DNA Polymerase Proofreading Defects: Consequences for Mutagenesis, Genome Instability and Cancer

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SCHOOL OF MEDICINE

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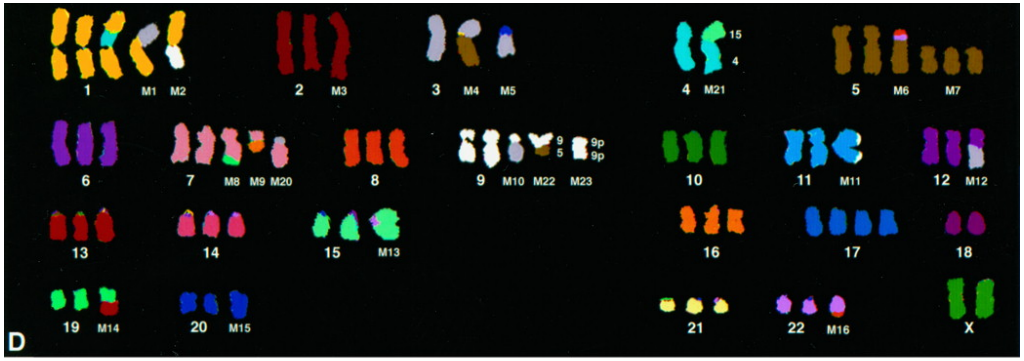
Chromosomal Instability



Microsatellite Instability

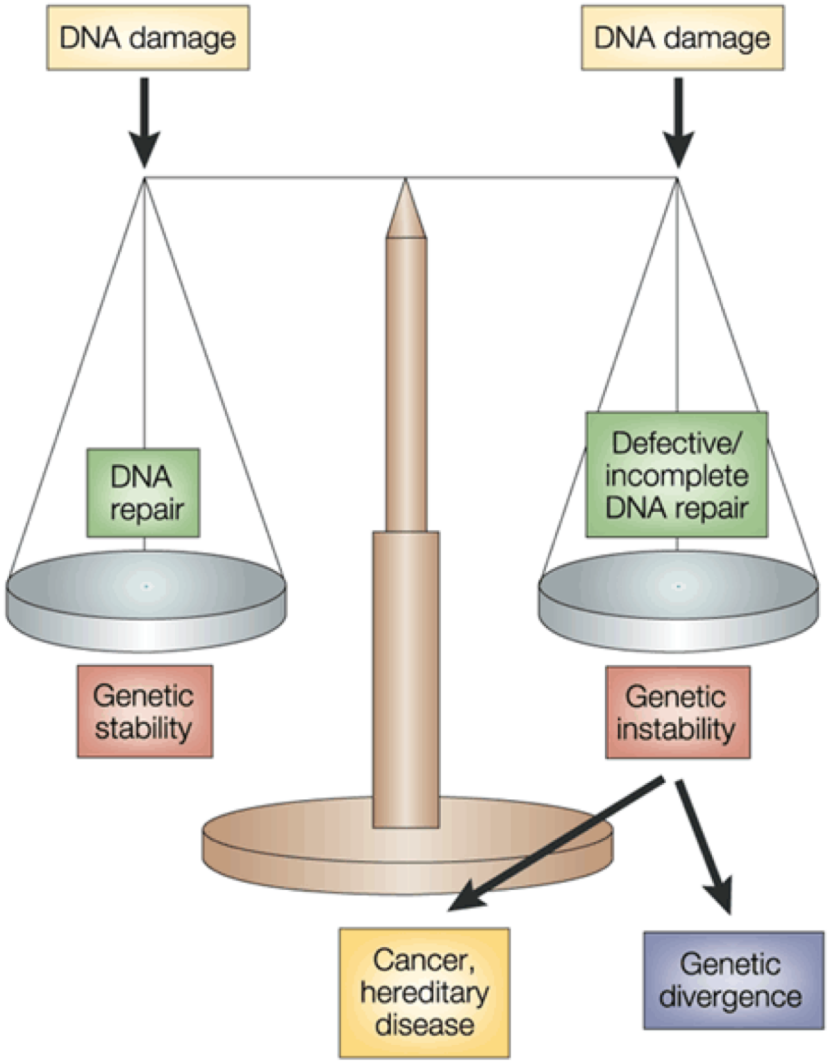
```
-TCGACACACACACACATCGA-
-AGCTGTGTGTGTGTGTAGCT-

-TCGACACACACACACACACACACATCGA-
-AGCTGTGTGTGTGTGTGTGTGTGTAGCT-
```

Point Mutation Instability

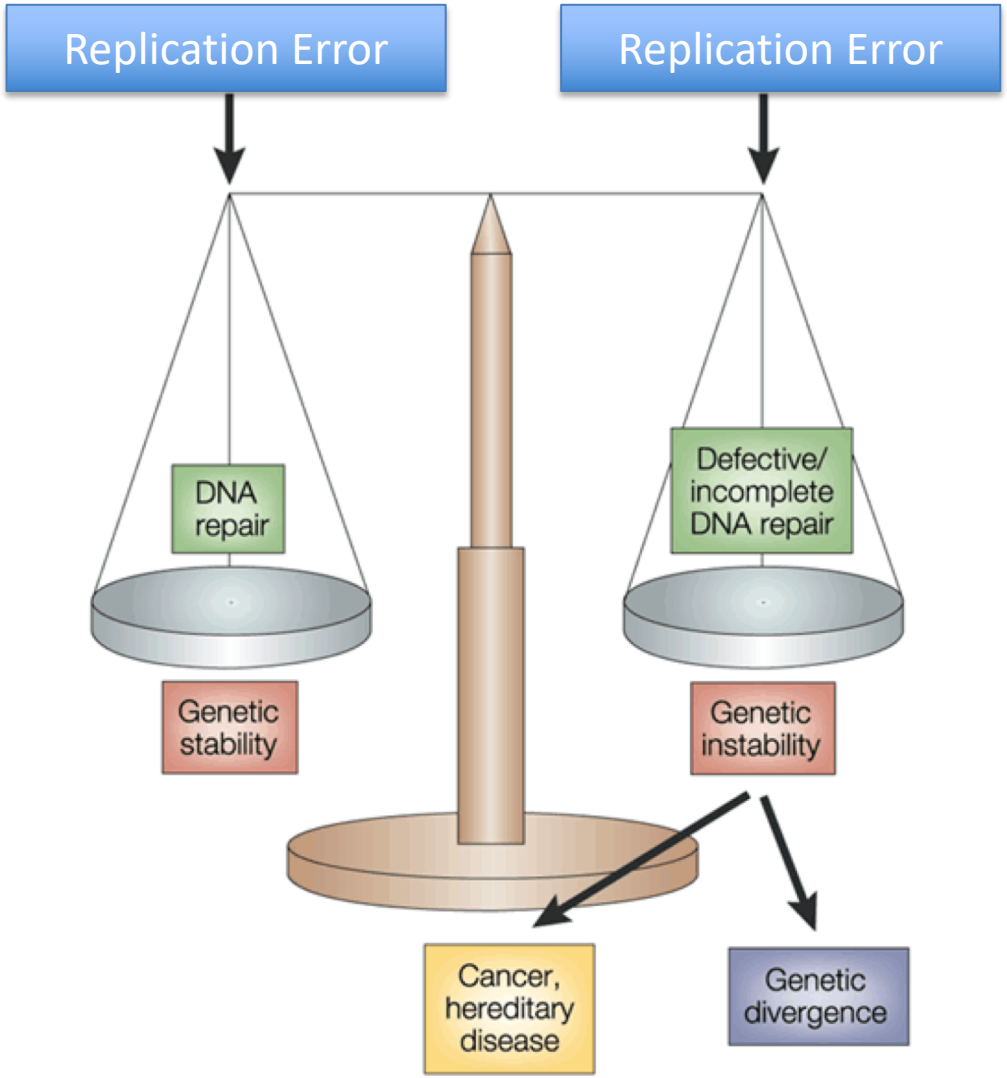
```
-CTG-        -CAG-
-GAC-   ➡    -GTC-
```

Life at the DNA Level Is a Balance Between Mutation and Correction



Friedberg EC *Nat Rev Cancer* 2001

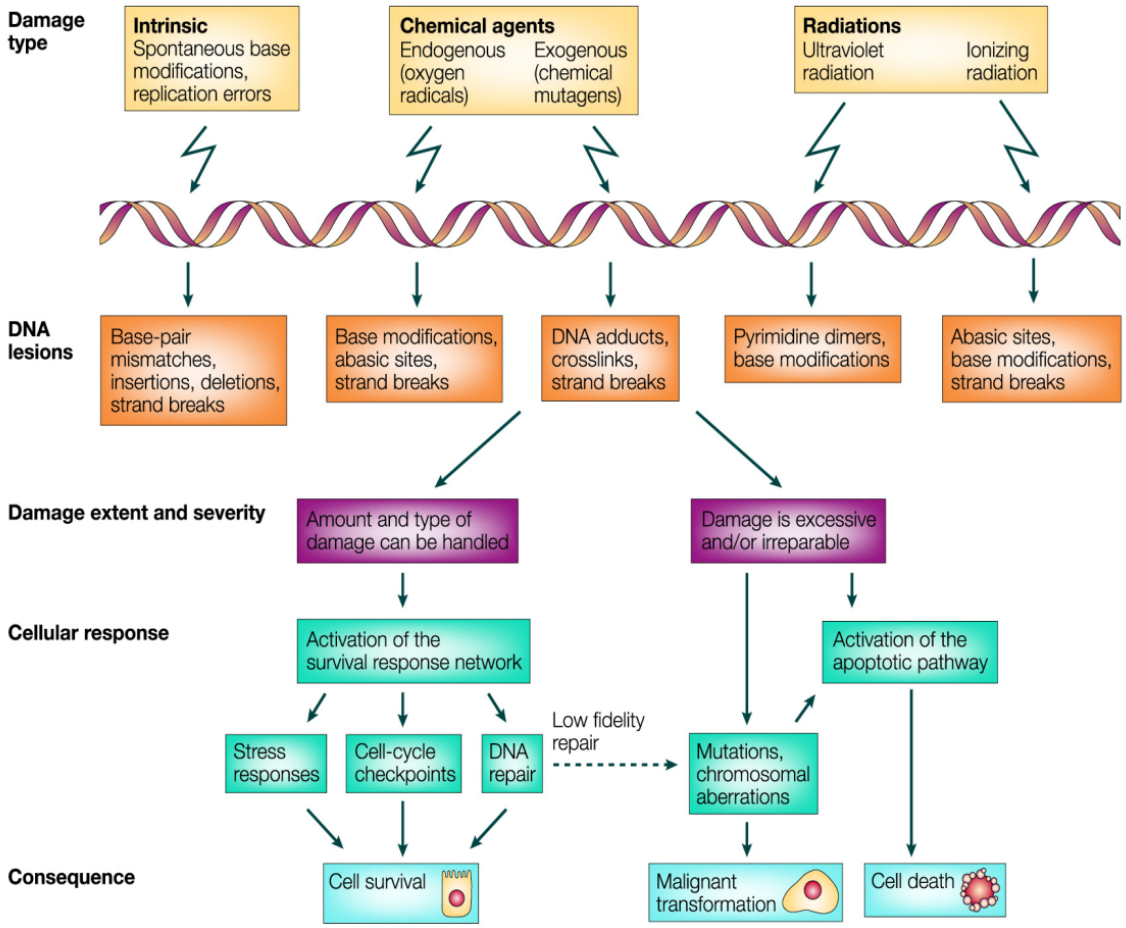
Life at the DNA Level Is Also Largely a Balance Between Mutation and Correction During Replication



