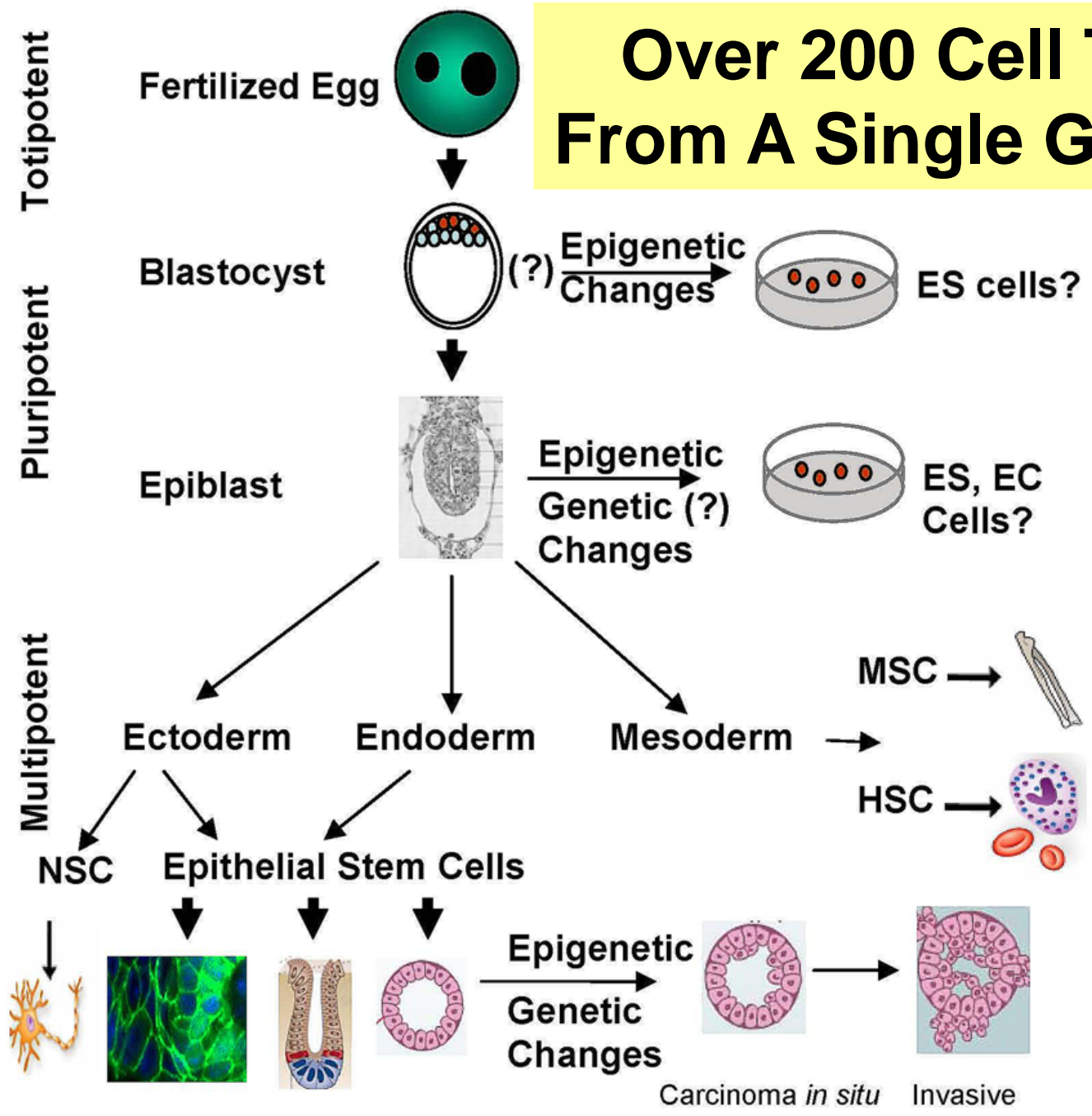


Epigenetics: DNA Methylation

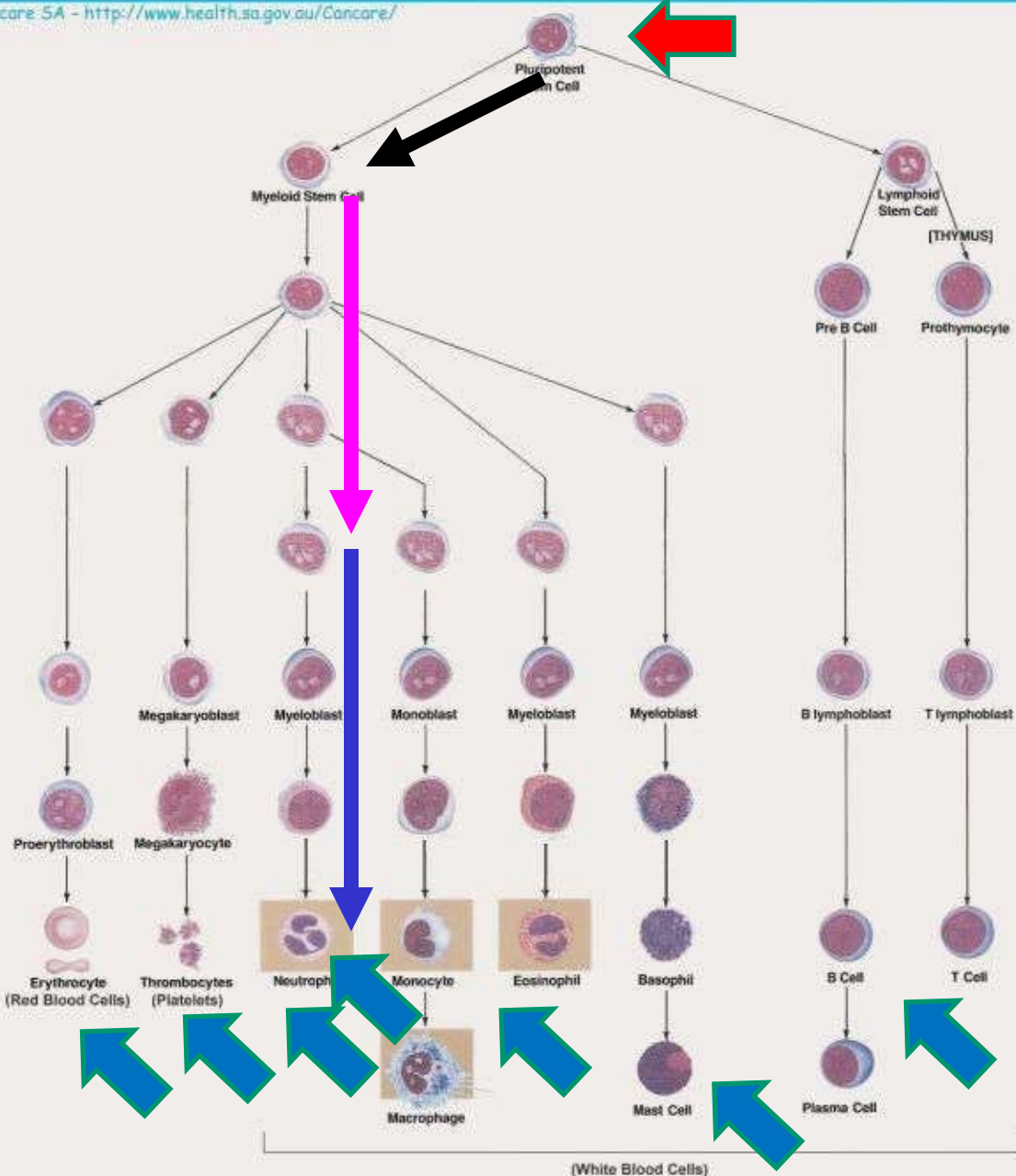
Darryl Shibata
University of Southern California
Keck School of Medicine

Over 200 Cell Types From A Single Genome!



HEMATOPOIESIS

Cancore SA - <http://www.health.sa.gov.au/Cancore/>



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)

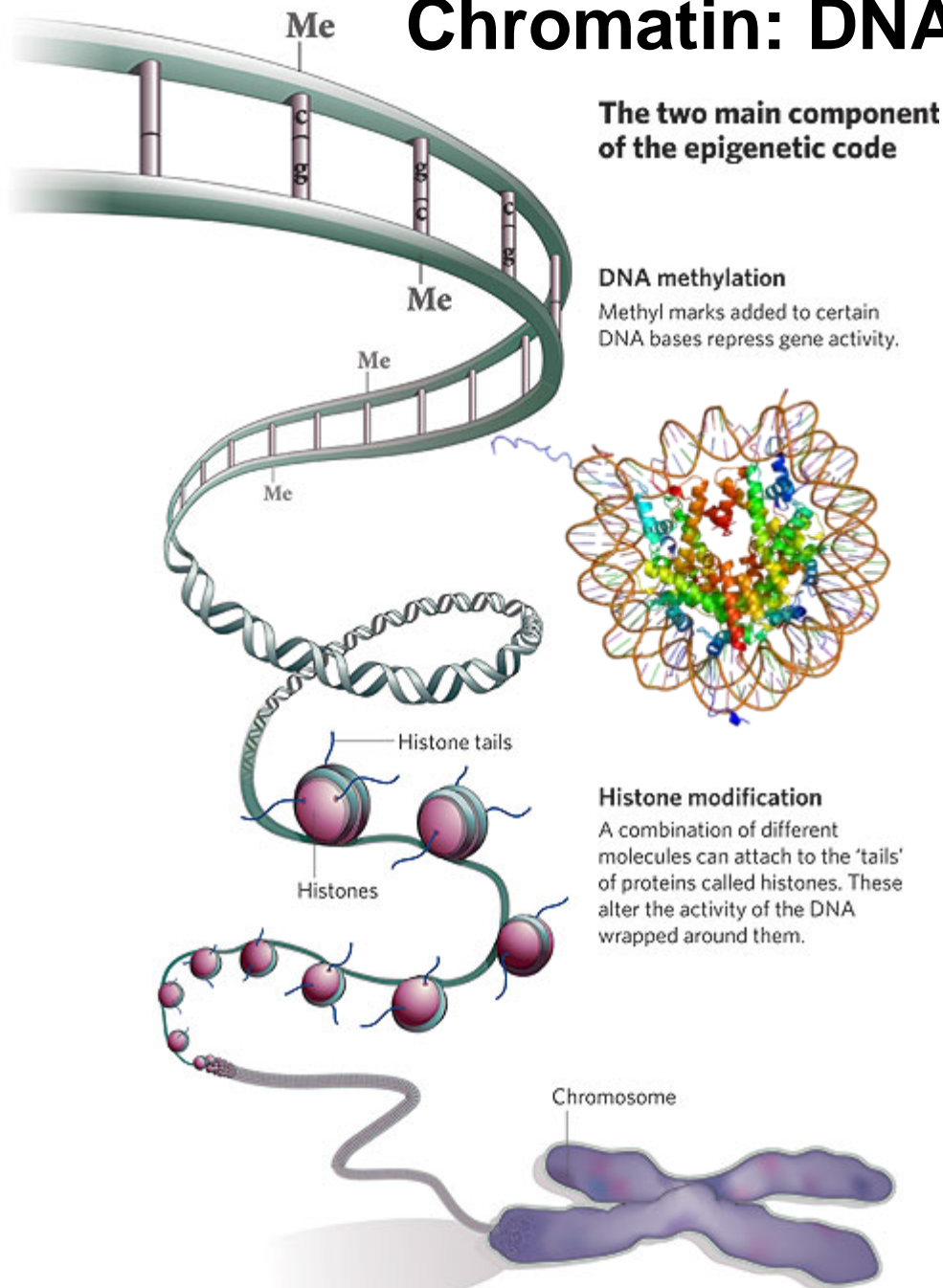
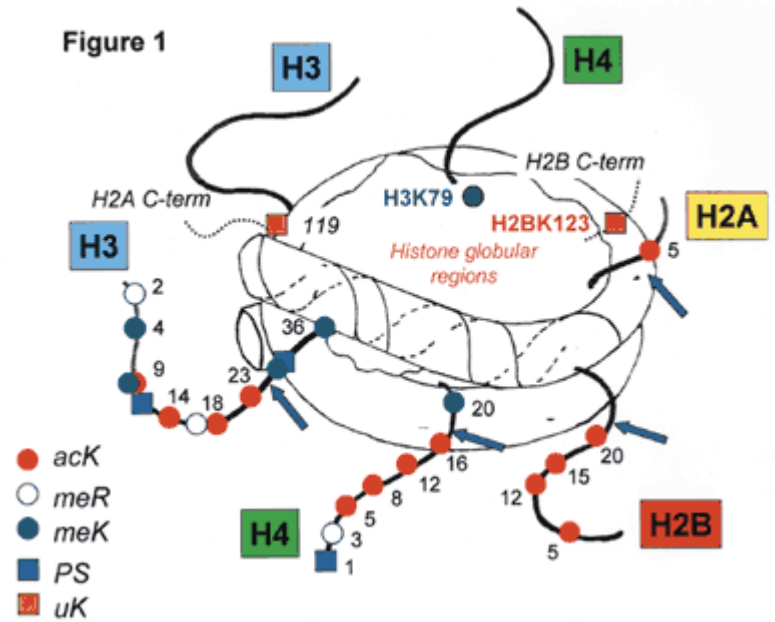
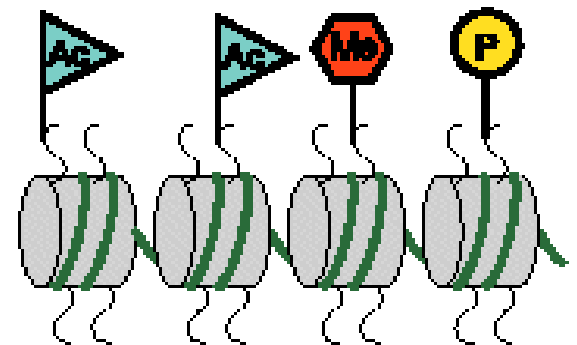


Figure 1

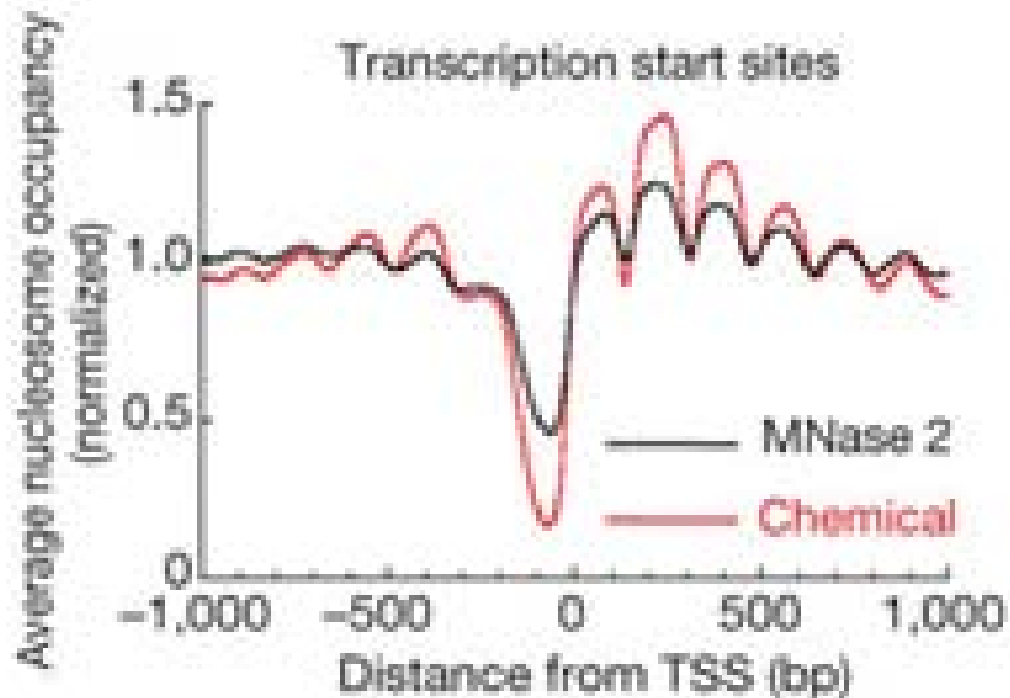


Histone Tails

The Histone Code



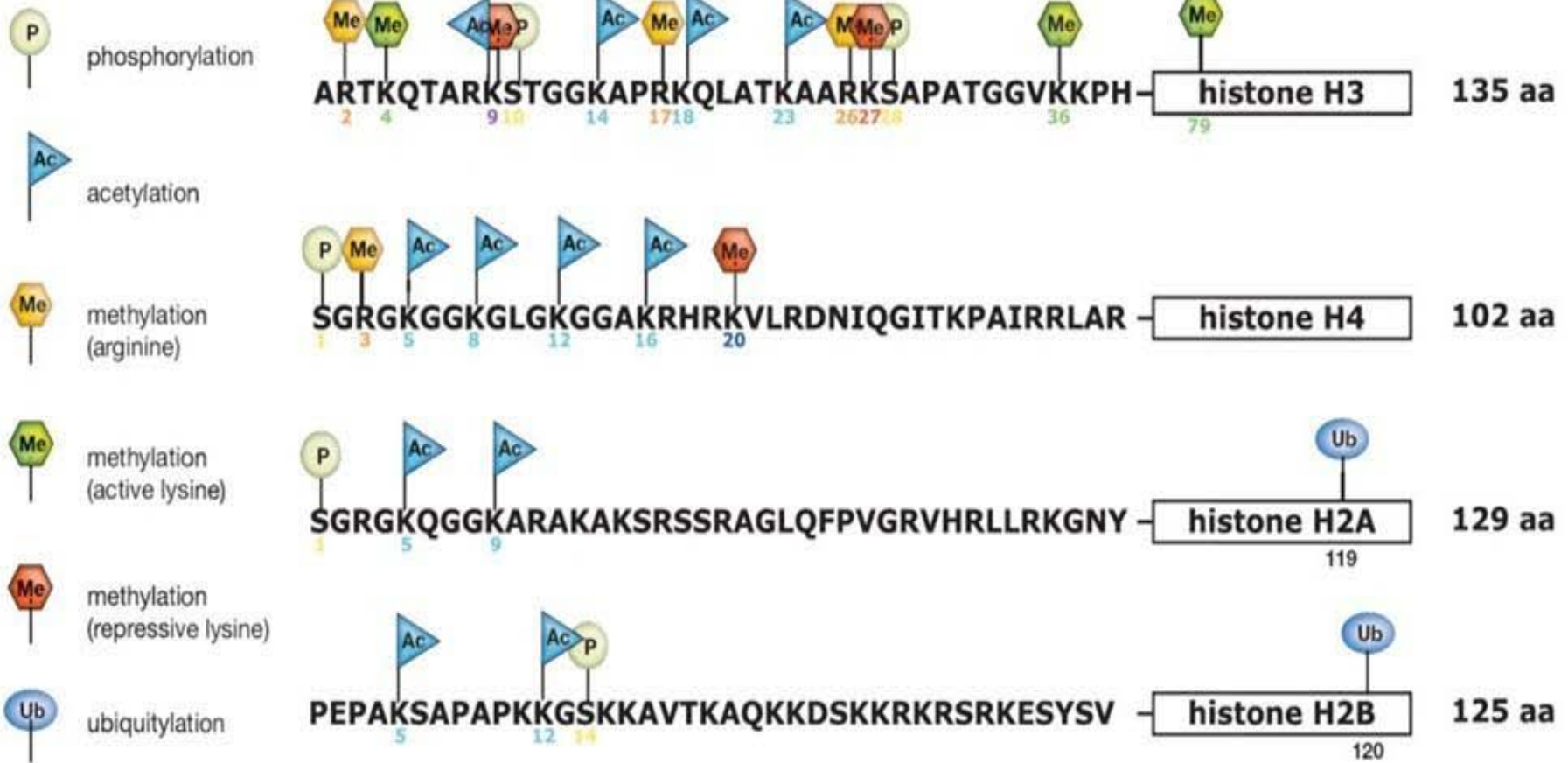
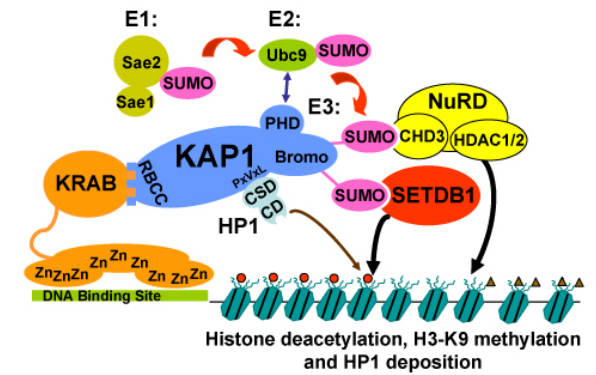
Nucleosome Positioning: Favored Locations



