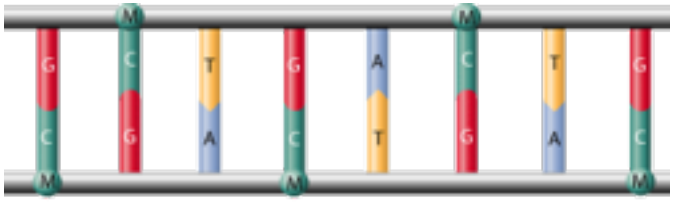
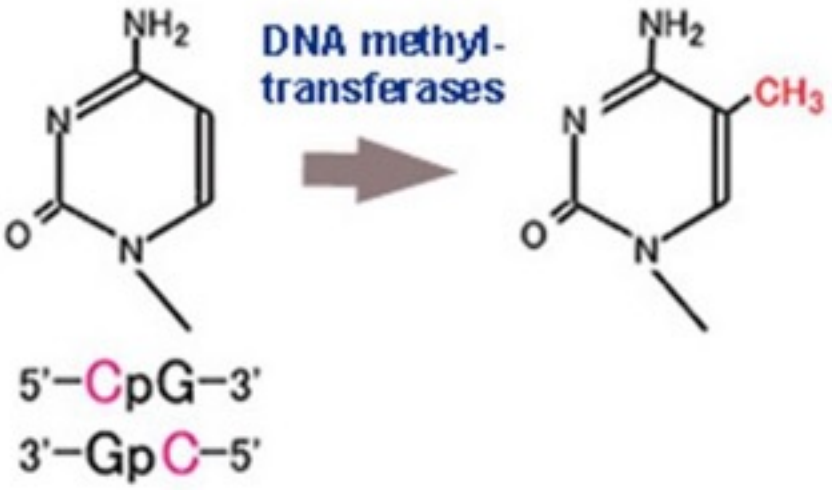
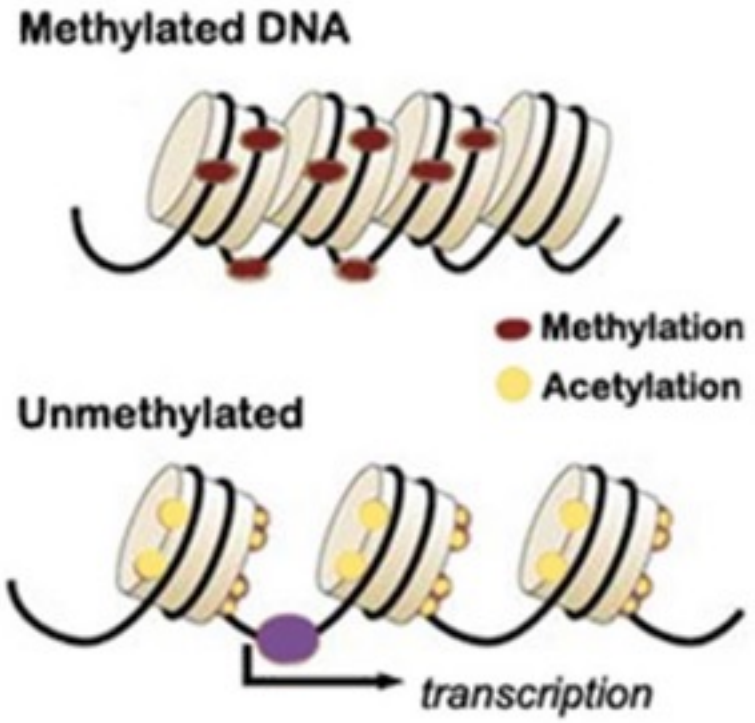
DNA methylation



Methylated bases, notably 5-methylcytosine, are an important feature of vertebrate genome and are related to a general **repression of transcription**





Actively transcribed vertebrate genes are marked by **CpG islands**

Deficiency in CpG results from an ineffective DNA repair process

The zygote contains methylated DNA inherited from the contributing sperm and egg cells, but the methyl groups are removed during embryogenesis

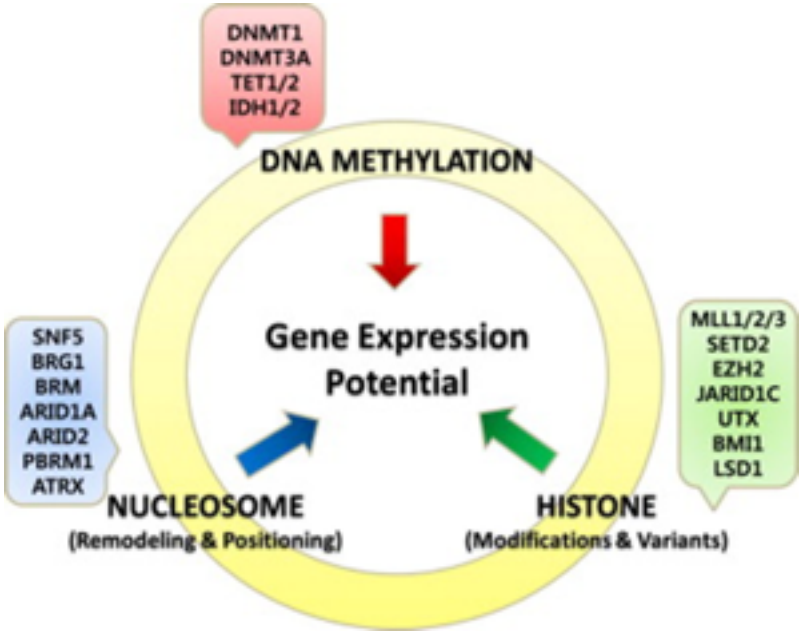
↓

Methylation = chromatin becomes closed and transcriptional inactive

It's function is still partly unknown

probably a primitive defense mechanism in procaryotic cells ?

- Cytosine-methyltransferases are able to recognize CpG sites
- CpGs are simmetric; after replication, the new sequences will maintain the same CpG methylation pattern



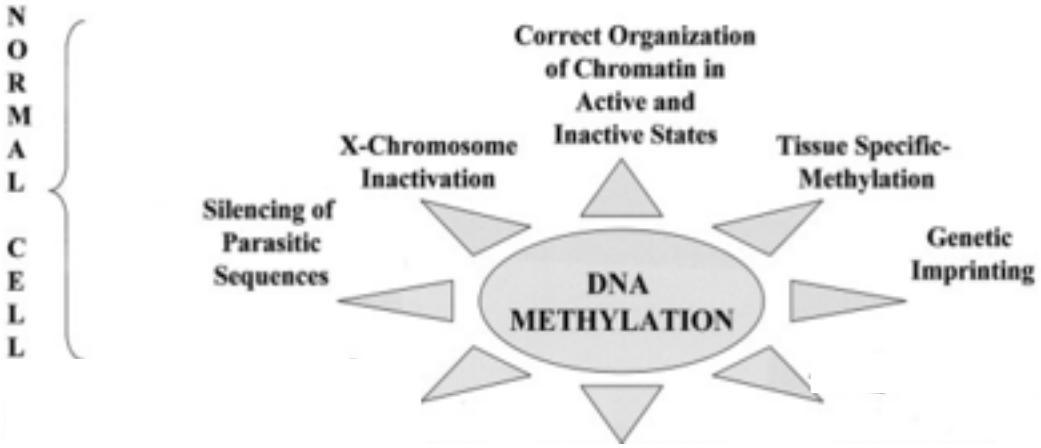
Epigenetic mechanisms are involved in the control of different gene expression patterns that in some cases can be **inherited**.....

for most of the genes the allelic expression is not inherited …

Non-methylated/methylated CpG islands

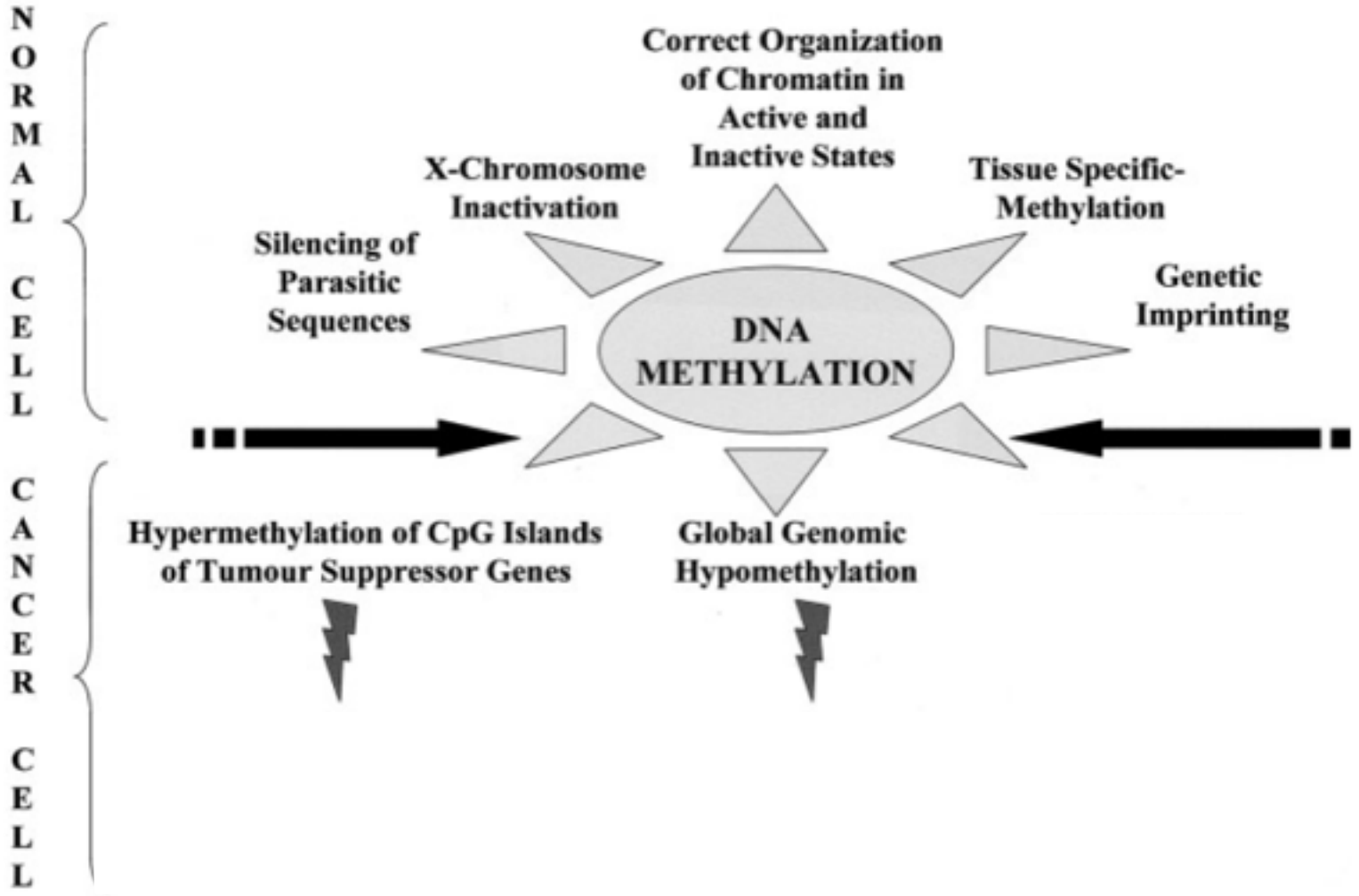
- few mammalian autosomal genes are unusual since the allelic expression depends on its parental origin, the so-called "imprinted genes"
- one of the female X chromosome is inactivated by methylation
- "transposon" sequences are methylated
- "repeated" sequences are methylated