Friedberg EC *Nat Rev Cancer* 2001

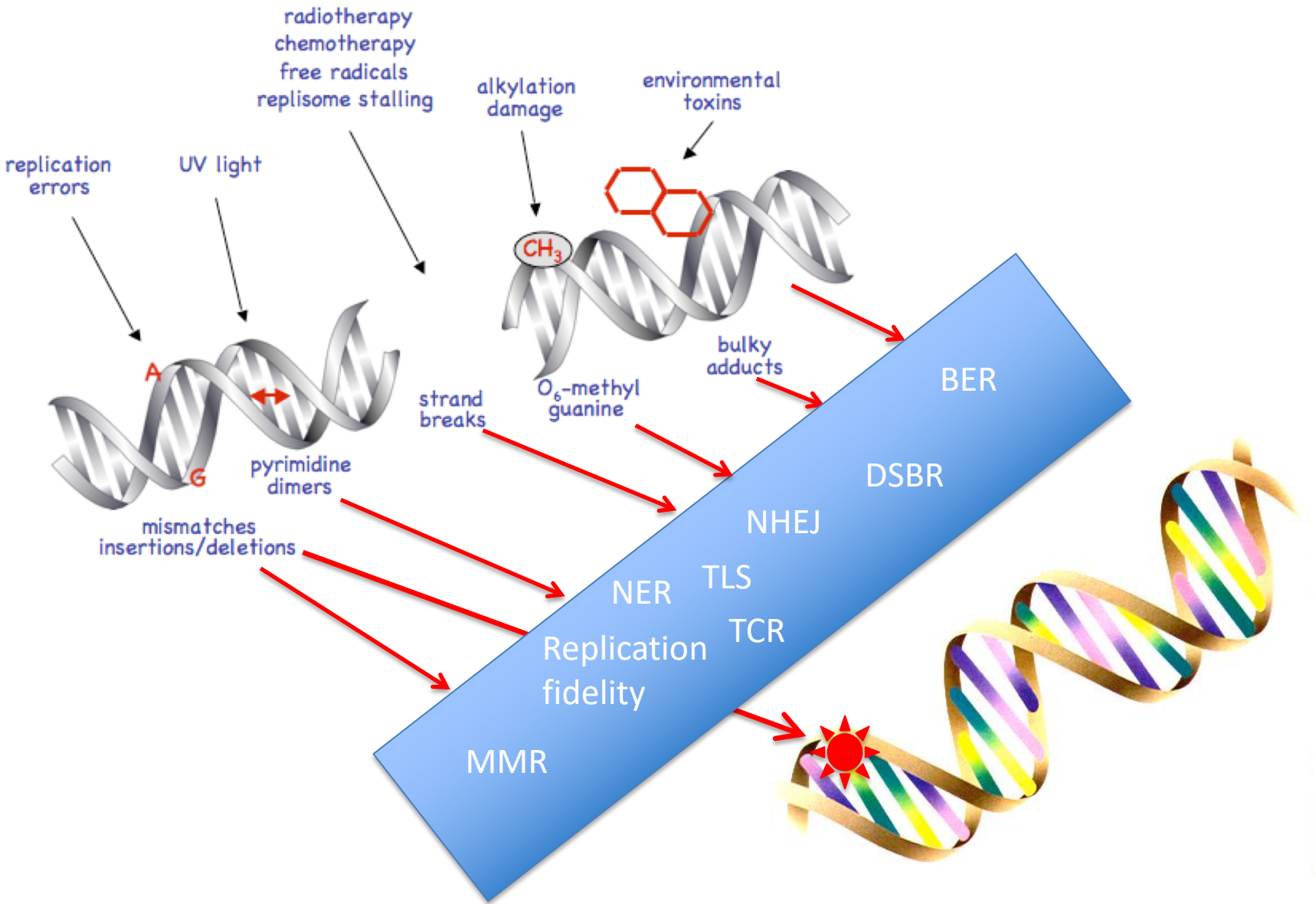
Cells are *constantly* exposed to agents that can cause damage to their genome.

In order to faithfully preserve this genetic material for transmission to subsequent generations, a series of intricate mechanisms have evolved to either repair the damage, tolerate the damage, or destroy the cell.



Y. Shiloh 3;155 2003

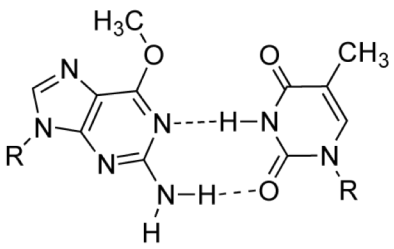
DNA Polymerases Play A Central Role In Responding To A Wide Array Of Continuous DNA Insults



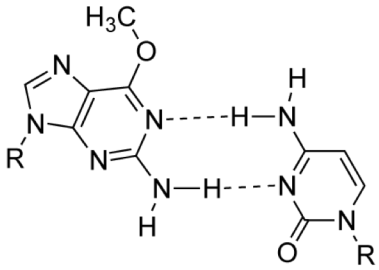
>20,000 Potential Mutations or Lethal Events In Each Cell, Each Day

Endogenous Source	No. of lesions in dsDNA
Hydrolysis	
Depurination	18,000
Depyramidation	600
Cytosine deamination	100
5-Methylcytosine deamination	10
Oxidation	
8-oxoG	~1,000-2,000
Ring-saturated purines (thymine glycol, cytosine hydrates)	~2,000
Lipid peroxidation products (M1G, etheno-A, etheno-C)	~1,000
Nonenzymatic methylation by *S*-adenosylmethionine	
7-Methylguanine	6,000
3-Methyladenine	1,200
Nonenzymatic methylation by nitrosated polyamines and peptides	
O^6-Methylguanine	20-100

Exogenous DNA Damage: The Good, The Bad…



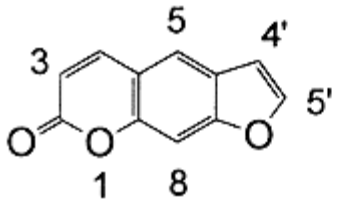
O(6)-MeG·T



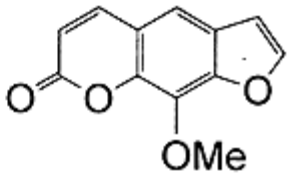
O(6)-MeG·C

Alkylation

SAM, temozolomide

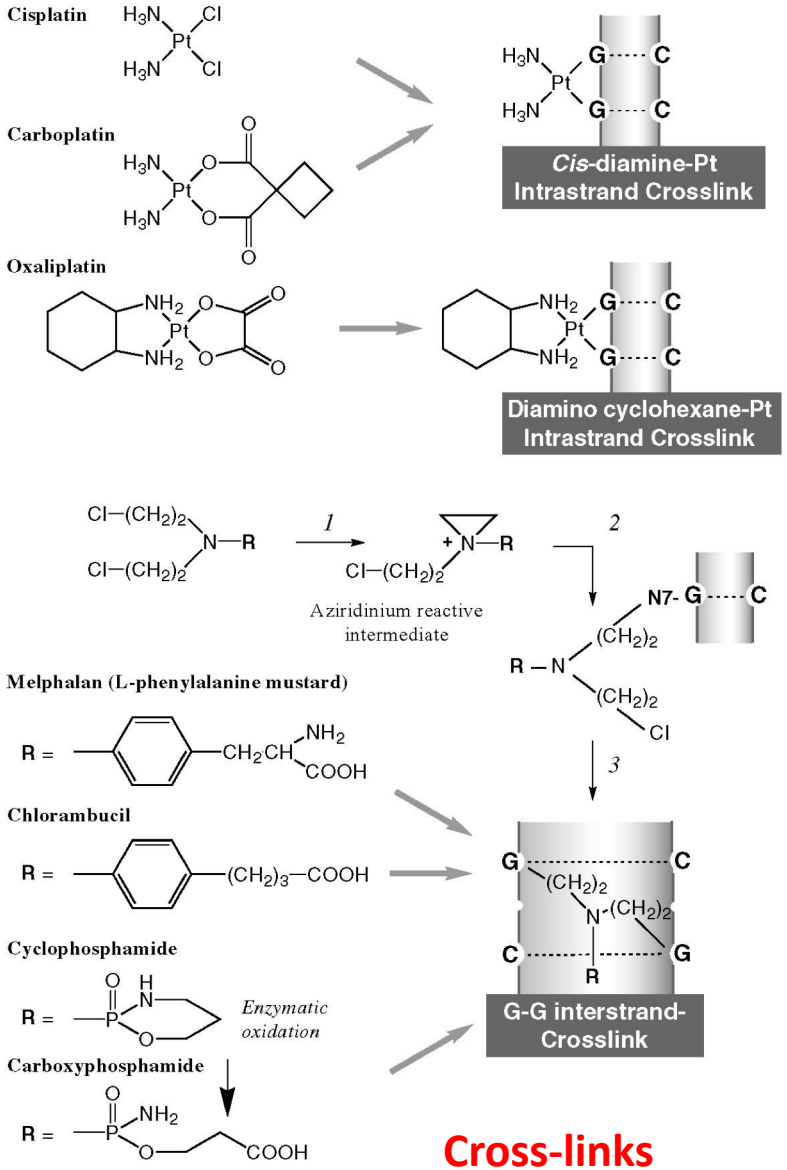


Psoralen



8-Methoxypsoralen
(8-MOP)

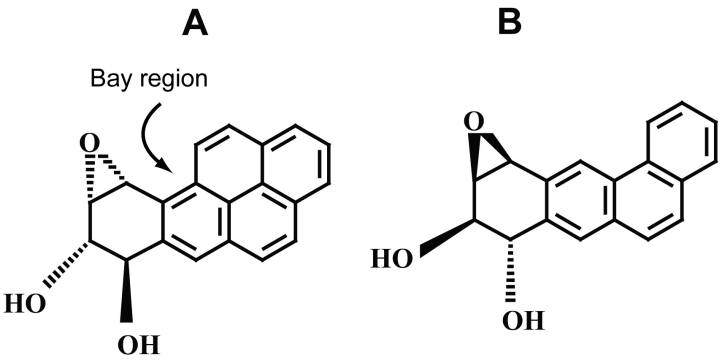
Intercalation



Cross-links

Exogenous DNA Damage: …and The Ugly

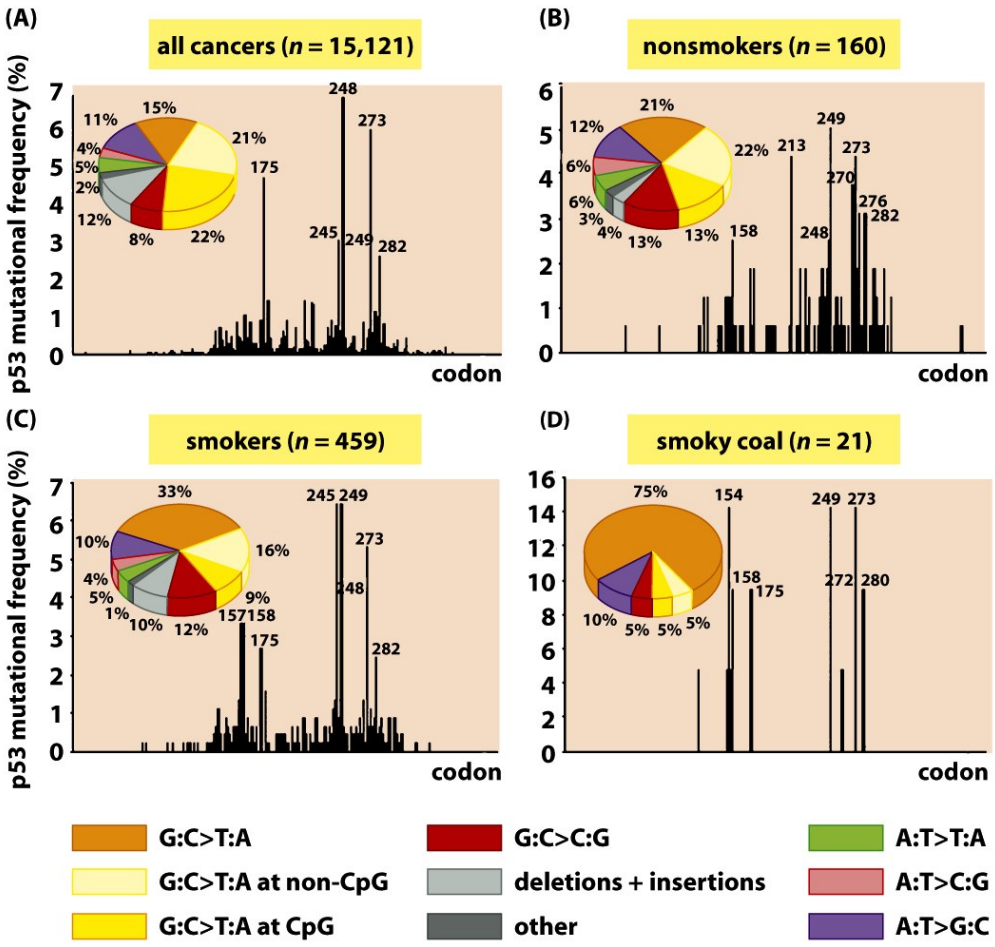
Polycyclic aromatic hydrocarbons



Significant environmental pollutant that are produced by many natural sources, but also by human activity

Non-polar and inert by themselves, they must be ‘activated’ by P450 metabolism to become DNA damaging agents

