Epigenetics: DNA Methylation

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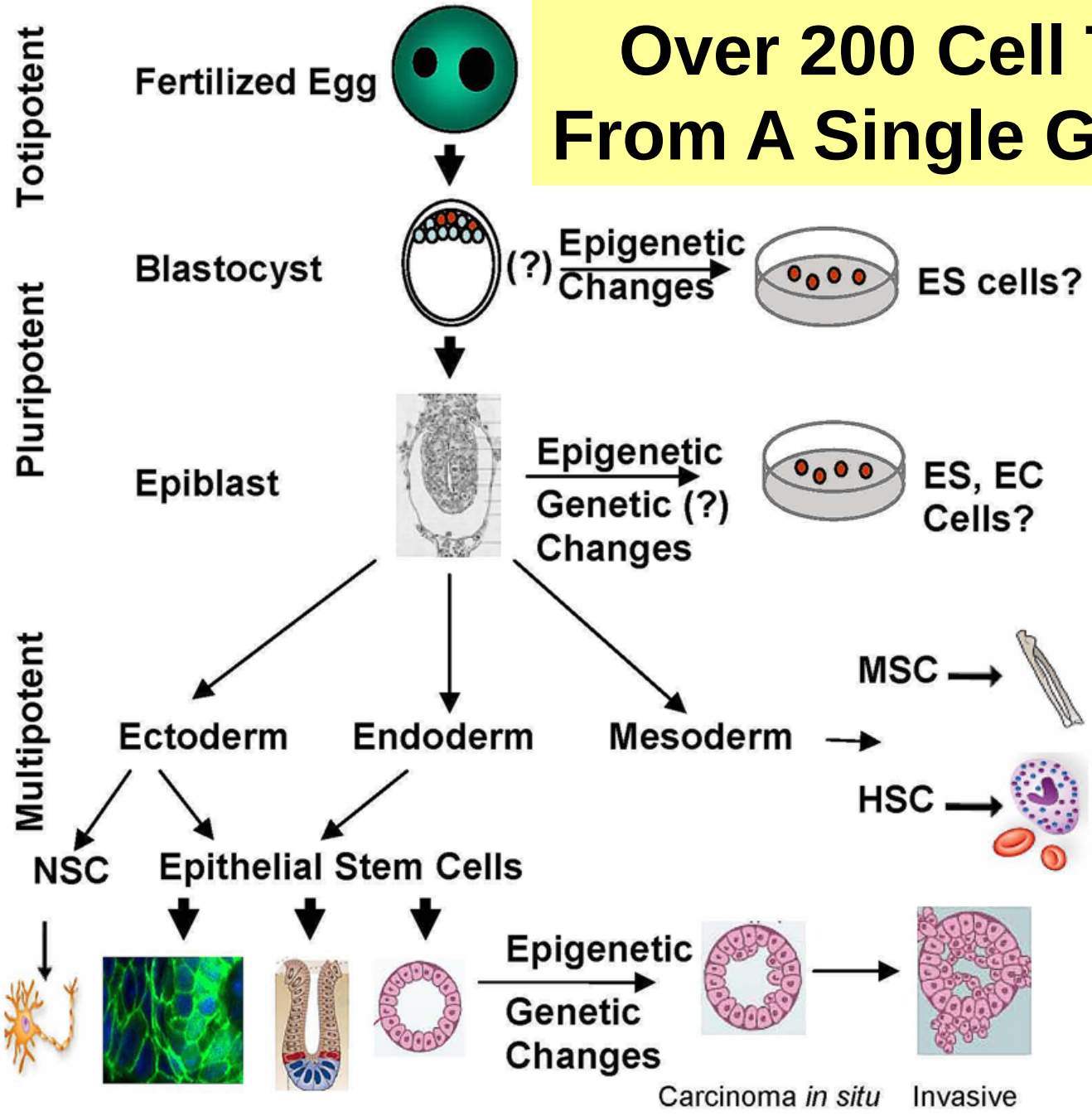
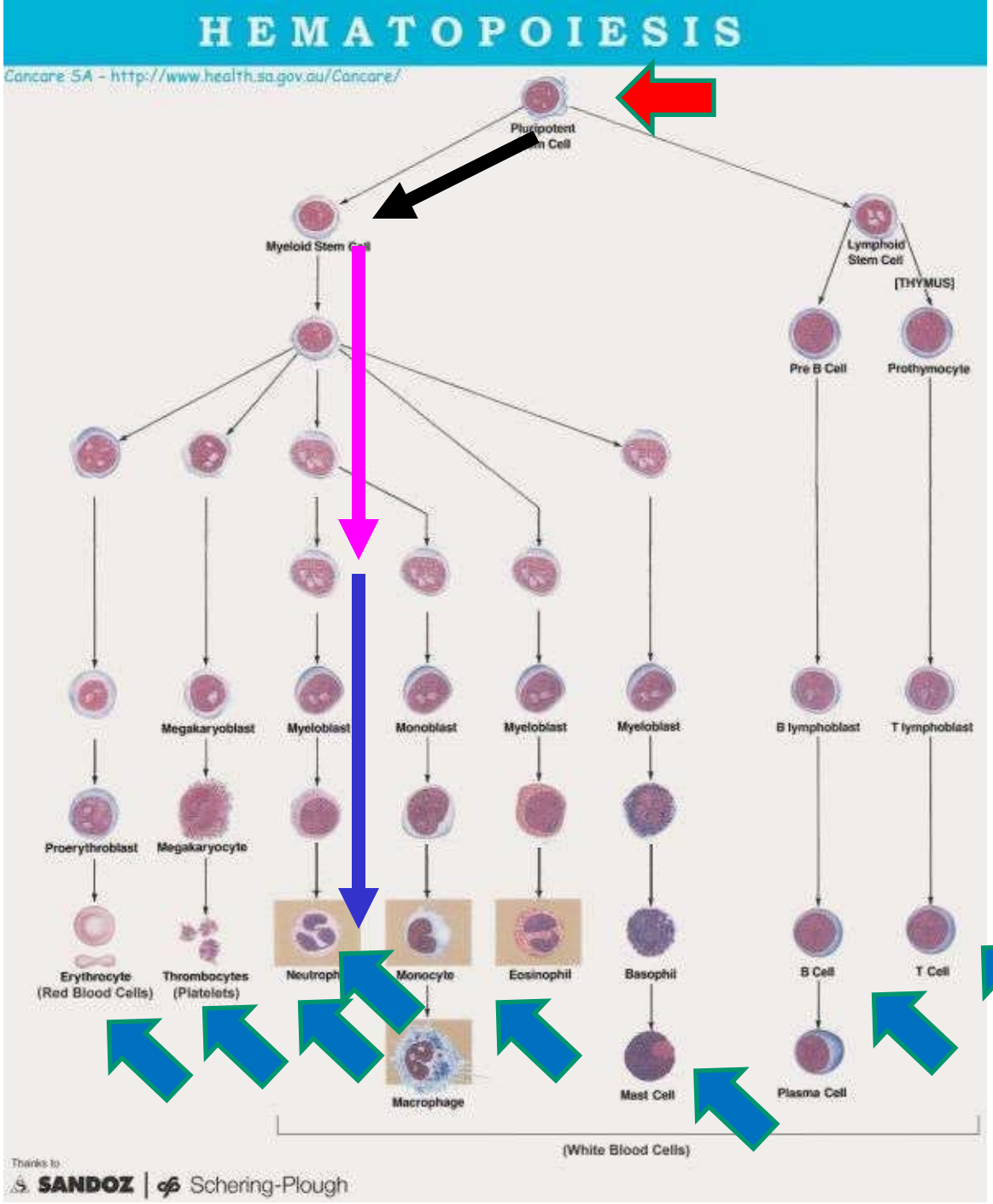
Over 200 Cell Types From A Single Genome!





hematopoietic stem cell
(few in number)

differentiation associated with cell division and sequential changes in gene expression: genes switched "on" and "off"

differentiated cells
(many in number)

Problem:
Different Cell Types But A Single Genome

Solution: Epigenetics

What is Epigenetics?

The broadest definition includes the transmission and perpetuation of information through meiosis or mitosis that is not based on the sequence of DNA

Epigenetic Modifications

Non-Covalent:

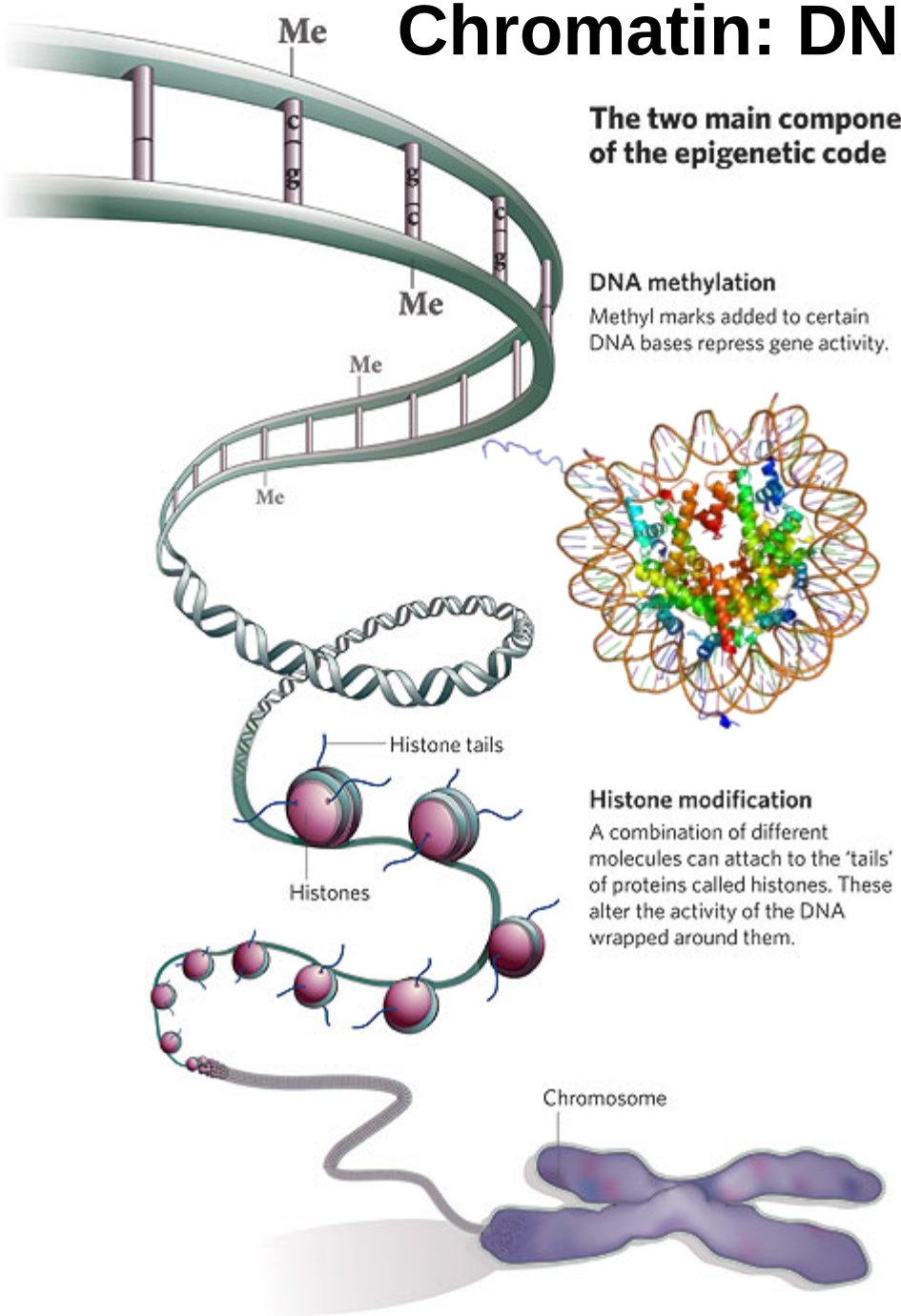
Proteins: Histones, Trithorax,

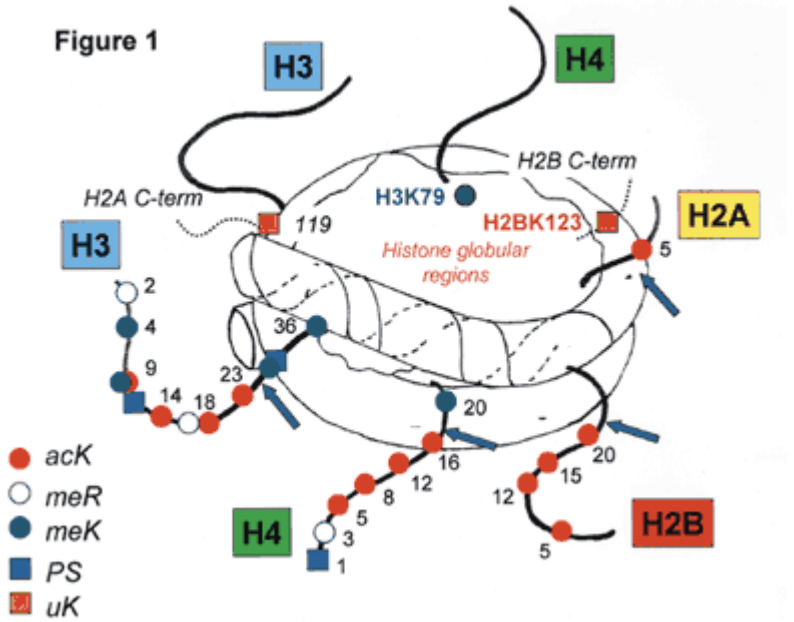
RNA: NC-RNA (X-chromosome Inactivation)

Covalent:

DNA Methylation

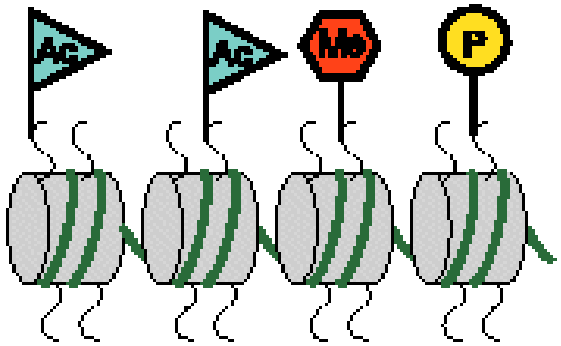
Chromatin: DNA + Histones (octomer)



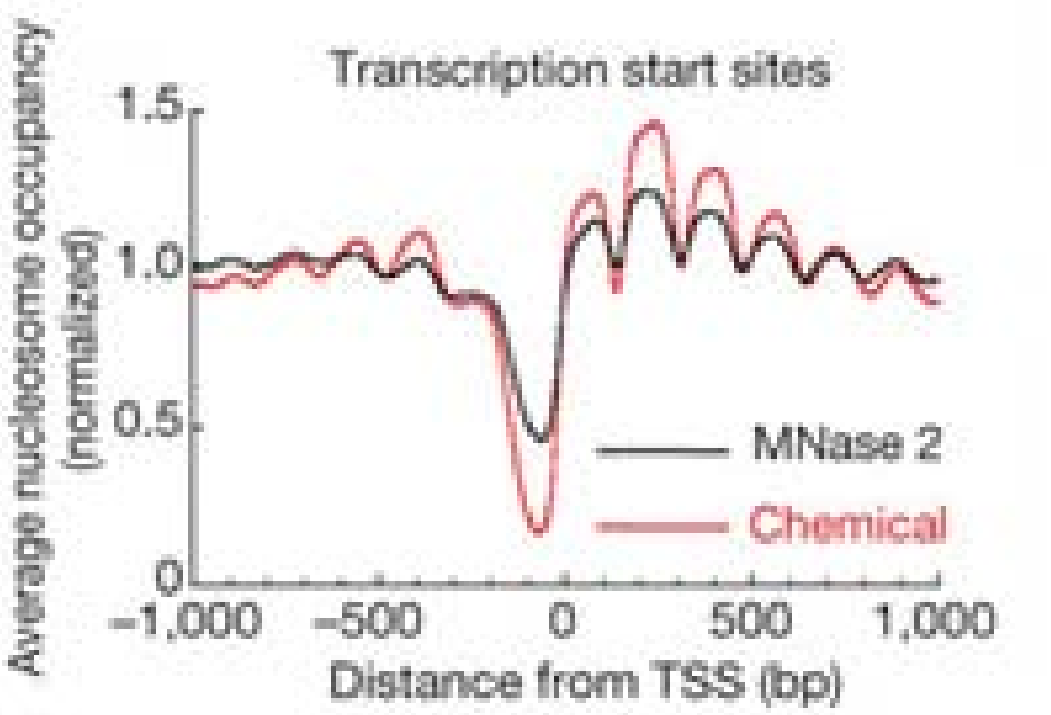


Histone Tails

The Histone Code

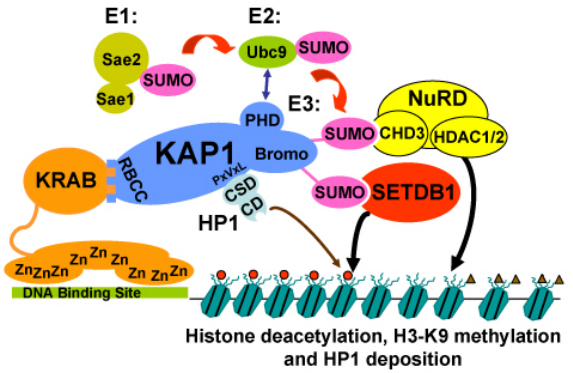


Nucleosome Positioning: Favored Locations



A map of nucleosome positions in yeast at base-pair resolution.
Brogaard K, Xi L, Wang JP, Widom J. Nature. 2012;486:496-501

Histone Code (combinatorial)



P phosphorylation

Ac acetylation

Me methylation (arginine)

Me methylation (active lysine)

Me methylation (repressive lysine)

Ub ubiquitylation

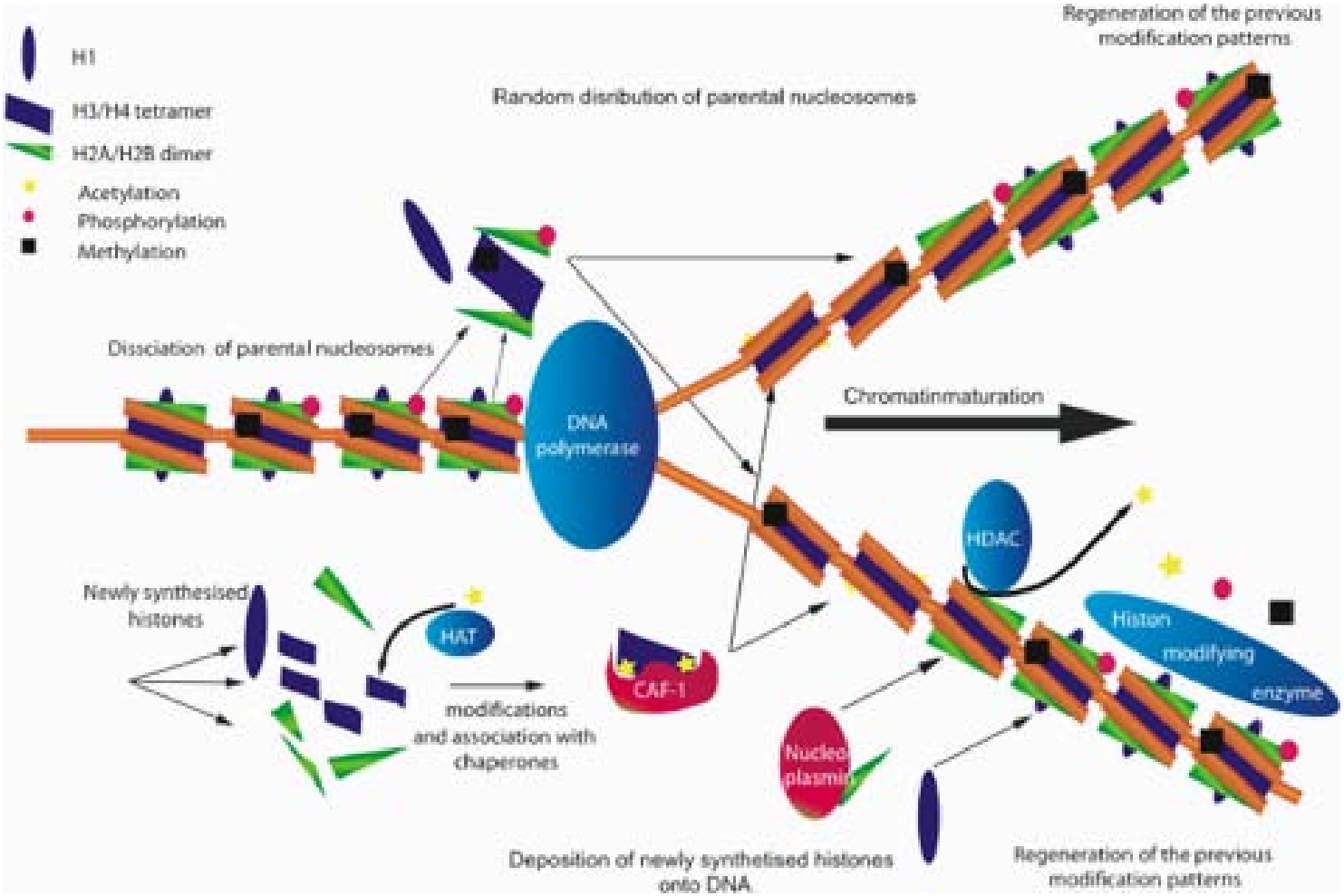
ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPH – histone H3 – 135 aa