Methylated CpG islands



J Pathol 2002; 196: 1–7

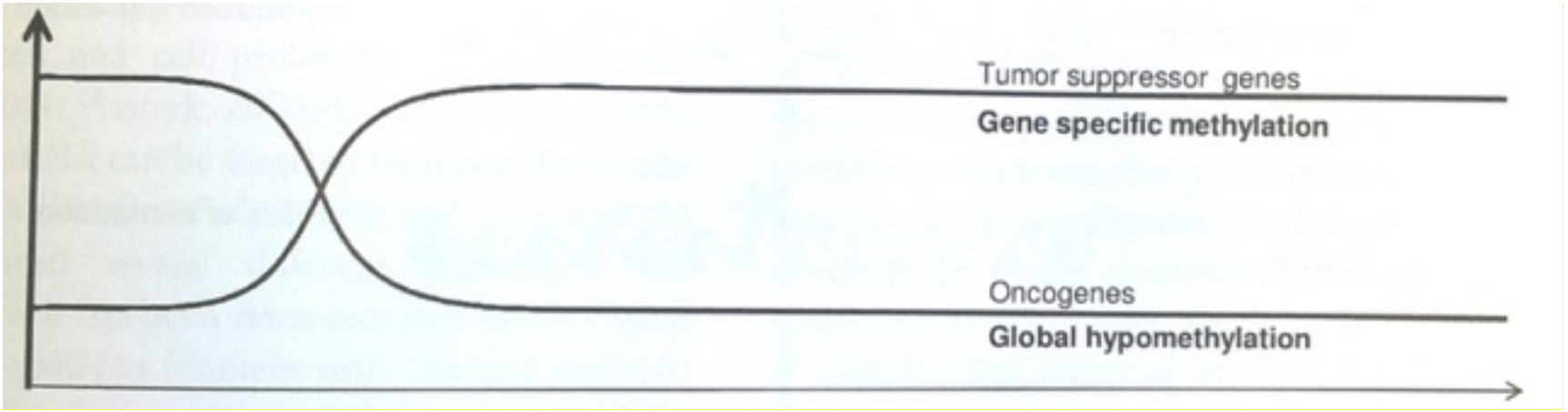
DNA hypo- and hypermethylation in cancer



J Pathol 2002; 196: 1–7

DNA Hypo- and hyper methylation in cancer progression

Met Levels



Tumor progression

DNA hypomethylation and colorectal cancer

- 1983 Goelz SE et al.

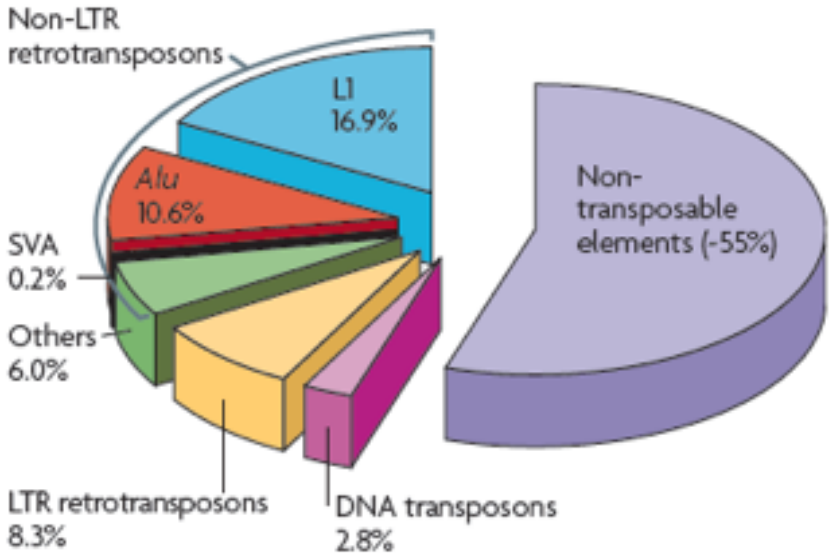
Hypomethylation of DNA from benign and malignant human colon neoplasms

- Since the end of '80

DNA hypomethylation can be involved in CRC by different mechanisms:

1. Proto-oncogene activation
2. Chromosomal Instability
3. Retrotransposon activation
4. Gene imprinting loss

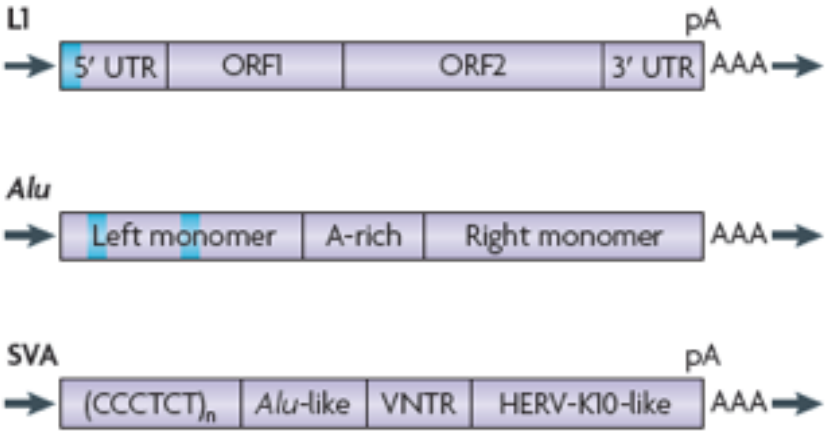
Members of the interspersed families of repeated sequences can be considered as transposable elements



Retrotransposon elements,

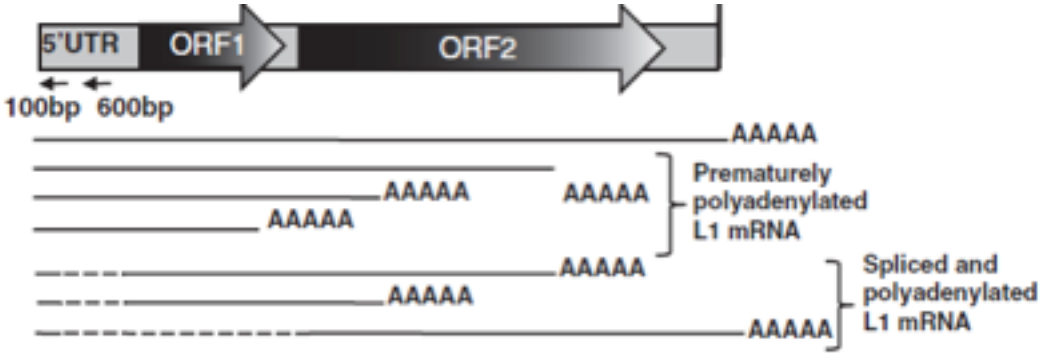
unstable DNA elements that can migrate to different regions of the genome

Their RNA transcript can be converted into a complementary DNA that can be reinsert back into chromosomal DNA



NATURE REVIEWS | **Genetics, 2009**

DNA hypomethylation of repeated sequences is associated with retrotransposition activation



Hypomethylation of long interspersed nuclear element-1 (LINE-1) leads to activation of proto-oncogenes in human colorectal cancer metastasis. Hur K

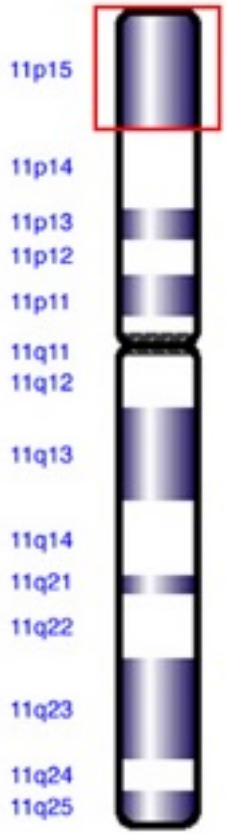
Somatic expression of LINE-1 elements in human tissues
Victoria P. Belancio

