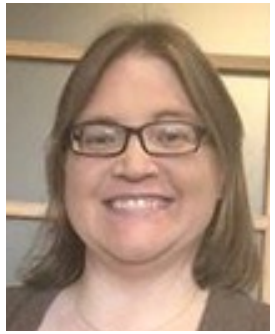
20.109 MOD1
Genomic Instability

Fall 2023
Day 1

Bevin P. Engelward, *Sc.D*.
Professor of Biological Engineering

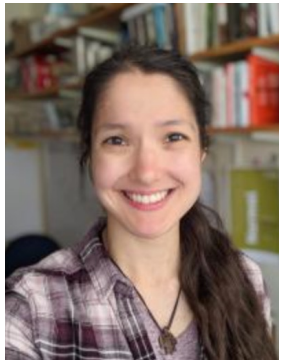
20.109 MOD1 Fall 2023 – The Fabulous Team

Dr. Noreen Lyell
Sr. Lecturer



Dr. Becky Meyer
Lecturer

Jamie Zhan
Instructor



Chiara Ricci-Tam
BE Communication Lab Manager & Lecturer

Sean Clarke
BE Communication Lab, Lecturer

Alexander Hostetler
TA

Chyna Mays
TA

Bryan Wong
TA

Objectives for Research Skills (Mod1)

Experimental Design

Quantitative Measurements

Experimental Variability

Data Interpretation and Presentation Skills

Statistics

Critical Data Interpretation

Written Communication

Basic Laboratory Skills

Sources of Error

Basic Laboratory Equipment

Mammalian Cell Culture

Immunohistochemistry

Image Analysis

Overall Course Conceptual Goals for Mod1

- Fundamental Biological Concepts of Molecular Pathways
- DNA Structure and DNA Replication
- Molecular Biology of DNA Repair
- Genomic Instability
- Inter-Individual Variation in Susceptibility to Cancer.
- Public Health
- Fundamental Engineering Concepts: Learn about harnessing engineering principles to translate an idea into a product.