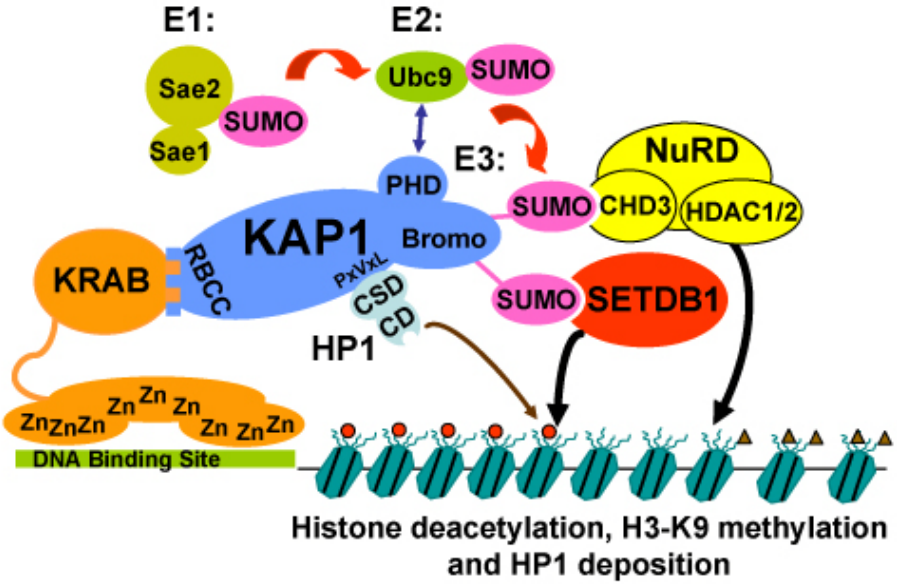
SGRGKGGKGLGKGGAKRHRKVLRDNIQGITKPAIRRLAR – histone H4 – 102 aa

SGRGKQGGKARAKAKSRSSRAGLQFPVGRVHRLLRKGNY – histone H2A – 129 aa

PEPAKSAPAPKKGSKKAVTKAQKKDSKKRKRSRKESYSV – histone H2B – 125 aa

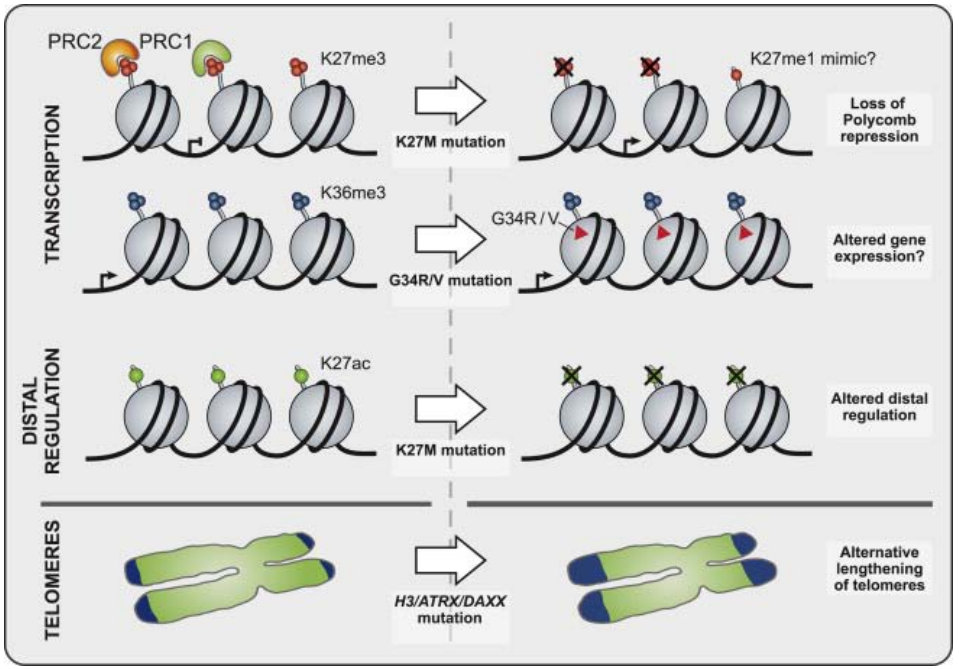
Chromatin Replication
(Genetic and Epigenetic Inheritance)





Mutations in Cancers:
Occur In Modifying Enzymes (Not Histones)

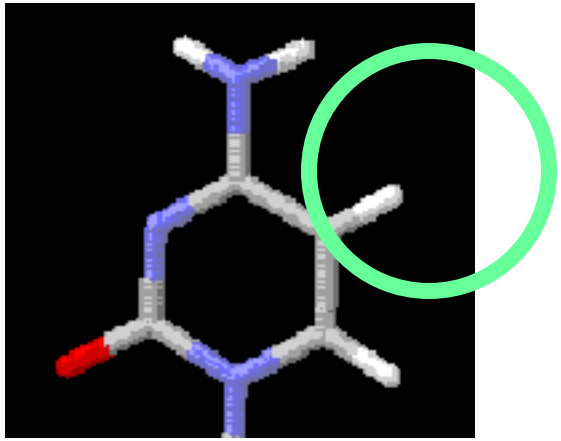
Cancer Mutations Result in Altered Transcription (Regulation)



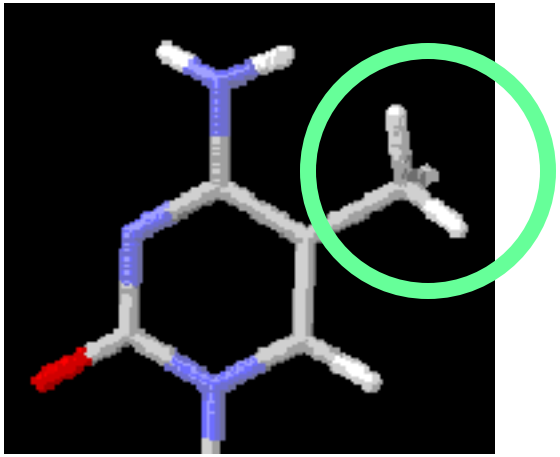
DNA Methylation

Epigenetic DNA Modifications: Heritable Changes That Do Not Change Genotype

1) Occurs On C's in CpG Sites
2) CpG Sites Cluster In CpG Islands
3) Most CpG Islands Are Unmethylated At Birth
4) Methylation Exhibits Somatic Inheritance



cytosine

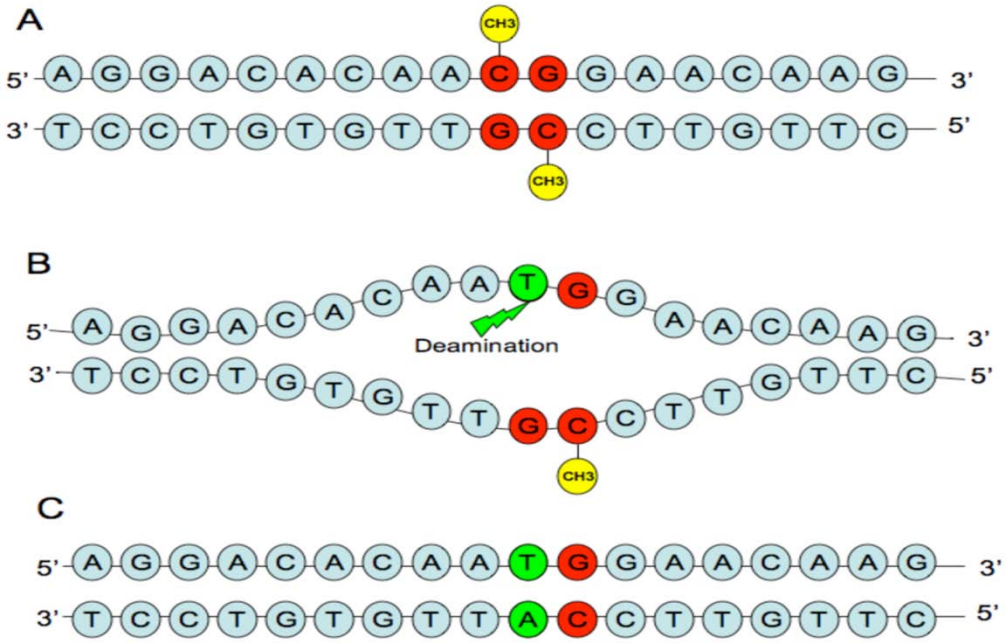


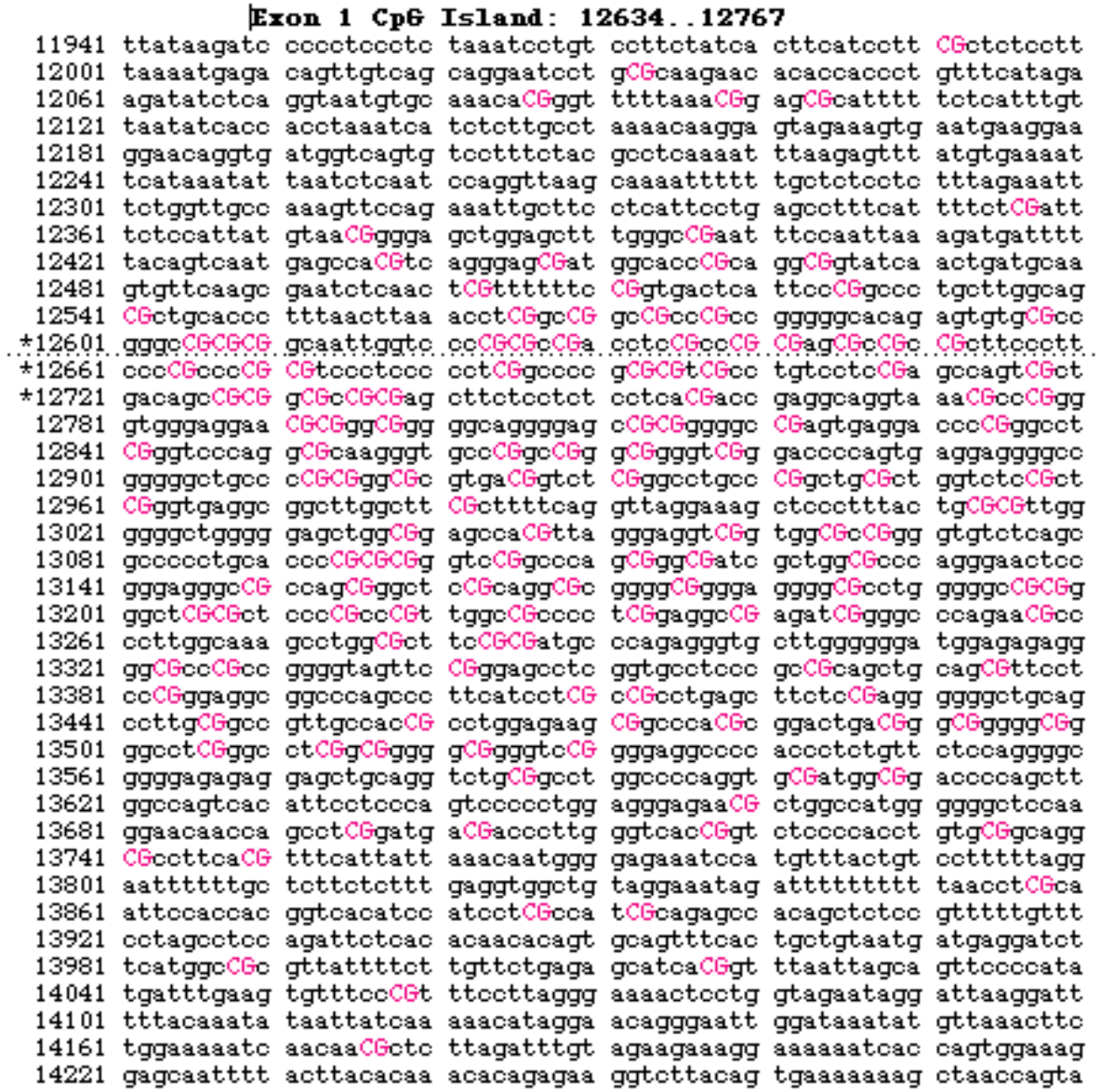
5-methyl cytosine

CpG Islands

1) Relative Deficit of CpG dinucleotides in Human Genome (1% versus expected 6.25%)
2) CpG dinucleotides Often Concentrated in CpG islands
3) Most CpGs Unmethylated in CpG islands
4) Most CpGs Methylated in non-CpG islands
5) Increased Mutation Rates (10X) at Methyl C compared to non-Methyl C

most common cancer mutation is CpG to TpG (due to DNA methylation)



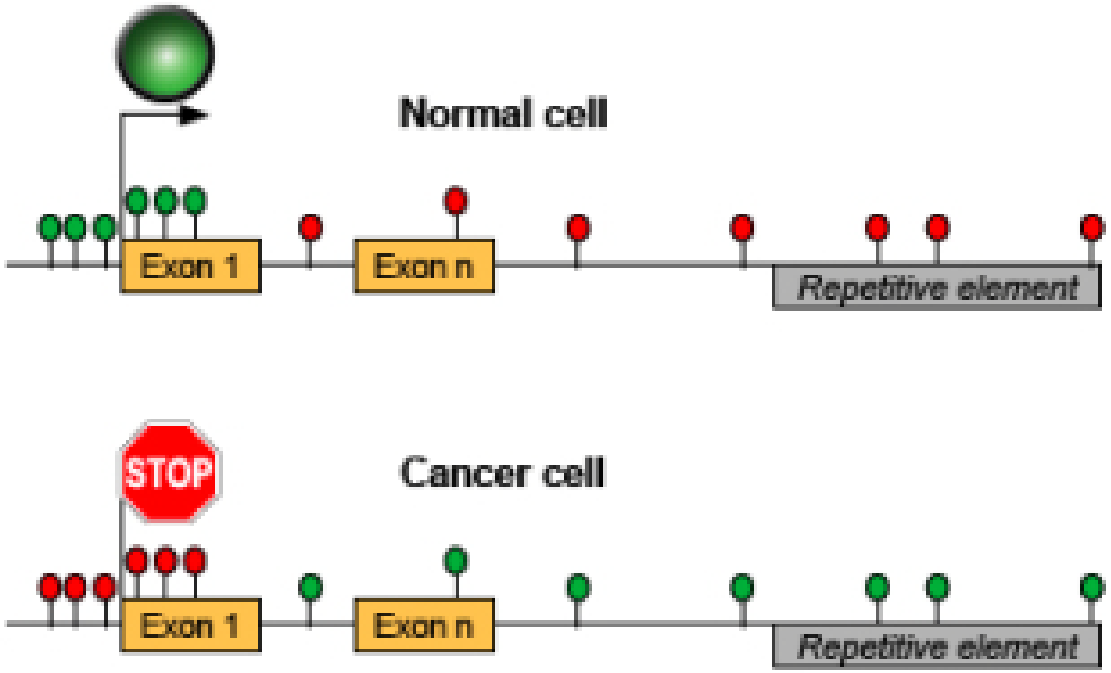


CpG Islands

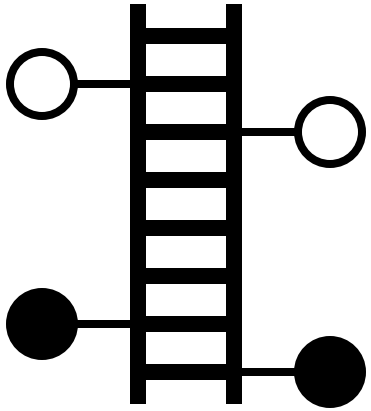
--increased CpG density

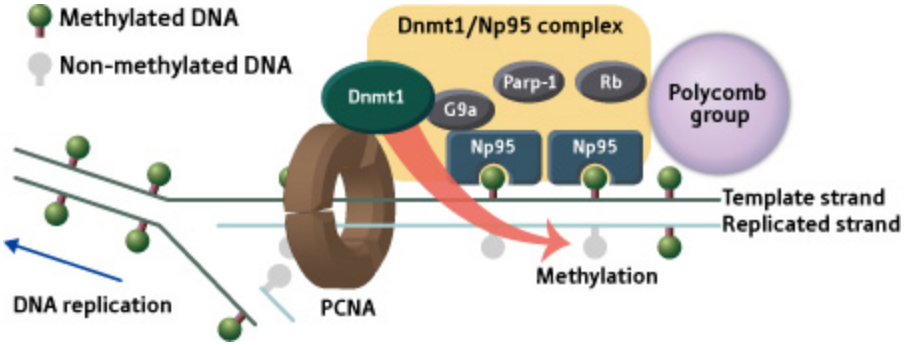
--often associated with promoters or repetitive elements

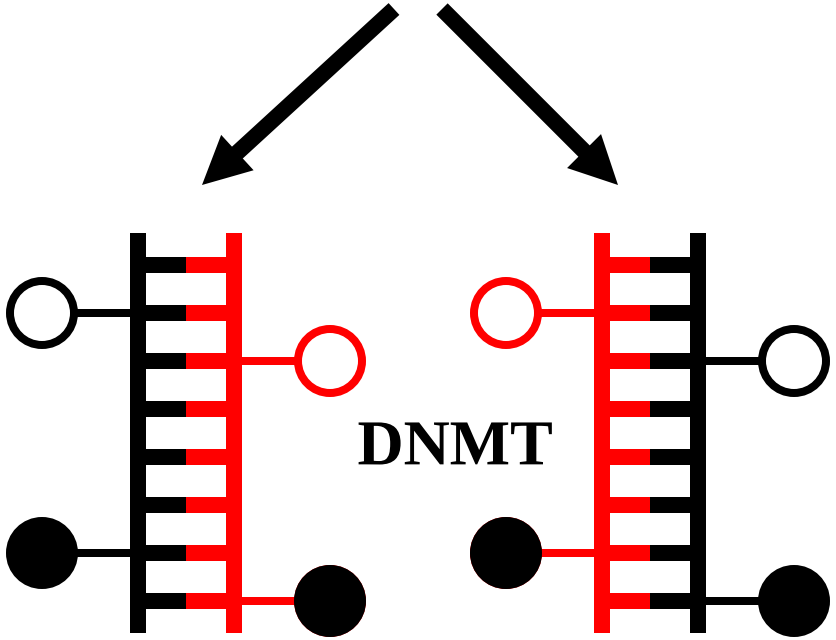
Promoter CpG Methylation Can Regulate Gene Expression (typically gene silencing)



Replication Clock





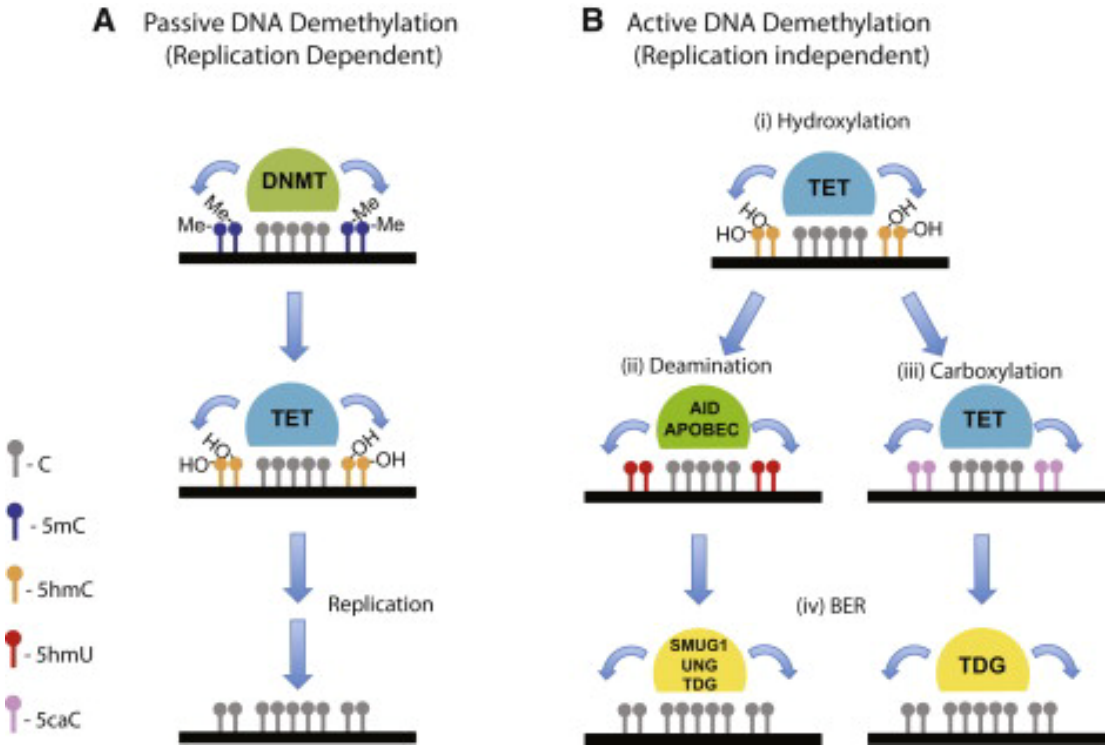


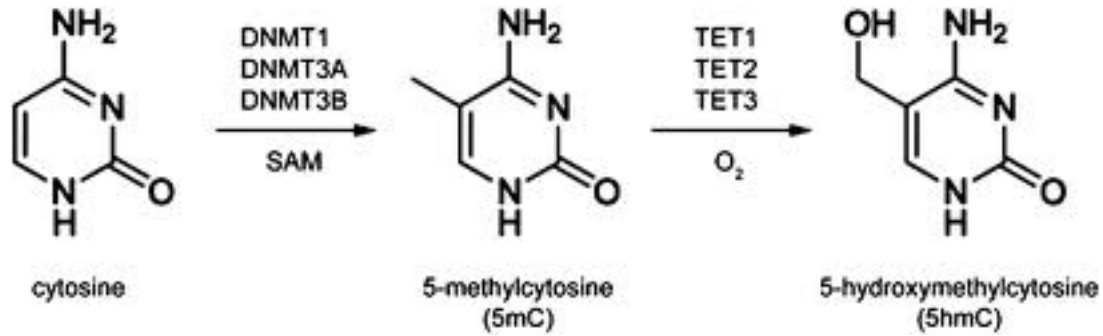
Genome Replication

Epigenetic Fidelity is less than Genetic Fidelity

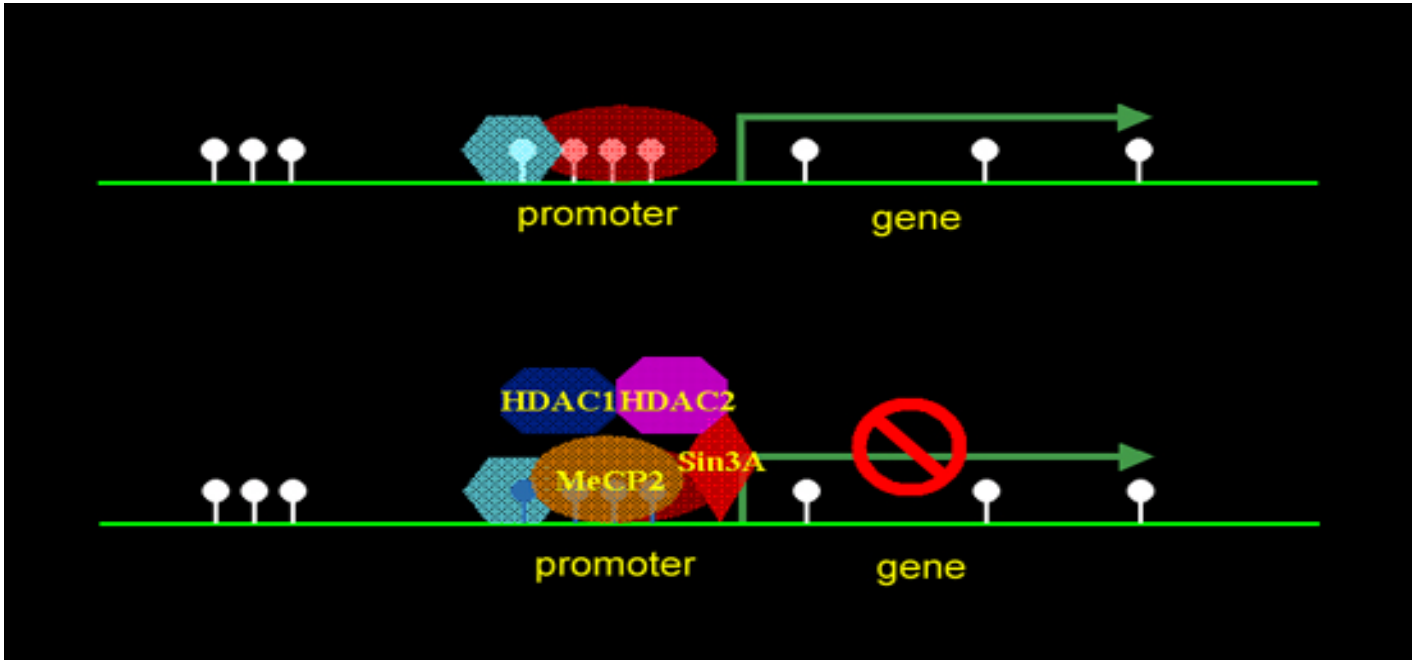
10^{-9} versus 10^{-5} errors per CpG per division

