GENETIC AND EPIGENETIC INSTABILITY IN CANCER

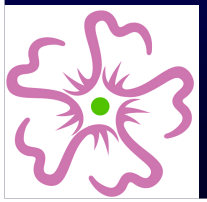
- **Genetic instability: Microsatellite instability & mutator phenotype**

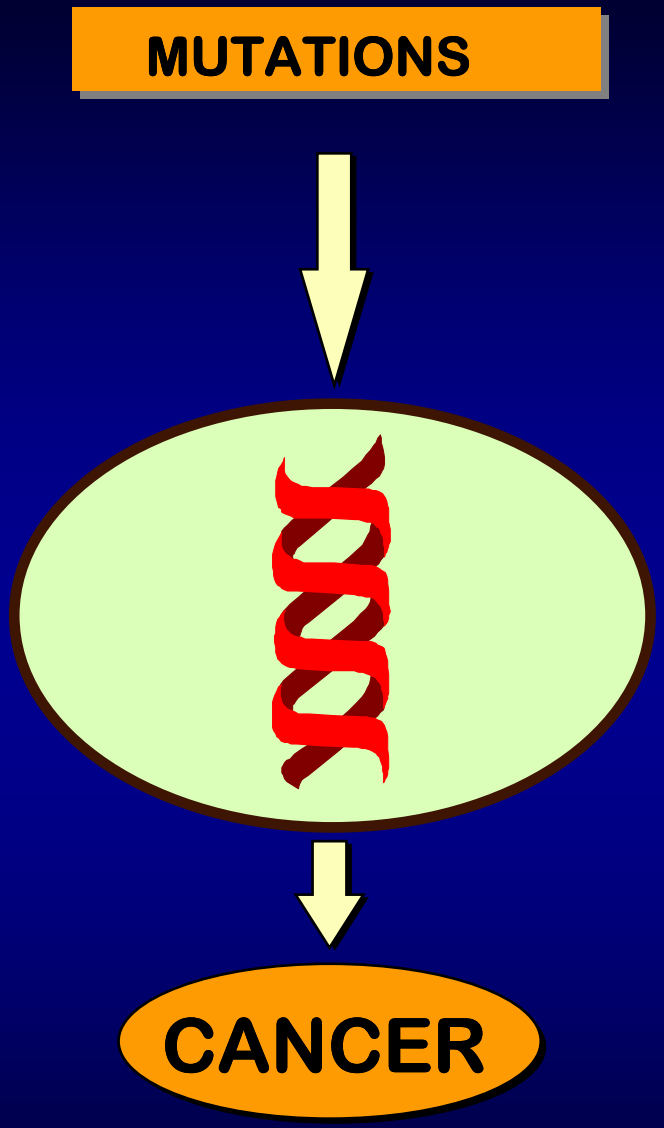
  ***a remote control oncogenic pathway*.**

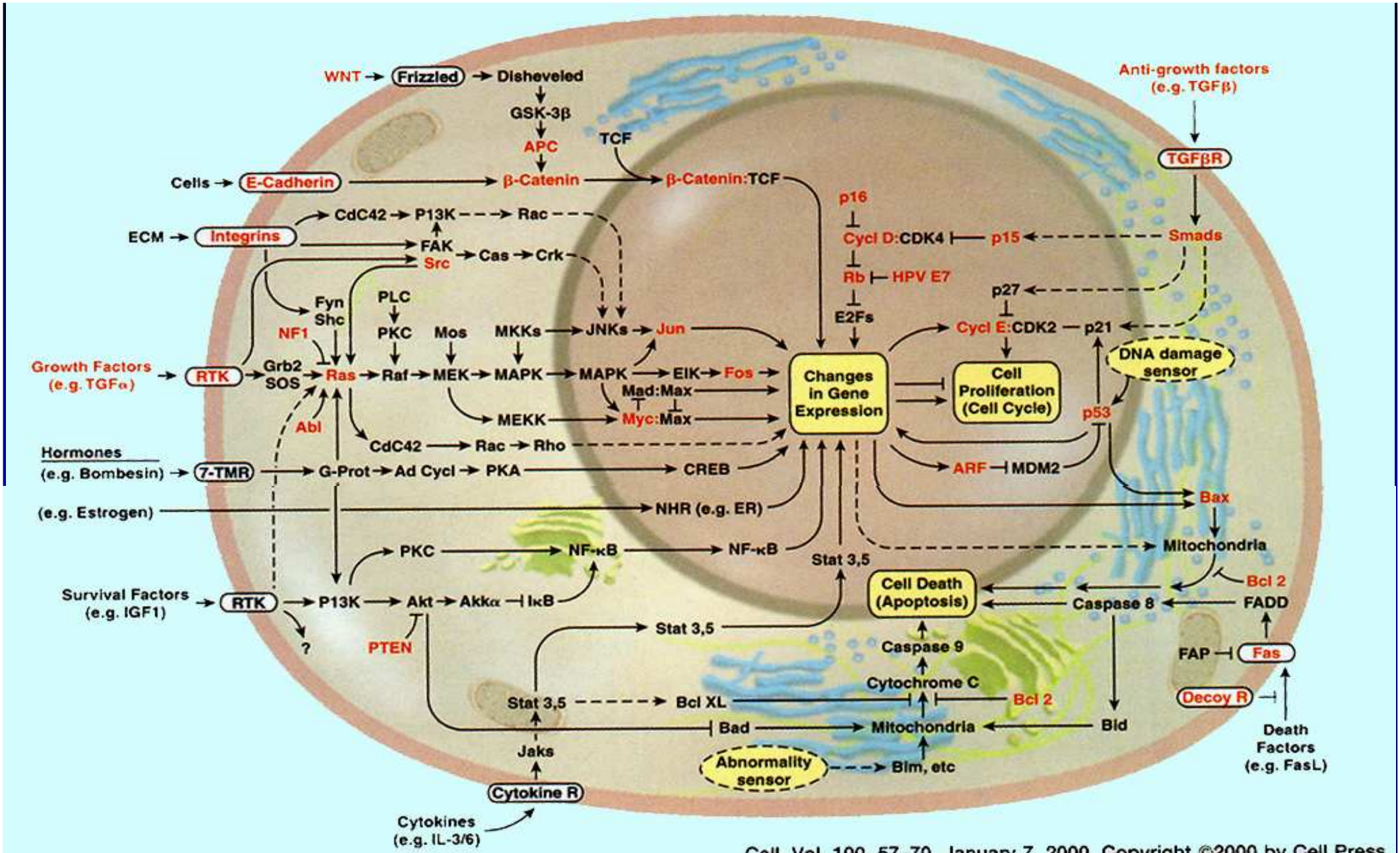
- **Epigenetic instability: DNA methylation alterations & 'methylator' phenotype**

  an ultraremote control oncogenic pathway?

- **Relationships between epigenetic and genetic instabilities in cancer.**




MUTATIONS
CANCER



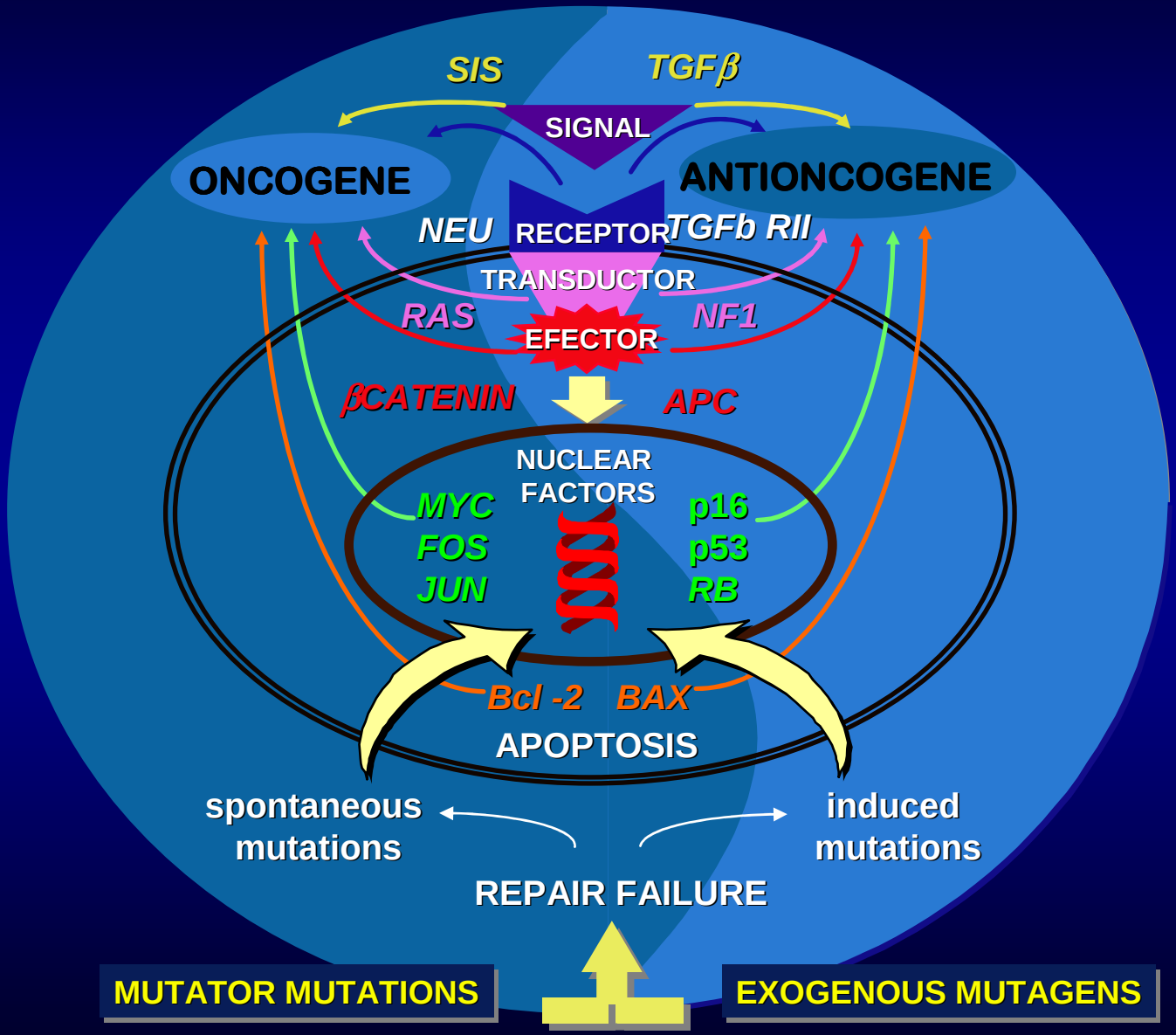
Cell, Vol. 100, 57–70, January 7, 2000, Copyright ©2000 by Cell Press

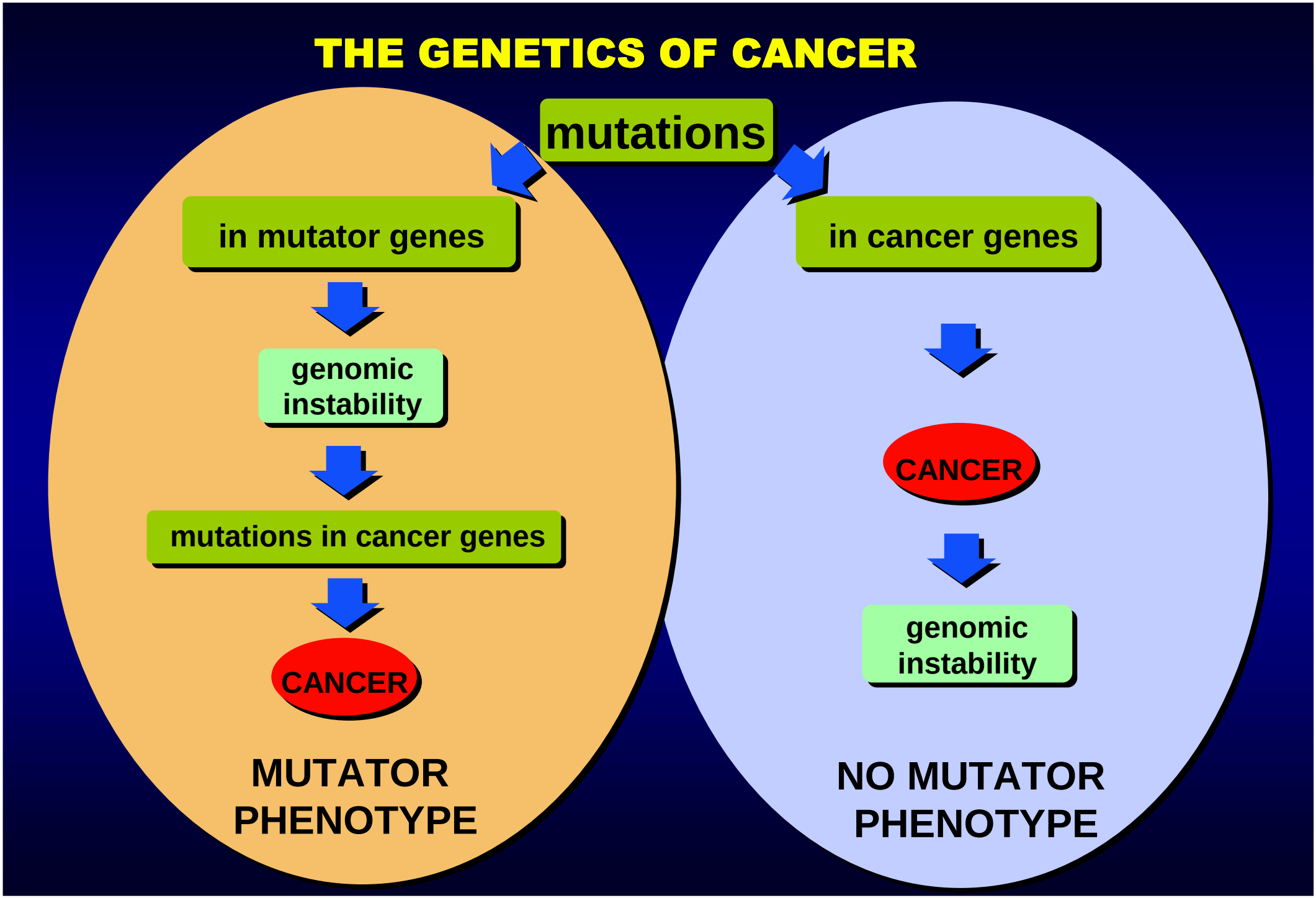
The Hallmarks of Cancer

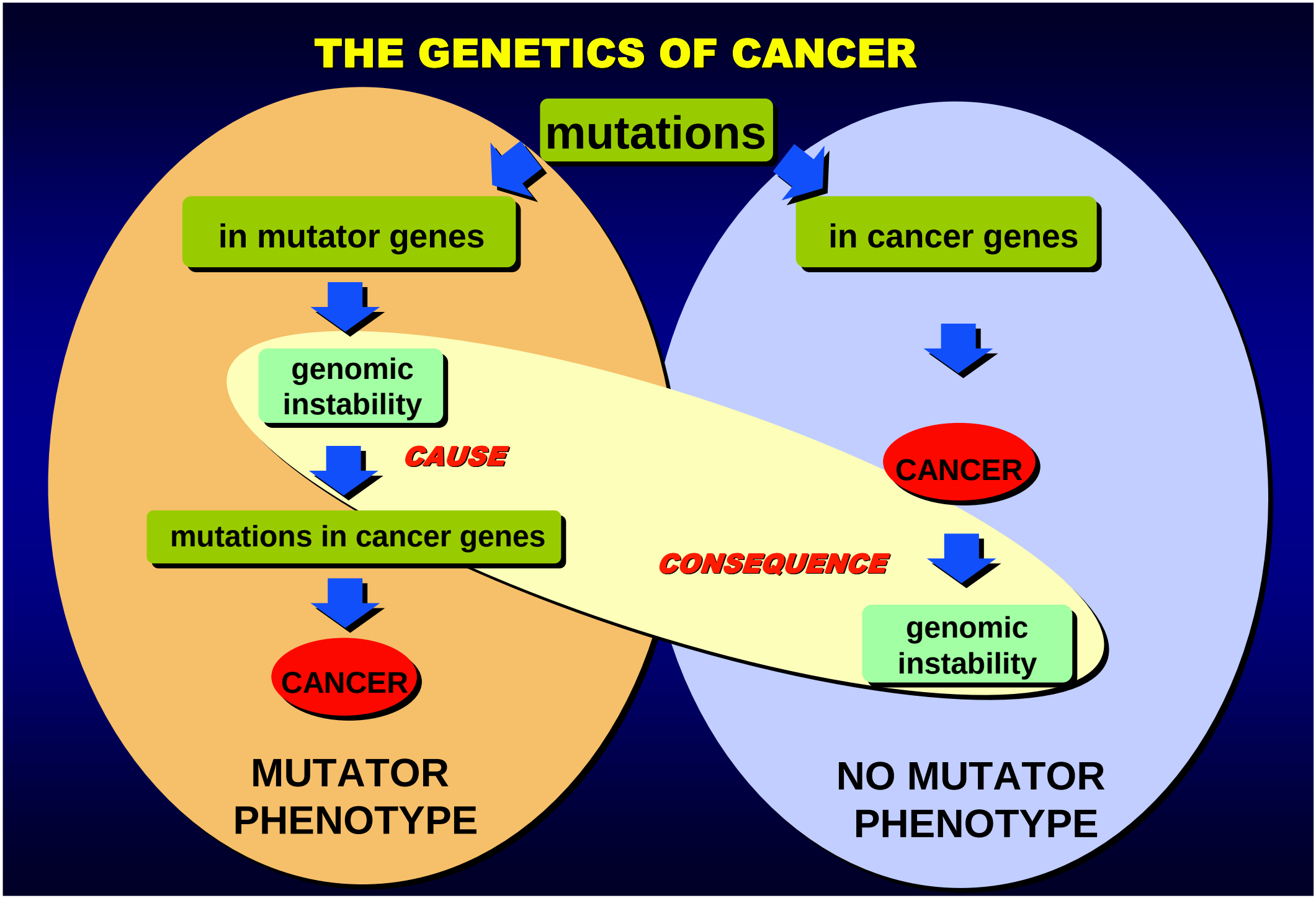
Douglas Hanahan* and Robert A. Weinbergt

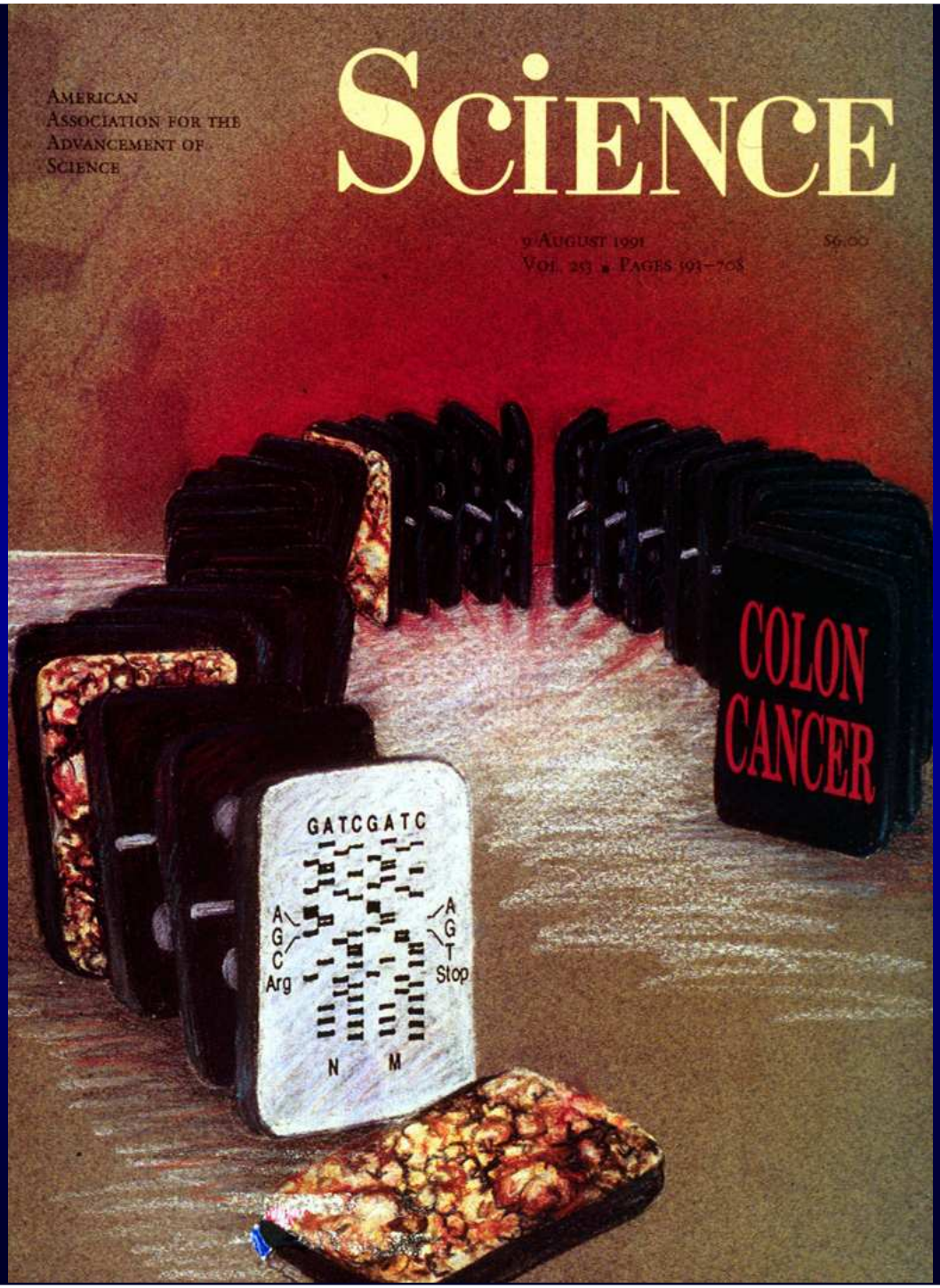
Highlighted in red some of the genes known to be functionally altered in cancer.

MOLECULAR GENETICS OF CARCINOGENESIS




THE GENETICS OF CANCER
mutations
in mutator genes
genomic instability
mutations in cancer genes
CANCER
MUTATOR PHENOTYPE
in cancer genes
CANCER
genomic instability
NO MUTATOR PHENOTYPE


THE GENETICS OF CANCER
mutations
in mutator genes
in cancer genes
genomic instability
CAUSE
CANCER
mutations in cancer genes
CONSEQUENCE
genomic instability
CANCER
MUTATOR PHENOTYPE
NO MUTATOR PHENOTYPE


American
Association for the
Advancement of
Science
SCIENCE
9 August 1991
Vol. 253 ■ Pages 191–708
$6.00
COLON
CANCER
GATCGATC
A
G
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Arg
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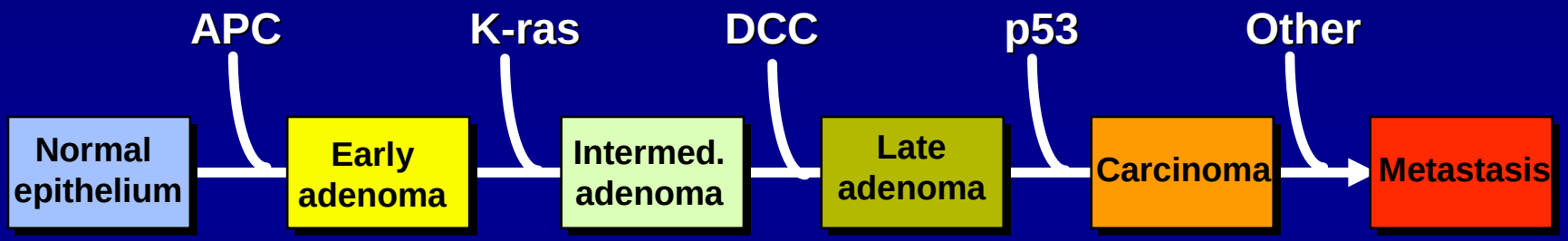
T
Stop
N
M

GENETIC ALTERATIONS AND COLON CANCER



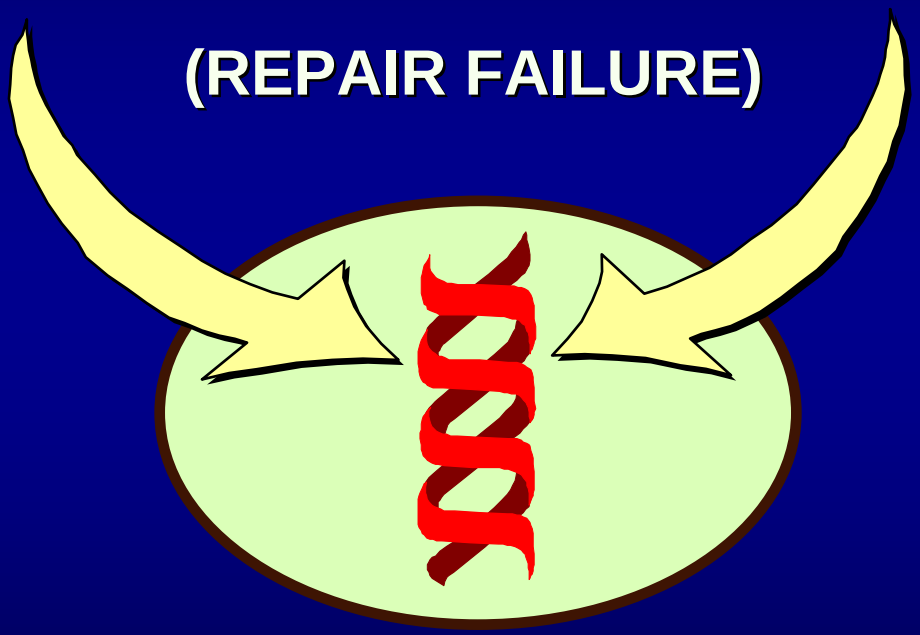
Fearon & Vogelstein. A Genetic model for colorectal tumorigenesis. Cell, 61, 759, 1990

But what was the cause of these oncogenic mutations?

