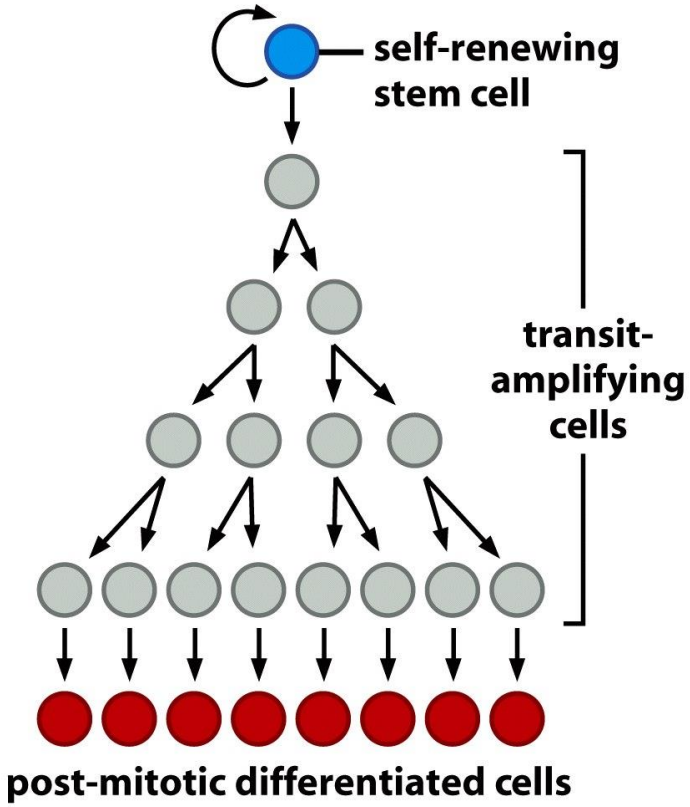
Crypt stem cells as the cells-of-origin of intestinal cancer

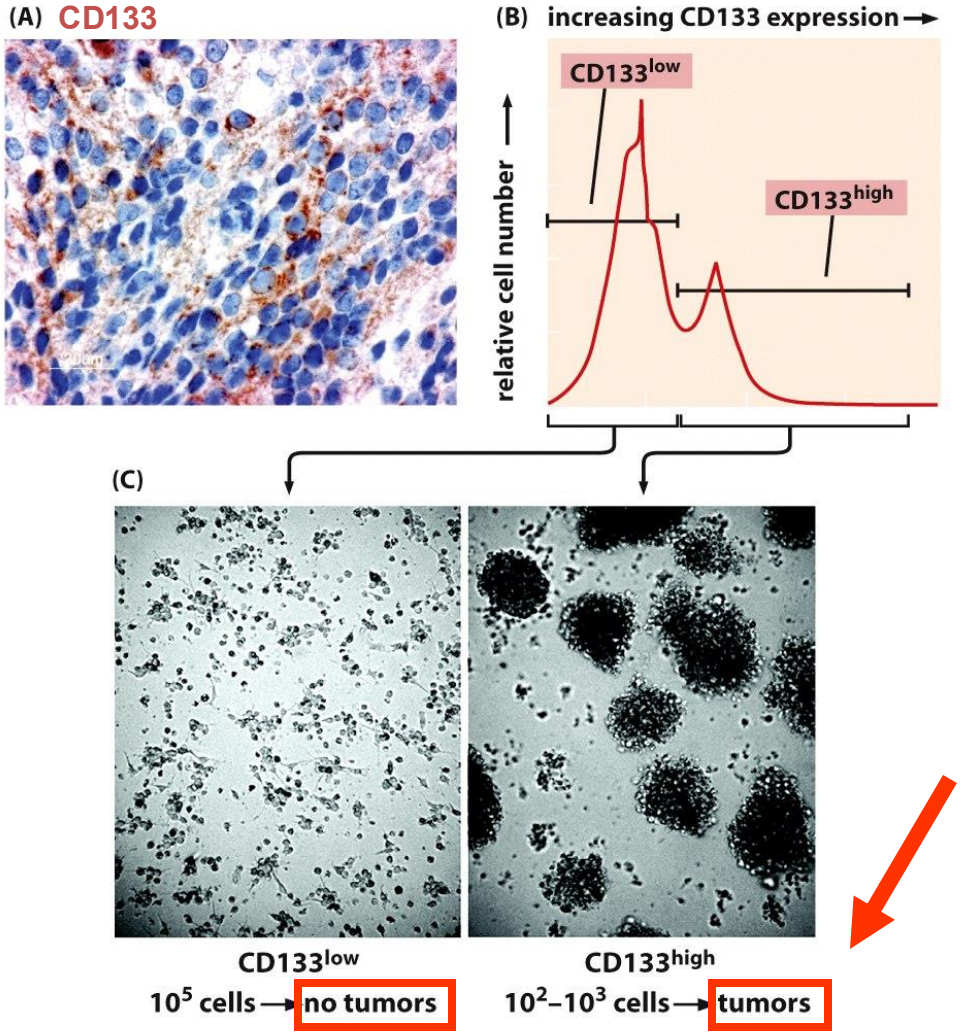
Nick Barker[1]*, Rachel A. Ridgway[2]*, Johan H. van Es[1], Marc van de Wetering[1], Harry Begthel[1], Maaike van den Born[1], Esther Danenberg[1], Alan R. Clarke[3], Owen J. Sansom[2] & Hans Clevers[1]



Cancer stem cells (CSCs) can be identified in many cancer types and express stem cell markers

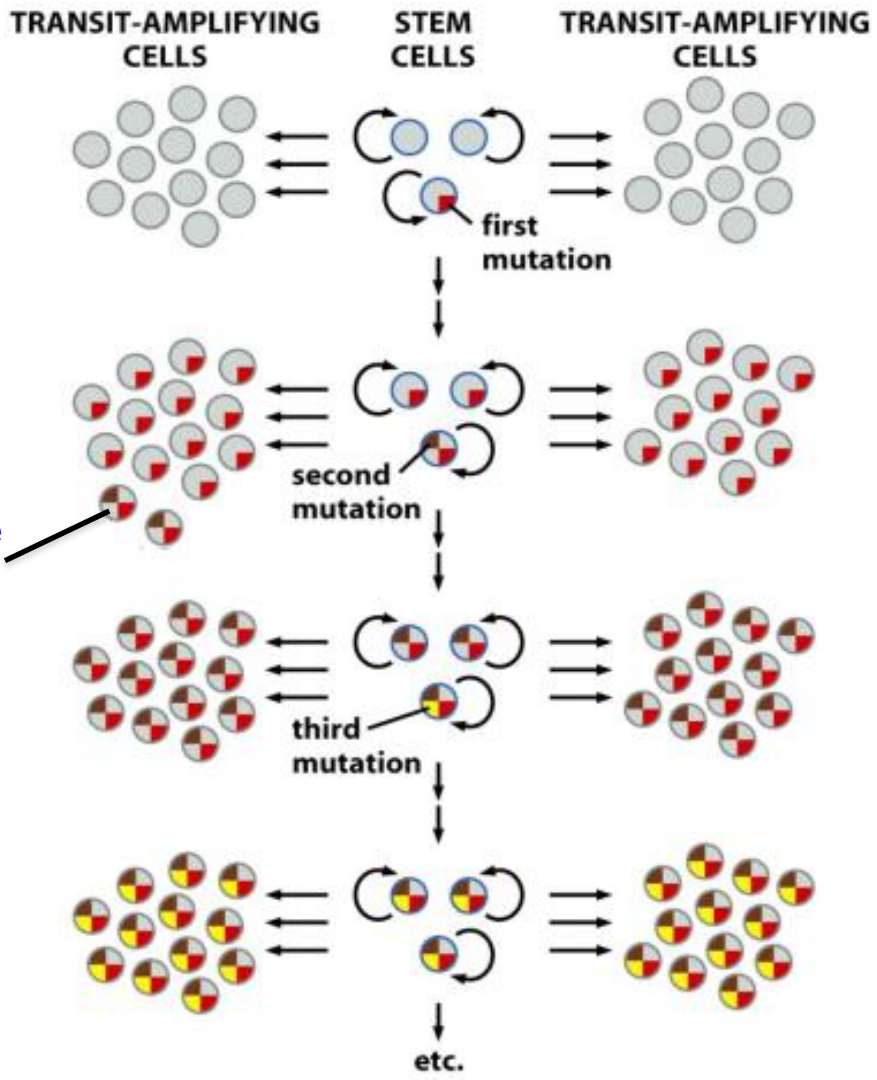
Identification of CSCs in glioblastoma





Based on the cancer stem cell hypothesis, only a minor proportion of the cancer cells in a tumor can support clonal expansion and tumor growth. So, mutations occurring in non-CSCs may be inconsequential to tumor progression (yet are detected by genome sequencing)