- dG-PAH pairs with A
- results in increase in G•C → T•A transversions



3 Types of Excision Repair

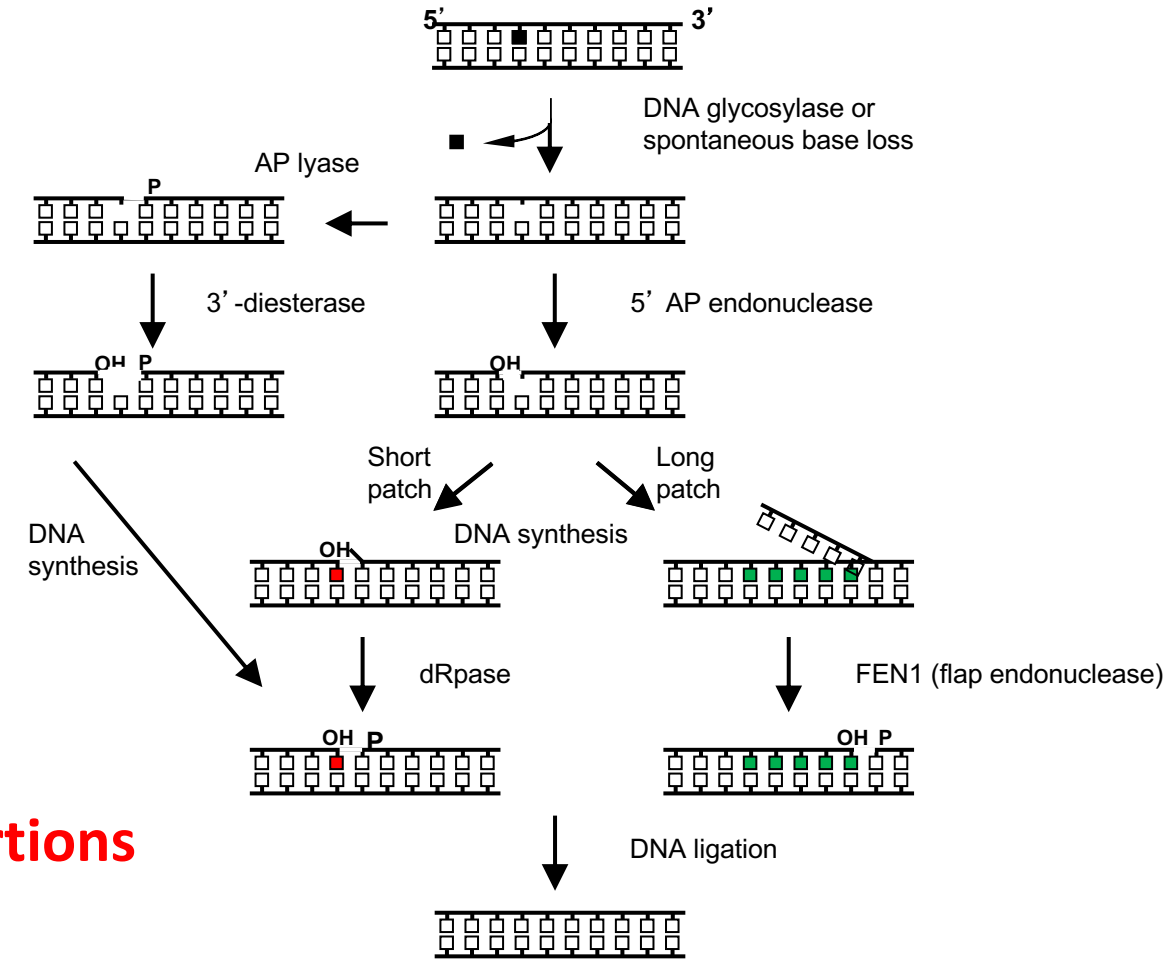
1. **Base Excision Repair**: excision of the single errant NT (e.g. uracil, oxidized base).

2. **Nucleotide Excision Repair**: Various bulky, helix-distorting lesions (particularly pyrimidine dimers), excision of a patch (oligonucleotide) surrounding lesion.

3. **Mismatch Excision Repair**: errors arising from DNA replication, excision of an extended region (up to 1 kb) surrounding the lesion.

2015 Nobel Prize in Chemistry: *DNA Repair!*

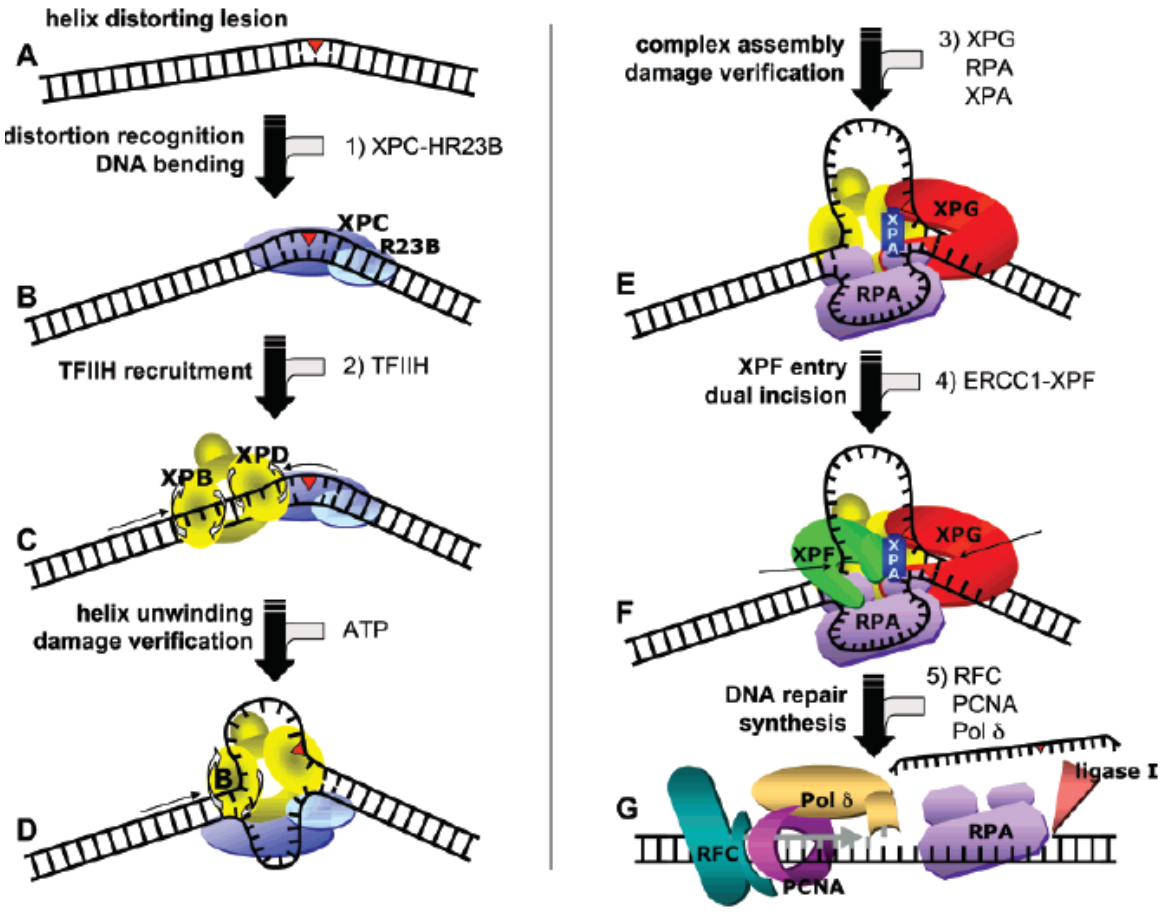
Base Excision Repair (BER)



Repair of Small Distortions Due to Base Damage

Unique Glycosylases Remove Various Lesions (e.g. Ung, Ogg1, etc.)

Nucleotide Excision Repair (NER)



HR23B = Human homolog of Rad23B

TFIIH = Transcription factor IIH complex (10 subunits, two of which are XPB and XPD)

RPA = replication protein A, a single stranded DNA binding protein

ERCC1-XPF = Excision repair cross-complementing protein 1

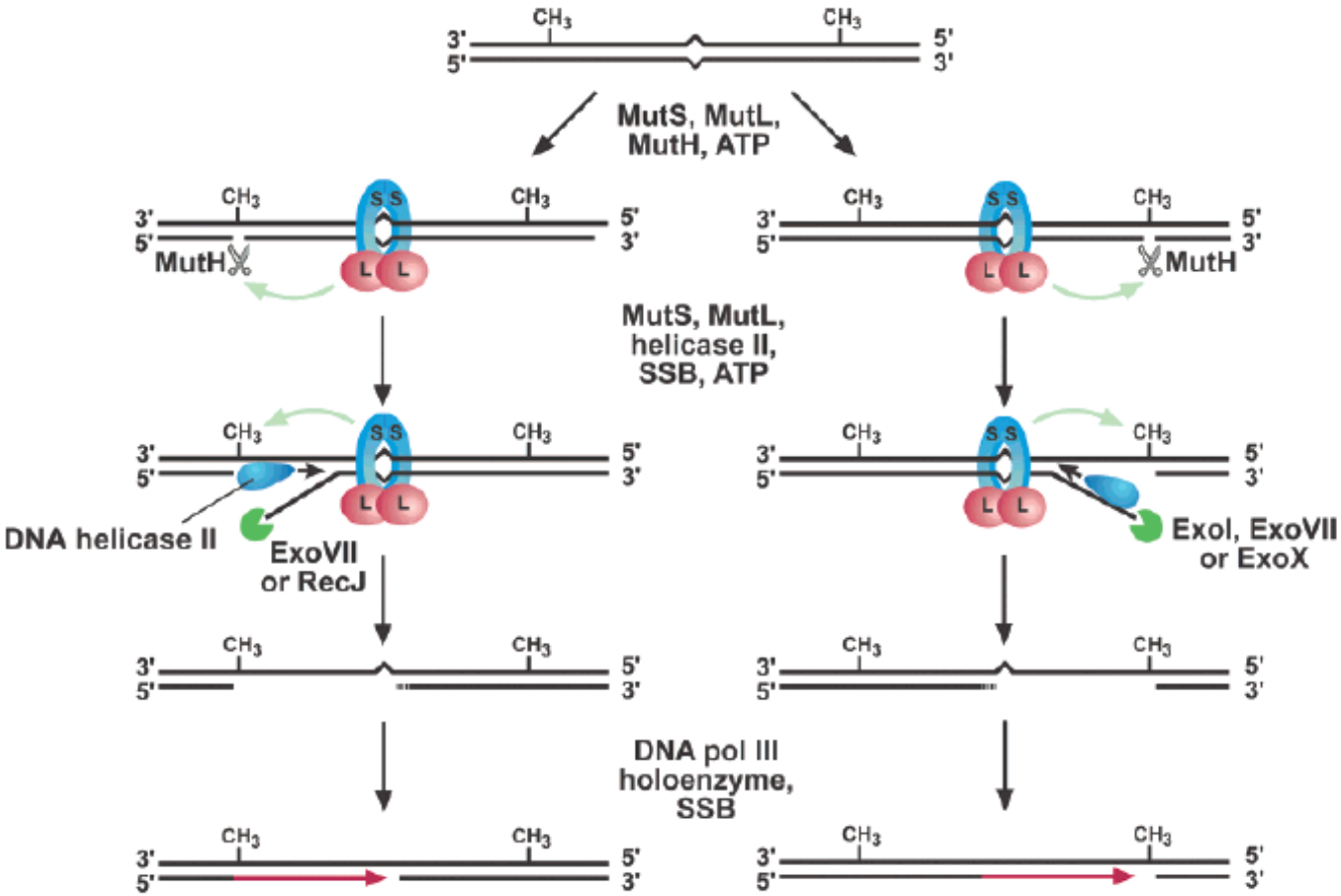
RFC = replication factor C, the clamp loader