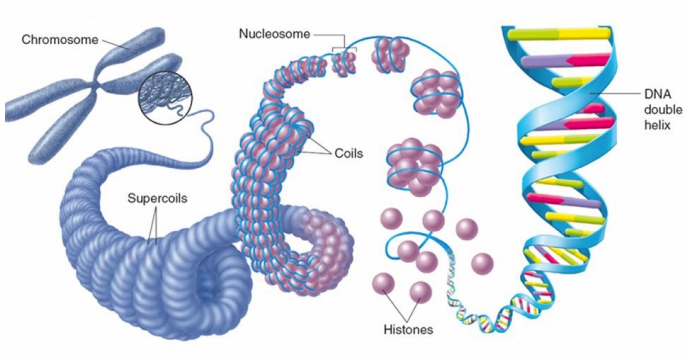
Causes of Cancer

Radiation & Chemotherapy

Targeted Chemotherapies

Antibody Affinity Maturation

Premature Aging



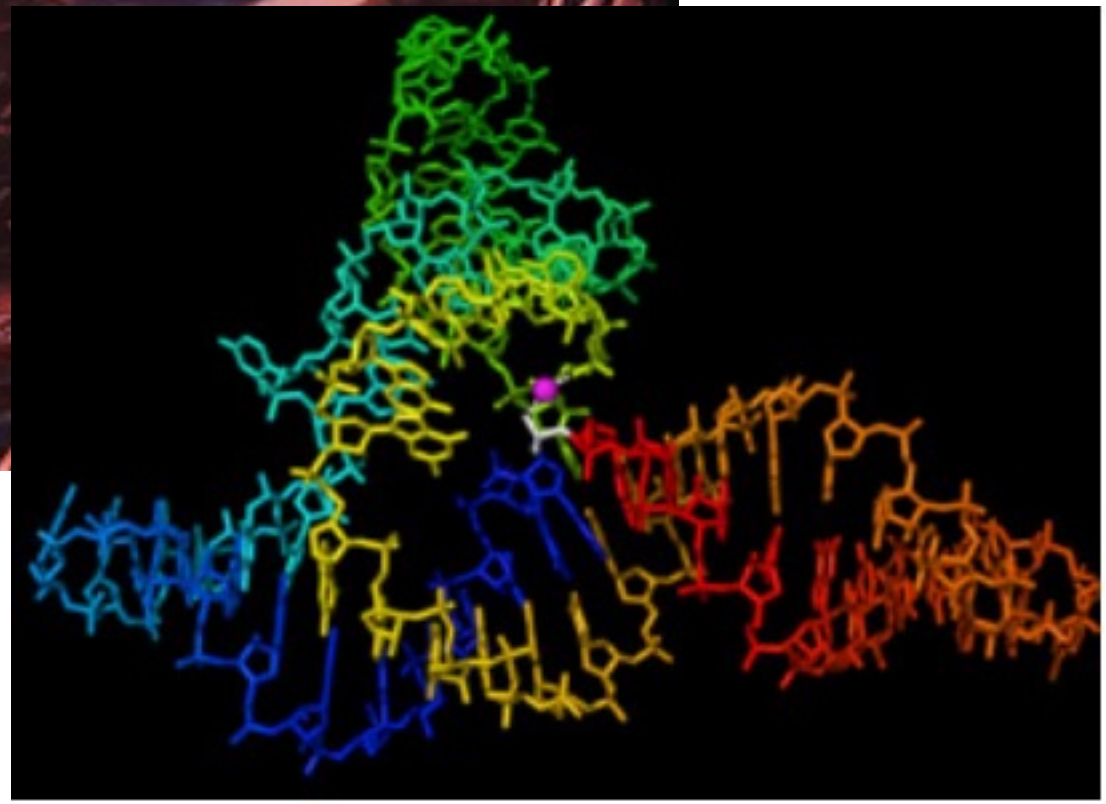
Injury Repair

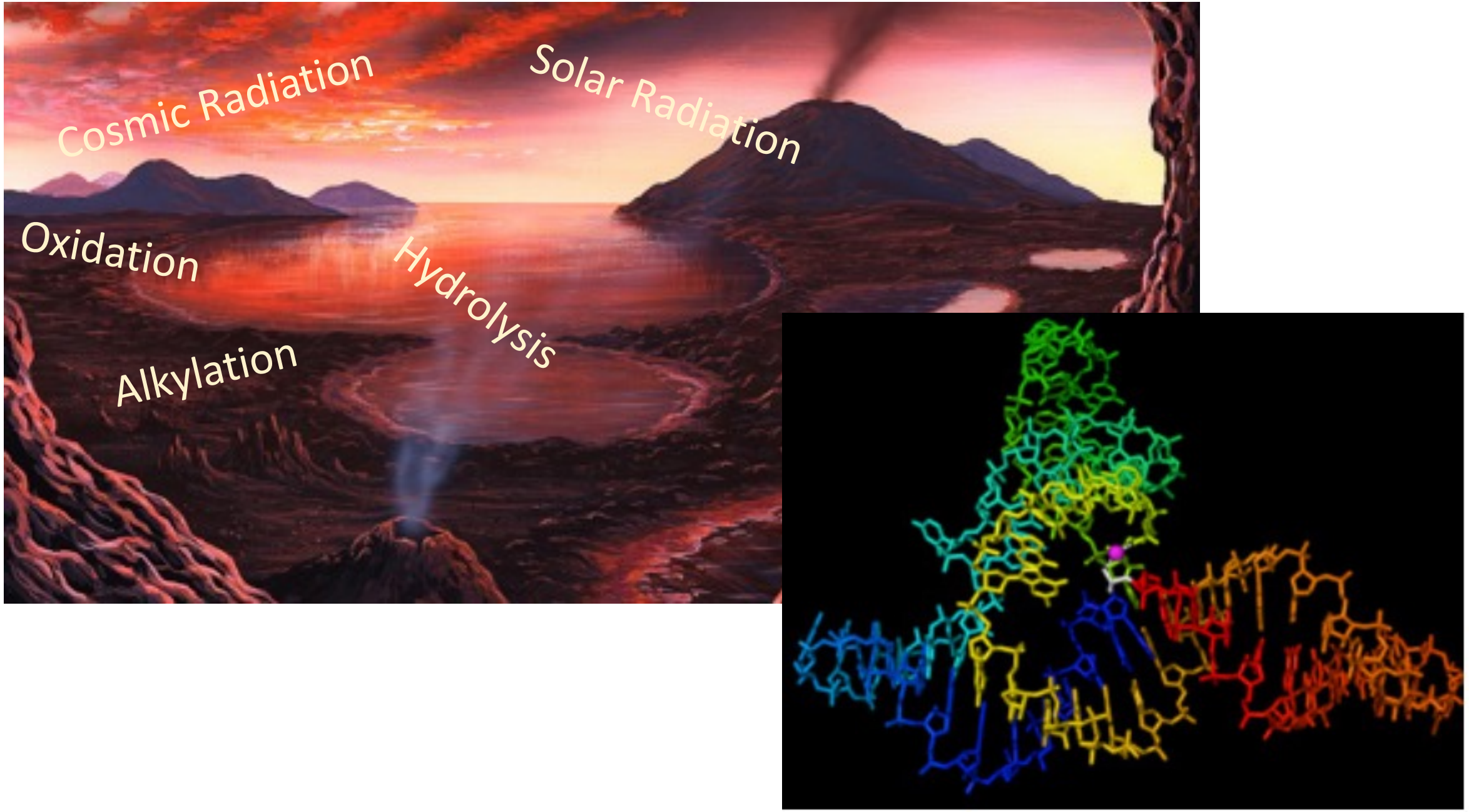
Neurological Disorders

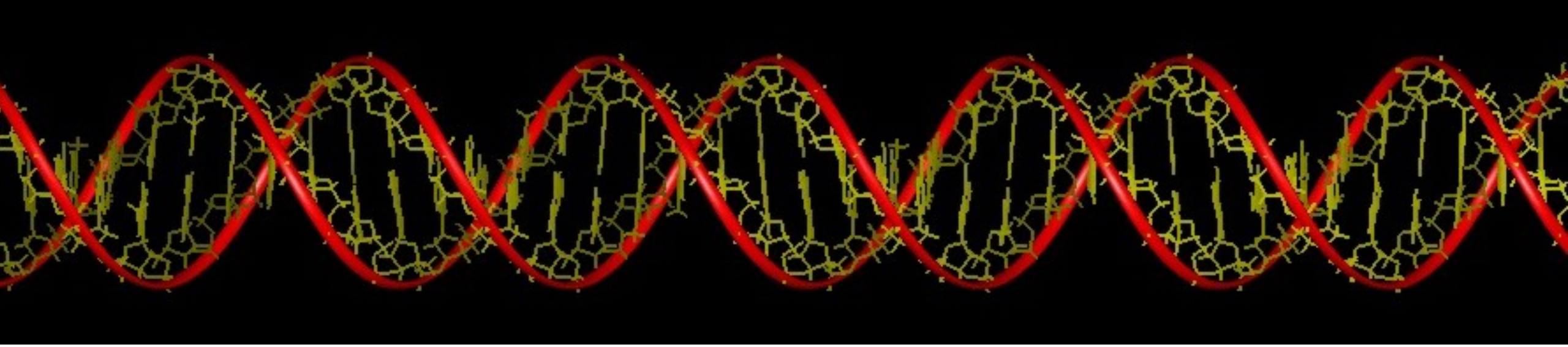
Gene Silencing

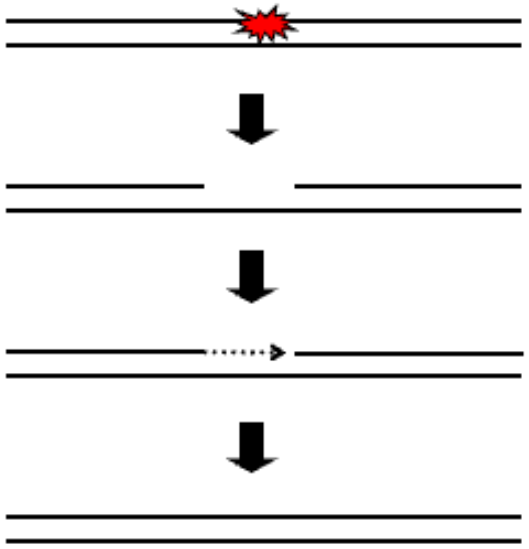
CRISPR KO's and Gene Editing

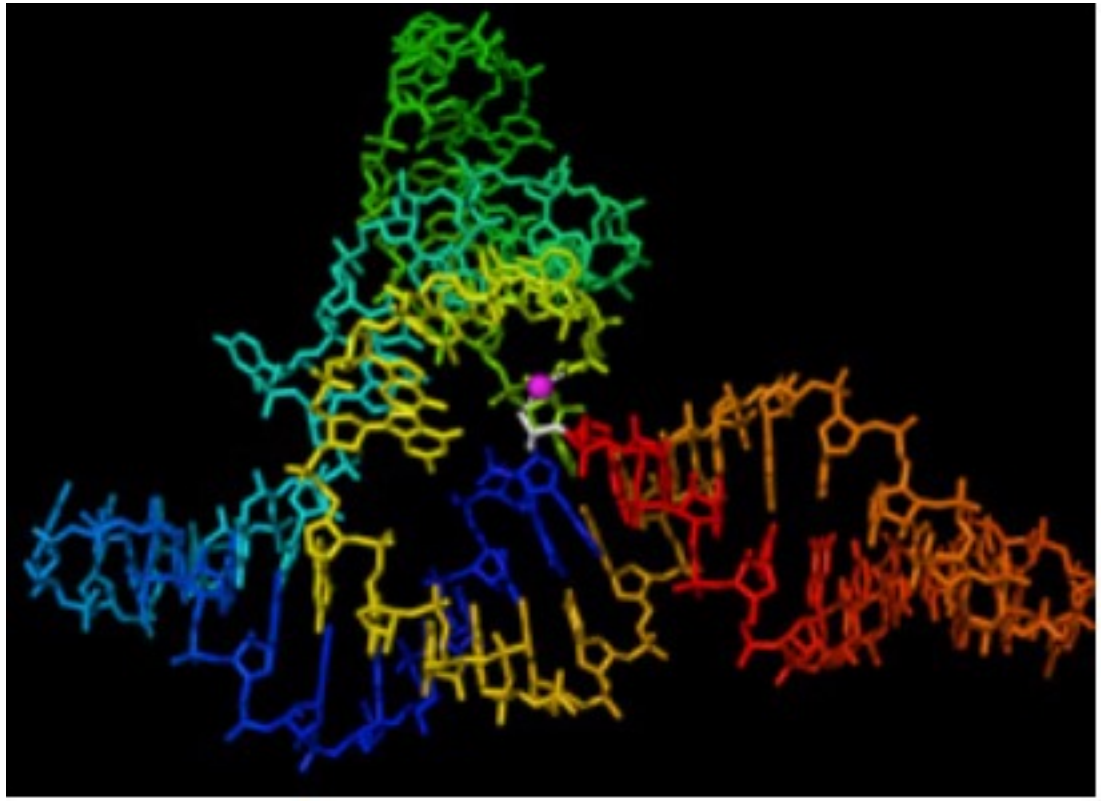
KOs (Gene Targeting) KDs (RNAi)




Cosmic Radiation
Solar Radiation
Oxidation
Hydrolysis
Alkylation







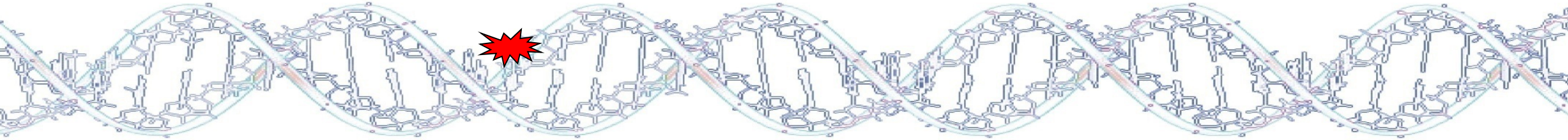
Cells need repairability as well as heritability.

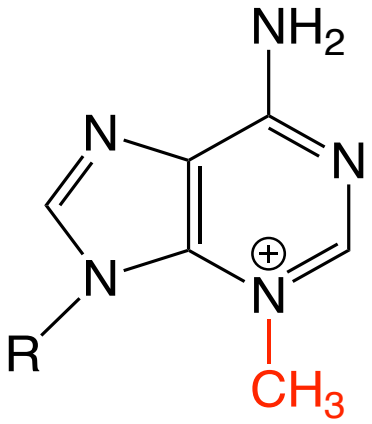
Genomic Instability Remains a Major Challenge

Steady State level of DNA lesions

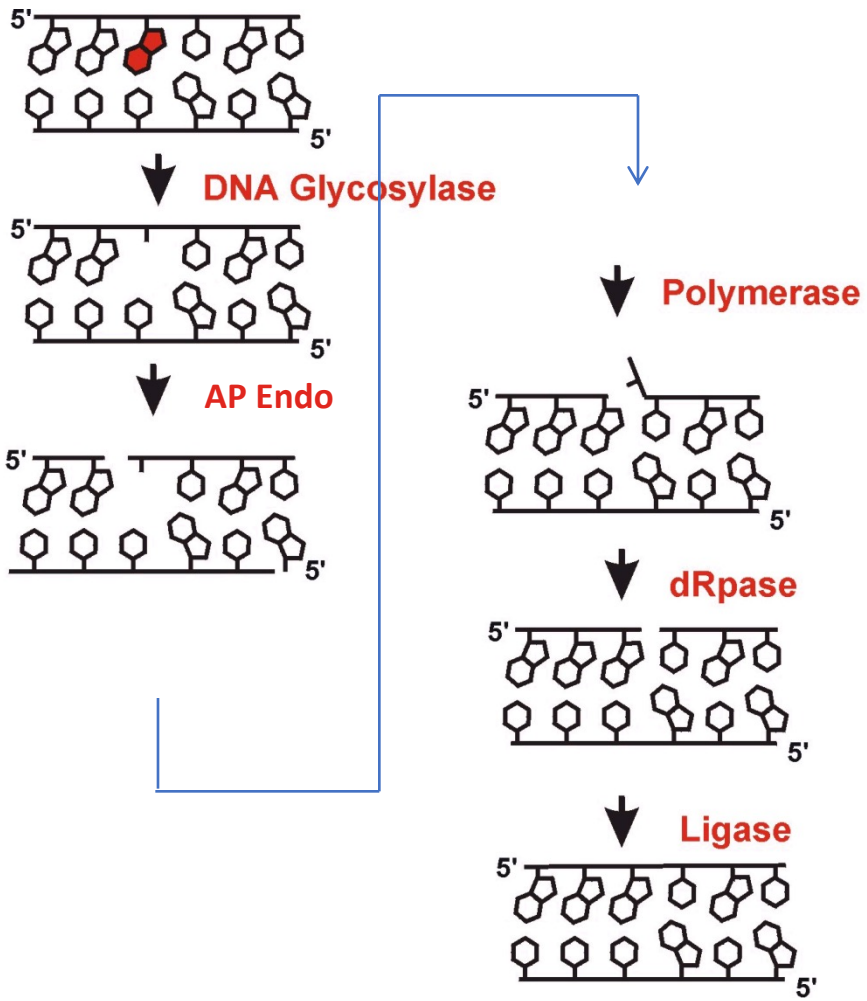
Single Strand Breaks	~1500
Double Strand Breaks	~5-10
Alkylated Bases	> 3,000
Oxidized Bases	100 - 1000

Fortunately, we have DNA Repair: Base Excision Repair





3MeA



You perform base excision repair more than a quadrillion times per day in your body

What happens if we have defects in DNA repair?