A revised model of multi-step progression and Darwinian selection



Any third mutation here is not propagated

CANCER

Inherited (rare) mutations, sporadic mutations, LOH, and genetic instability, all contribute to the onset of cancer

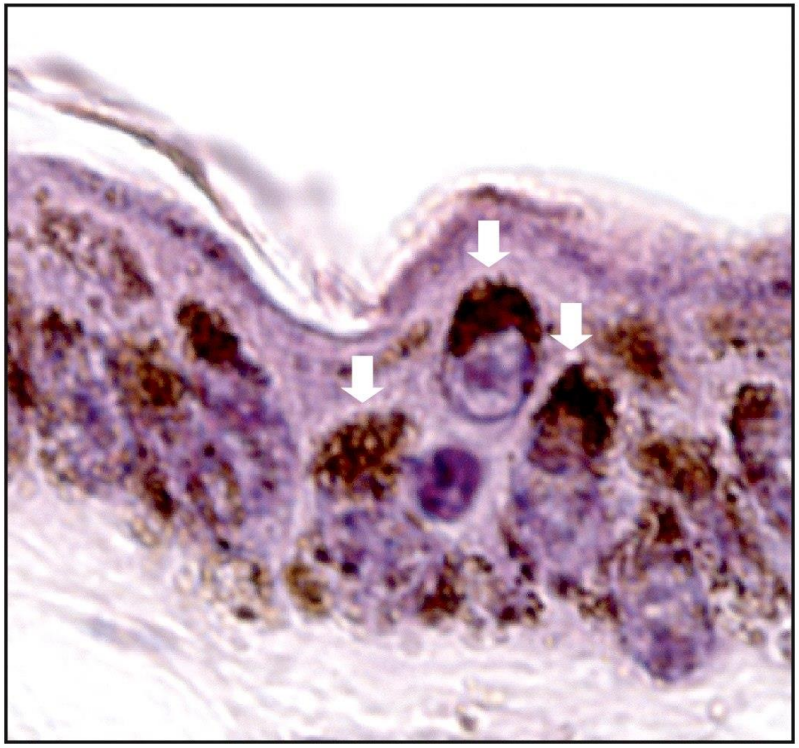
How do our cells cope with this?

Low rates of stem cell divisions

Physical and biochemical protection from mutagenic events

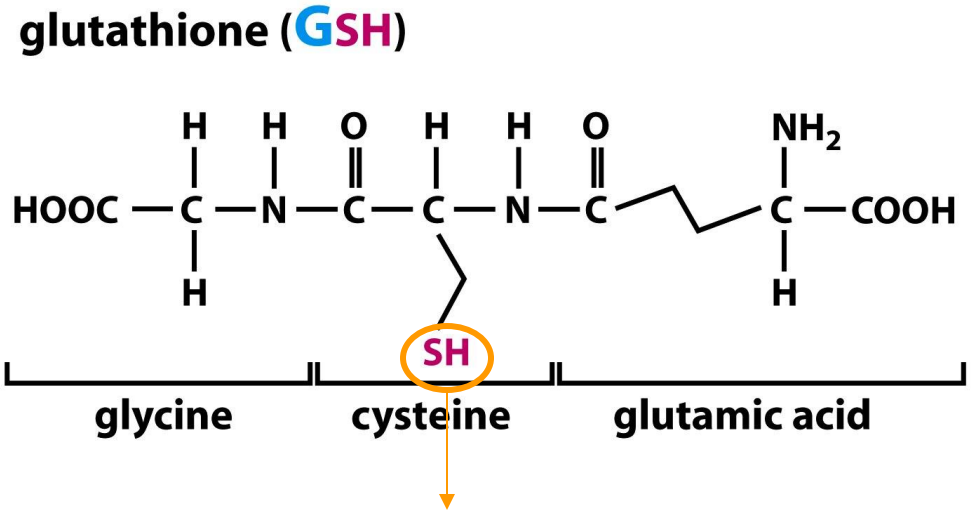
DNA repair enzymes

Physical shielding of keratinocyte nuclei from UV radiation



Keratinocytes are protected from the UV light by melanin pigments, which are transferred from melanocytes to the keratynocytes

Glutathione-S-transferases (GSTs)



Sulphydryl group reacts with reactive compounds

- Glutathione is a tripeptide that is used by GSTs to “neutralize” toxic molecules (eg. aflatoxins)
- Some cancers downregulate GSTs via promoter methylation; 90% of prostate adenocarcinomas have low GST levels
- Individuals with two null alleles of one key GST have 4-fold higher risk of developing myelodysplastic syndrome (MDS) compared to homozygous individuals.

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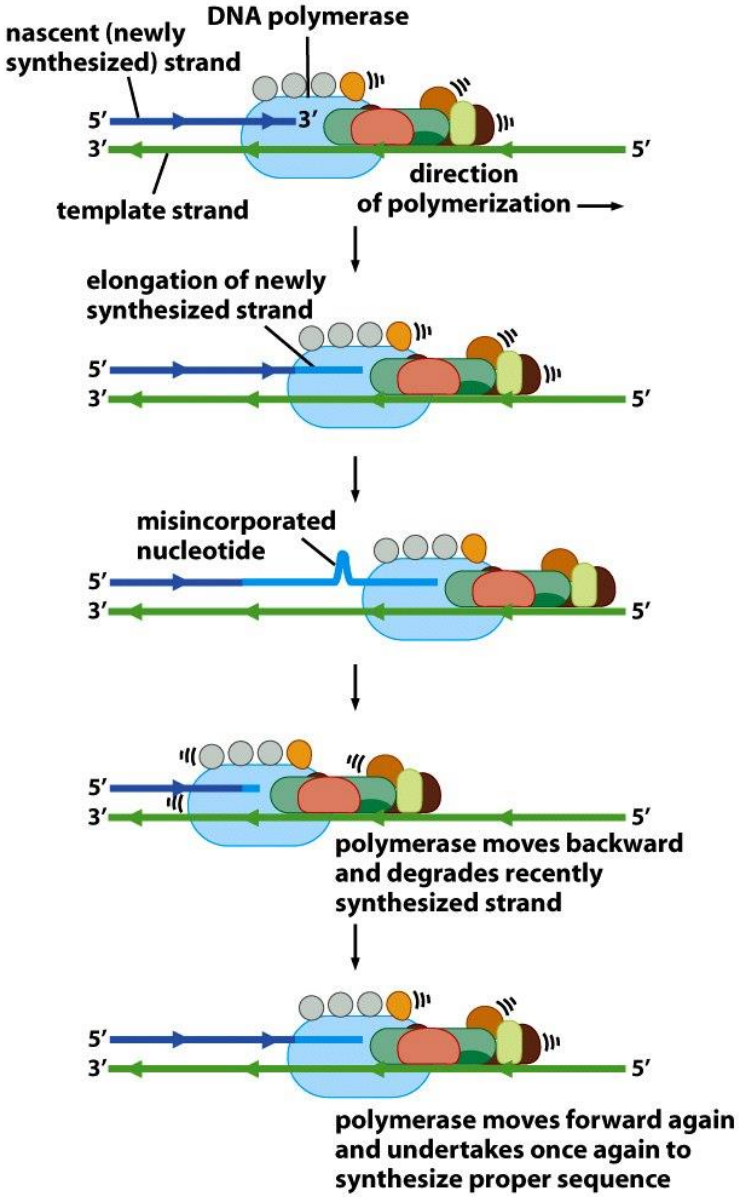
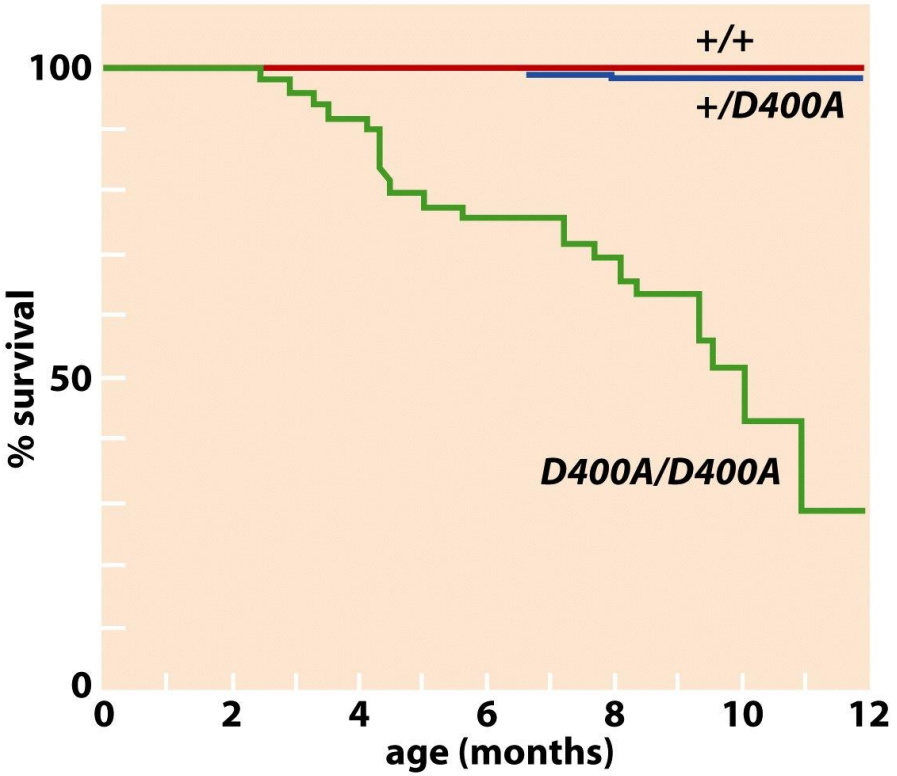
DNA repair enzymes