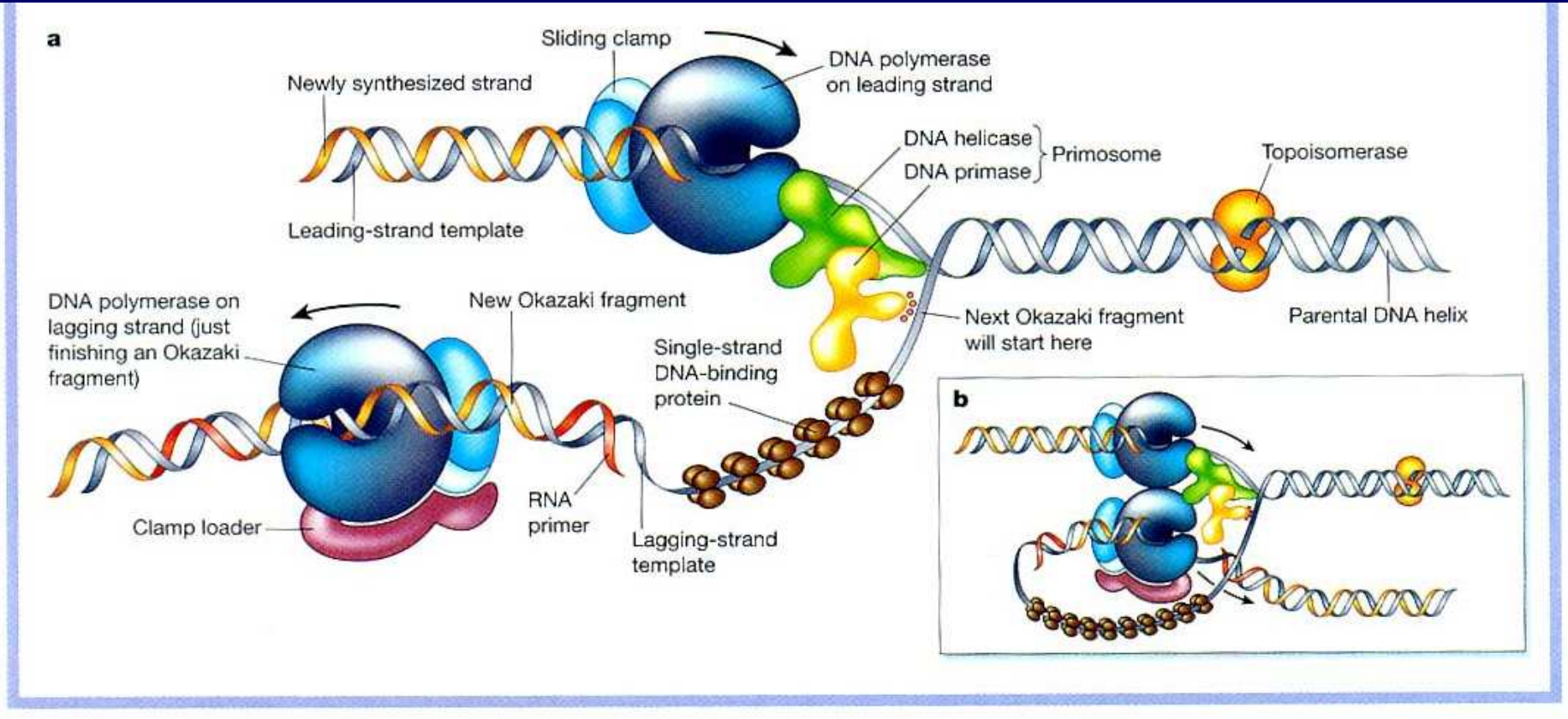
Spontaneous mutations

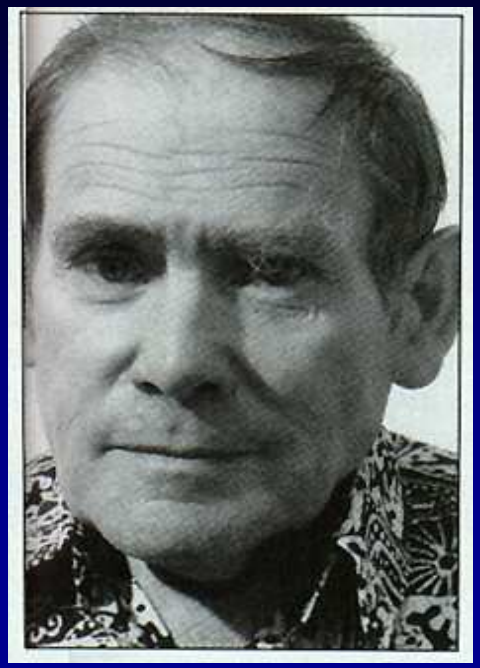
Induced mutations

(REPAIR FAILURE)



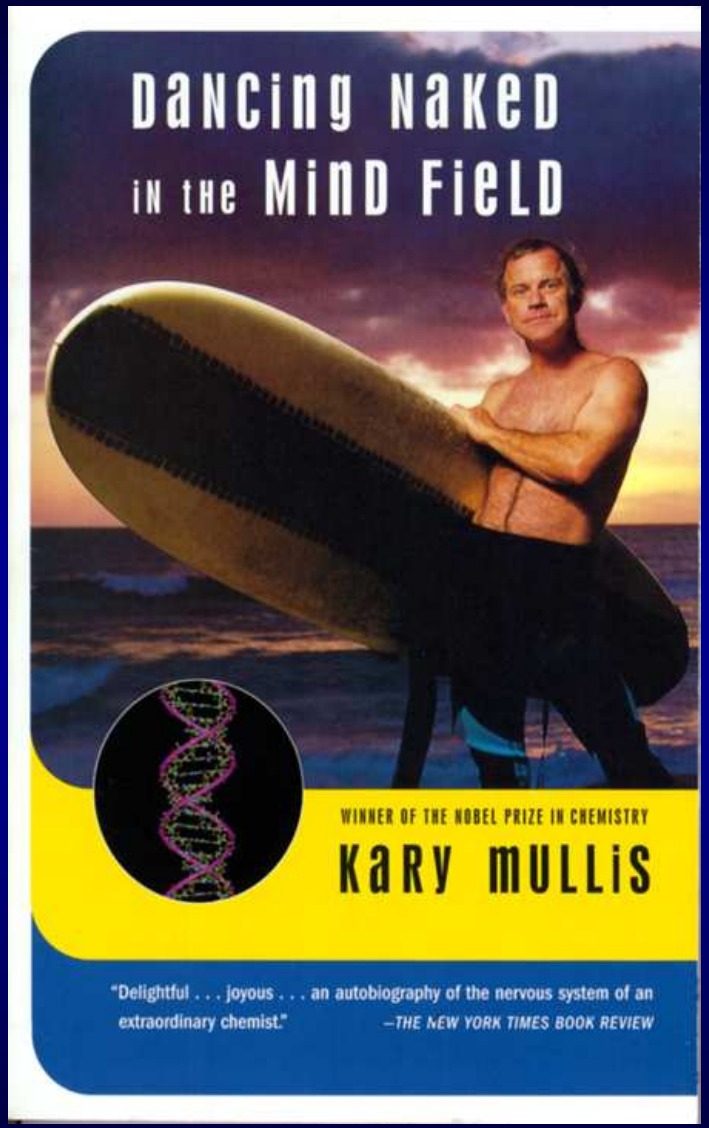
CANCER


a
Sliding clamp
DNA polymerase on leading strand
Newly synthesized strand
DNA helicase
DNA primase
Primosome
Topoisomerase
Leading-strand template
DNA polymerase on lagging strand (just finishing an Okazaki fragment)
New Okazaki fragment
Next Okazaki fragment will start here
Parental DNA helix
Single-strand DNA-binding protein
Clamp loader
RNA primer
Lagging-strand template
b

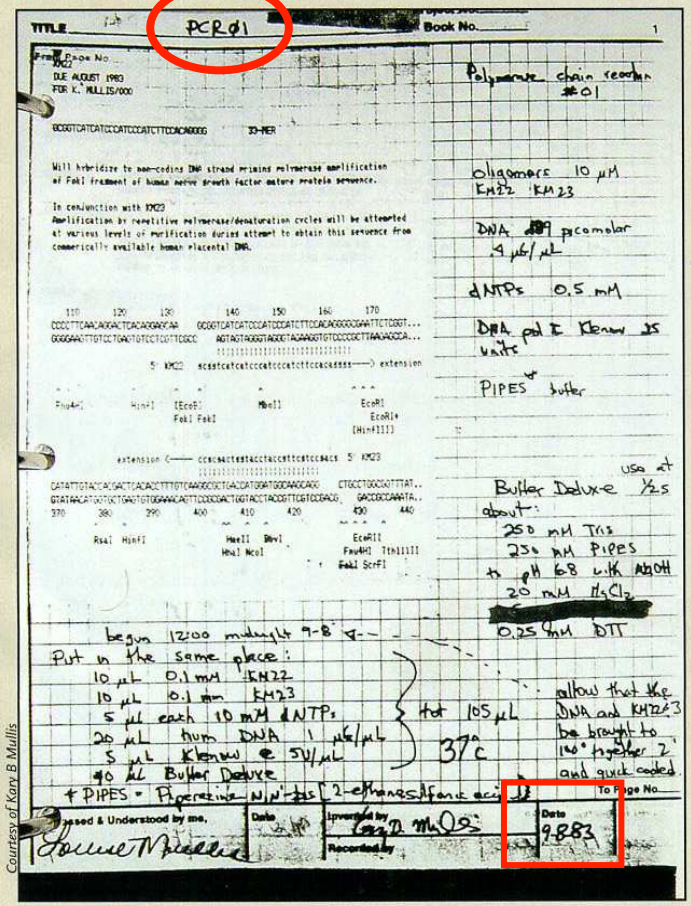


PROGRESS IN SCIENCE DEPENDS ON NEW TECHNIQUES, NEW DISCOVERIES AND NEW IDEAS, PROBABLY IN THAT ORDER

Sidney Brenner, 1980.



FOUNDATIONS



The First Polymerase Chain Reaction

"This page from my notebook lists the chemicals which I put together into a single, purple-capped tube on September 8th, 1983, in a reaction I labeled PCR01. No cycling, only one tube, no variations, no controls, and anyone familiar with PCR conditions used today will recognize very little here, except the idea.

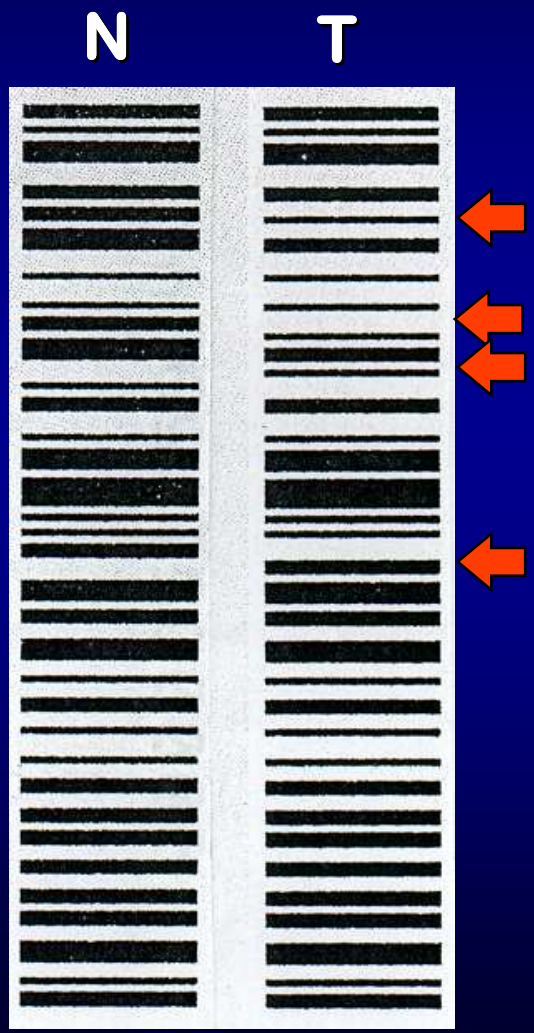
I wasn't positive that the reaction would not cycle itself. I knew that any chemical equilibrium had some finite value, meaning that some portion of any nominally double-stranded DNA would be single-stranded. And to increase the initial population of single strands, I had to cut the template DNA with a restriction enzyme. And the primers were there in sumptuous abundance. I was certainly not a proponent of doing things the hard way if there were any other possibilities.

You might conclude that it was a long-shot experiment. I agreed, so [at midnight] I poured myself a cold Becks into a prechilled 500 ml beaker from the isotope freezer for luck, and went home.

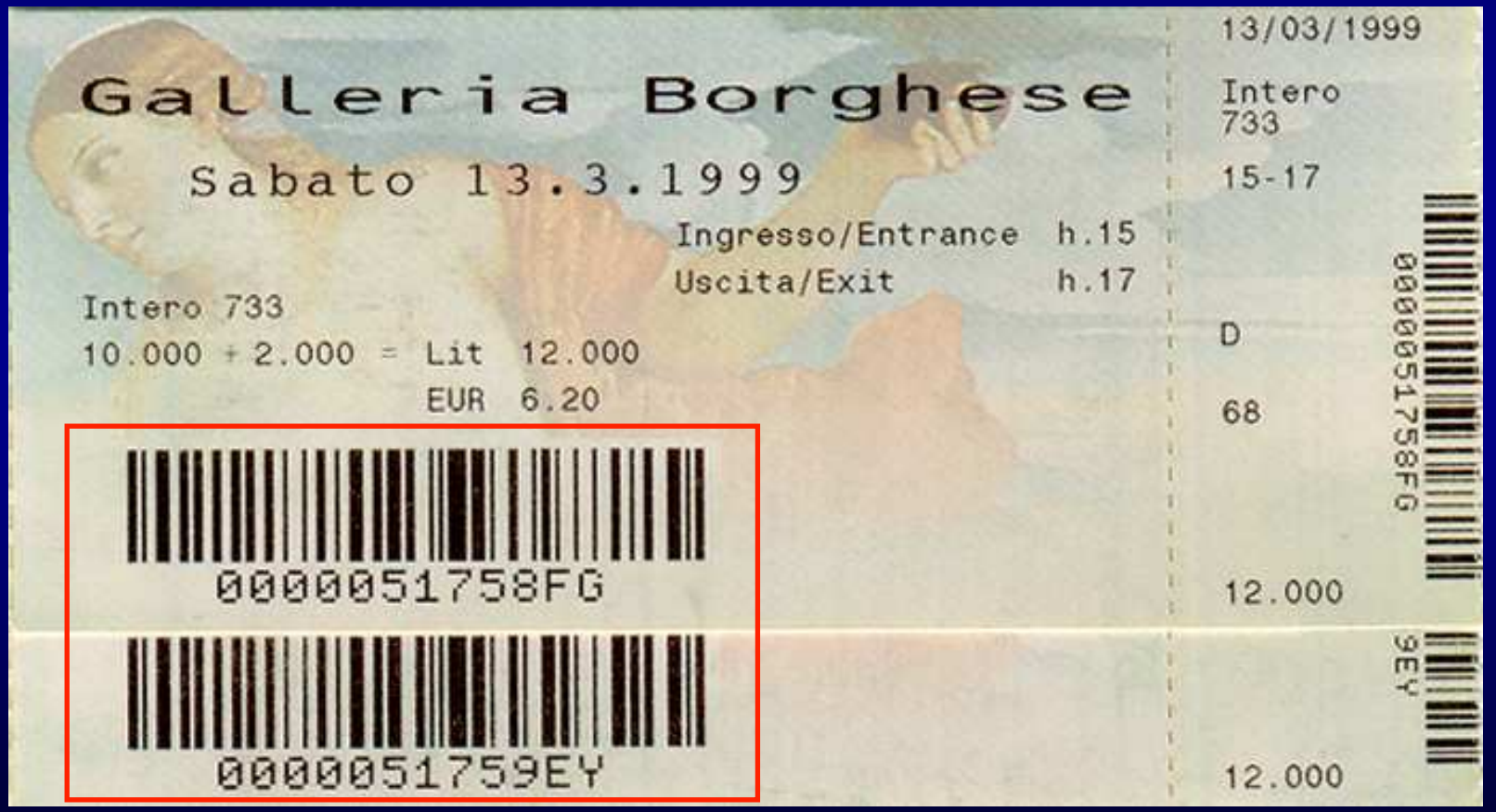
I ran a gel the next afternoon [and] stained it with ethidium. It took several months to arrive at conditions [that] would produce a convincing result."

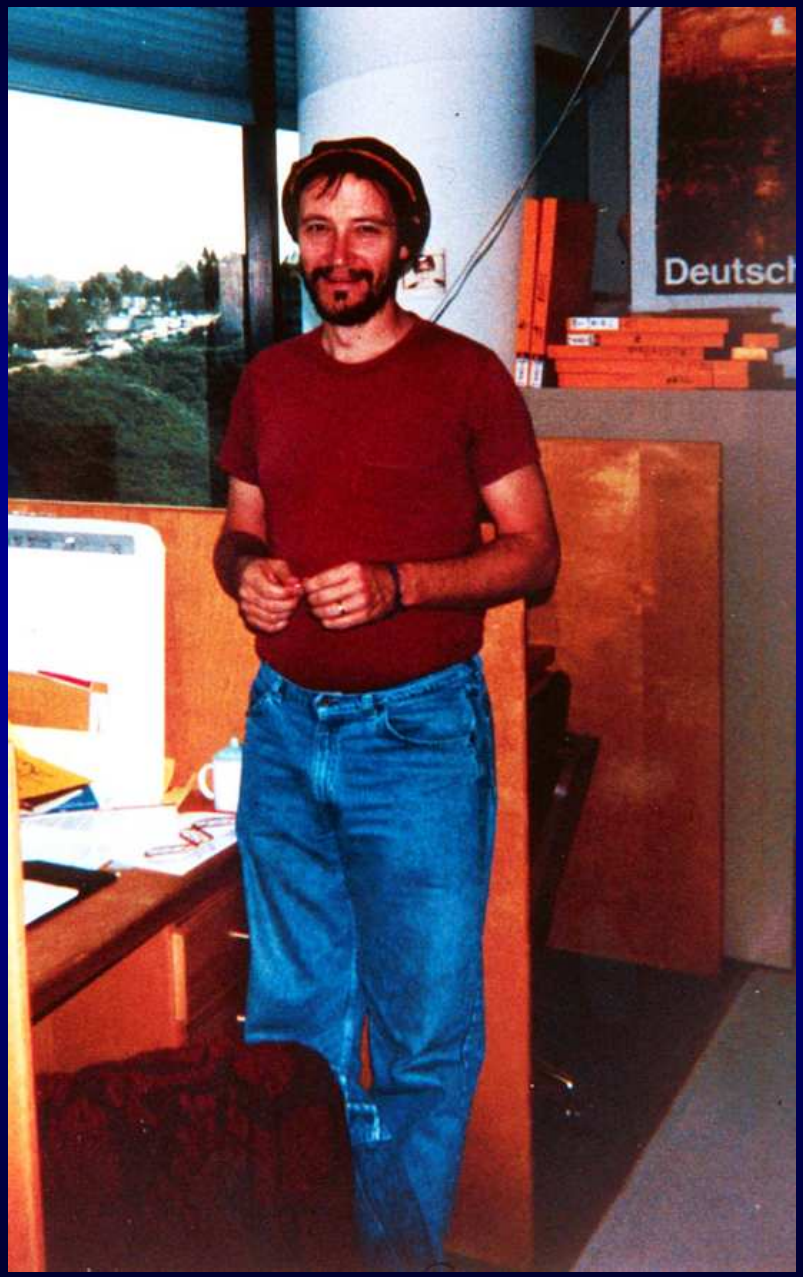
—Kary B. Mullis received the Nobel Prize in chemistry in 1993 for his discovery of the PCR method.

PRINCIPLE OF DNA FINGERPRINTING



PRINCIPLE OF DNA FINGERPRINTING





Nucleic Acids Research, Vol. 18, No. 24 7213

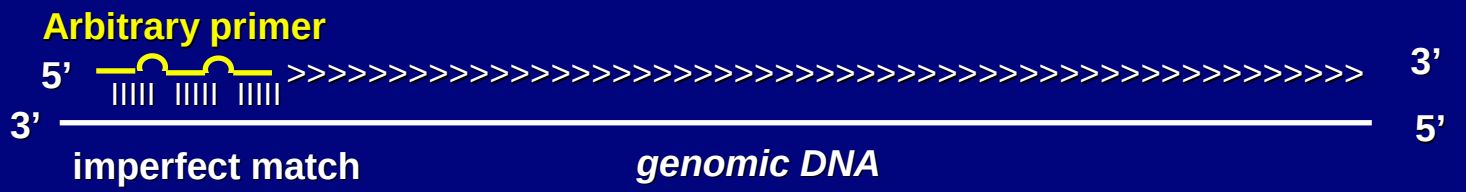
Fingerprinting genomes using PCR with arbitrary primers*

John Welsh and Michael McClelland
California Institute of Biological Research, 11099 North Torrey Pines Road, La Jolla, CA 92037 USA

THE ARBITRARILY PRIMED PCR (AP-PCR)

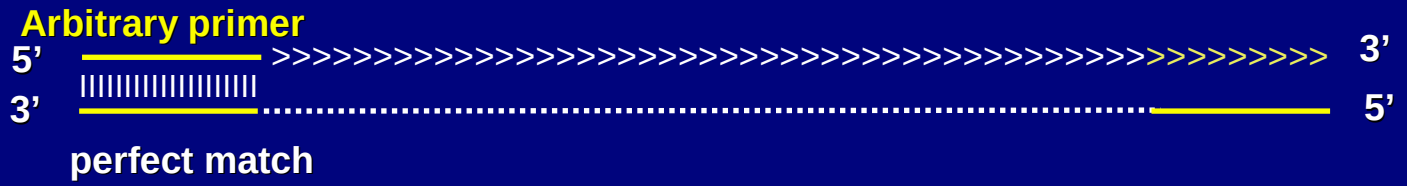
John Welsh & Michael McClelland. Nucleic Acid Res. 18, 7213, 1990.

CYCLE 1: Low annealing temperature (about 45°C) and thermostable polymerase.
The primer makes imperfect but sufficiently good match in many sites of genomic DNA.



CYCLE 2: Heat to 95°C then low annealing temperature (normally about 45°C)
The primer makes imperfect but sufficiently good matches in a few products from CYCLE 1.

CYCLE 3: Heat to 95°C then high annealing temperature (about 65°C).
Perfect matches for all successful priming events from CYCLES 1 and 2.