Our focus is on the relationship between DNA repair and cancer.

More subtle differences may make a difference to cancer risk.

A small increase in cancer risk for a large number of people is a big problem.

Skin cancer (NER)

Colon Cancer (MMR)

Breast Cancer (low HR)

Ovarian Cancer (low HR)

Leukemia (high HR)

Anemia (FANC)

Amyotrophic Lateral Sclerosis (possibly BER)

Microcephaly (NHEJ)

Xeroderma Pigmentosum. NER Defect. 2000X Increased Risk of Cancer

In addition to genetics, we also need to think about exposures

Pharmaceuticals

Industrial chemicals

Household chemicals

Food

Pollutants

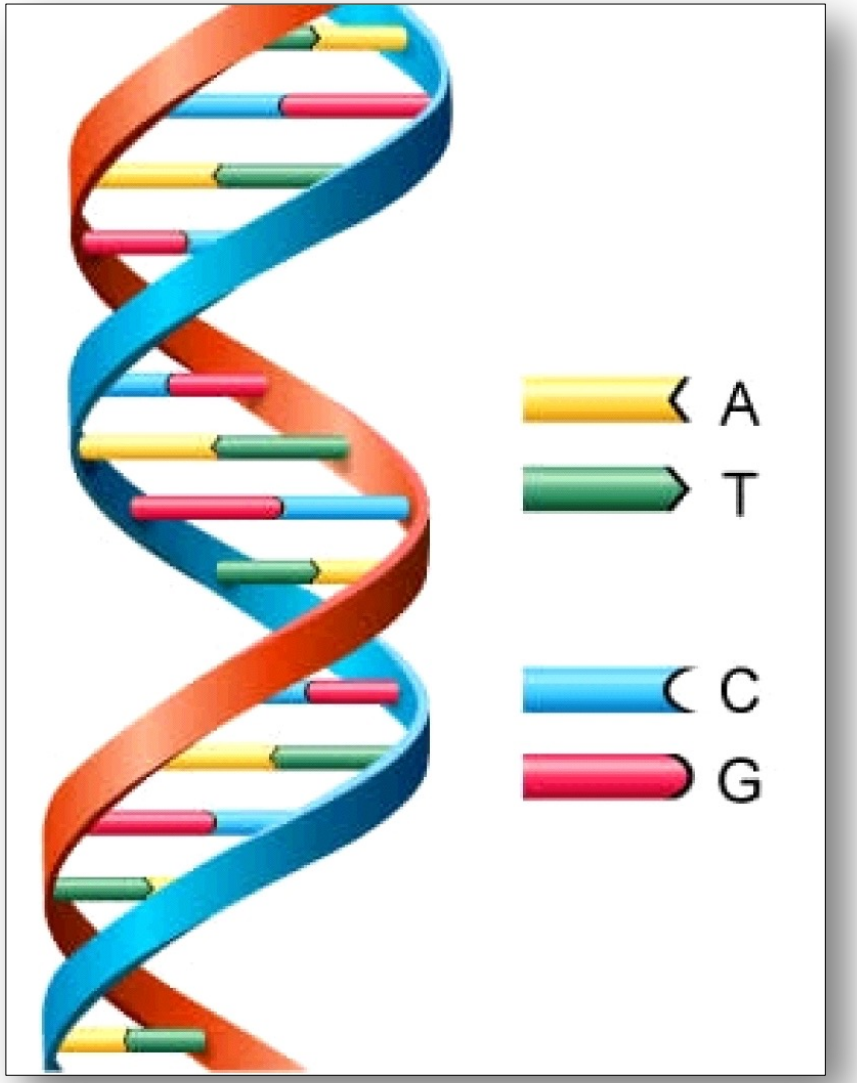
Cosmetics

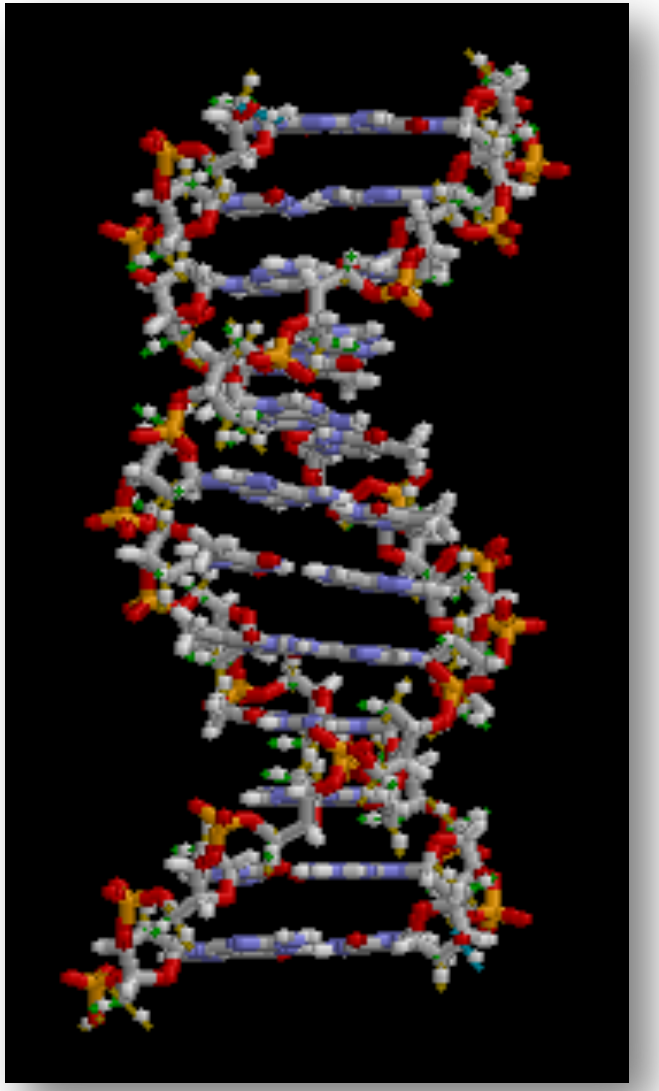
Disease can be a product of Gene-Environment Interactions

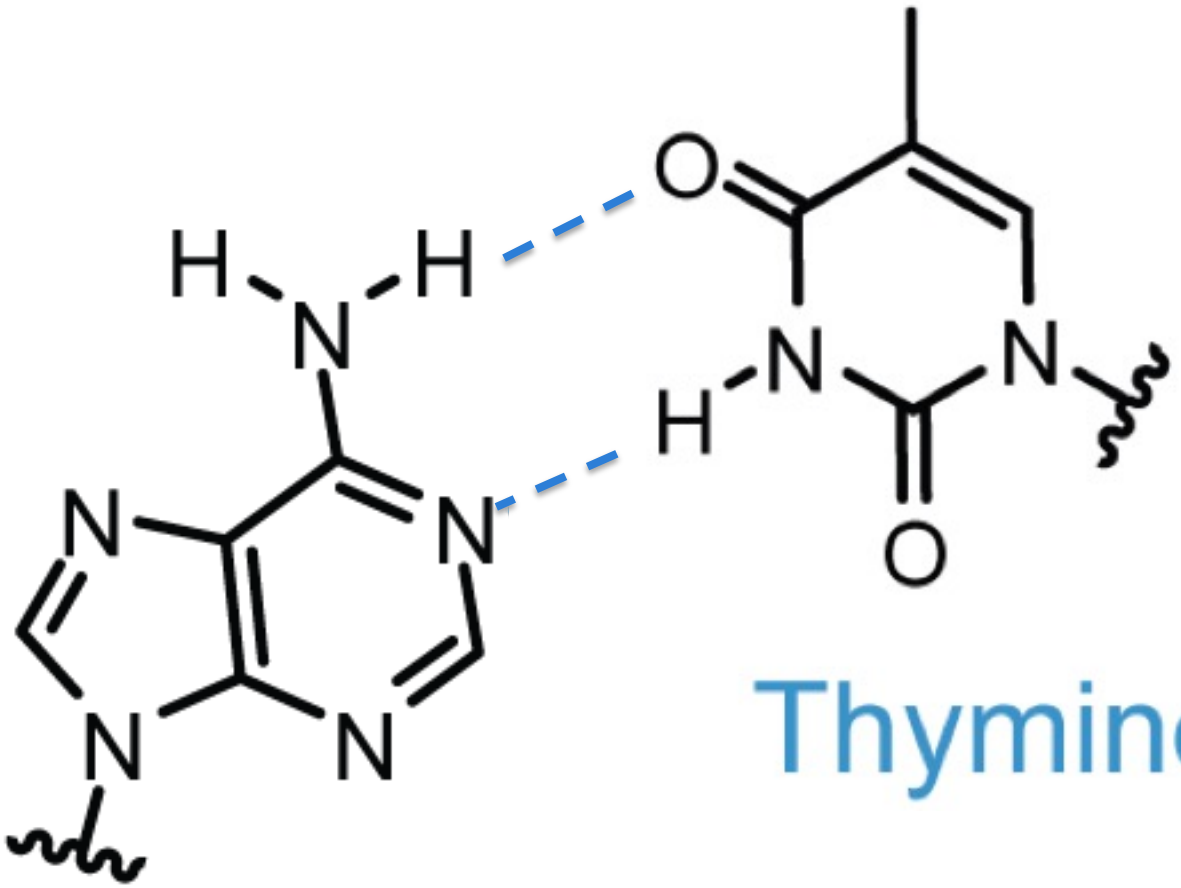
Xeroderma Pigmentosum
NER Defect
2000X Increased Risk of Cancer

Skin cancer is mostly in sunlight exposed skin.