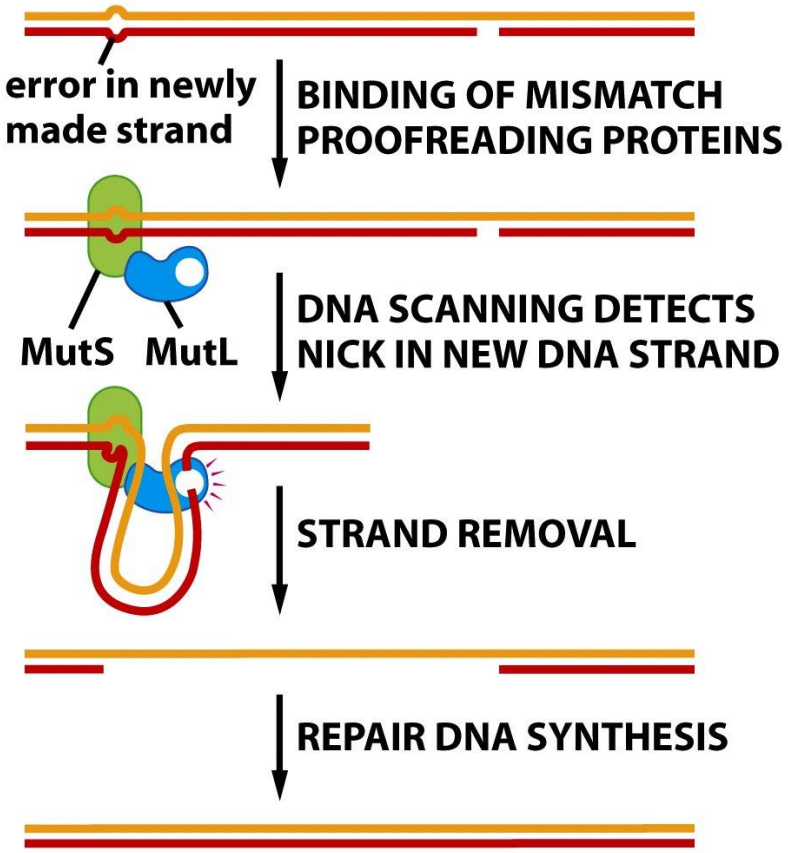
Repair of mutated DNA: Exo-nuclease activity of DNA polymerases



Mice carrying a DNA pol with inactivated exo-nuclease activity



Mismatch repair (MMR) enzymes



These enzymes correct mis-matches (wrong pairing) introduced by DNA polymerases

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DNA polymerase mismatches: 10^{-5}

+ repair: 3' -> 5' exonucl. activity: 10^{-7}

+ repair: MMR enzymes: 10^{-9}

MutS homologs (eg. *MSH2/MSH6*)
MutL homolog (eg. *MLH1/PMS1*)

Mutations in the MMR enzymes can be recognized by increased microsatellite instability

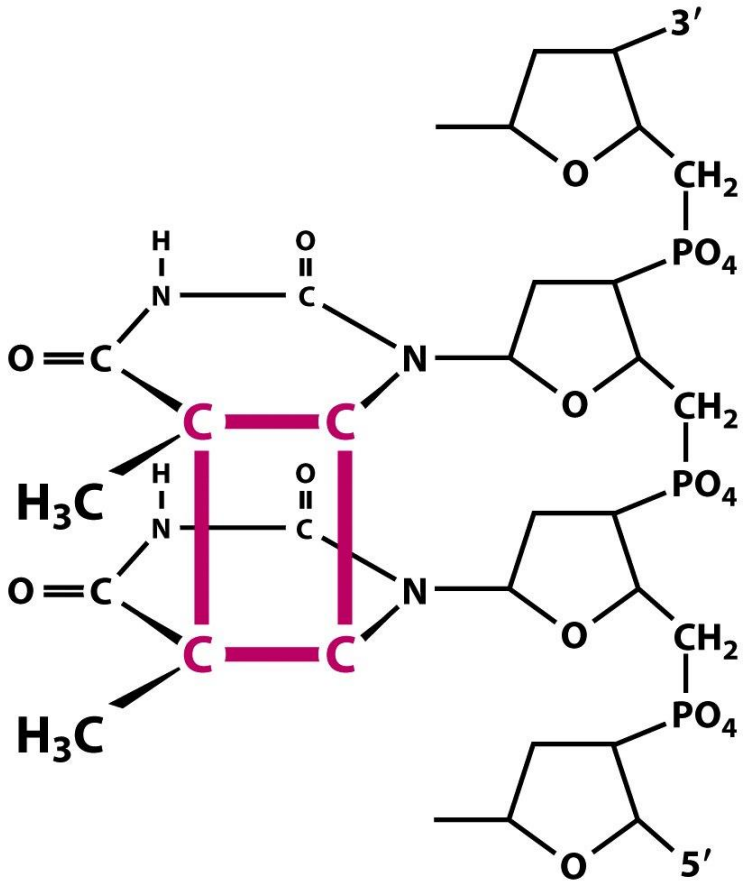
Nucleotide excision repair (NER)

The NER system is usually employed to correct pyrimidine dimers and DNA adducts. P53 activates several genes involved in NER