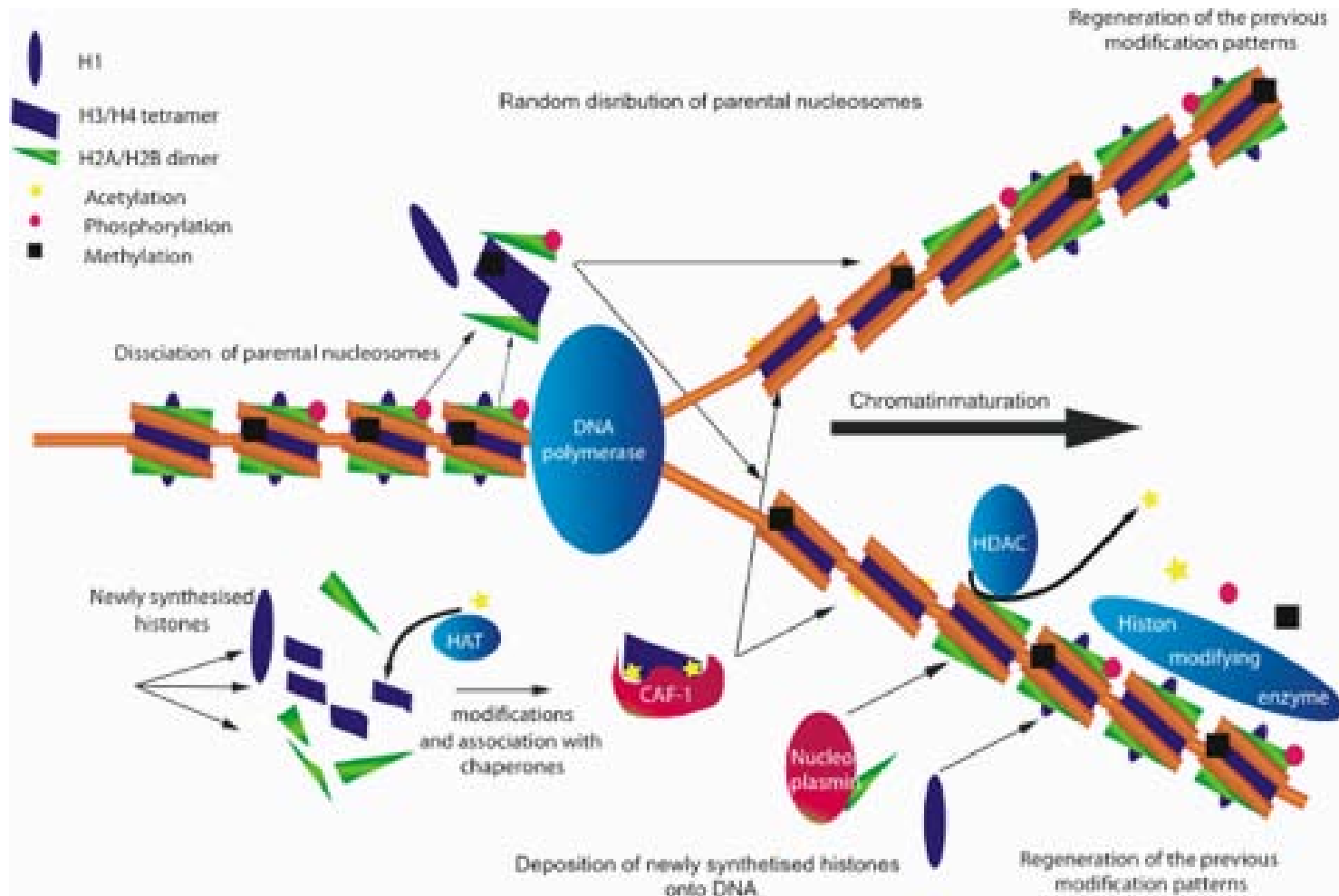
[A map of nucleosome positions in yeast at base-pair resolution.](#)

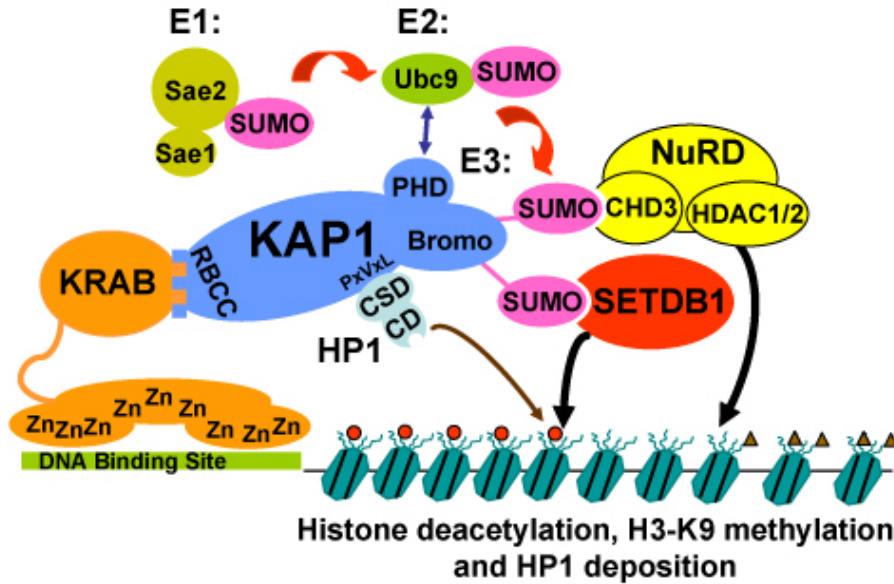
Brogaard K, Xi L, Wang JP, Widom J. Nature. 2012;486:496-501

Histone Code (combinatorial)



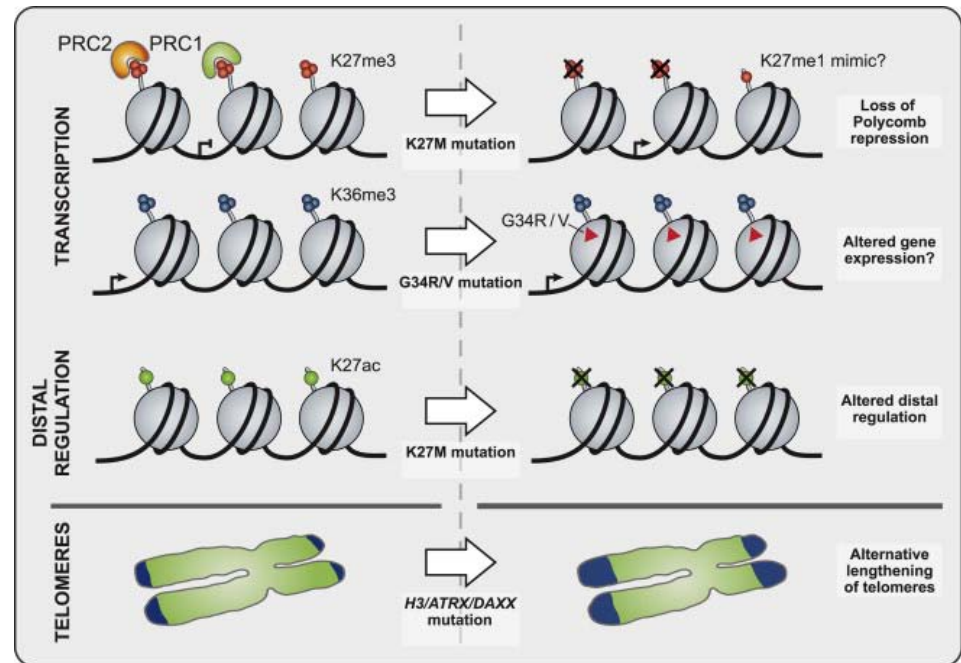
Chromatin Replication (Genetic and Epigenetic Inheritance)





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

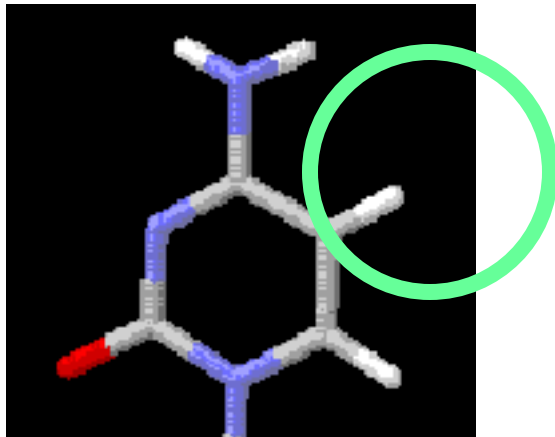
**Cancer Mutations Result
 in
 Altered Transcription
 (Regulation)**



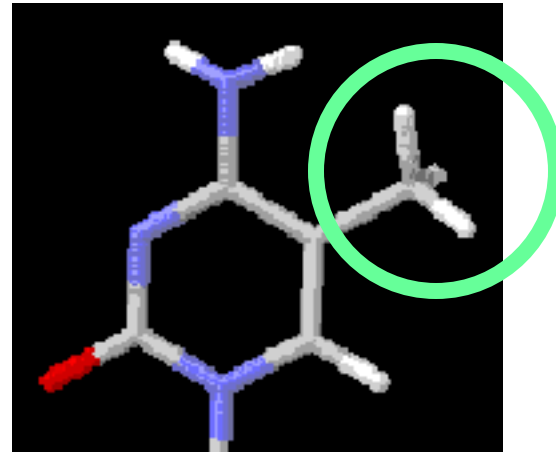
DNA Methylation

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

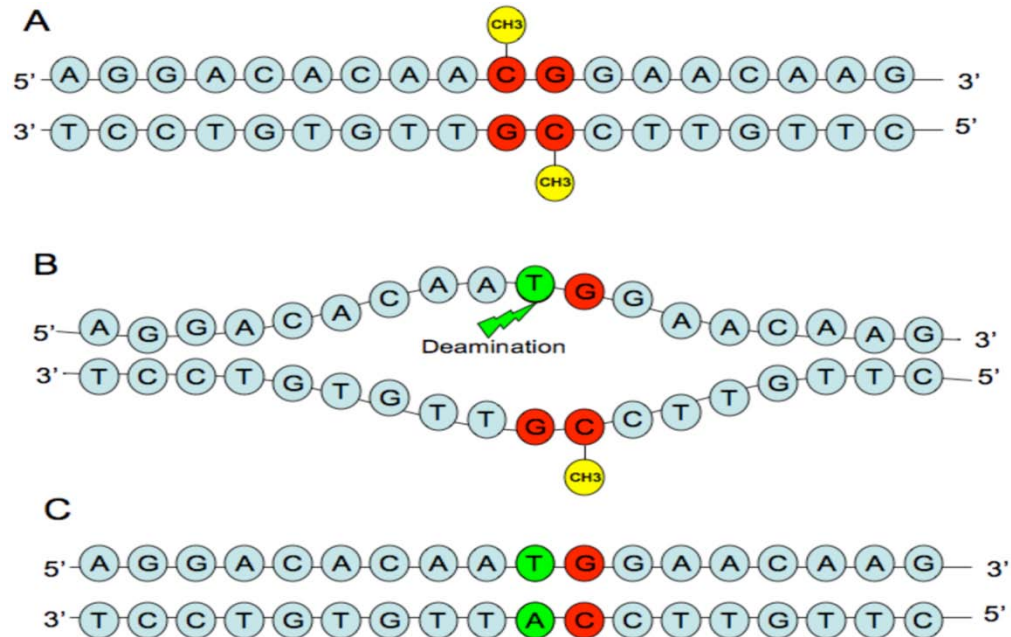


5-methyl cytosine

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)