All known life forms are based on DNA

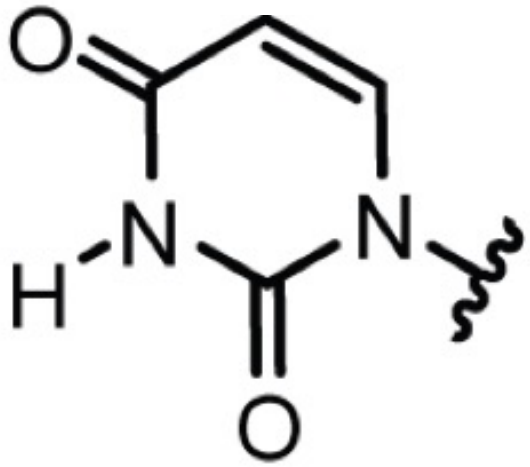


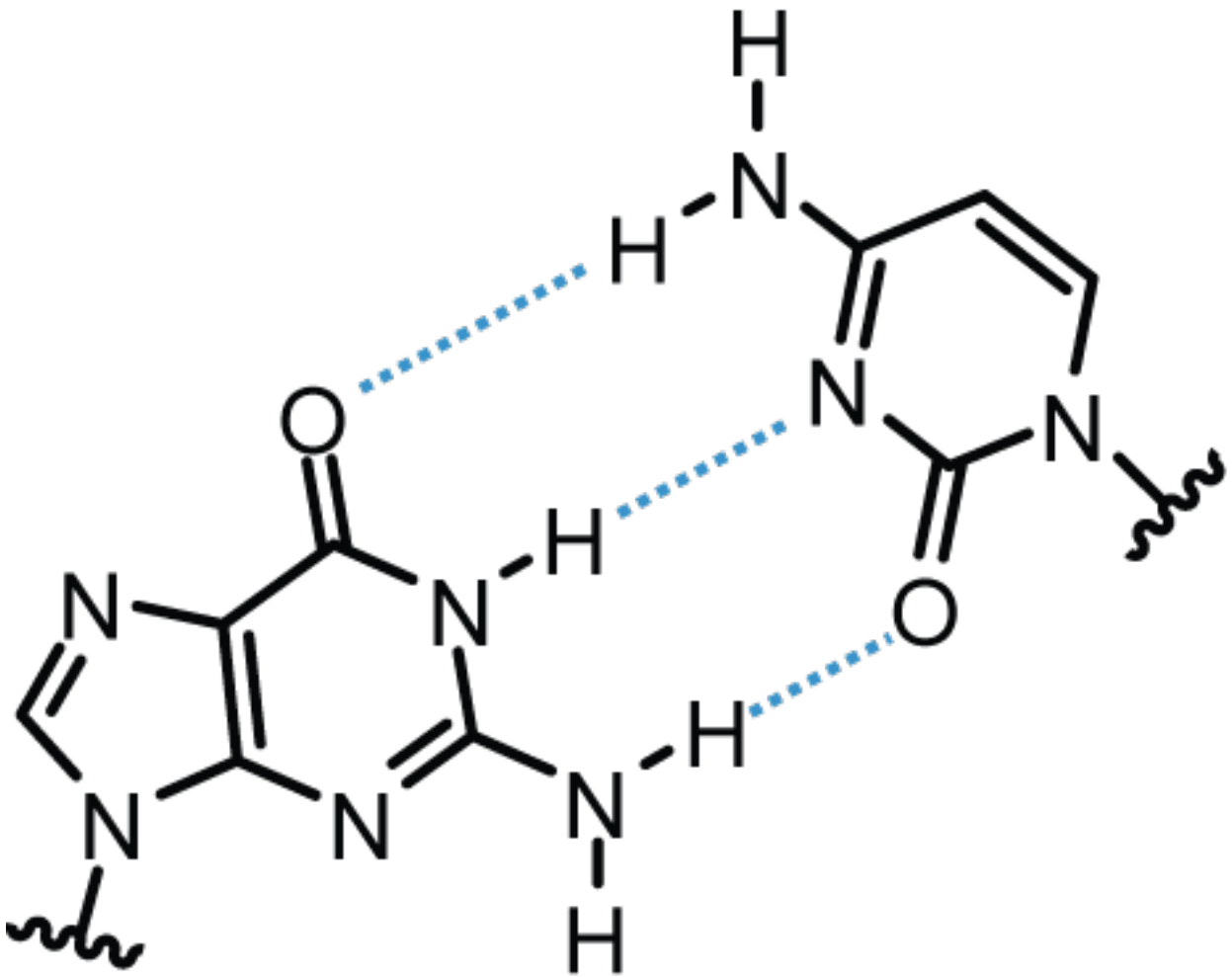




Adenine

Thymine




H
N
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O
H
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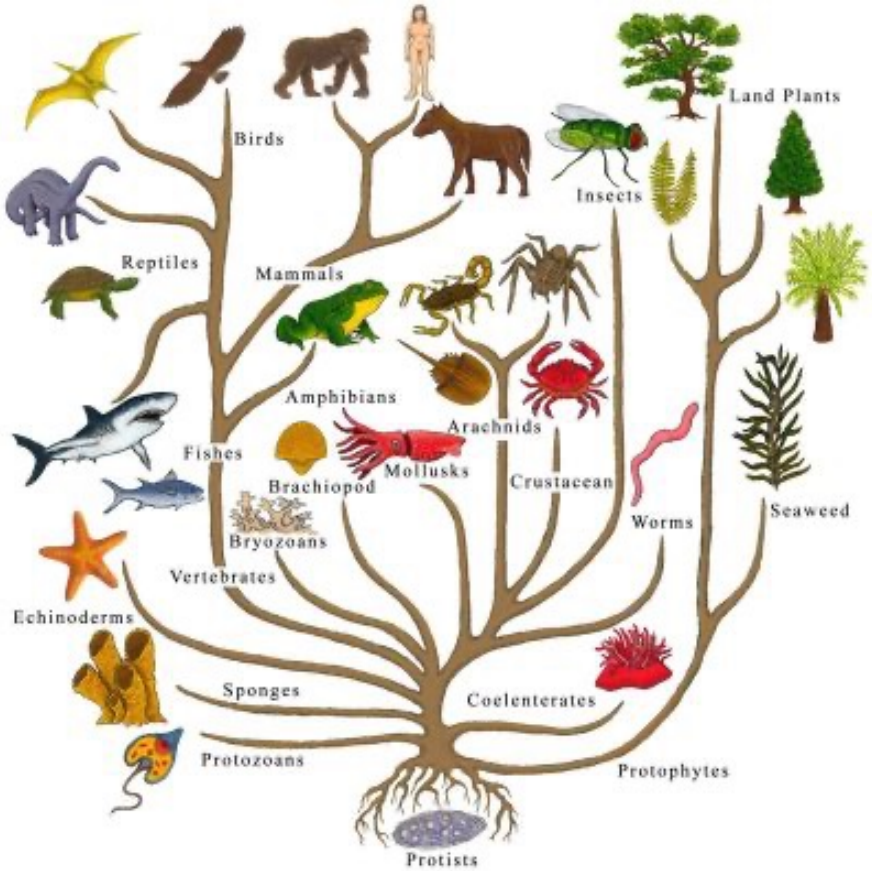
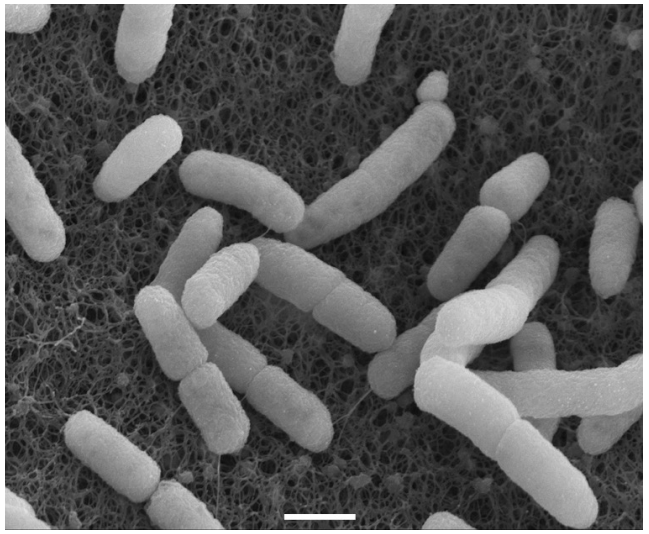
H