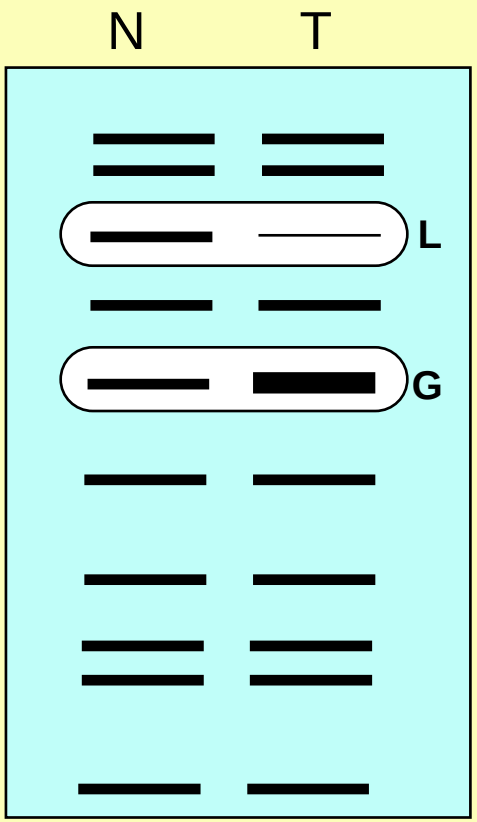


CONTINUE 30 CYCLES

CANCER PATHWAYS

SUPPRESSOR

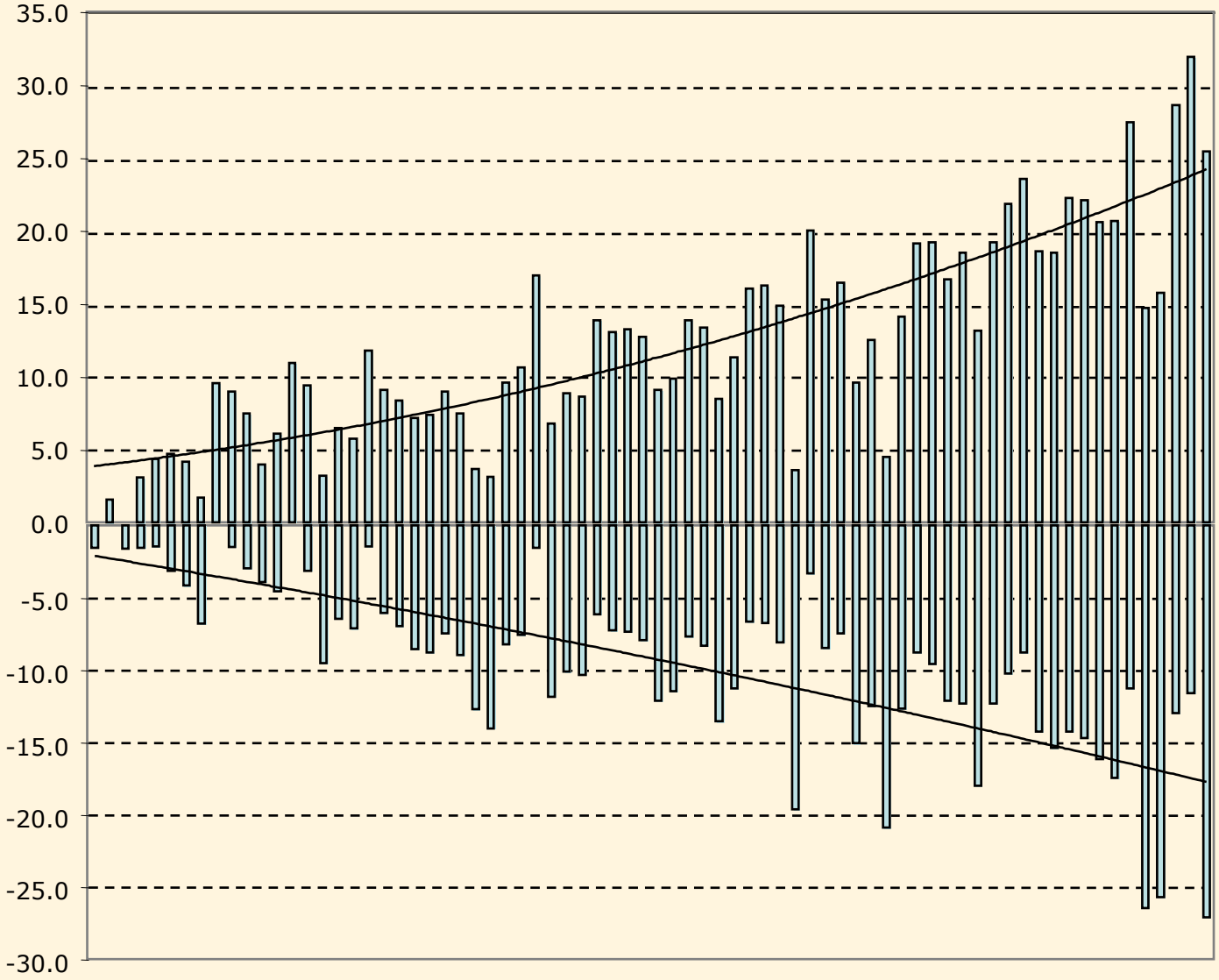
quantitative changes



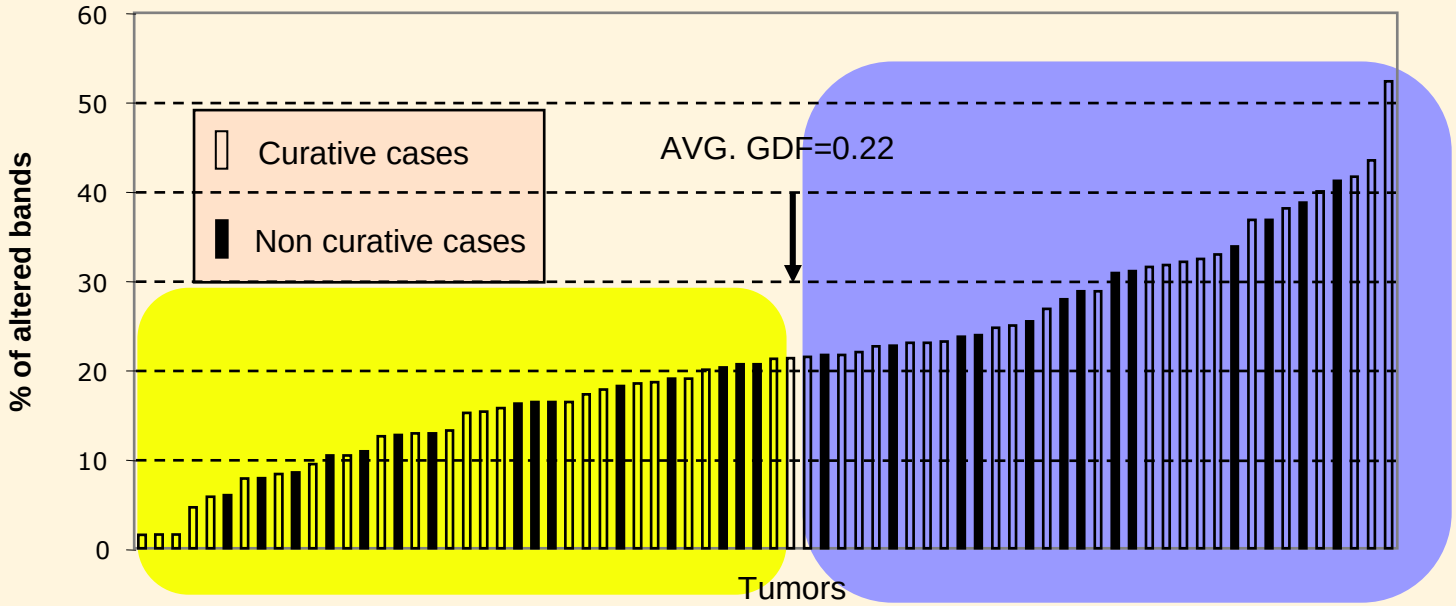
ANEUPLOID PHENOTYPE

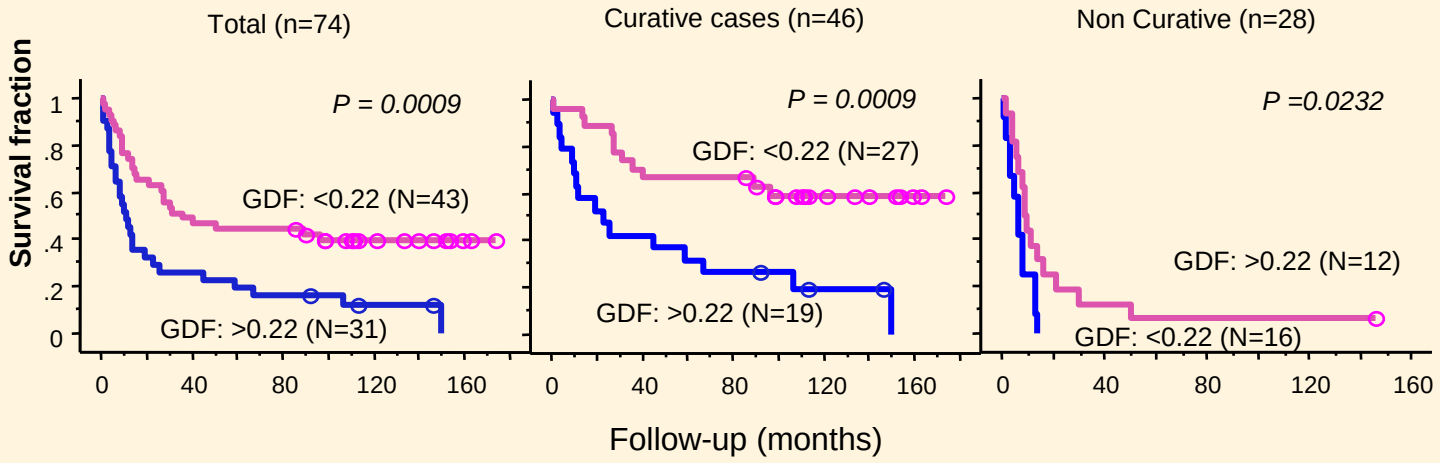
(N: NORMAL; T: TUMOR; L: LOSSES; G: GAINS)

Genetic alterations in gastric cancer by AP-PCR fingerprinting

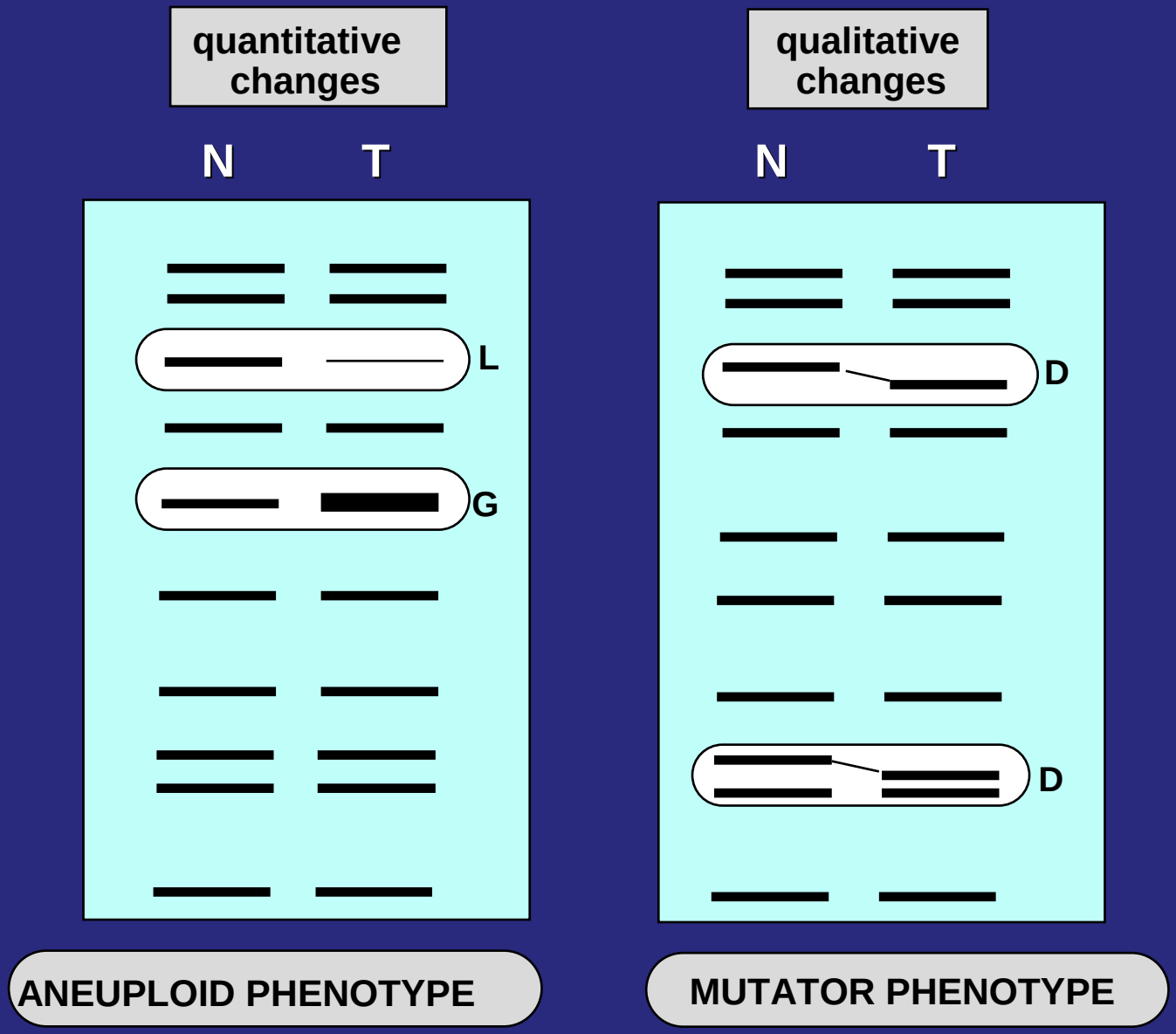


GDF & curative vs. non curative gastric cancer



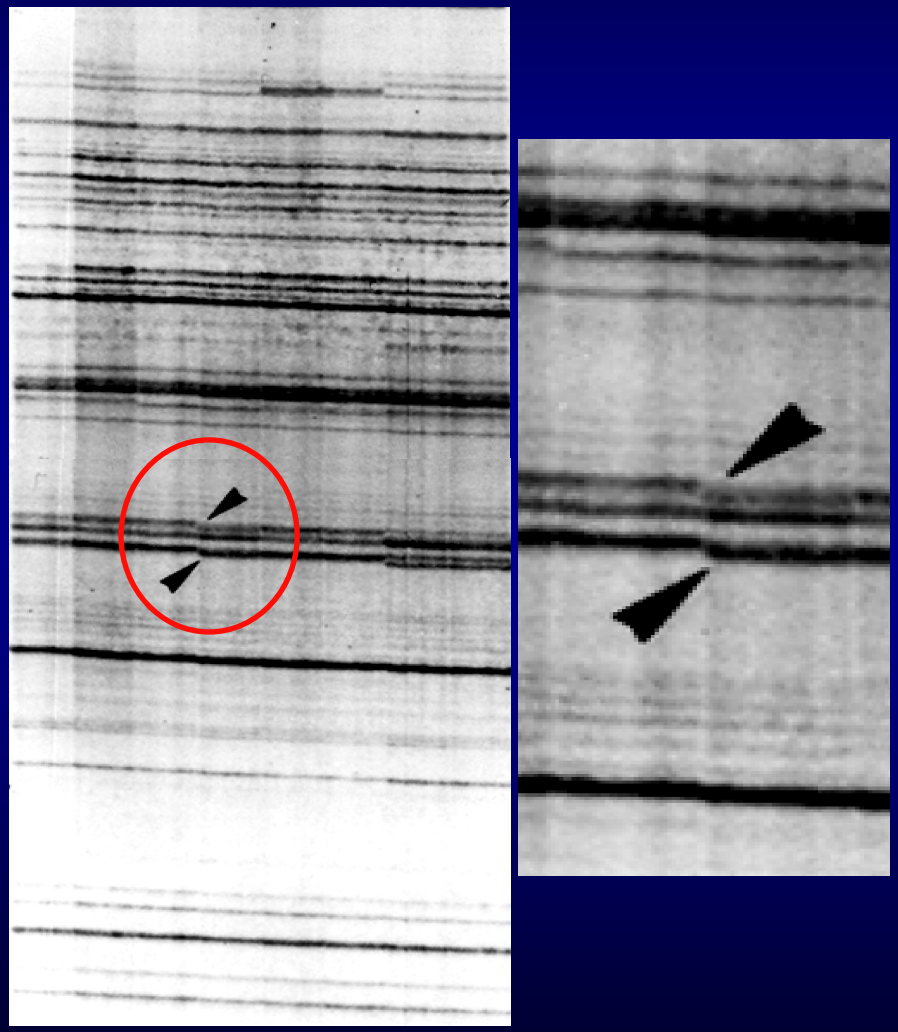


AP-PCR & the discovery of microsatellite instability (MSI)



(N: NORMAL; T: TUMOR; L: LOSSES; G: GAINS;) D: DELETIONS

MSI & colon cancer of the microsatellite mutator phenotype



AP-PCR DNA FINGERPRINT

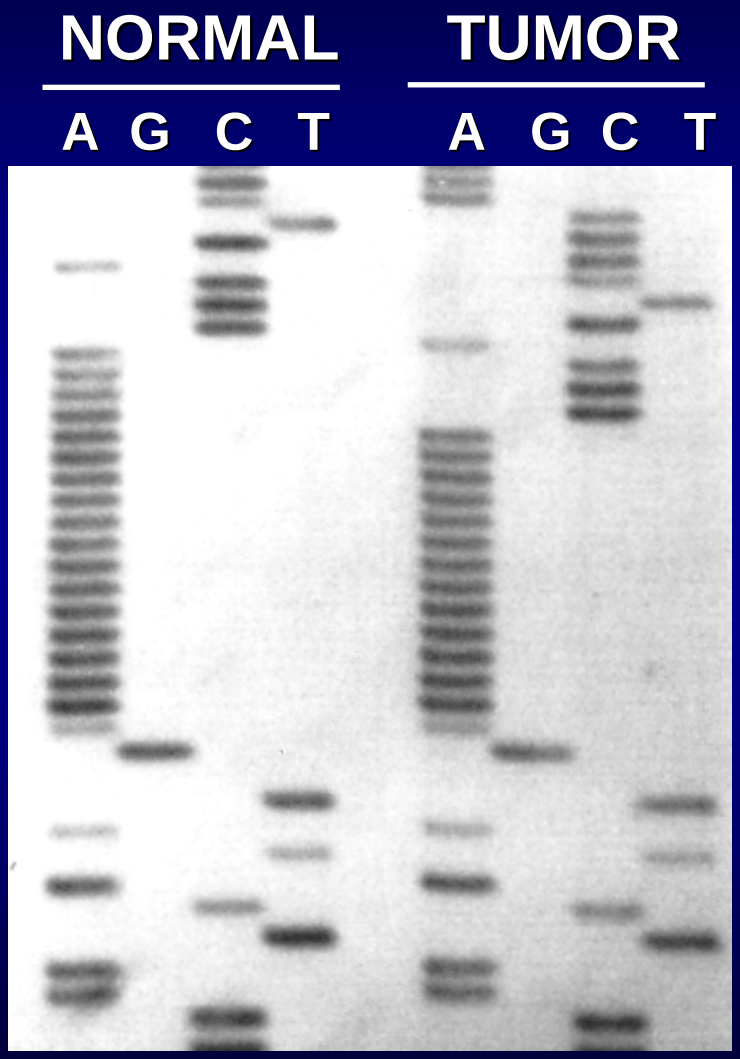
One or two bands in the fingerprints from about 13% of unselected colon tumors exhibited mobility shifts due to mutations in microsatellite sequences.

The arbitrary nature of AP-PCR permitted to estimate that the mutations in these tumors surpassed hundreds of thousands:

$$\text{Number of mutations} = \frac{\text{\# of total base pairs (bp) in the genome}}{\text{\# of total bp in the AP-PCR fingerprints}} = \frac{3 \times 10^9}{\sim 3 \times 10^4} = \sim 10^5$$

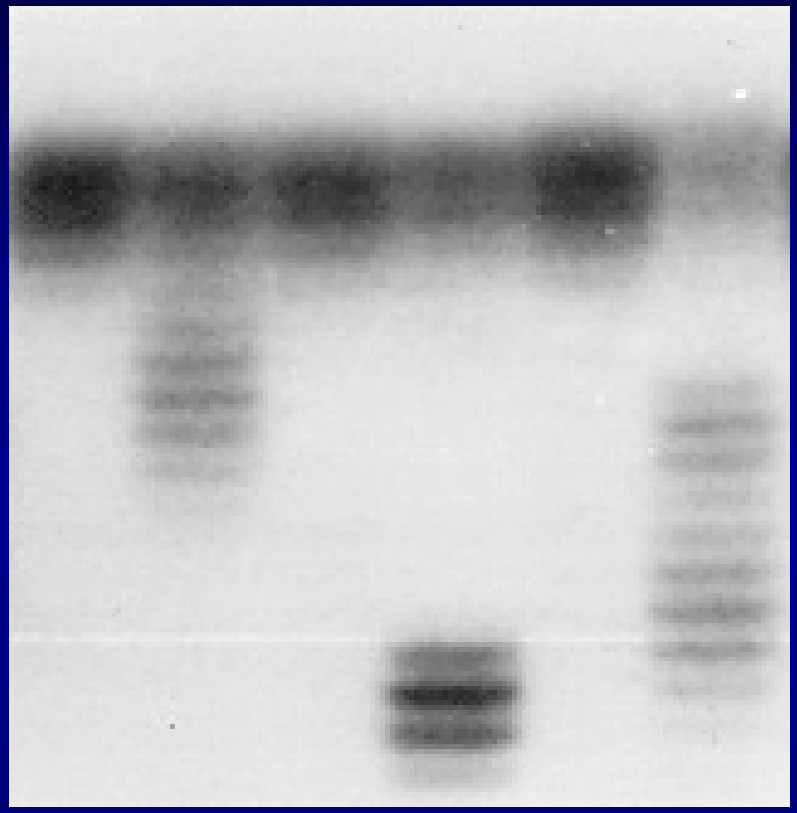
Ionov et al Nature 363, 558-561, 1993

THE MICROSATELLITE MUTATOR PATHWAY FOR COLON CANCER



Cloning and sequencing revealed that the mobility shifts were due to somatic deletions of a few nucleotides in mononucleotide **microsatellite repeats**, i.e. poly(A)n tracts.

MICROSATELLITE INSTABILITY



N T N T N T

MSI is easily detectable by a simple PCR reaction of a long mononucleotide repeat.

Frameshift Mutations and the Genetic Code

This paper is dedicated to Professor Theodosius Dobzhansky on the occasion of his 66th birthday.

GEORGE STREISINGER, YOSHIMI OKADA, JOYCE EMRICH, JUDITH NEWTON
AKIRA TSUGITA[1], ERIC TERZAGHI*,[1] AND M. INOUYE[1]

Institute of Molecular Biology, University of Oregon, Eugene, and Institute of Molecular Genetics, University of Osaka, Japan[1].

Cold Spring Harb. Sympos. Quant. Biol. vol 31, 77-84, 1966.

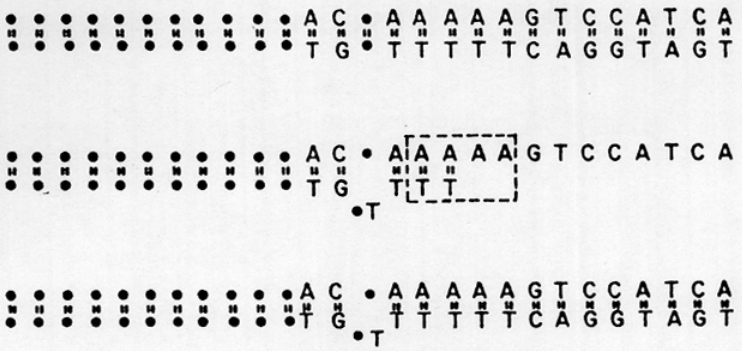
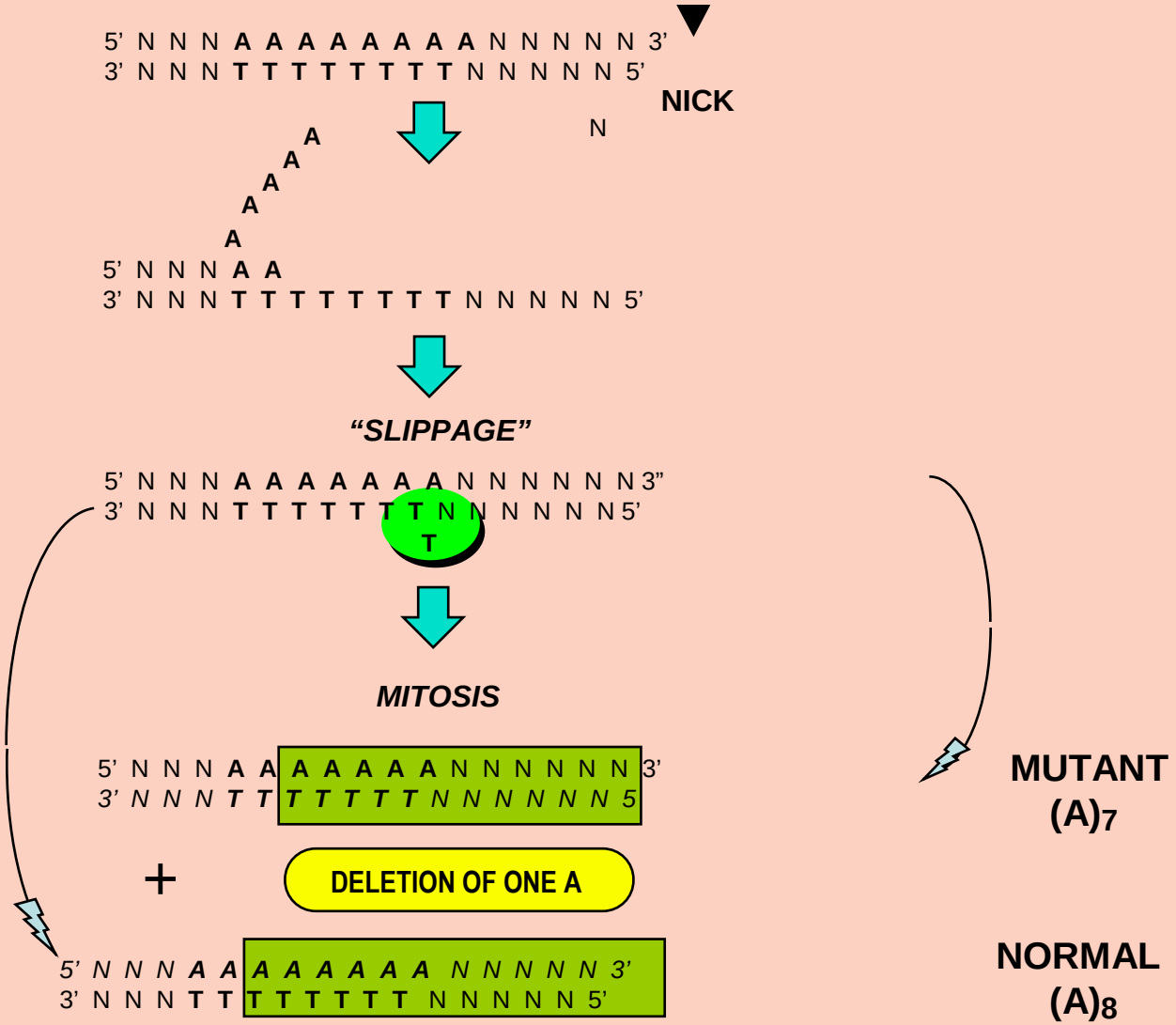


FIGURE 5. (a) Origin of a frameshift mutation at the end of a molecule. Line 1 shows the normal end of a molecule, line 2 shows an end in which one chain has been digested by an exonuclease followed by mispairing, and line 3 shows the appearance of the molecule after resynthesis of the digested chain.