PCNA = polymerase clamp

Excised region is ~27 nucleotides long

Repair of Helix Distorting Lesions, particularly CPDs and 6-4 photoproducts

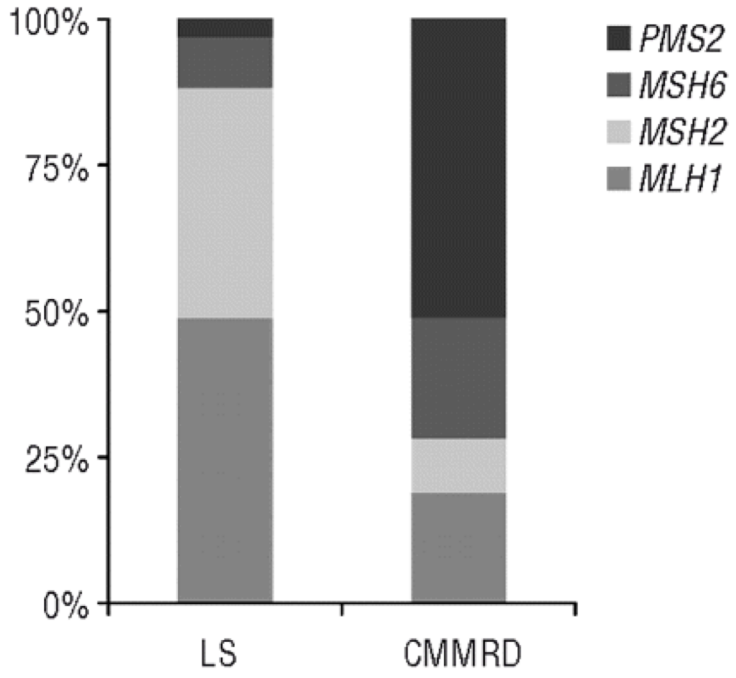
MMR Pathway Corrects Replication Errors



Repair of Replication Errors

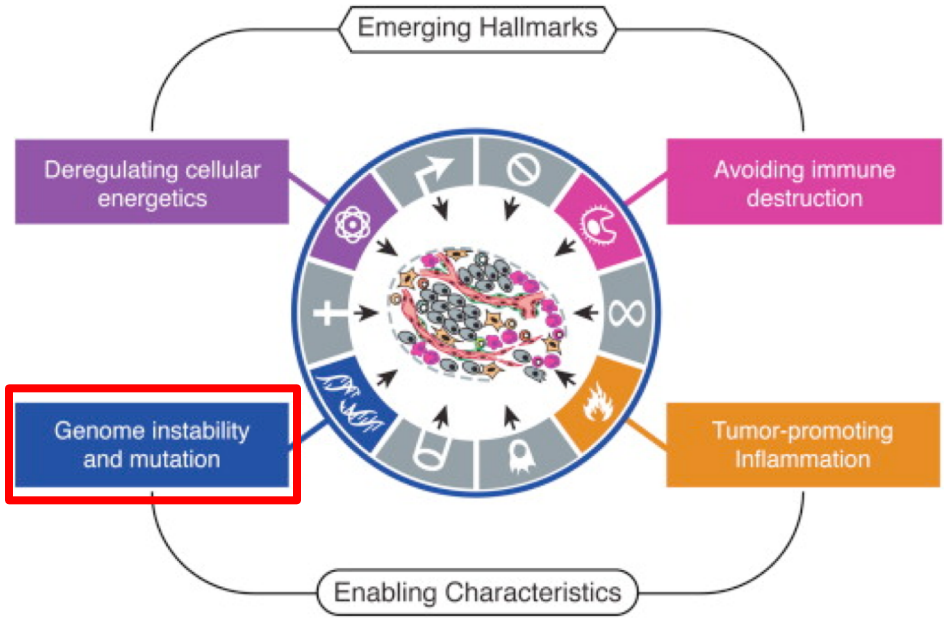
Compromised MMR Increases Cancer Risk and Accelerates Age of Onset, Particularly for Colorectal and Endometrial

Cancer Type	General population risk	Lynch syndrome (MLH1 and MSH2 heterozygotes)	
		Risk	Mean age of onset
Colon	5.5%	52-82%	44-61 years
Endometrium	2.7%	25-60%	48-62 years
Stomach	< 1%	6-13%	56 years
Ovary	1.6%	4-12%	42.5 years
Hepatobiliary tract	< 1%	1.4-4%%	Not reported
Urinary tract	< 1%	1-4%	~55 years
Small bowel	< 1%	3-6%	49 years
Brain/central nervous system	< 1%	1-3%	~50 years
Sebaceous neoplasms	< 1%	1-9%	Not reported



- Loss of Mlh1 most common, either through mutation or epigenetic silencing, followed by Msh2 mutation
- Inherited defects: Lynch syndrome (HNPCC), bMMRD, FAP, AFAP, FJP, CD
- Spontaneous/Sporadic as well

Genome Instability Both Influences and Is Influenced By Alterations to Cell Physiology During Cancer Progression



Normal	Mutation Frequency ($x10^{-8}$)	Neoplastic	Mutation Frequency ($x10^{-8}$)
Squamous epithelium	<1	Ovarian carcinoma	75
Renal cortex	<1	Perirenal liposarcoma	65
Colonic Mucosa	<1	Colonic adenocarcinoma	475
Inflamed renal cortex	4	Renal carcinoma	270
Skeletal Muscle	<1	Pleomorphic carcinoma	141
Untreated	2	ENU-treated	175

From 65-475-fold increase in mutation frequency observed in tumors

Hanahan and Weinberg. Cell 2011.

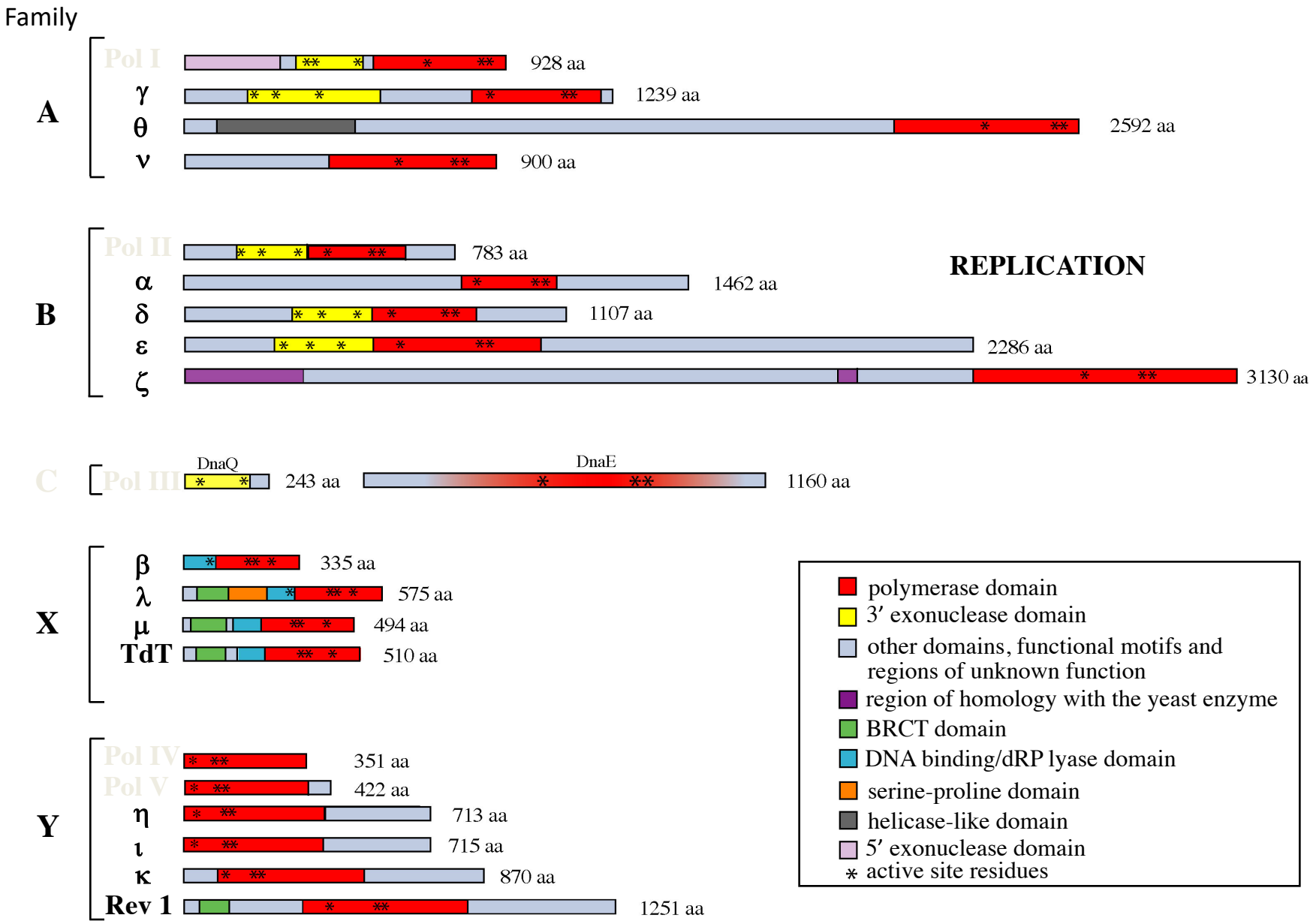
Bielas et al. PNAS. 2006 **103** (48) 18238-18242.

But Replication Is Normally Extremely Accurate

Only ~1 spontaneous error per genome duplication

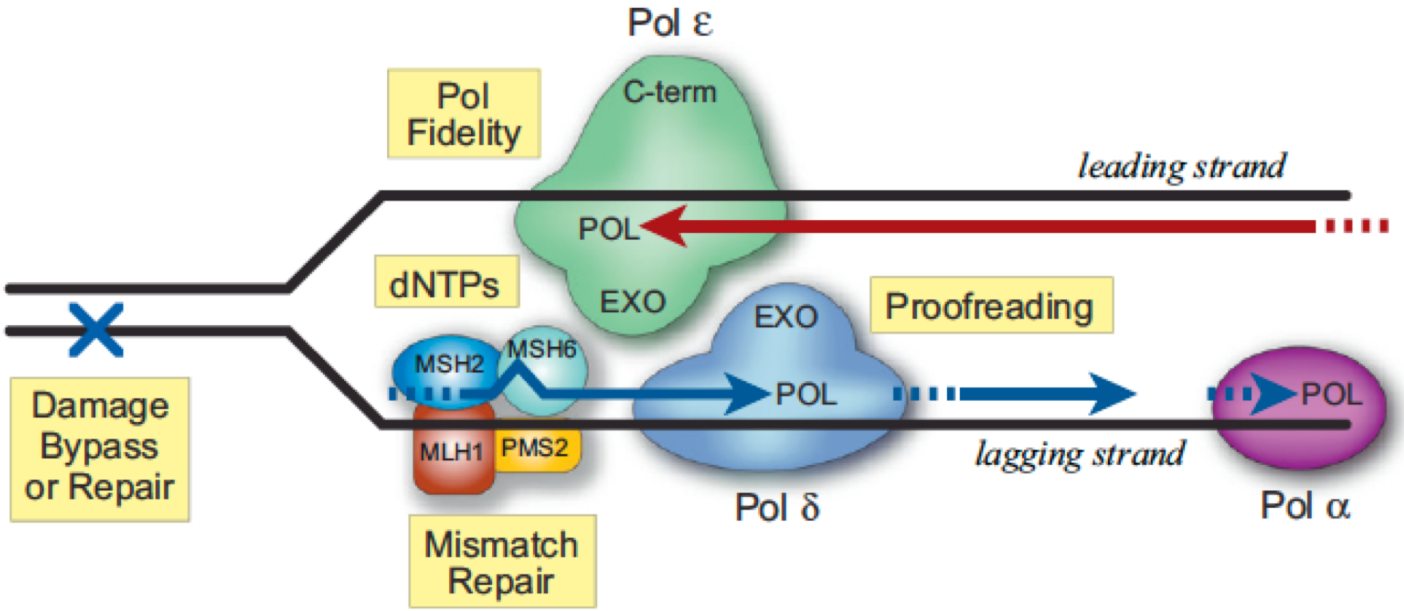
Your Equivalent: Type the equivalent of 5 x 10^3 novels
In 8 hours
While making only 1 typo!

DNA Polymerases: DNA Replication & DNA Repair

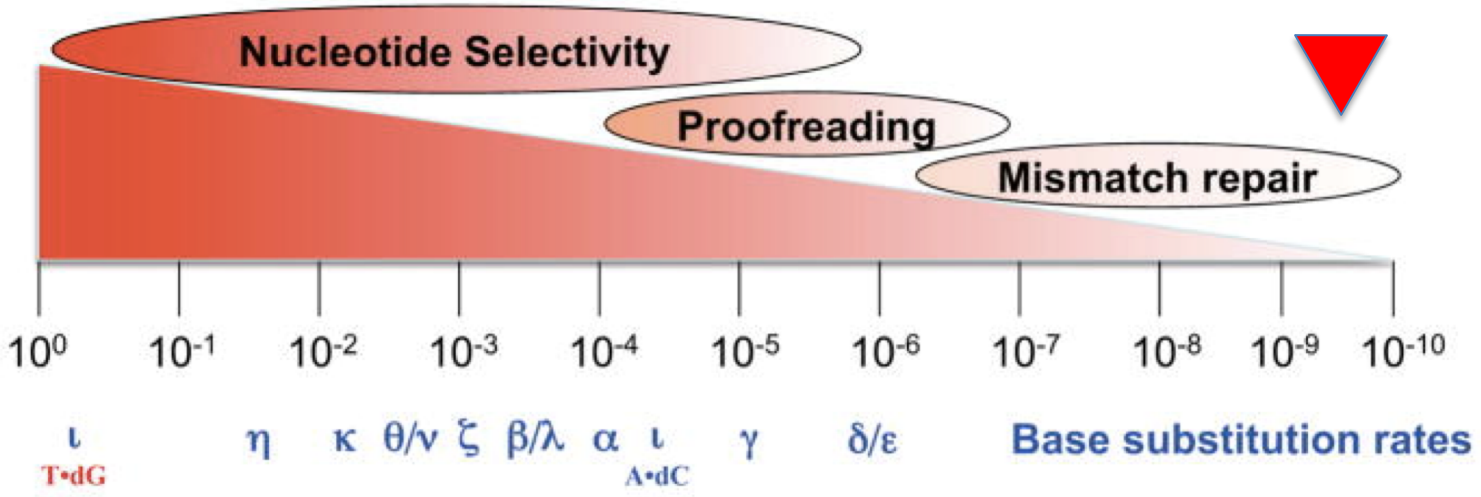


Bebenek et al 2005 Adv in Prot Chem

Decreases In Replication Fidelity Lead To Genome Instability



Eukaryotic Cells



Preston BD et al. Semin Cancer Biol. 2010 Oct;20(5):281-93.