DNA Demethylation: Passive and Active

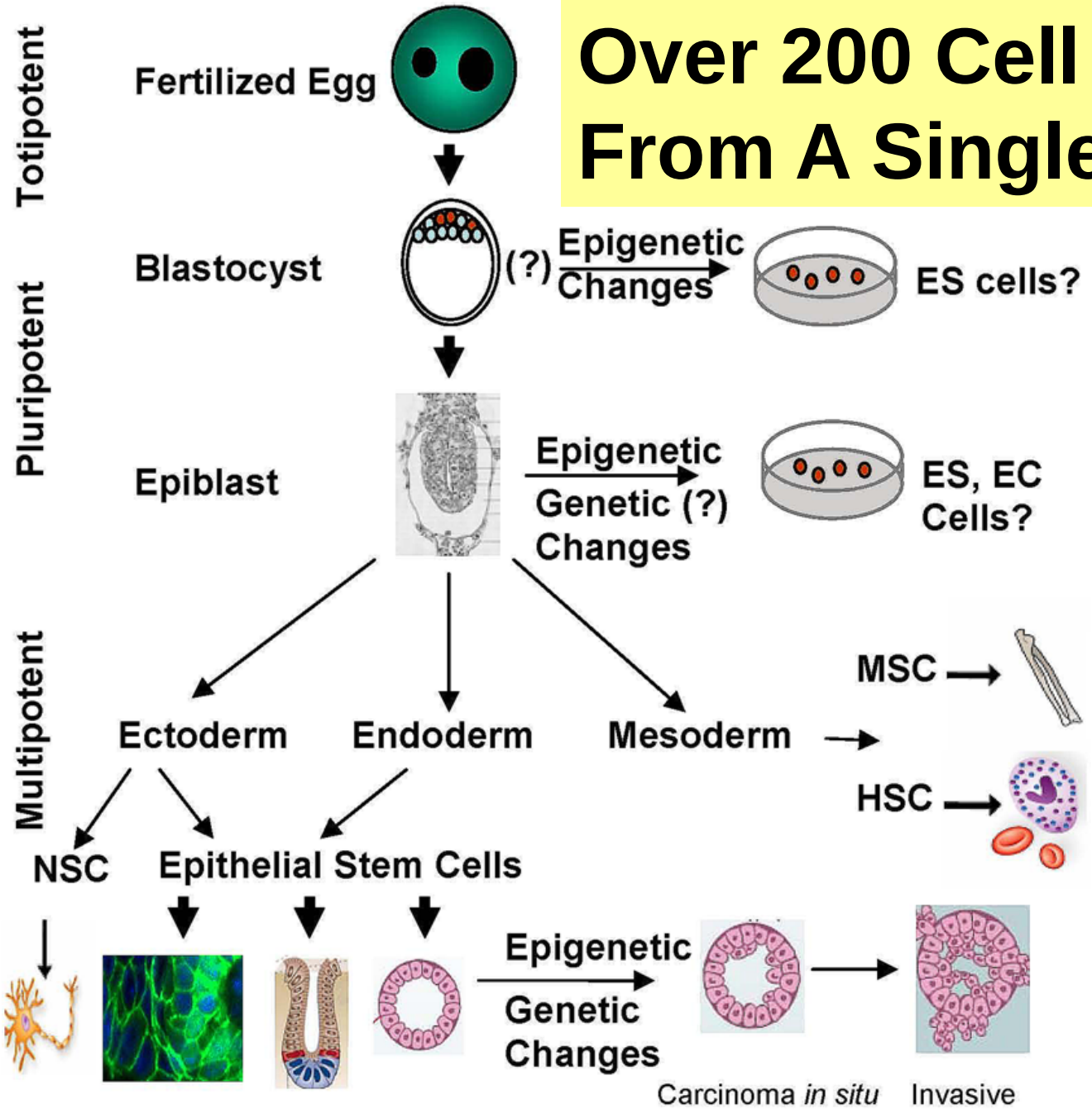




Promoter CpG Methylation Can Regulate Gene Expression

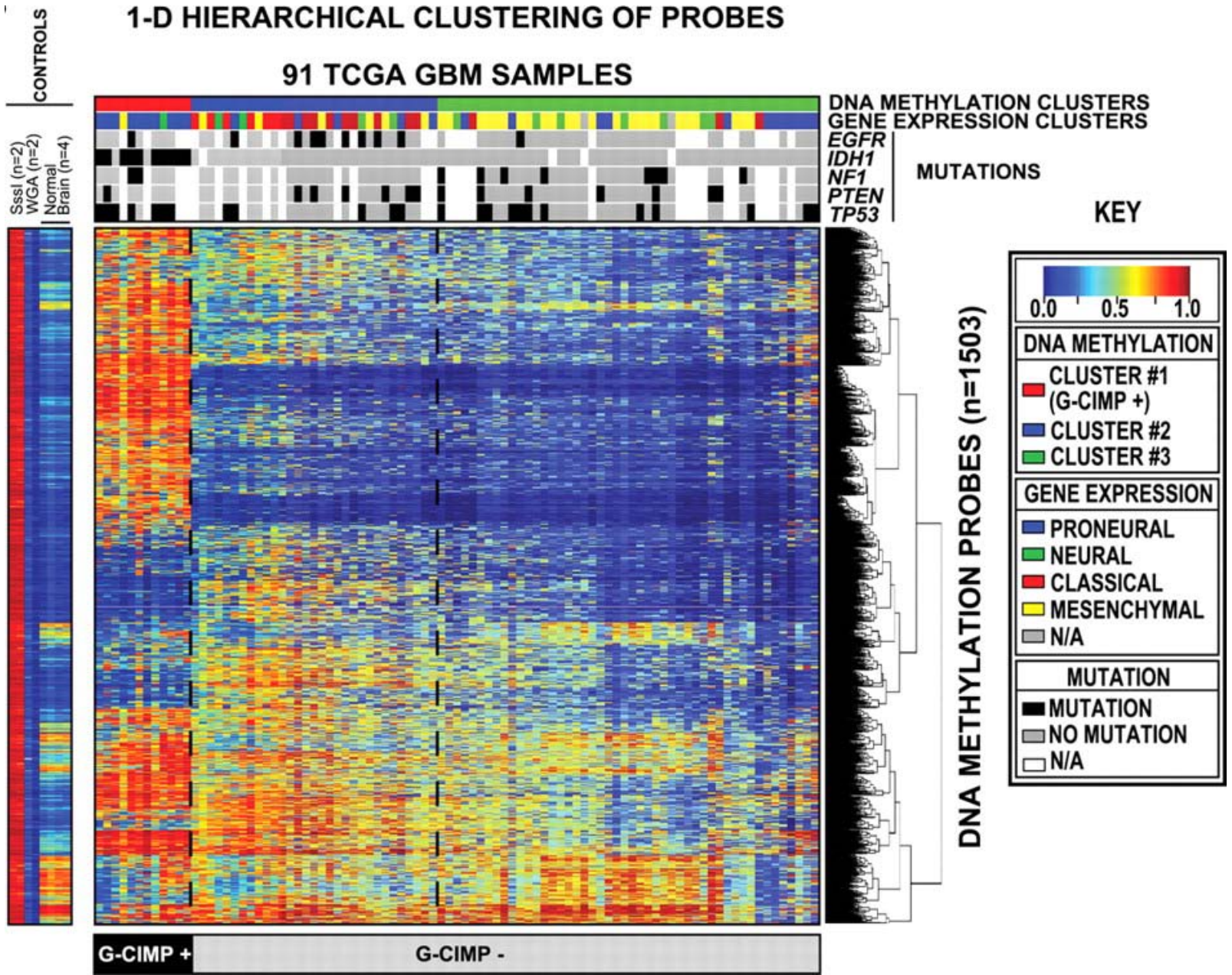


Over 200 Cell Types From A Single Zygote

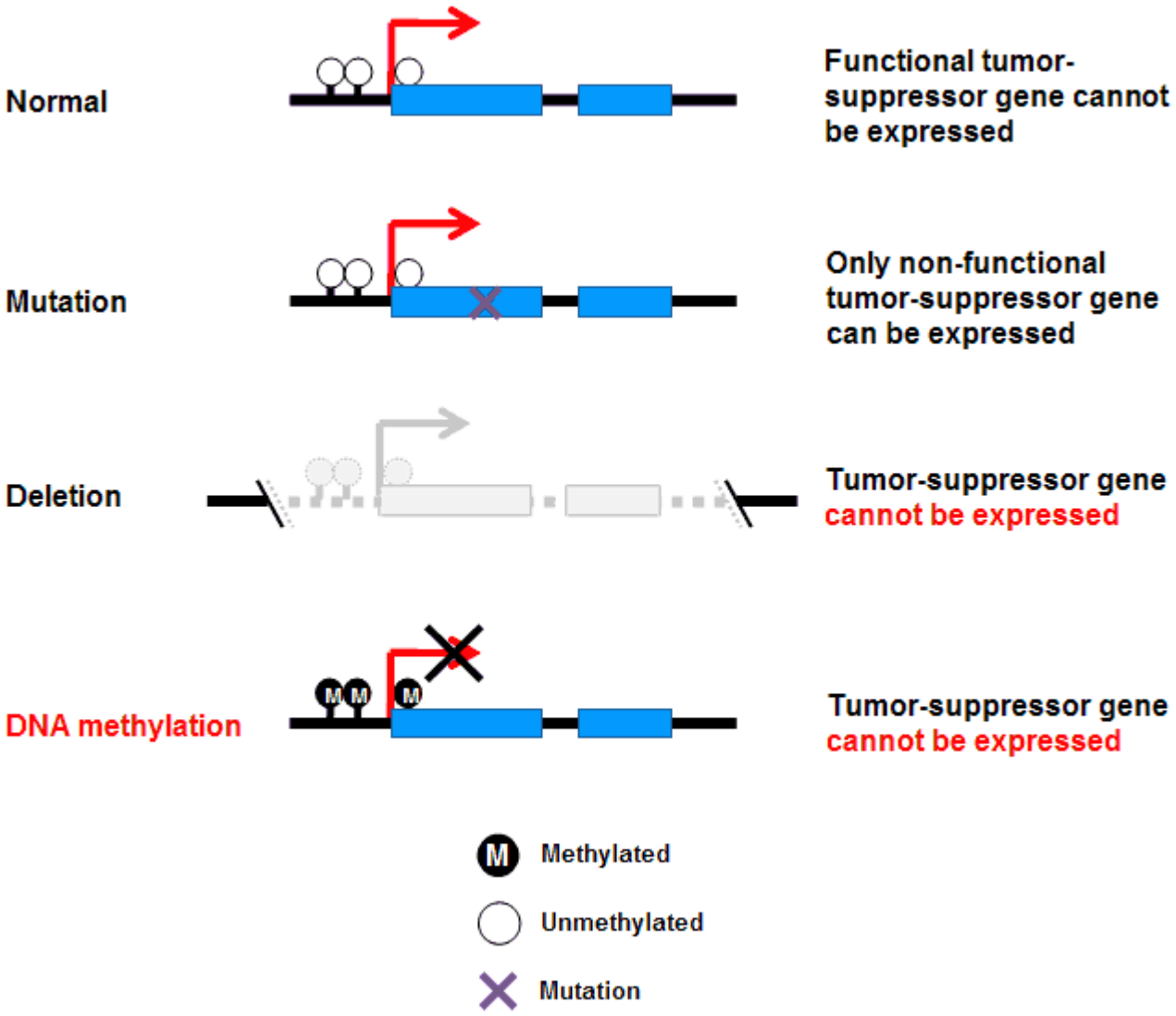


Measuring DNA methylation
Bisulfite Sequencing

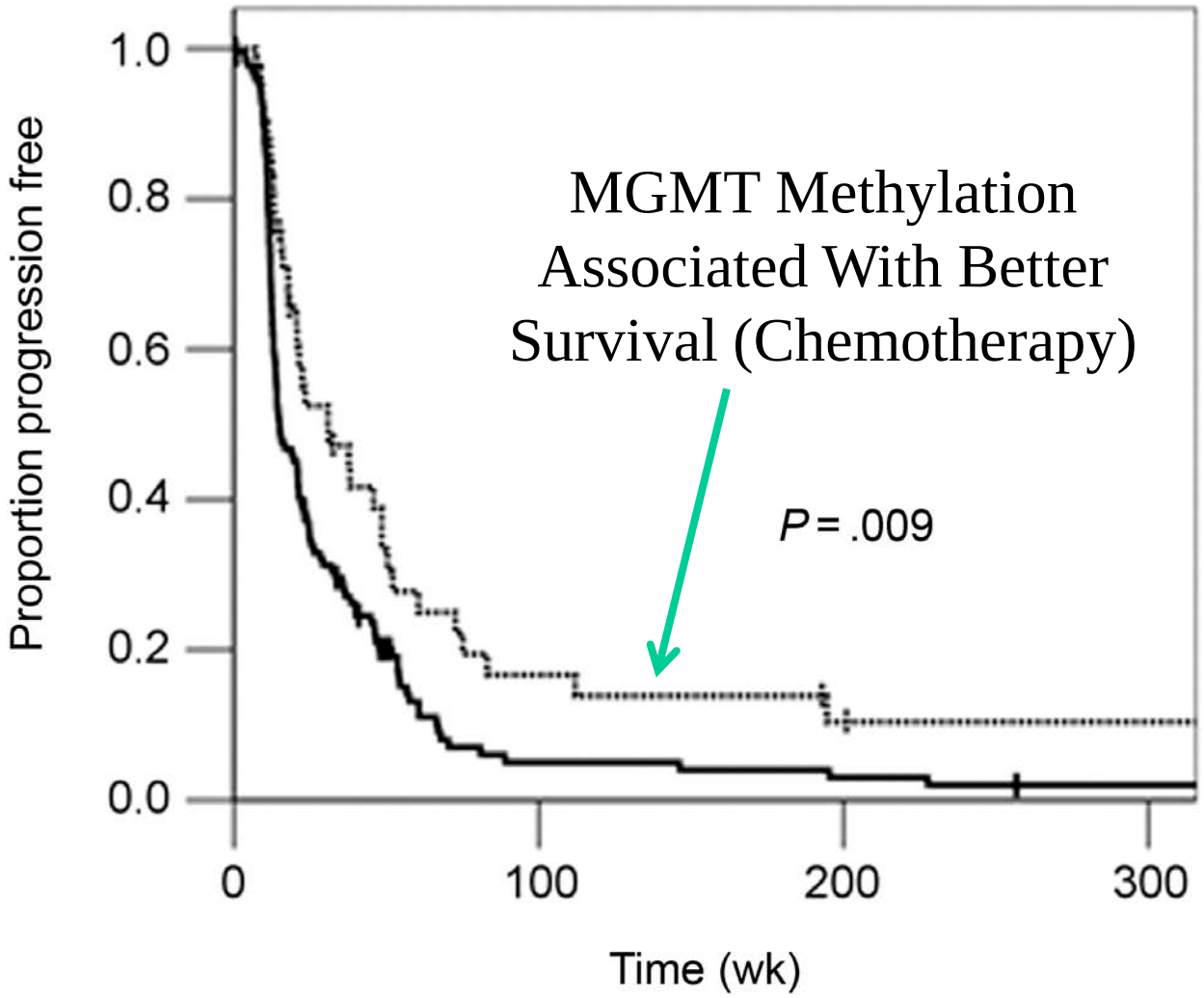
DNA Methylation Is Altered In Tumors



Alternative Methods To Silence Tumor Suppressor Genes



Progression-free survival and MGMT promoter methylation status.



Rivera A L et al. Neuro Oncol 2010;12:116-121

Neuro-Oncology

Summary: DNA Methylation in Cancer

1. General Overall Hypomethylation
2. Focal Hypermethylation Of Many CpG Rich Promoters
3. Can Result in Silencing of Critical Tumor Suppressor Genes

How Does Altered DNA Methylation Occur in Cancer?

1. Generally No Mutations in DNA Methyltransferases or "Demethylases"
2. Difficult to Find Consistent DNA Methylation Alterations
   (ie same cancer types have many different genes methylated)
3. Like Mutations, Many Genes With DNA Methylation May Be "Passenger" Changes (Neutral)
4. Hence "No One Knows"

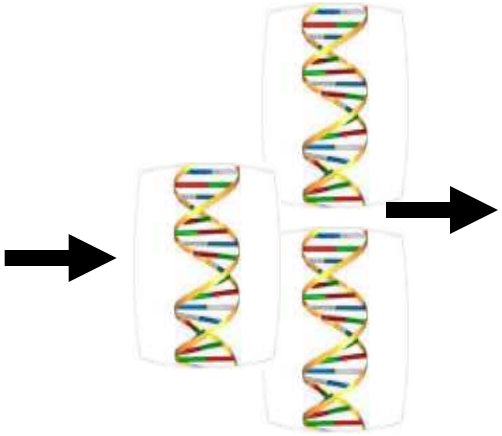
Reconstructing Human Somatic Cell "Evolution" With Molecular Clocks

Problem: Difficult To Study Human Somatic Cell Changes

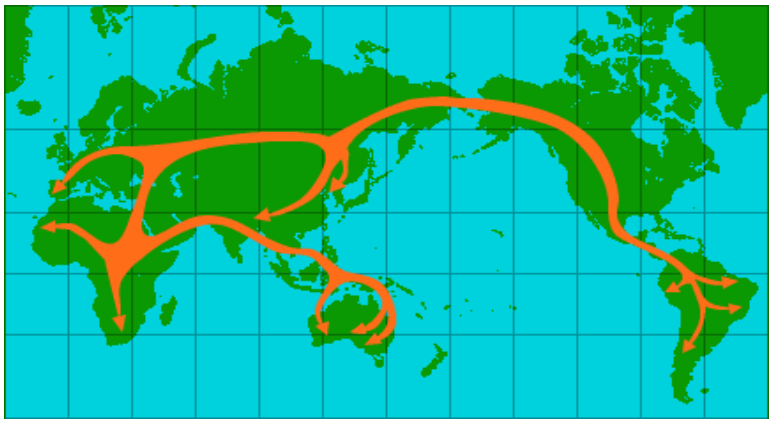
1) Long Human Lifetimes
2) Inability To Perform Human "Experiments"
3) Potential Solution: Genome Comparisons
   (Historical Documents---copies of copies)