STREISINGER'S SLIPPAGE BY STRAND MISALIGNMENT FOR GENERATION OF MICROSATELLITE MUTATIONS



Yurij Ionov*, Miguel A. Peinado*†, Sergei Malkhosyan*, Darryl Shibata‡ & Manuel Perucho§

* California Institute of Biological Research, 11099 North Torrey Pines Road, La Jolla, California 92037, USA
† Department of Pathology, University of Southern California School of Medicine, Los Angeles, California 90033, USA

SPONTANEOUS errors in DNA replication were proposed to be substantial in transformation to explain the chromosomal alterations of cancer cells[1]. A replication-defective factor could generate an enhanced error rate in the clonal variants arising during tumour progression. But increased mutation rate in tumour cells has not been demonstrated[2]. Using unbiased genomic fingerprinting we show that somatic deletions in poly(dA·dT) and other simple repeats occur in 12% of colorectal carcinomas in large numbers. These mutations are clustered in tumours with distinctive genotypic and phenotypic features and ubiquitous in neoplastic regions of synchronous tumours from the same patient, including adenomas. These microdeletions represent a discrete molecular pathway for colon cancer involving a mutator mutation with an active role in oncogenesis and that may have an inherited predisposition.

Spontaneous errors in DNA replication have been suggested to explain the chromosomal alterations seen in cancer cells[1]. Mutations in a replication factor could increase the error rate in tumour cells, but despite intensive efforts, no increase in the tumour cell mutation rates has ever been shown[2]. Here we use an unbiased genomic fingerprinting technique to show that 12% of colorectal carcinomas carry somatic deletions in poly dA:dT sequences and other simple repeats. We estimate that some tumours may carry more than 10^5 such mutations. Only tumours with affected poly dA:dT sequences carry mutations in the other simple repeats examined, and such mutations can be found in all neoplastic regions of synchronous tumours from the same patient, including adenomas. Tumours with these mutations show distinctive genotypic and phenotypic features. Certain patients may therefore have an inherited predisposition to produce an altered DNA replication factor of reduced fidelity which plays an active role in colorectal oncogenesis.

Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis

Yurij Ionov*, Miguel A. Peinado*†, Sergei Malkhosyan*, Darryl Shibata‡ & Manuel Perucho*§

* California Institute of Biological Research, 11099 North Torrey Pines Road, La Jolla, California 92037, USA
‡ Department of Pathology, University of Southern California School of Medicine, Los Angeles, California 90033, USA

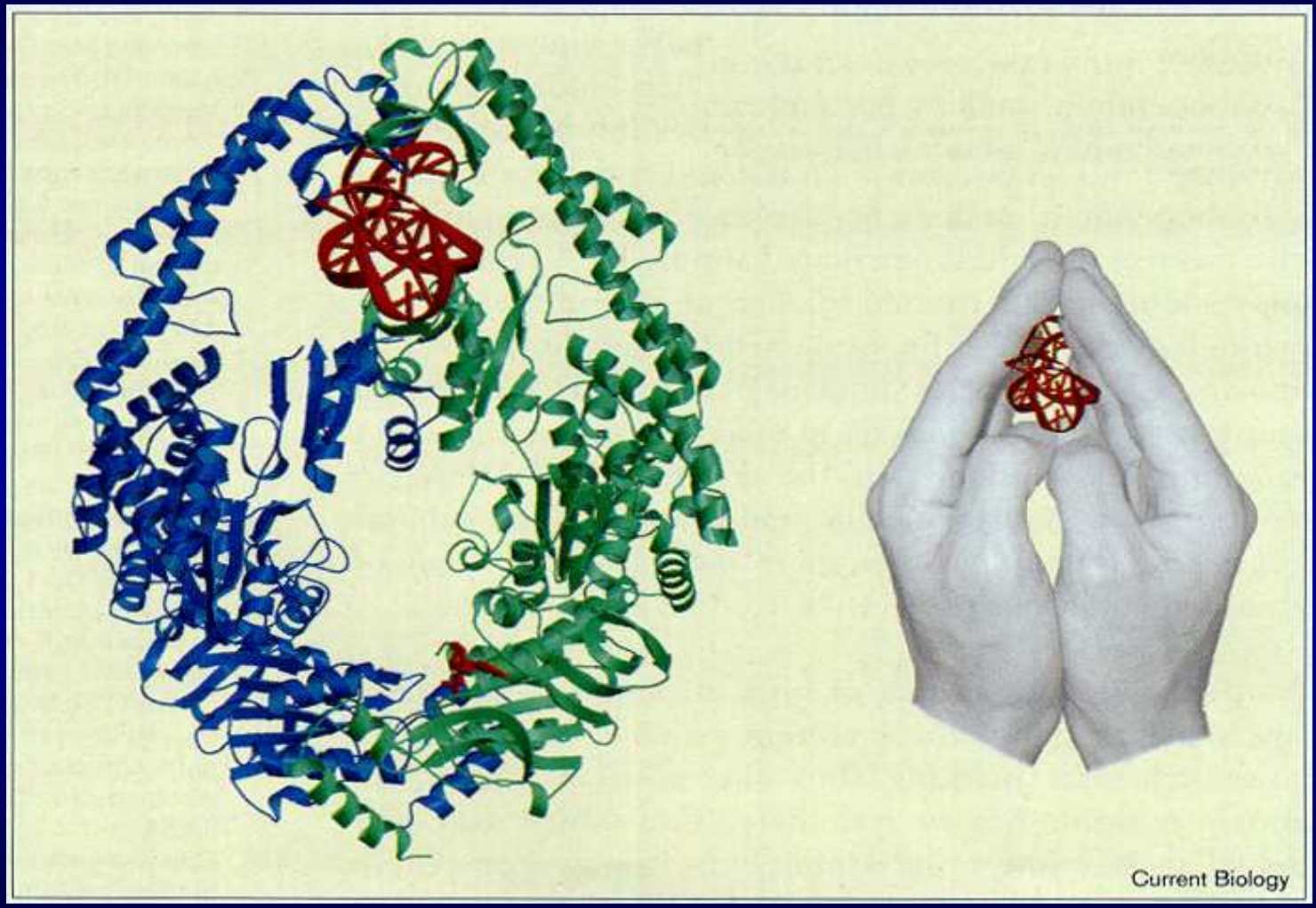
† Present address: Institut de Recerca Oncologica, Hospital Duran i Reynals, Autovia de Castelldefels, Km 2,7 Hospitalet, 08907 Barcelona, Spain.
§ To whom correspondence should be addressed.

SPONTANEOUS errors in DNA replication have been suggested to play a significant role in neoplastic transformation and to explain the chromosomal alterations seen in cancer cells[1]. A defective replication factor could increase the mutation rate in clonal variants arising during tumour progression, but despite intensive efforts, increases in tumour cell mutation rates have not been unambiguously shown[2]. Here we use an unbiased genomic fingerprinting technique[3] to show that 12 per cent of colorectal carcinomas carry somatic deletions in poly(dA · dT) sequences and other simple repeats. We estimate that cells from these tumours can carry more than 100,000 such mutations. Only tumours with affected poly(dA · dT) sequences carry mutations in the other simple repeats examined, and such mutations can be found in all neoplastic regions of multiple tumours from the same patient, including adenomas. Tumours with these mutations show distinctive genotypic and phenotypic features. We conclude that these mutations reflect a previously undescribed form of carcinogenesis in the colon (predisposition to which may be inherited) mediated by a mutation in a DNA replication factor resulting in reduced fidelity for replication or repair (a 'mutator mutation').

Tumor cells with hundreds of thousands of somatic microsatellite mutations had a much higher mutation rate than normal cells. That is, they displayed a

mutator phenotype.

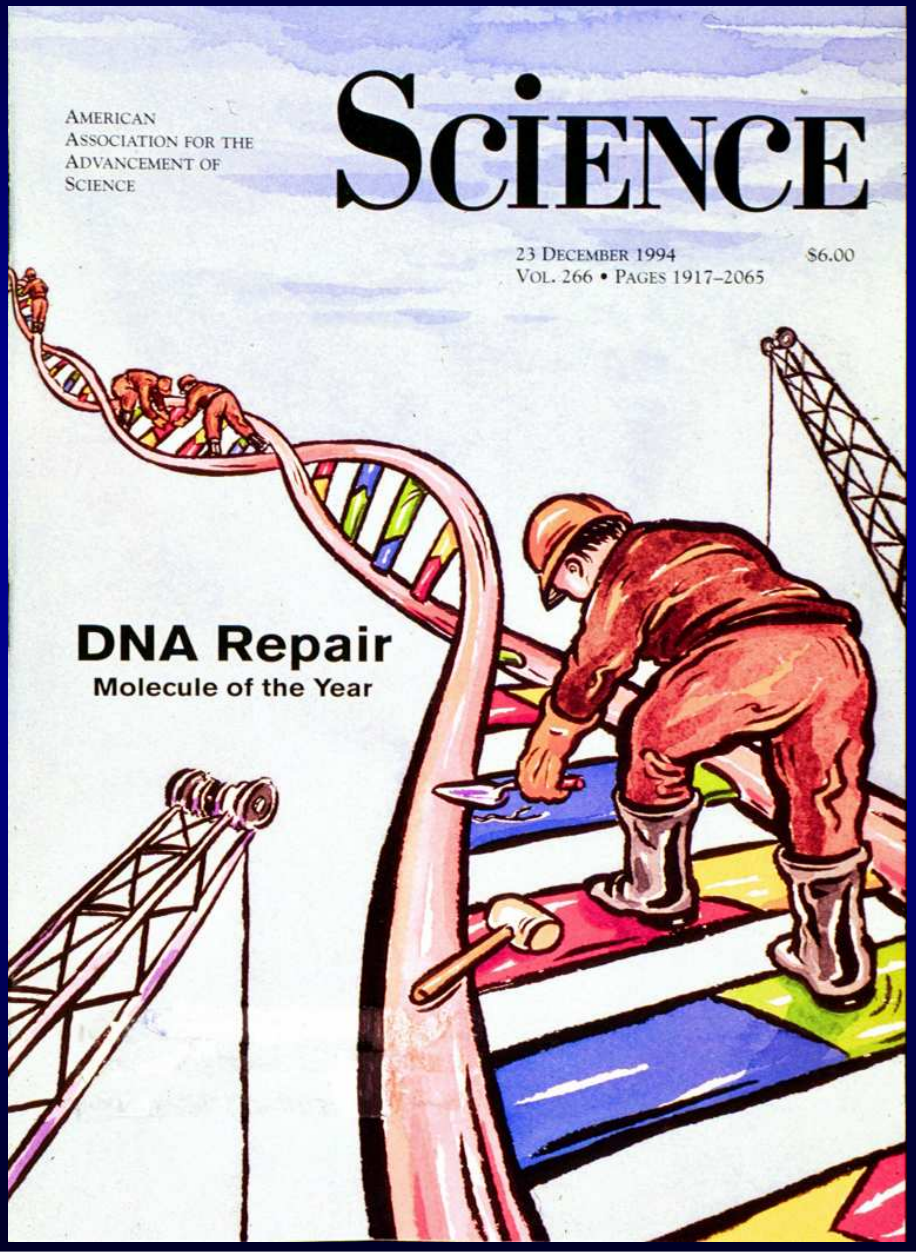
A MUTATOR GENE



Structure of *E. coli* Mut S homodimer bound to DNA

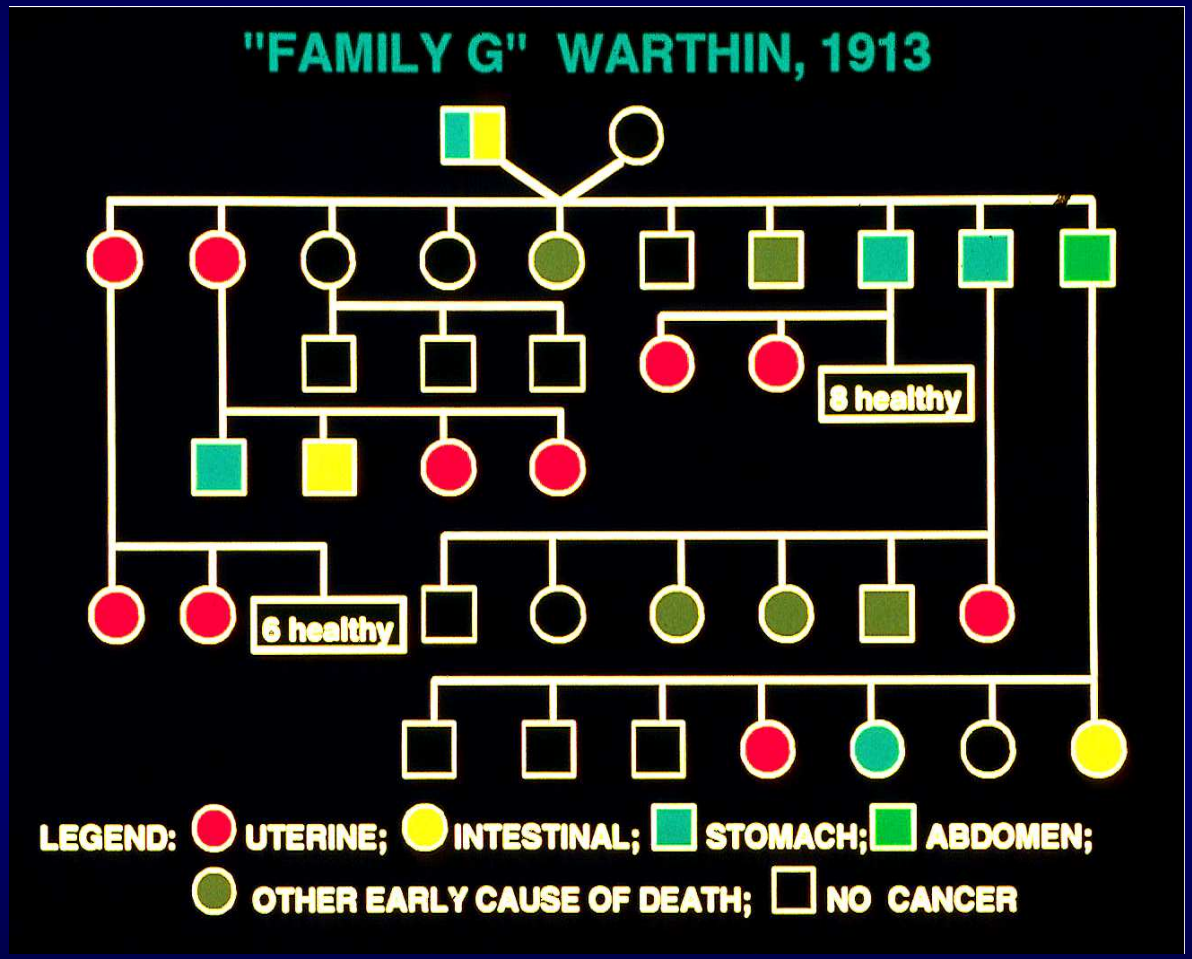
MSI & cancer of the mutator phenotype pathway

Defects in the DNA MMR system underlie a majority of hereditary non polyposis colon cancers (HNPCC),

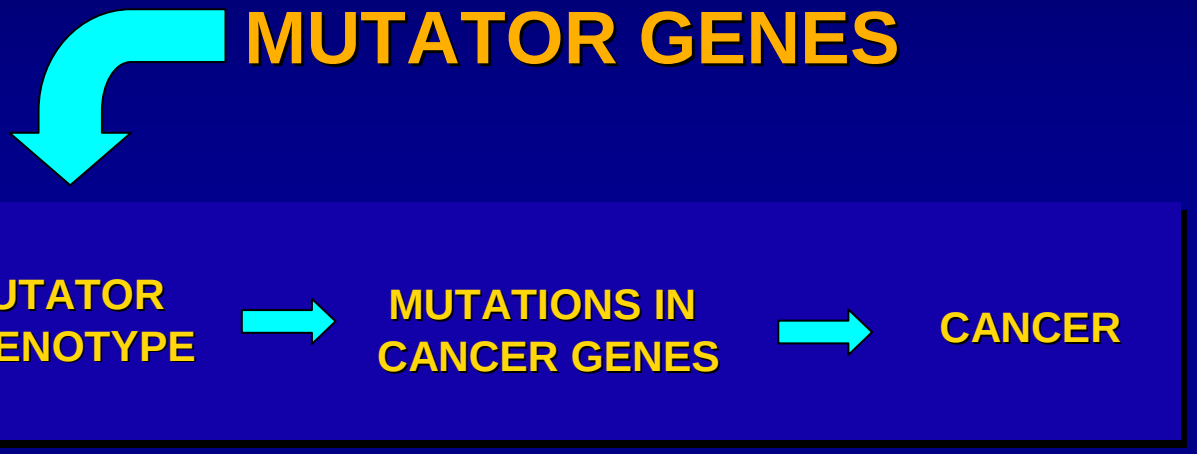


and a minority of sporadic colon tumors and other tumors from the gastrointestinal tract.

HNPCC (Lynch syndrome) represents the most common hereditary cancer syndrome



MICROSATELLITE INSTABILITY DISCLOSED THE EXISTENCE OF A REMOTE CONTROL MECHANISM FOR CANCER DEVELOPMENT



A MUTATOR GENE IS NOT

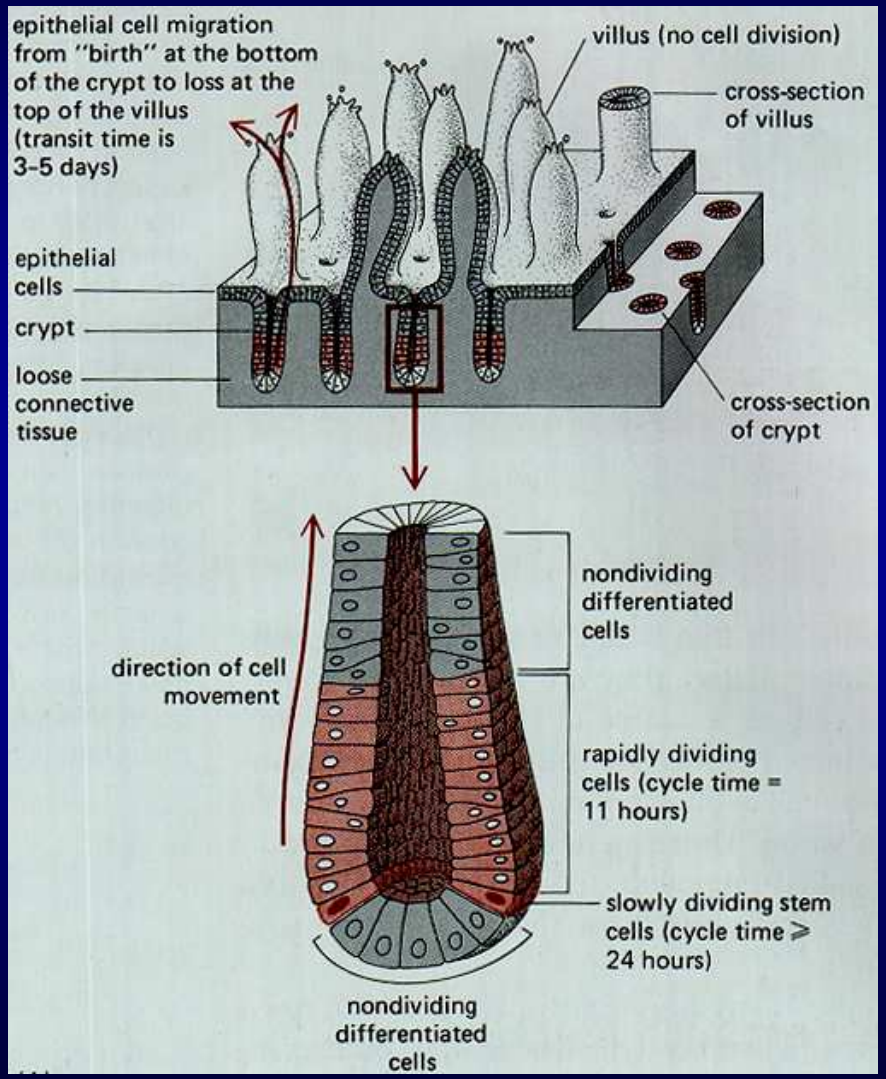
AN ONCOGENE

IT DOES NOT CONFER A NEOPLASTIC PHENOTYPE,
ONLY MAY CONFER A MUTATOR PHENOTYPE

A TUMOR SUPPRESSOR

IT DOES NOT SUPPRESS THE NEOPLASTIC PHENOTYPE,
ONLY SUPPRESSES GENOME DISINTEGRATION

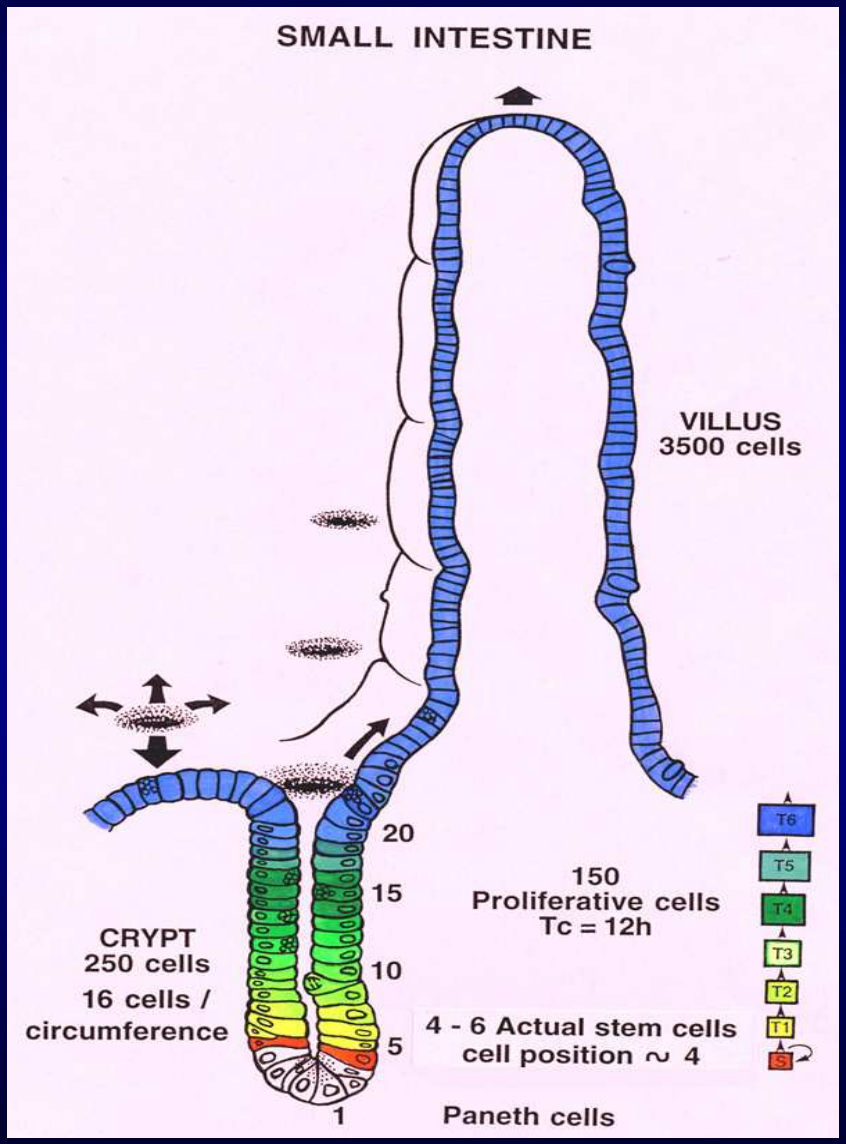
Introduction: Discovery of microsatellite instability by AP-PCR fingerprinting.