Kunkel TA, Cold Spring Harbor Symp Quant Biol 2009

All Tumors Have Multiple Genomic Mutations, Though The Frequency Varies Considerably

3,000,000 -

300,000 -

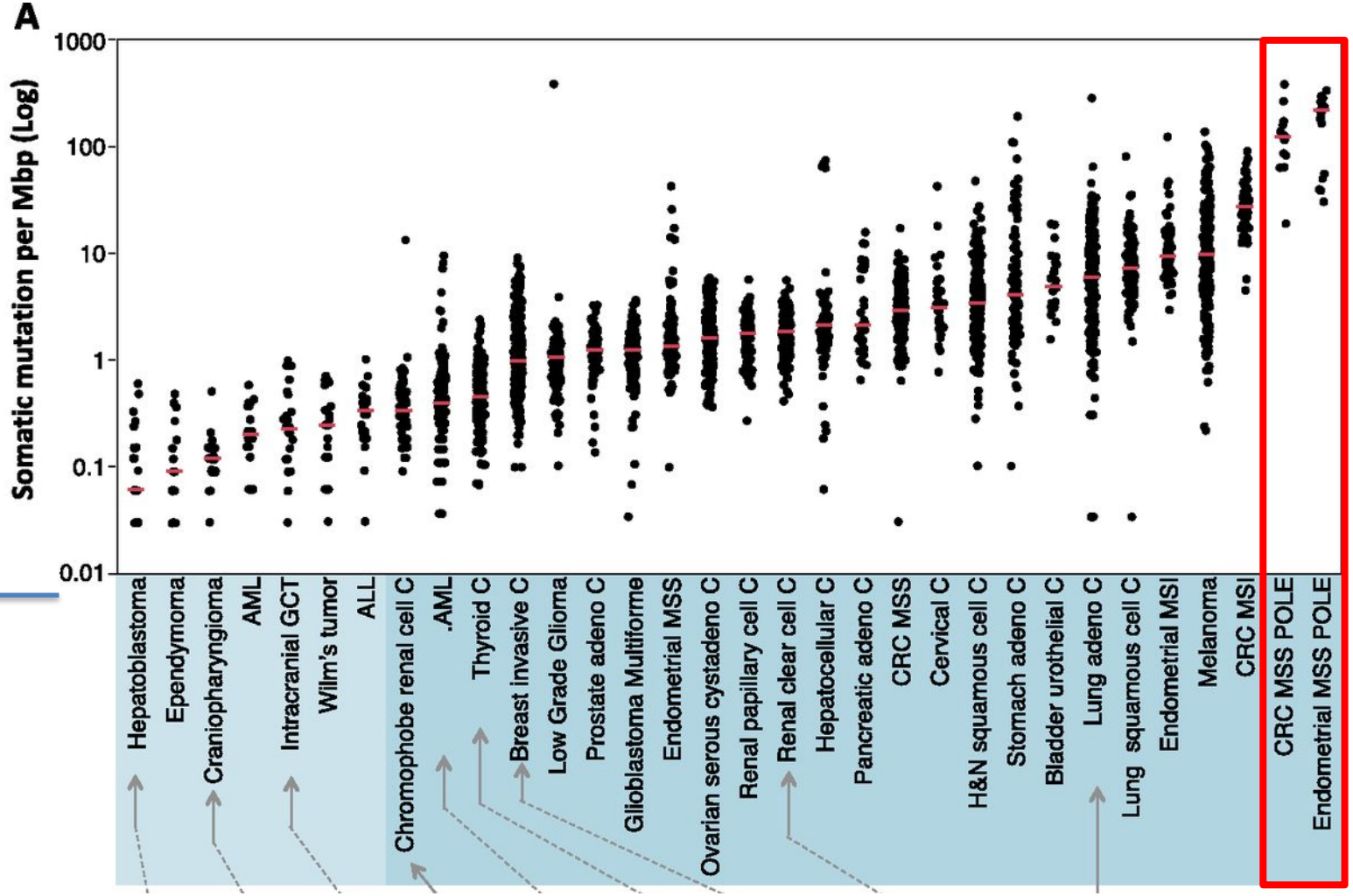
30,000 -

3,000 -

300 -

30 -

Mutations per Haploid Genome!



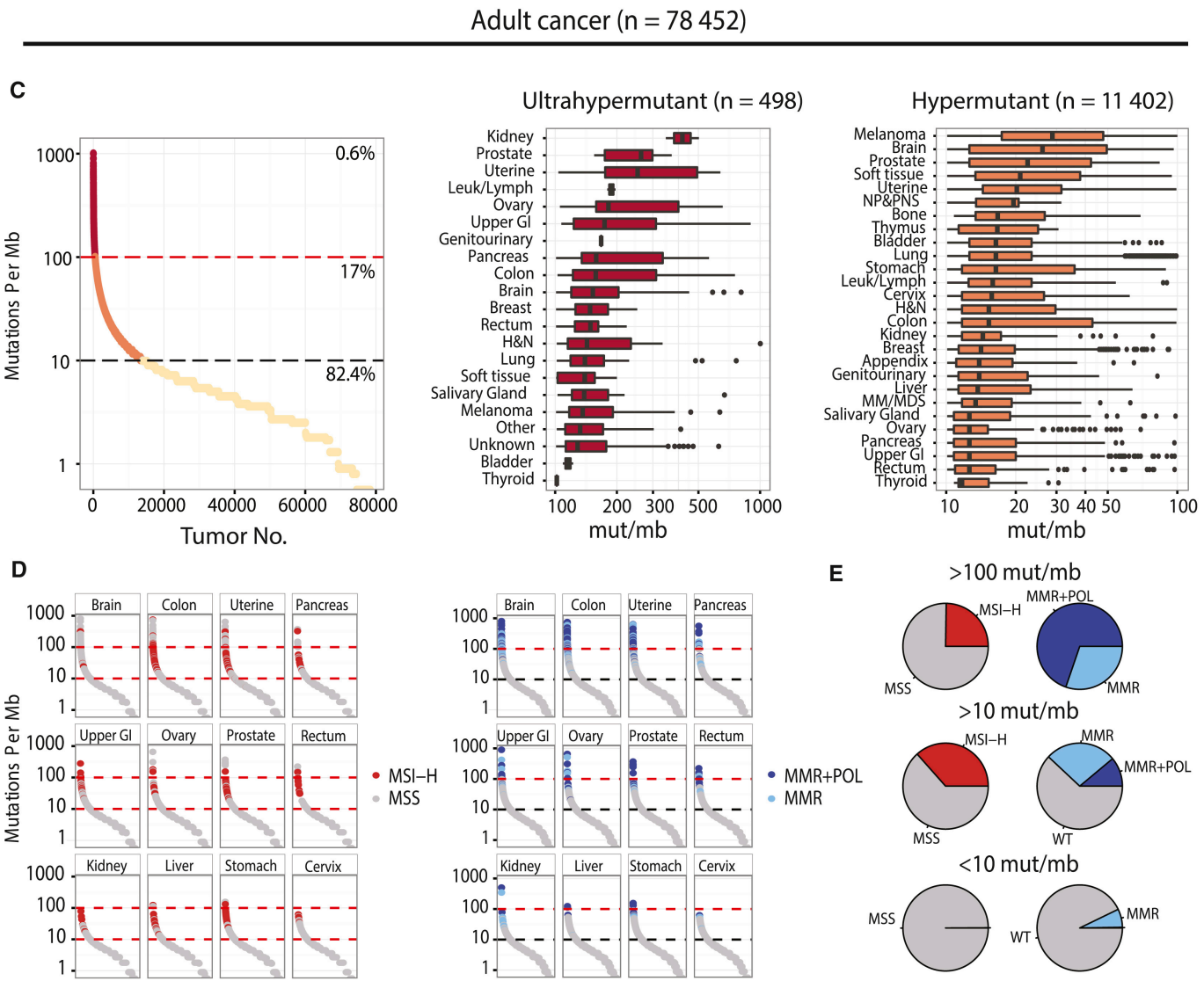
DNA Polymerase Mutant Tumors Have Highest Mutation Burden Measured

Wheeler D A , and Wang L Genome Res. 2013;23:1054-1062

For You Future MD-Type Doctors: Understanding Genomic Variants Is Rapidly Becoming Part of Your Standard Care

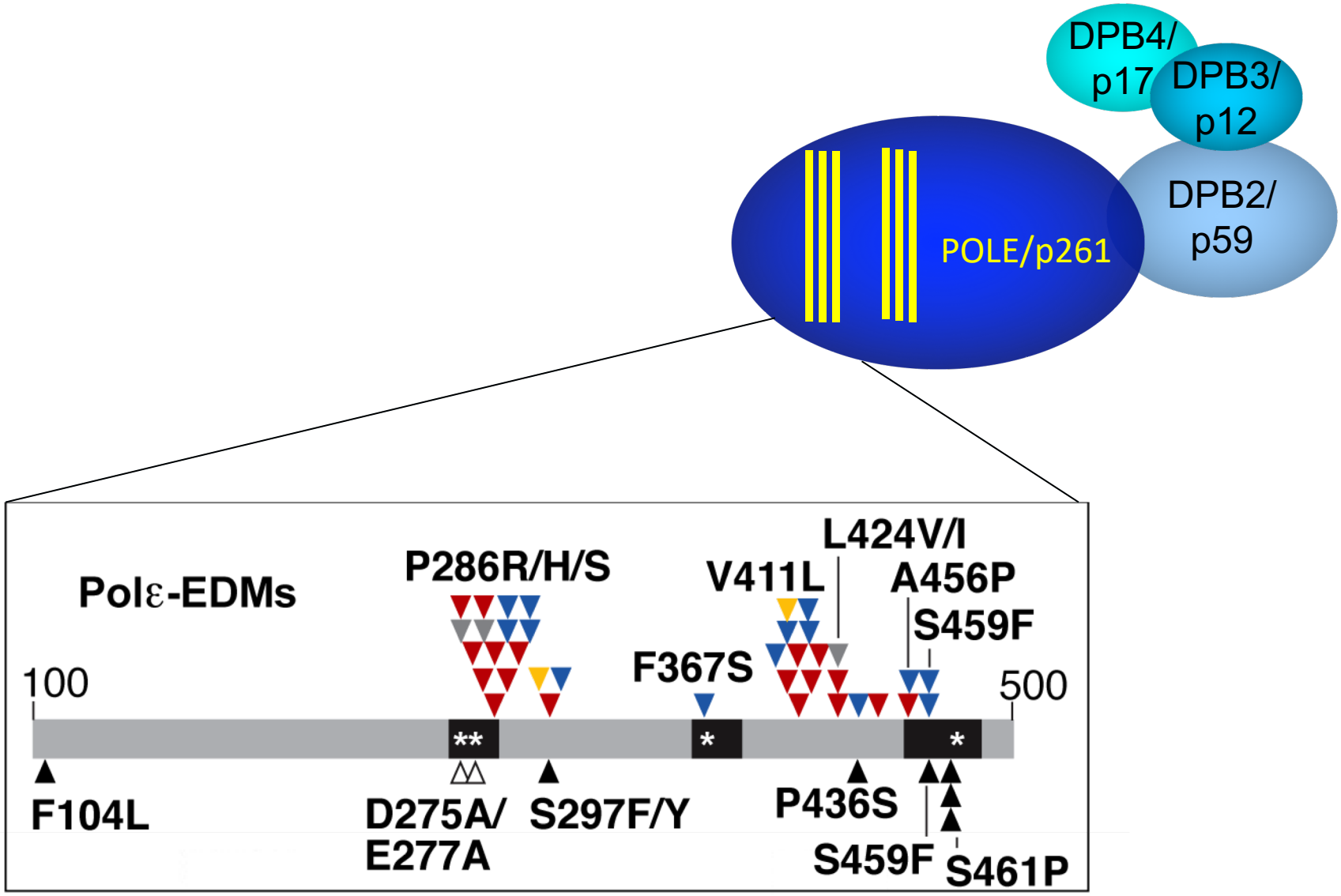
We were part of a recent study that sequenced a portion of of the genome from tumors from *80,000 (!!!)* patients.