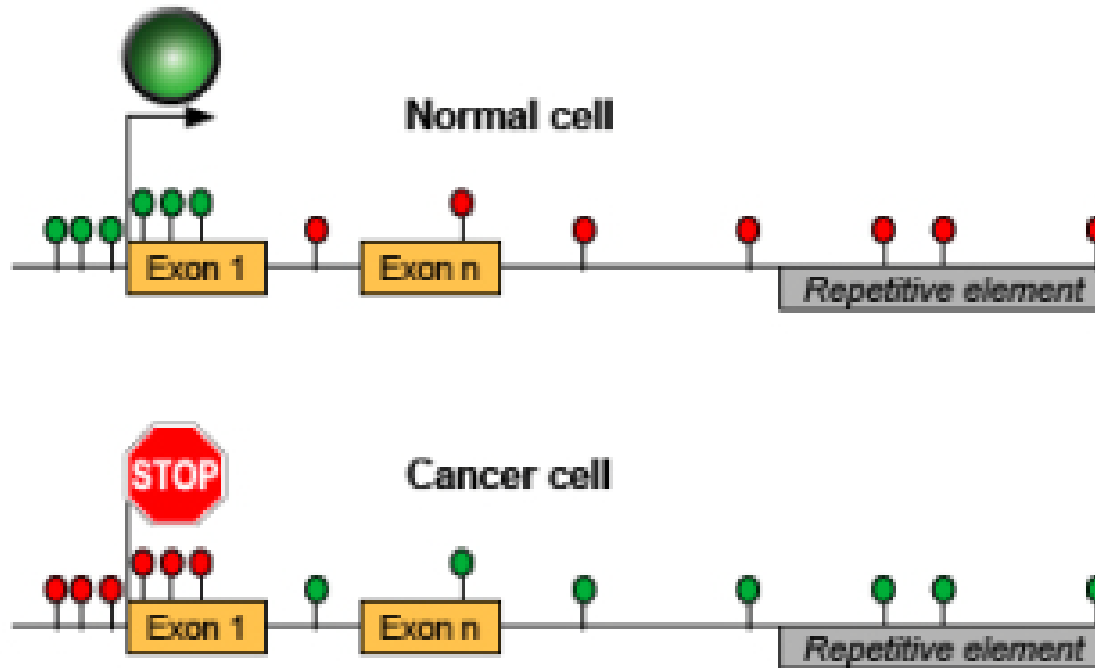
Exon 1 CpG Island: 12634..12767

```
11941 ttataagatc cccctccctc taaatcctgt ccttotatca cttcctcctt CGctctcctt
12001 taaaatgaga cagttgtcag caggaatcct gCGcaagaac acaccaccct gtttcataga
12061 agatatctca ggtaatgtgc aaacaCGgggt ttttaaaCGg agCGcatttt totcatttgt
12121 taatatcacc acctaaatca totottgcct aaaacaagga gtagaaagtg aatgaaggaa
12181 ggaacaggtg atggtcagtg toctttctac gctcaaaaat ttaagagttt atgtgaaaat
12241 tcataaatat taatctcaat ccaggttaag caaaattttt tgcctctctc tttagaat
12301 totggttgcc aaagttccag aaattgcttc ctcattcctg agcctttcat tttctCGatt
12361 totccattat gtaaCGggga gctggagctt tgggcCGaat ttccaattaa agatgatttt
12421 tacagtcaat gagccaCGtc agggagCGat ggcaccCGca ggCGgtatca actgatgcaa
12481 gtgttcaagc gaatotcaac tCGttttttt CGgtgactca tccCGgccc tgcttggcag
12541 CGctgcaccc ttttaactta acctCGgcCG gcCGccCGcc gggggcacag agtgtgCGcc
*12601 gggcCGCGCG gcaattggtc ccCGCGCGa cctcCGccCG CGagCGcCGc CGcttccctt
*12661 cccCGcccCG CGtccctccc cctCGgcccc gCGCGtCGcc tgtcctcCGa gccagtCGct
*12721 gacagcCGCG gCGcCGCGag cttctcctct cctcaCGacc gaggcaggta aaCGccCGgg
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13201 ggctCGCGct cccCGccCGt tggcCGcccc tCGgaggcCG agatCGgggc ccagaaCGcc
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14161 tggaaaaatc aacaaCGctc ttagatttgt agaagaaagg aaaaaatcac cagtggaaag
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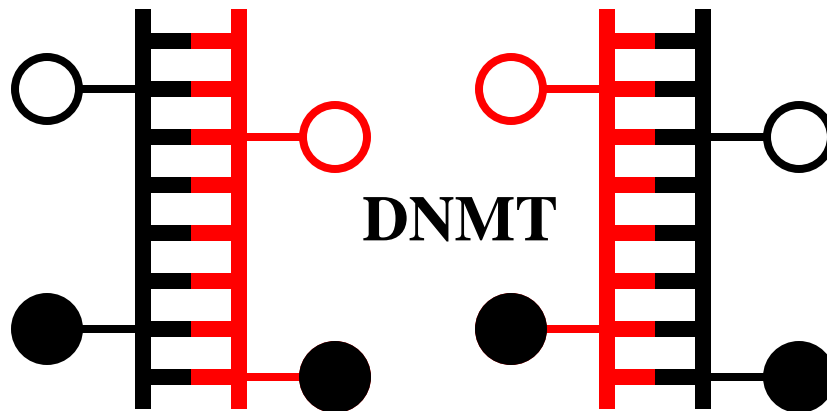
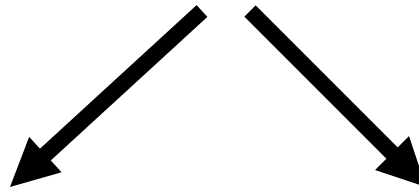
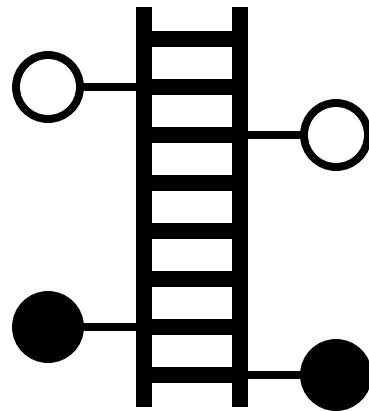
CpG Islands

- increased CpG density
- often associated with promoters or repetitive elements

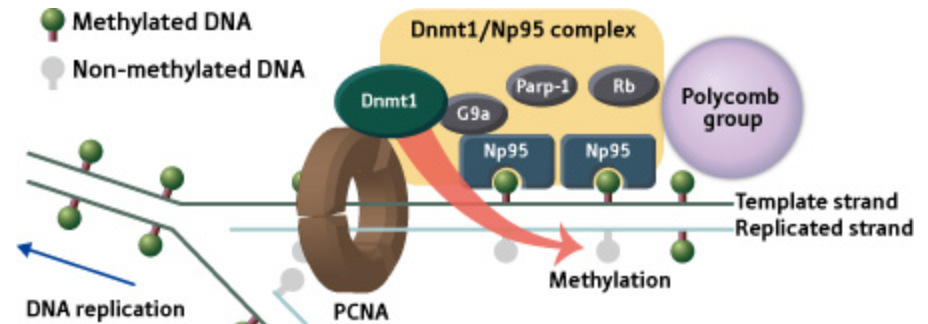
Promoter CpG Methylation Can Regulate Gene Expression (typically gene silencing)



Replication Clock



Genome Replication



Epigenetic Fidelity