Molecular Clocks: Genomes Record Ancestry

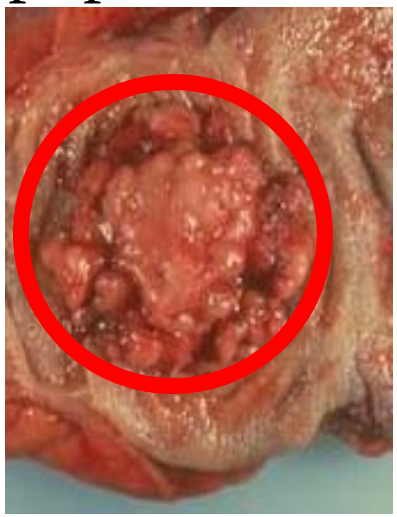
populations



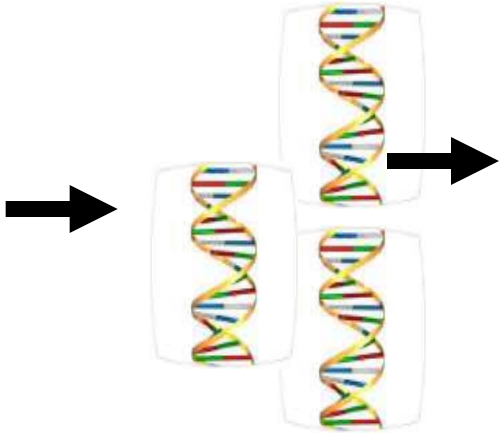
compare genomes

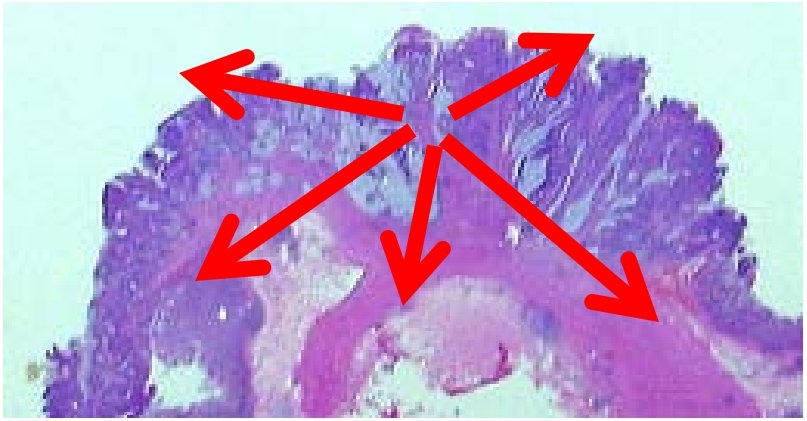


infer ages and migrations patterns

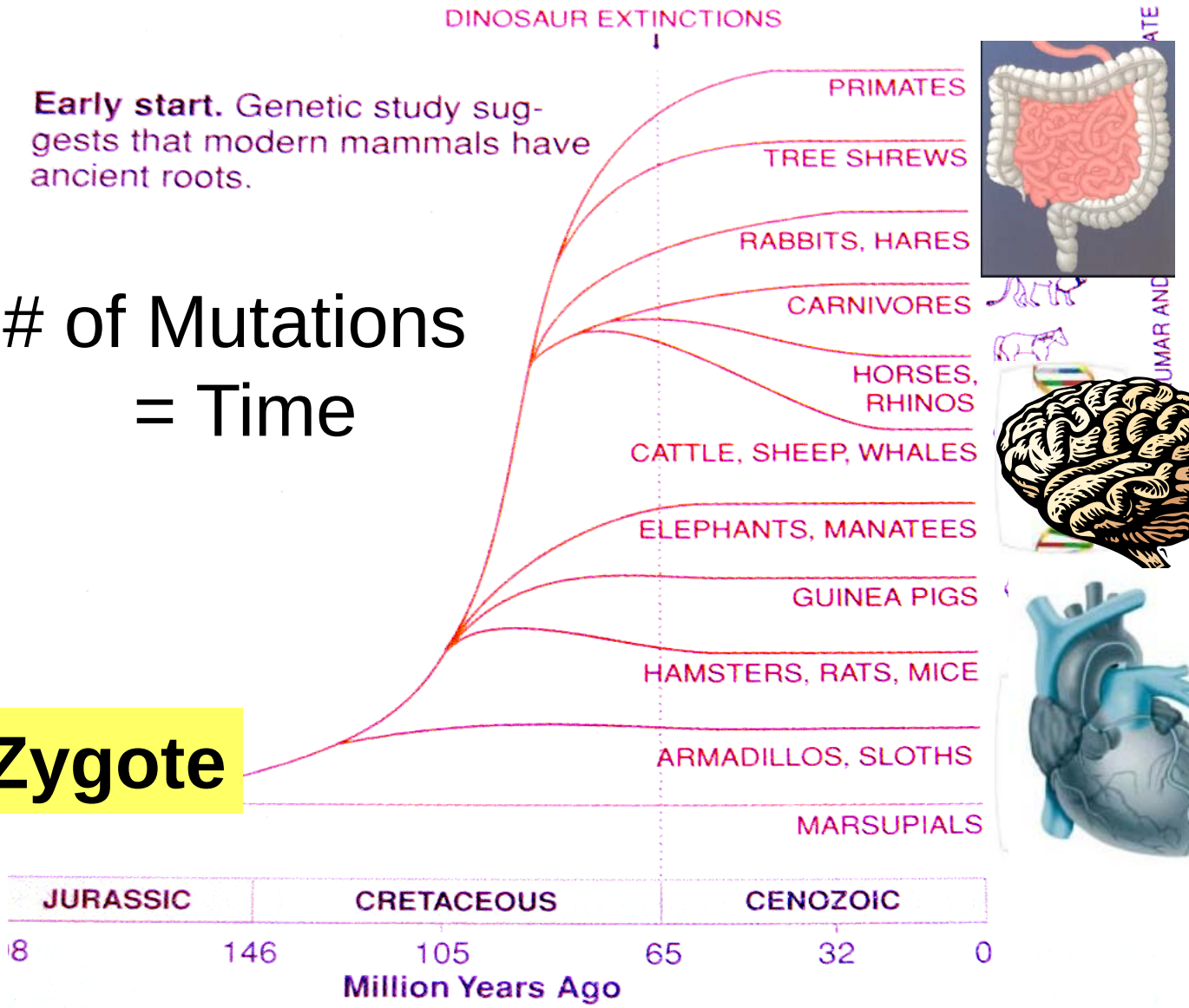


billions of cells





How To Study Biology Passively: Molecular Clocks



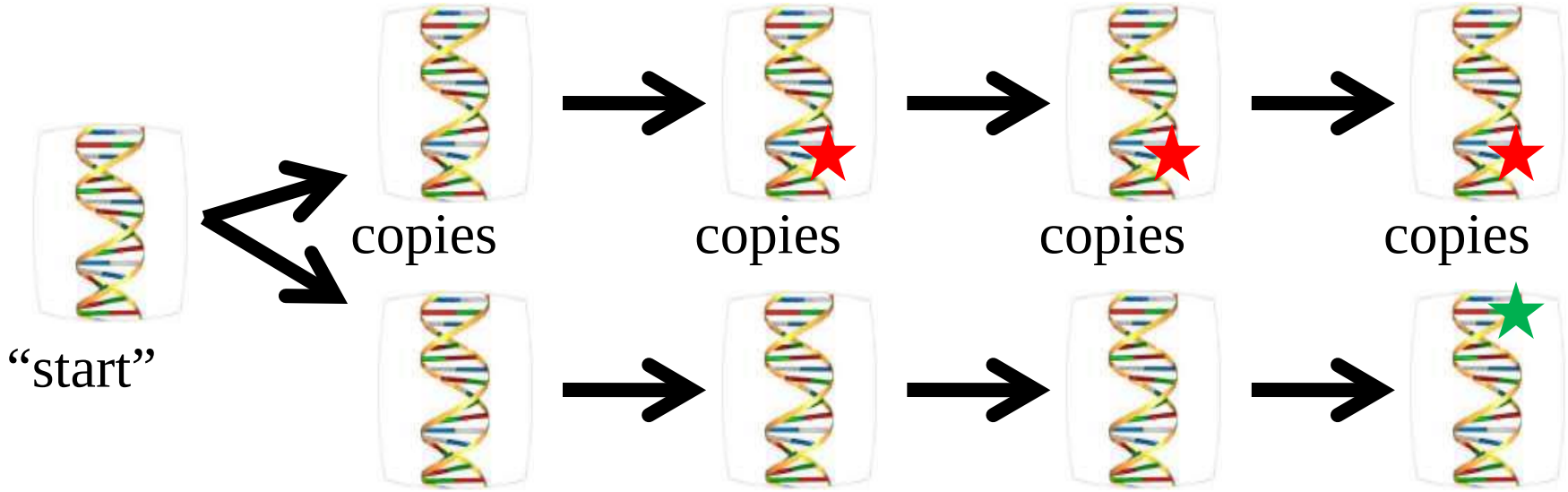
# of Mutations = Time

Random Drift Over Millions of Years

Zygote

The Approach: “Molecular Clocks” (Coalescence Theory)

Genomes Are Almost Perfect Copies of Copies



The Greater The Time or Copies Since A Common Ancestor,
The Greater Their Differences (pairwise distance or PWD)

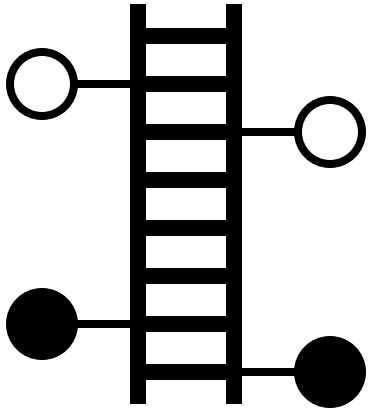
Low PWD → (more replication errors) → High PWD

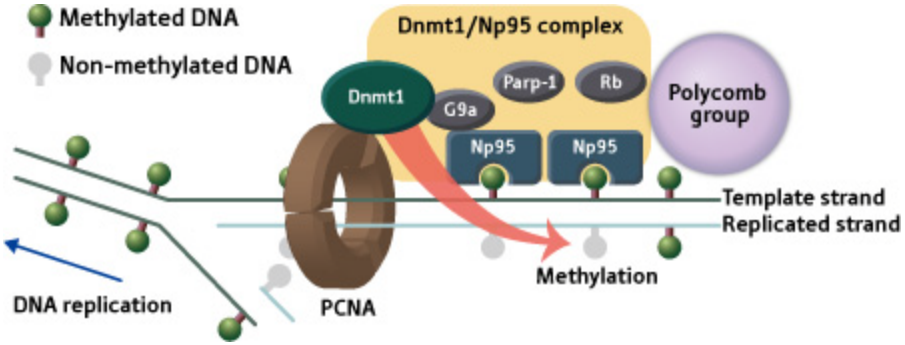
Problems With "Genetic" Somatic Cell Clocks

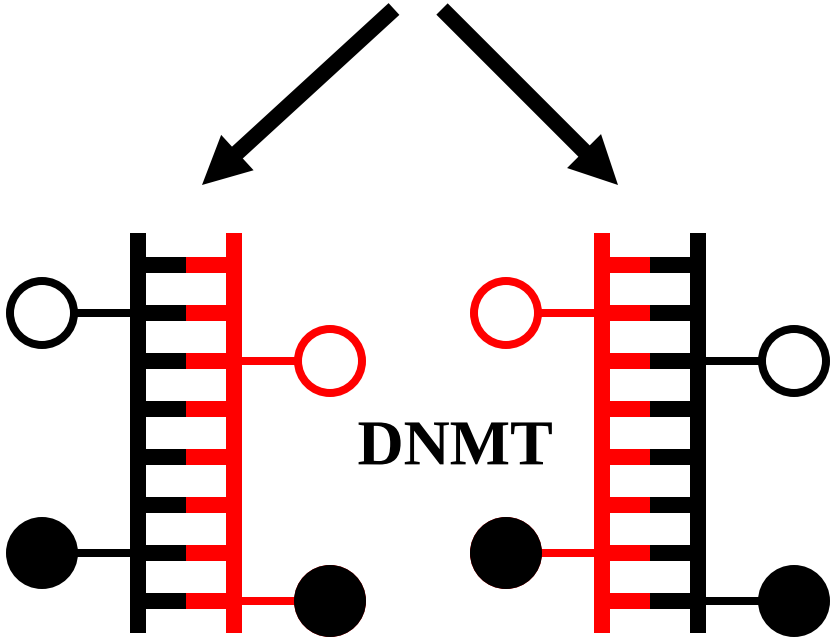
1. Somatic Mutations Are Relatively Rare
   (<1 per 100,000 bases even in cancers)
2. Modern High Throughput DNA Sequencers
   Have Relatively High Error Rates
   (~1 per 100 to 1 per 1,000 bases)
3. Can Use High Coverage (10-fold) To Detect
   Clonal (Cancer) Mutations But More Difficult
   to Detect Subclones (minor variants)

Potential Solution: Epigenetic Molecular Clock

Replication Clock





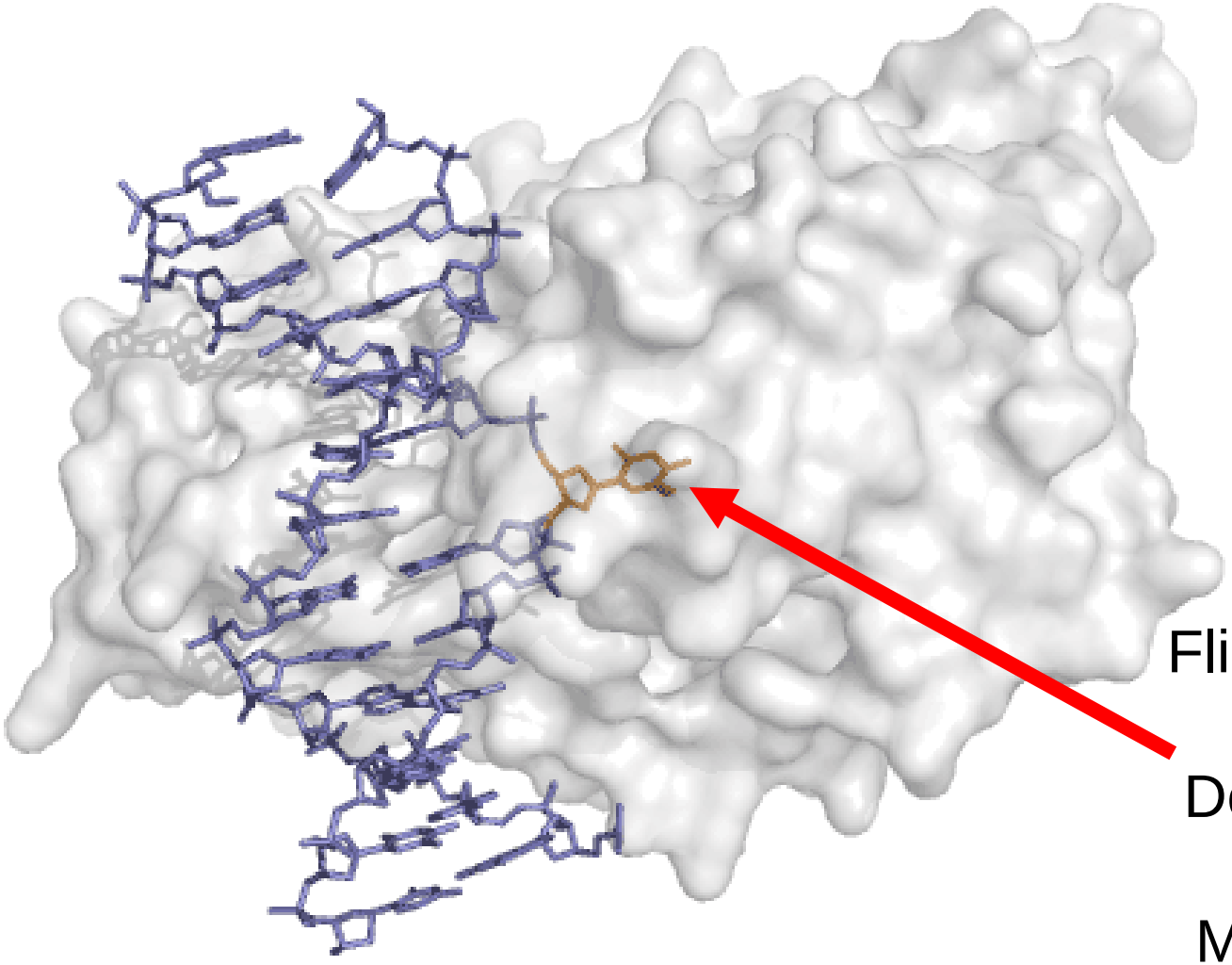


Genome Replication

Epigenetic Fidelity is less than Genetic Fidelity

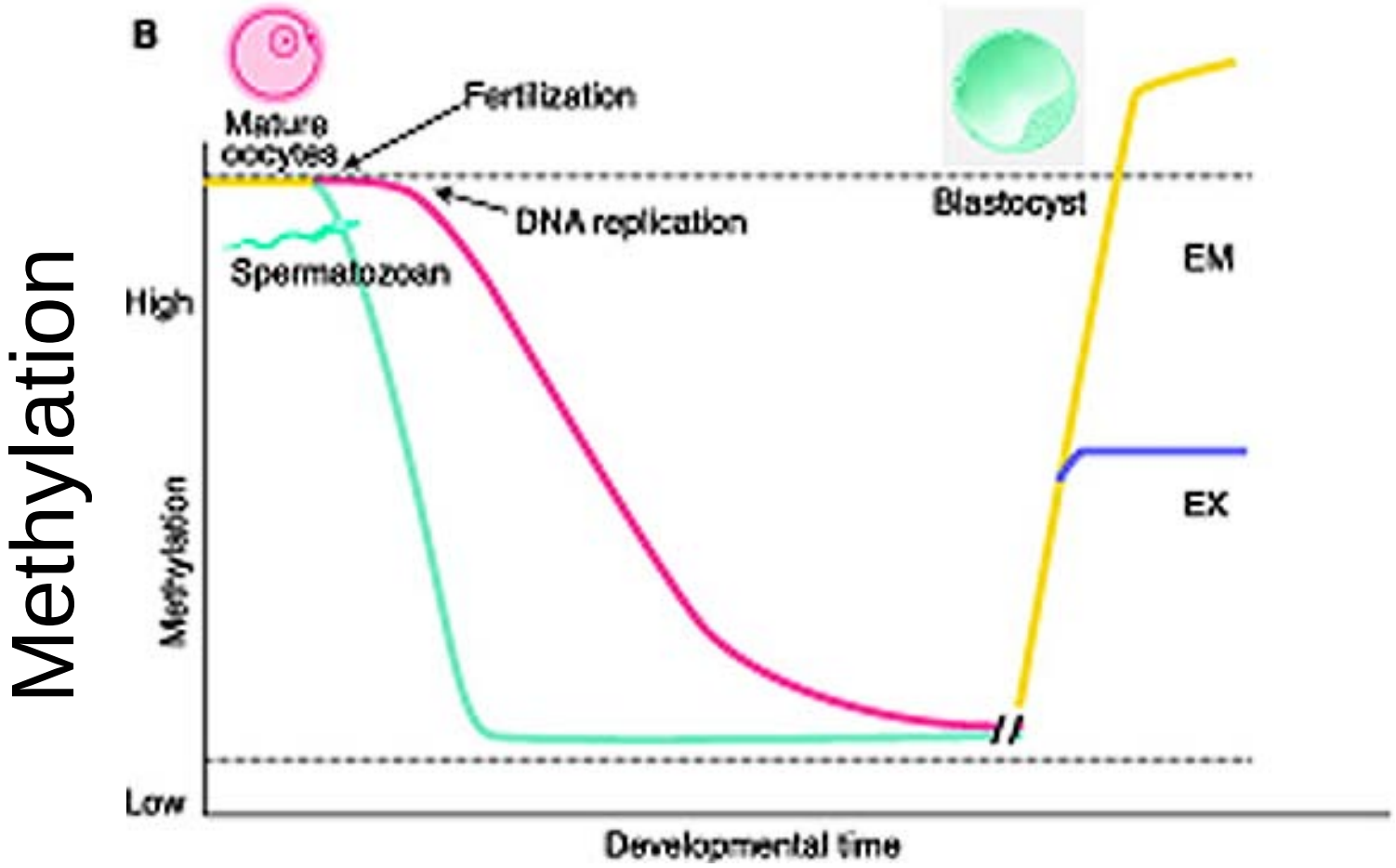
10^{-9} versus 10^{-5} errors per CpG per division