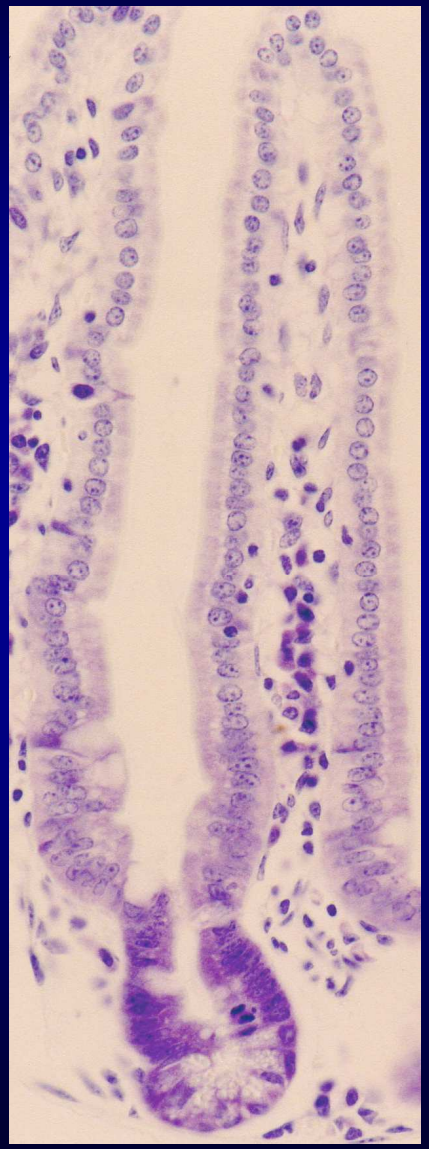


The simplest hypothesis to explain the staggering amount of clonal mutations in these tumors is that the mutator mutation occurs in a *stem cell* of the colon crypts *before* transformation

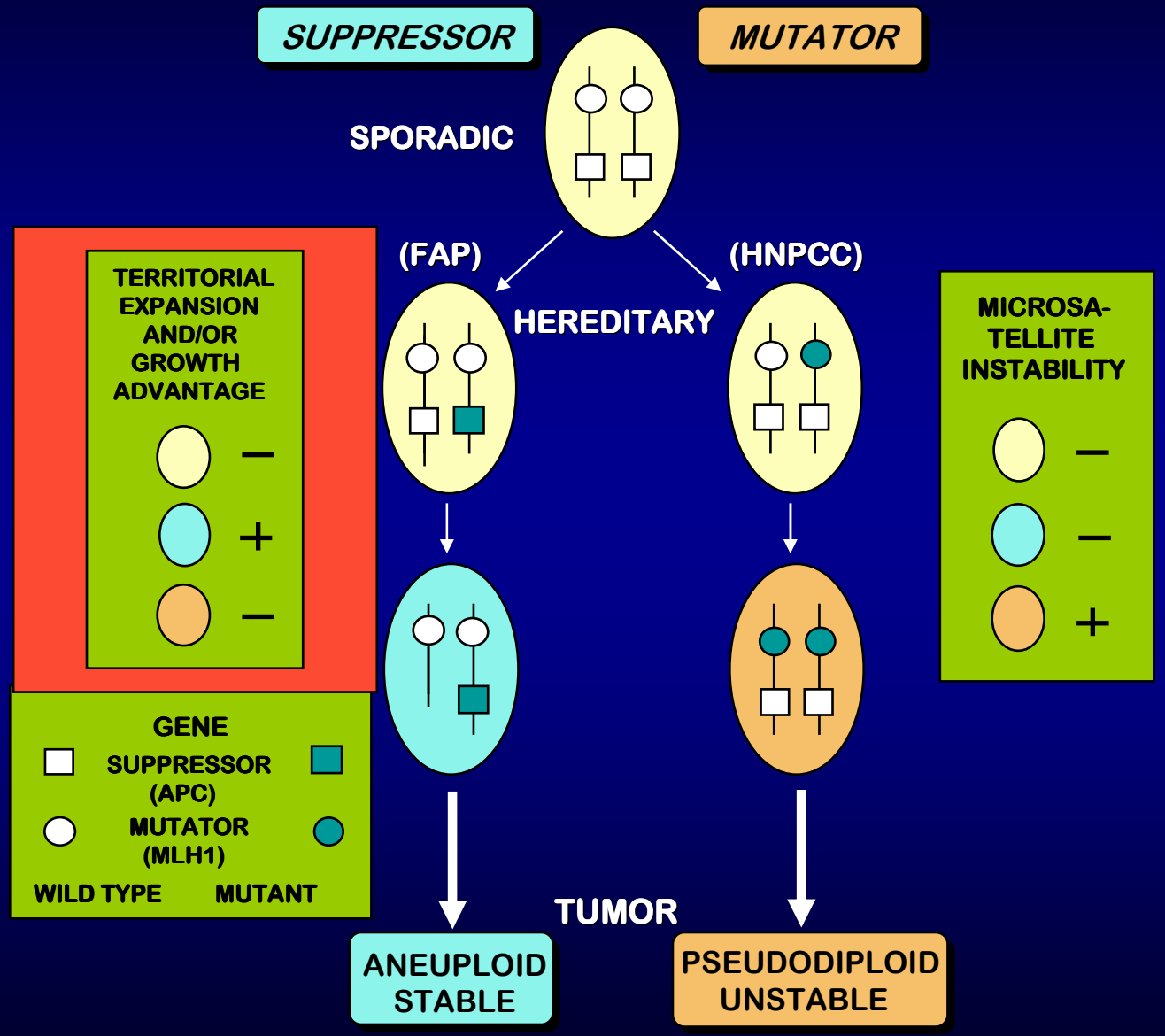
This hypothesis is also based on the assumption that mutator mutations do not confer growth advantage.

Crypt Stem Cell Lineage



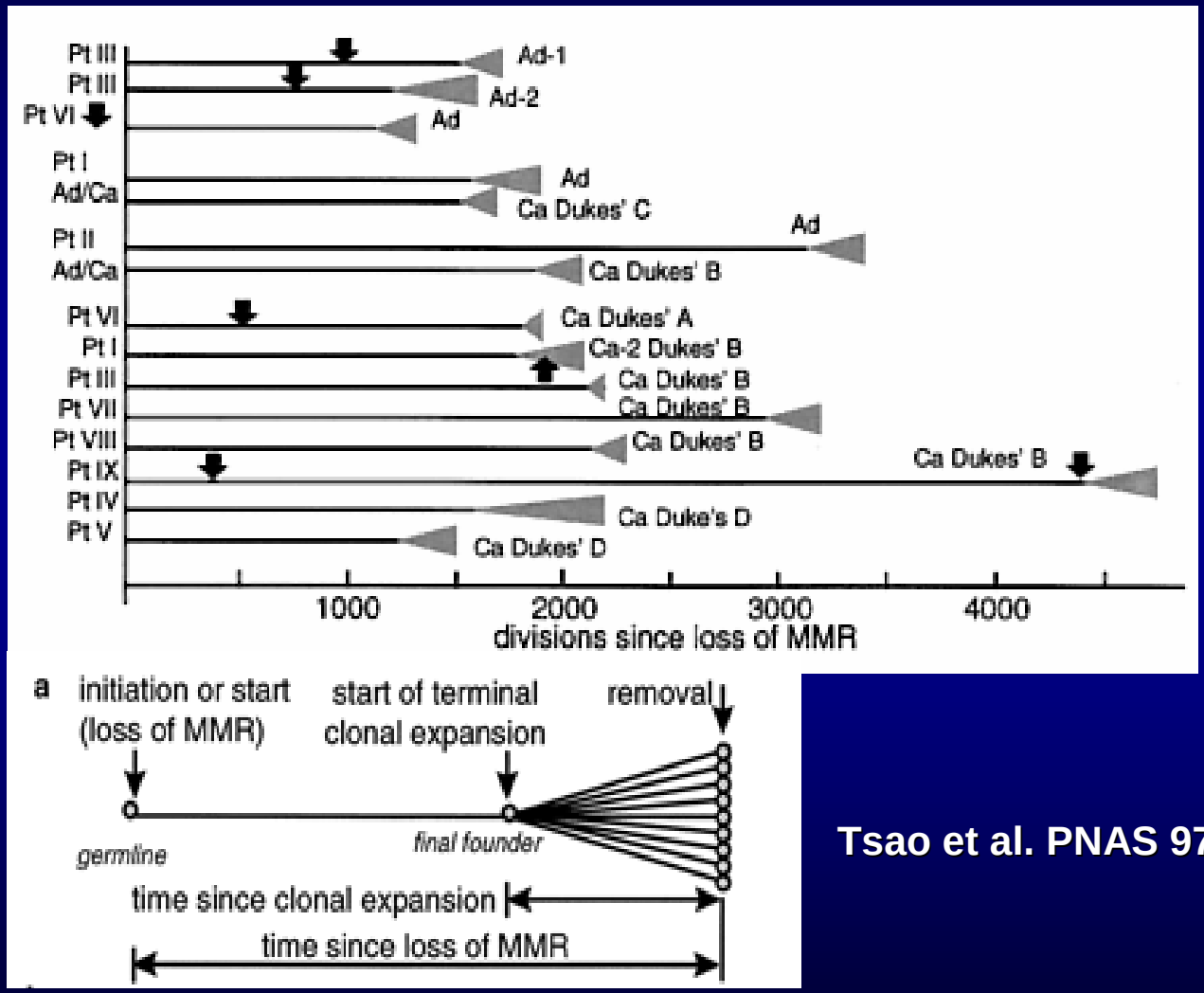


MOLECULAR GENETIC PATHWAYS FOR COLON CANCER



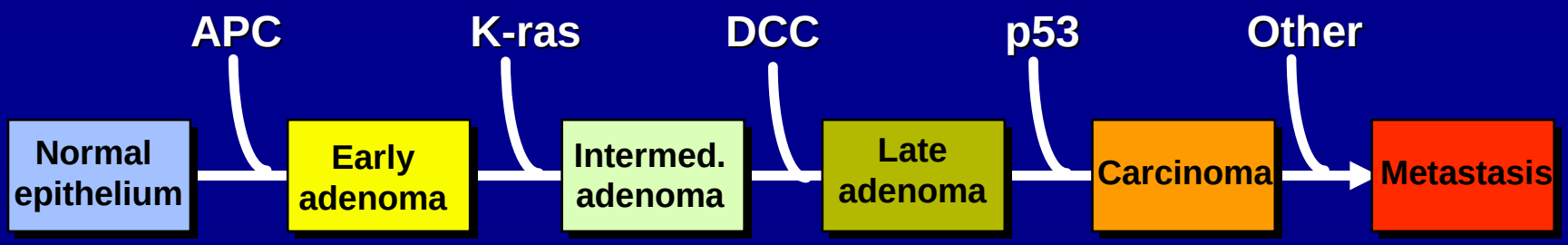
Perucho et al., CSHSQB. 59, 339, 1994

Genetic reconstruction of individual colorectal tumor histories.



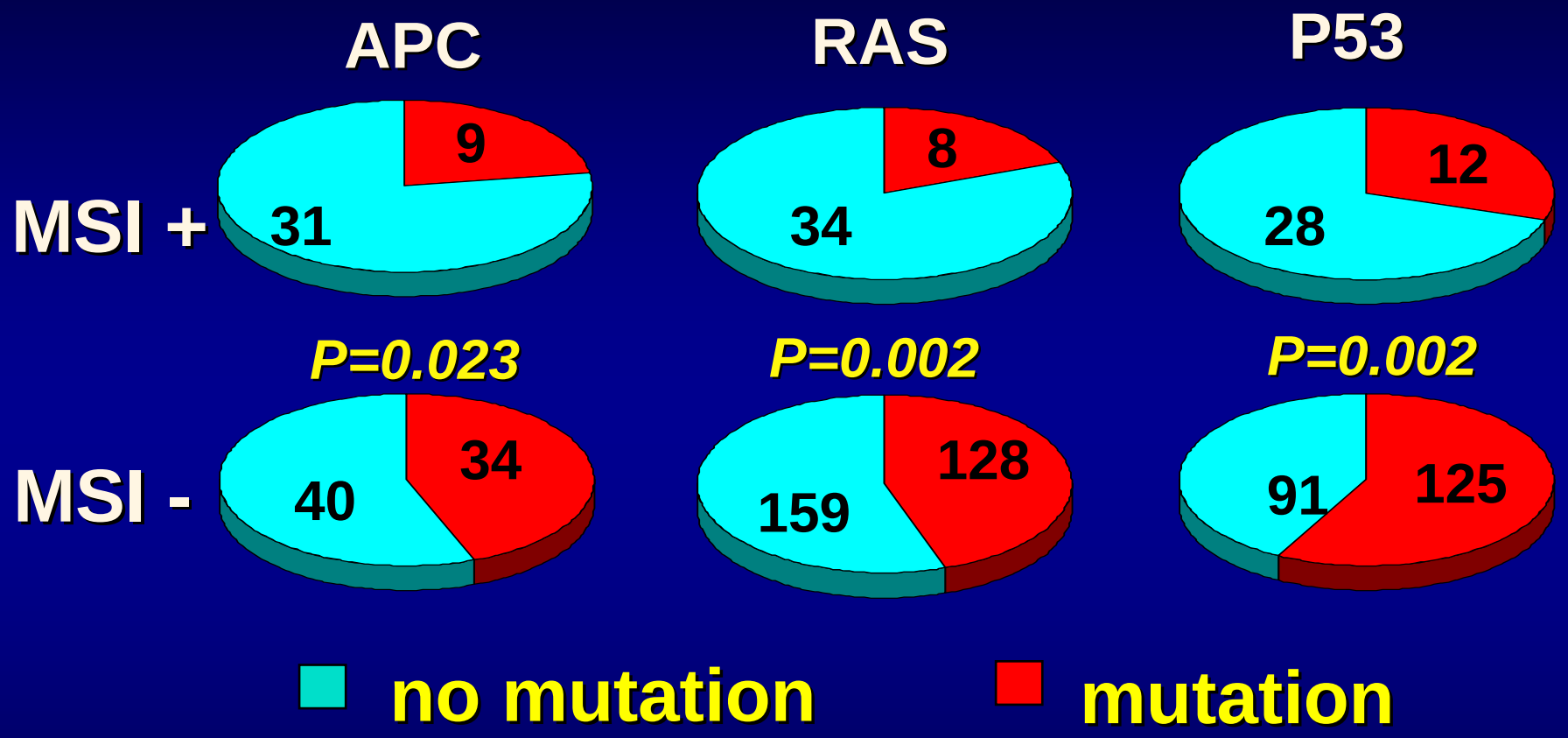
Tsao et al. PNAS 97:1236-41, 2000

THE MOLECULAR GENETICS OF COLON CANCER



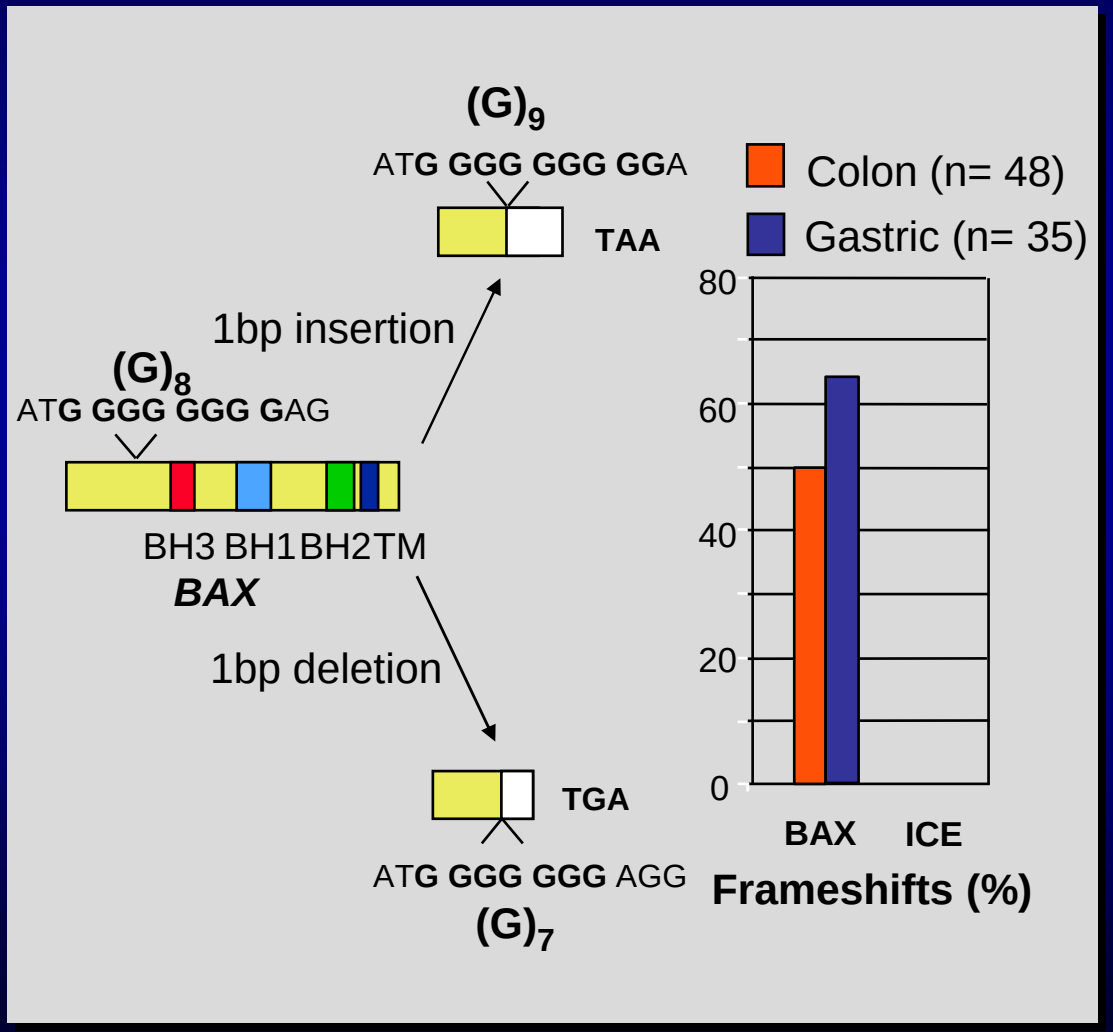
Fearon & Vogelstein. A Genetic model for colorectal tumorigenesis. Cell, 61, 759, 1990

Tumors with microsatellite instability have fewer mutations in the prototypical cancer genes for colon cancer

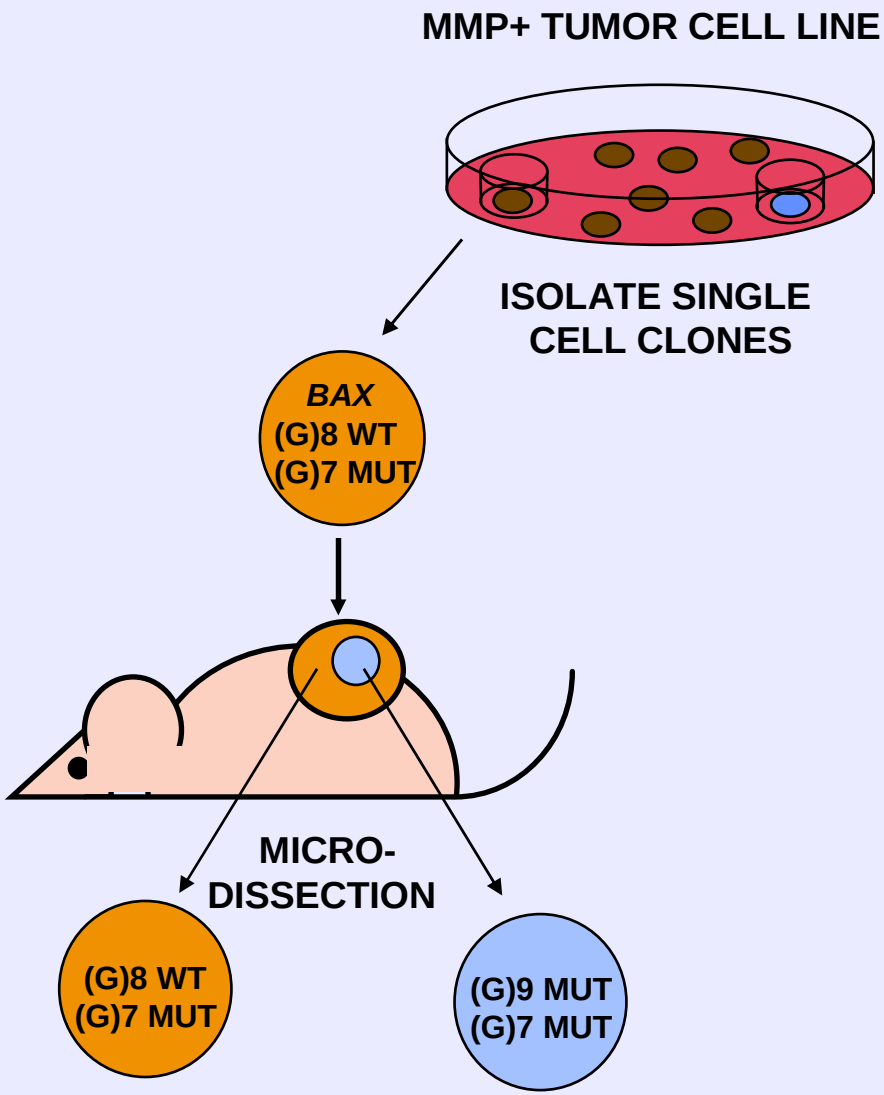


On the other hand, they harbor a large number of other mutated cancer genes in the same oncogenic networks (TGFβRII, Bax, etc)

BAX mutations in gastrointestinal cancer of the microsatellite mutator phenotype pathway

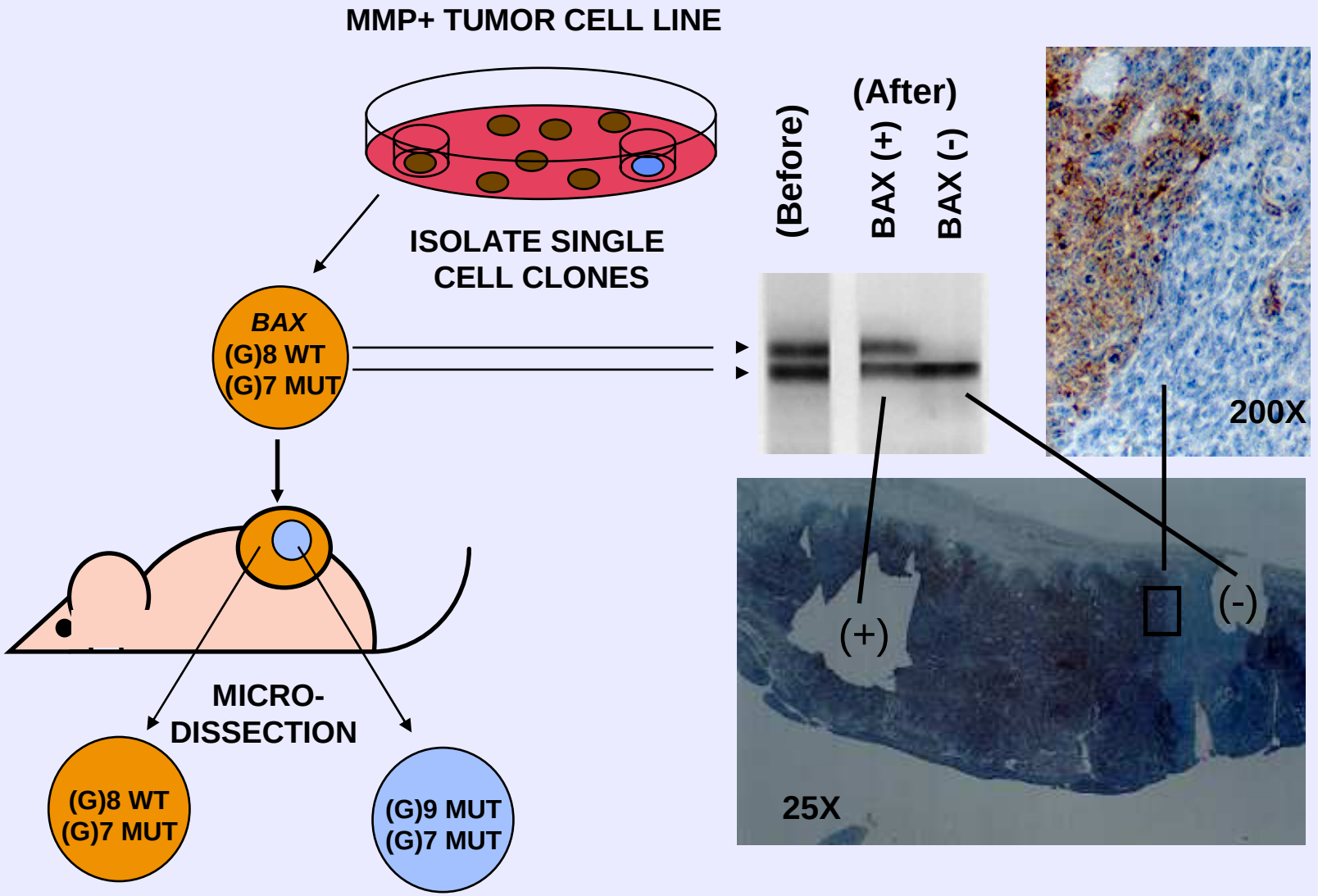


In vivo selection for *BAX* mutational inactivation



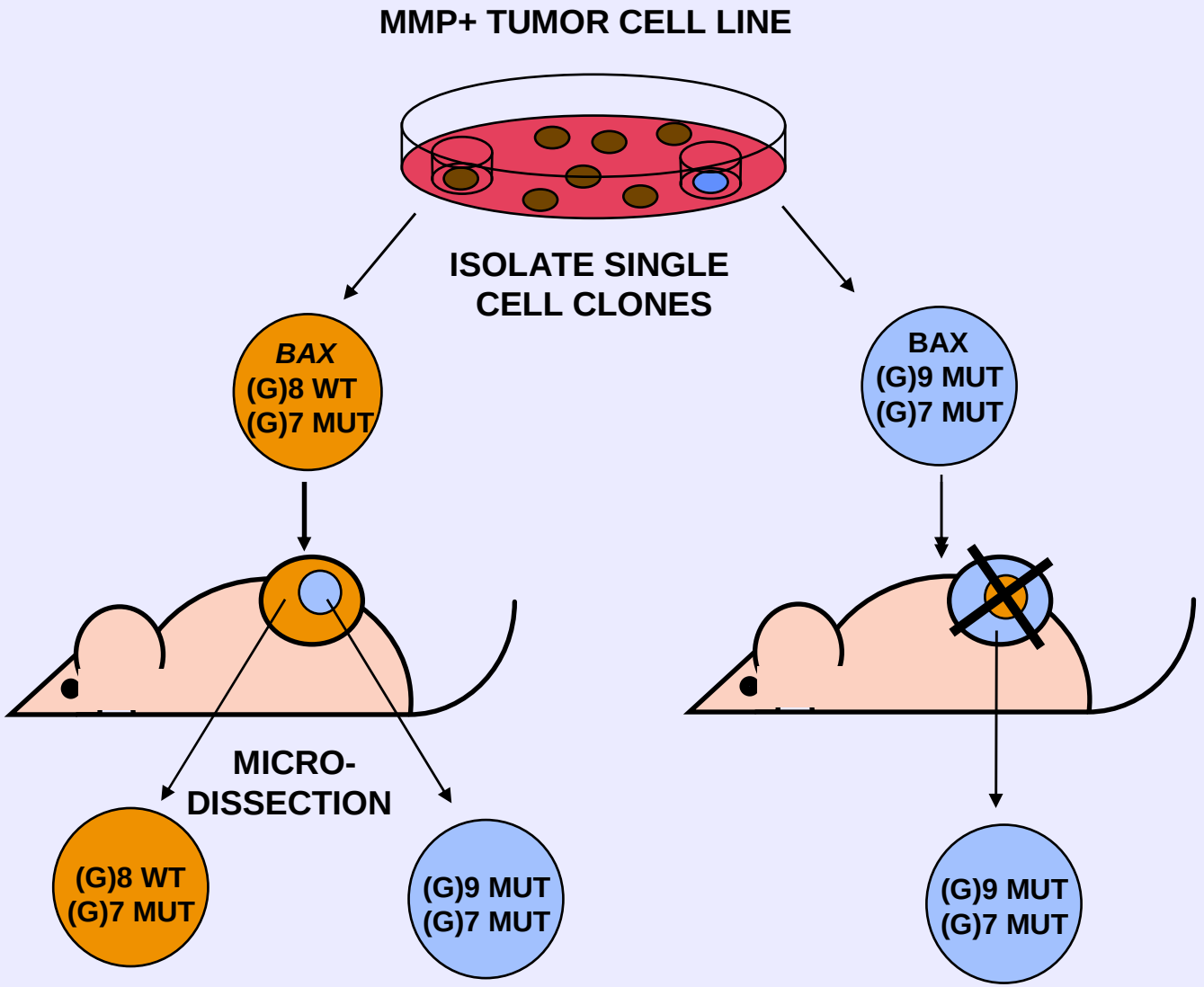
Y. Ionov, H. Yamamoto, S. Krajewski, J. Reed & M. Perucho, PNAS 97: 10872 (2000)