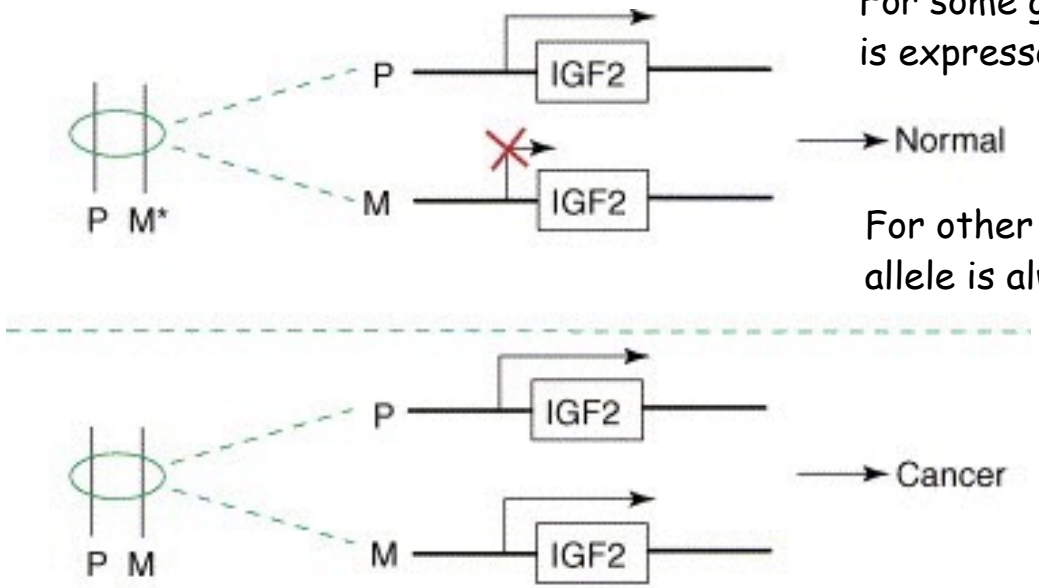
1988 2008 2010 2014

Unit-length line-1 transcripts in human teratocarcinoma cells.
J Skowronski

Activation and transposition of endogenous retroviral elements in hypomethylation induced tumors in mice.
Howard G

Gene imprinting loss as a cause of cancer





For some genes the maternal allele is expressed in specific cells

For other genes the paternal allele is always expressed

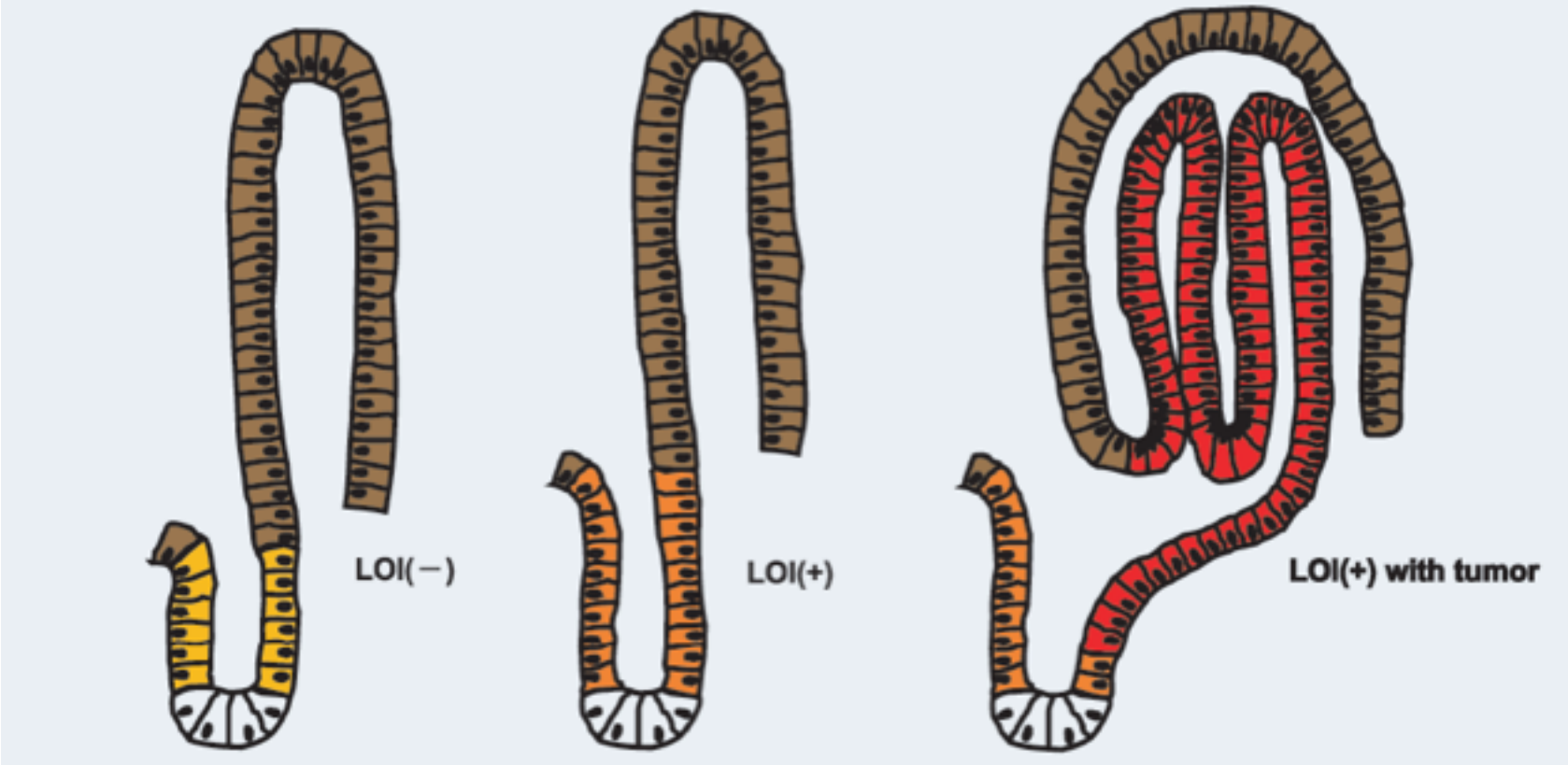
Hypomethylation of maternal allele

insulin-like growth factor type 2, IGF2

- Wilms' Tumour (Nature 1993; 362:747-9)
- Colorectal cancer
- Prostate cancer
- Lung cancer

Loss of Imprinting of *IGF2*: A Common Epigenetic Modifier of Intestinal Tumor Risk

Atsushi Kaneda and Andrew P. Feinberg



Cancer Res 2005; 65: (24). December 15, 2005

DNA hypermethylation : colorectal cancer and CIMP phenotype

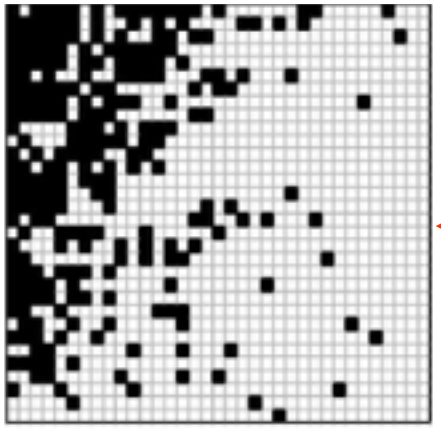
Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 6870–6875, June 1998
Genetics

Incidence and functional consequences of *hMLH1* promoter hypermethylation in colorectal carcinoma

JAMES G. HERMAN*†‡, ASAD UMAR†§, KORNELIA POLYAK*¶, JEREMY R. GRAFF*, NITA AHUJA*, JEAN-PIERRE J. ISSA*, SANFORD MARKOWITZ¶‖, JAMES K. V. WILLSON‖, STANLEY R. HAMILTON*, KENNETH W. KINZLER*, MICHAEL F. KANE**, RICHARD D. KOLODNER**, BERT VOGELSTEIN*¶, THOMAS A. KUNKEL§, AND STEPHEN B. BAYLIN*

genes

cases



Simultaneous hypermethylation of several **tumor-suppressor genes**

Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 8681–8686, July 1999
Medical Sciences

CpG island methylator phenotype in colorectal cancer

MINORU TOYOTA, NITA AHUJA, MUTSUMI OHE-TOYOTA, JAMES G. HERMAN, STEPHEN B. BAYLIN, AND JEAN-PIERRE J. ISSA*

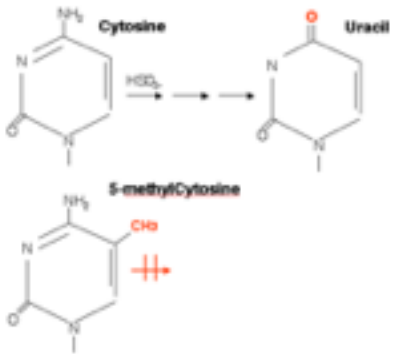
CpG Island Methylation in Colorectal Cancer: Past, Present and Future

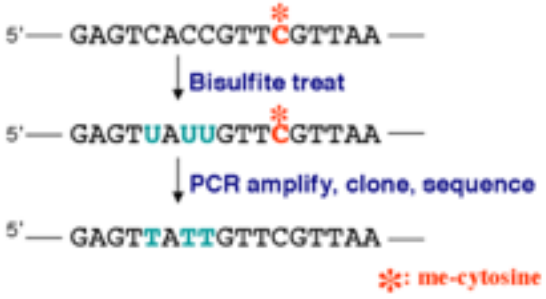
Table 1: A history of CIMP panels used to assess CpG island methylation in colorectal cancer.