DNA Methyltransferase



Flips cytosine
Out of
Double helix
For
Methylation

DNA is Demethylated Early in Development: Epigenetic “Clocks” Start Unmethylated

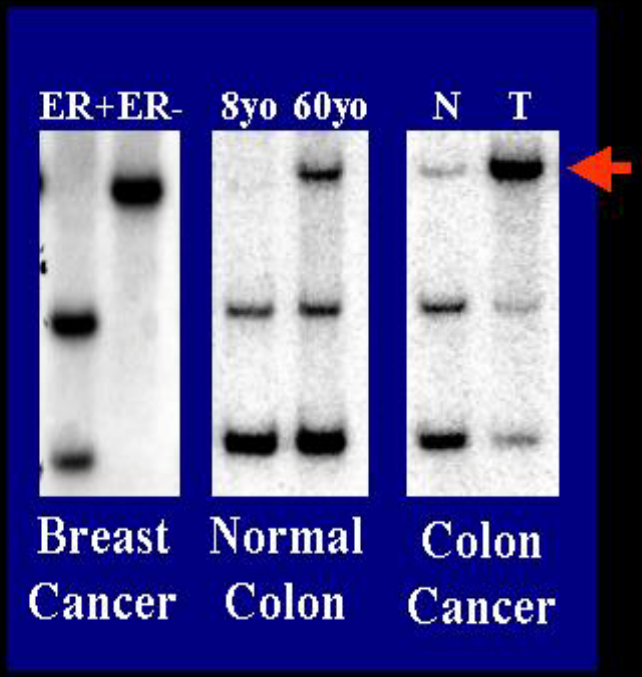


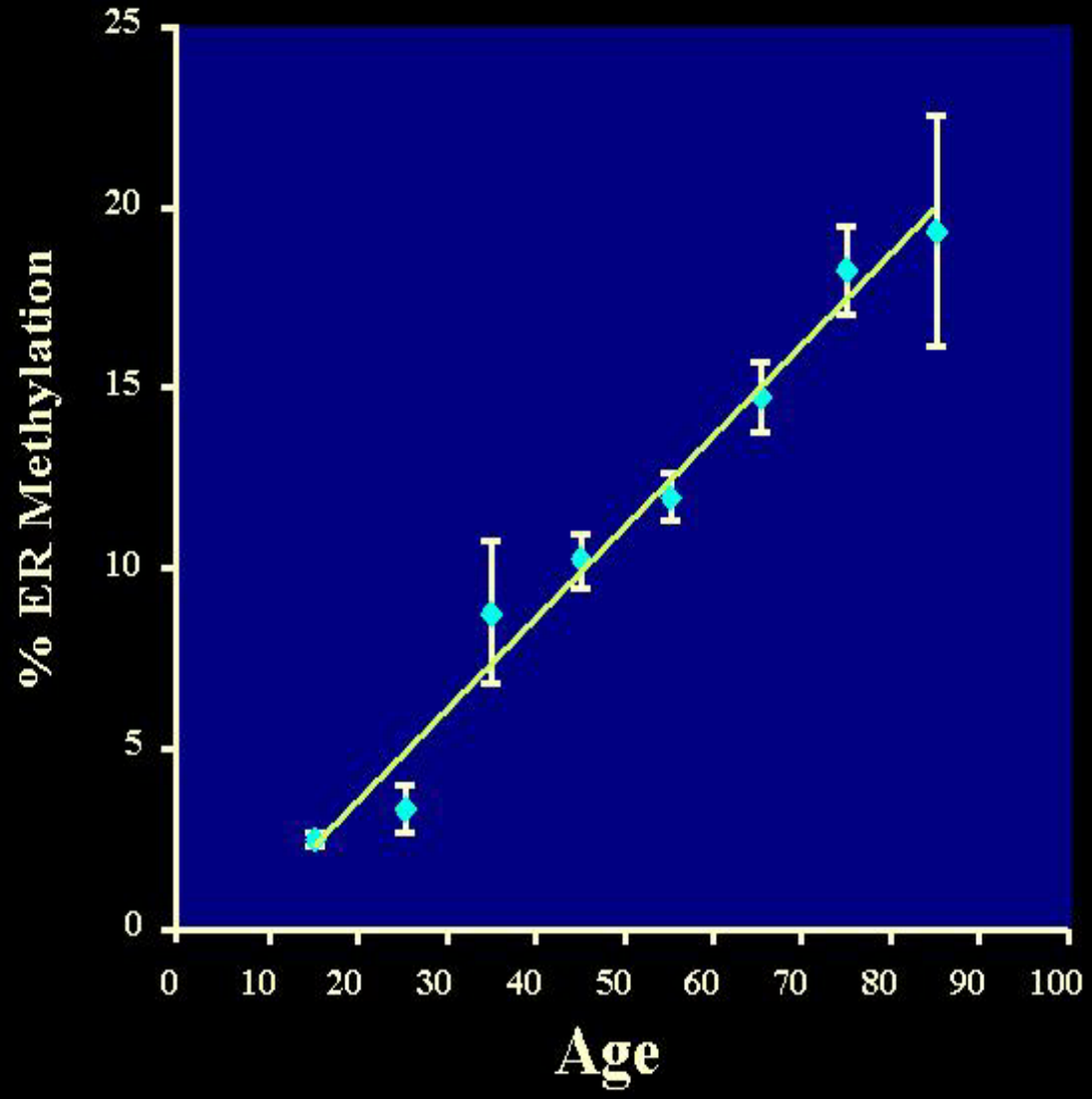
Methylation

Zygote

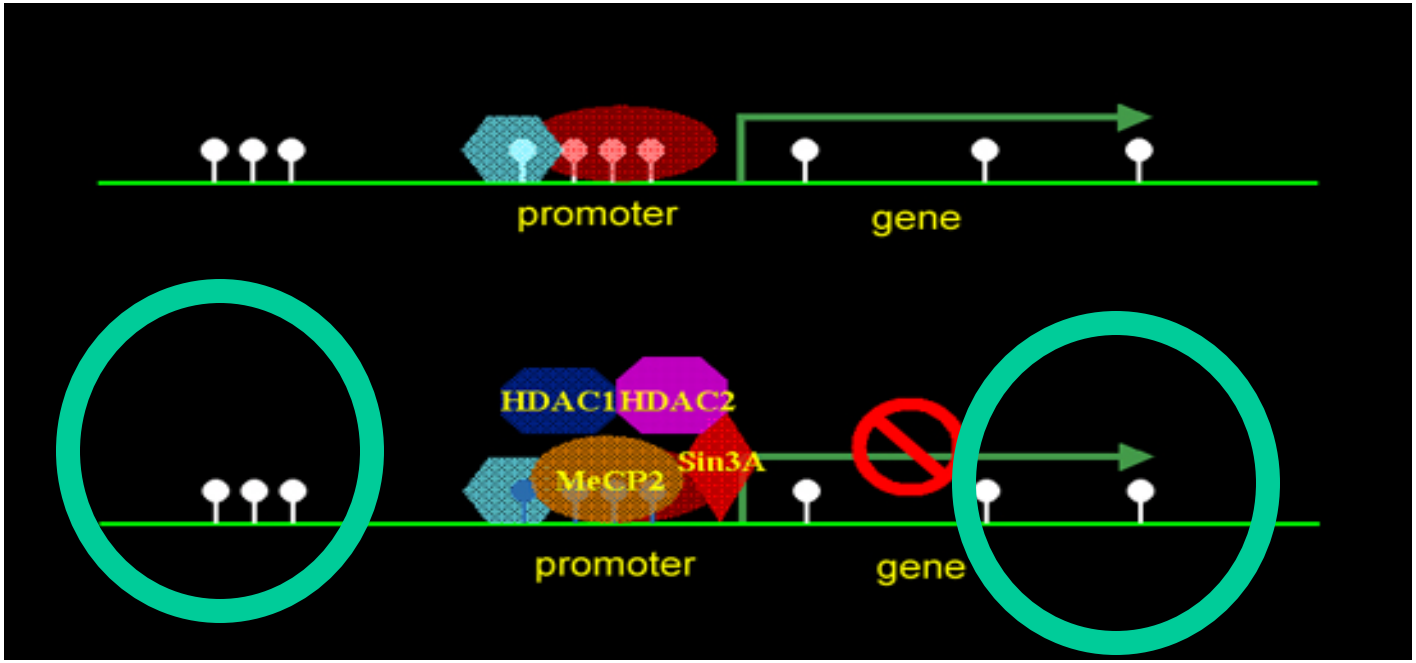
Implantation

ER Methylation and Age in Normal Colon



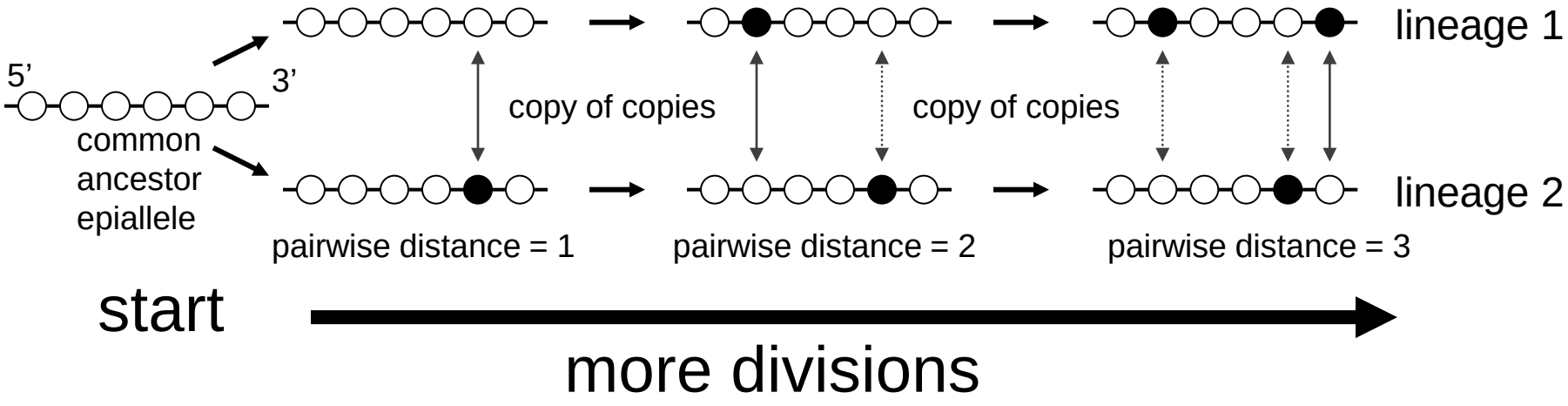


Promoter CpG Methylation Can Regulate Gene Expression



Epigenetic Clocks: Selectively Neutral
(not in promoters, in genes not expressed
in the tissue of interest, ie random
“passenger” replication errors)

Passenger Methylation Pattern Diversity May Represent Replication Errors (Drift)



--- Passenger Epigenetic Clocks on X-chromosome

(not expressed in colon)

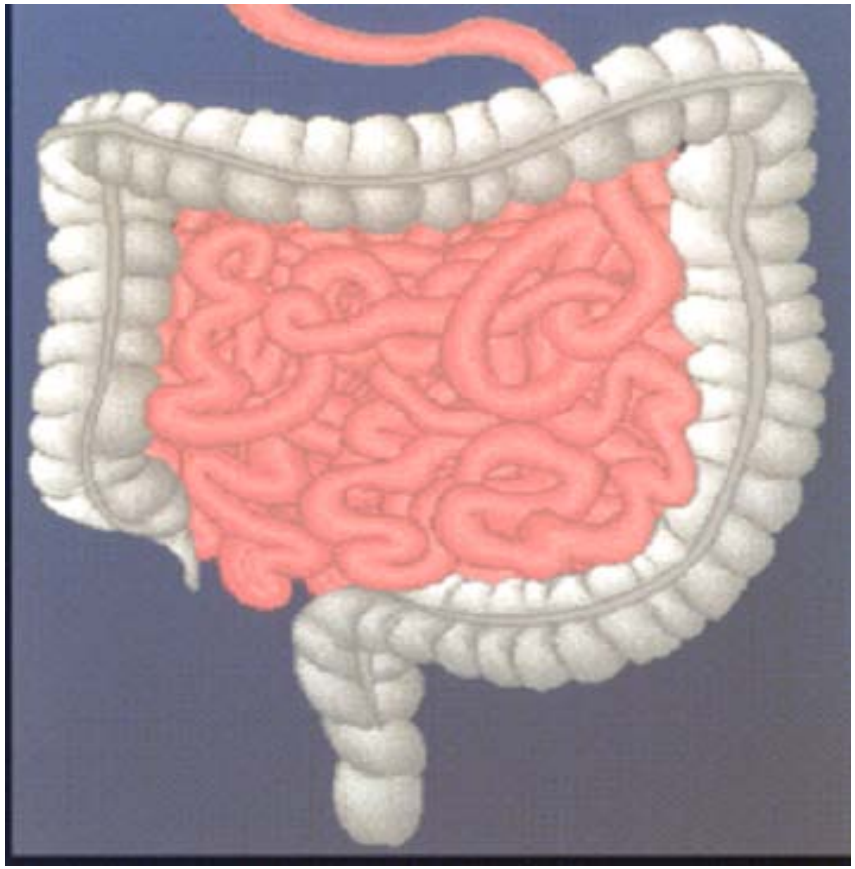
---Samples From Male Patients

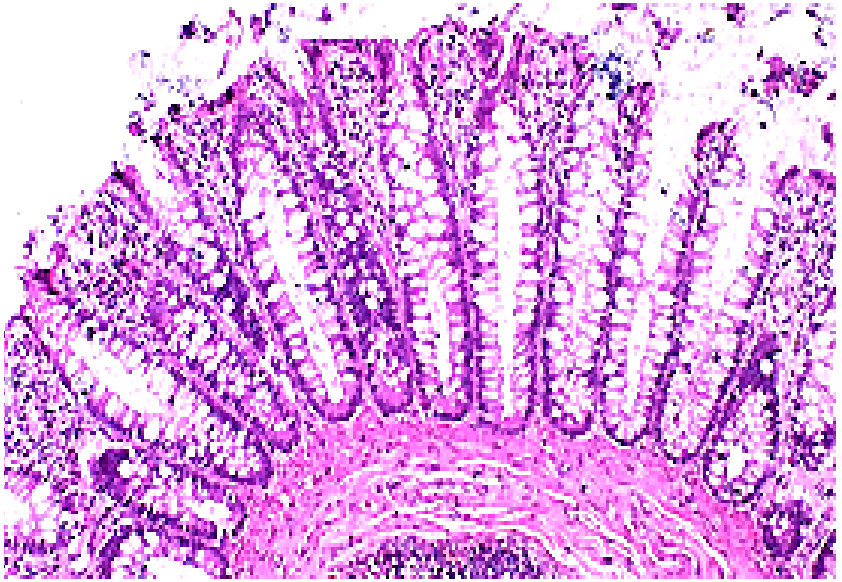
(single epiallele per cell)

BGN “clock” (9 CpG sites, Chr Xq28)

TTTaggagtgagtagTtgTtttCGgtTCGTCGgaTaTaTCGgaTa
gataga**CG**tg**CG**ga**CG**gTTTaTTaTTTTagTT**CG**TTaaTtagt
TagTTtg**CG**TTtgg**CG**TTtTTTTtTtTTaggtagggTtggT

Example: Human Colon Crypts





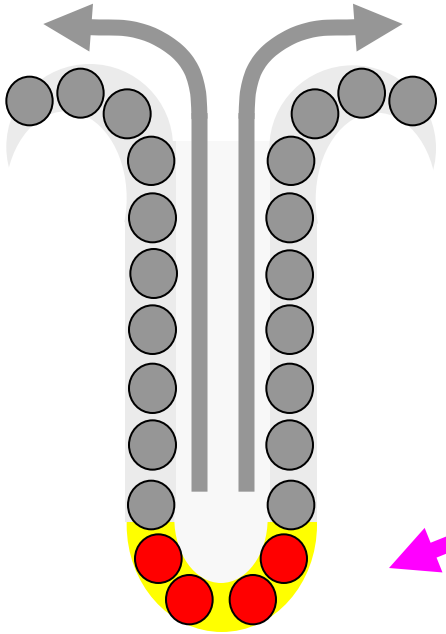
--- about 15 million crypts
--- multiple stem cells per crypt
--- potential to divide everyday

5% Lifetime Risk of Cancer by Age 100 Years

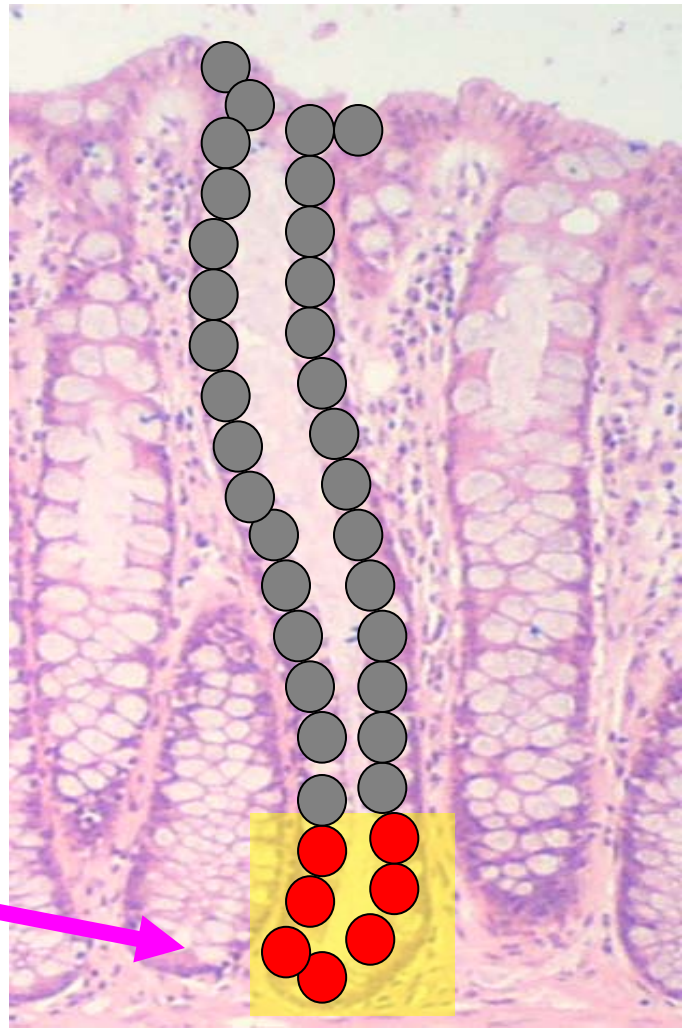
Human Colon Crypts: Mitotic With Constant Cell Replacement

1 crypt = 2,000 cells
All cells but stem cells die in a week

die in 1 week

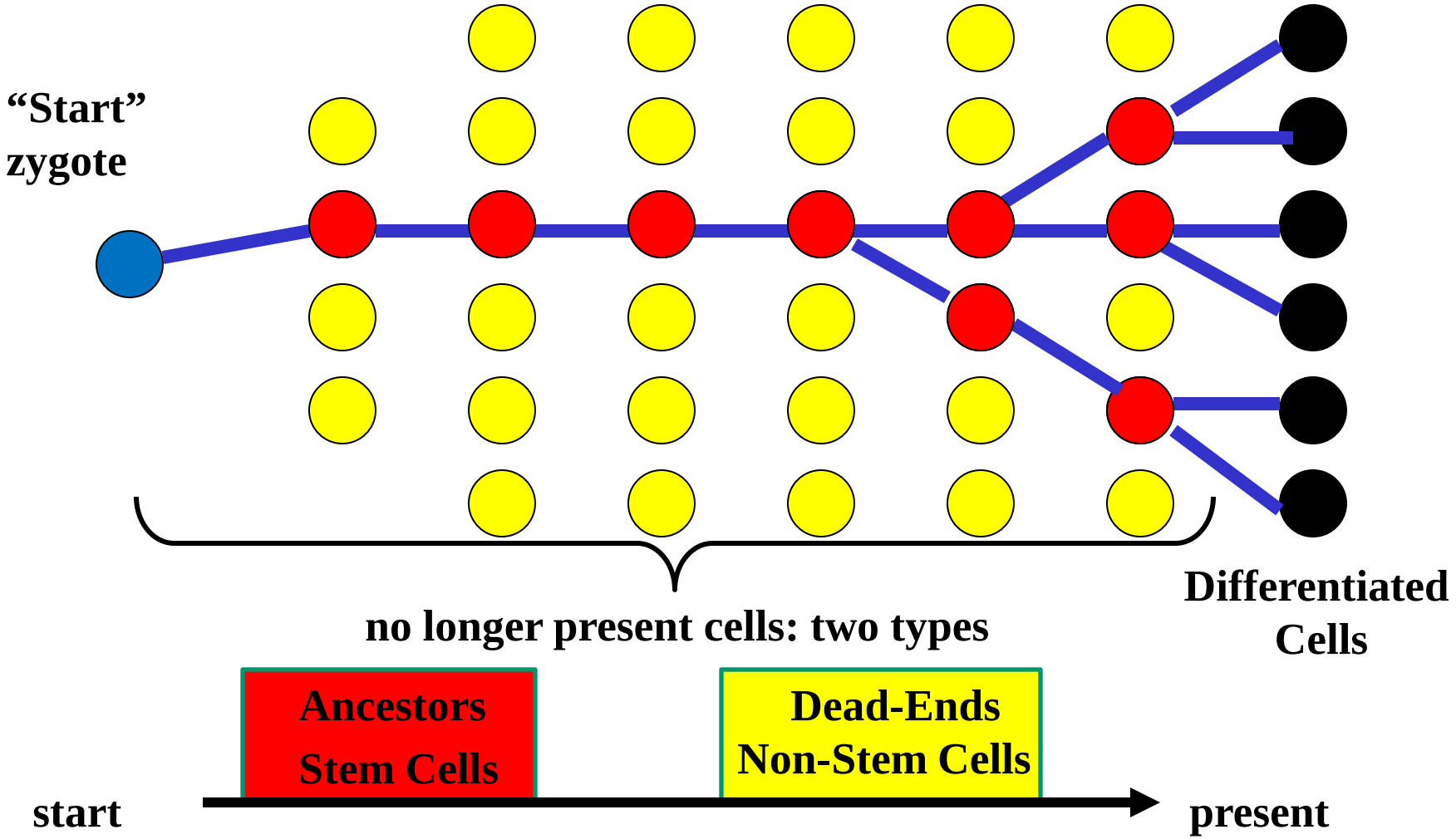


multiple stem cells



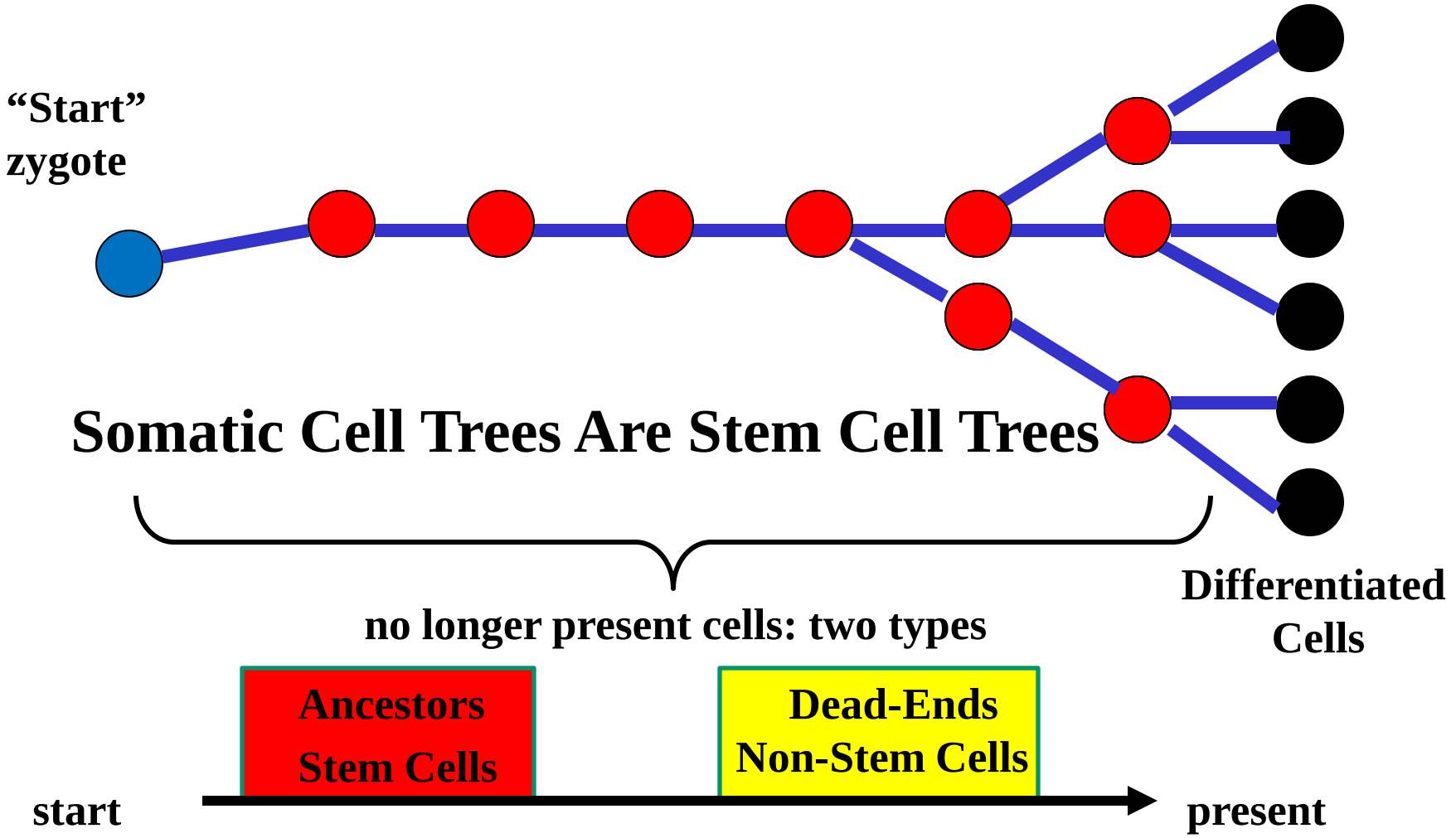
Human Somatic Cell Ancestral Trees

(just 4 kinds of cells)