In vivo selection for *BAX* mutational inactivation



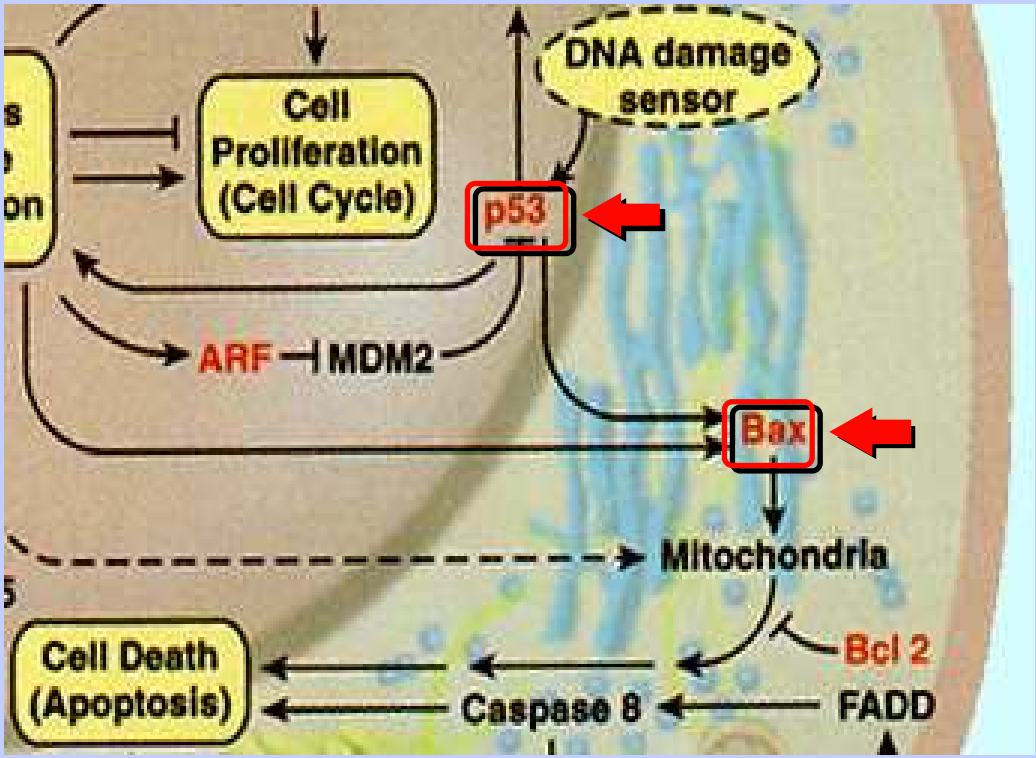
Y. Ionov, H. Yamamoto, S. Krajewski, J. Reed & M. Perucho, PNAS 97: 10872 (2000)

In vivo selection for *BAX* mutational inactivation



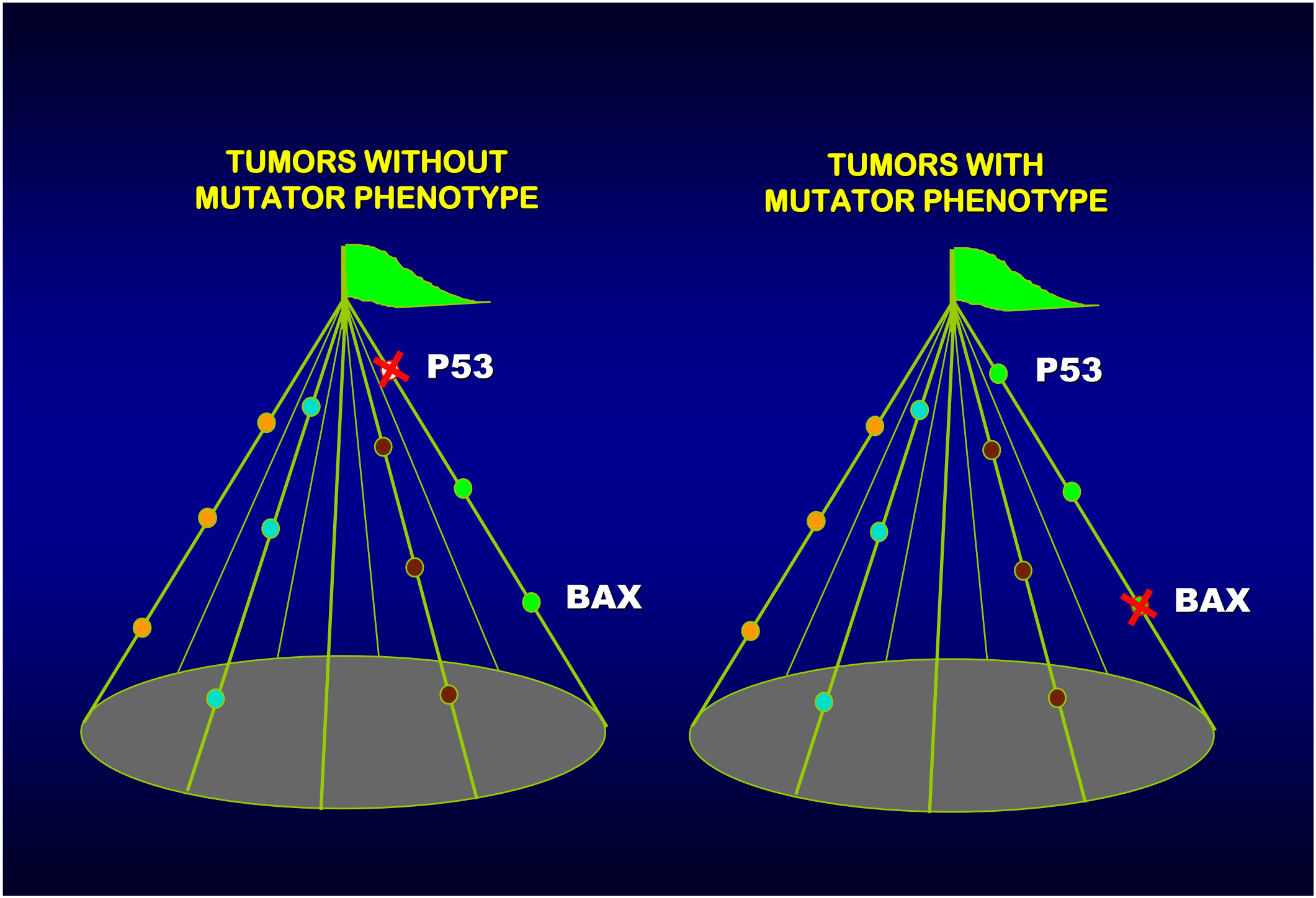
Y. Ionov, H. Yamamoto, S. Krajewski, J. Reed & M. Perucho, PNAS 97: 10872 (2000)

BAX MUTATIONS RELEASE THE PRESSURE FOR P53 MUTATIONS IN GASTROINTESTINAL CANCER OF THE MMP

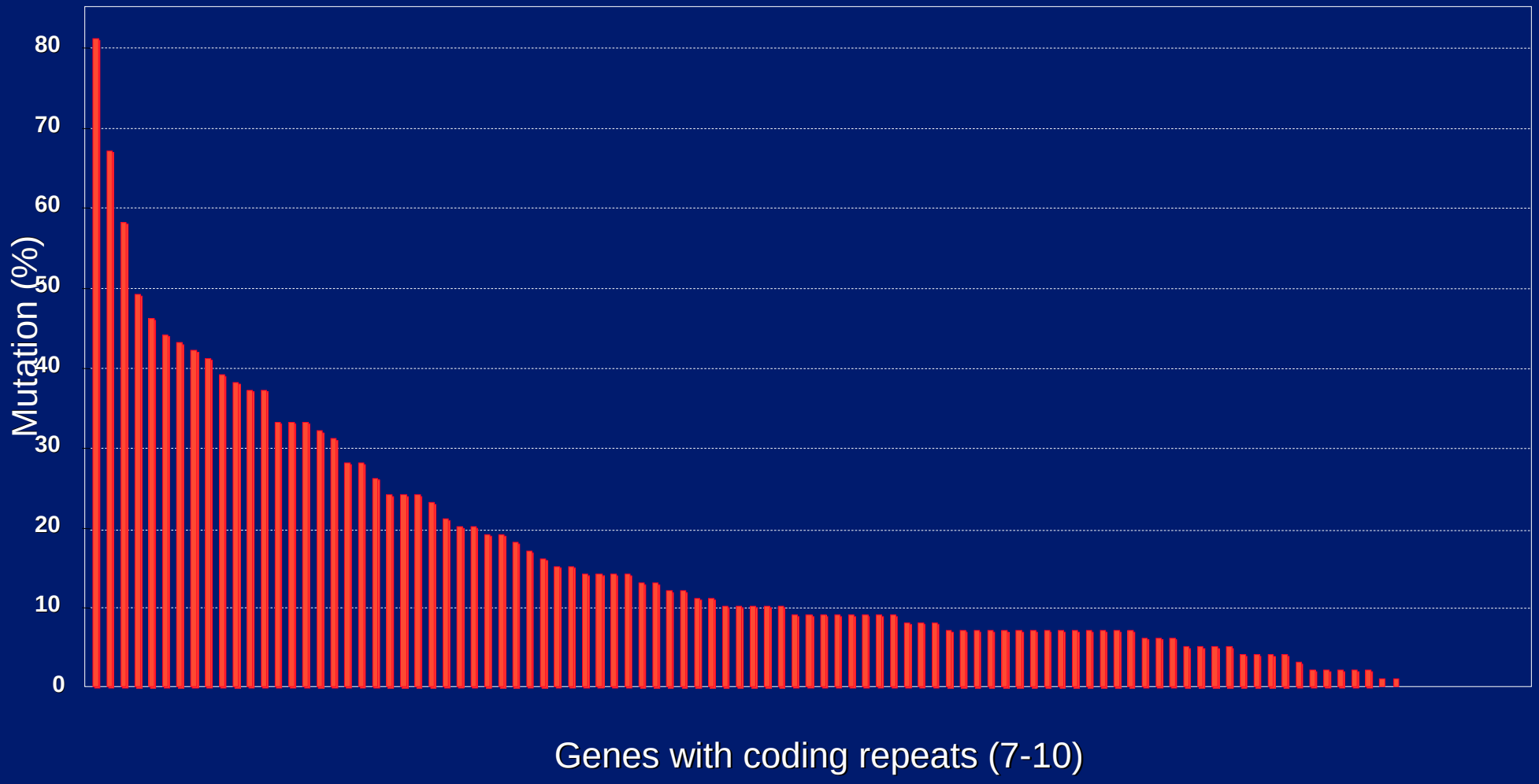


From Hanahan & Weinberg. Cell, 100, 57-70, 2000.

Once the mutator phenotype unfolds, mutations in hotspot repeats within some cancer genes (i.e. BAX) occur sooner than in other cancer genes without these repeats (i.e. p53)


TUMORS WITHOUT MUTATOR PHENOTYPE
P53
BAX
TUMORS WITH MUTATOR PHENOTYPE
P53
BAX

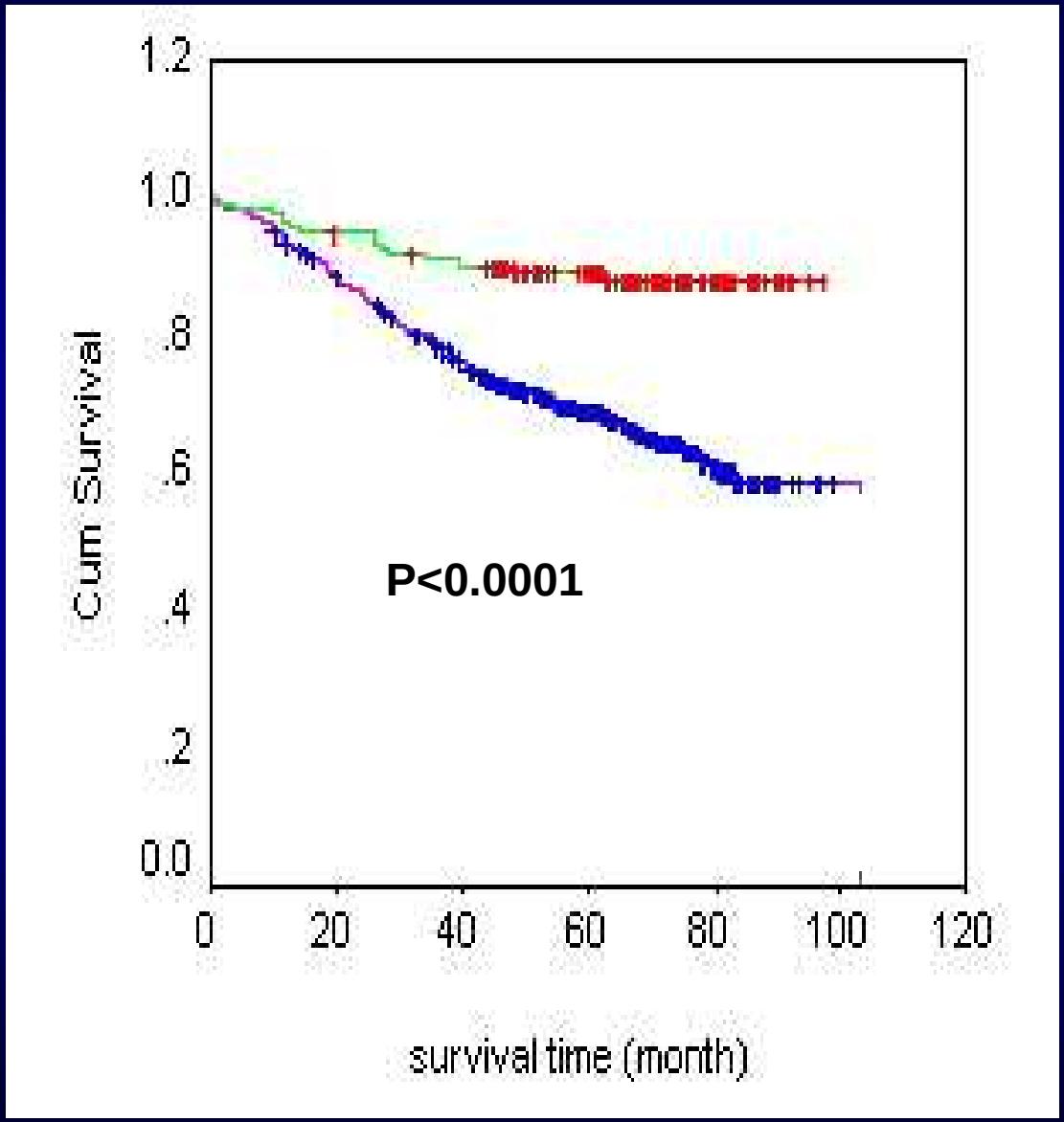
MUTATED TARGET GENES IN COLON CANCER OF THE MICROSATELLITE MUTATOR PHENOTYPE



Woerner *et al*. Oncogene. 2003.

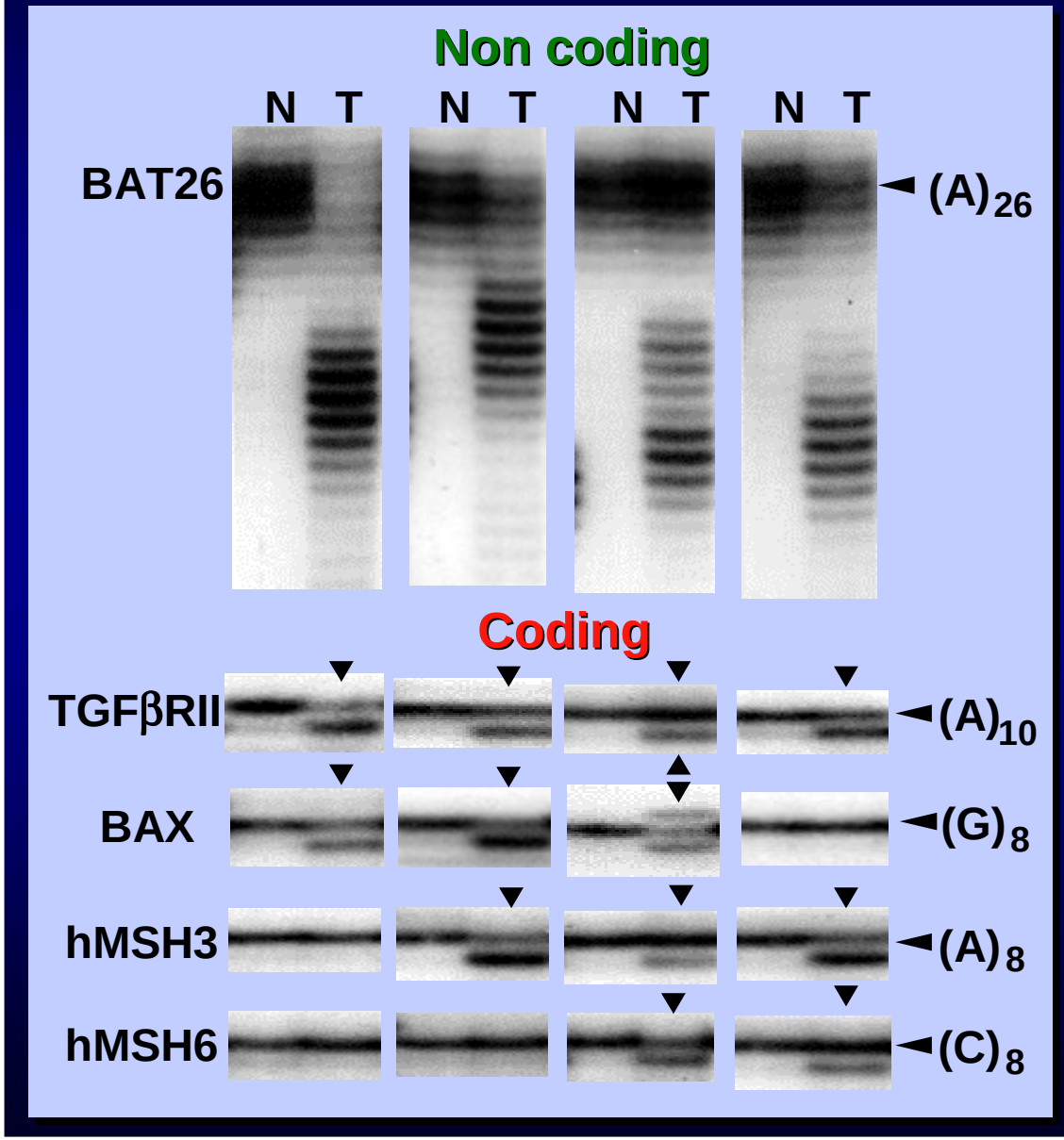
MSI & survival of 714 stage II & III colorectal cancers

Tumors with MSI are very different in genotype & phenotype relative to tumors without MSI



These differences include patient survival

BIALLELIC AND MONOALLELIC MUTATIONS IN CANCER OF THE MUTATOR PATHWAY

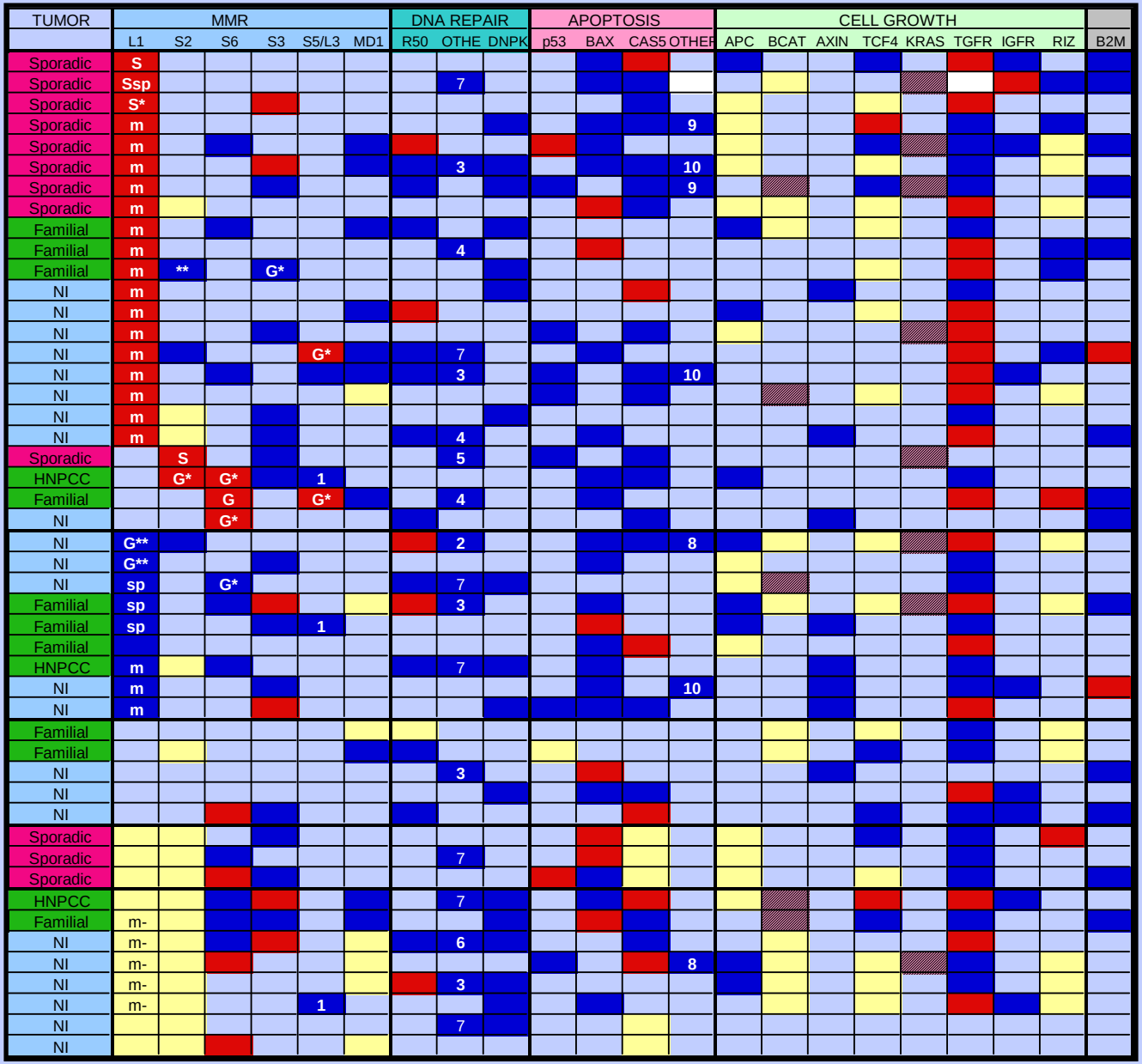


Tumor cells of the microsatellite mutator phenotype present another paradox:

They accumulate many **biallelic** mutations in neutral (non coding) sequences,

but also many **monoallelic** mutations in functional (coding) sequences.