All known life forms are based on DNA

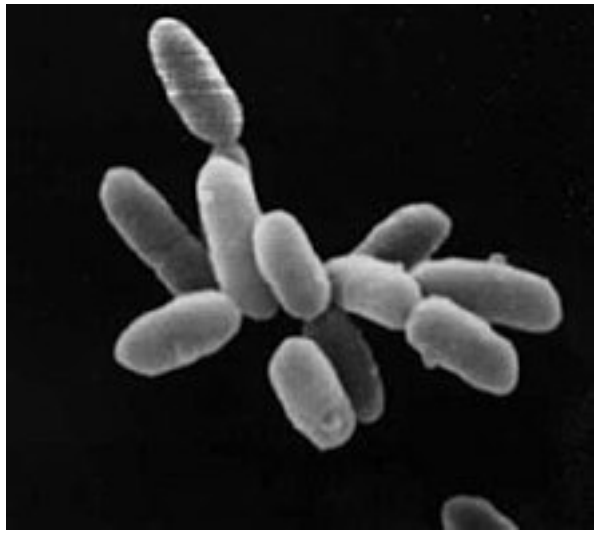


The cell is generally considered to be the basic unit of life

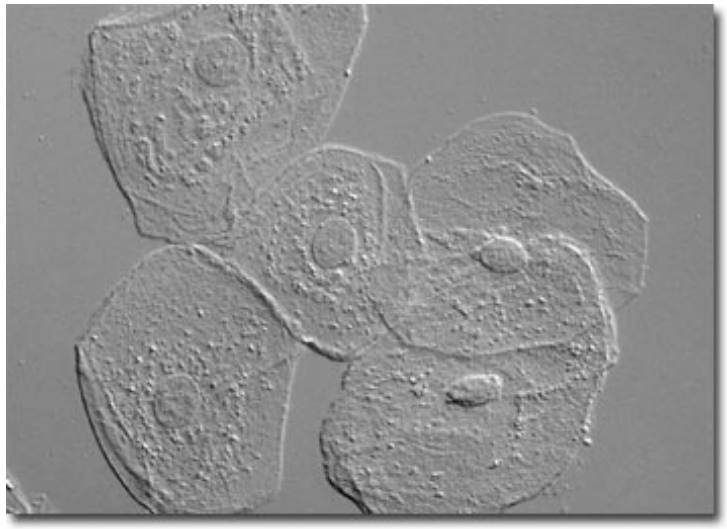
Bacteria



Archaea



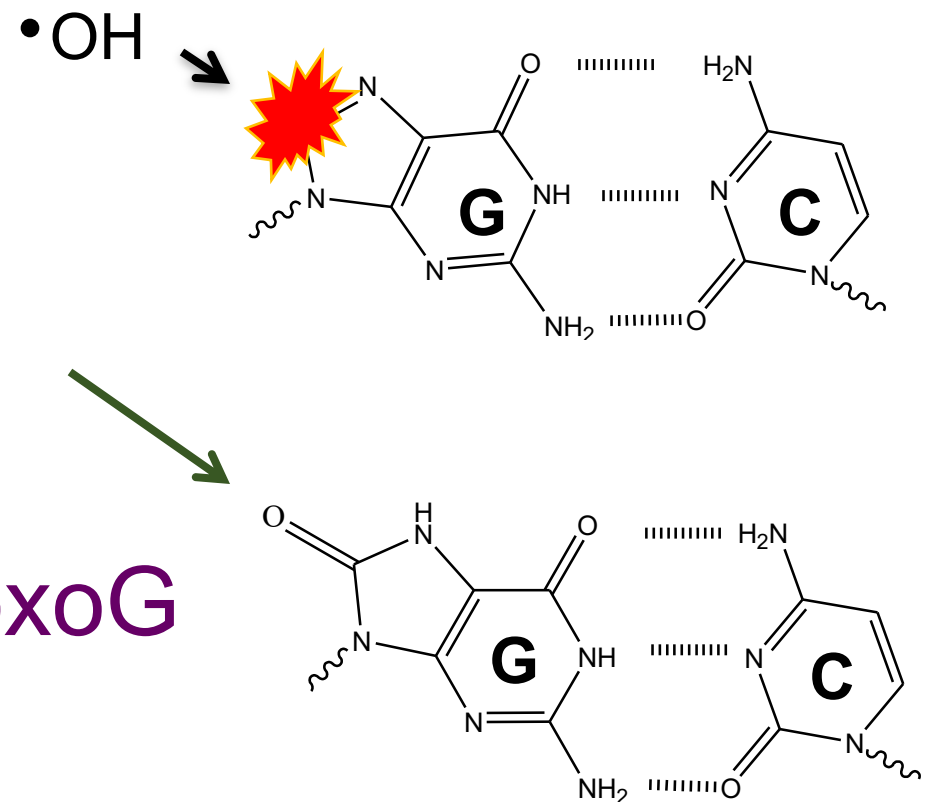
Eukaryota

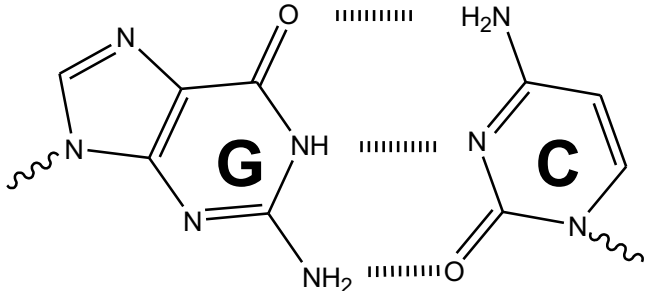


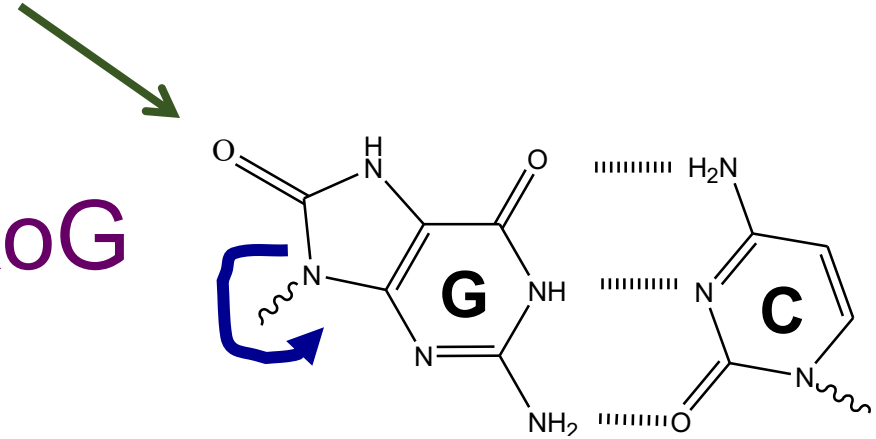
The Three Domains of Life

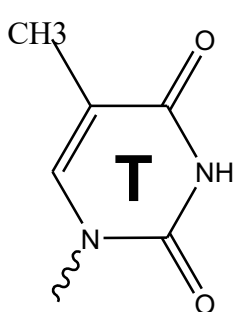
Structure is Information

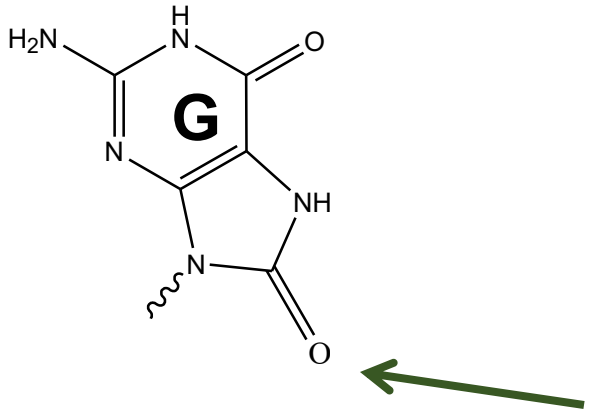
8oxoG


•OH
8oxoG
G
C
NH
NH2
H2N
O
N
H


O
N
H2N
G
NH
N
C
N
N
NH2
O


8oxoG
O
H
N
O
H2N
N
G
NH
N
C
N
N
NH2
O


CH3
O
T
NH
N
O


H2N
H
N
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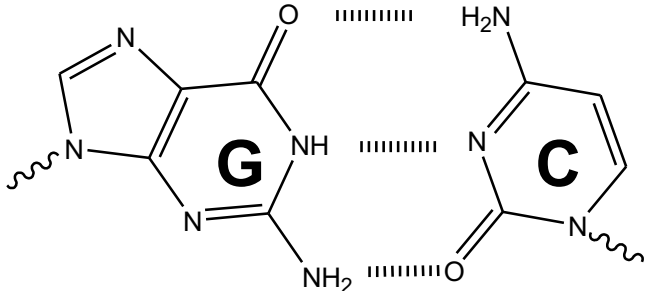
G
N
NH
N
O


O
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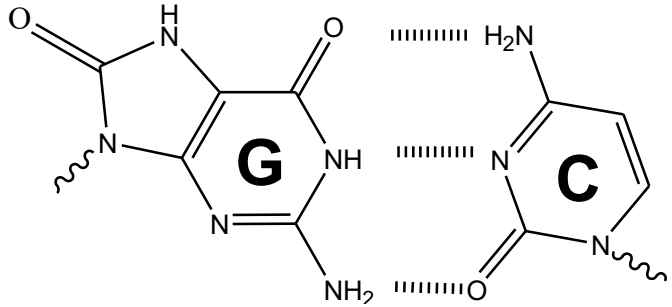
G
NH
N
NH2
H2N
N
C
N
O