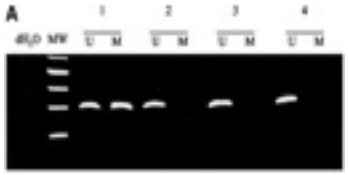
Study	CIMP panel markers	Notes
Toyota et al 1999	*CDKN2A (p16)*, MINT1, MINT2, MINT12, MINT17, MINT25, MINT27, MINT31, *MLH1*, *THBS1*	Pioneering work to identify markers that distinguish CIMP from age-related methylation
Park et al. 2003	*CDKN2A*, MINT1, MINT2, MINT31, *MLH1*	So-called "classic" or traditional panel
Weisenberger 2006	*CACNA1G*, *IGF2*, *NEUROG1*, *RUNX3*, *SOCS1*	"New" panel based on stepwise screen of 195 markers
Ogino et al. [2006	*CACNA1G*, *CDKN2A*, *CRABP1*, *MLH1*, *NEUROG1*	Selected markers to distinguish high-level from low-level methylation
Shen et al. [2007	CIMP1: MINT1, *MLH1*, *RIZ1*, *TIMP3*, *BRAF* mutation; CIMP2: MINT2, MINT27, MINT31, Megalin, *KRAS* mutation	Examined 27 CpG sites, proposed optimal epigenetic and genetic markers to identify CIMP1, CIMP2, or CIMP-
Tanaka et al. 2010	*CACNA1G*, *CDKN2A*, *CHFR*, *CRABP1*, *HIC1*, *IGF2*, *IGFBP3*, *MGMT*, MINT1, MINT31, *NEUROG1*, *p14*, *RUNX3*, *SOCS1*, *WRN*	Correlation structures of markers and CIMP differ by *KRAS* and *BRAF* status
Ang et al. 2010	Total of 202 CpG sites differentially methylated between tumor and normal	Comprehensive DNA methylation profiling in 807 cancer genes
Kaneda and 2011	Group 1: *IGF2*, *LOX*, MINT1, MINT2, MINT31, *MLH1*, *RUNX3*, *SOCS1*; Group 2: *ADAMTS1*, *DUSP26*, *EDIL3*, *ELMO1*, *FBN2*, *HAND1*, *IGFBP3*, *NEUROG1*, *RASSF2*, *STOX2*, *THBD*, *UCHL1*	Comprehensive DNA epigenotyping of genomewide regions indentified two groups (high and intermediate to low methylation)

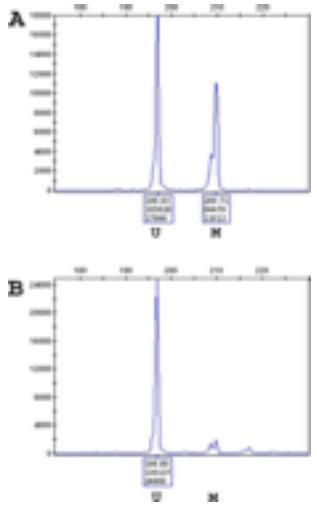
Pathology Research International
Volume 2011, Article ID 902674, 1

Bisulfite analysis for methylation studies



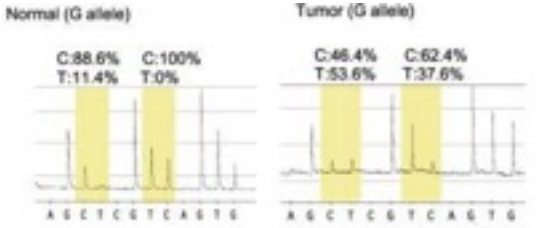


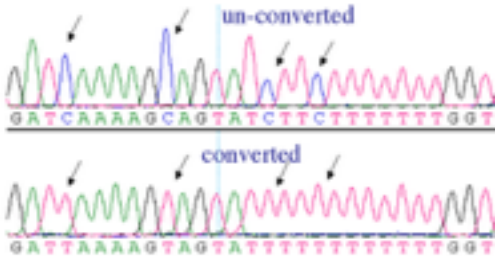




| Summary of methods using sodium bisulfite treatment.

Method	Author
Ligation-mediated PCR	Pfeifer et al. (1989)
Bisulfite sequencing	Frommer et al. (1992)
MS-PCR	Herman et al. (1996)
MS-SNuPE	Gonzalgo and Jones (1997)
MS-SSCA	Bianco et al. (1999)
MS-HRM	Wojdacz and Dobrovic (2007)
Bisulfite treatment to create new restriction sites	Sadri and Hornsby (1996)

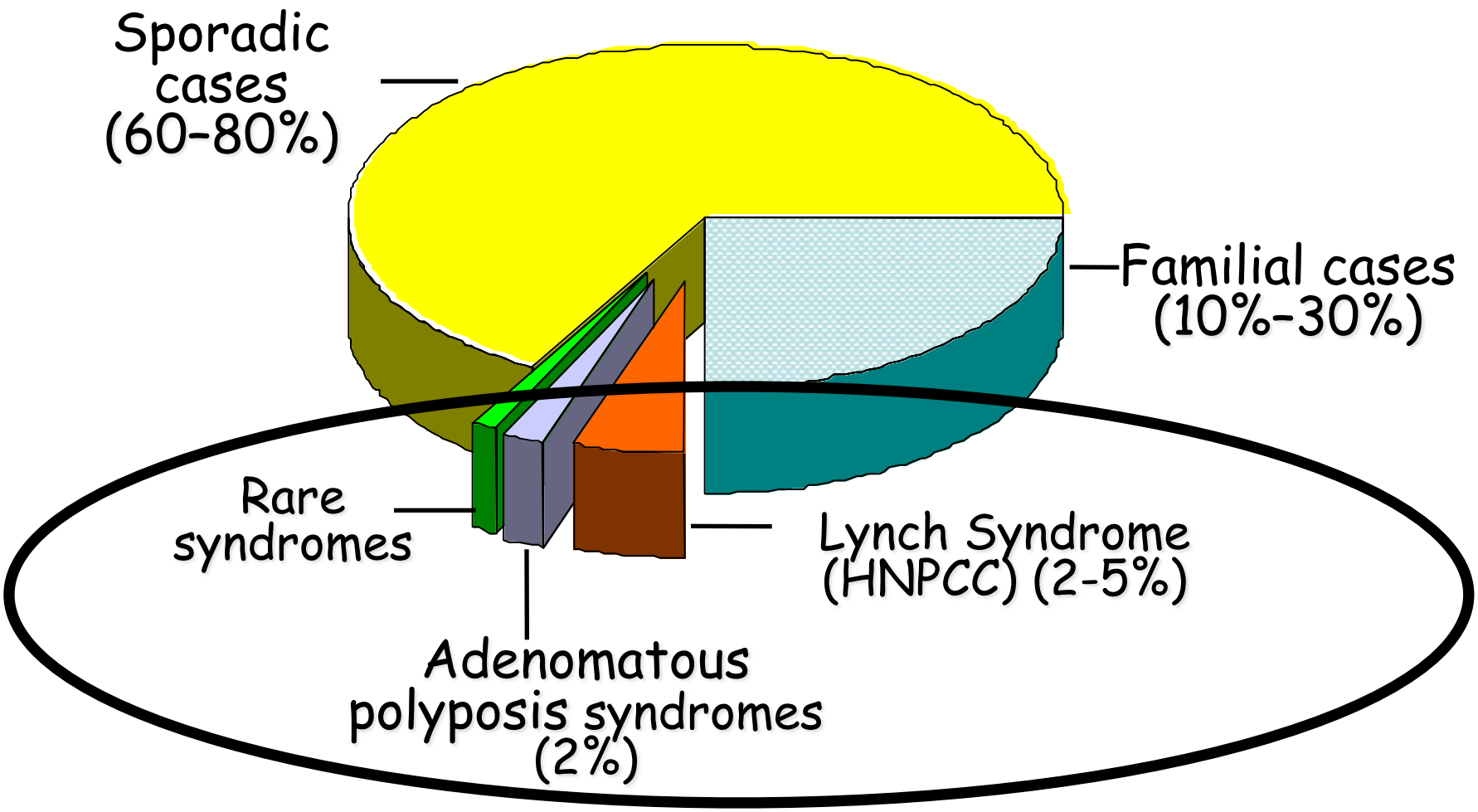




DNA methylation: a timeline of methods and applications

frontiers in GENETICS
REVIEW ARTICLE

Colorectal cancer



CCR hereditary syndromes as models of sporadic colorectal tumorigenesis

Familial adenomatous polyposis

Adenoma-carcinoma sequence
Vogelstein's model

MUTYH-associated polyposis

DNA-oxidative pathway

Lynch syndrome or HNPCC

Hypermutability pathway

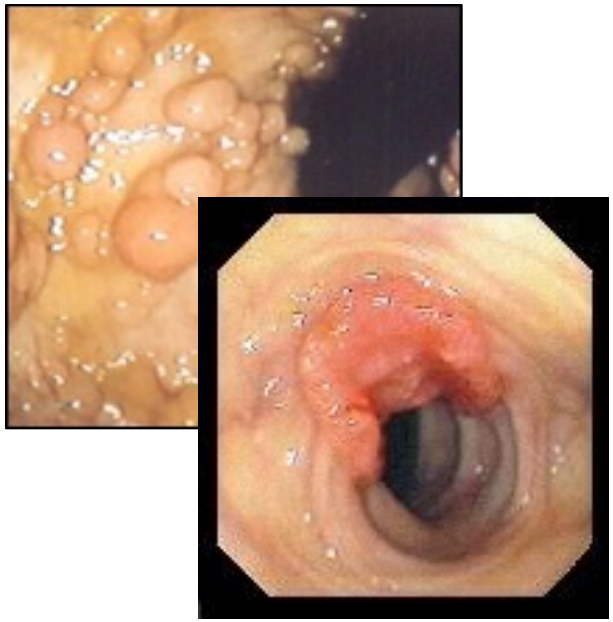
Epigenetic germline alterations ?