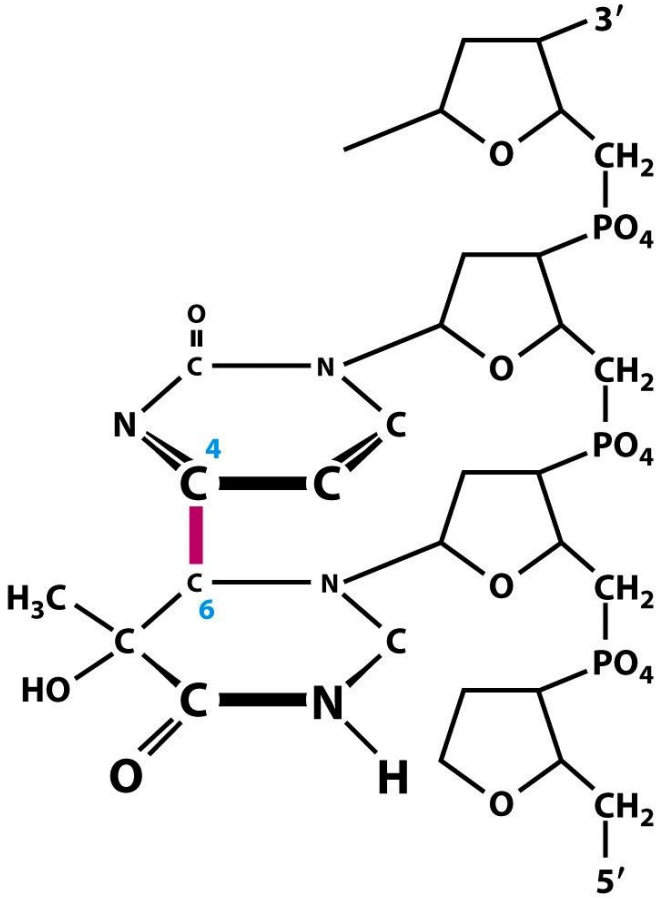
Pyrimidine dimers

- UV light induces intra-strand pyrimidine dimers
- UV induces 60% TT, 30% TC dimers, and 10% CC -> frequent in p53
- These are stable mutations that can be repaired by NER enzymes

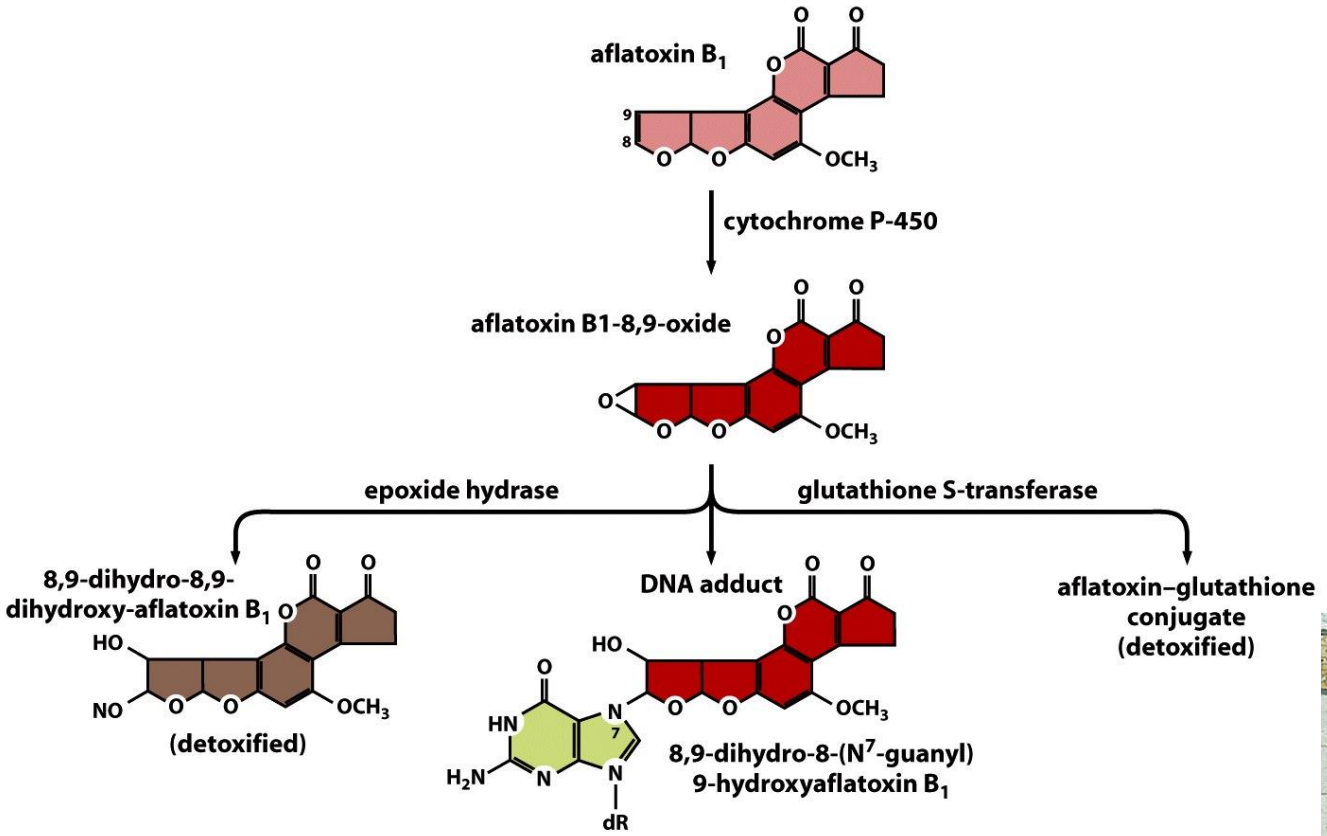
Cyclobutane pyrimidine dimers

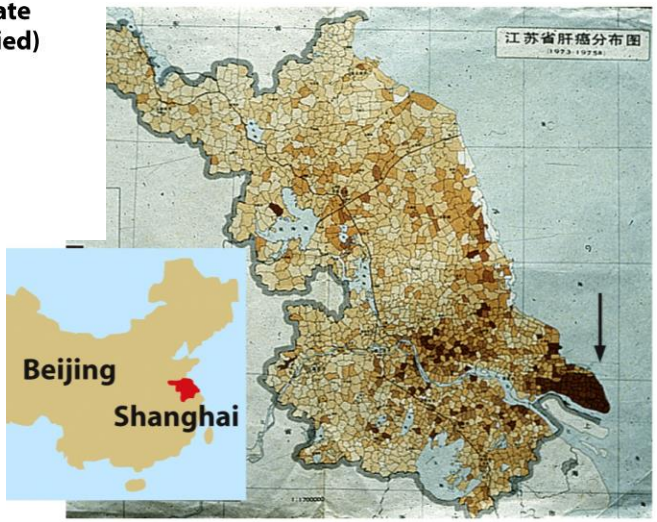


Pyrimidine pyrimidinone



DNA adducts (aflatoxin B1)