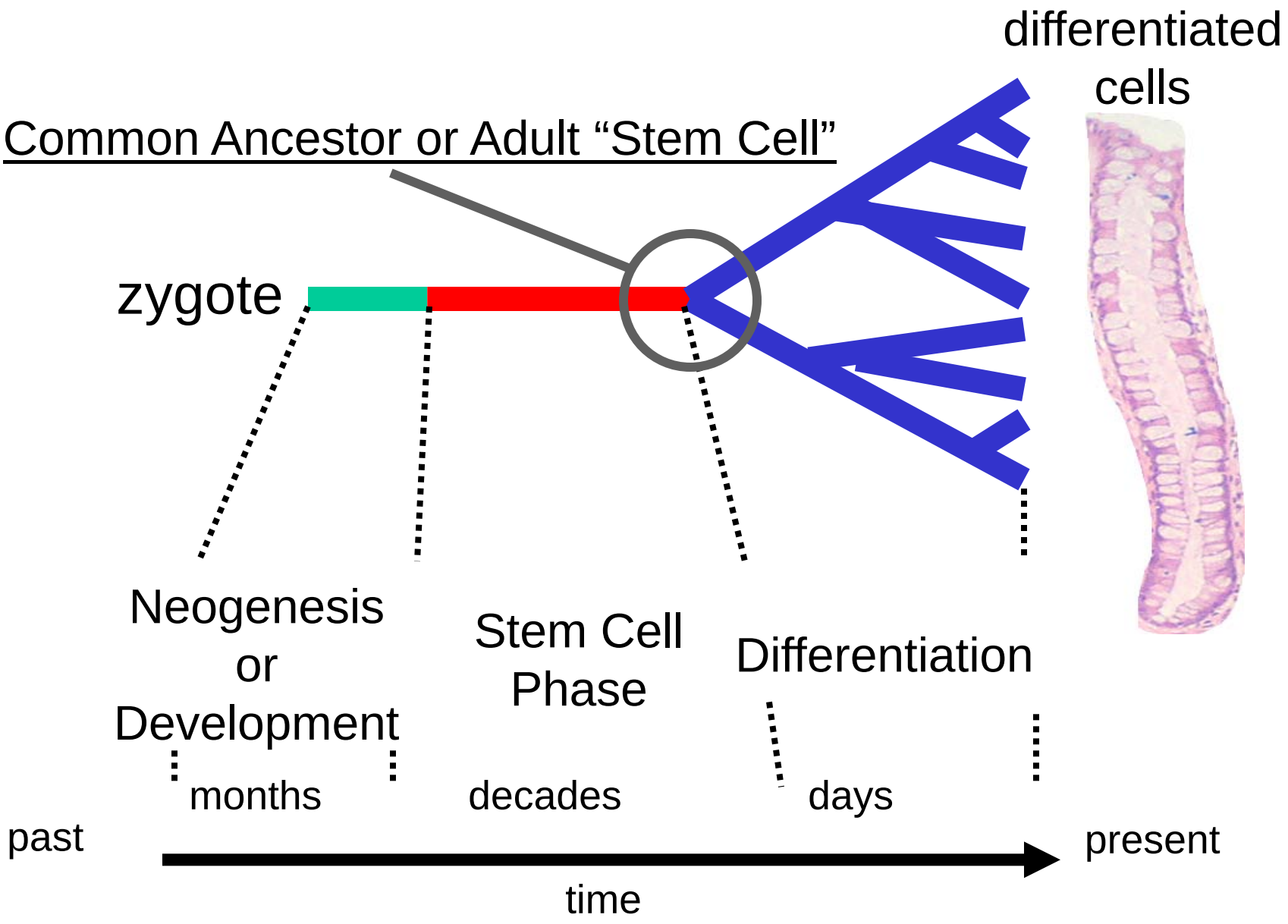


Human Somatic Cell Ancestral Trees

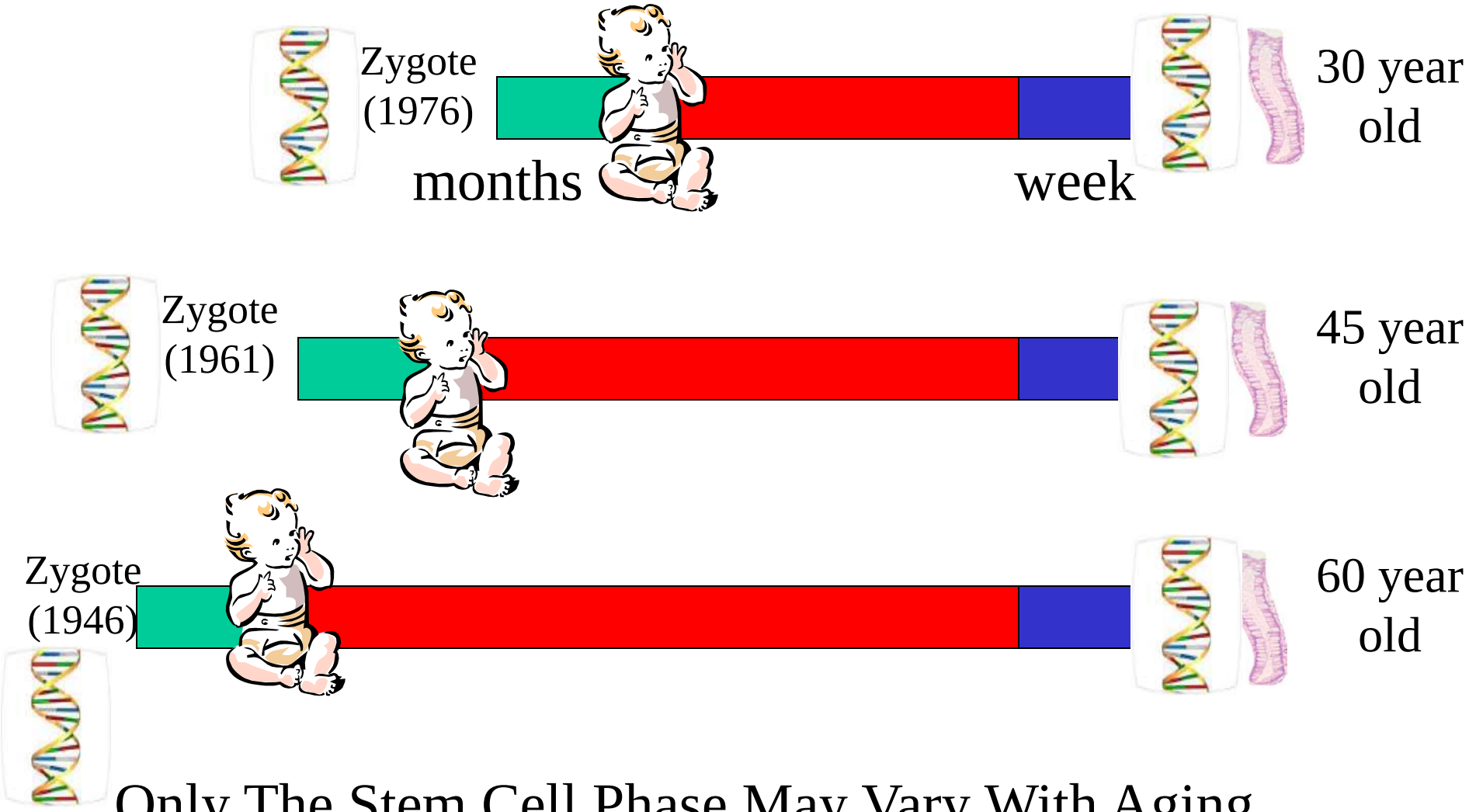
(just 4 kinds of cells)



Human Ancestral Trees (Genealogy)



Many Somatic Cell Genealogies Are Stem Cell Trees



Only The Stem Cell Phase May Vary With Aging

Stem Cells: Two Models

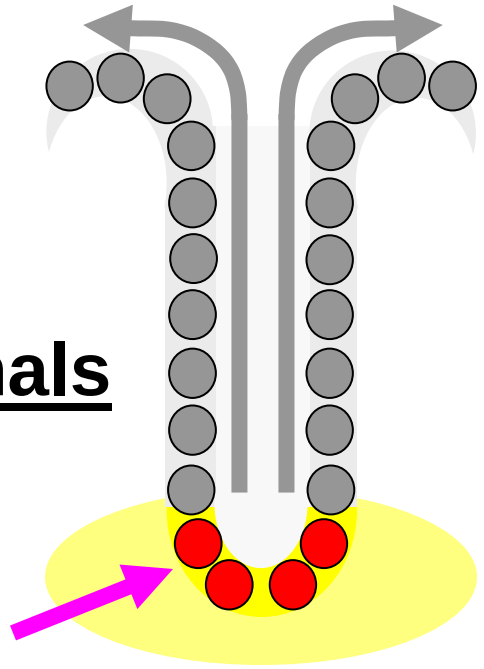
1) Immortal Stem Cell Lineages (Intrinsic)

2) Stem Cell Niches (Two Components)

--- Epithelial Stem Cells

--- Surrounding Stroma

(Extrinsic Signals)



<u>Stroma Niche Signals</u>

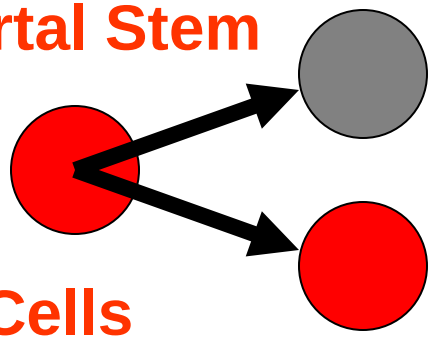
Wnt Pathway?

TGFRII Pathway?

COX2 Pathway?

<u>Cells That Leave A Niche Are No Longer Stem Cells</u>

Types of Cell Division: (Always Binary)



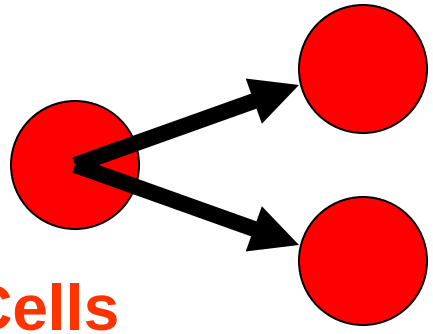
Immortal Stem Cells

Niche Stem Cells

Differentiated Cell

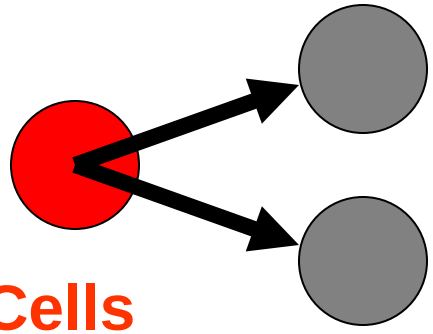
Stem Cell

Asymmetric **Replacement**



Niche Stem Cells

Symmetric **Expansion**

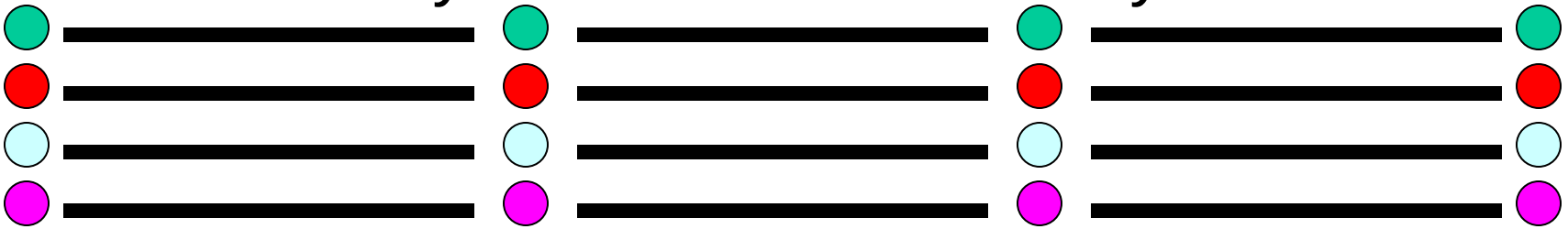


Niche Stem Cells

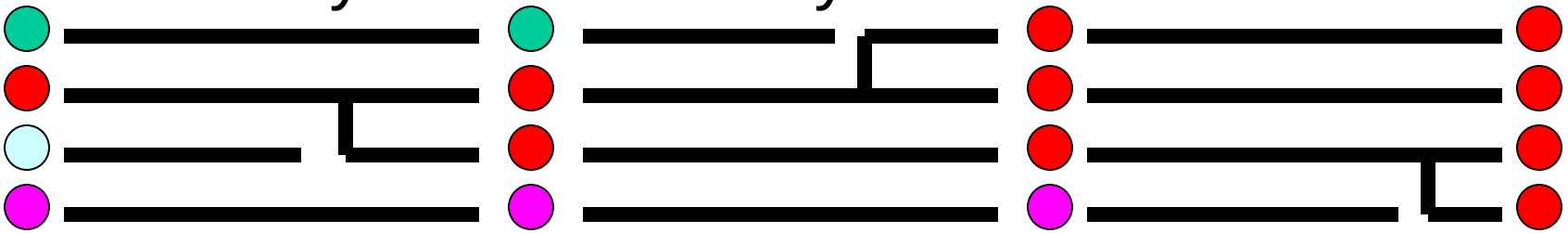
Symmetric **Extinction**

Immortal Vrs Niche Stem Cell Lineages

Immortal: Asymmetric Divisions Only

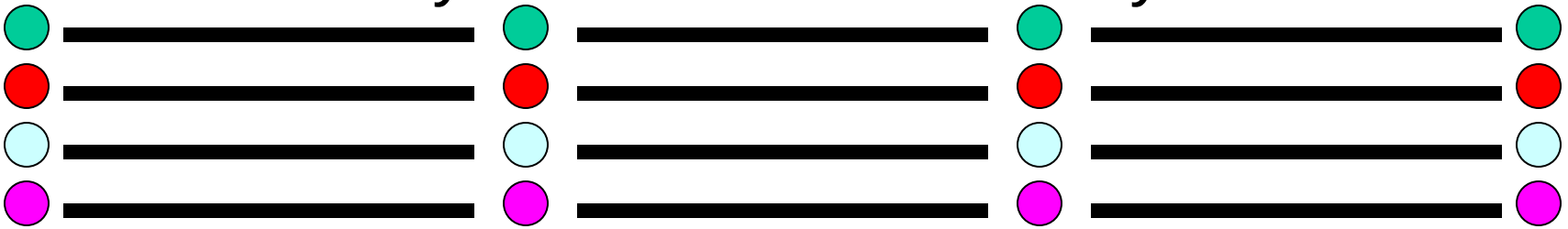


Niche: Asymmetric And Symmetric Divisions

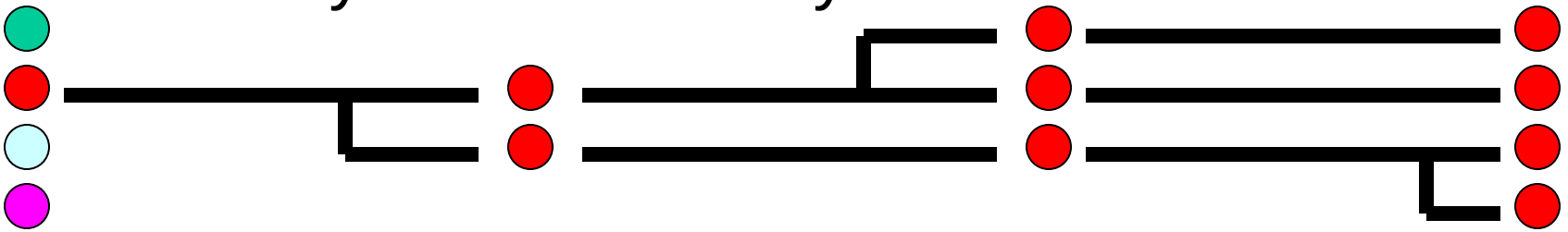


Immortal Vrs Niche Stem Cell Lineages

Immortal: Asymmetric Divisions Only



Niche: Asymmetric And Symmetric Divisions



Appears All Mammalian Stem Cells Are Maintained By Niches (Stem Cell Clonal Evolution)

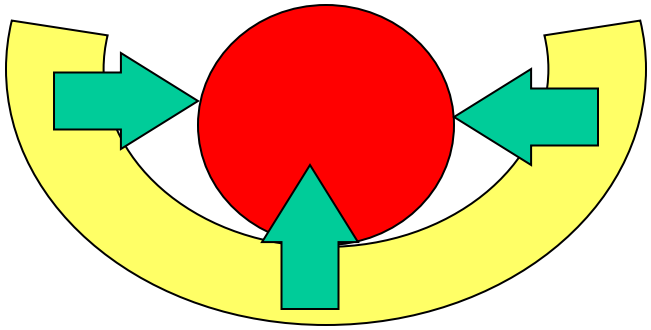
Niche Stem Cells

A Niche Has Two Parts:

--- Mesenchymal

--- Epithelial “Stem Cells”

WNT And Other Factors

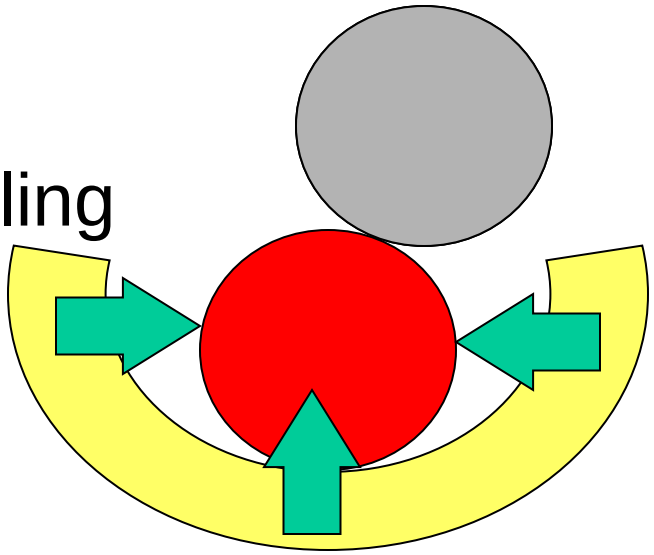


Niche Stem Cells

NICHE STEM CELLS

1) Extrinsically Defined
2) Will Differentiate Outside of Niche

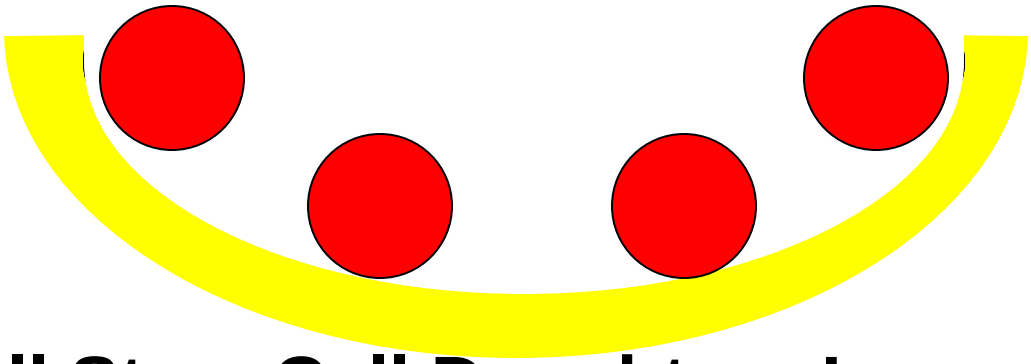
Wnt Signaling Pathway and Others



Stem Cell Niche Dynamics

Stem Cell Niche = Multiple Dividing Stem Cells and Random Loss With Replacement

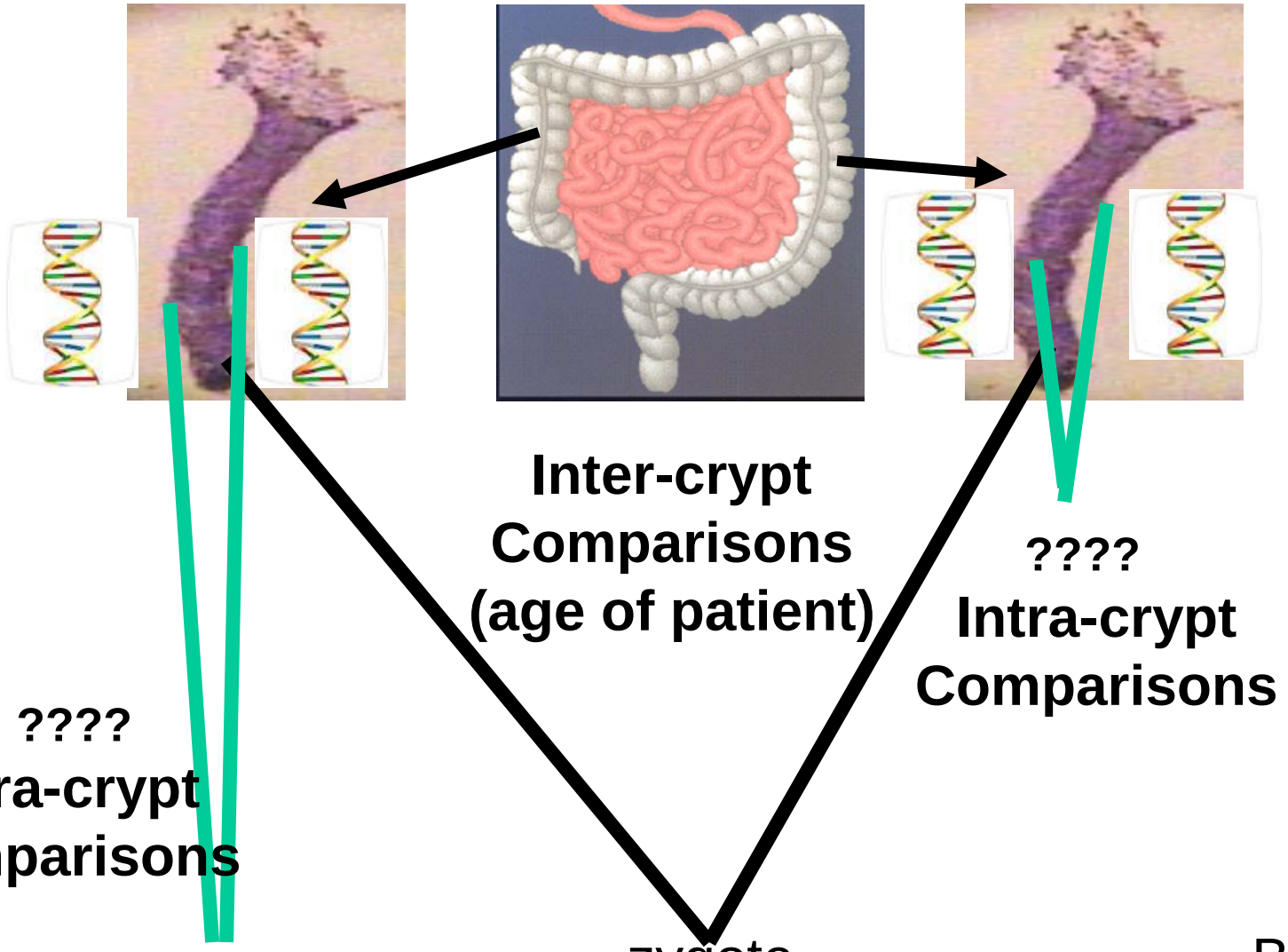
Loss of All Lineages Except One = Stem Cell Clonal Evolution