Hypermethylation pathway

Familial adenomatous polyposis

- autosomic dominant condition in linkage with mutations on APC tumour-suppressor gene

- hundreds-thousands adenomas that confer a higher CRC risk at young age (about 40 aa)



- variable phenotype

affected individuals develop:

retinic lesions, desmoids, osteomas

In addition to this **"classical polyposis"** there is:

Attenuated familial adenomatous polyposis (AFAP)

- less < 100 adenomas, polyps and cancer can onset later and the expression of extra-colonic manifestations is limited

Familial adenomatous polyposis

APC is a tumour-suppressor gene

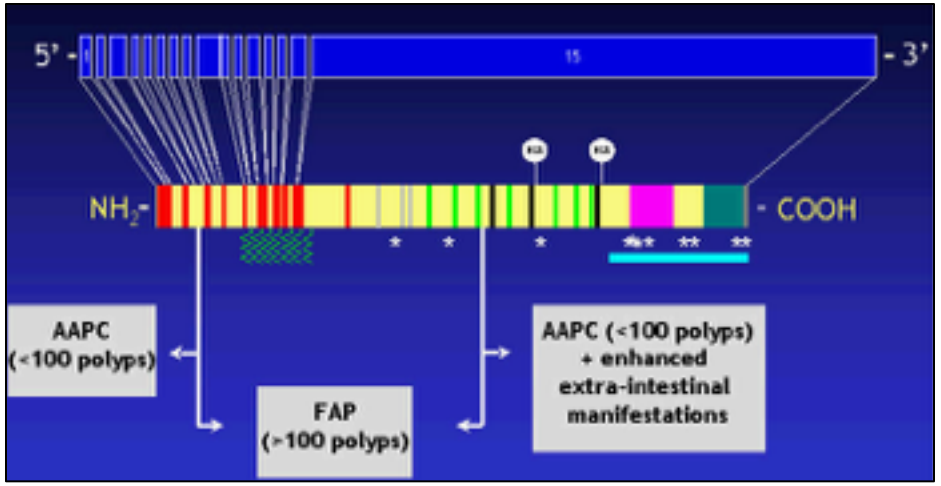
- identified with linkage analysis and positional cloning on 5q21
- 100 Kb, 15 exons, 2843 aa

APC is a highly penetrant gene

Most (95%) of the alterations identified so far lead to a truncated protein

Mutations are identified

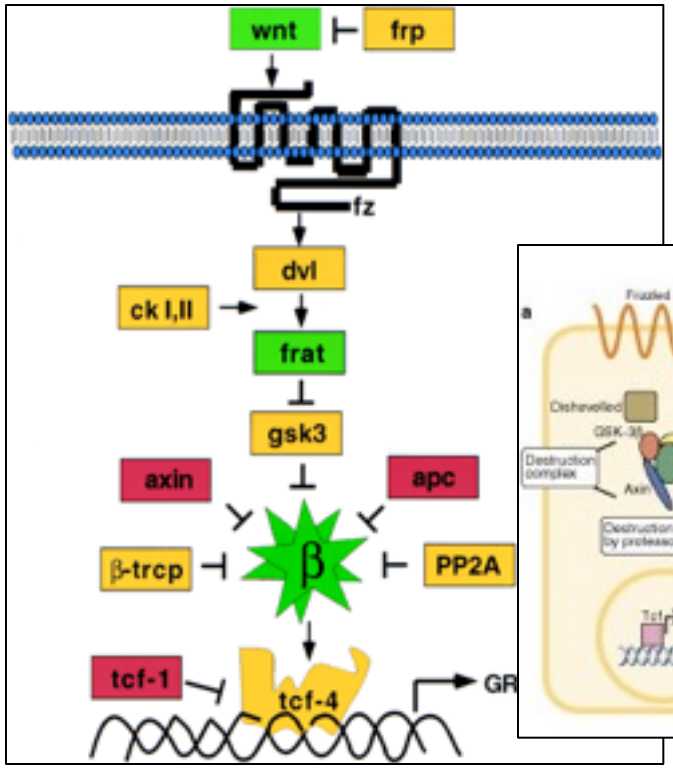
- in 70% of classical polyposis patients
- in 10% of AFAP patients
- there is genotype-phenotype correlation



Familial adenomatous polyposis

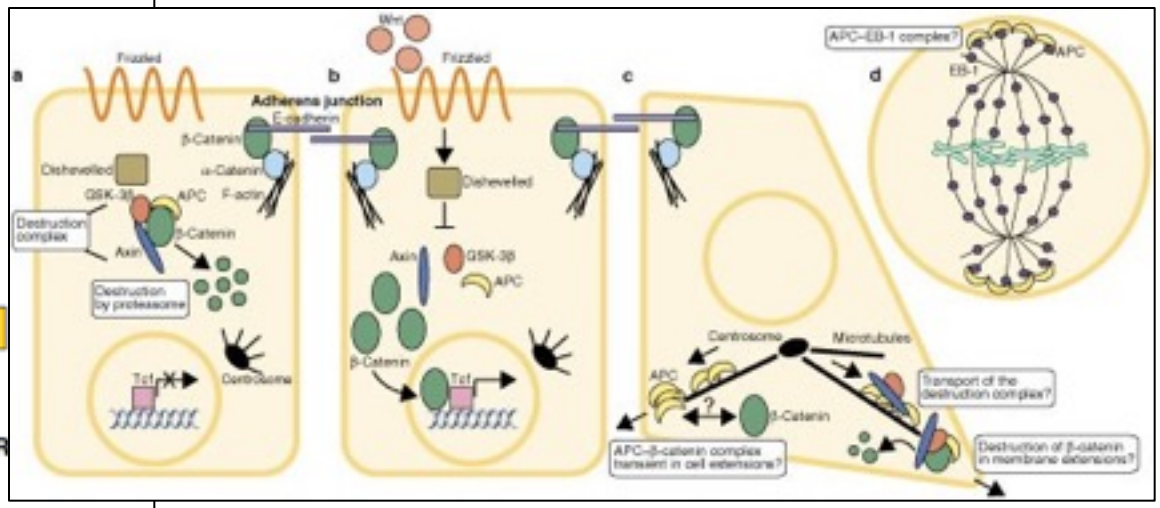
APC is a multifunctional protein taking part to different cellular processes

The main role is to regulate the intracellular ß-catenin level in the Wnt pathway



adhesion

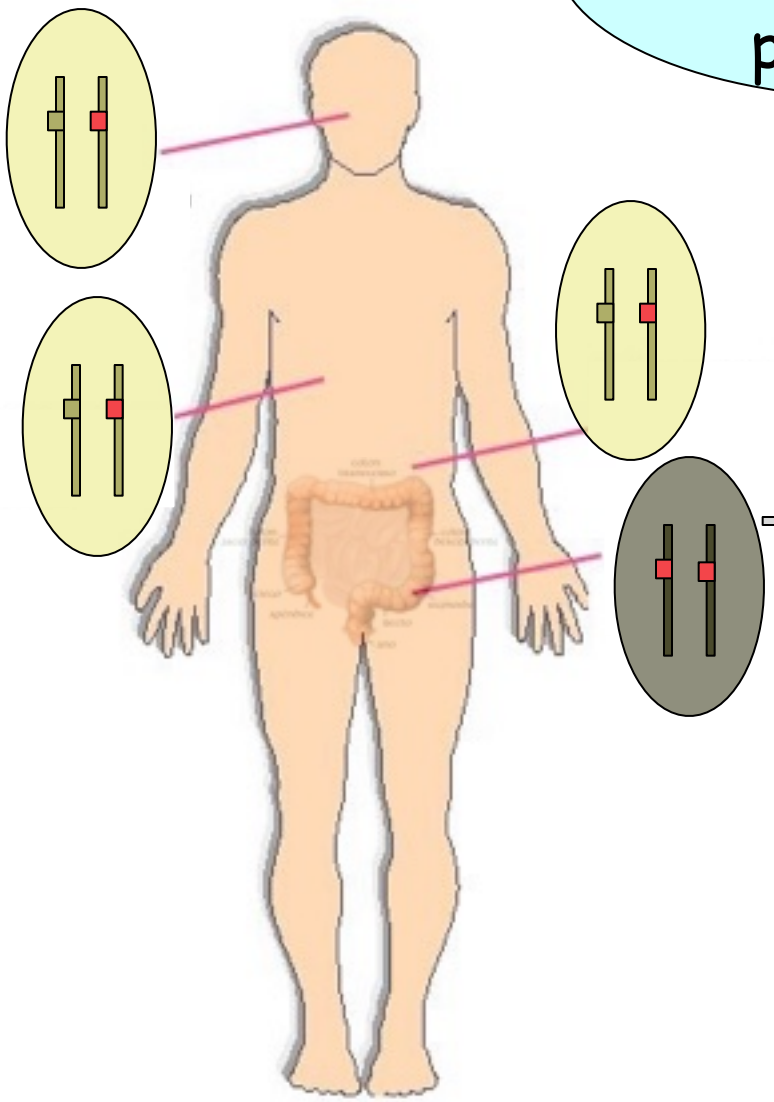
mitosis



apoptosis

migration

Familial adenomatous polyposis



FAP/AFAP:
two "hits" model,
the **first constitutive**,
the **second somatic**

TUMORIGENESIS
FIRST STEPS

PROGRESSION NEEDS
THE ACCUMULATION
OF OTHER GENETIC
SOMATIC MUTATIONS

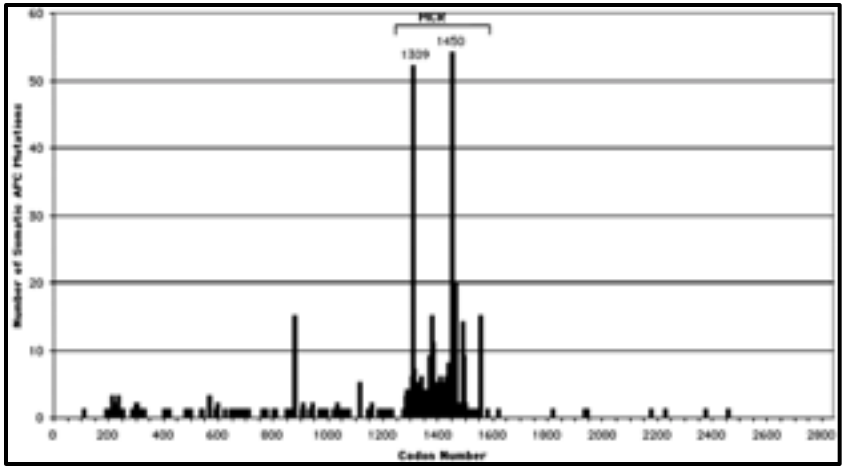
Familial adenomatous polyposis

APC role

APC is a tumour-suppressor genes

Most of APC somatic mutations lead to truncating proteins

60% of this mutations onset in the Mutation Cluster Region (MCR) between codons 1286 and 1513 in the binding domain to ß-catenin



Thierry Soussi APC database at hptt://perso.curie.fr.