O
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G
NH
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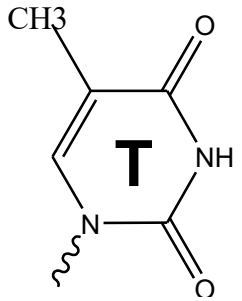
NH2
H2N
N
C
N
O


CH3
O
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NH
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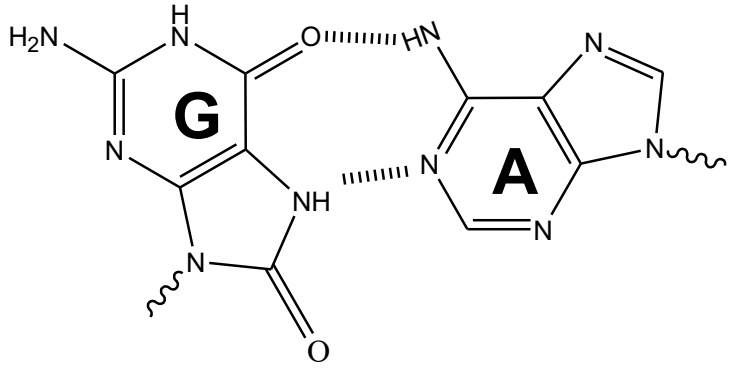

H2N
H
N
O
HN
N
G
N
N
A
NH
N
N
O

GC →TA

What is cancer?

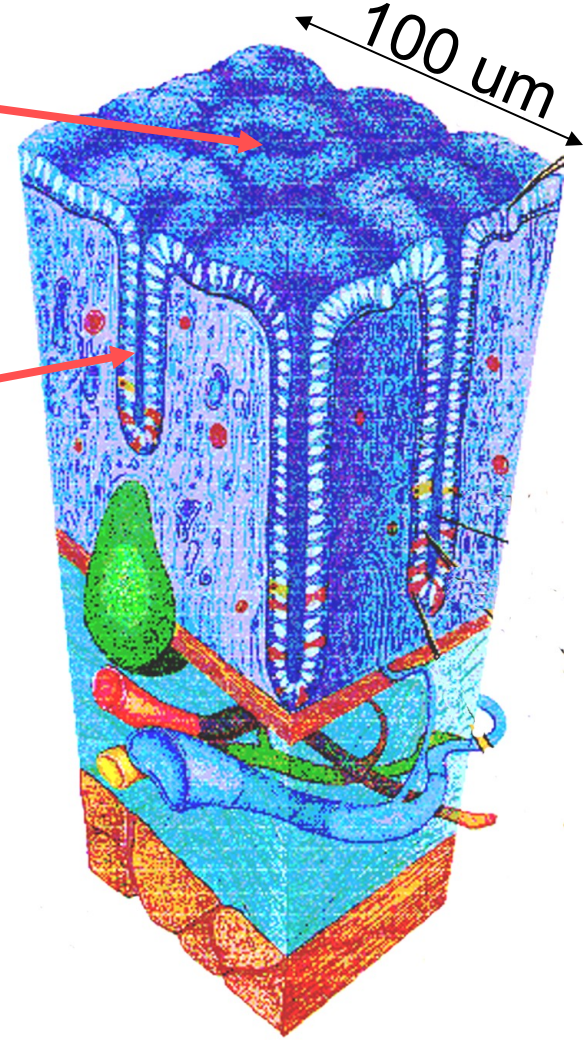
Why are mutations important?

Normal Colon Tissue

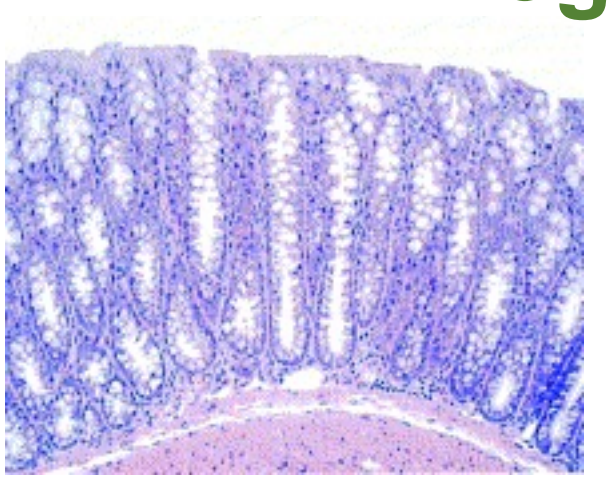
TOP: Inner surface of the colon

Divits = "Crypts"