is less than
Genetic Fidelity

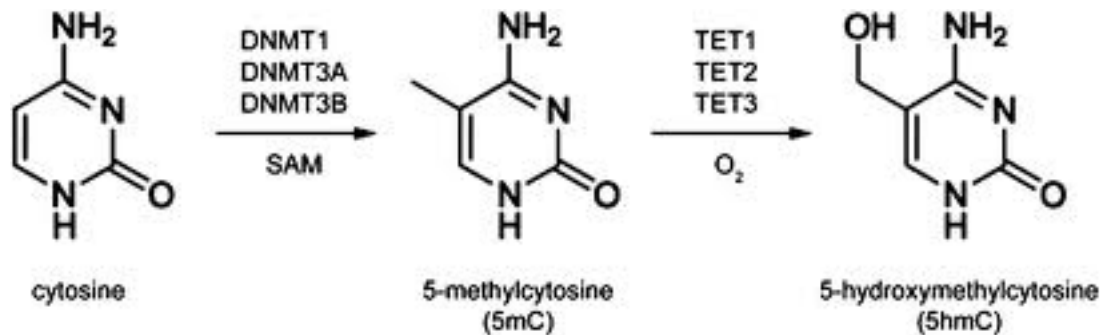
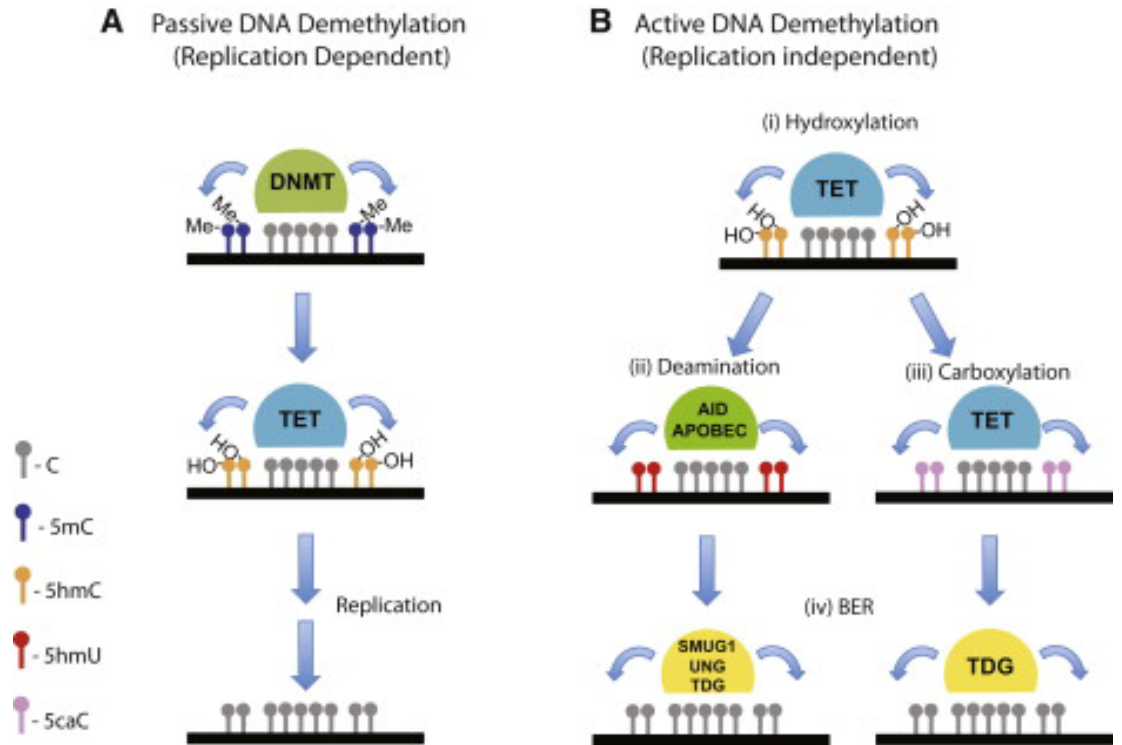
10^{-9}

versus

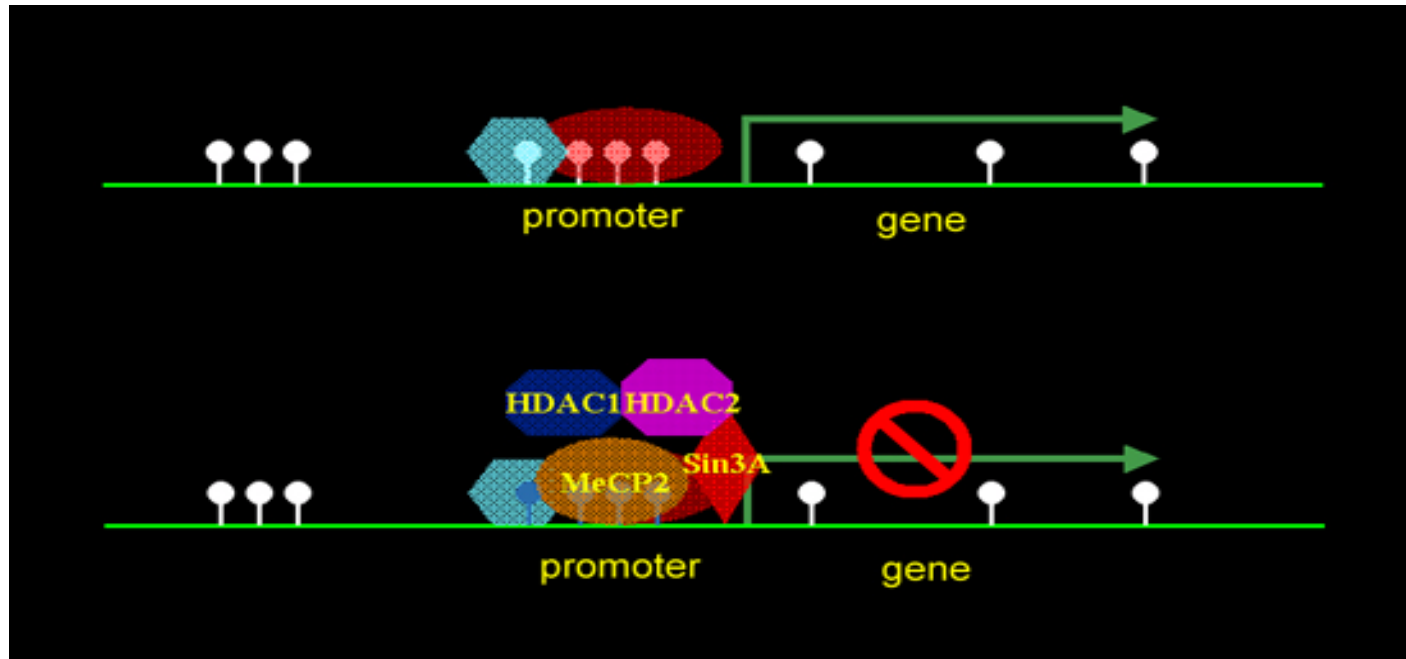
10^{-5}

errors per CpG per division

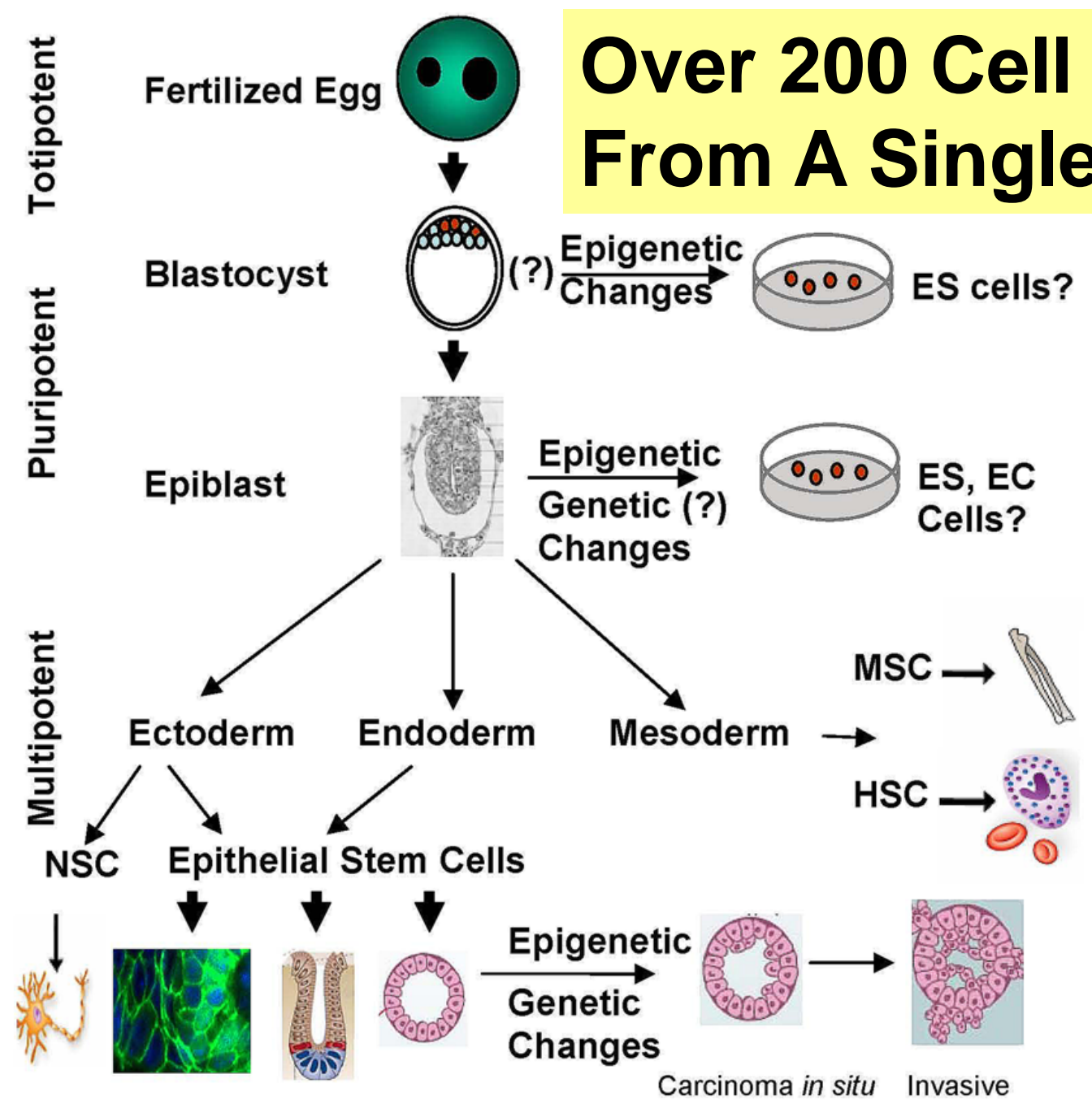
DNA Demethylation: Passive and Active



Promoter CpG Methylation Can Regulate Gene Expression



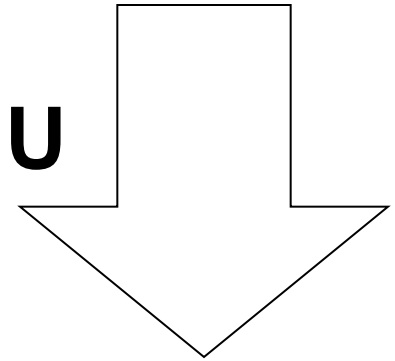
Over 200 Cell Types From A Single Zygote



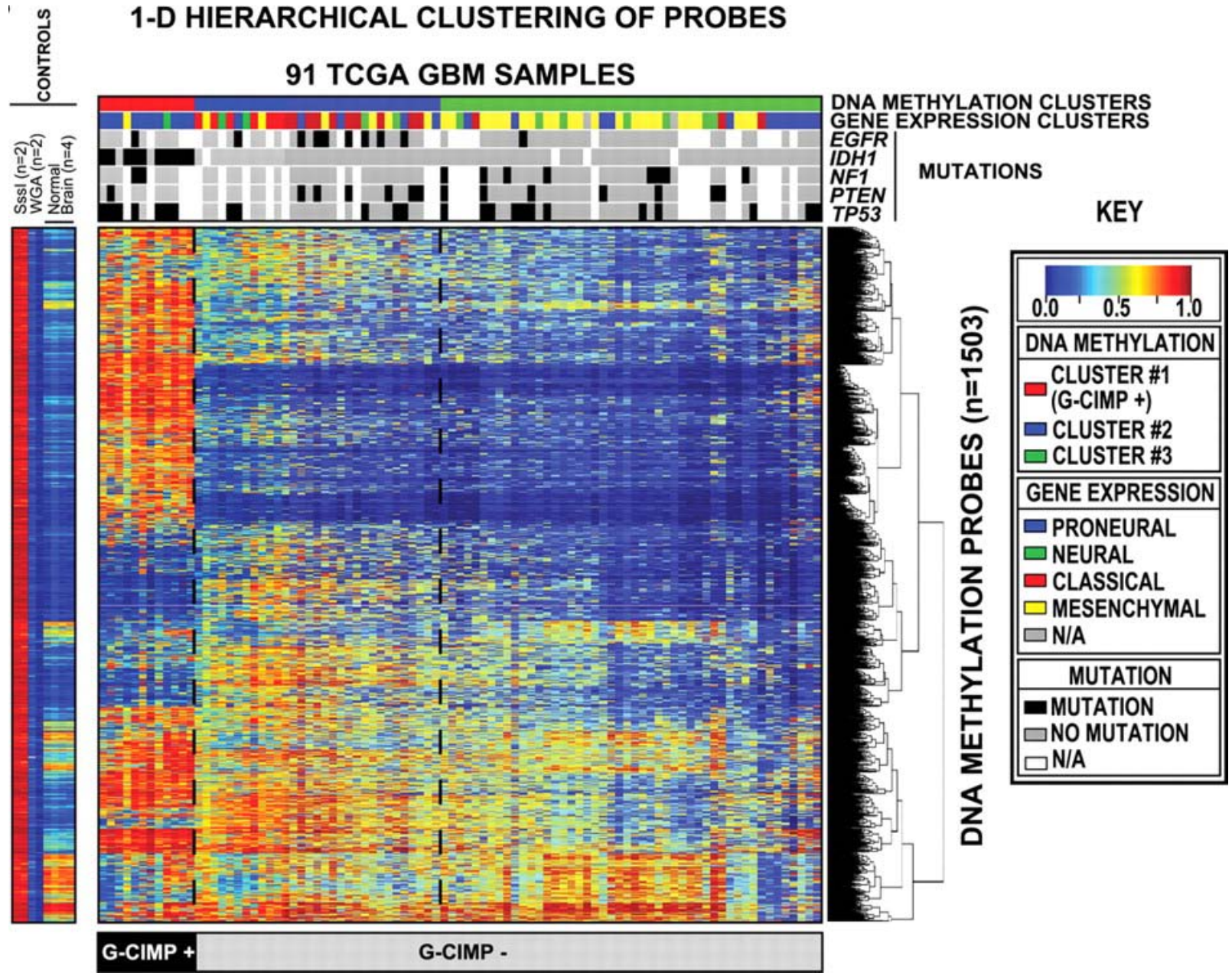
Measuring DNA methylation Bisulfite Sequencing



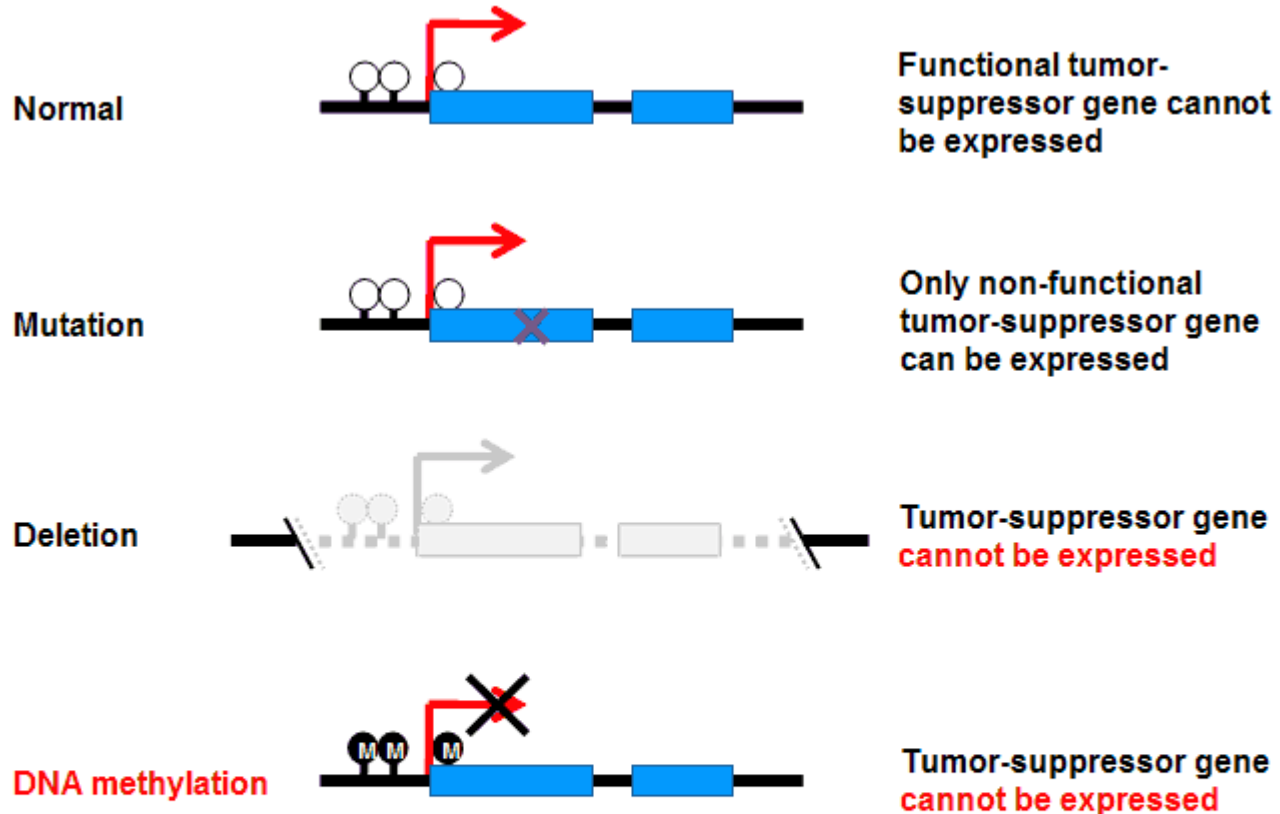
**Sodium Bisulfite Converts C to U
But MeC is not Converted**



DNA Methylation Is Altered In Tumors

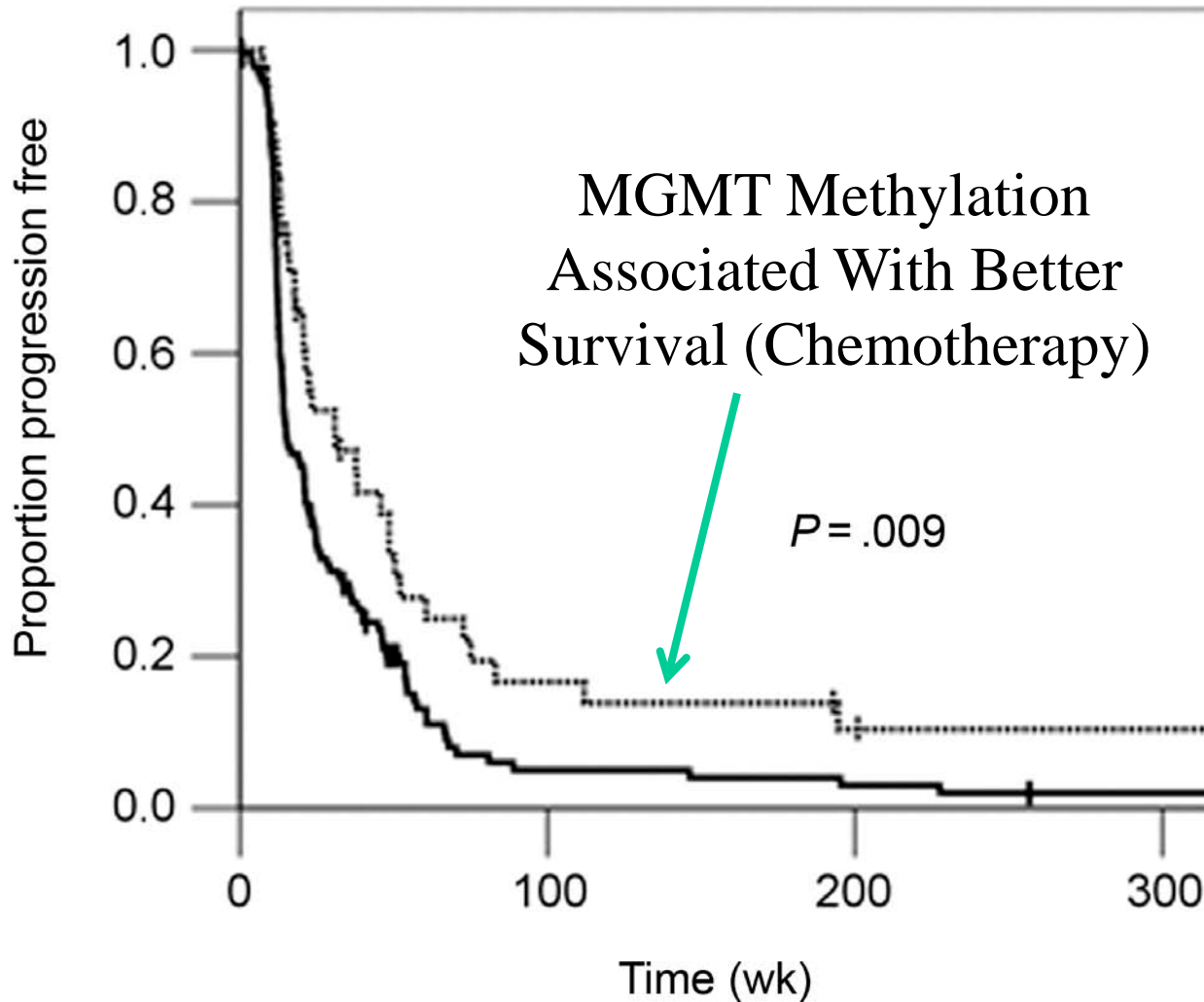


Alternative Methods To Silence Tumor Suppressor Genes



- M** Methylated
- Unmethylated
- X** Mutation

Progression-free survival and MGMT promoter methylation status.



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

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

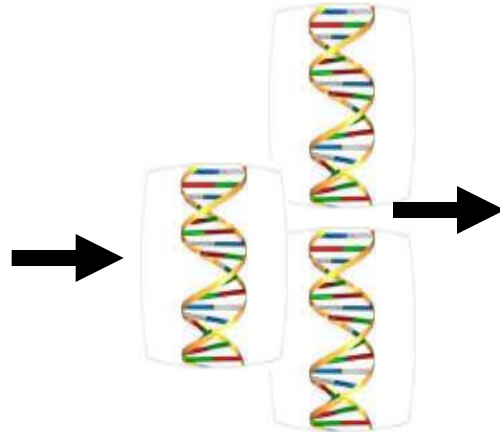
Problem: Difficult To Study Human Somatic Cell Changes

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

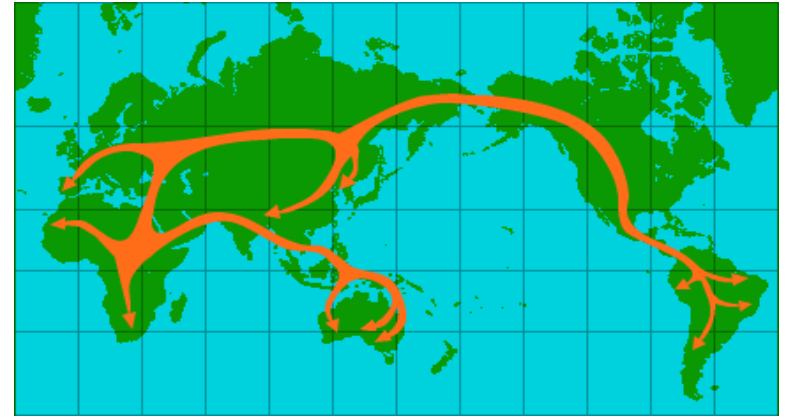
Molecular Clocks: Genomes Record Ancestry



populations



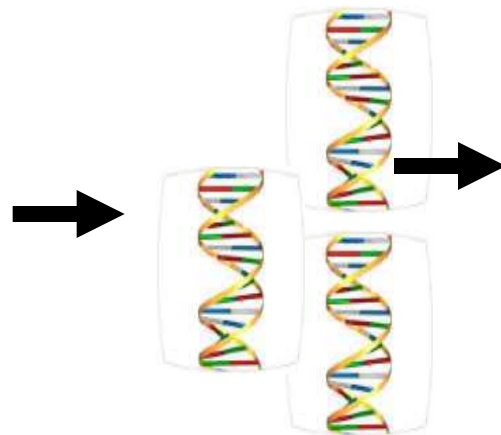
compare genomes



infer ages and
migrations patterns



billions of cells



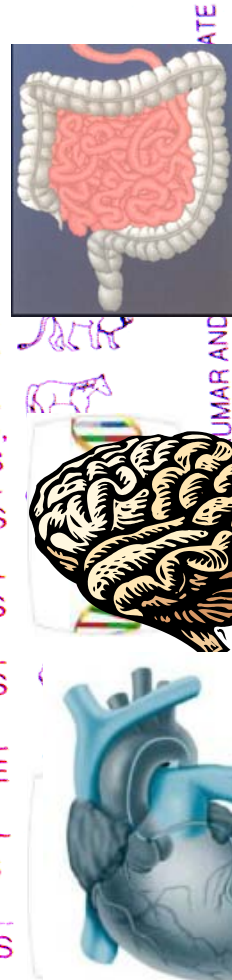
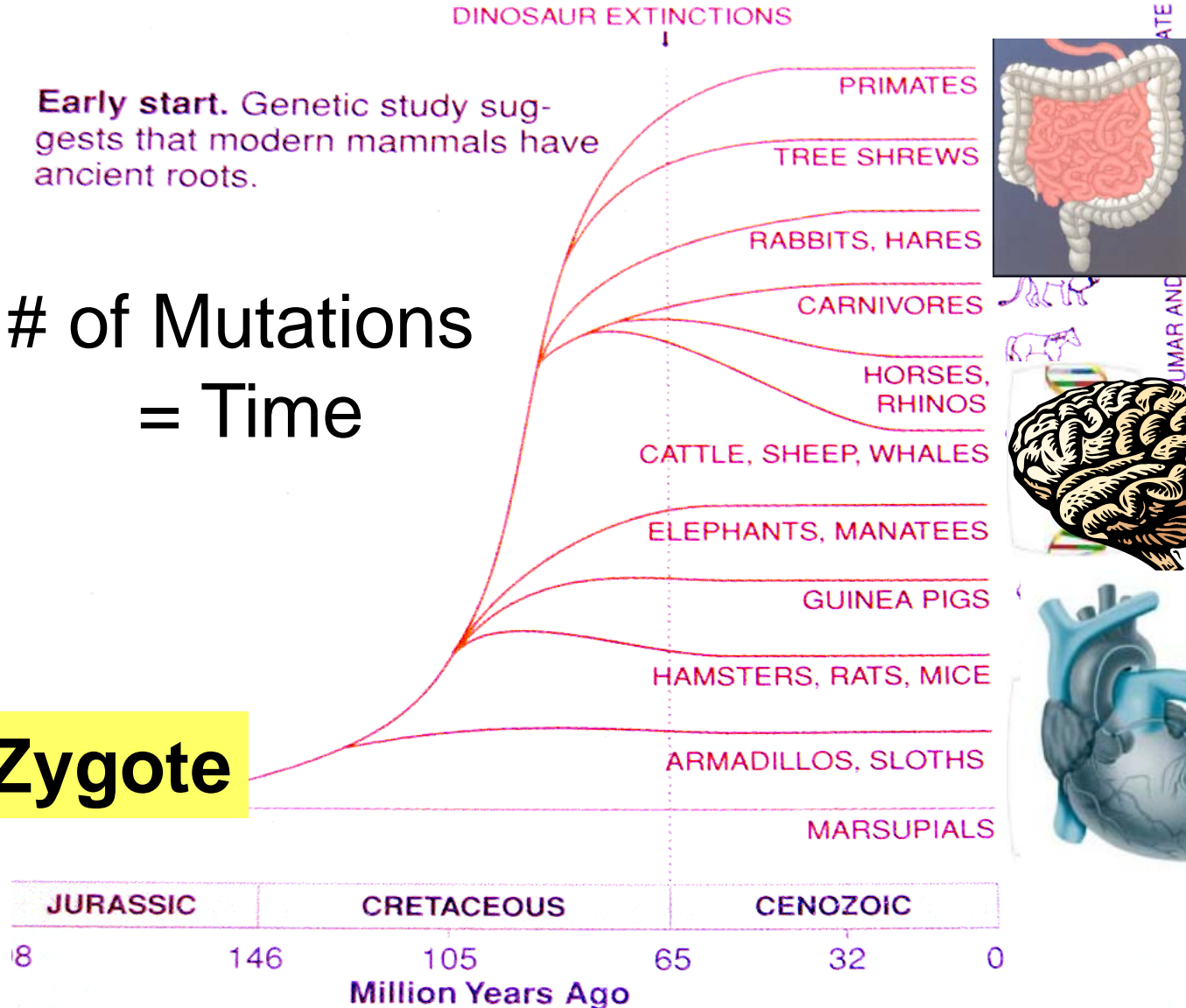
How To Study Biology Passively: Molecular Clocks

DINOSAUR EXTINCTIONS

Early start. Genetic study suggests that modern mammals have ancient roots.

of Mutations
= Time

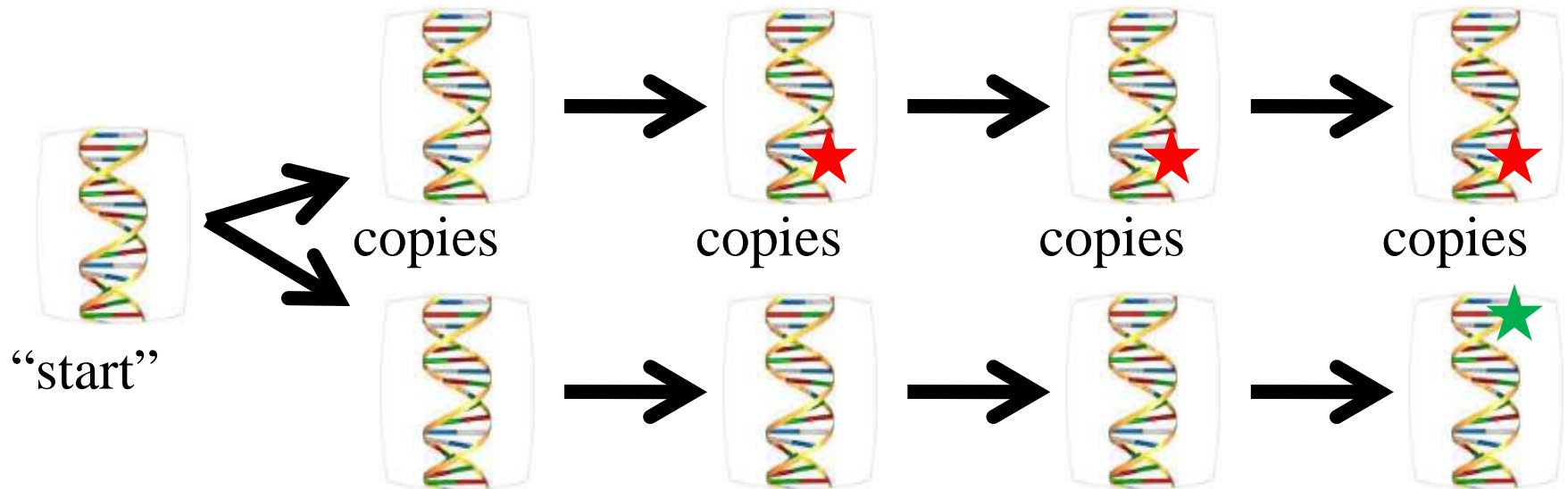
Zygote



Random
Drift Over
Millions
of
Years

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)

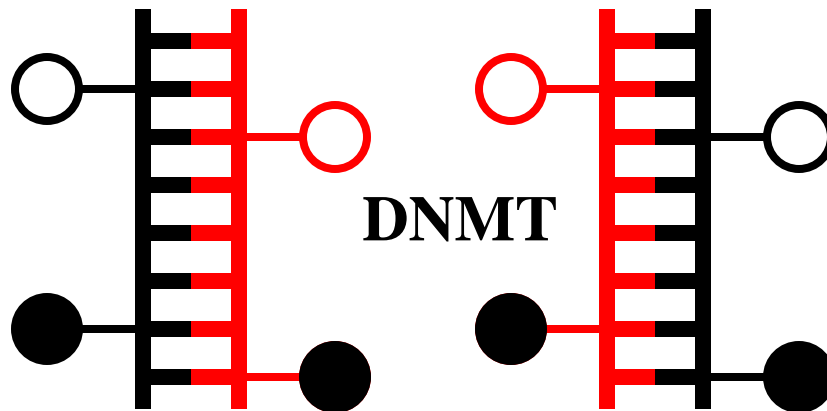