MCR mutations confer a selective advantage to the transforming cells

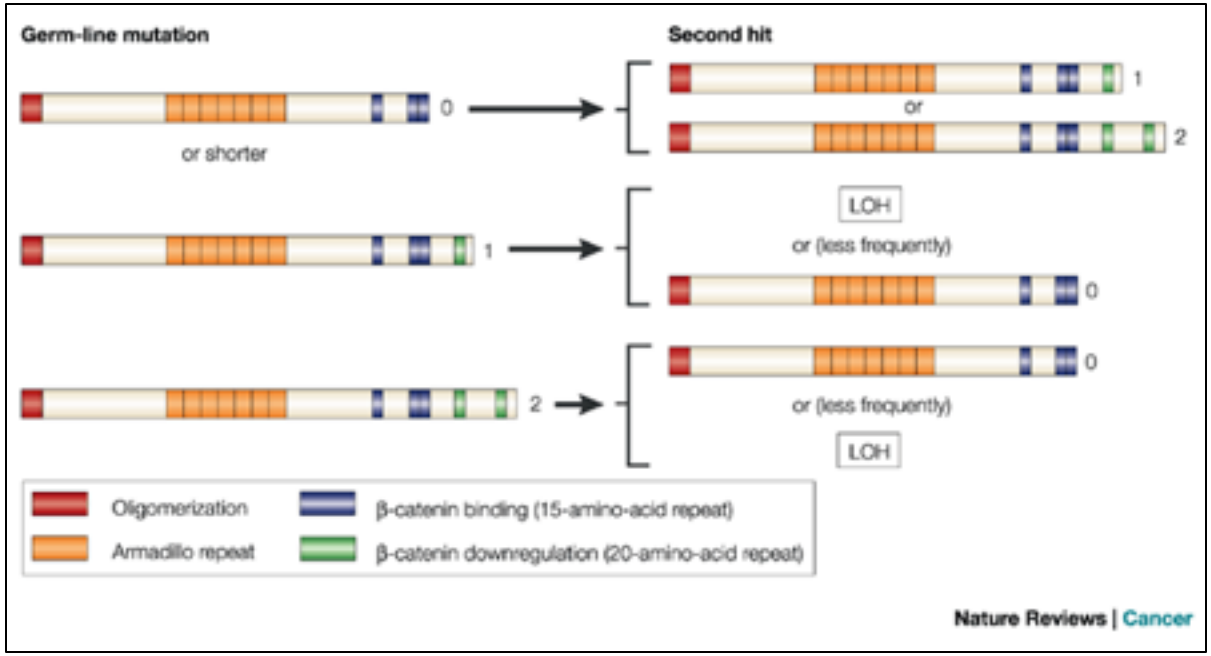
Just-right signaling model

Albuquerque et al., 2002

Familial adenomatous polyposis

APC is a characteristic tumour-suppressor gene

Two "hits" interdependence



APC mutations are selected according to their proliferative advantage

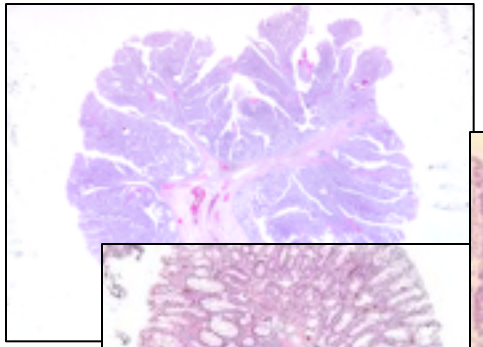
This advantage is due to the cytoplasmatic β-catenin level

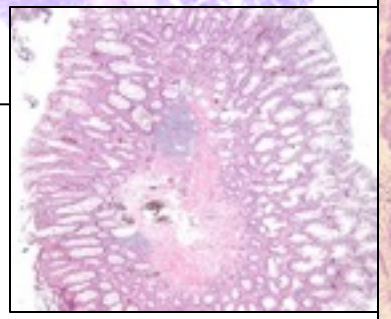
Just-right signaling model

Familial adenomatous polyposis

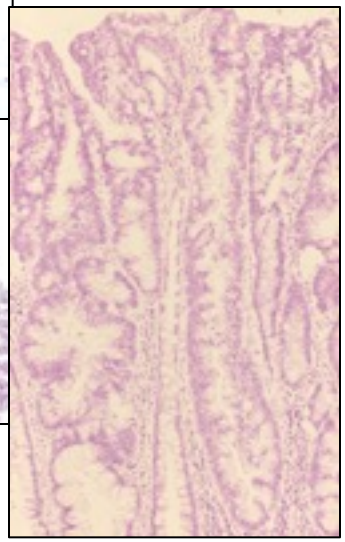
Genotype-phenotype correlation

FAP patients show a considerable variable phenotype





hyperplastic polyps



serrated adenomas

- variable number of adenomas
- presence of carcinomas
- extra-colonic manifestations
- variable polyp histotype

Modifier genes ?

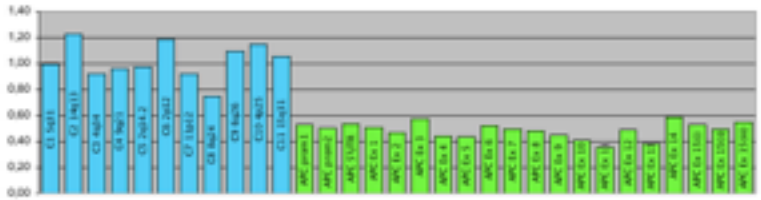
"Alternative genes" to APC in linkage with the syndrome ?

Familial adenomatous polyposis

Genotype-phenotype correlation

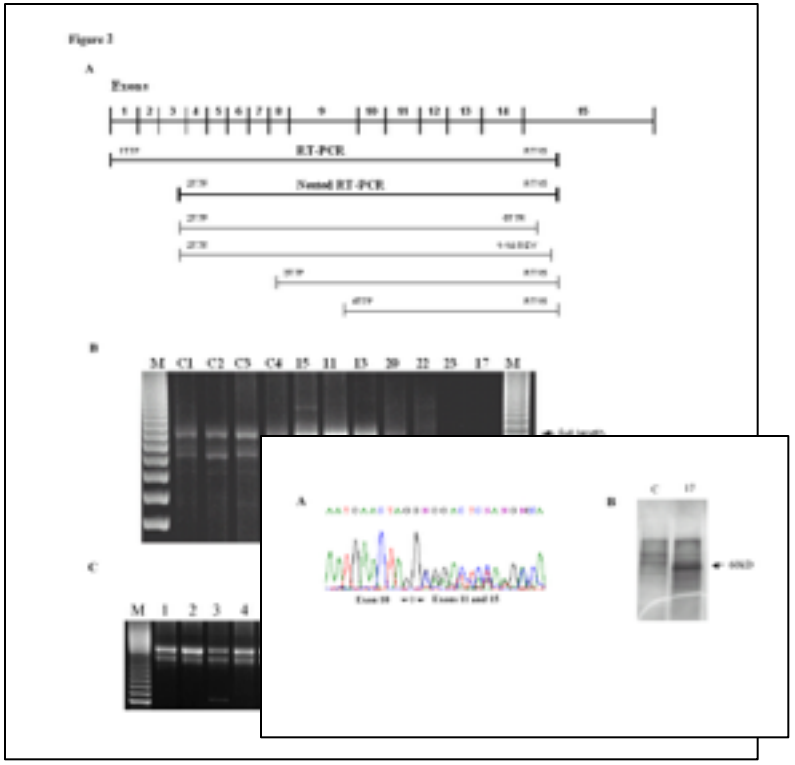
APC mutation **type** can play as modifier

- **Constitutional deletions** are associated with a more aggressive phenotype



Lab Invest 83: 1859-66 (2003)