Half of All Stem Cell Daughters Leave The Niche

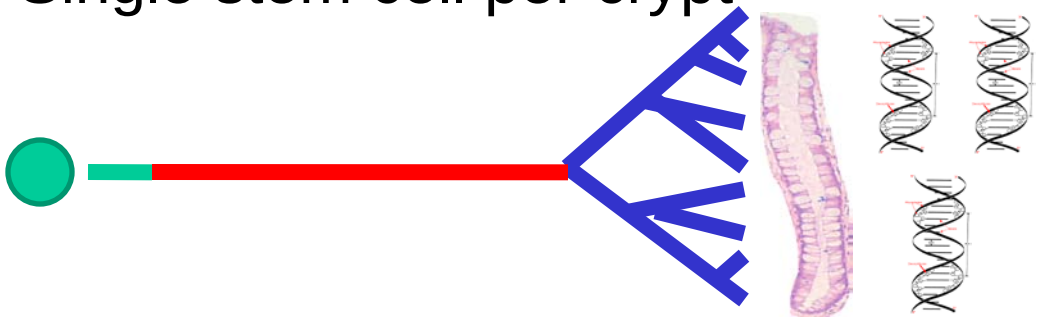
Experimental Strategy:

Characterizing Human Crypt Stem Cells From Their Genomes

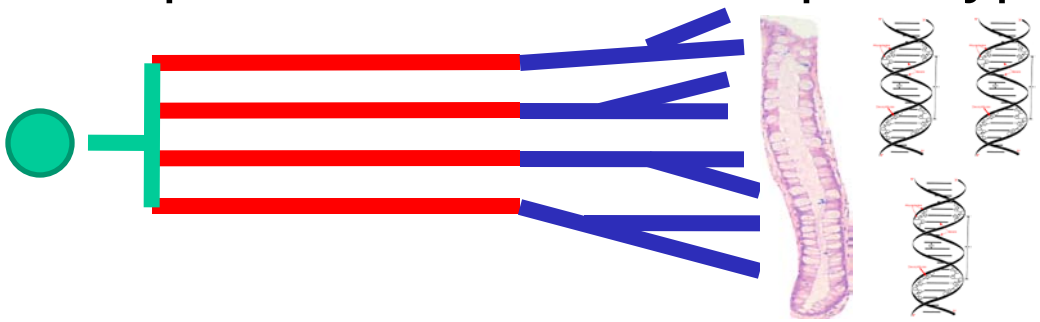


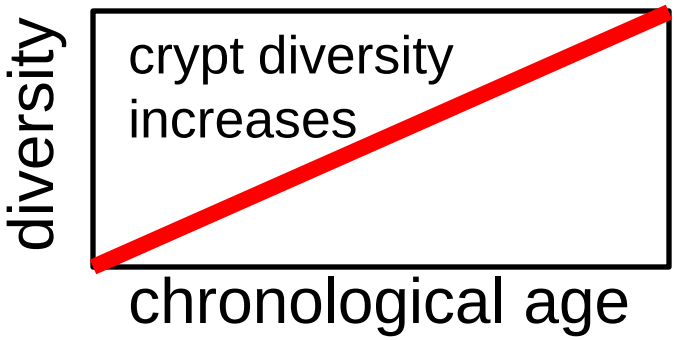
Distinguishing Between Crypt Stem Cell Models

Single stem cell per crypt

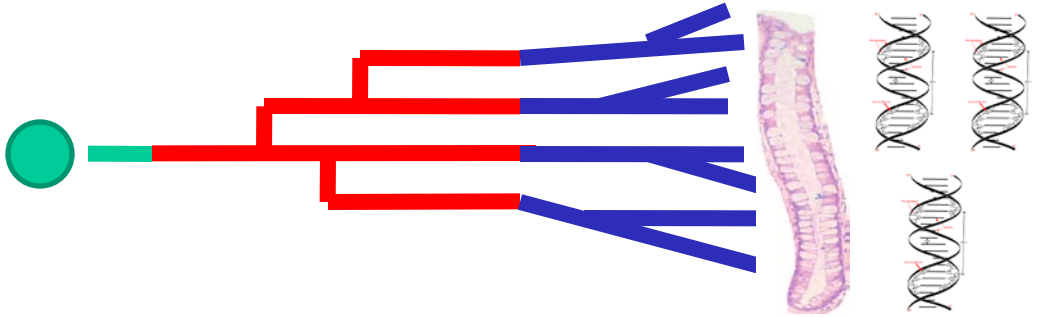


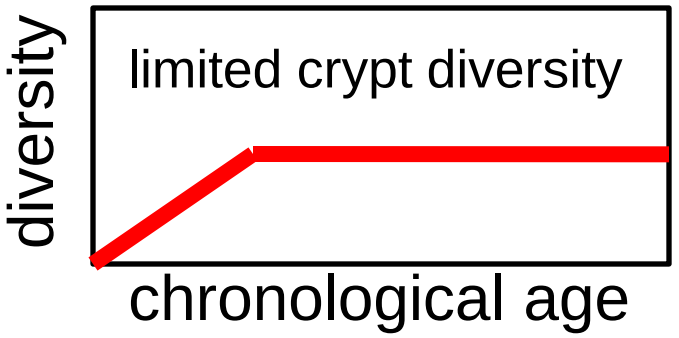
Multiple immortal stem cell per crypt





Multiple niche stem cell per crypt

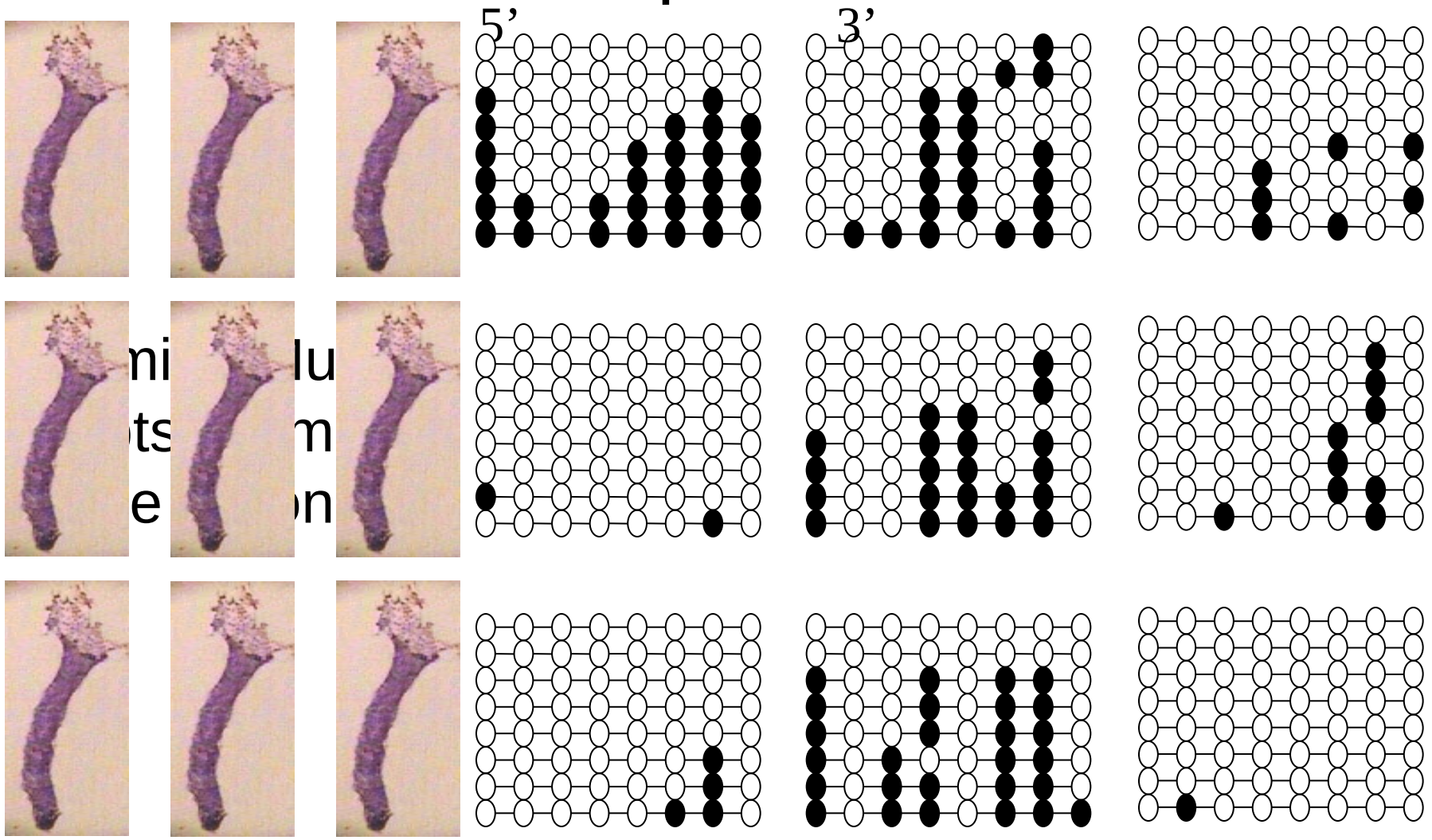




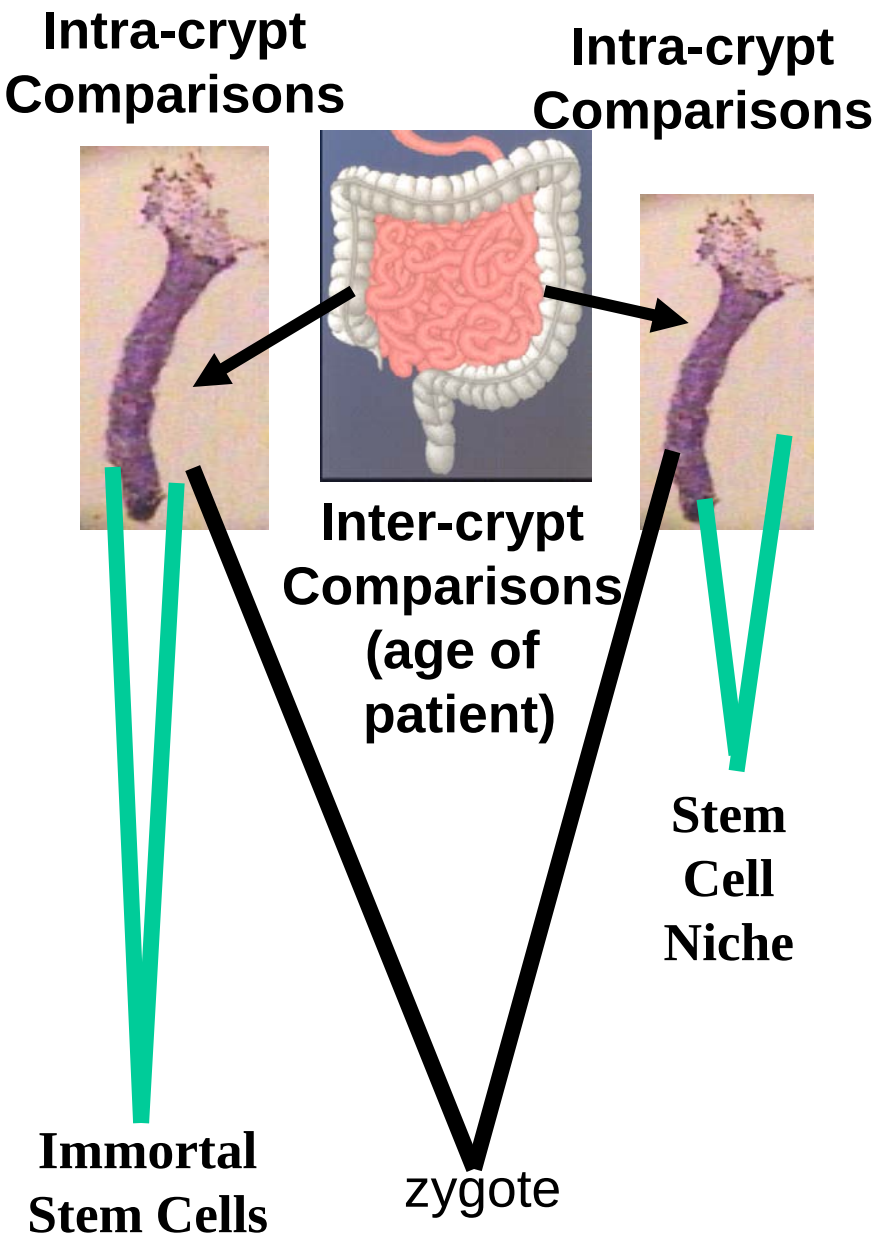
Experimental Plan

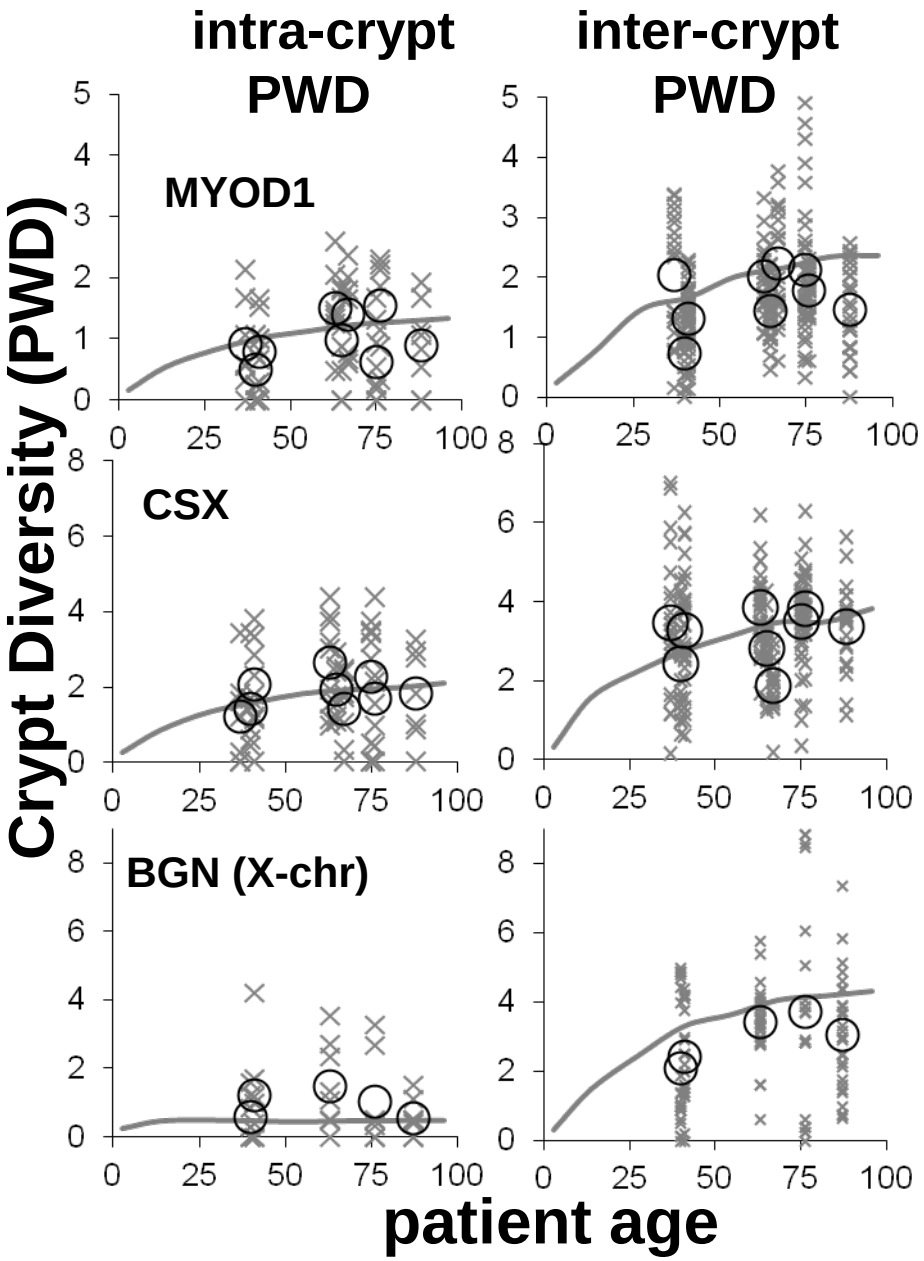
--Colectomy
--Take 1-2 CM^2 of Normal Mucosa
--Isolate Single Crypts (2,000 cells)
--Isolate DNA
--Bisulfite Treat
--PCR, Clone, Sequence Clones
--MYOD1, CSX, BGN
(X-chromosome)

Random Replication Errors

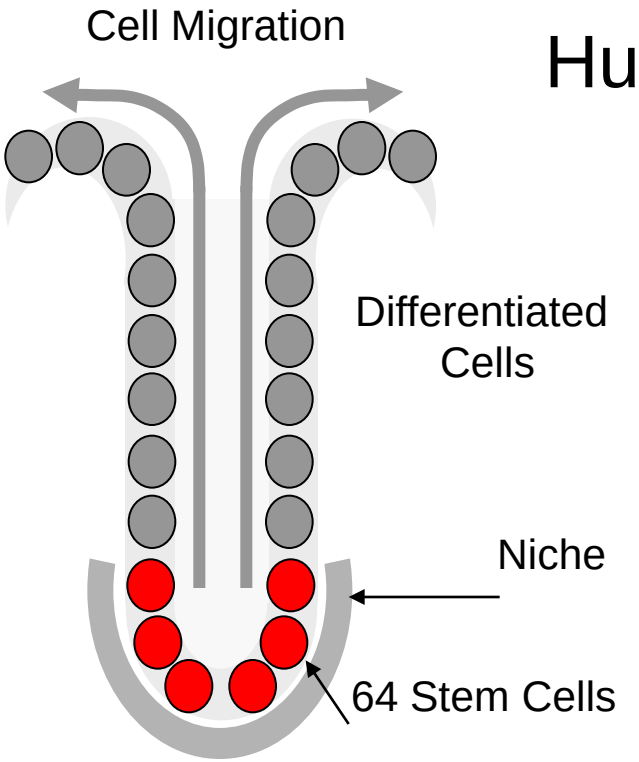


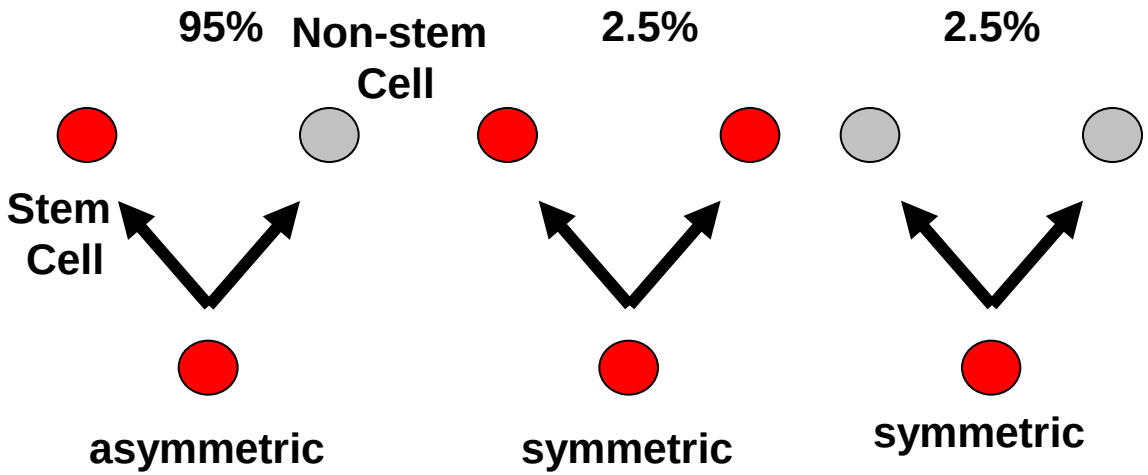
Stem Cell Niches vrs Immortal Stem Cells?