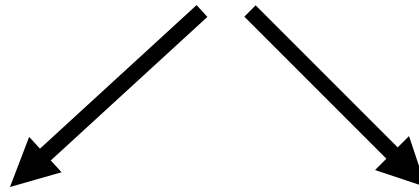
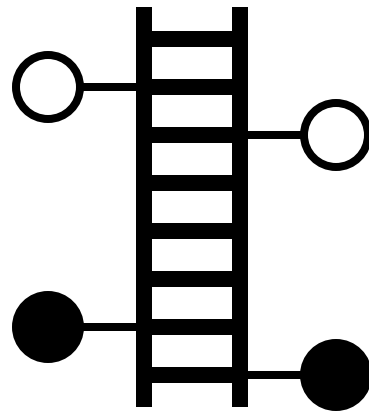
Low PWD (more replication errors) → High PWD

Problems With “Genetic” Somatic Cell Clocks

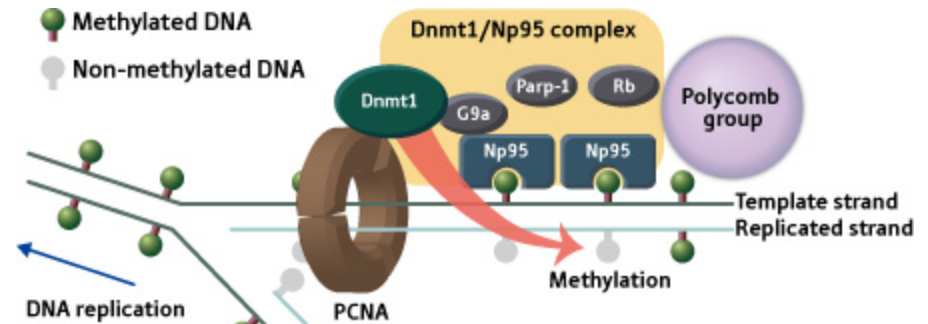
1. Somatic Mutations Are Relatively Rare
(<1 per 100,000 bases even in cancers)
2. Modern High Throughput DNA Sequencers
Have Relatively High Error Rates
(~ 1 per 100 to 1 per 1,000 bases)
3. Can Use High Coverage (10-fold) To Detect
Clonal (Cancer) Mutations But More Difficult
to Detect Subclones (minor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

Potential Solution: Epigenetic Molecular Clock

Replication Clock



Genome Replication



Epigenetic Fidelity
is less than
Genetic Fidelity

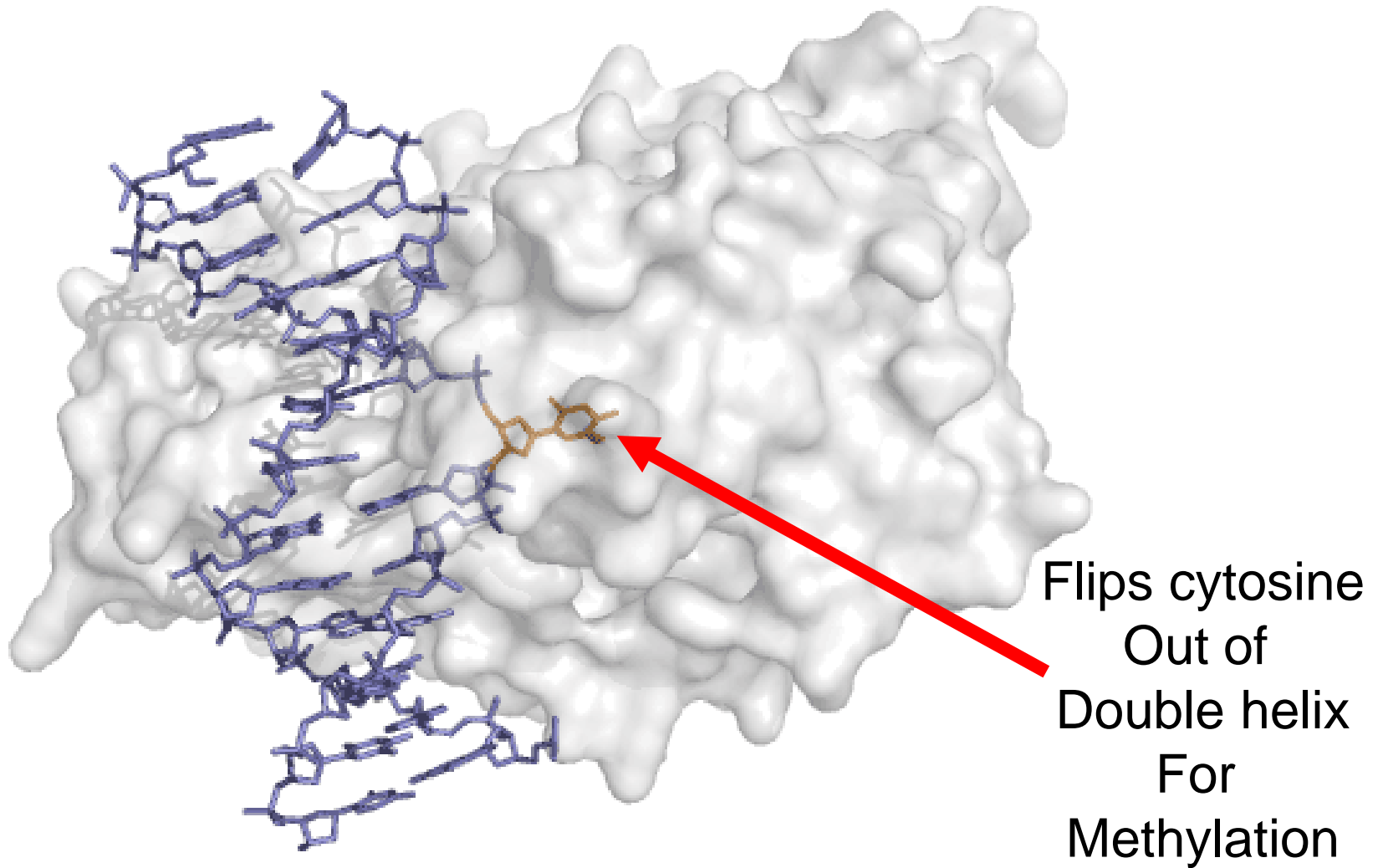
10^{-9}

versus

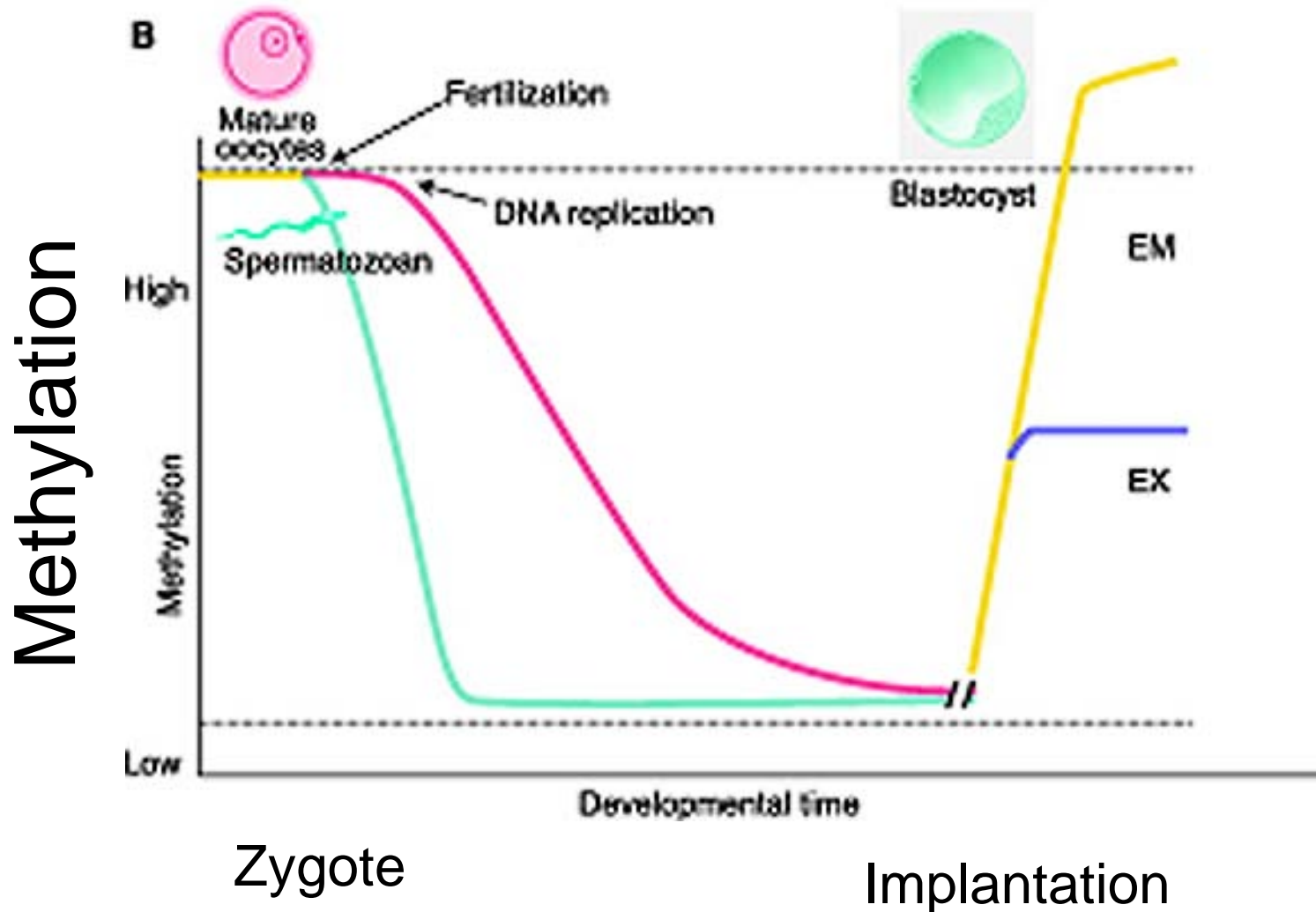
10^{-5}

errors per CpG per division

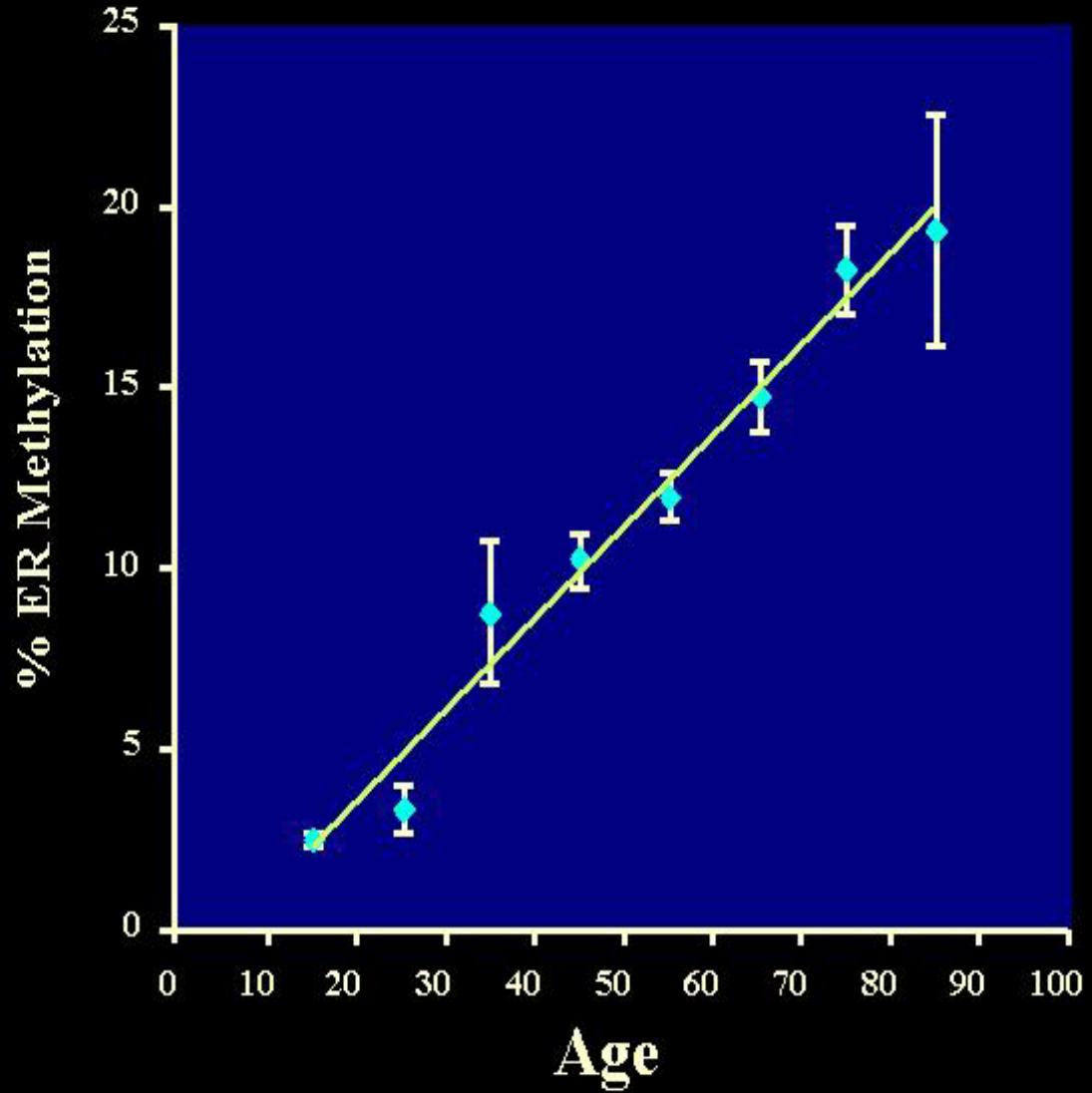
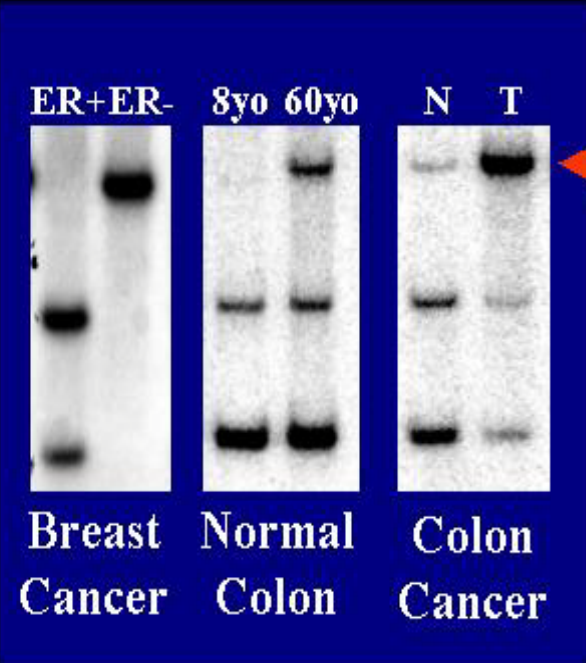
DNA Methyltransferase



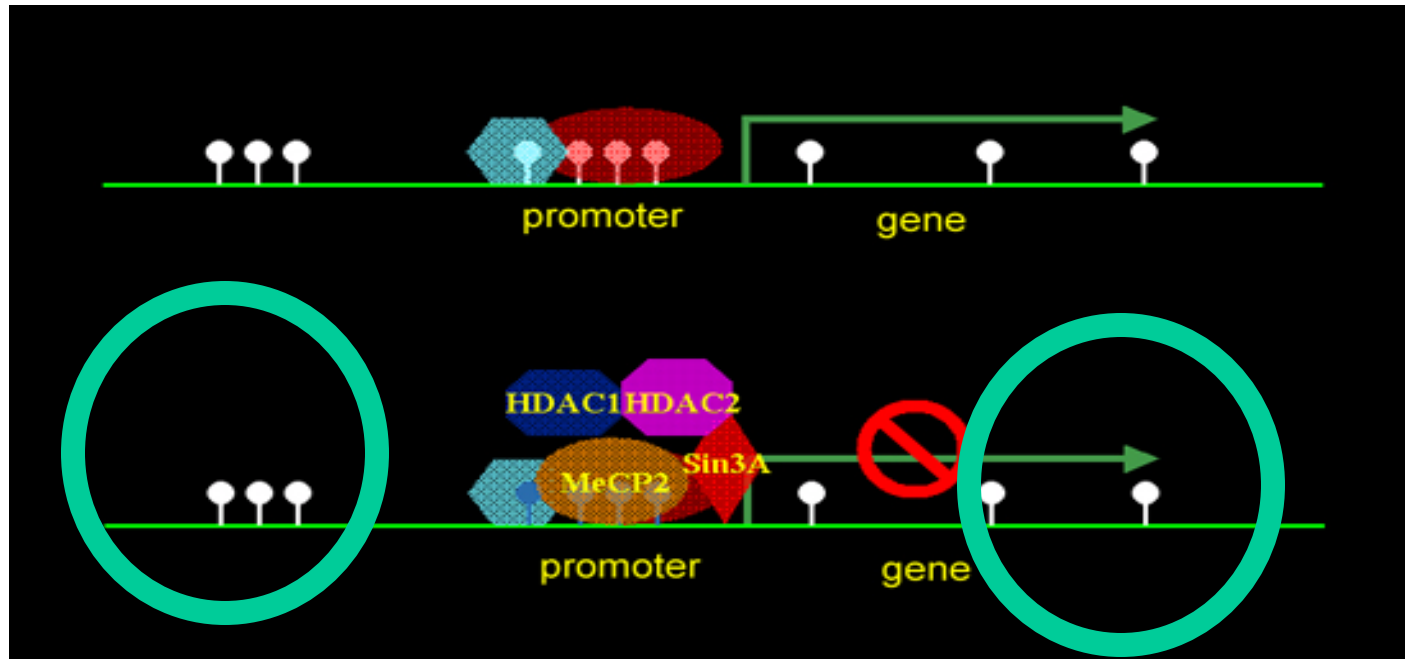
DNA is Demethylated Early in Development: Epigenetic “Clocks” Start Unmethylated



ER Methylation and Age in Normal Colon

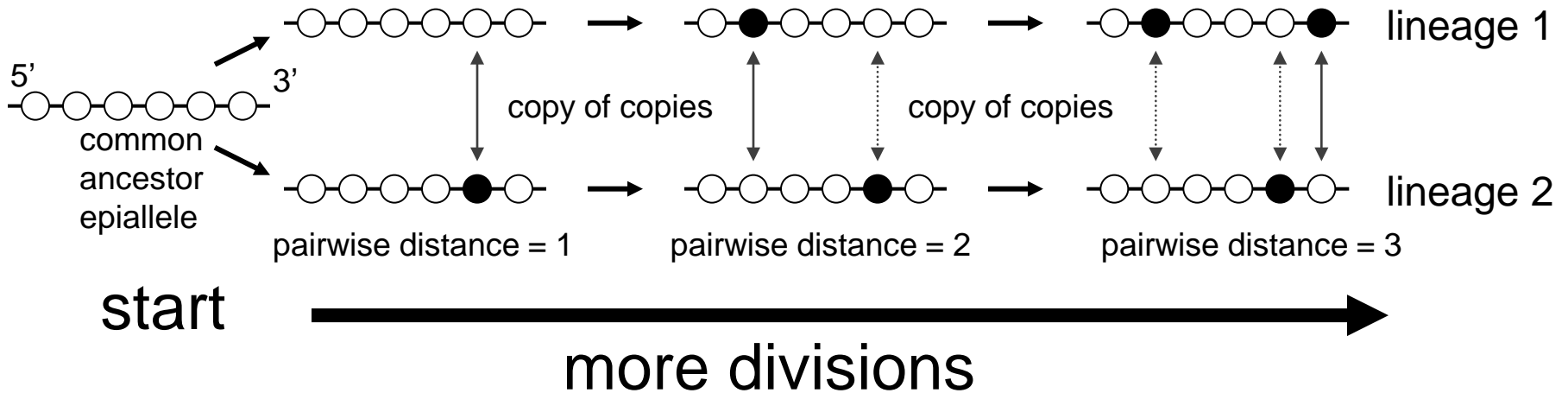


Promoter CpG Methylation Can Regulate Gene Expression



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)

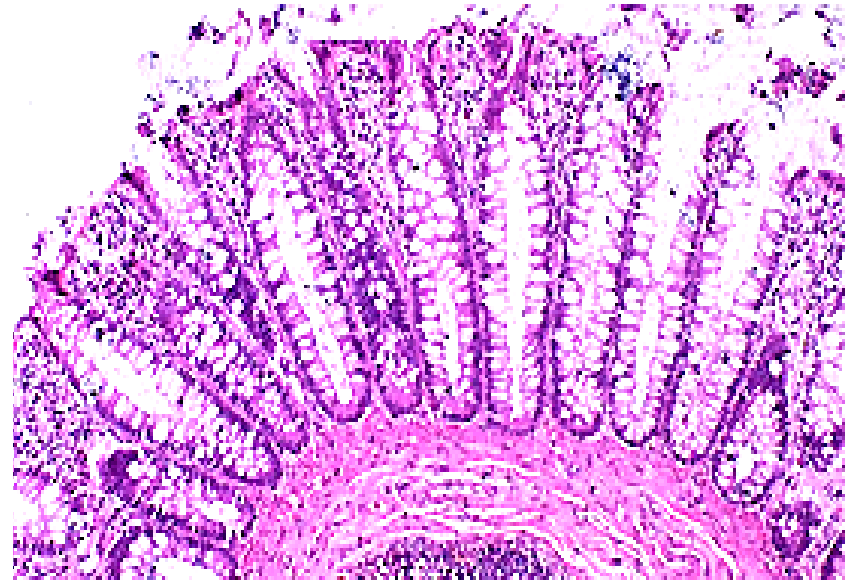
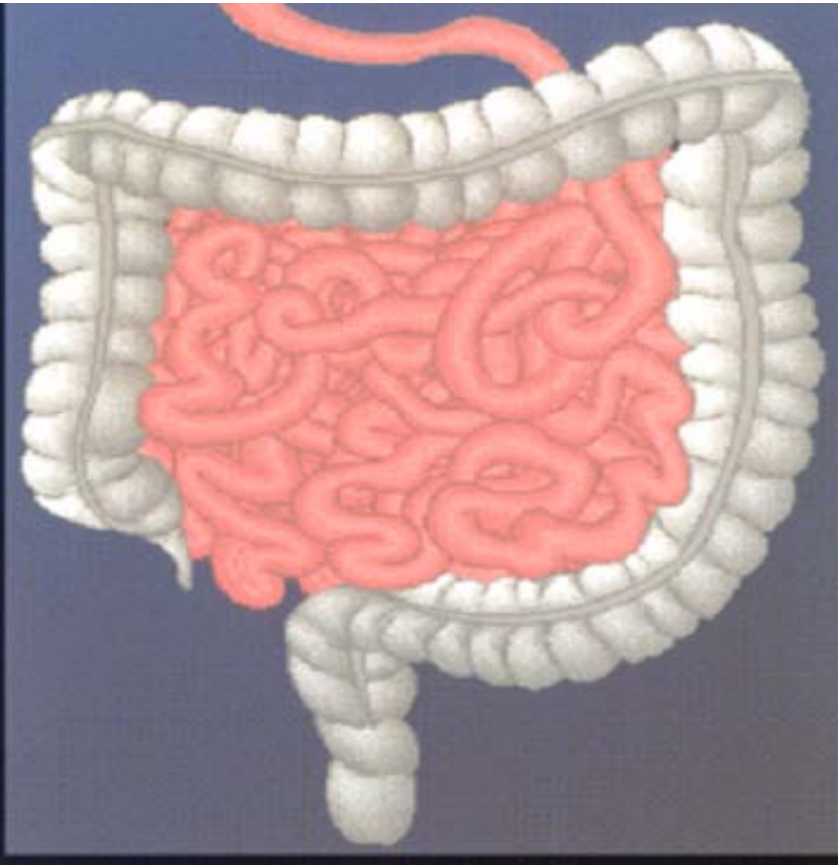


- Passenger Epigenetic Clocks on X-chromosome
(not expressed in colon)
- Samples From Male Patients
(single epiallele per cell)

BGN "clock" (9 CpG sites, Chr Xq28)

TTTaggagtgagtagTtgTttCGgtTCGTCGgaTaTaTCGgaTa
 gatagaCGtgCGgaCGgTTTaTTaTTTTagTTCGTTaaTtagt
 TagTTtgCGTTtggCGTTtTTTTtTTTaggtagggTtggT

Example: Human Colon Crypts



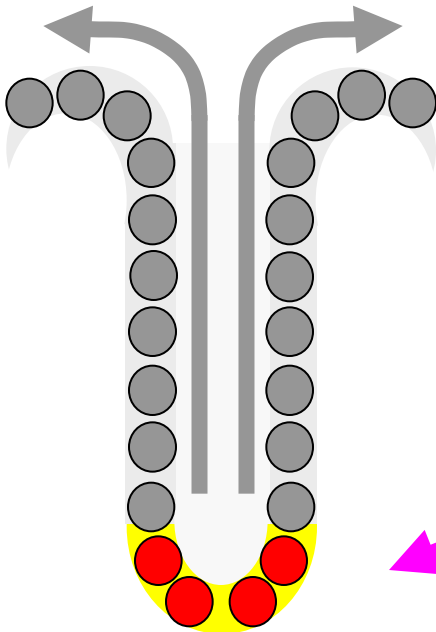
- about 15 million crypts
- multiple stem cells per crypt