- **Alternative splicing/isoforms** in the first 14 exons associated with AFAP

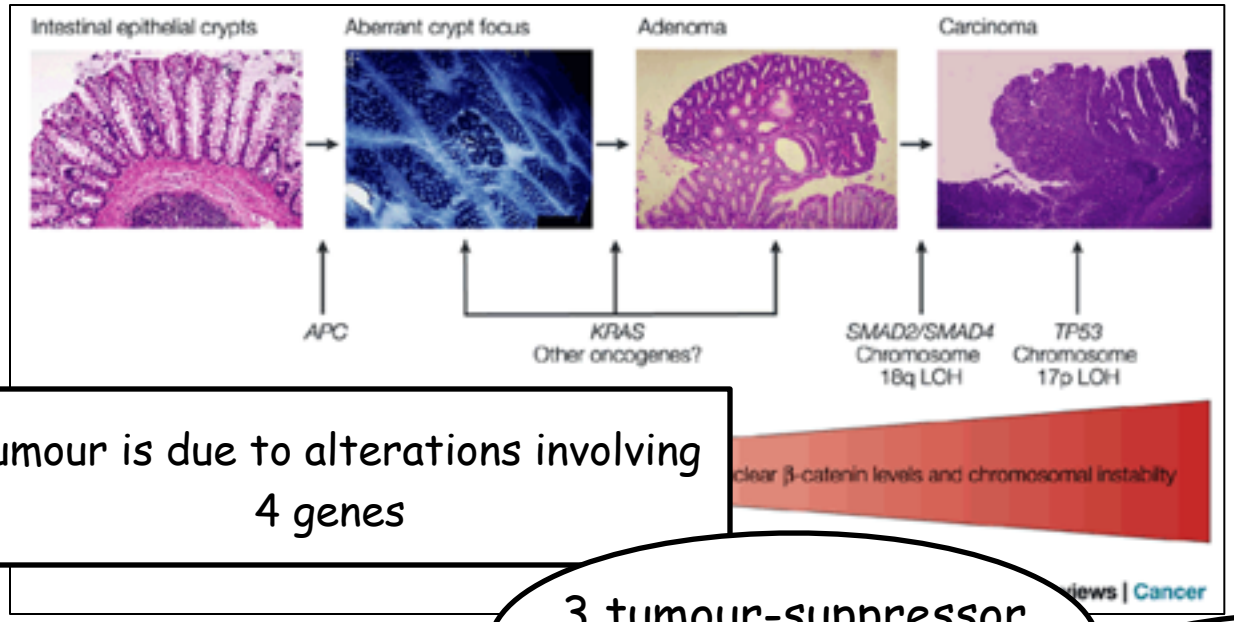


J Mol Med 85: 301-8 (2007)

Familial adenomatous polyposis

Adenoma-carcinoma sequence
Vogelstein's model

Histologic progression is related to the acquisition of genetic alterations



Most of sporadic colorectal tumours follow this model

A model also for other type of neoplasia

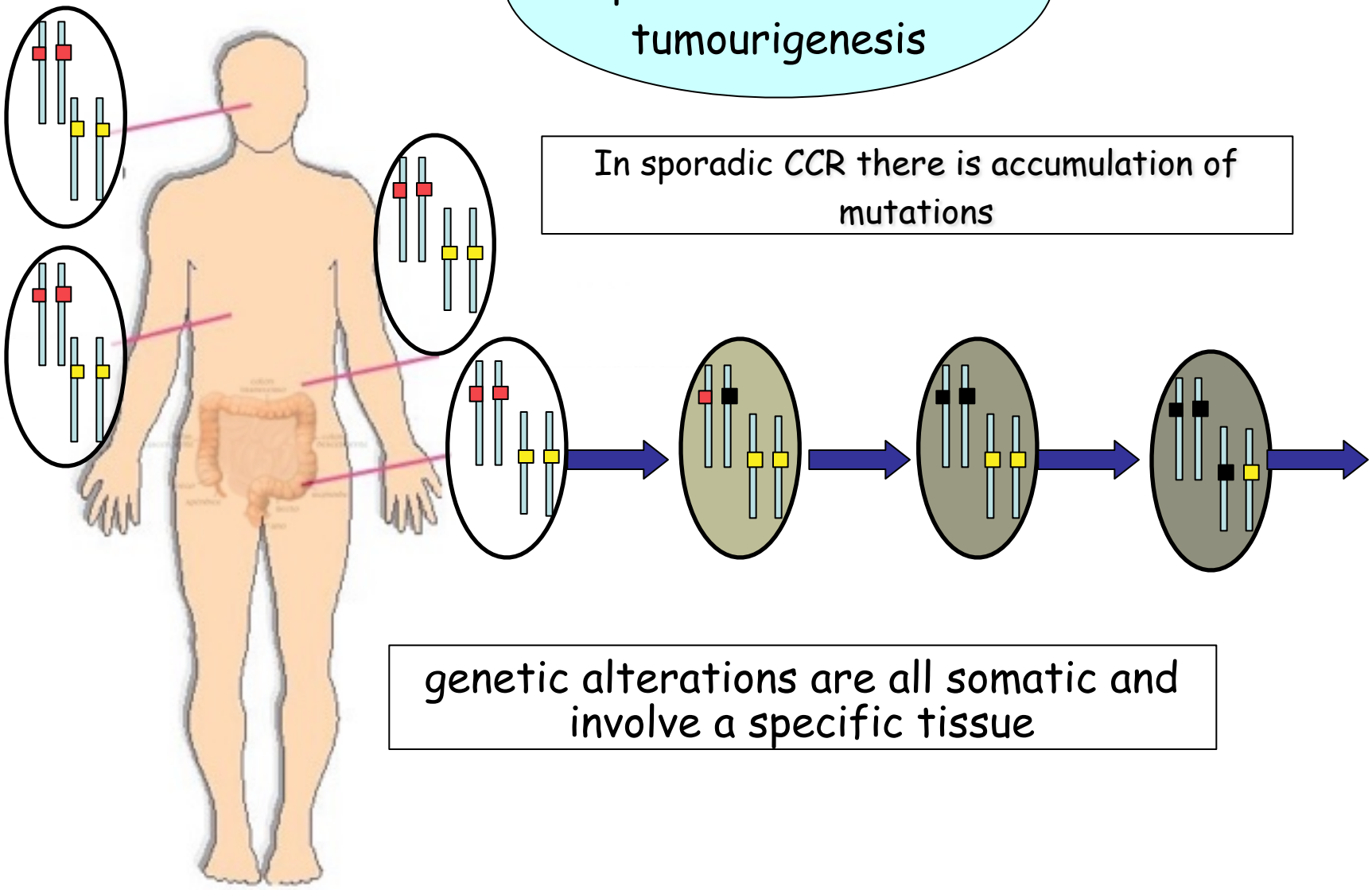
Tumour is due to alterations involving 4 genes

3 tumour-suppressor genes and 1 oncogene

APC is the gatekeeper of the colon

FAP is the model of sporadic colorectal tumourigenesis

In sporadic CCR there is accumulation of mutations

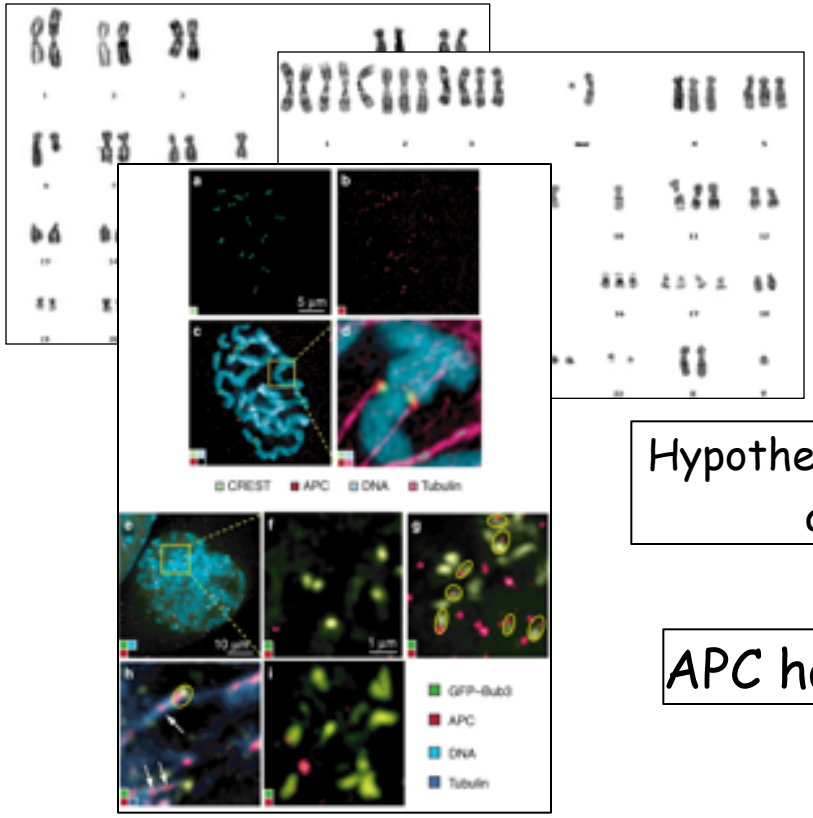


genetic alterations are all somatic and involve a specific tissue

FAP is the model of sporadic colorectal tumourigenesis

Adenoma-carcinoma sequence
Vogelstein's model

- CRC following this model show a lot of chromosomal alterations



Chromosomal instability
CIN

What molecular mechanisms ?

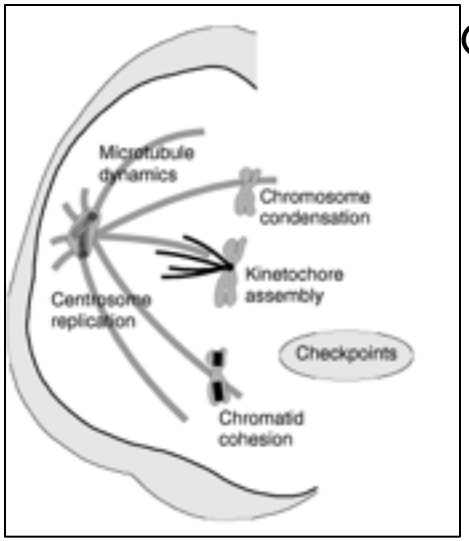
Hypothesis : qualitative and quantitative chromosomal alterations during mitosis segregation

APC has a role in CIN

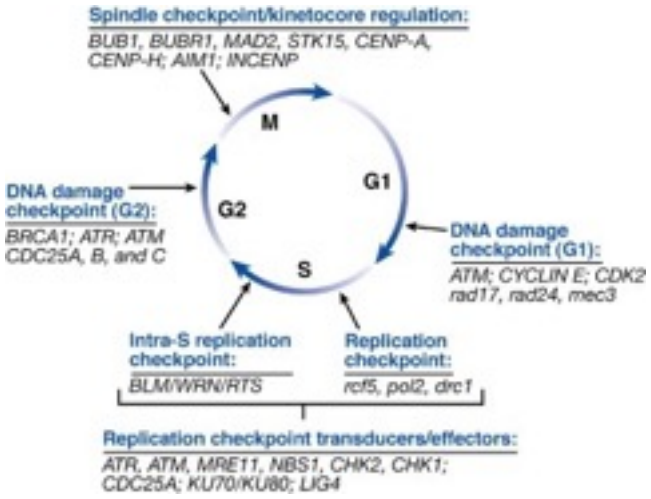
J Pathol, 2004; 204:193-9

Chromosomal instability (CIN) is a feature of most of the sporadic CRC

What molecular mechanisms?