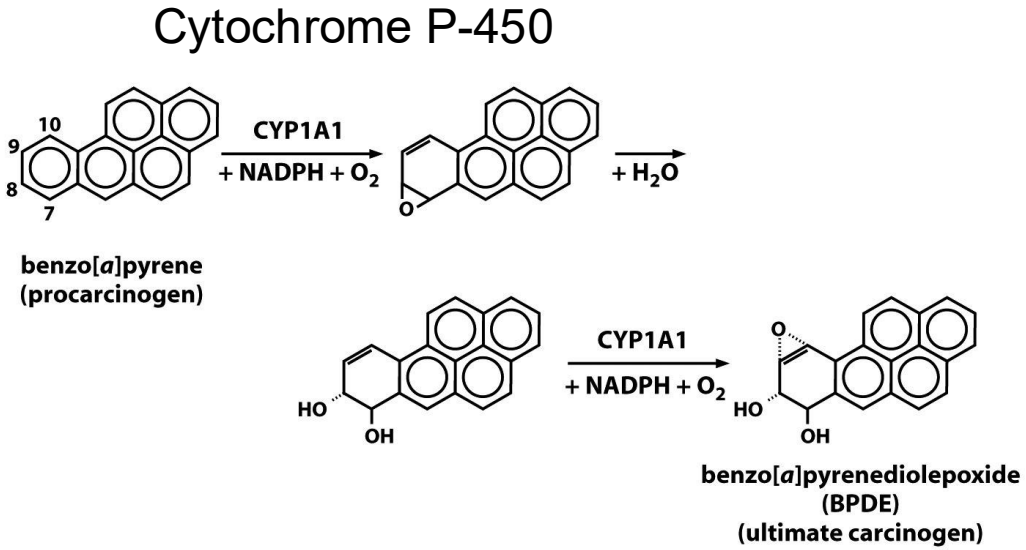


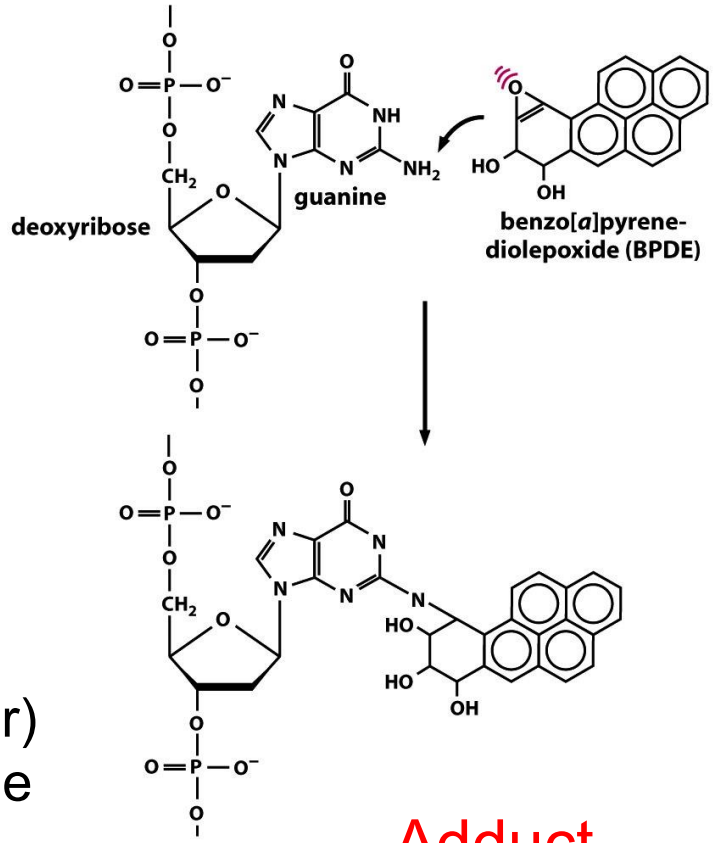


Many of the HCC cases in the Jiangsu province were registered in the Qidong peninsula (arrow), a very humid area

Produced by *Aspergillus flavus,* which grows in corn/peanuts/grain stores of humid areas. Induces HCC (commonly a G -> T transversion in *p53*)

DNA adducts (benzopyrene)



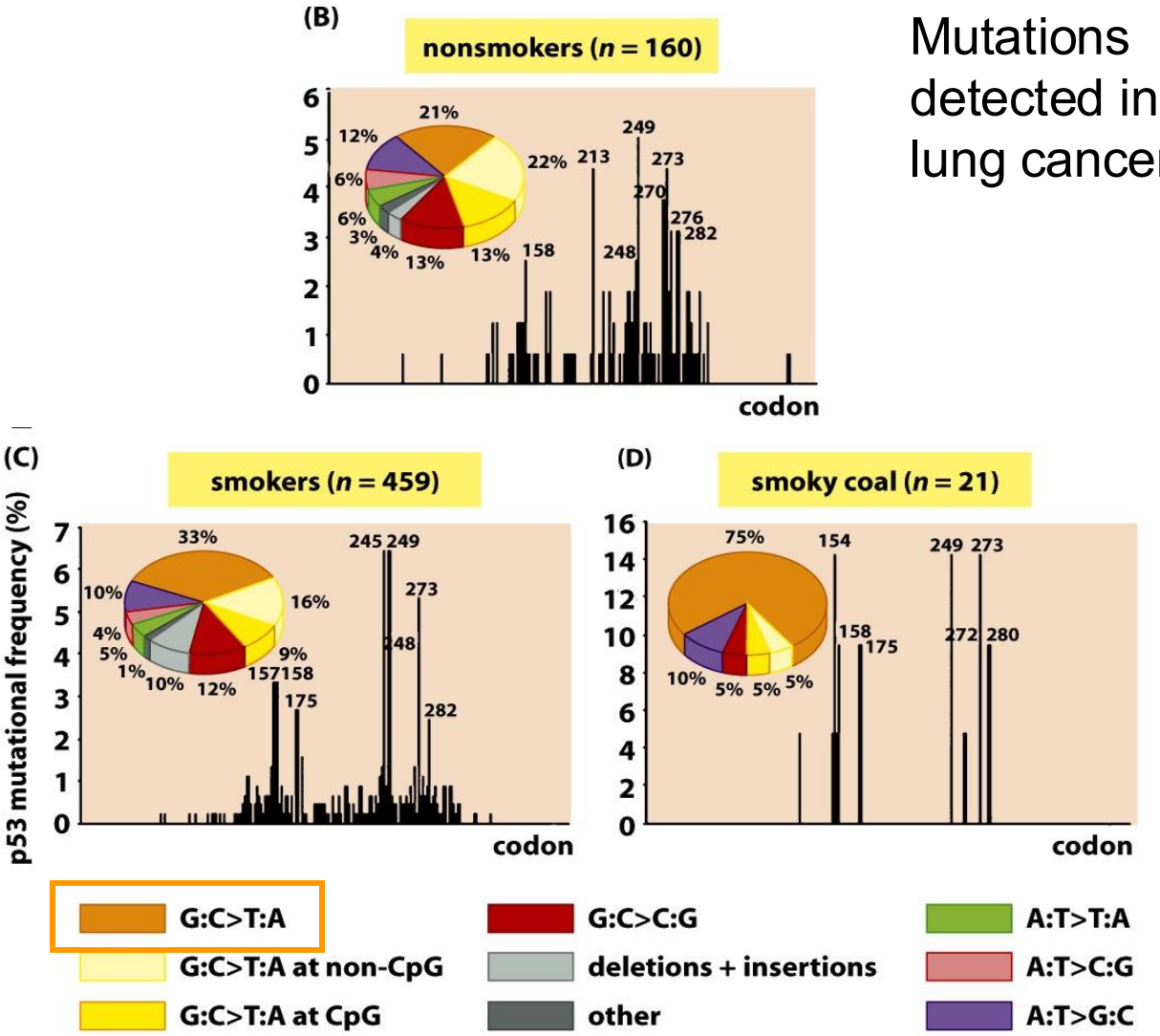


Benzo(a)pyrene (contained in cigarette tar) is a pro-carcinogen. It is converted into the carcinogen BPDE by CytP-450. BPDE reacts with the amine group of G. This adduct can lead to a G -> T transversions

Adduct

p53 mutations in lung cancer implicate benzopyrene as a carcinogen

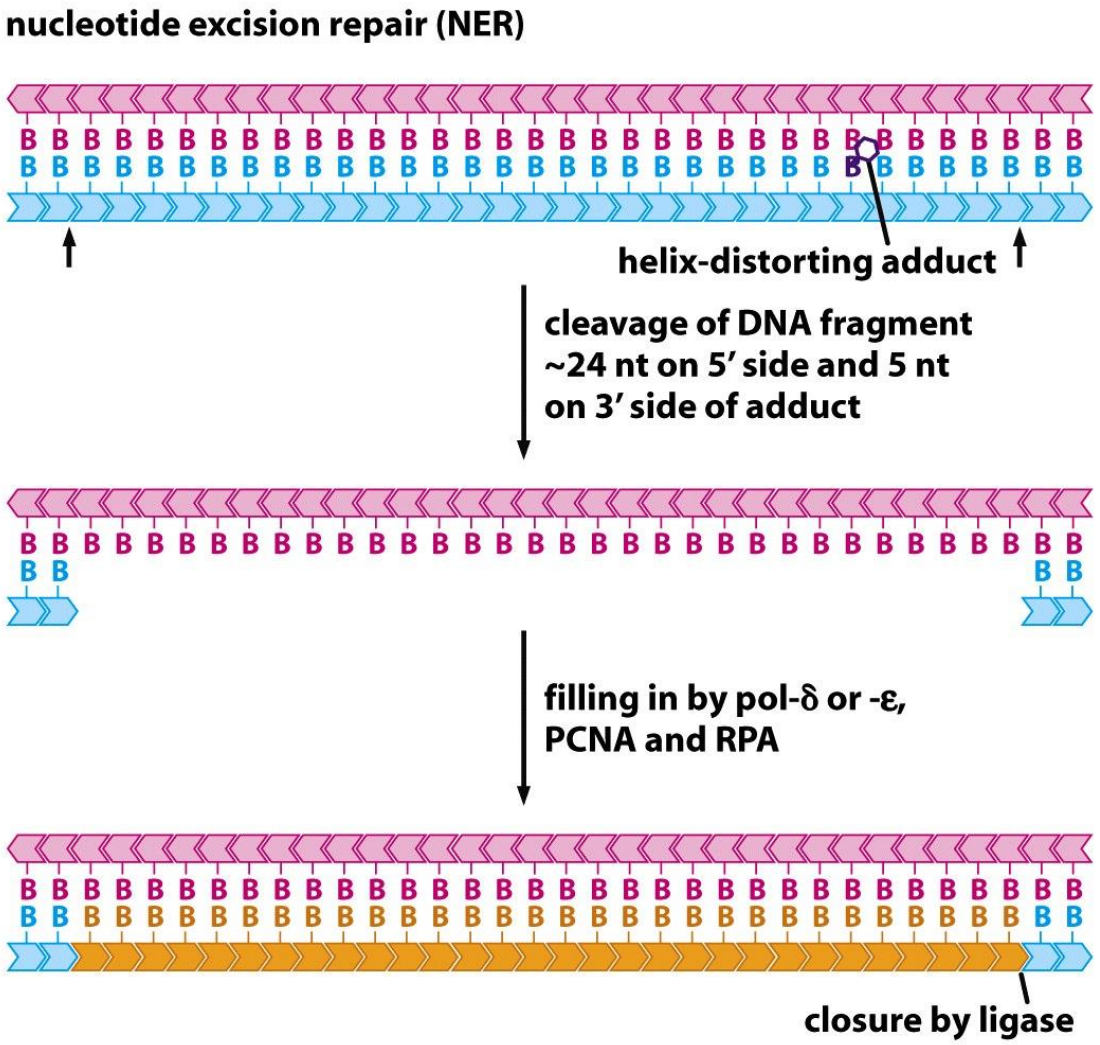
G->T transversions are caused by benzopyrene. Their frequency in *p53* alleles is increased in the lung cancers of smokers or people exposed to smoky coal