- potential to divide everyday

5% Lifetime Risk of Cancer by Age 100 Years

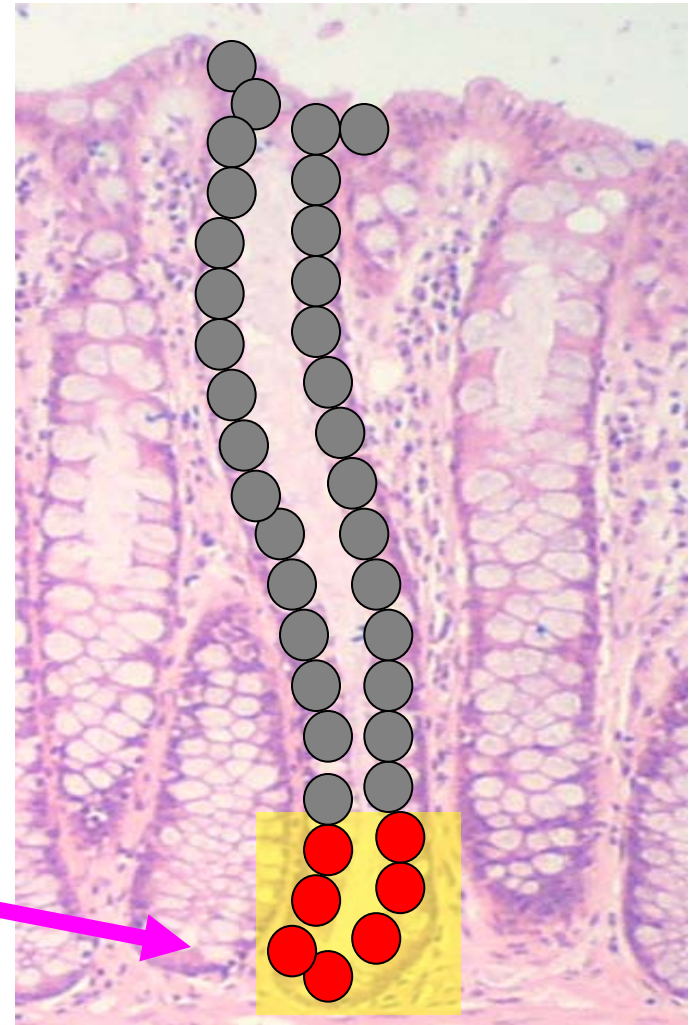
Human Colon Crypts: Mitotic With Constant Cell Replacement

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

die in 1 week

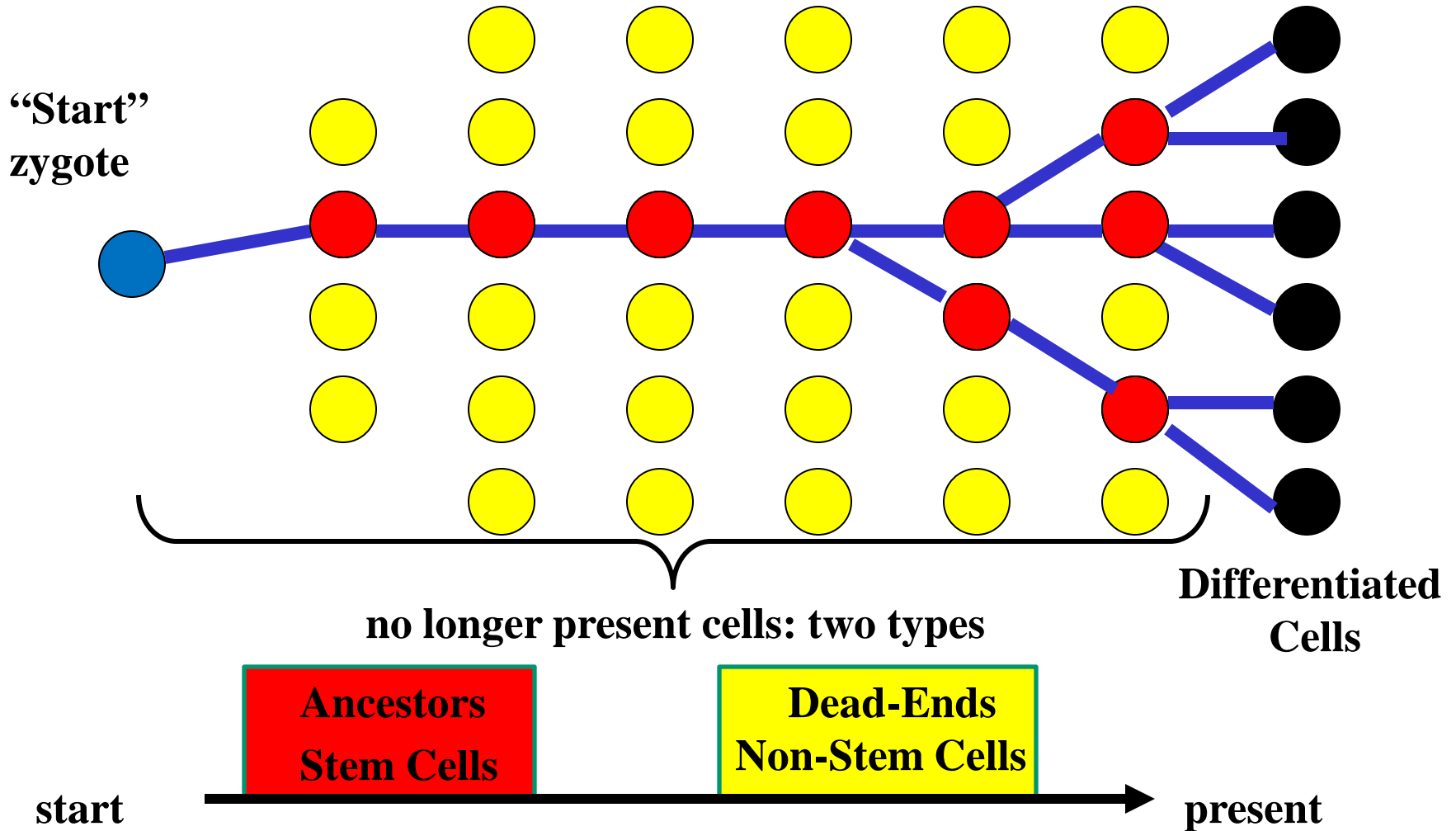


multiple
stem cells



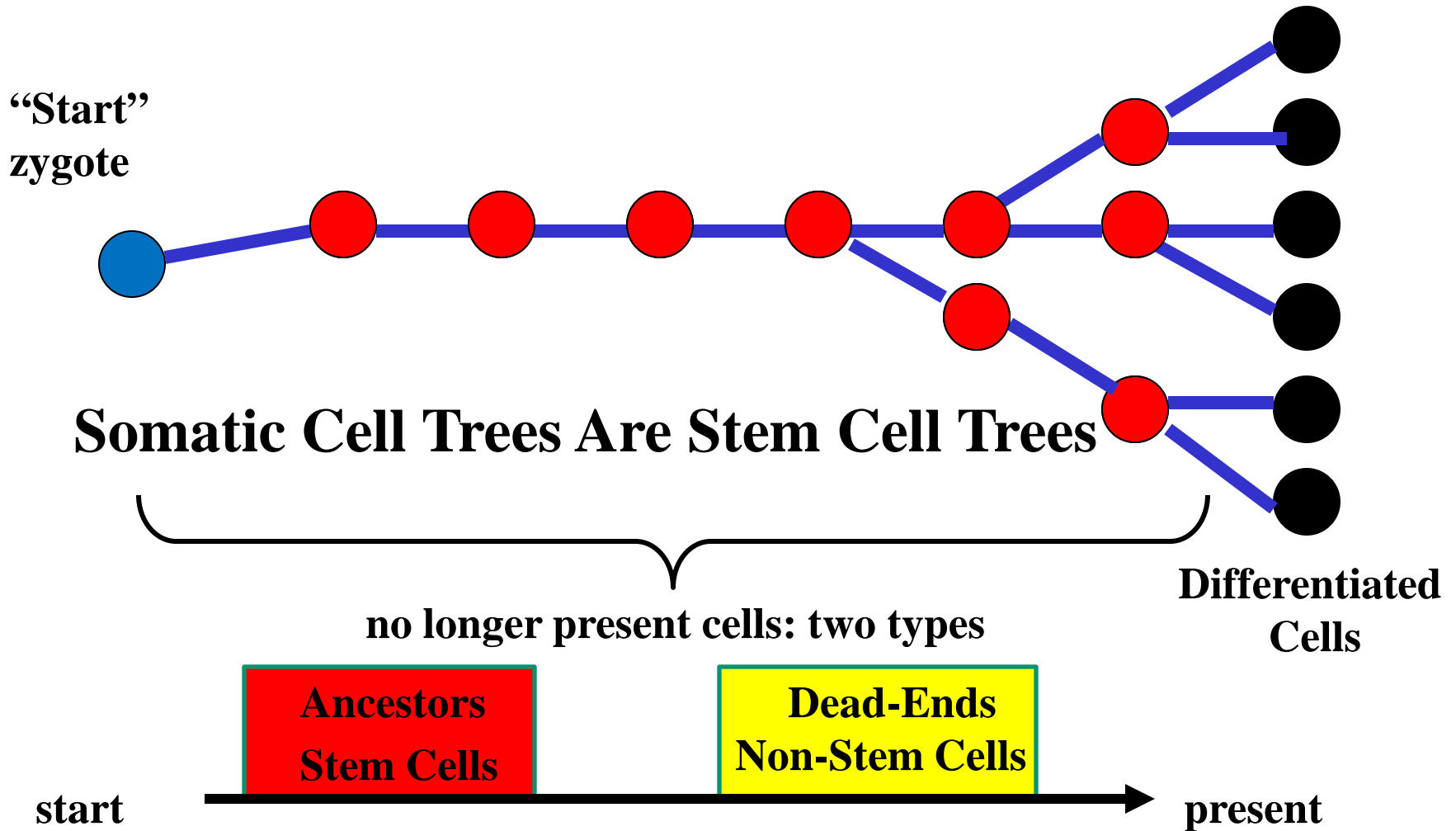
Human Somatic Cell Ancestral Trees

(just 4 kinds of cells)

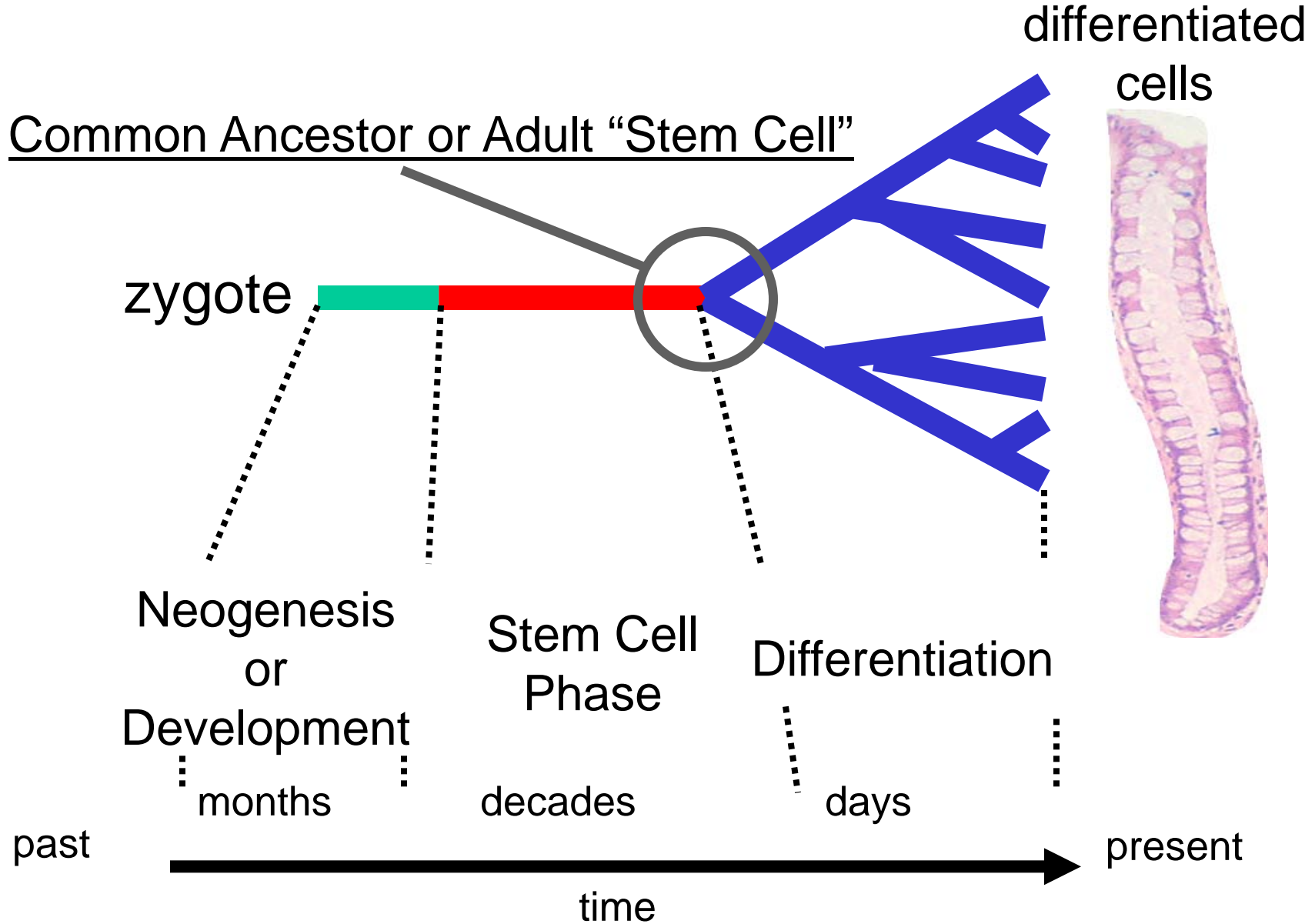


Human Somatic Cell Ancestral Trees

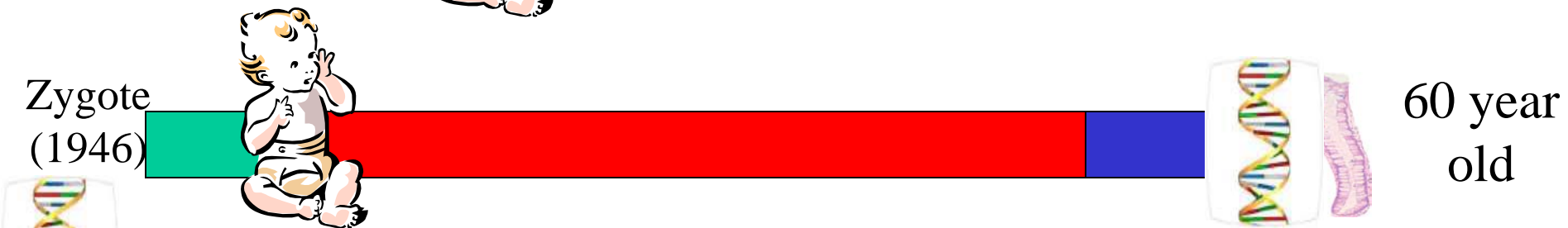
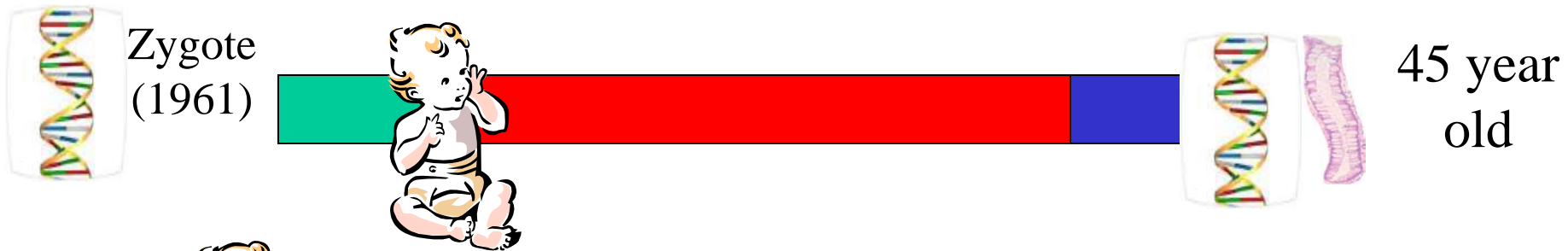
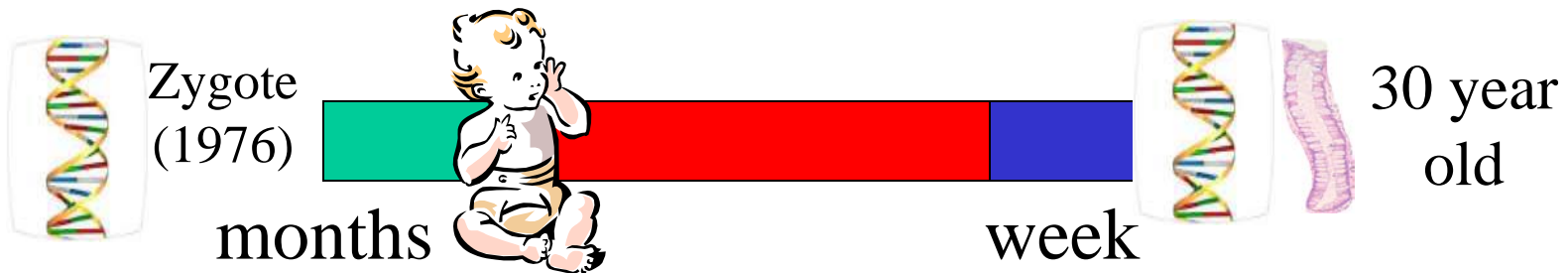
(just 4 kinds of cells)



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

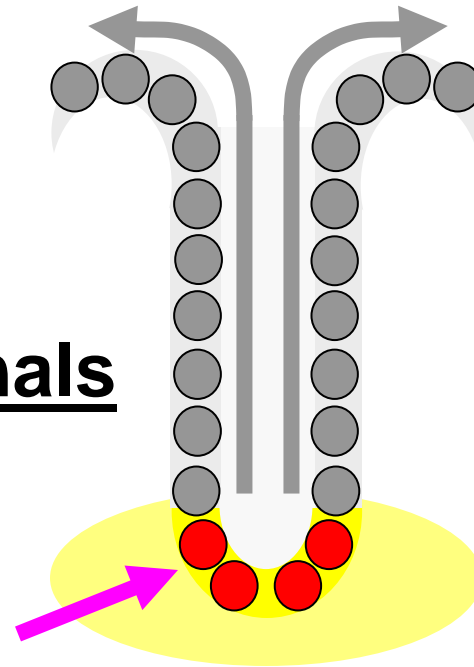
- 1) Immortal Stem Cell Lineages (Intrinsic)
- 2) Stem Cell Niches (Two Components)
 - Epithelial Stem Cells
 - Surrounding Stroma (Extrinsic Signals)

Stroma Niche Signals

Wnt Pathway?

TGFR II Pathway?

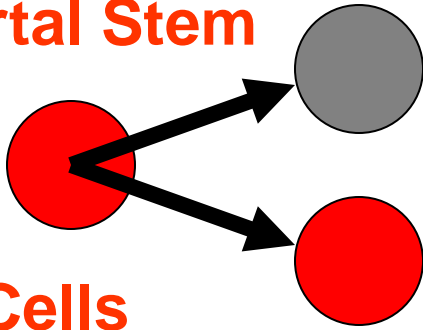
COX2 Pathway?



Cells That Leave A Niche Are No Longer Stem Cells

Types of Cell Division: (Always Binary)

Immortal Stem Cells



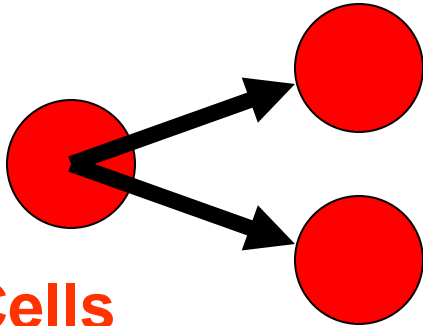
Differentiated Cell

Asymmetric Replacement

Niche Stem Cells

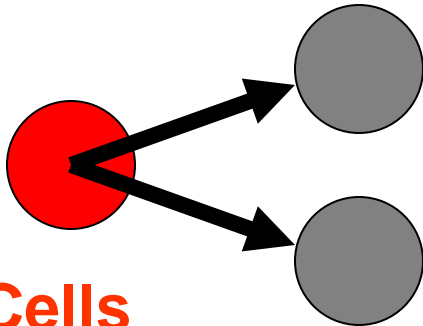
Stem Cell

Niche Stem Cells



Symmetric Expansion

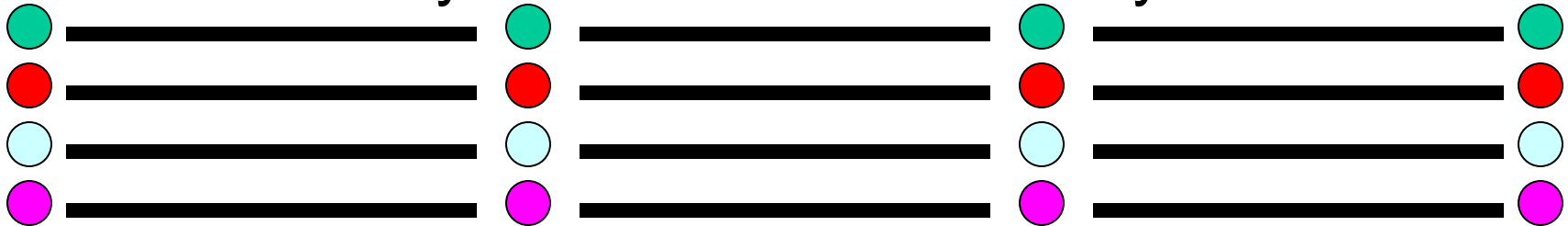
Niche Stem Cells



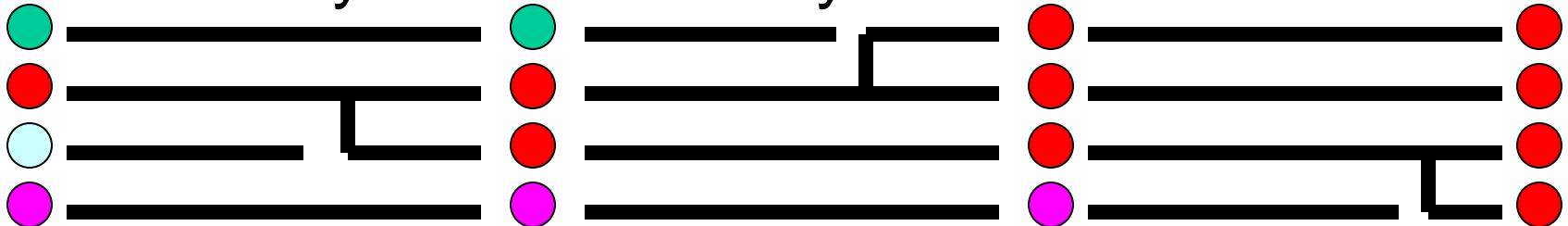
Symmetric Extinction

Immortal Vrs Niche Stem Cell Lineages

Immortal: Asymmetric Divisions Only

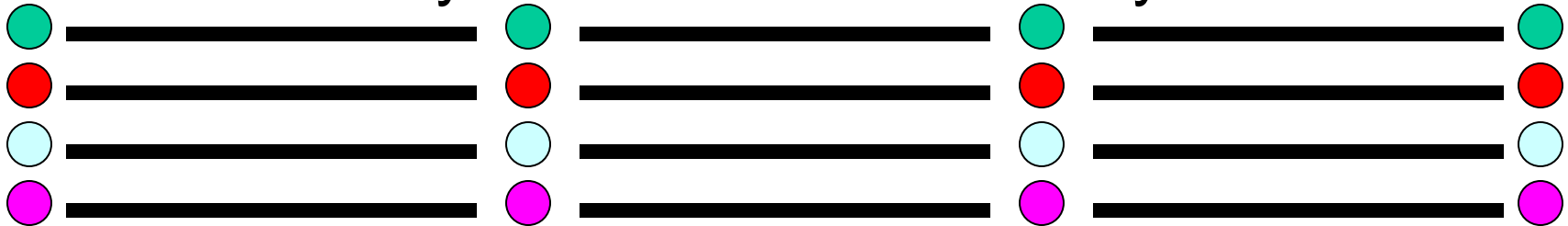


Niche: Asymmetric And Symmetric Divisions

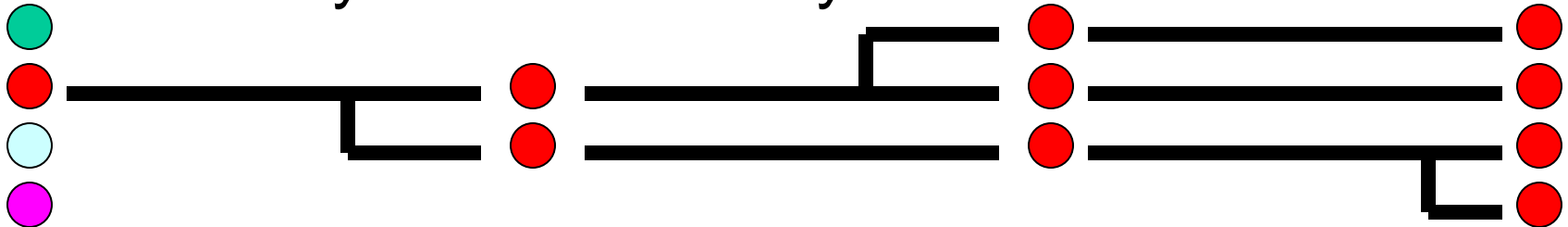


Immortal Vrs Niche Stem Cell Lineages

Immortal: Asymmetric Divisions Only



Niche: Asymmetric And Symmetric Divisions



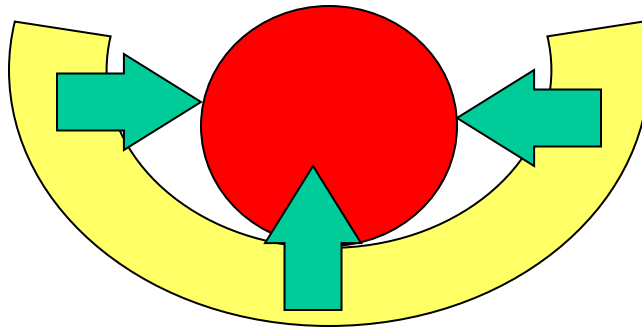