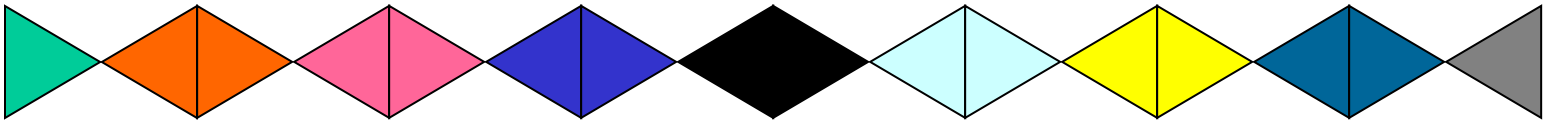


Human Colon Crypt Niche: Summary

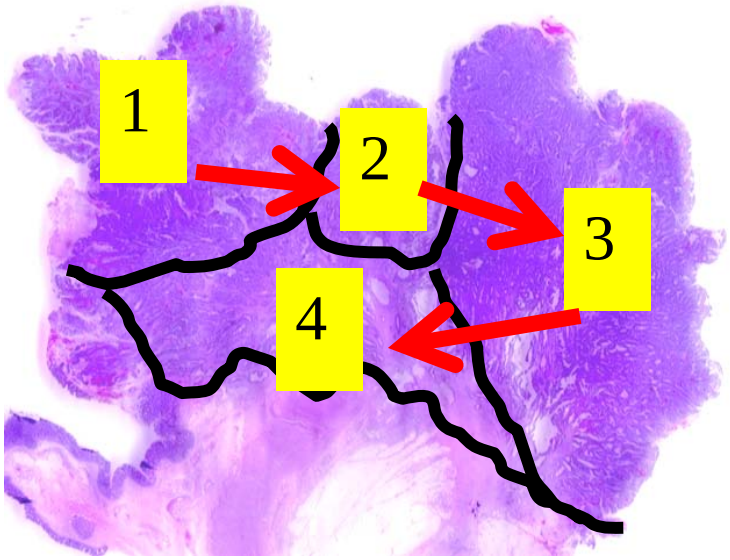




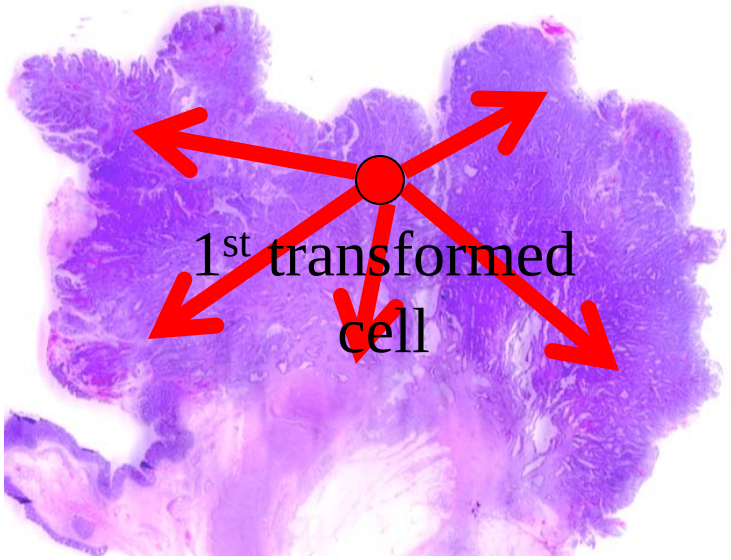
1) More Consistent With Niche Rather Than Immortal Stem Cells
2) Multiple Non-quiescence Stem Cells Per Crypt
3) Most Stem Cell Divisions Are Asymmetric (95%)
4) Crypt Niche Succession Recurs About Every 8 Years



A Problem: The Evolution of Any Individual Human Cancer is Unknown



Sequential or
Clonal Evolution

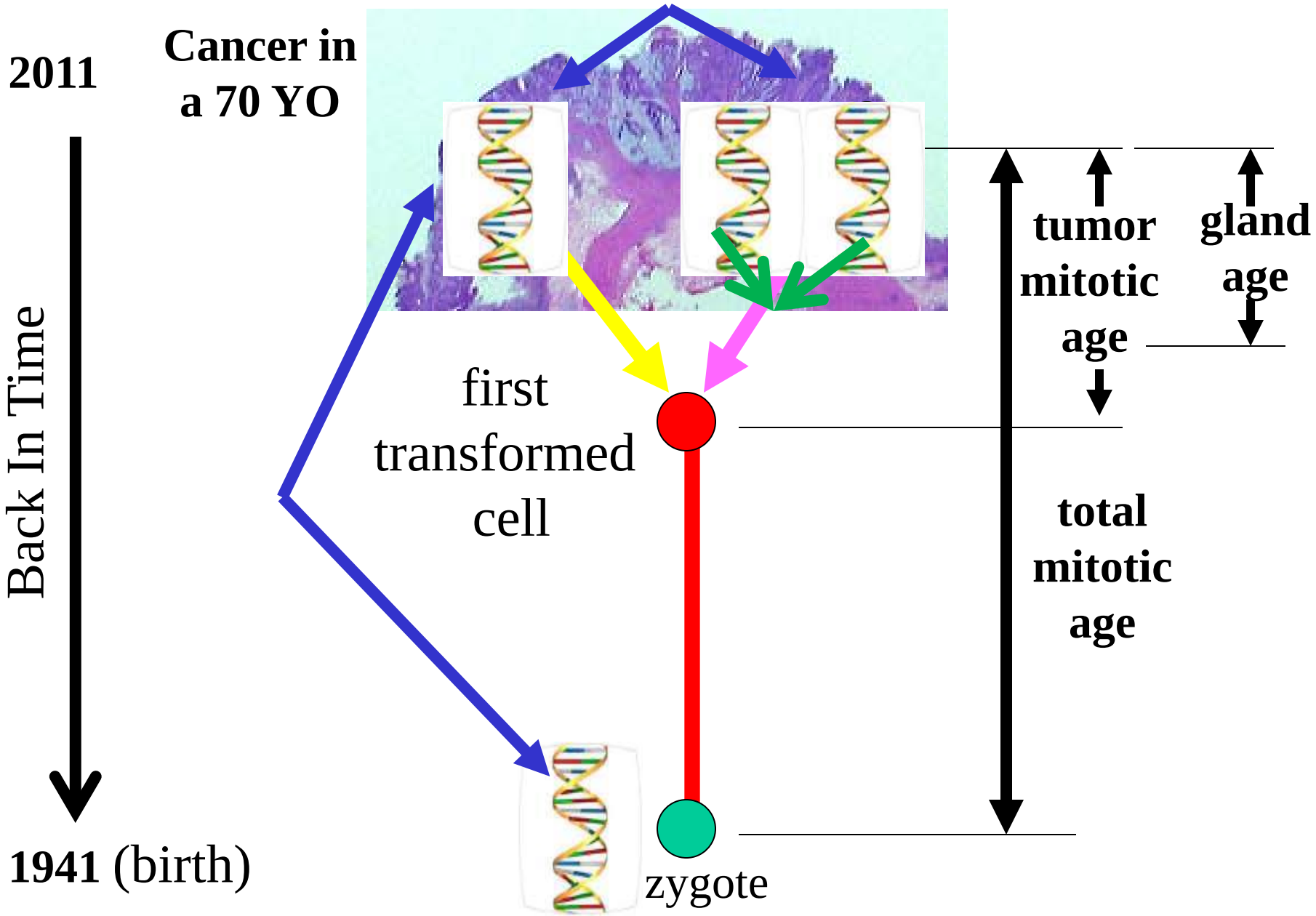


"Big Bang" (full malignant potential at transformation)

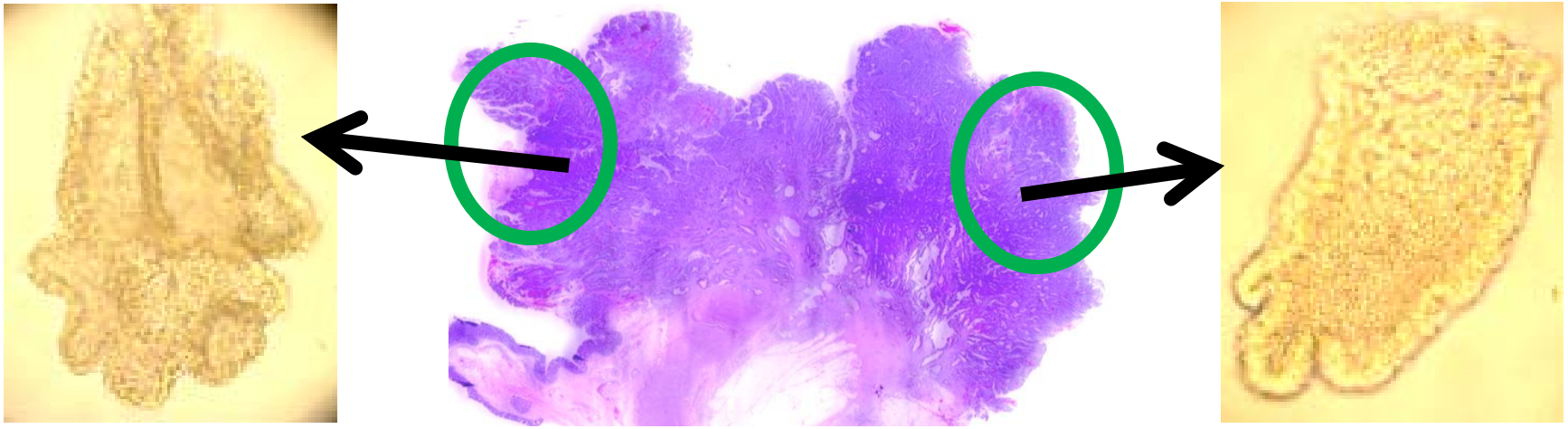
Older Parts More Diverse

Uniform Diversity

Basic Cancer Ancestry Reconstruction



Human Colorectal Cancer: Intratumoral Heterogeneity



six cancer glands

left side

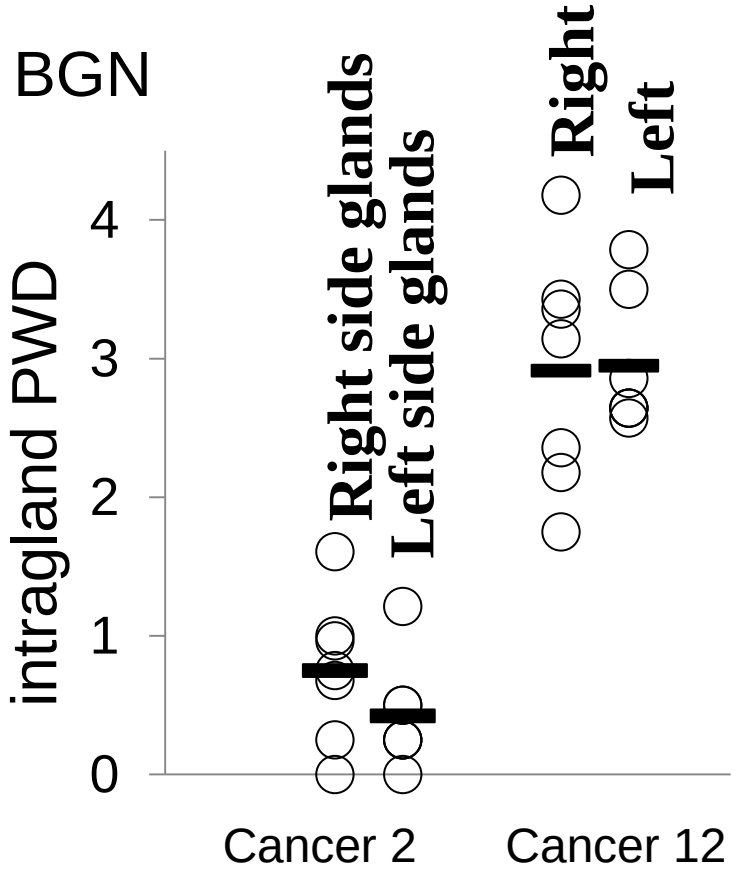
5’ 3’ 5’ 3’ 5’ 3’

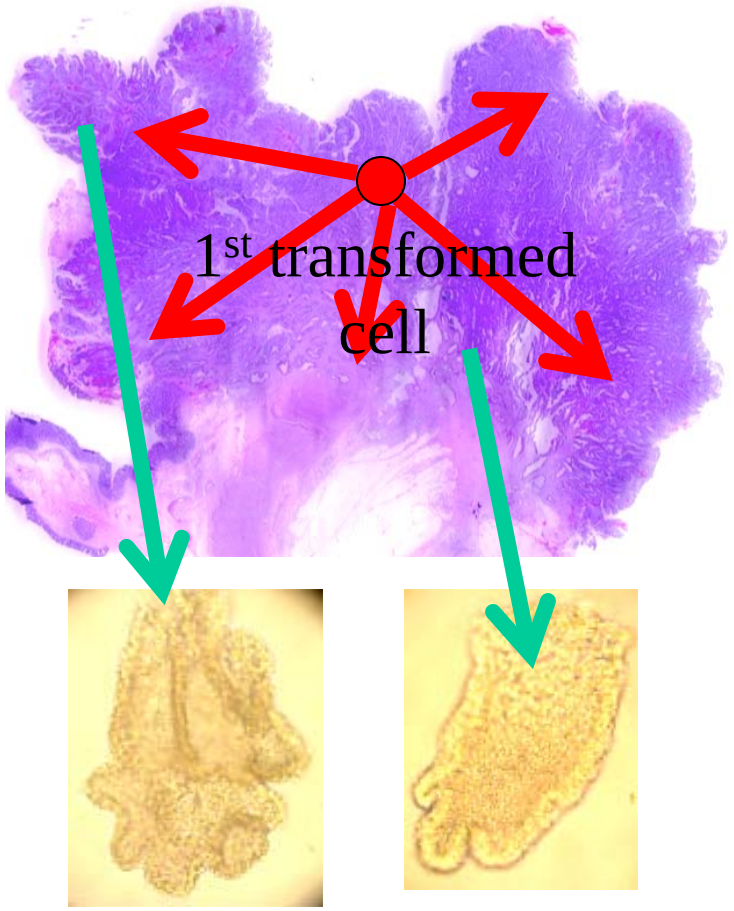
six cancer glands

right side

5’ 3’ 5’ 3’ 5’ 3’

Simple Models of Tumor Growth

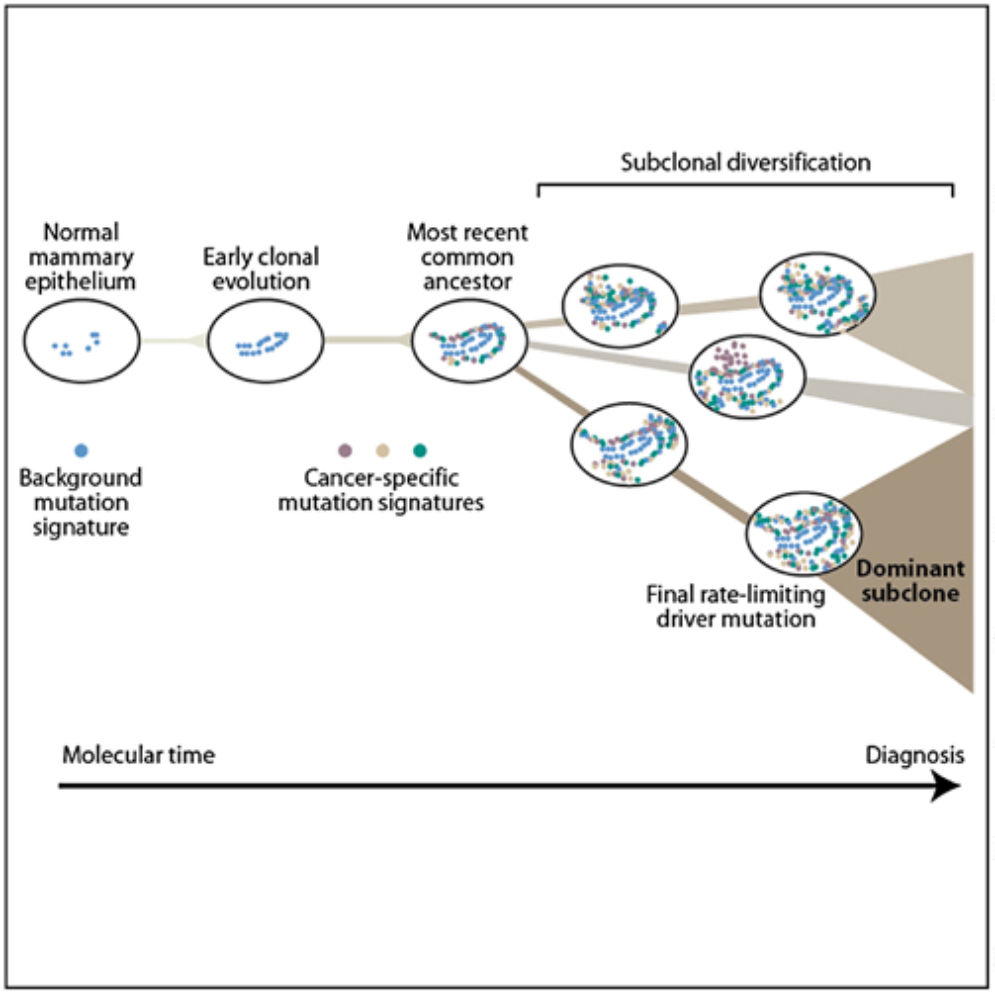


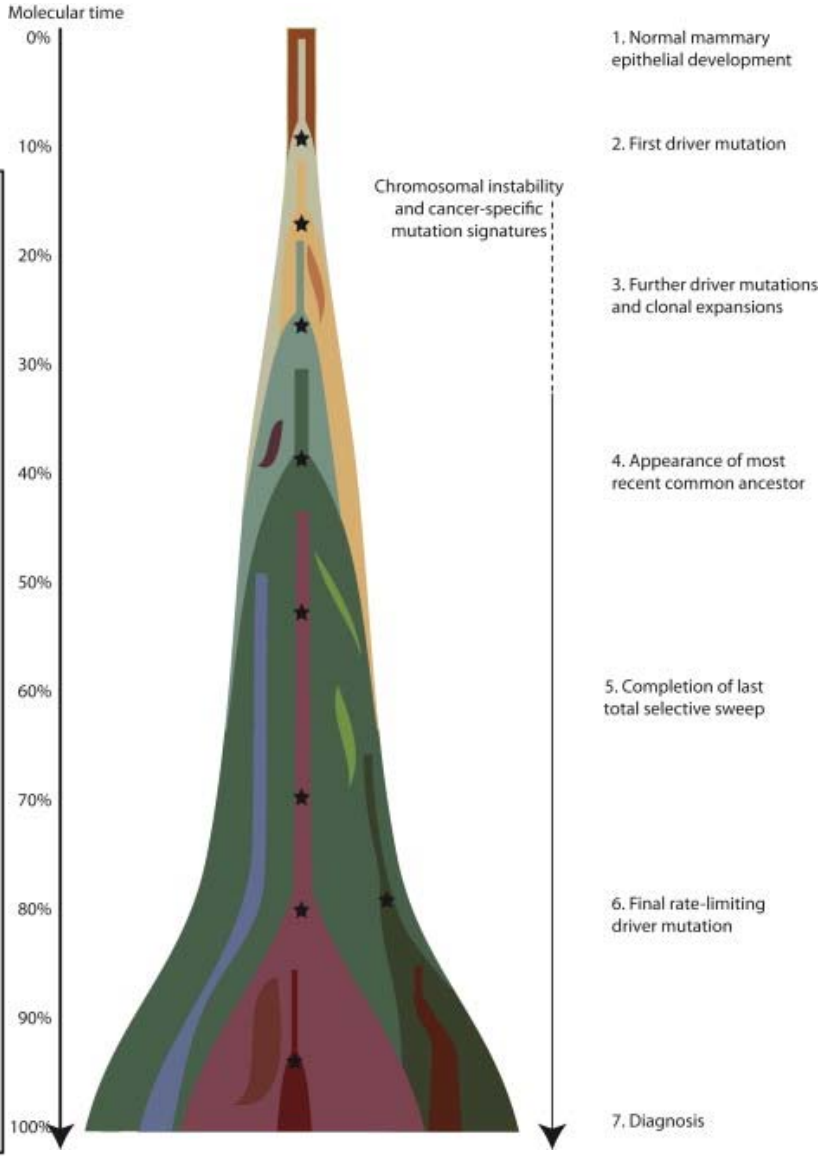


glands within a single cancer have similar ages or PWDs

Cancer Ancestry From DNA Sequencing

The life history of 21 breast cancers.
Cell. 2012 May 25;149(5):994-1007.





Summary

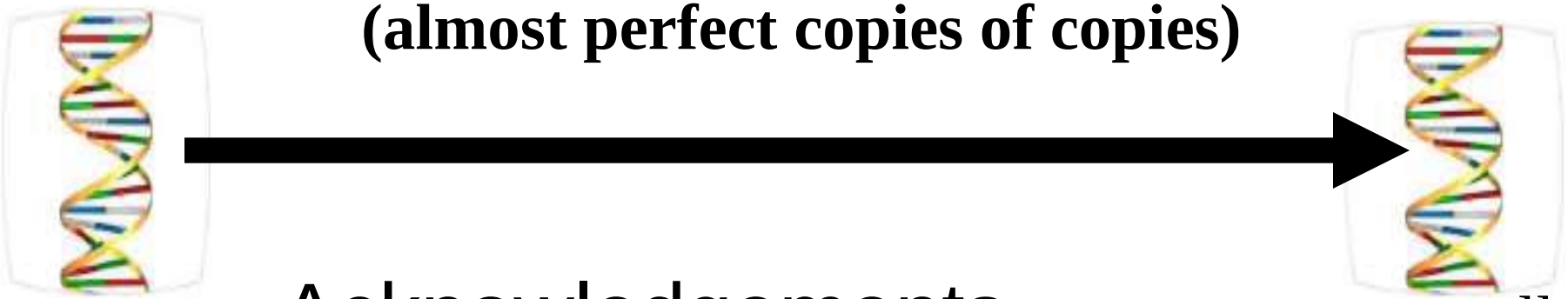
- Genomes Are “Historical” Documents (copies of copies)
- Exact Methods and Measurements Uncertain

  (lack of alternative ethical methods)
- Should Be Possible To Reconstruct Histories Of Human Tissues And Cancers From Their Genomes
- Somatic Cell Genomes Reflect Stem Cell Behaviors

  (neutral drift or random stem cell turnover)
- Different Human Cancers Have Different Histories
- Technological Improvements (High Throughput DNA Sequencers) Will Facilitate Studies
- Potential To Offer Personalized Medicine

  (Histories of Individual Aging and Cancers)

Genomes Are “Historical” Documents (almost perfect copies of copies)



zygote (start)

current cell (end)

Acknowledgements

- Yasushi Yatabe
- Kyoung-Mee Kim
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- Peter Calabrese
- Kim Siegmund
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