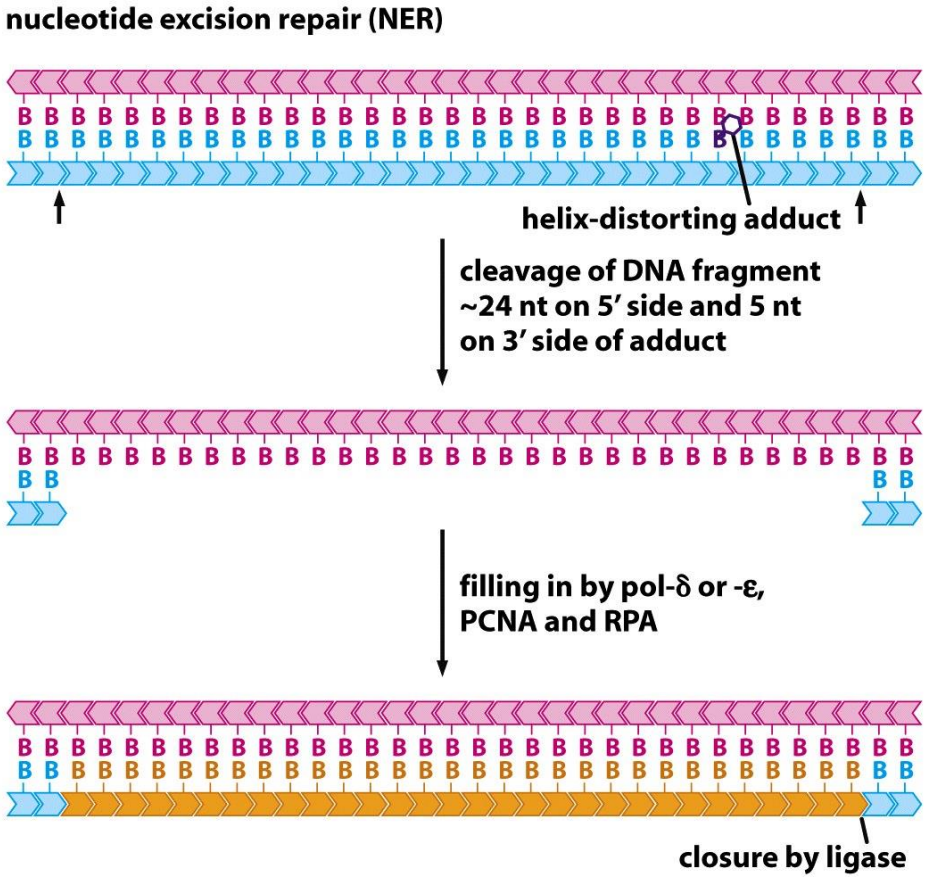
Mutations detected in lung cancers

Nucleotide excision repair (NER)

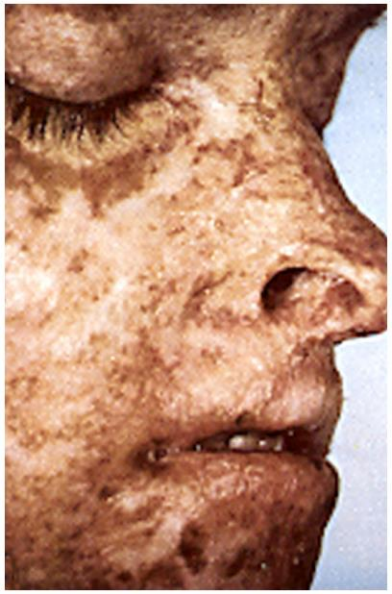
The NER system is usually employed to correct pyrimidine dimers and DNA adducts. P53 activates several genes involved in NER



Defects in nucleotide excision repair (NER)



- The NER system is usually employed to correct pyrimidine dimers and DNA adducts.
- P53 activates several genes involved in NER
- Defects in NER increase incidence of epithelial tumors (eg., skin SCC and BCC)
- *Xeroderma pigmentosum* is caused by inherited mutations in the NER genes