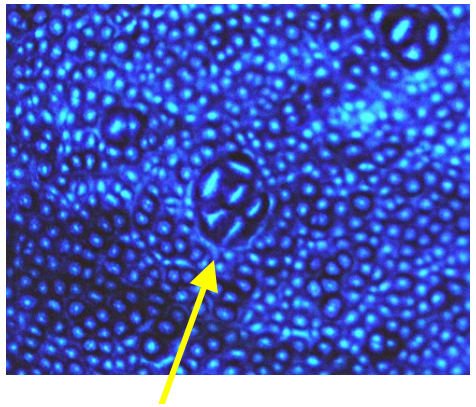
Cell Lining = Epithelial Cells



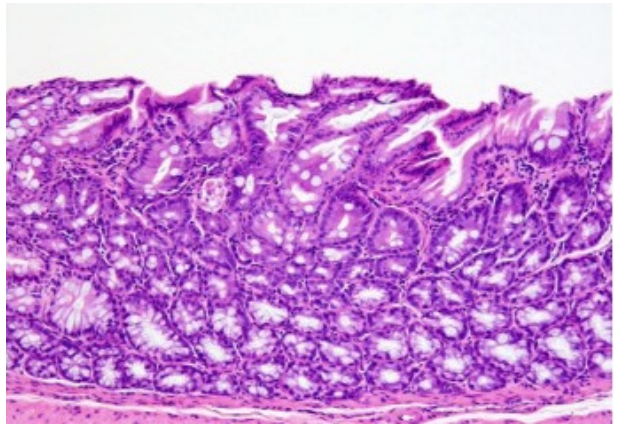
Progression



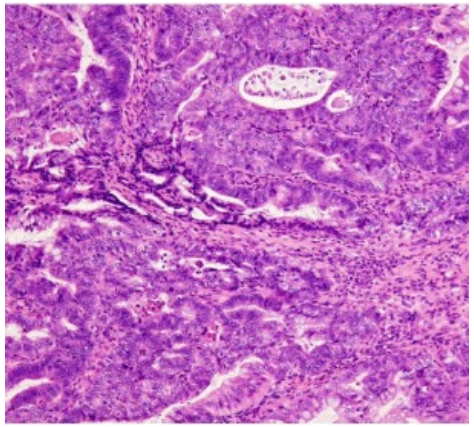
Normal Colonic Epithelium



Dysplasic Crypt

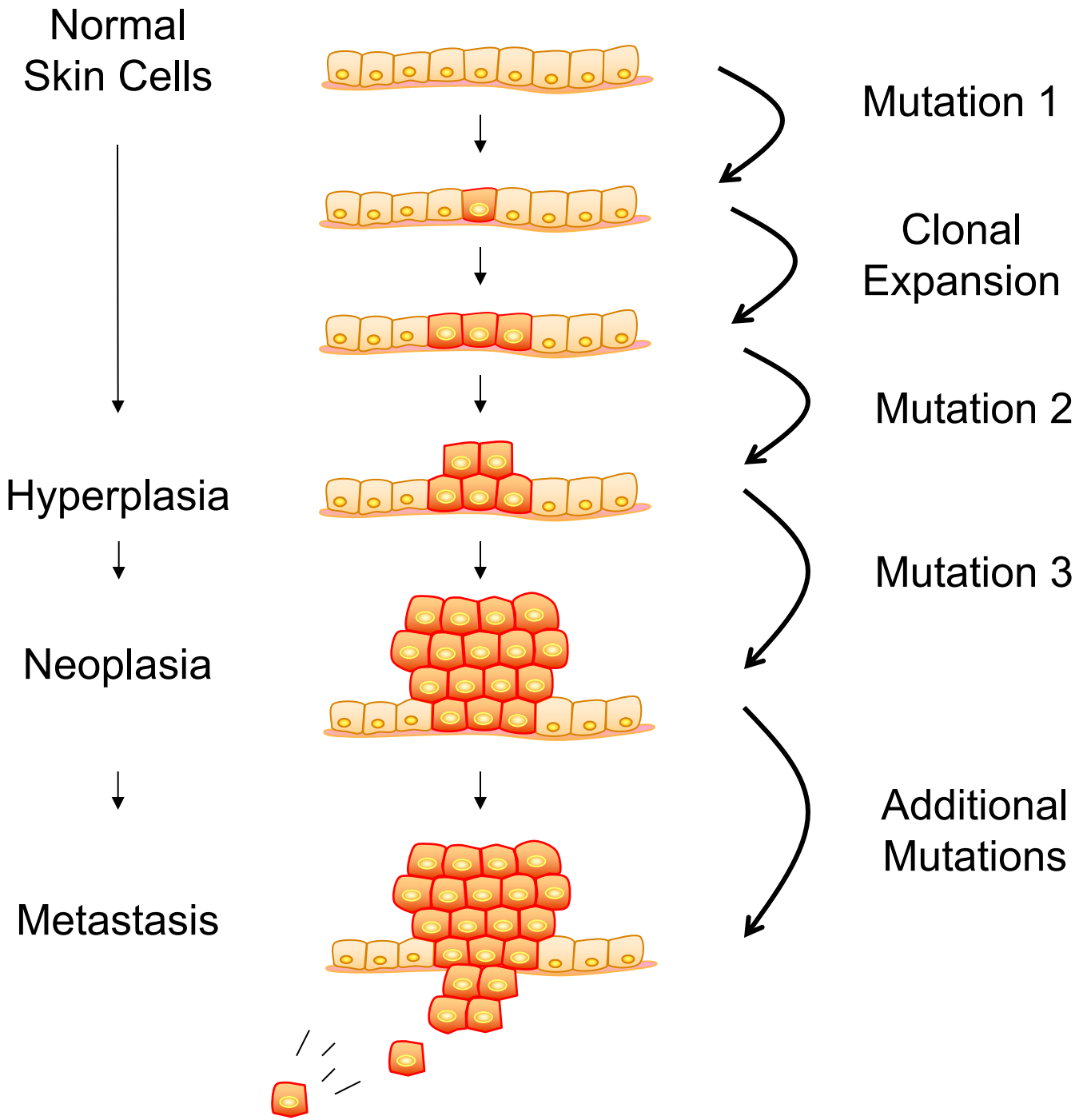


Mild Dysplasia

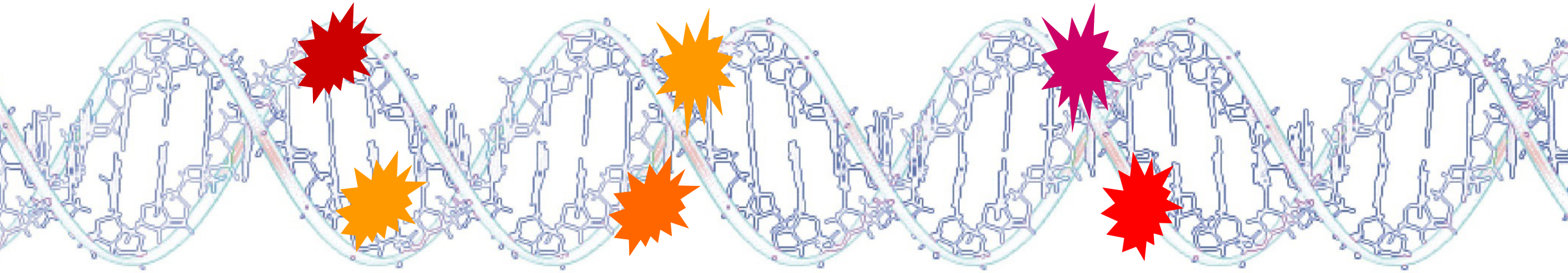


Cancer

What are the genetic steps?
What does a cancer cell need to be able to do?

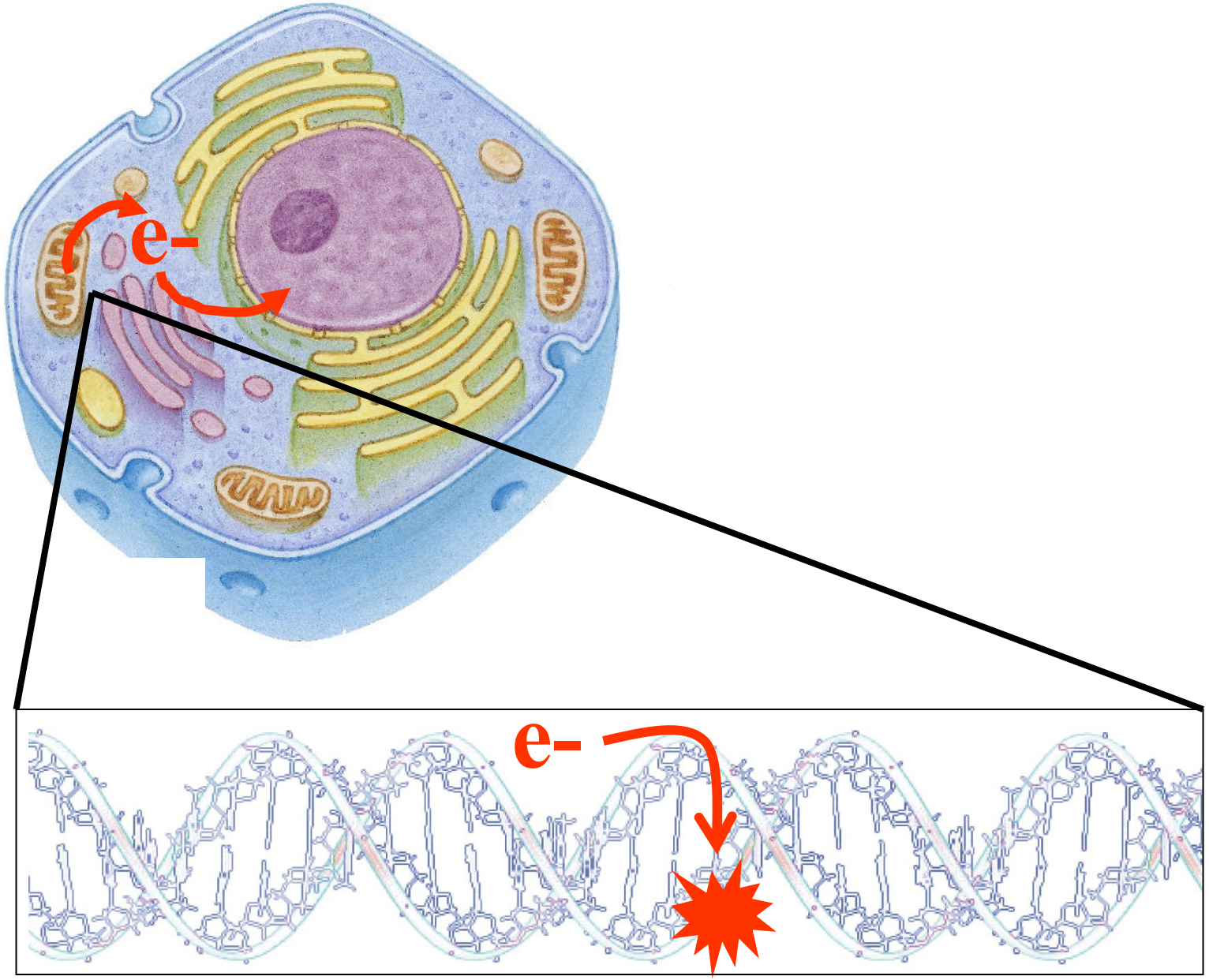

Normal
Skin Cells
Hyperplasia
Neoplasia
Metastasis
Mutation 1
Clonal
Expansion
Mutation 2
Mutation 3
Additional
Mutations

Where do mutations come from?