Disease biomarkers, Pregnancy issues, Etc.



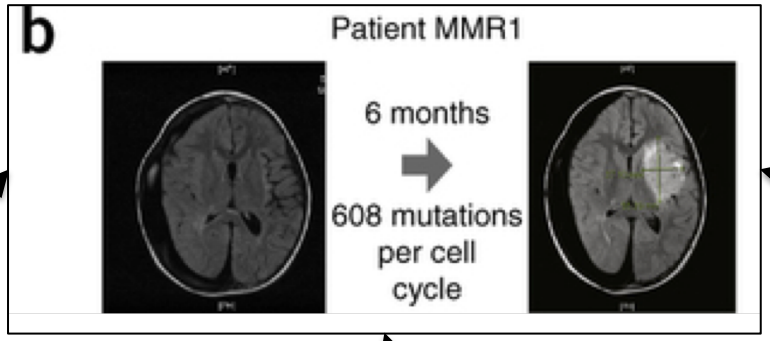
Cell 2017 171, 1042-1056.e10DOI: (10.1016/j.cell.2017.09.048)

How Do We Make Sense of All These Mutations/VUS/Etc.?
One Example: My Lab - Mutant DNA Polymerase ε in Tumors

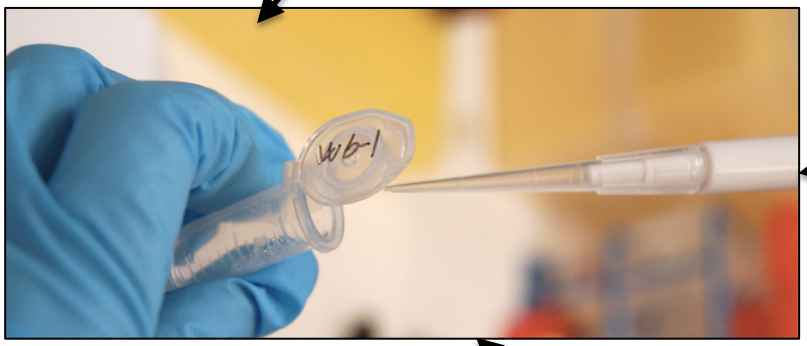


Multidisciplinary Approach To Modeling Polymerase-Associated Mutagenesis

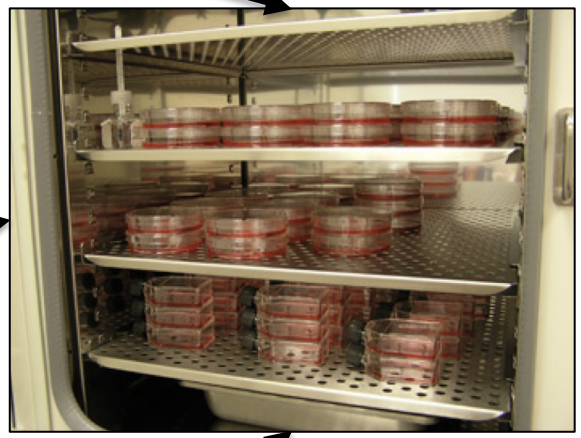
Patient Data



Biochemistry

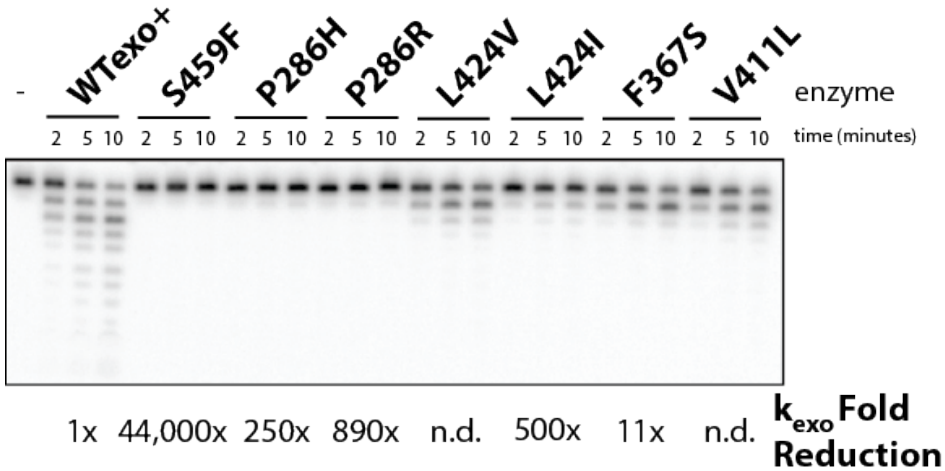


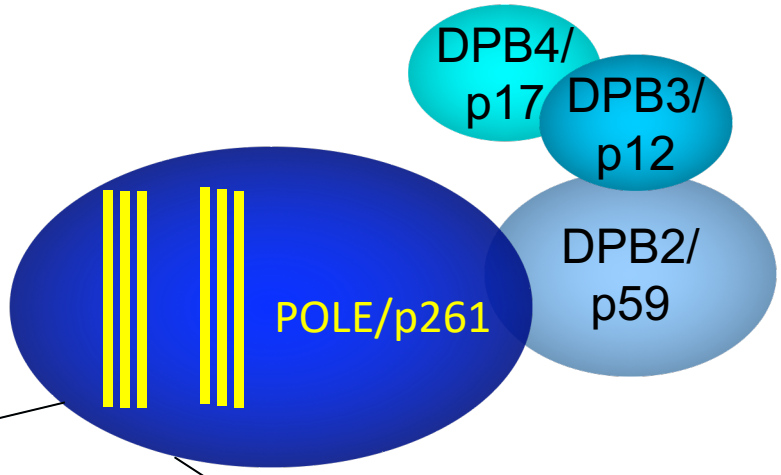
Cell Model

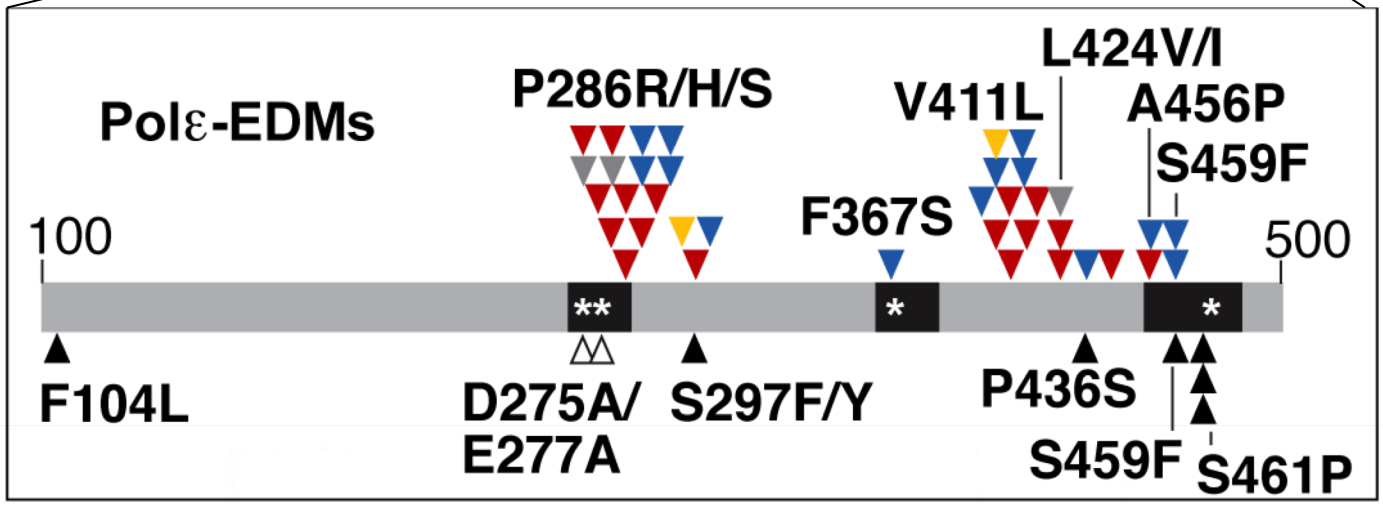


Mouse Model

Mutant POLE Alleles Cluster in Exonuclease Domain And Compromise Proofreading Acitivity

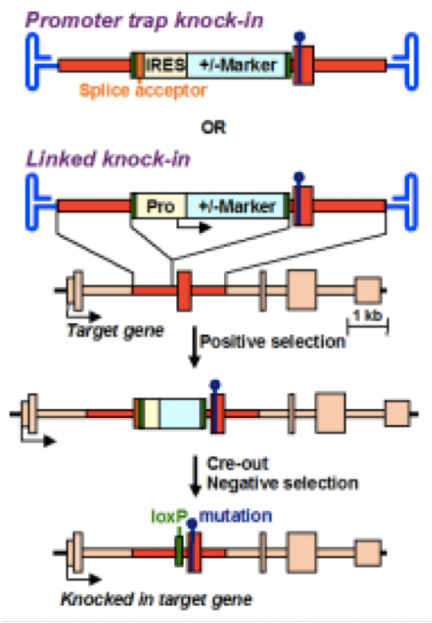




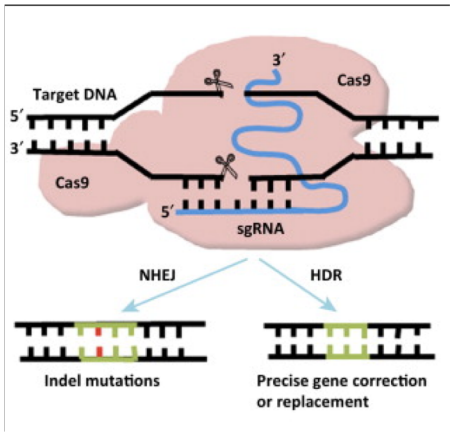


Modeling Polymerase Mutators In Cells Using Gene Editing

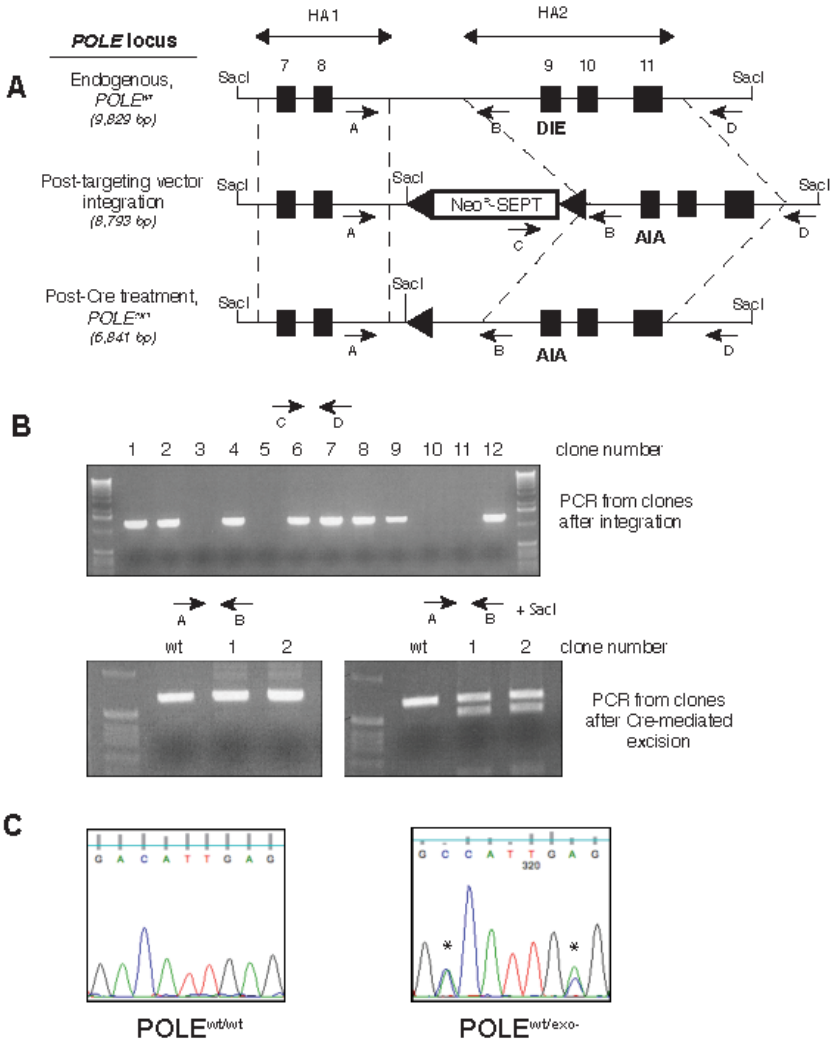
rAAV



CRISPR (Karl Hodel)