ACCUMULATIVE HAPLOINSUFFICIENCY MODEL FOR CANCER OF THE MMP



BIALLELIC MUTATION

MONOALLELIC MUTATION

DOMINANT MUTATION

NOT ANALYZED

NO MUTATION

S TWO SOMATIC MUTATIONS

G GERMLINE+SOMATIC MUTATION

G GERMLINE MUTATION

* MISSENSE MUTATION/VARIANT

** AMINO ACID DELETION

sp SPLICING MUTATION

m METHYLATION +/- LOH

m- NEGATIVE FOR METHYLATION

1 *MLH3*

2 *BRCA1*

3 *HELICASE*

4 *ERCC5*

5 *BRCA2*

6 *BLOOM*

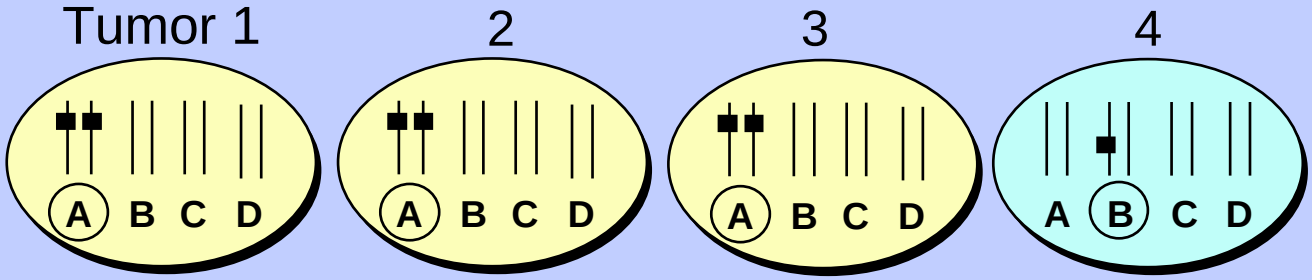
7 *ATR*

8 *FAS*

9 *APAF-1*

10 *BCL10*

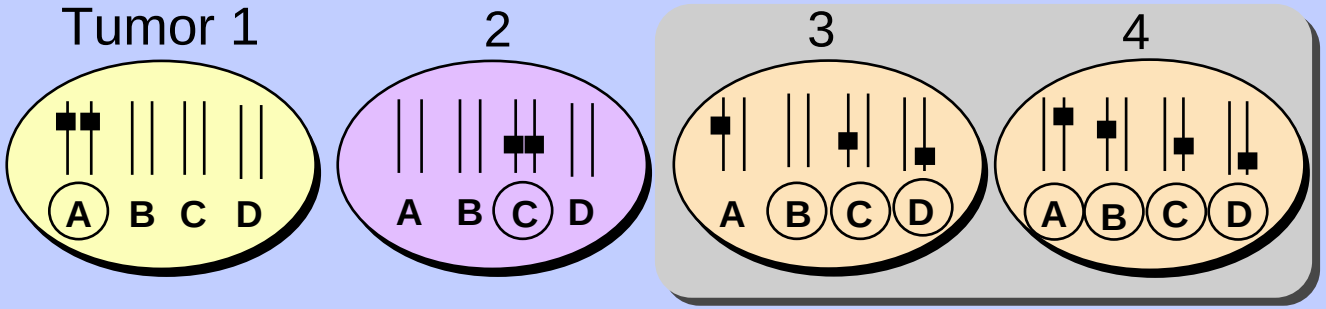
Tumors without mutator phenotype



Few mutated cancer genes: i.e., APC (A), β-catenin (B).
High mutation incidence of individual cancer genes
under strong selection during tumorigenesis (i.e., APC).
Biallelic mutations.

• • • •

Tumors with mutator phenotype



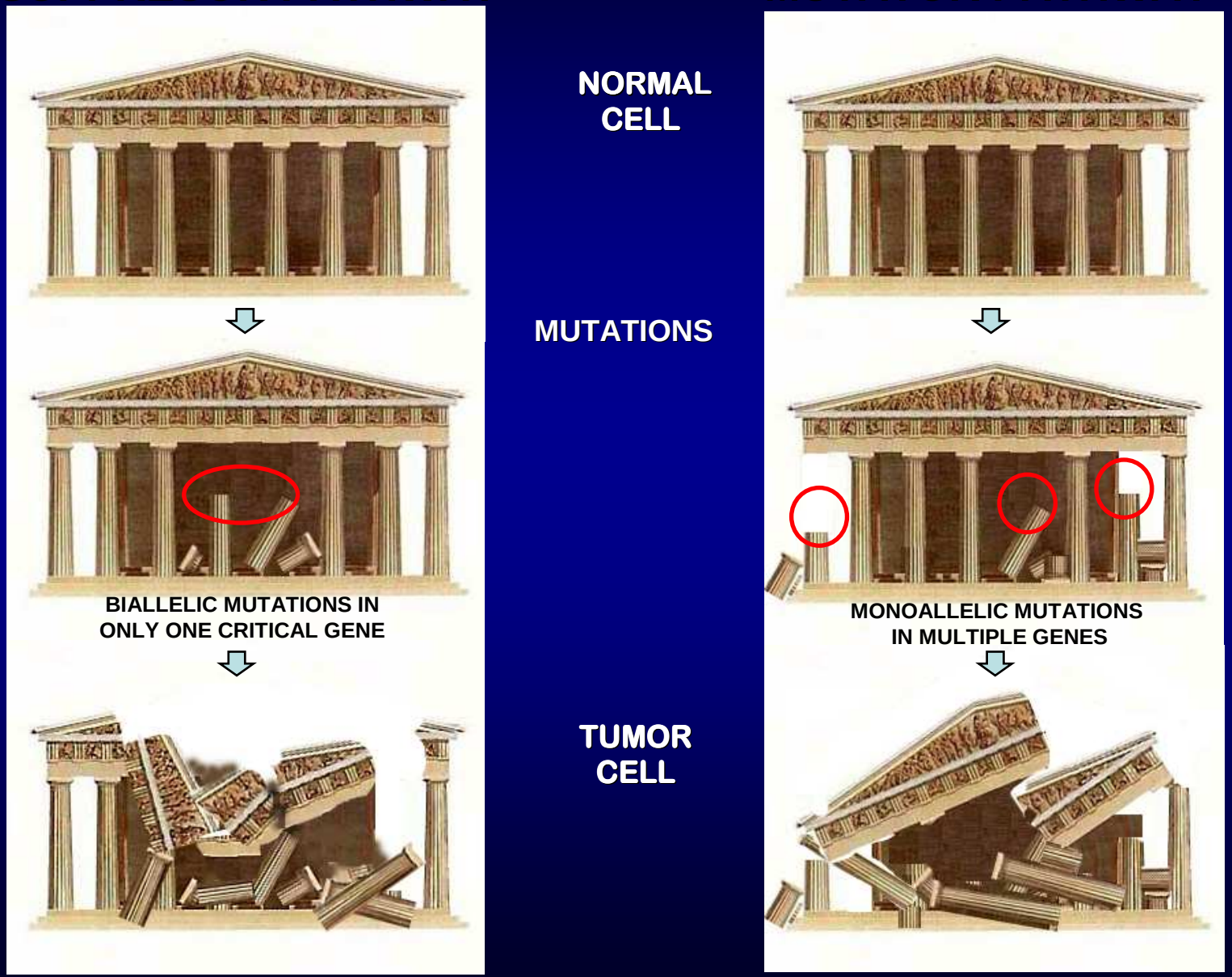
Several mutated cancer genes of the same network.
Low mutation incidence of each individual gene
i.e., APC (A), Axin (B), TCF-4 (C), etc.
Biallelic and monoallelic mutations.

A: APC
B: β-catenin
C: Axin
D: TCF-4
E: etc.

MODEL OF ACCUMULATIVE HAPLOINSUFICIENCY FOR COLON CANCER OF THE MICROATELLITE MUTATOR PHENOTYPE

SUPPRESOR PATHWAY

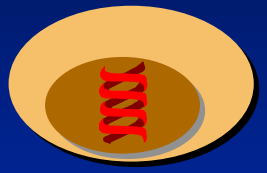
MUTATOR PATHWAY



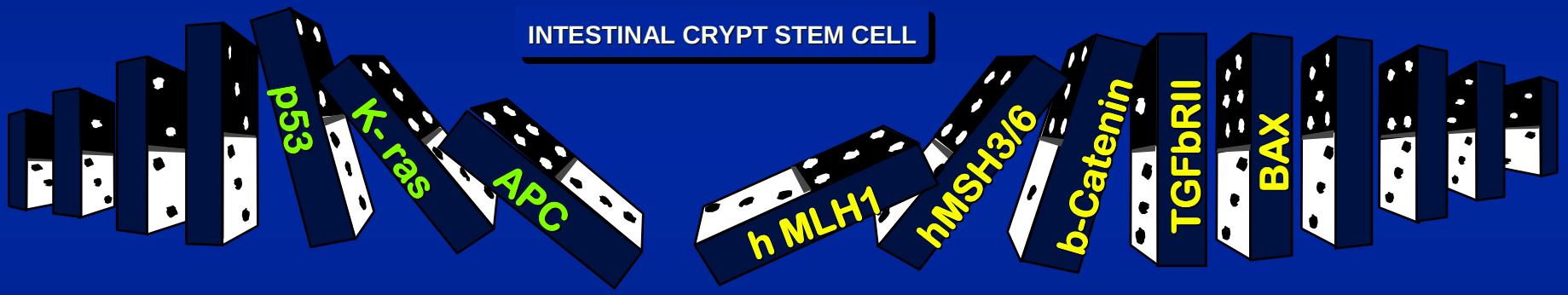
THE MICROSATELLITE MUTATOR PHENOTYPE PATHWAY FOR COLON CANCER

ALTERNATIVE MUTATIONAL PATHWAYS

SUPRESSOR PATHWAY



MUTATOR PATHWAY



Biallelic mutations in a few cancer genes without targets for the mutator phenotype

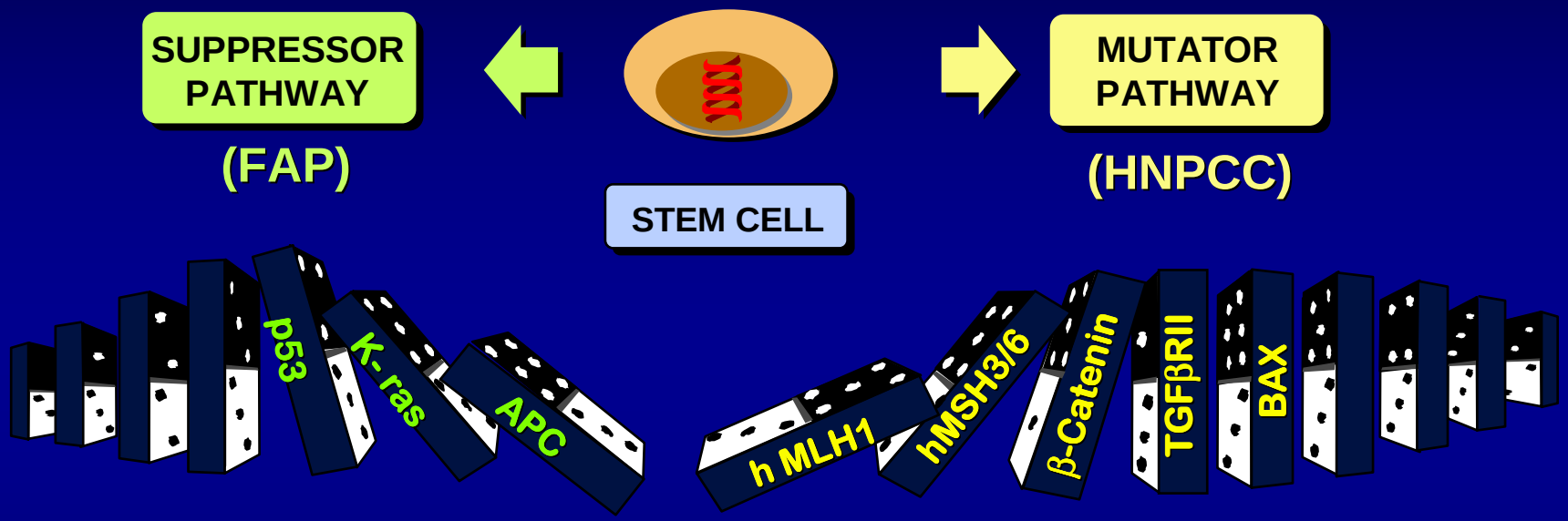
Initial rare genetic or epigenetic biallelic (sporadic) or monoallelic (hereditary) inactivation of suppressor (FAP) or mutator (HNPCC) gene.

Biallelic and monoallelic mutations in many cancer genes with targets for the mutator phenotype

Manuel Perucho. The Burnham Institute

General Motors Foundation, June 1999

ALTERNATIVE GENETIC PATHWAYS FOR COLON CANCER

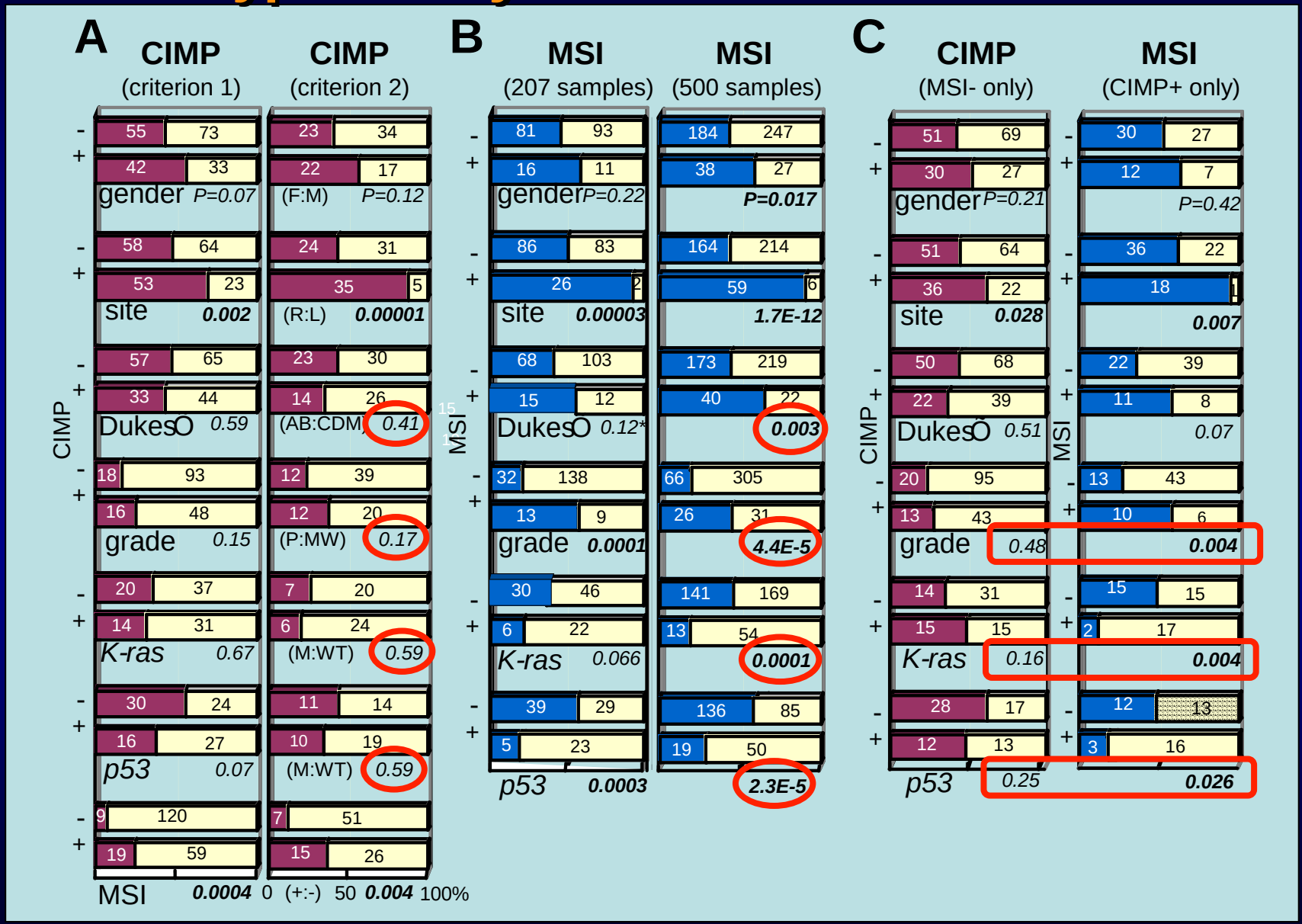


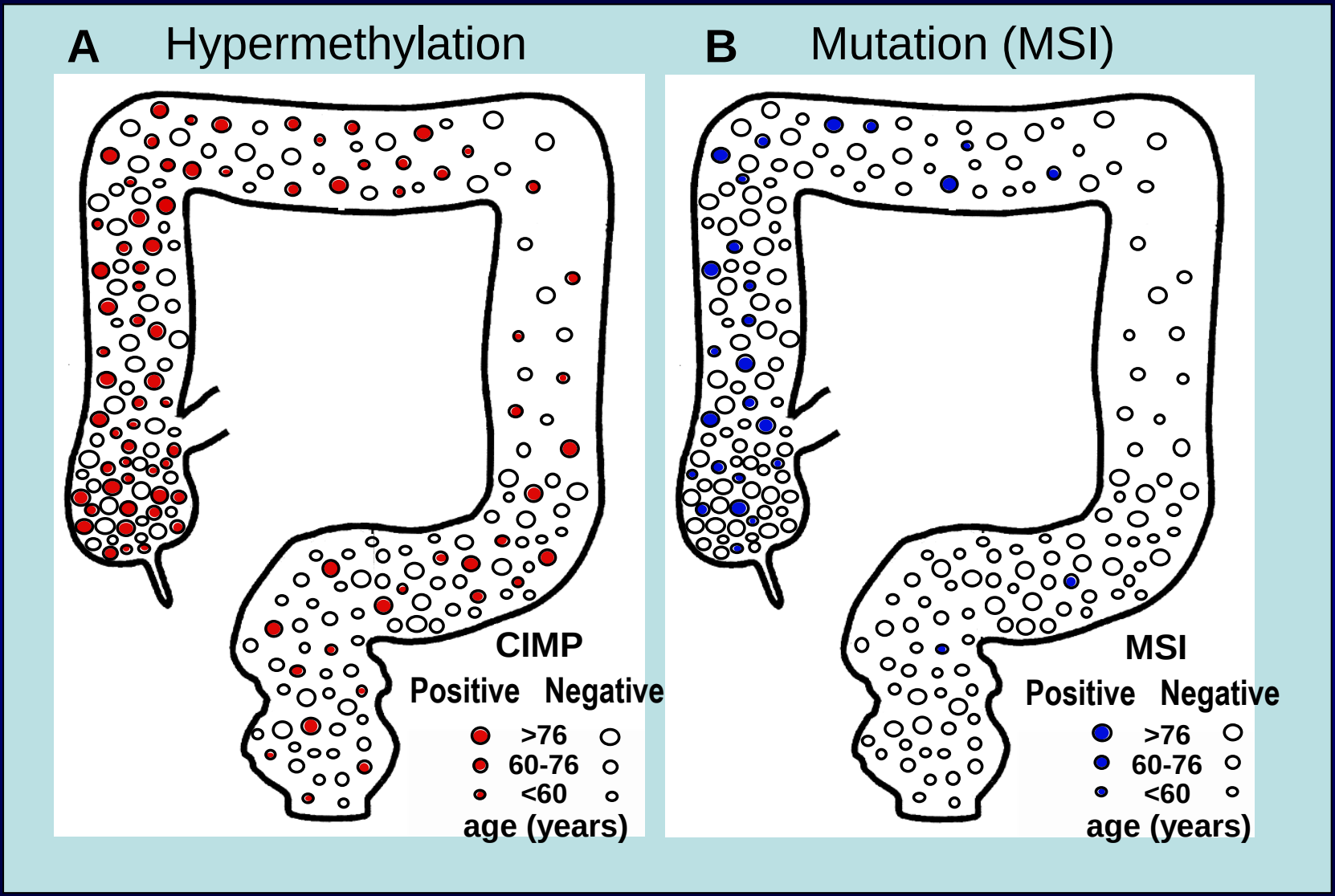
Mutations in cancer genes **without** targets for the mutator phenotype

Initial genetic or epigenetic biallelic (sporadic) or monoallelic (hereditary) inactivation of mutator or suppressor genes.

Mutations in cancer genes **with** targets for the mutator phenotype

Mutator phenotype (MSI) is dominant over DNA hypermethylation in colorectal cancer

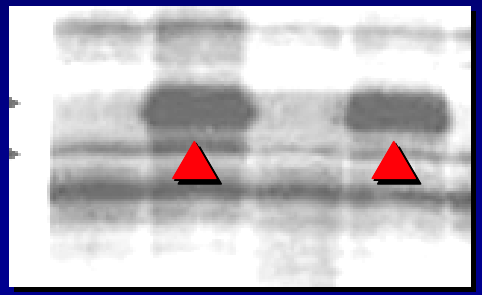


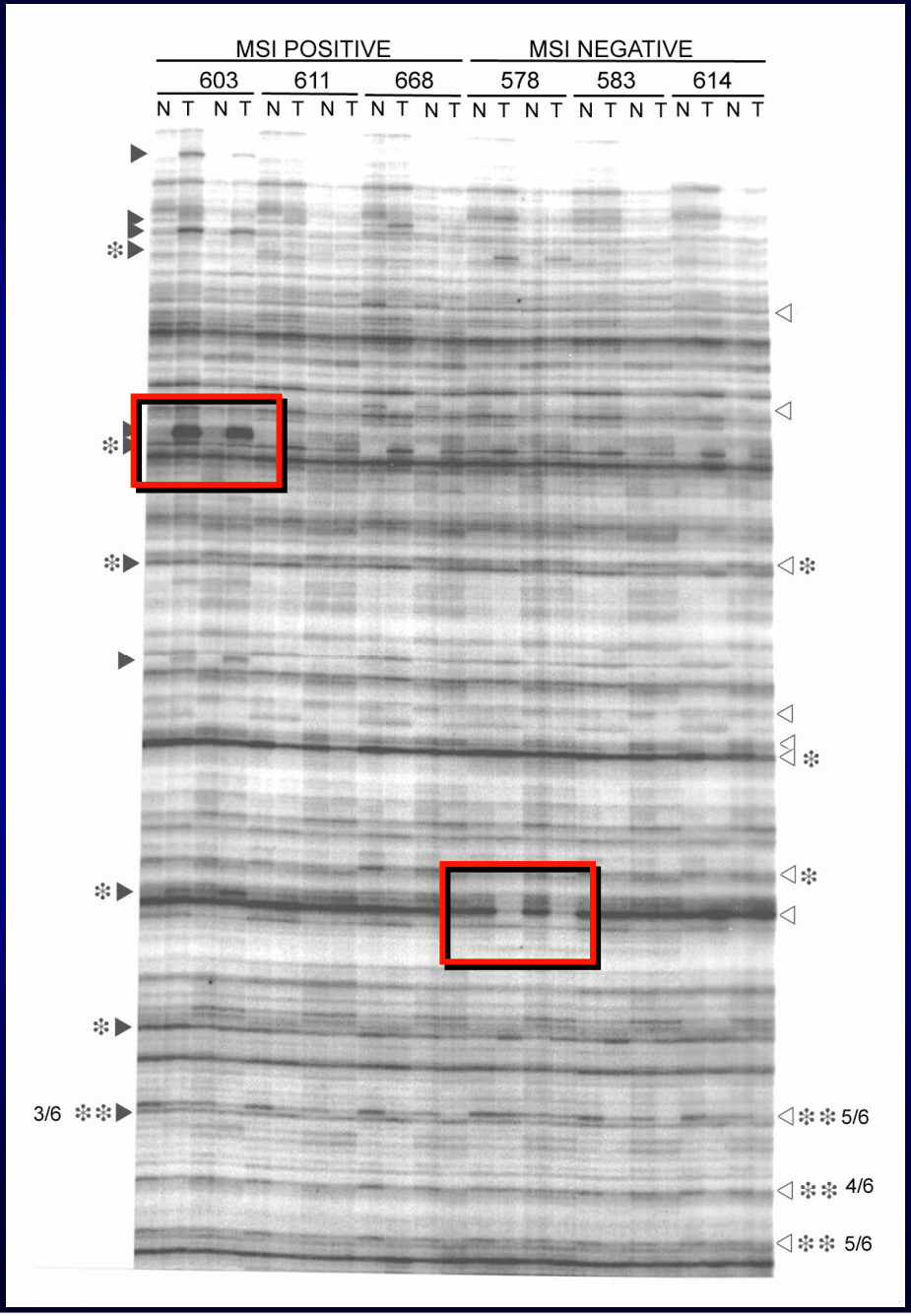

A Hypermethylation
B Mutation (MSI)
CIMP
Positive Negative
>76
60-76
<60
age (years)
MSI
Positive Negative
>76
60-76
<60
age (years)