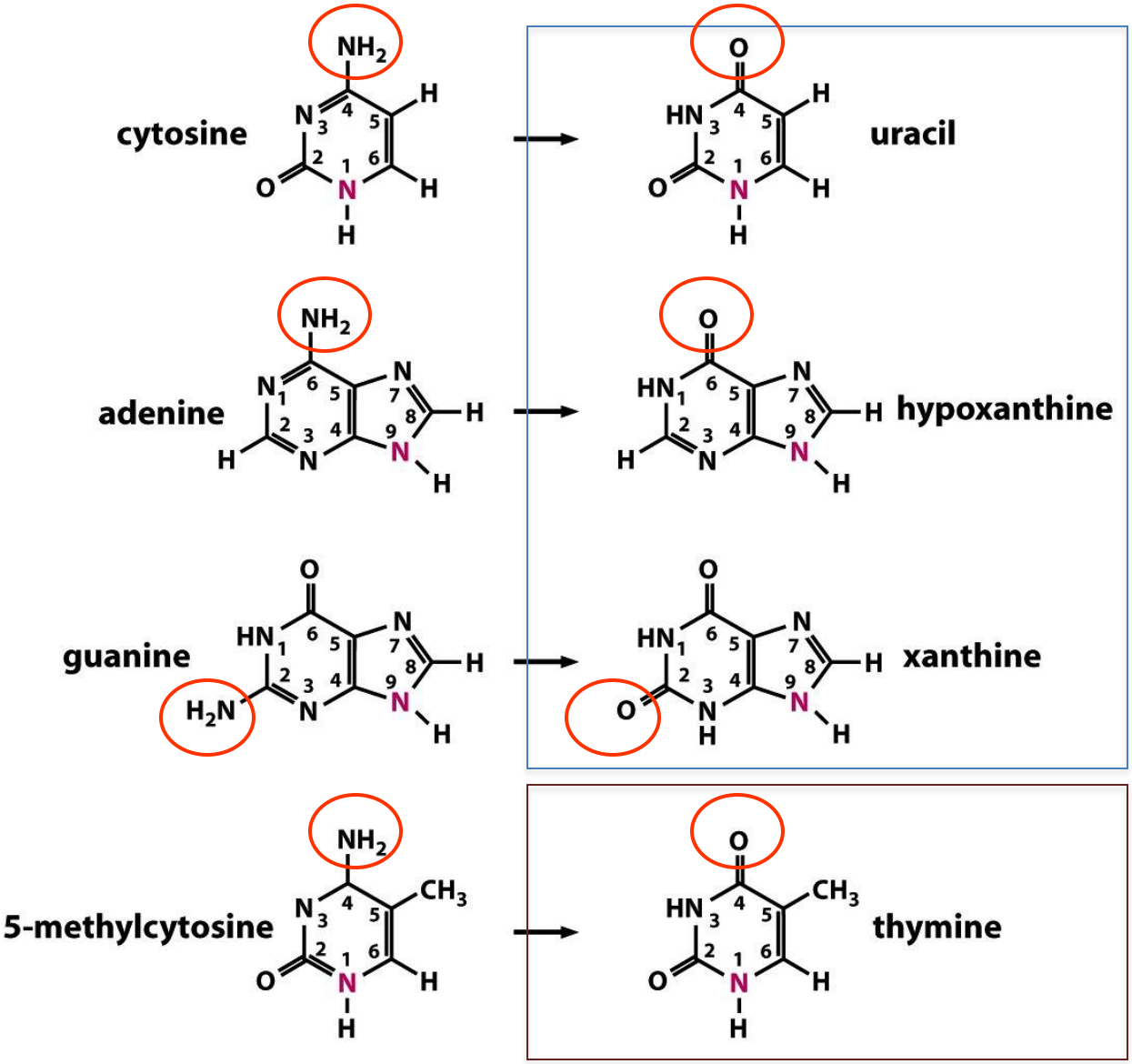


Base excision repair (BER)

The BER system is usually employed to correct deamination

Deamination

- Spontaneous mutations occurring in the genome

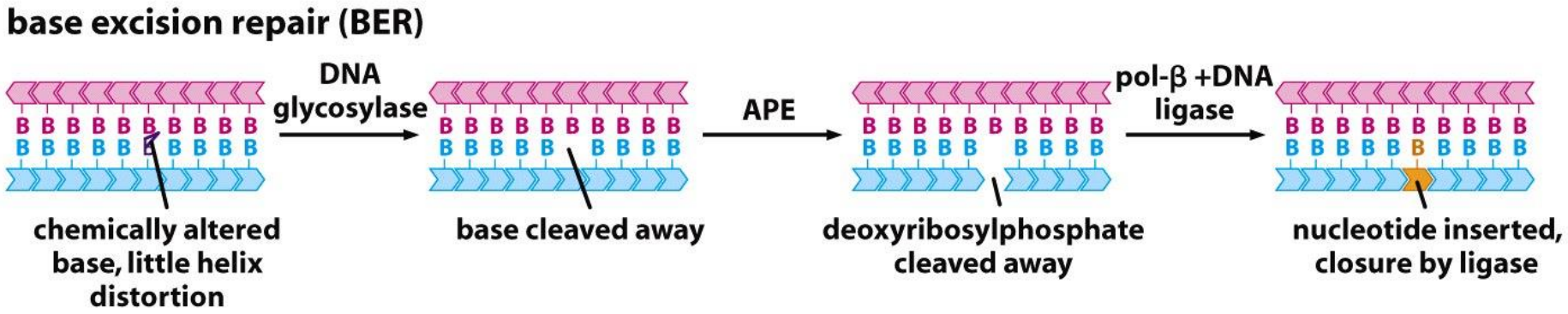


Recognized by the DNA repair machinery (anomalous bases in the DNA)

Not recognized as abnormal nucleotide!! This is the most frequent mutation in tumors of visceral organs (67%)

Base excision repair (BER)

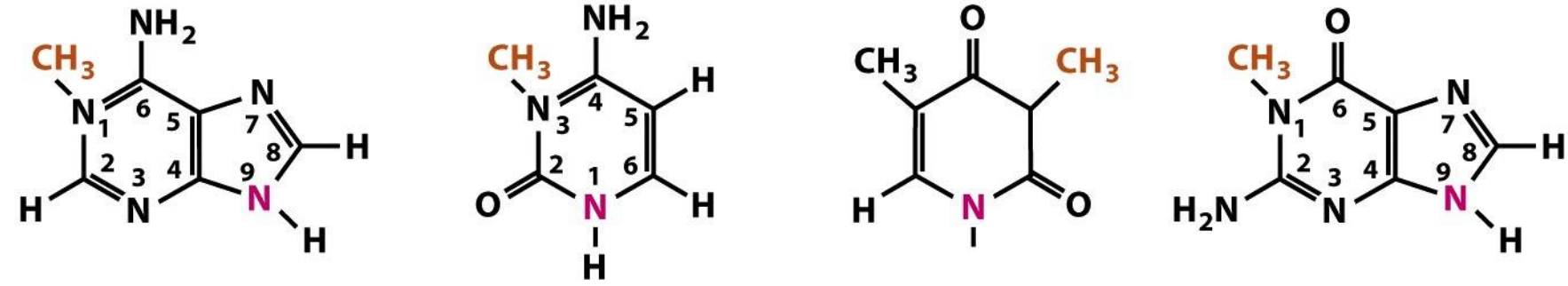
The BER system is usually employed to correct deamination



Restoration of normal base structure by DNA alkyltransferases

• Alkylating agents, such as ENU and N-ethyl-N-nitrosurea (very mutagenic), cause A->T transitions and AT→GC or GC → AT transitions

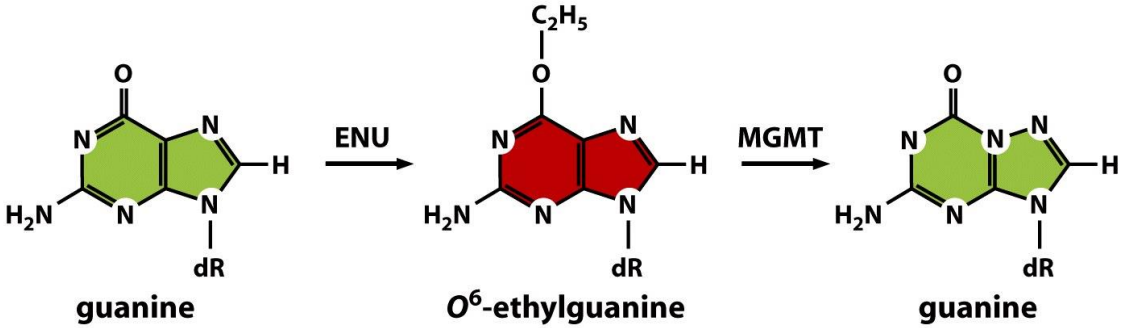
Alkylation

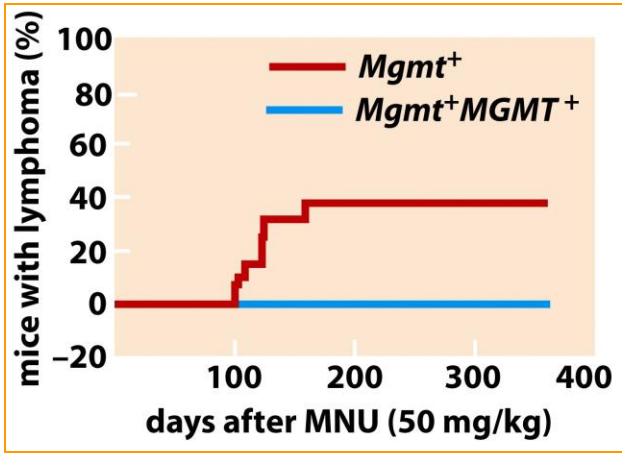


- Caused by environmental alkylating agents
- Present in many industrial products and also used as chemical weapons in the first world war (sulfur mustard)
- Misread during DNA replication, induce mutations that can promote cancer.