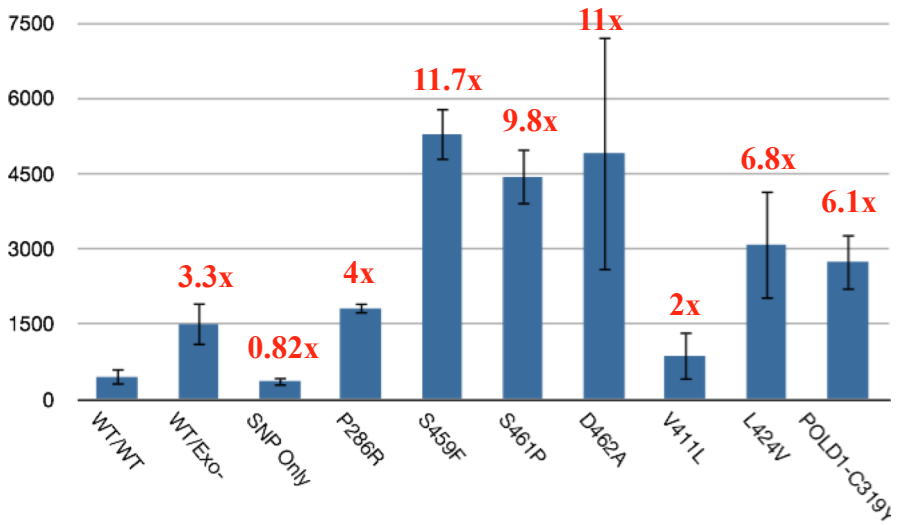


Heterozygous POLE-EDM



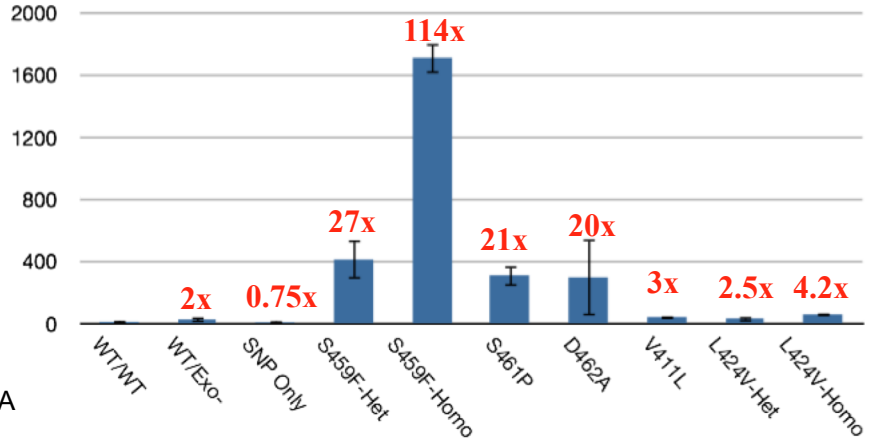
Heterozygous POLE Cancer Mutants Are Mutators *In Vitro*

MMR-
(HCT116-WT)



HPRT1 Mutant Frequency (x10 $^{-6}$)

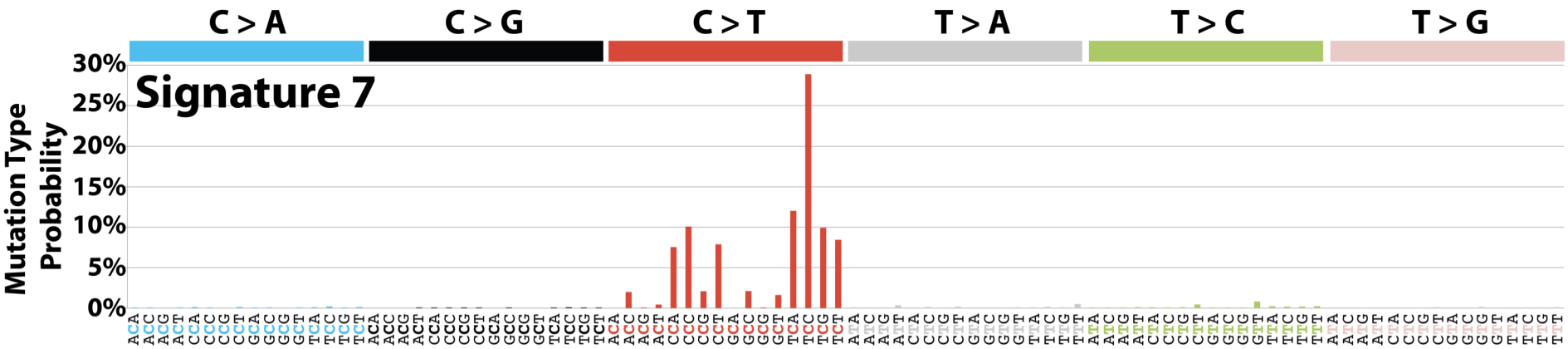
MMR+
HCT116+MLH1



HPRT1 Mutant Frequency (x10 $^{-6}$)

"exo-" = D275A/E277A

Tumor Mutation Signatures Can Help Understand Molecular Mechanism(s) of Mutagenesis



Signature 7 found enriched in high UV exposed cancers

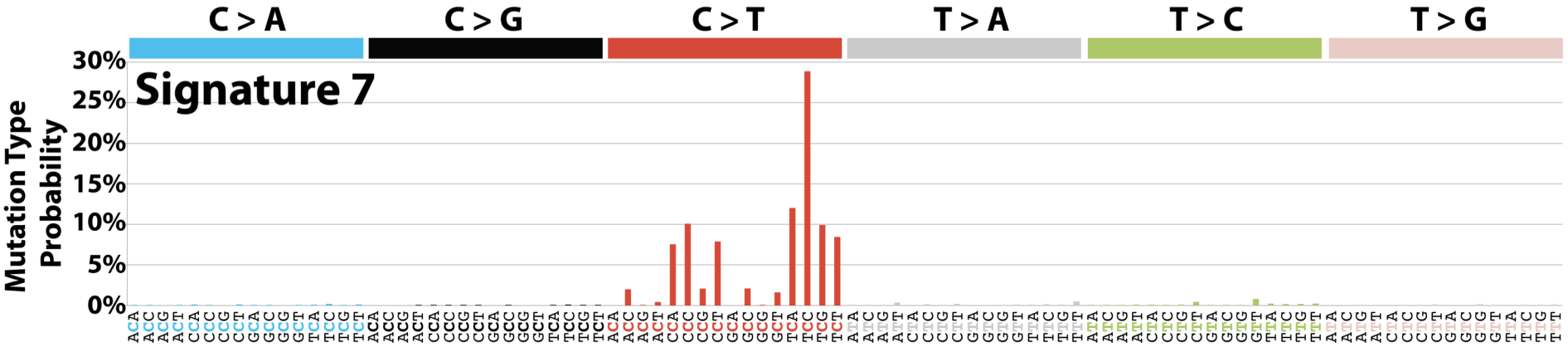
- Skin cancers
- head & neck, oral squamous

Strong Bias towards CC>CT and TC>TT

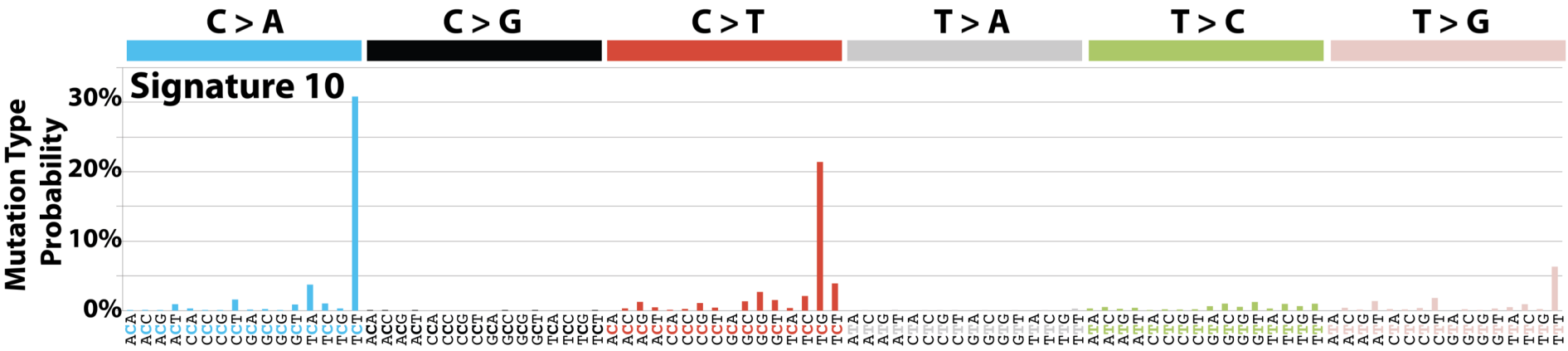
- consistent with UV-induced DNA lesions
- cyclopyrimidine dimers & 6-4 photoproducts

Tumor Mutation Signatures Can Help Understand Molecular Mechanism(s) of Mutagenesis

UV-Associated Signature 7



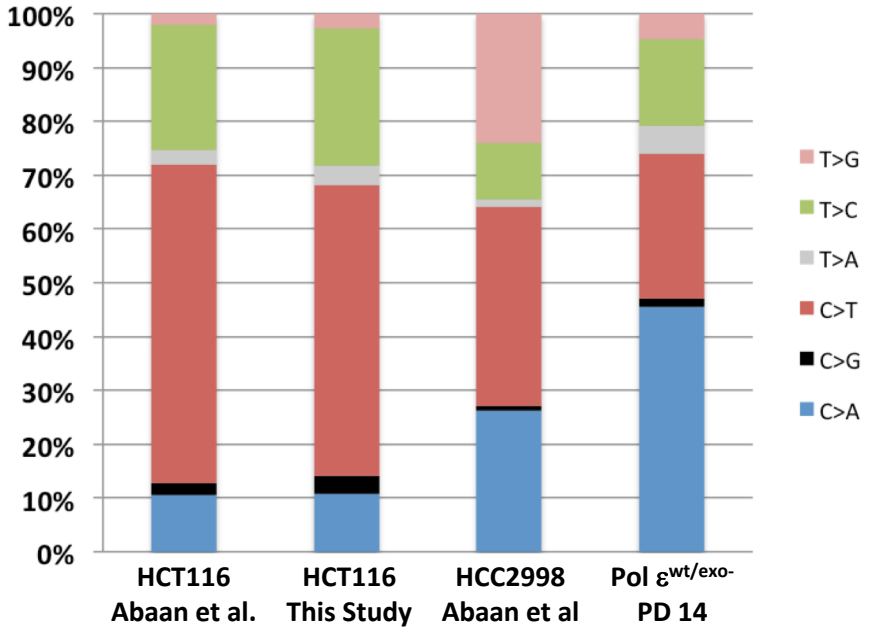
POLE-Associated Signature 10

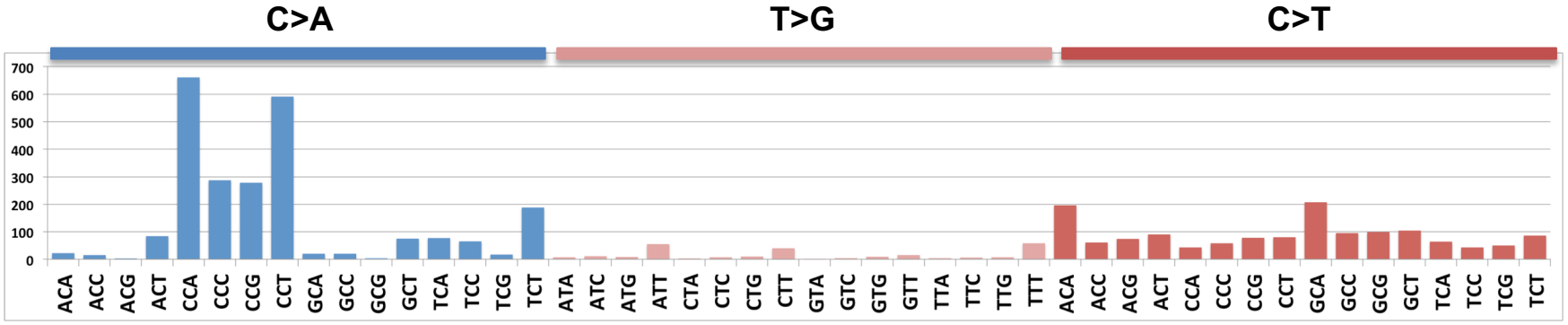


Very specific subset of mutations in POLE-mutated tumors

Pole$^{wt/exo-}$ Cells Accumulate 377* Genomic Mutations Per Round of Replication