**Appears All Mammalian Stem Cells
Are Maintained By Niches
(Stem Cell Clonal Evolution)**

Niche Stem Cells

A Niche Has Two Parts:

- Mesenchymal
- Epithelial “Stem Cells”

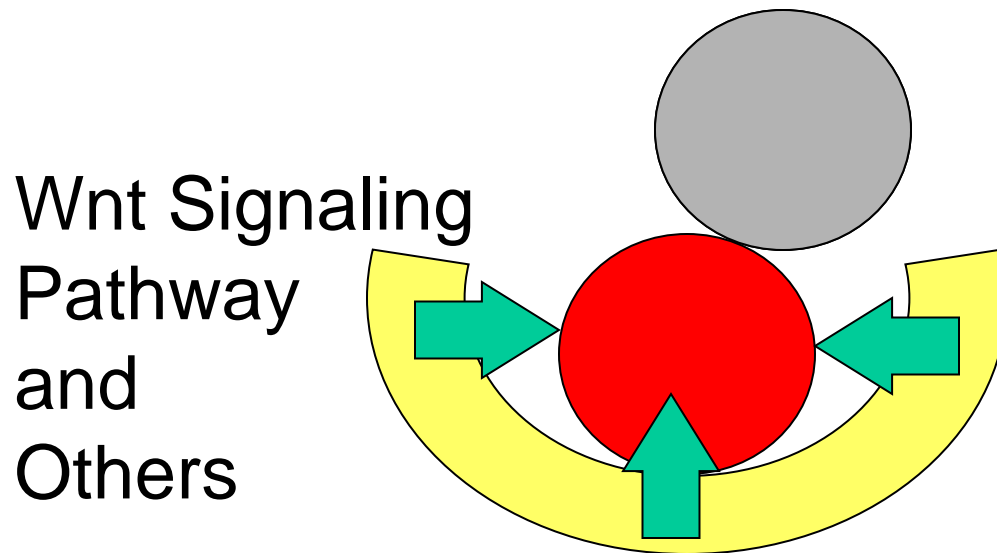
**WNT
And
Other
Factors**



Niche Stem Cells

NICHE STEM CELLS

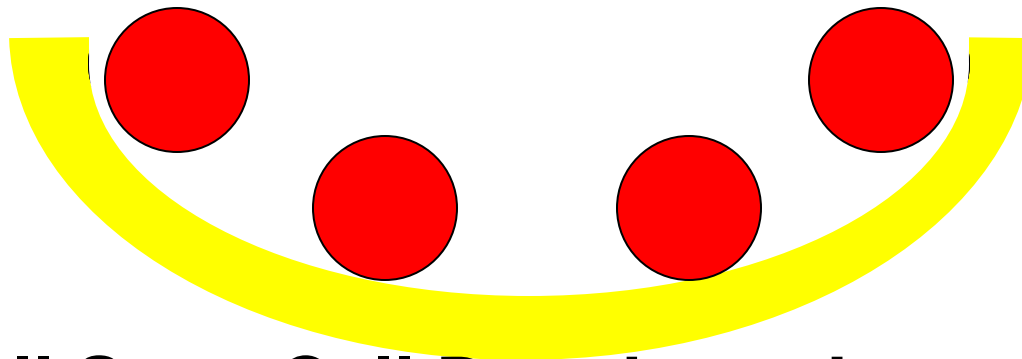
- 1) Extrinsically Defined
- 2) Will Differentiate Outside of Niche



Stem Cell Niche Dynamics

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

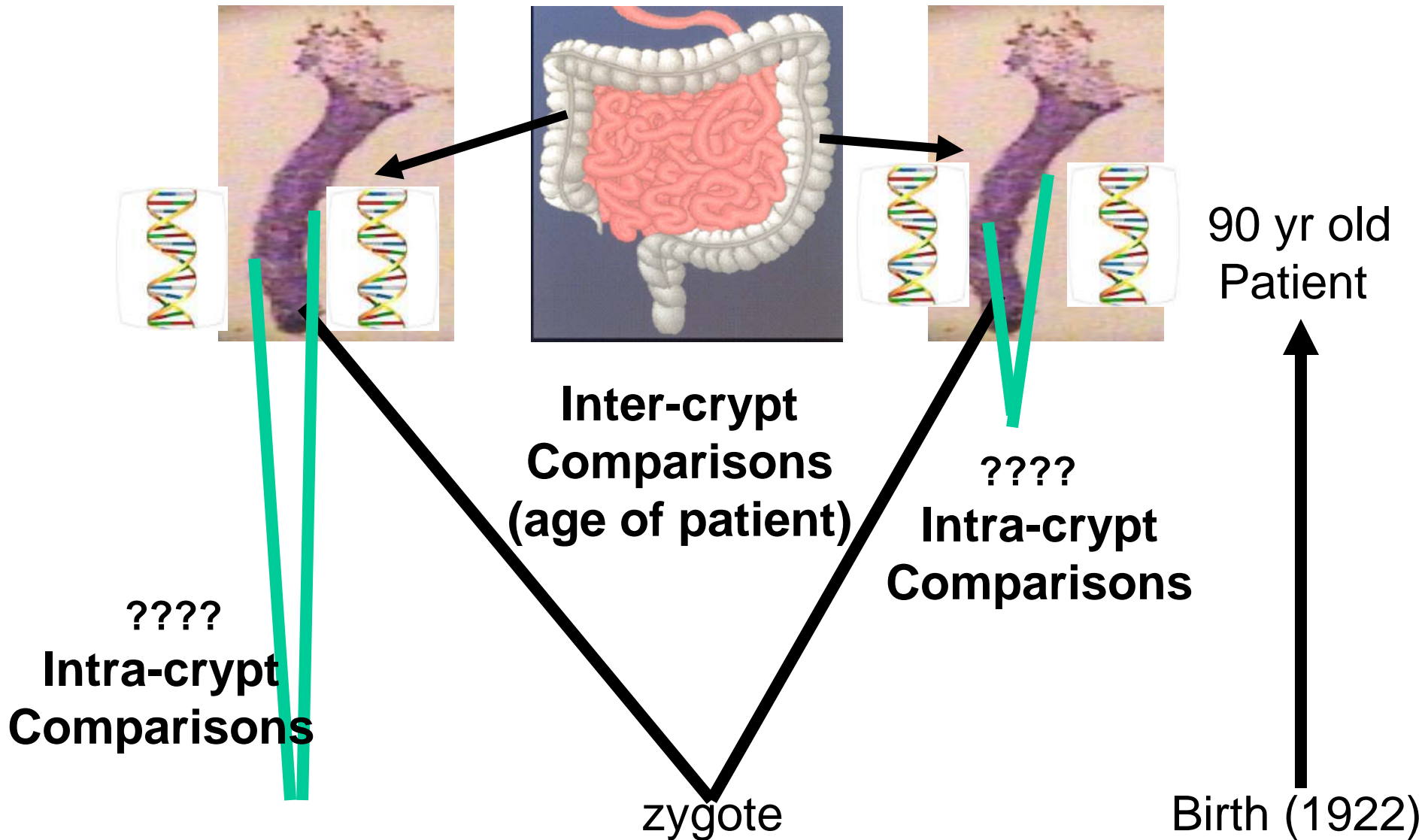
Loss of All Lineages Except One = Stem Cell Clonal Evolution



Half of All Stem Cell Daughters Leave The Niche

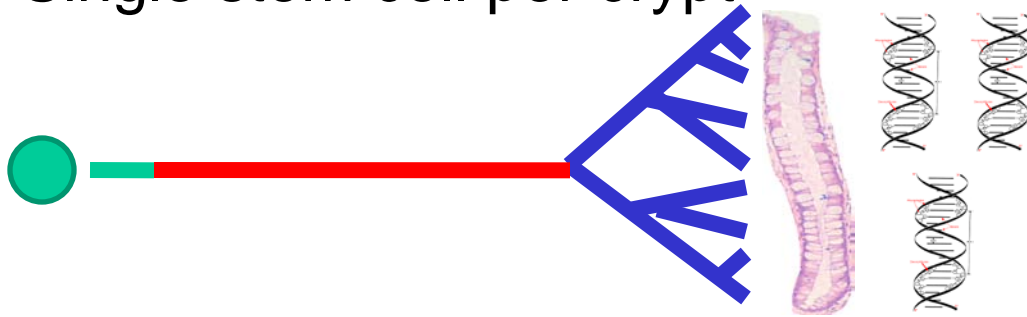
Experimental Strategy:

Characterizing Human Crypt Stem Cells From Their Genomes

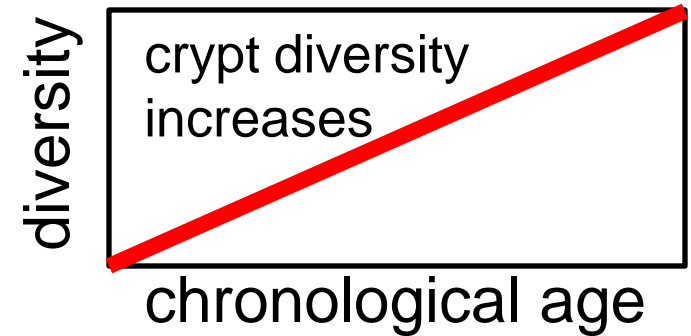
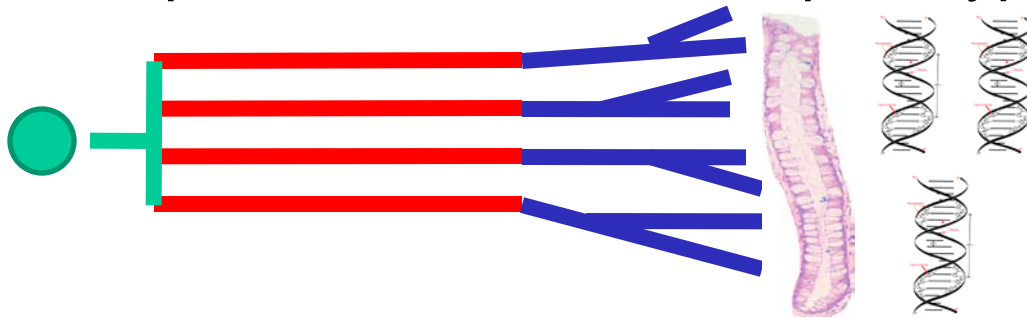


Distinguishing Between Crypt Stem Cell Models

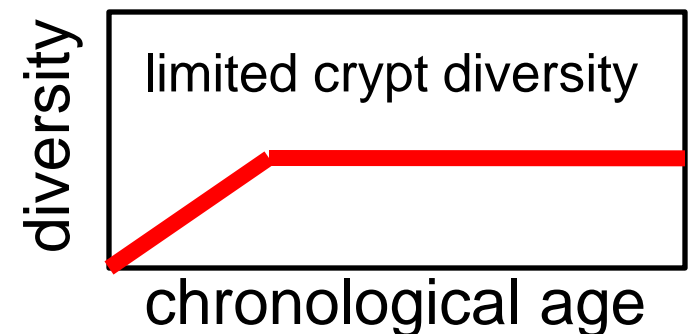
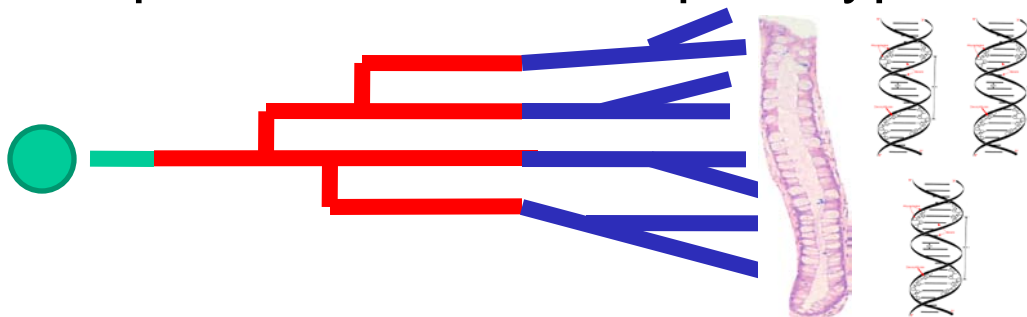
Single stem cell per crypt



Multiple immortal stem cell per crypt



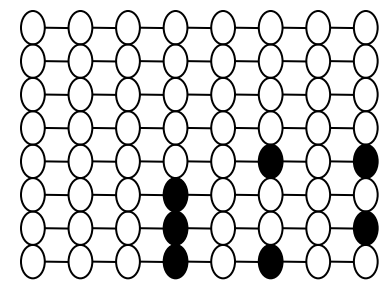
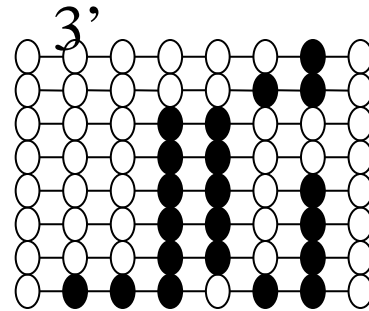
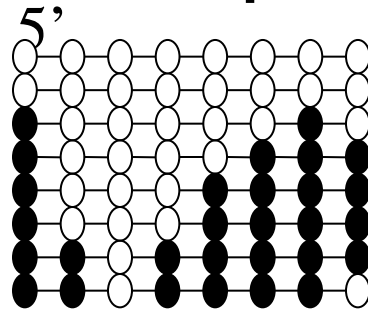
Multiple niche stem cell per crypt



Experimental Plan

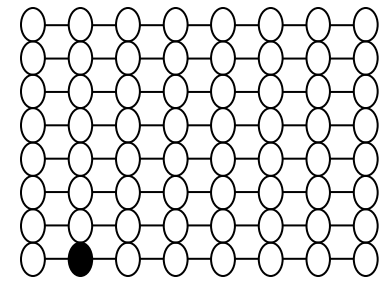
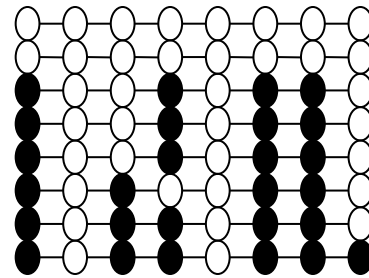
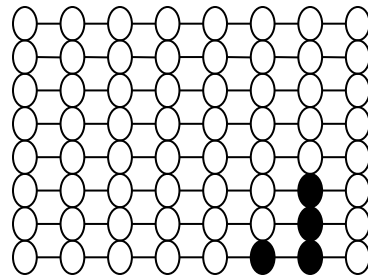
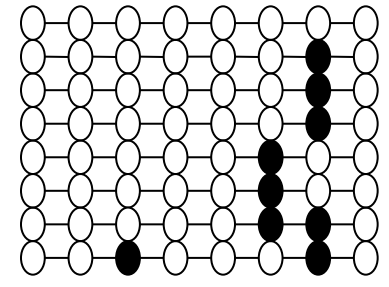
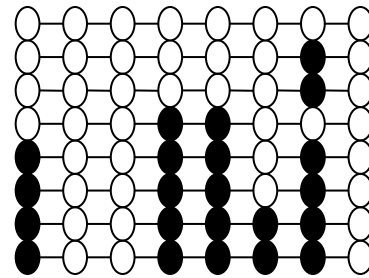
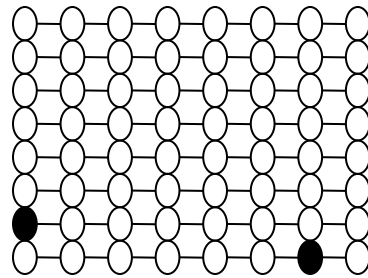
- Colectomy
- Take 1-2 CM² of Normal Mucosa
- Isolate Single Crypts (2,000 cells)
- Isolate DNA
- Bisulfite Treat
- PCR, Clone, Sequence Clones
- MYOD1, CSX, BGN
(X-chromosome)

Random Replication Errors



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Stem Cell Niches vrs Immortal Stem Cells?

Intra-crypt Comparisons