Mutations, Toxicity, Cellular Defects

Cancer, Aging, Heritable Diseases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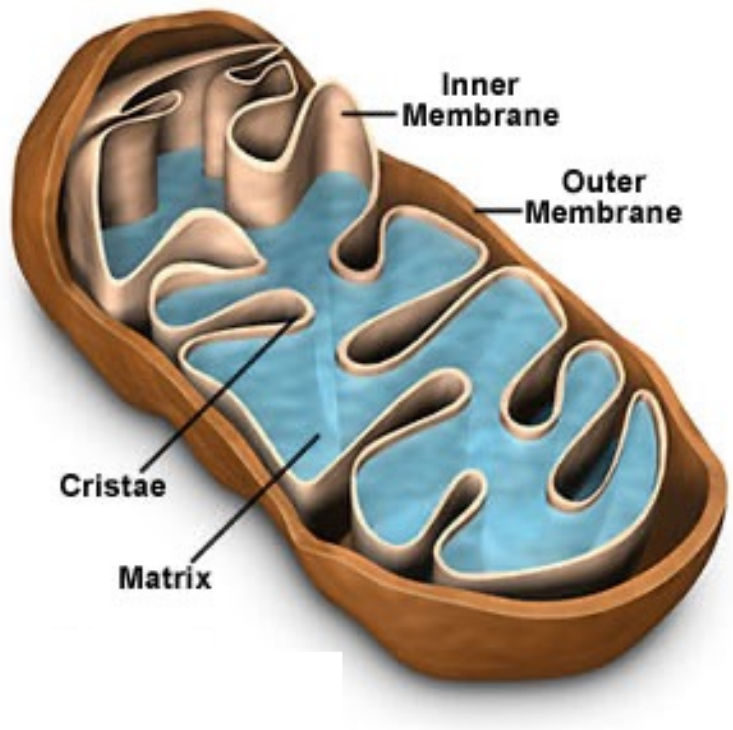
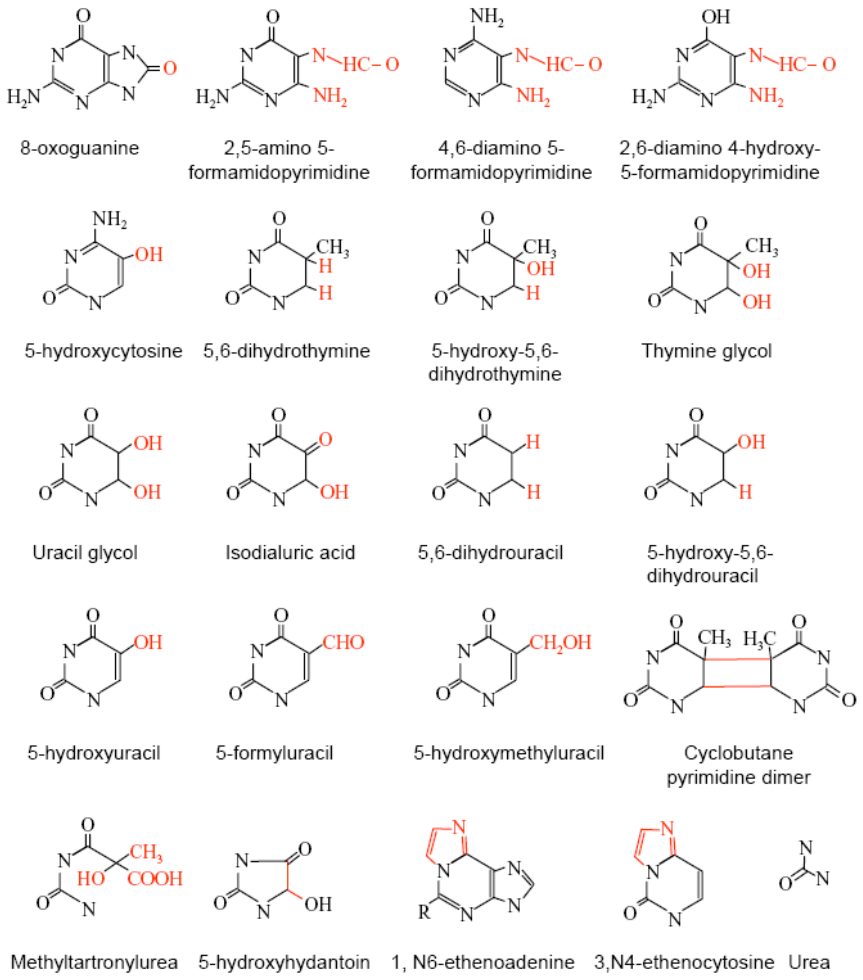
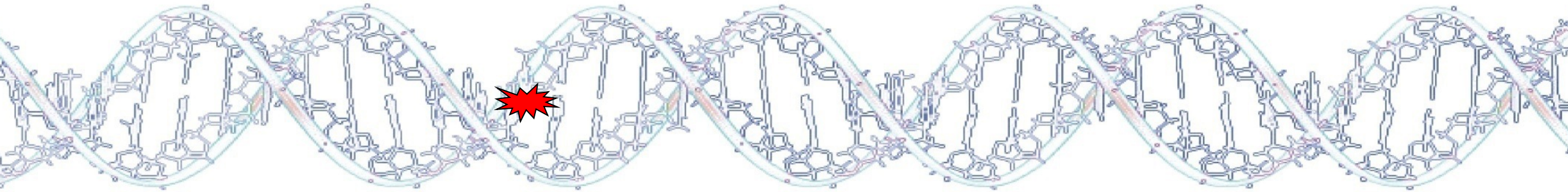
e-

Reactive Oxygen Species Damage DNA Bases

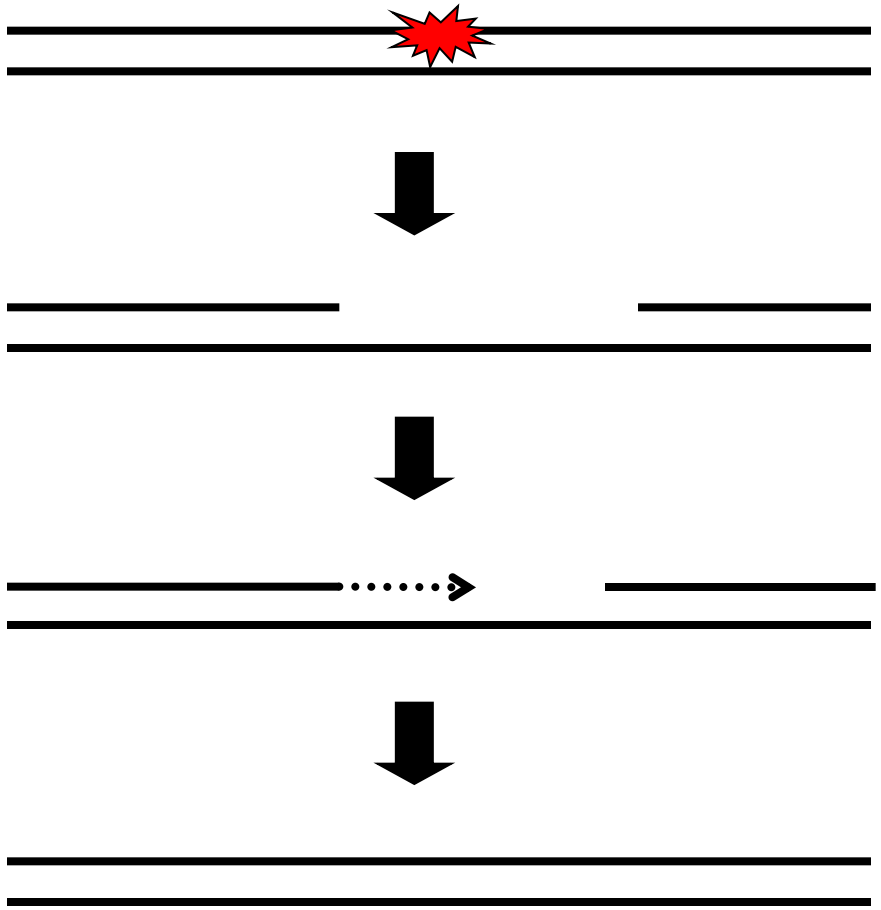


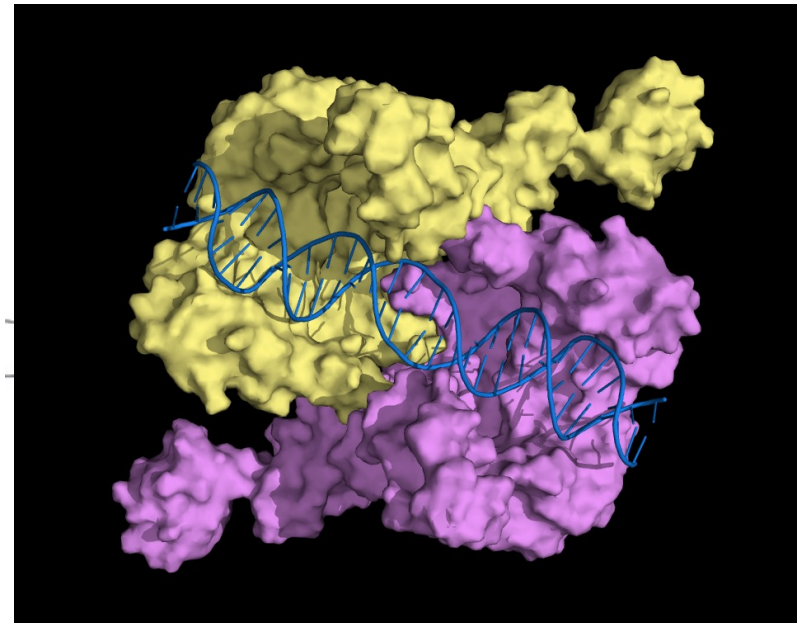


From Slupphaug, Kavli and Krokan



Sunlight-Induced DNA Damage can be Repaired

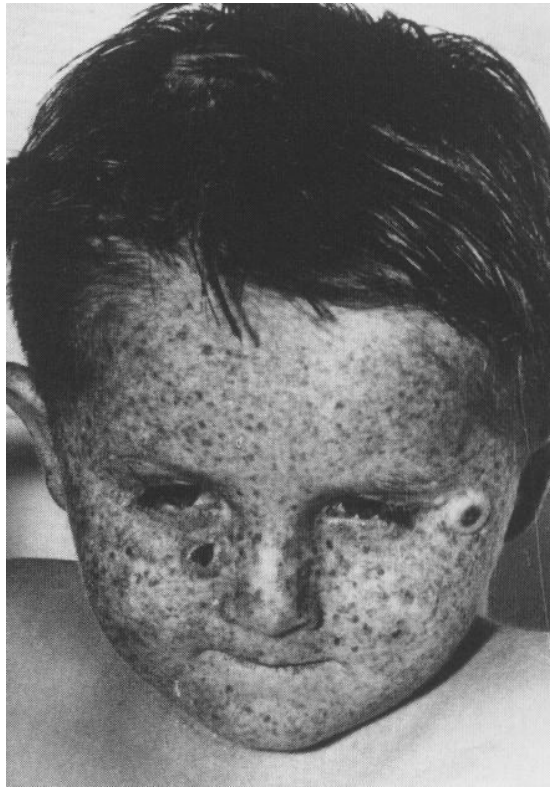




Nucleotide Excision Repair

Marcin Nowotny

DNA Repair impacts Risk of Cancer



People lacking repair of UV dimers have a 2000X increased risk of skin cancer.

Xeroderma Pigmentosum – A rare human disease

Get to Know your Cells

Do they like crowding? How low can you plate your cells? What is “too crowded”?

How fast do they divide? How long does it take for them to start dividing after being split?

Are they immortal? If not, how long can you culture them?

What do they look like when they are healthy?

How often do they like to have their media changed?

Are they mycoplasm free?

How do you pick which cells to study?

Primary
Transformed
Cell Line
Isogenic
Genomic Stability
Sources
Differentiation State

Things you need to know about your cells…

Lag phase

Log phase

Doubling time

Optimal Maximum Density

Optimal Minimum Density

Plating Efficiency

Contact inhibition