DNA METHYLATION ALTERATIONS IN COLON CANCER DETECTED BY MS-AFLP

HYPOMETHYLATION

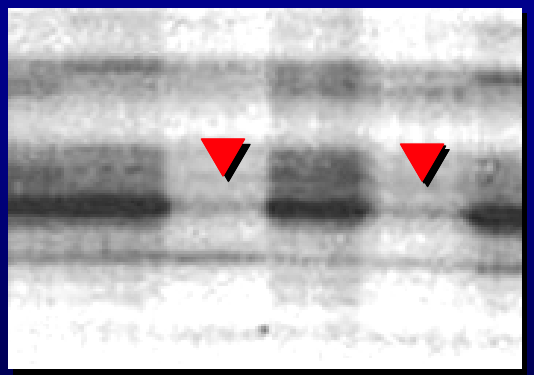
N T N T





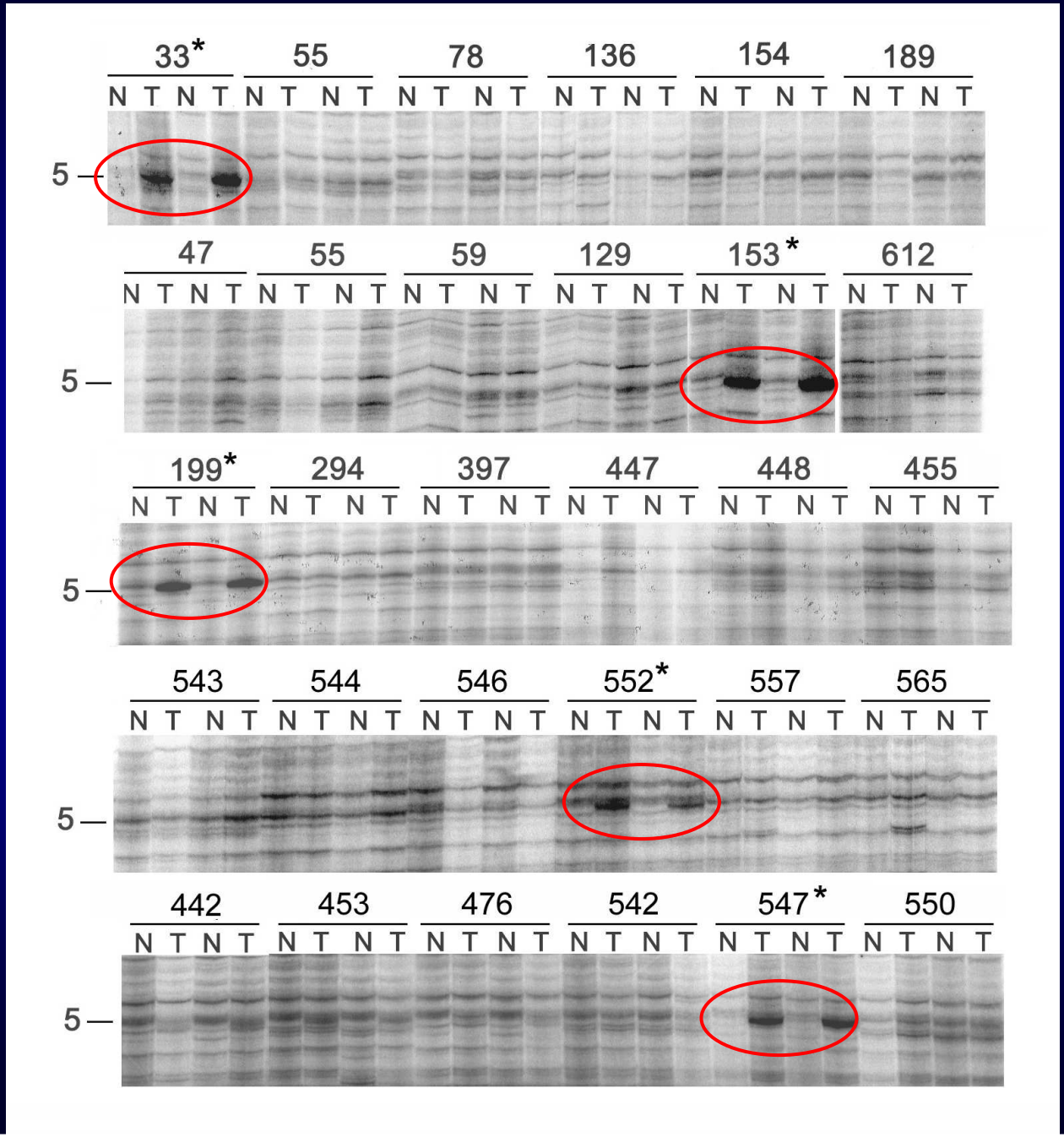
HYPERMETHYLATION

N T N T



Yamashita et al. Cancer Cell 4, 121-131, 2003.

Detection of hypomethylation alterations by MS-AFLP

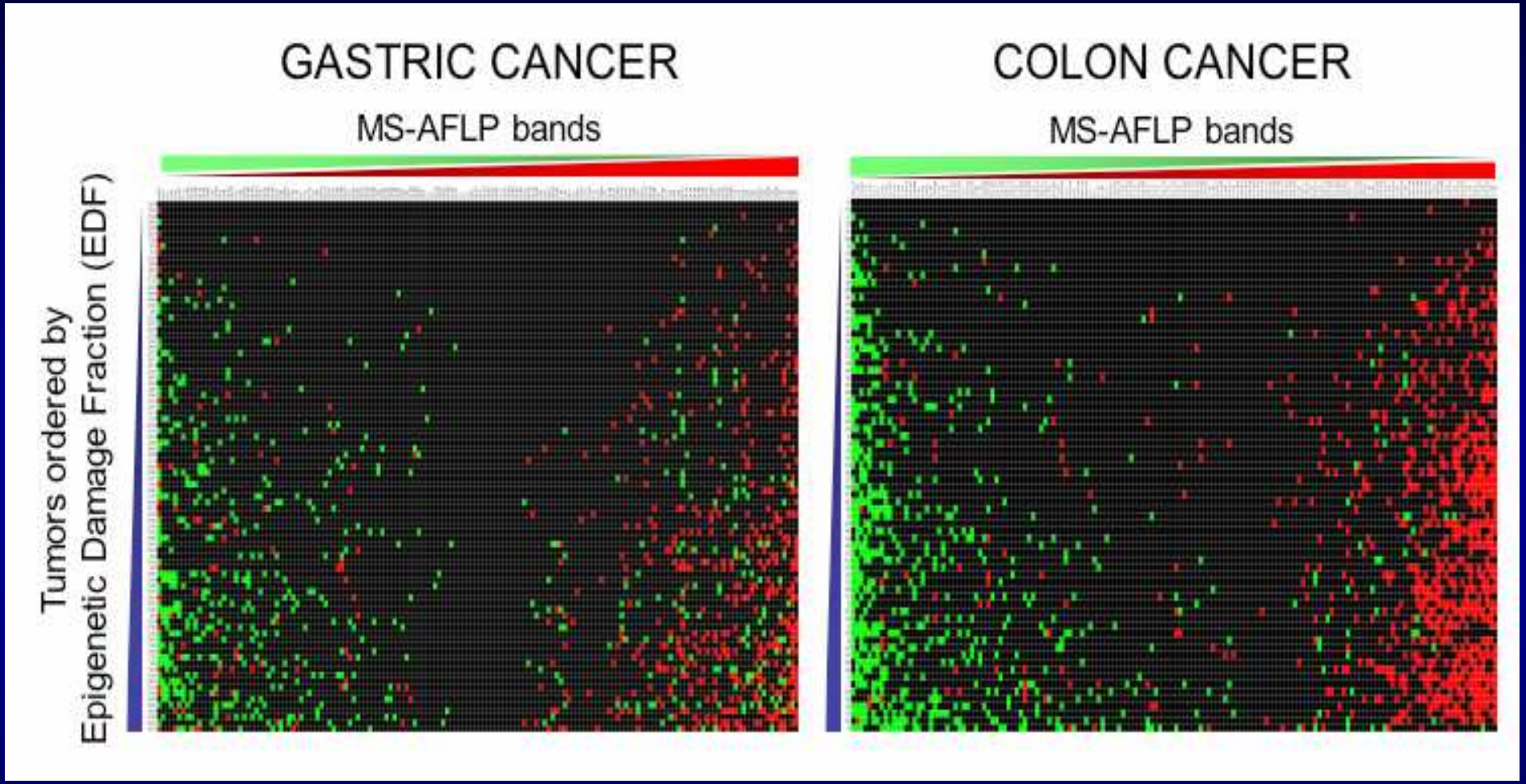


COLON

Hypermethylation
% of altered Not I sites
0
2
4
6
8
10
12
14
16
18
1 5 9 13 17 21 25 29 33 37 41 45 49 53 57 61 65 69 73
Tumors ordered by hypermethylation alteration

Hypomethylation
% of altered Not I sites
0.0
5.0
10.0
15.0
20.0
25.0
30.0
1 5 9 13 17 21 25 29 33 37 41 45 49 53 57 61 65 69 73
Tumors ordered by hypomethylation alteration

Distribution of methylation alterations in gastric & colon cancers



HYPOMETHYLATION

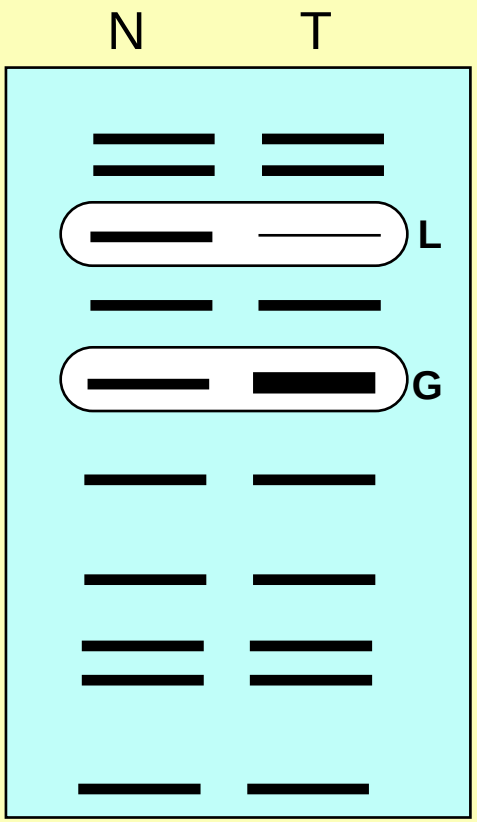
HYPERMETHYLATION

No evidence for bimodal distribution of somatic hypermethylation or hypomethylation alterations in colon and gastric cancer

CANCER PATHWAYS

SUPPRESSOR

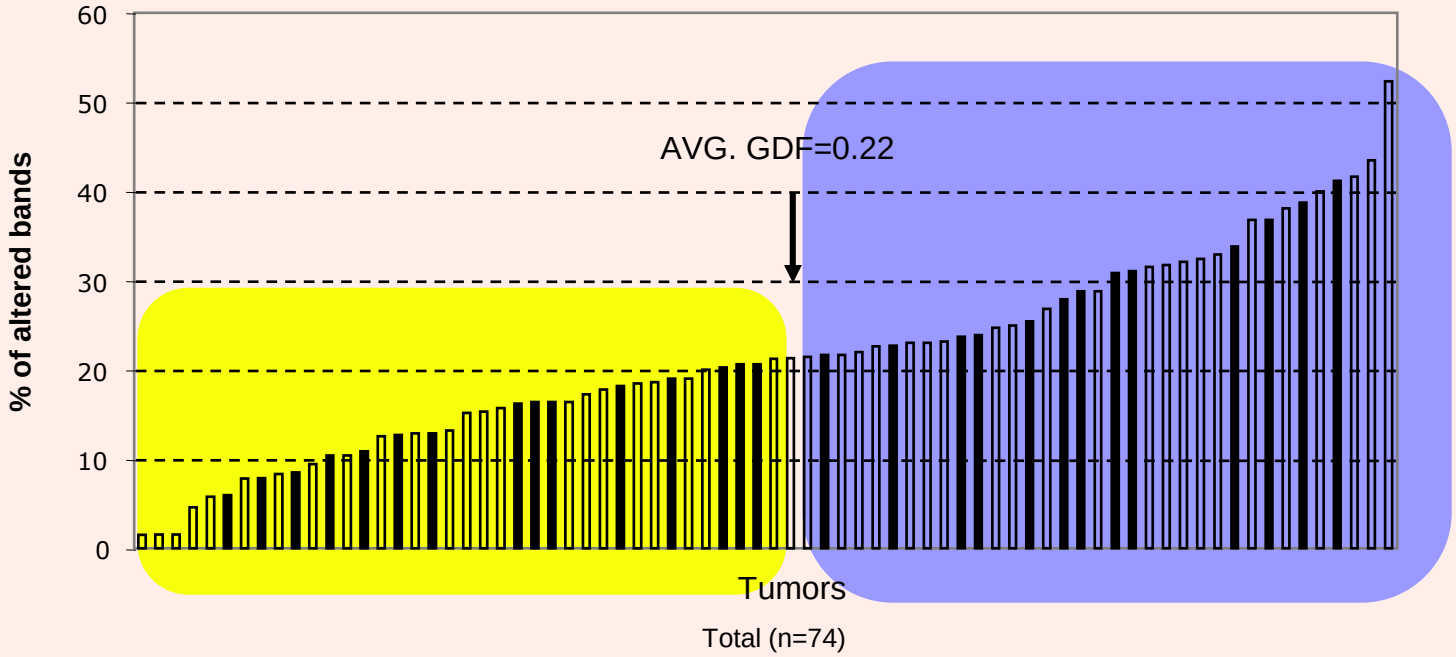
quantitative changes

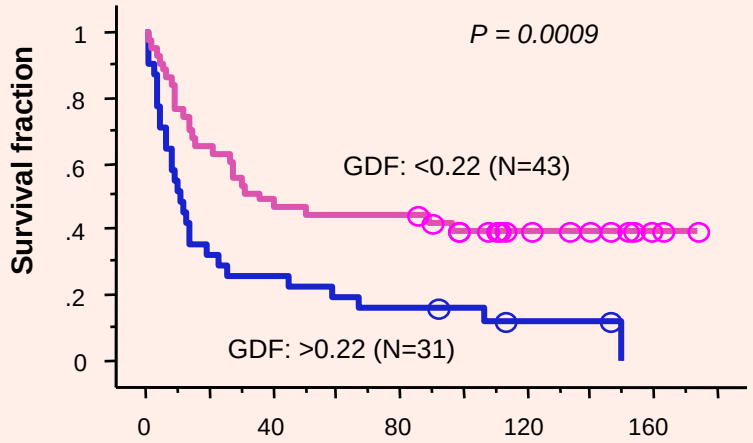


ANEUPLOID PHENOTYPE

(N: NORMAL; T: TUMOR; L: LOSSES; G: GAINS)

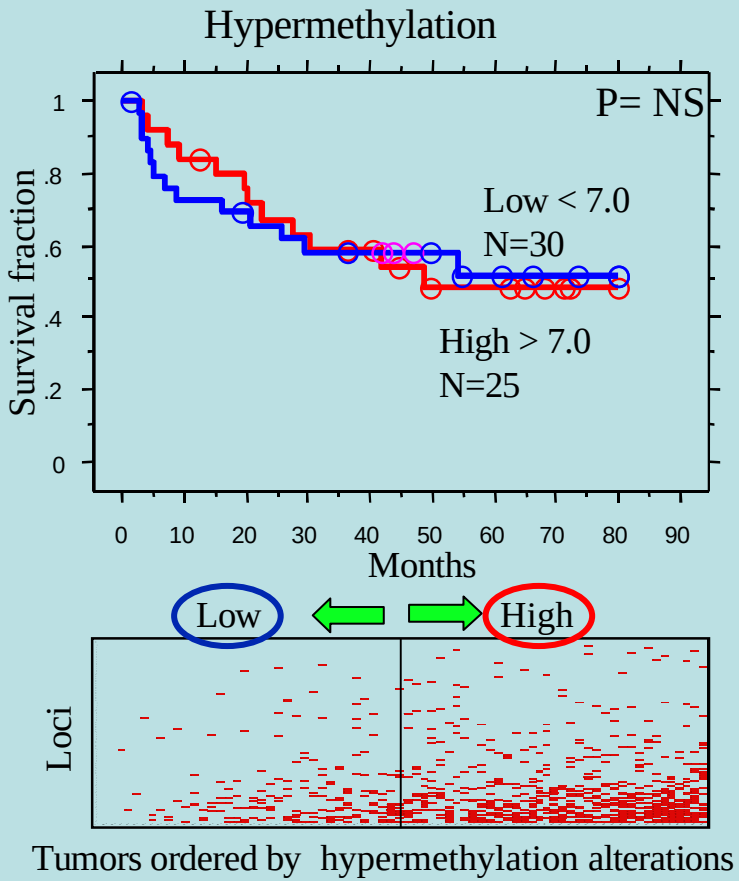
Genomic Damage Fraction (GDF) & gastric cancer survival

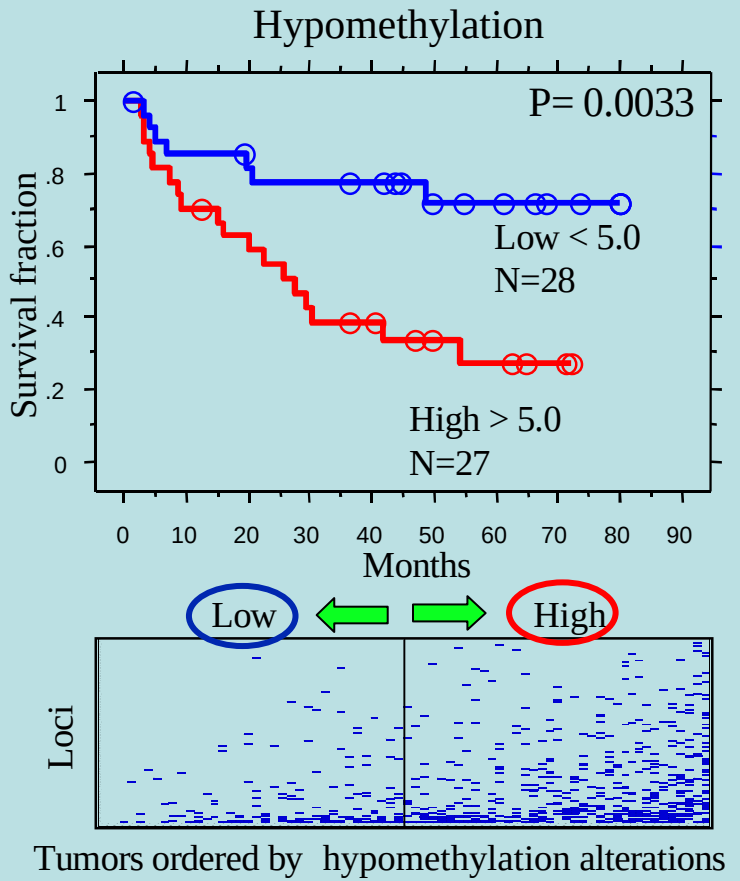




Suzuki et al. *Gastroenterology* 125, 1330-1340, 2003.

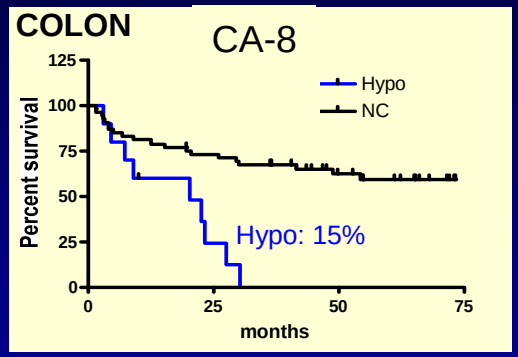
Methylation alterations and survival in colon cancer



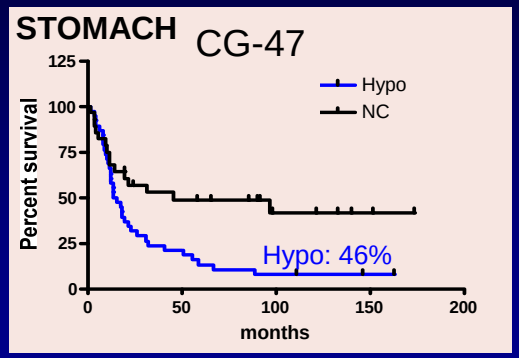


Survival according to methylation status of some of the most frequently altered MSAFLP bands.

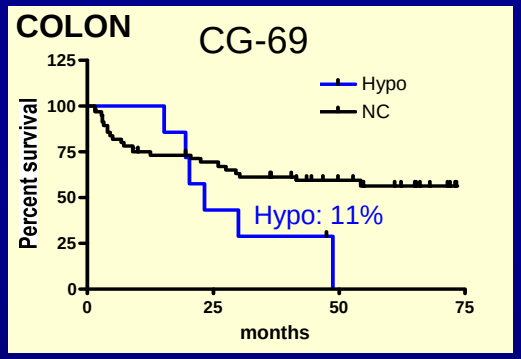
HYPOMETHYLATION



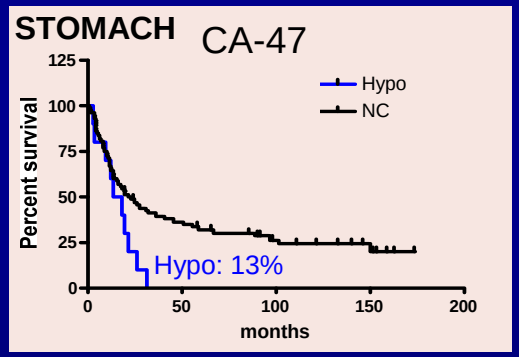
RAPGEF. ras activator.



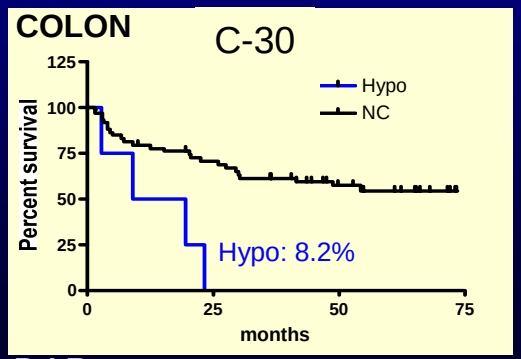
Multiple locations



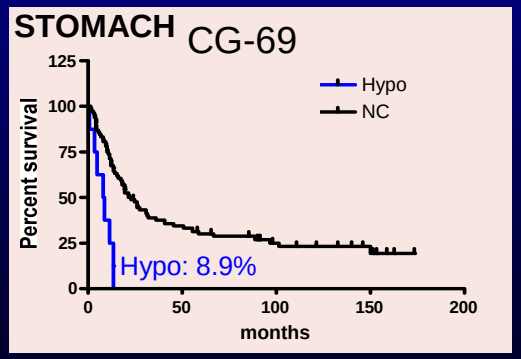
TUBGCP3. Gamma tubulin complex.



Unknown



DAP. Death associated protein.



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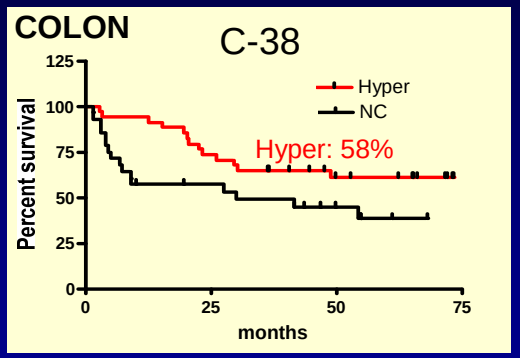
HYPERMETHYLATION



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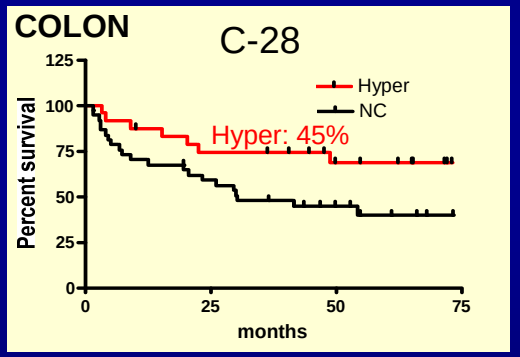
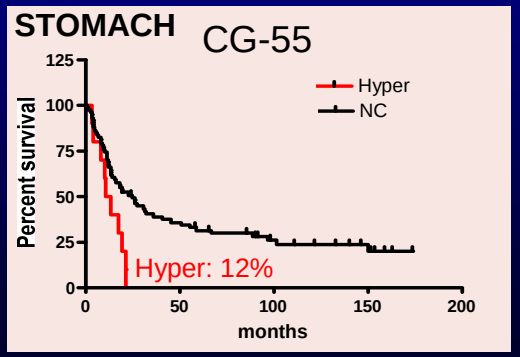


PTPRN2 Receptor tyrosine-prot. phosphatase



Unknown

THE ALTERATIONS IN CPG ISLAND METHYLATION ARE NOT DUE TO A METHYLATOR OR DEMETHYLATOR PHENOTYPE.

THEN, WHY ARE THEY OCURRING?

(BECAUSE NOTHING HAPPENS WITHOUT A CAUSE…)