Restoration of normal base structure by DNA alkyltransferases

• Alkylating agents, such as ENU and N-ethyl-N-nitrosurea (very mutagenic), cause A->T transitions and AT→GC or GC → AT transitions
• O6-methylguanine DNA metyltransferase (**MGMT**) removes alchyl groups.
• This gene is silenced in 40% of gliomas and colorectal carcinomas and 25% of non-small cell carcinomas via promoter methylation



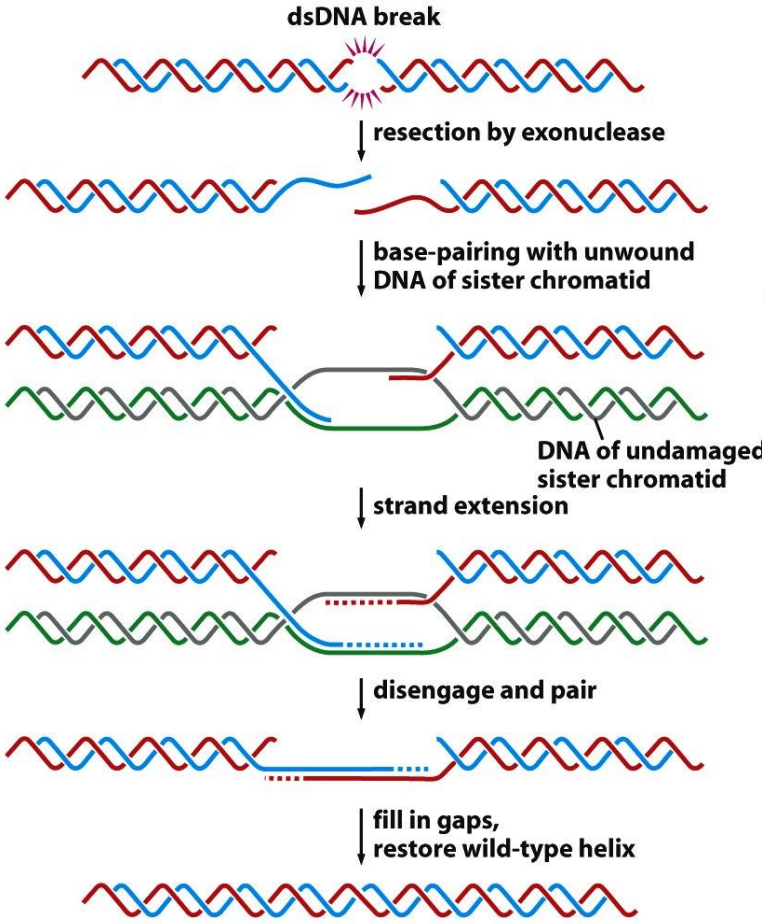


Mice engineered to overexpress MGMT do not manifest lymphoma upon treatment with alkylating agents

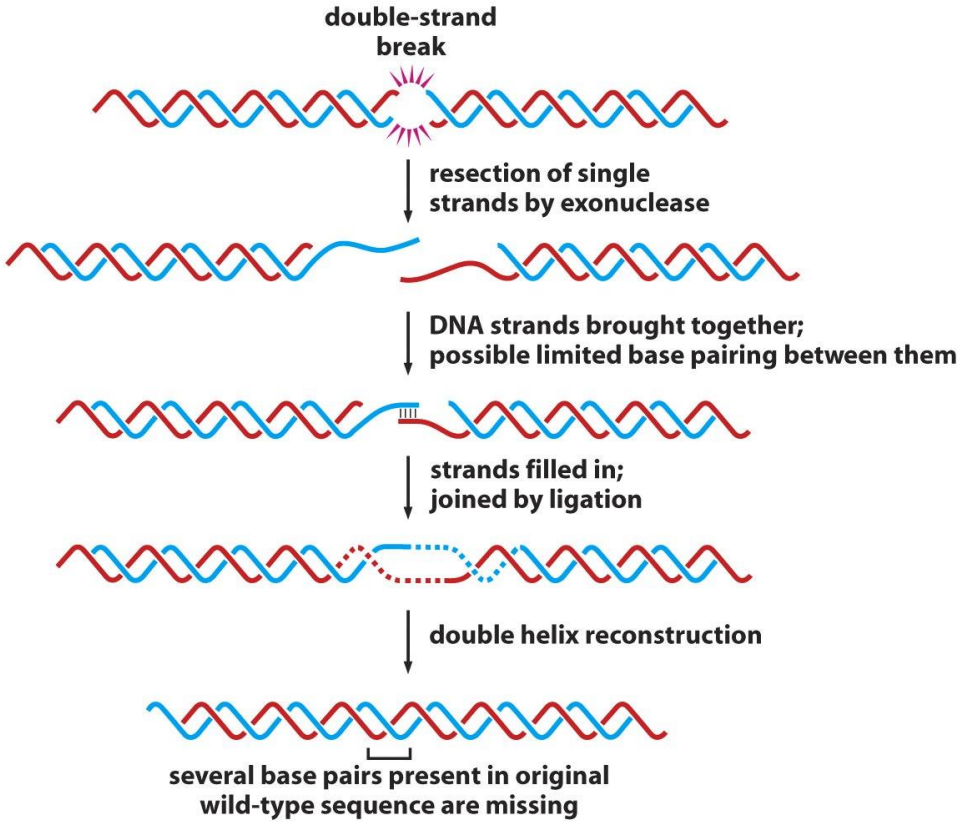
Homology directed repair (HDR) and non-homologous end joining (NHEJ)

These systems are used to repair dsDNA breaks

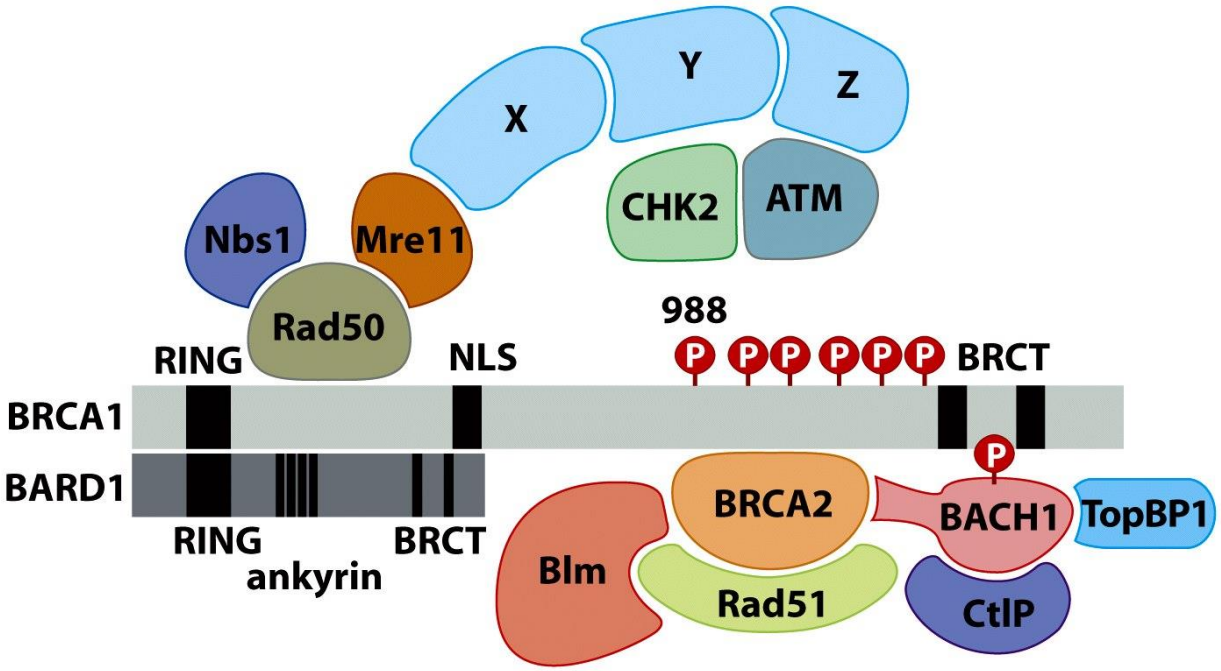
HDR (S and G2)
high-fidelity



NHEJ (G2)
error prone – salvage mechanism



Breast cancer susceptibility 1 (BRCA1)



- BRCA1 is part of a large protein complex that binds DNA
- BRCA1 repairs double-strand DNA breaks.
- BRCA1 participates in HDR to repair damaged DNA
- One mutation is inherited, a second is acquired by LOH

7-10% of women will develop breast cancer; some BRCA1 mutations may increase the risk to 60%.

Ovarian cancer risk increases from 15% to 40%.

Prophylactic bilateral mastectomy reduces breast cancer risk significantly in patients with BRCA mutations

More insight into HDR and NHEJ in next class!!