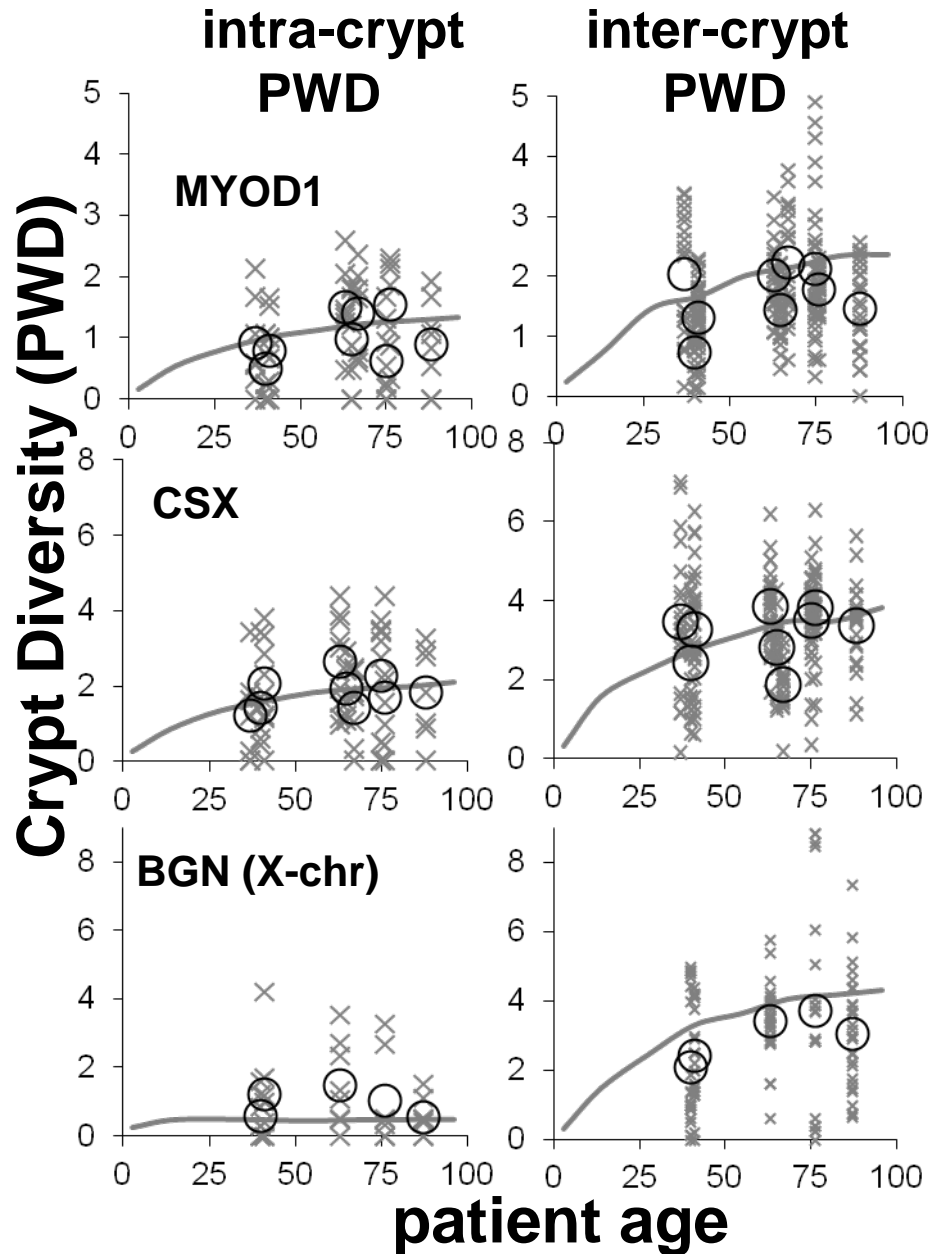
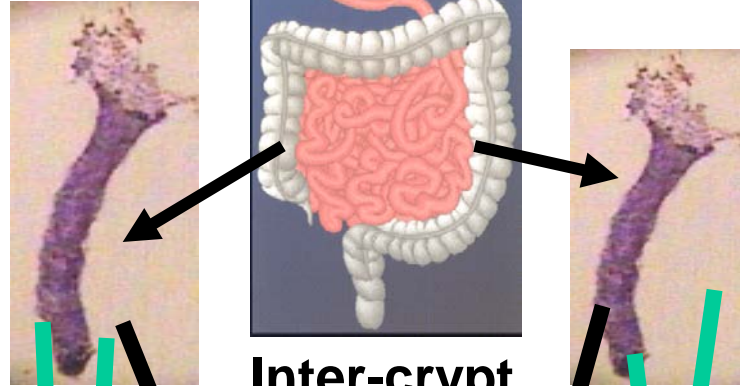
Intra-crypt Comparisons

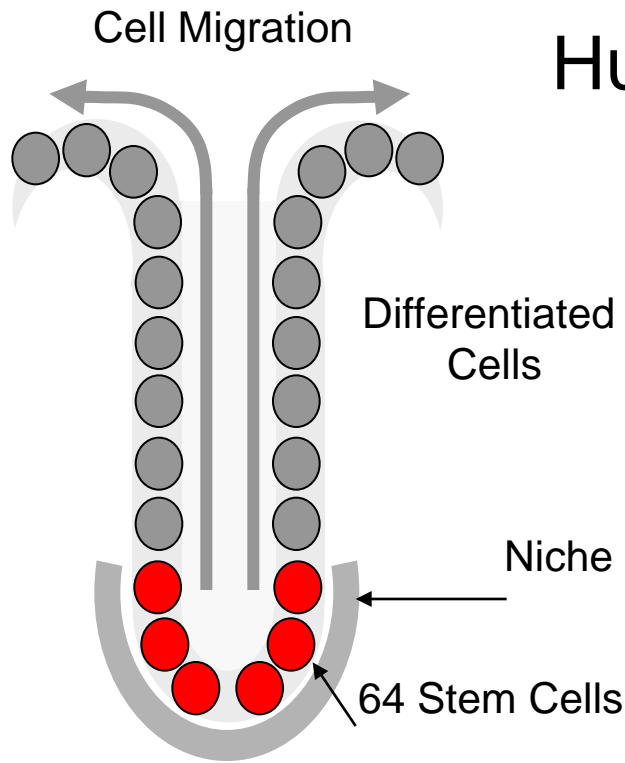
**Inter-crypt Comparisons
(age of patient)**

**Immortal
Stem Cells**

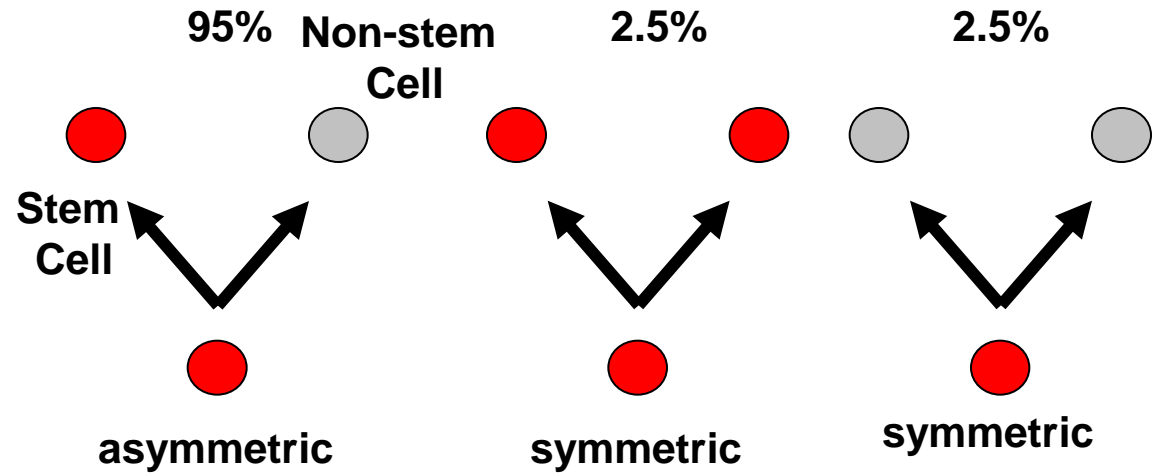
zygote

**Stem
Cell
Niche**

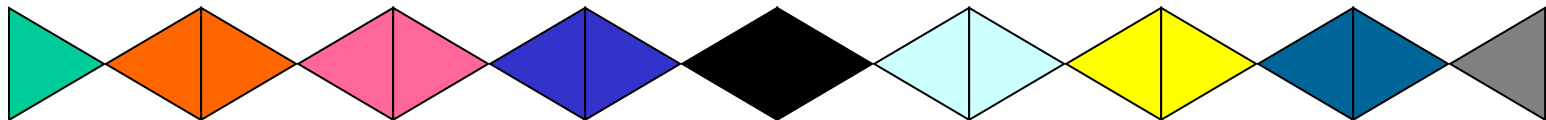




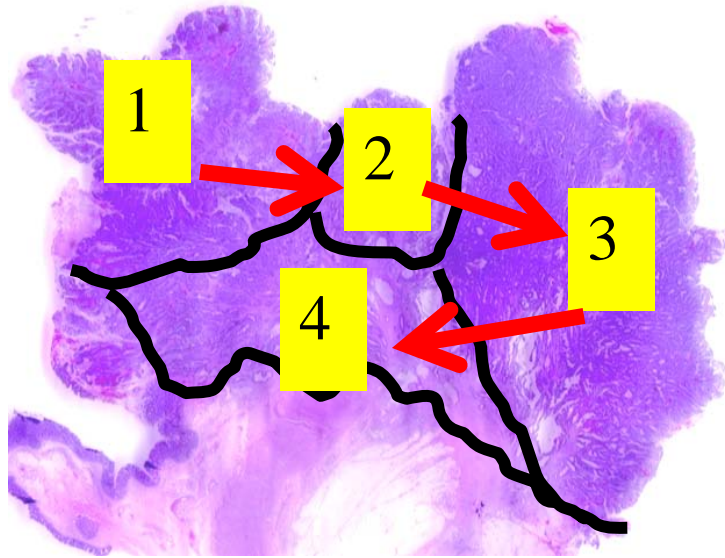
Human Colon Crypt Niche: Summary



- 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

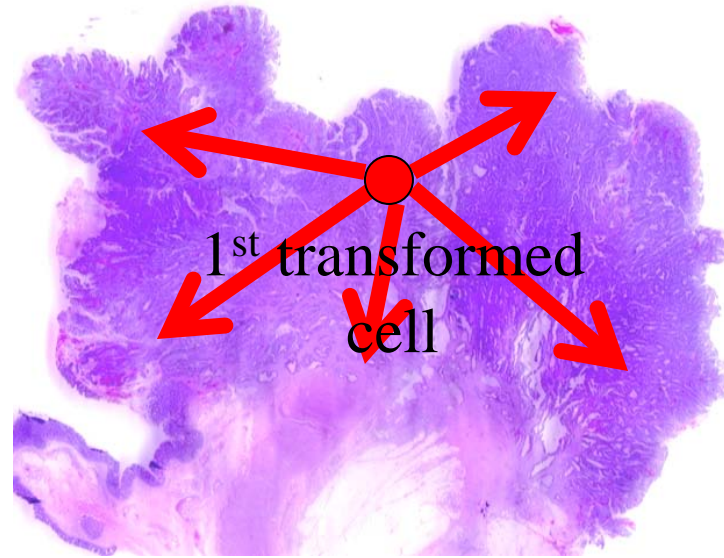


A Problem: The Evolution of Any Individual Human Cancer is Unknown



Sequential or
Clonal Evolution

Older Parts More Diverse



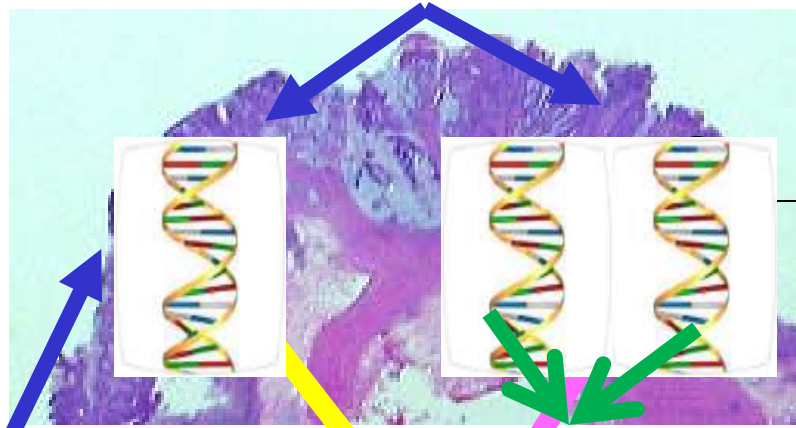
“Big Bang” (full malignant
potential at transformation)

Uniform Diversity

Basic Cancer Ancestry Reconstruction

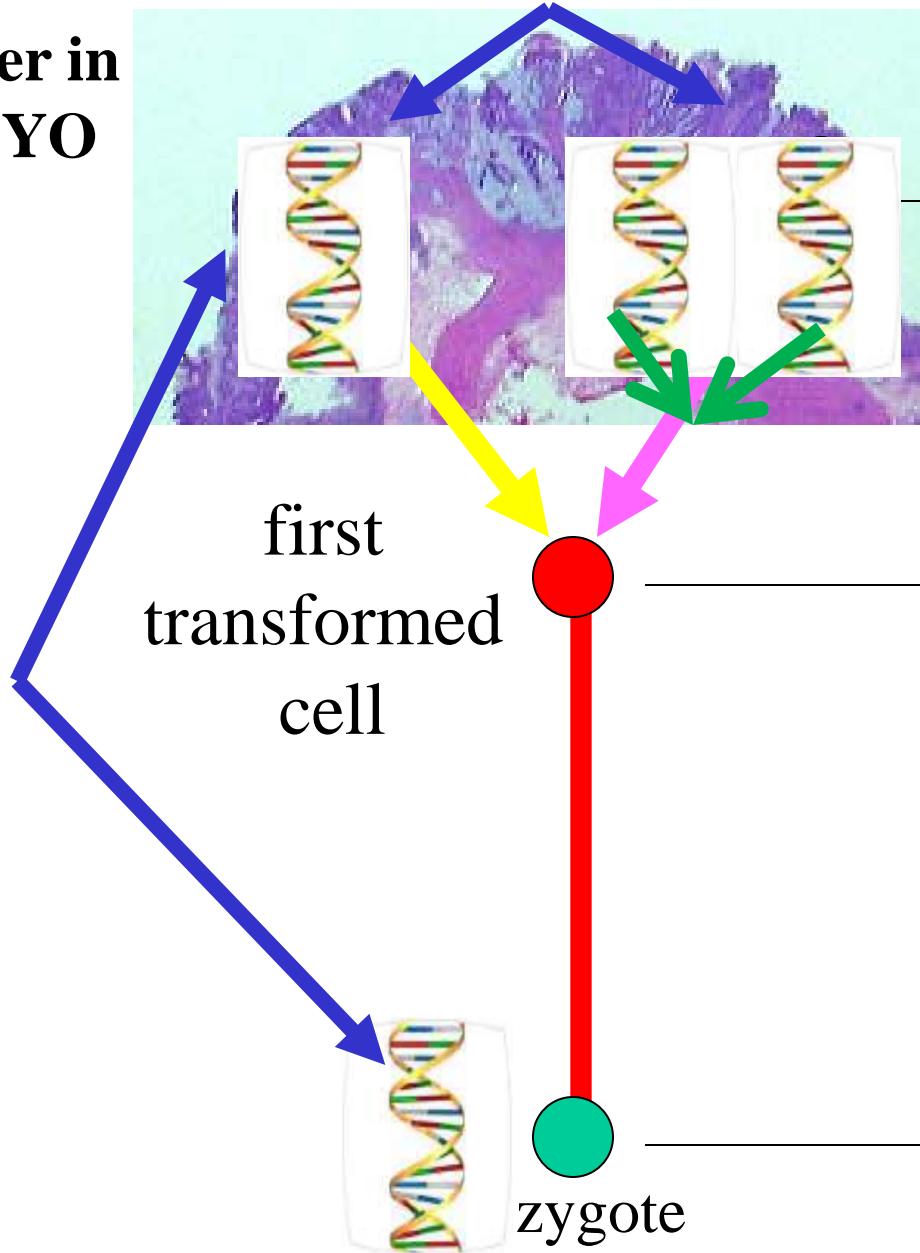
2011

Cancer in
a 70 YO



Back In Time

1941 (birth)

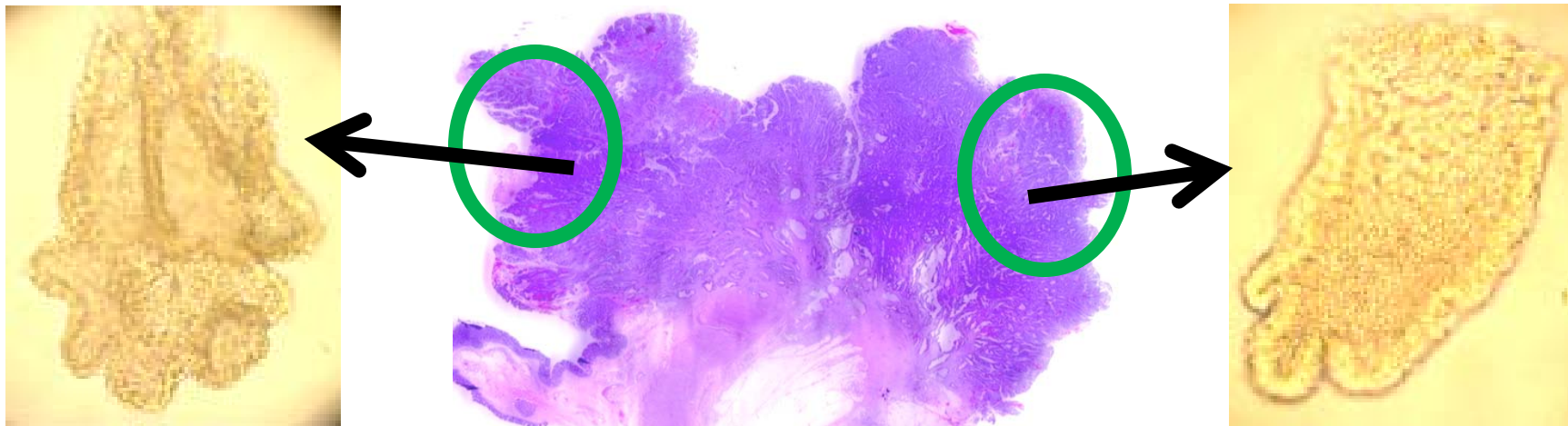


tumor
mitotic
age

gland
age

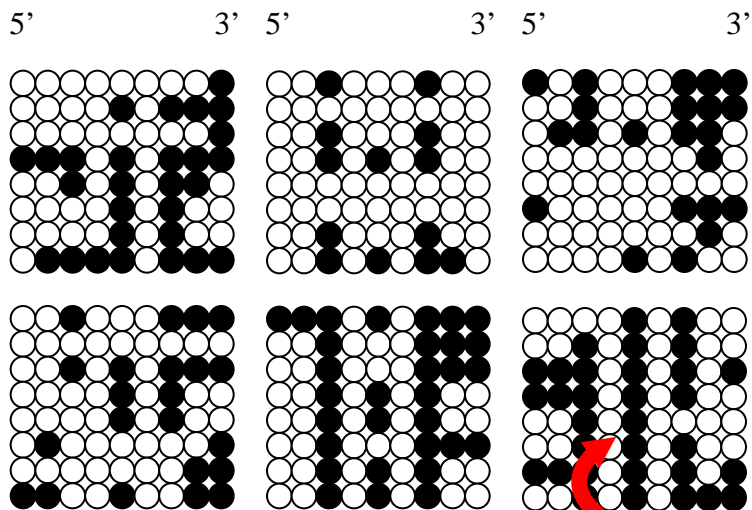
total
mitotic
age

Human Colorectal Cancer: Intratumoral Heterogeneity



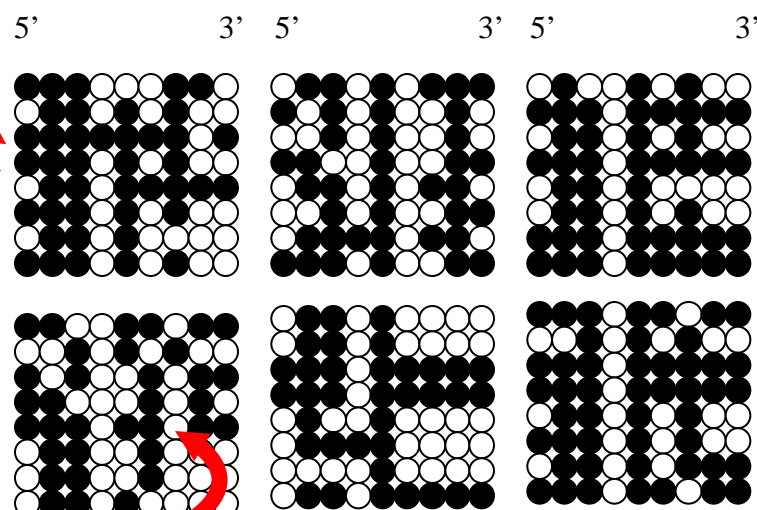
six cancer glands

left side

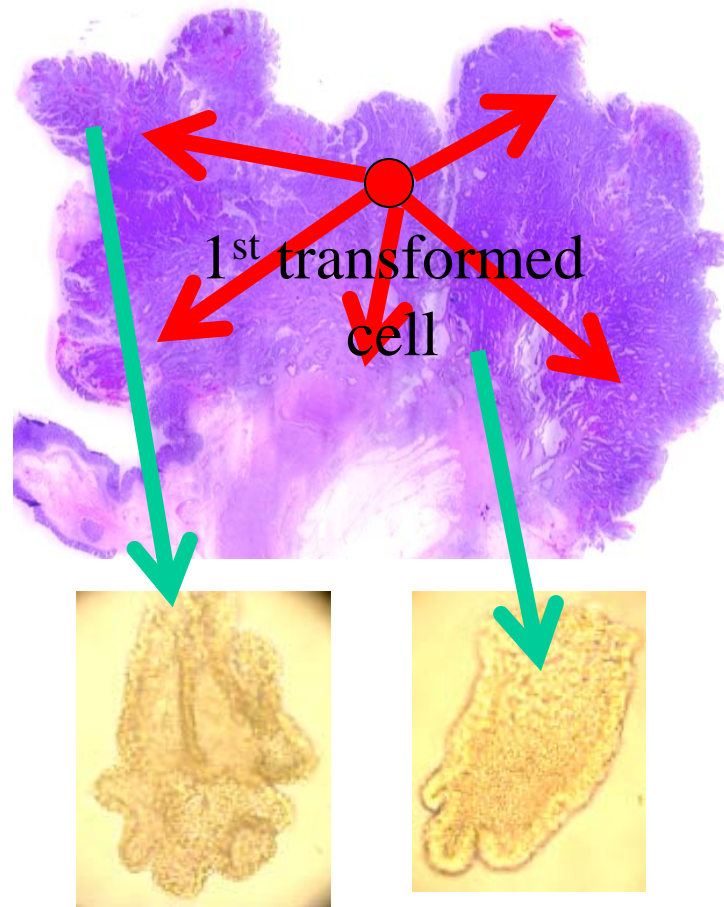
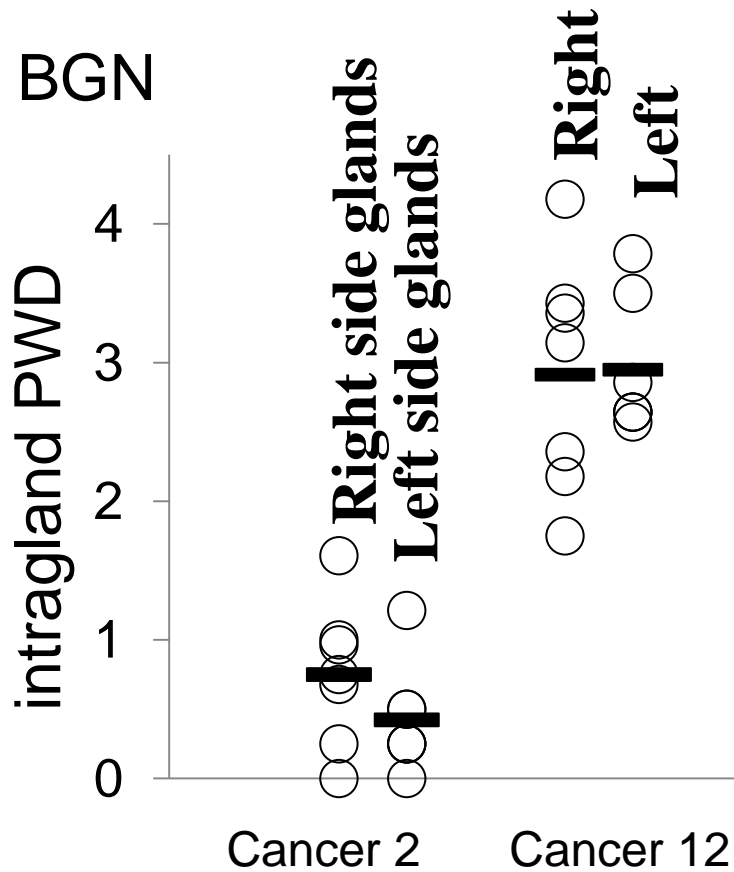


six cancer glands

right side



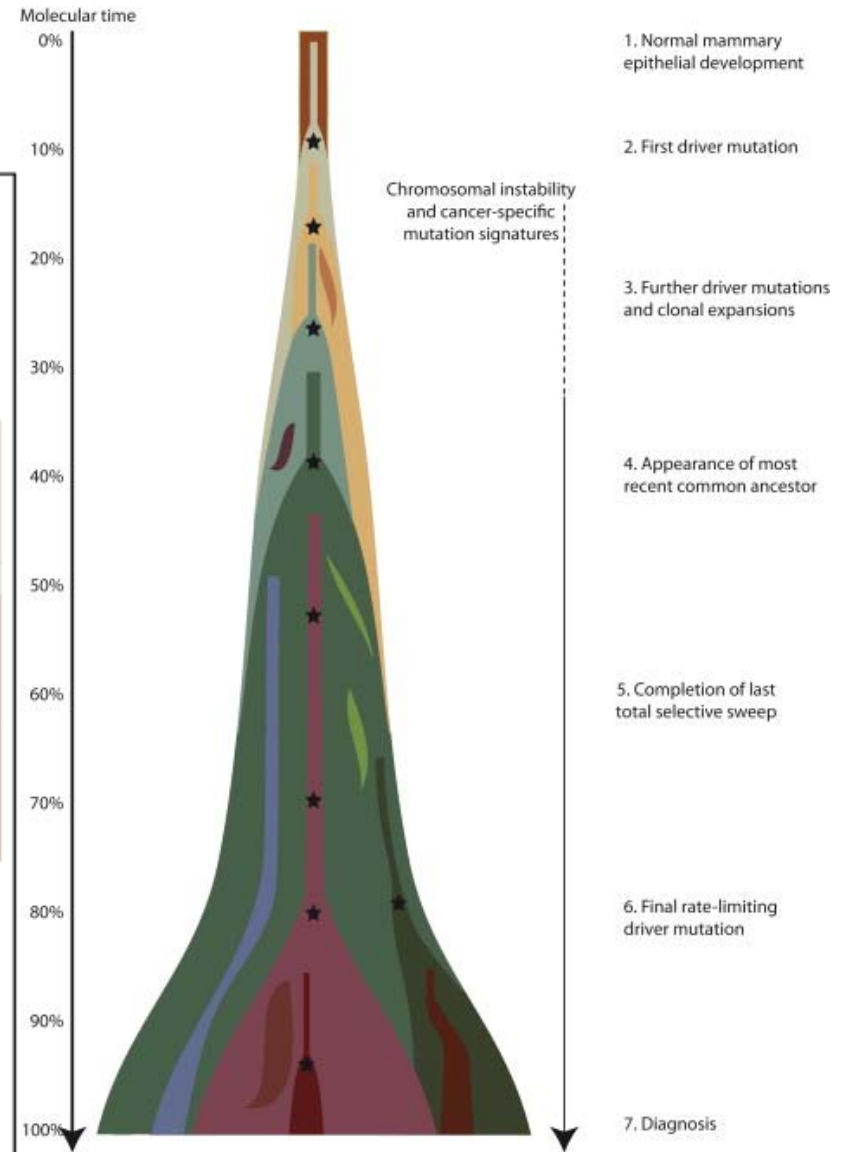
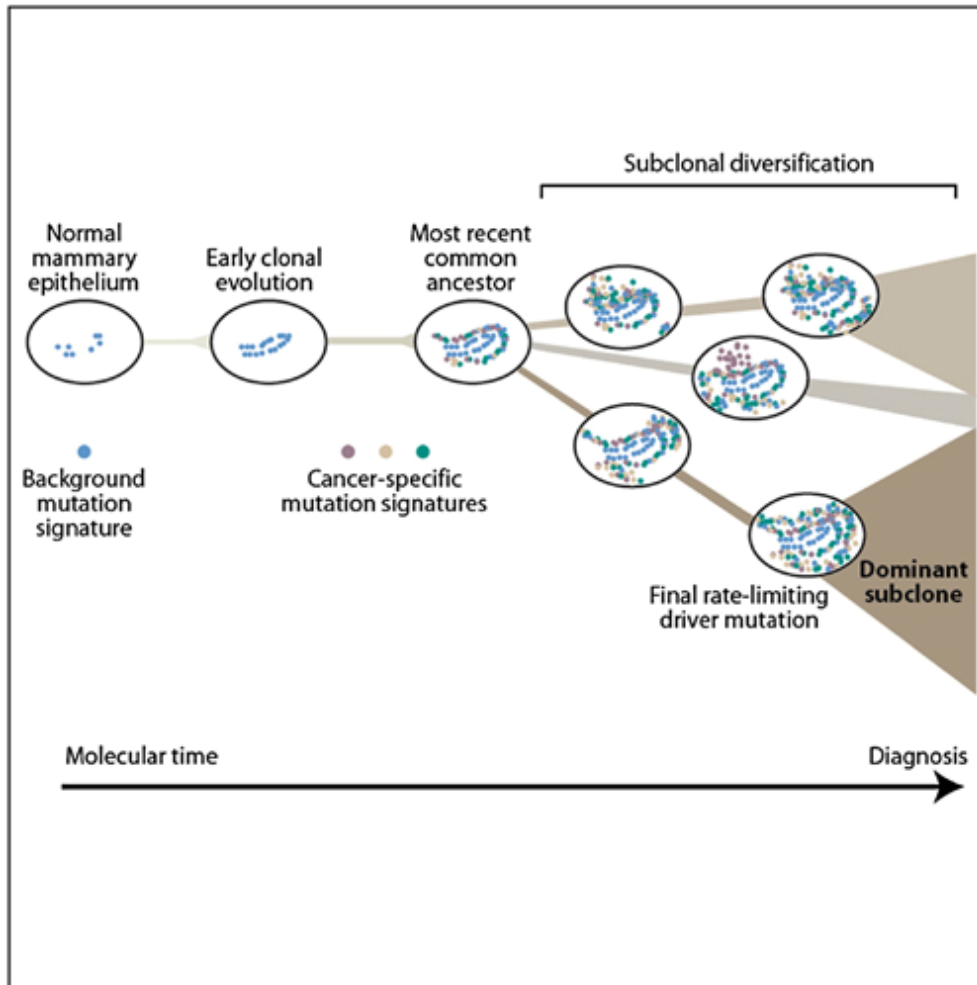
Simple Models of Tumor Growth



glands within a single cancer have similar ages or PWDs

Cancer Ancestry From DNA Sequencing

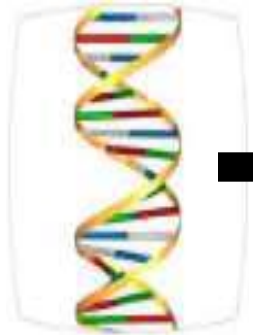
[The life history of 21 breast cancers.](#)
Cell. 2012 May 25;149(5):994-1007.



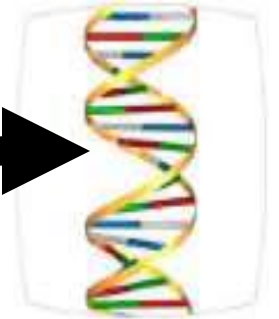
Summary

- 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
- Jung Yeon Kim
- Aimee Kang
- Peter Calabrese
- Kim Siegmund
- Paul Marjoram
- Simon Tavaré