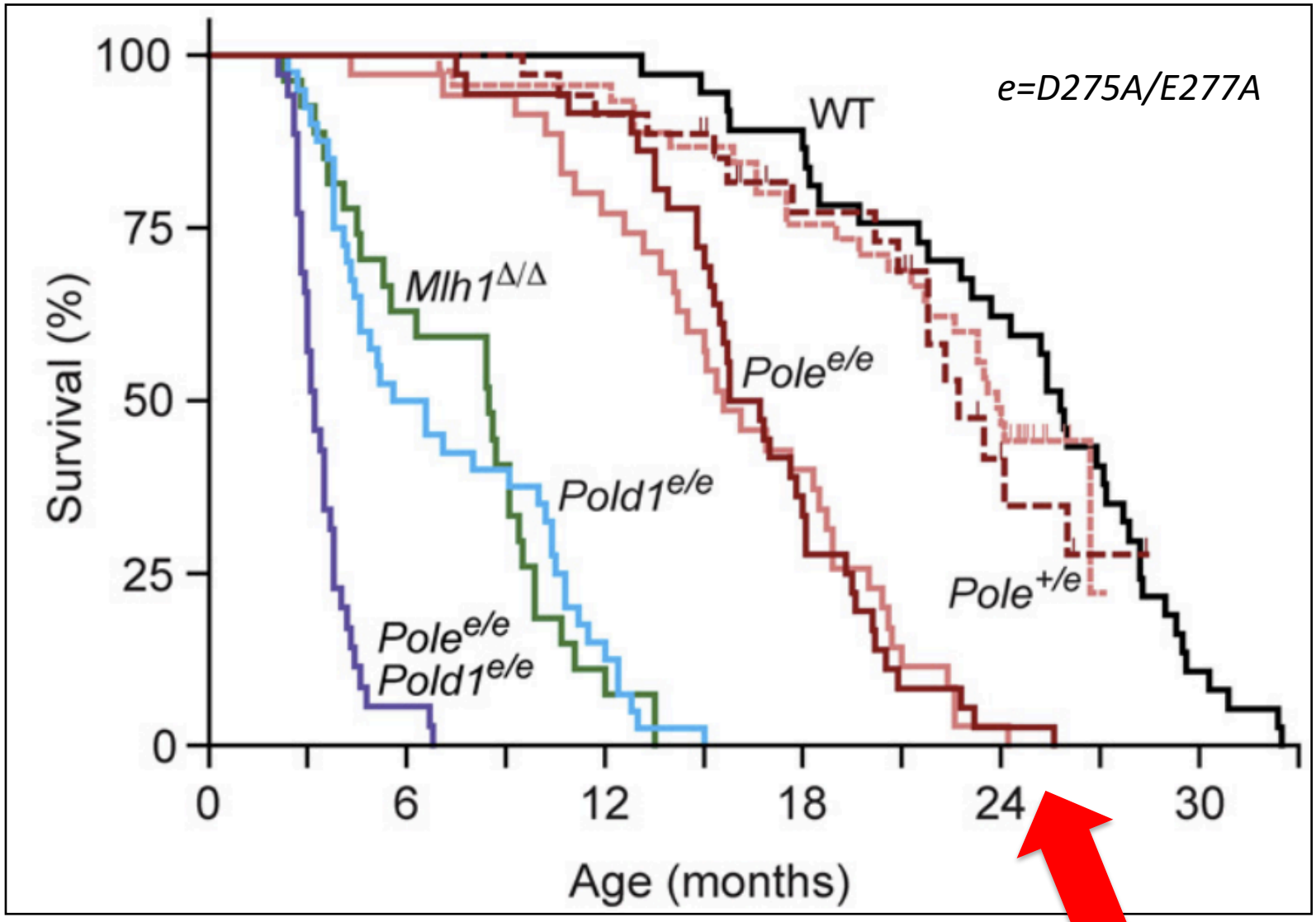
Cell Line	Pol $\varepsilon^{wt/exo-}$ - MLH1
Population Doubling	14
WGS SNVs	5,282
Mut/bp/Doubling	1.40×10^{-7}
μ_{BS} (from HPRT1)	0.23×10^{-7}
Transversions	
C:G>A:T	2406
C:G>G:C	78
T:A>A:T	271
T:A>G:C	245
Transitions	
C:G>T:A	1428
T:A>C:G	854





Hodel et al. eLife 2018

Paradox: Human POLE Patients Are All Heterozygous… But Previous POLE Exonuclease-Deficient Mouse Model Drives Tumorigenesis Only When Homozygous!



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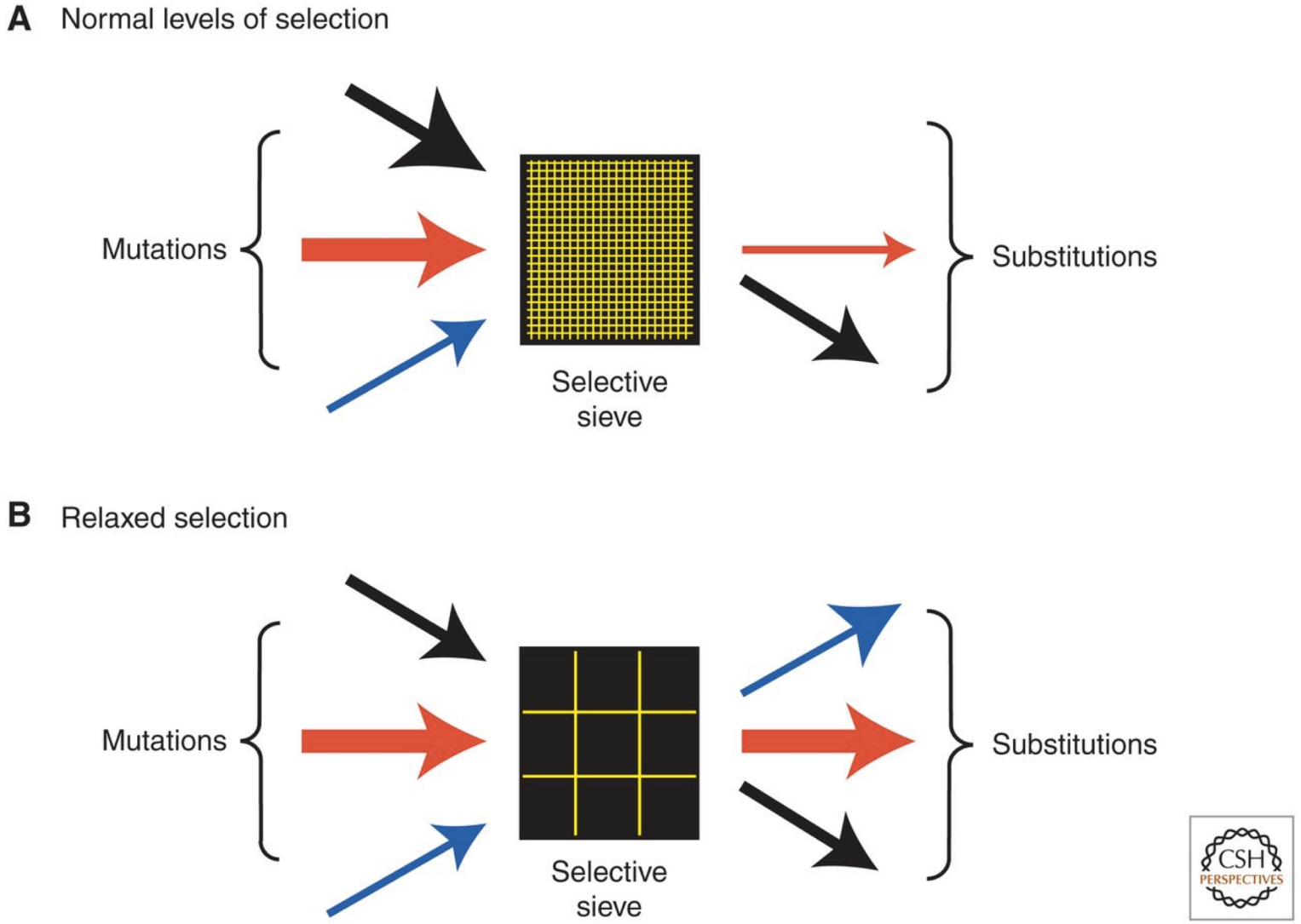
Brittany Campbell
Richard de Borja

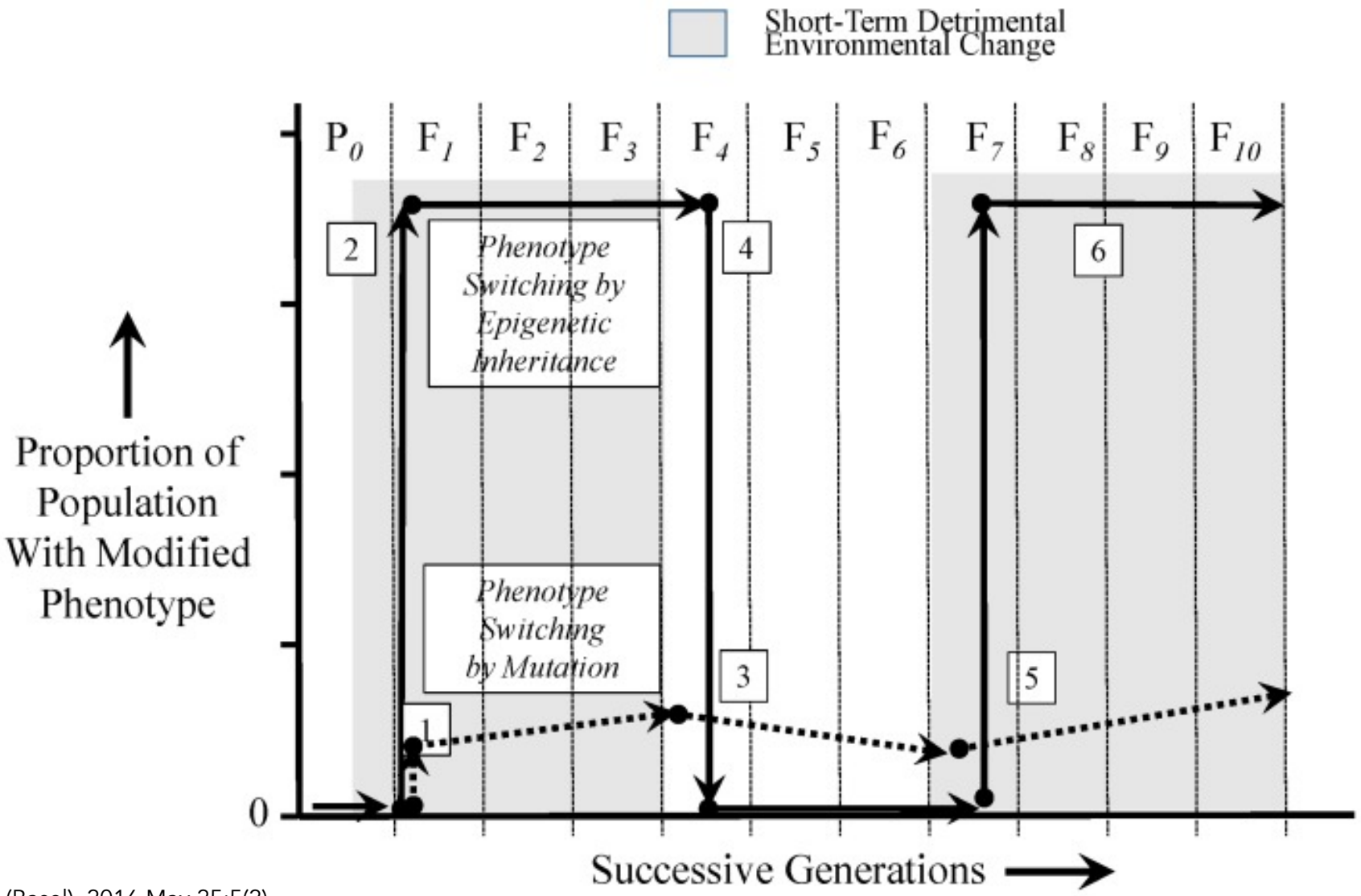
Baylor College of Medicine
David Wheeler
Eve Shinbrot

Genetic Variation is Required for Evolutionary Changes; Mutation is the Source of This Genetic Variation



Hershberg R. Cold Spring Harb Perspect Biol. 2015 Sep 1;7(9):a018077.

Epienetic Variation is Well Suited at Population Level to Respond to Environmental Stressors



Biology (Basel). 2016 May 25;5(2).