Qualitative and quantitative chromosomal alterations during mitosis segregation



- Gene-specific hypothesis
- Non gene-specific hypothesis

APC plays a role in CIN

Adenoma-carcinoma sequence
Vogelstein's model

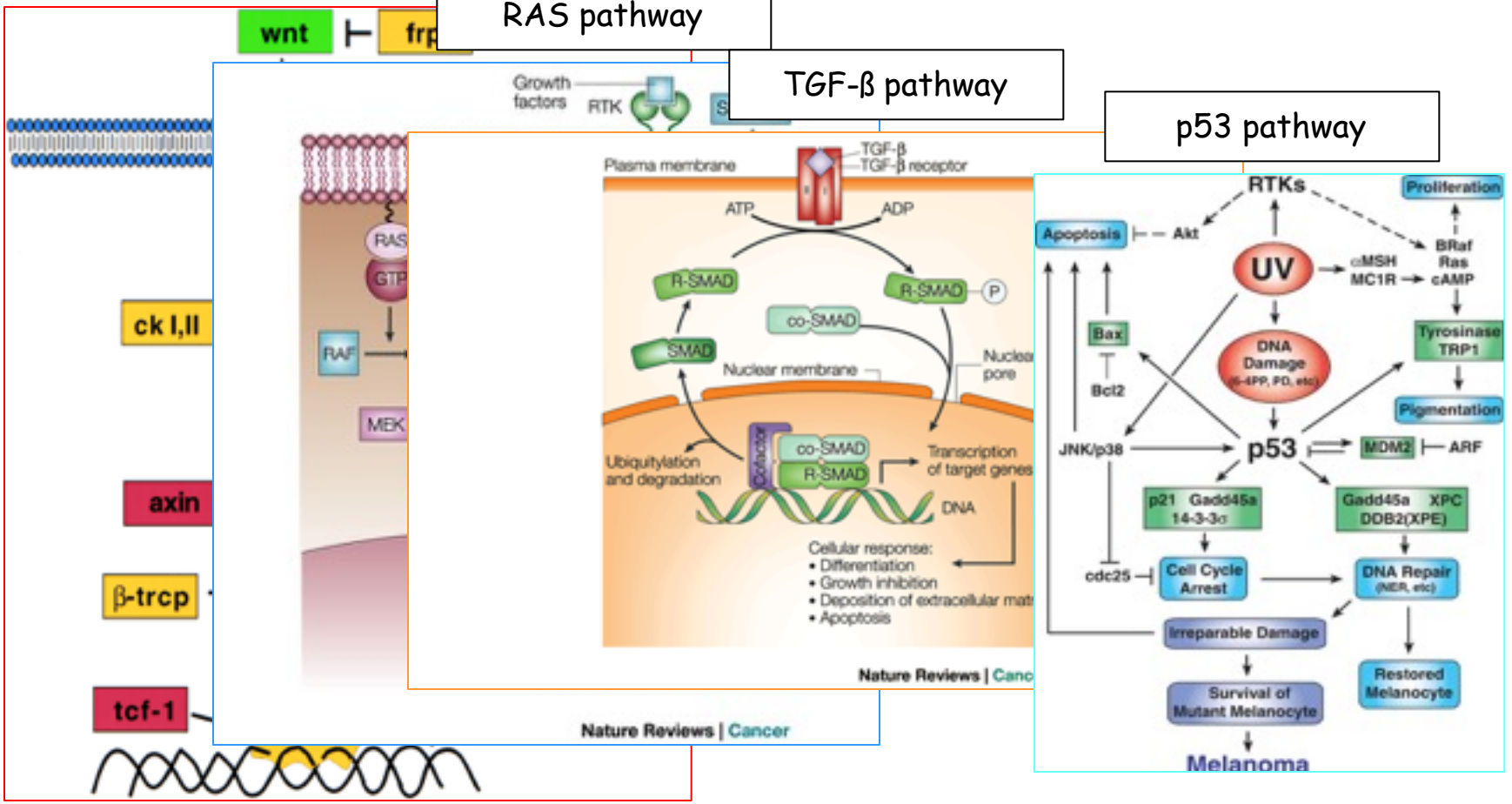
Involved pathways

Wnt pathway

RAS pathway

TGF-ß pathway

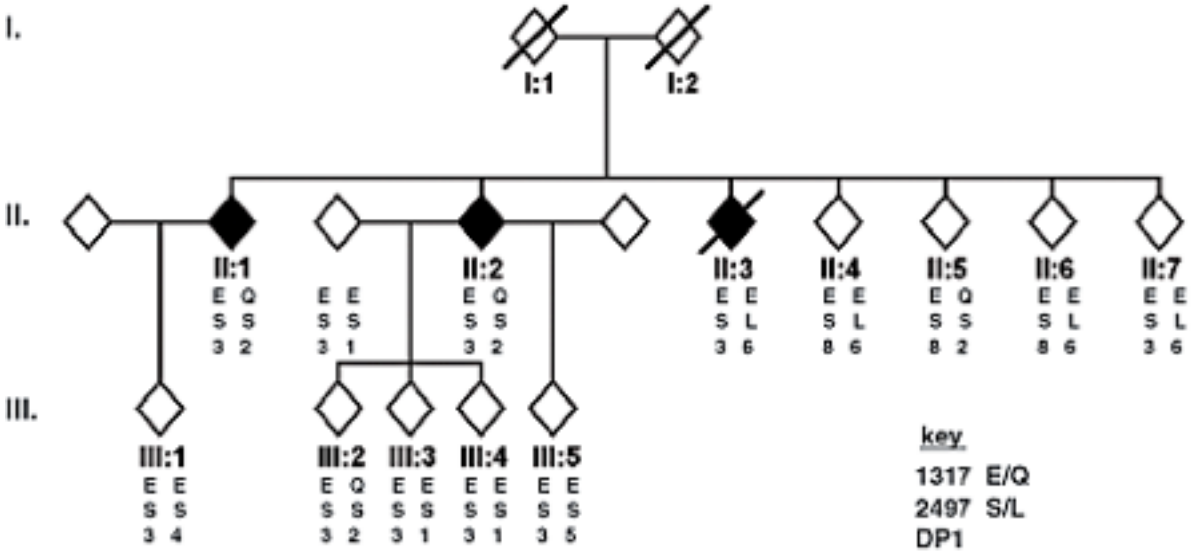
p53 pathway



MUTYH-associated polyposis
MAP

The other player…

Al-Tassan et al.,Nature Genet, 2002



Famiglia N

An alternative gene to APC is involved in the susceptibility to polyposis and CRC

Al-Tassan et al., Nature Genet. 30, 227-232

APC somatic analysis

APC ORF was sequenced in 11 adenomas and 1 carcinoma

15/18 G:C > T:A

Found more **G>T** transversions than sporadic CRC or FAP tumors

Alterations in genes involved in BER system ?

Table 1 • Somatic *APC* mutations identified in family N

Sample[a]	Nucleotide change	Amino-acid change	No. of clones (*x*/*y*)[b]	Sequence context[c]
A1	2602G→T	Glu868X	2/6	AGAAAAT
	4351G→T	Glu1451X	2/6	AGAAGTA
A2	721G→T	Glu241X	NA	AGAAGCA
	4381G→T	Glu1461X	2/6	TGAAAAG
A3	4717G→T	Glu1573X	4/5	TGAAATA
	NI	NI		
A4	423-1G→T[d]	NA	2/2	NA
	4351G→T	Glu1451X	6/6	AGAAGTA
A5	601G→T	Glu201X	NA	GGAAGAA
	4348C→T	Arg1450X	3/6	NA
B2	3331G→T	Glu1111X	7/10	AGAAACA
	LOH	LOH		NA
B4	3586C→A	Ser1196X	3/7	TGAAAAT
	3856G→T	Glu1286X	4/5	TGAAATA
B5	604G→T	Glu202X	3/6	AGAACAA
	3850G→T	Glu1284X	6/6	TGAAGAT
B6	2863G→T	Glu955X	5/7	AGAATAC
	3949G→T	Glu1317X	4/6	TGAAGAT
C2b	1495C→T	Arg499X	3/6	NA
	NI	NI		
C1a[e]	NI	NI		

[a]For somatic *APC* mutations, we analyzed five adenomas from sibling II:1 (A1–5), four adenomas from sibling II:2 (B2, B4, B5, B6), and one adenoma (C2b) and one carcinoma (C1a) from sibling II:3. Mutations are described according to the established nomenclature system[29]. Biallelic mutations were found to be on opposite alleles in all tumors except A2 and A5, for which this could not be determined. [b]Number of clones, where *x* represents the number with the mutation and *y* represents the total number from that allele. In general, mutations were found in only a proportion of clones. Nonmutated clones from the same allele most probably represent contaminating normal tissue. All mutations were confirmed by an independent assay on a fresh PCR product. [c]Sequence context surrounding the coding region G:C→T:A mutations (underlined); the sequence of the nontranscribed strand is shown except for the Ser1196X variant in B4. [d]423-1G→T was shown to cause skipping of exon 4 of *APC* and is predicted to terminate the reading frame at the seventh codon of exon 5. [e]C1a did not contain any identified *APC* mutations, despite re-sequencing of the ORF in DNA from a second micro-dissected tumor sample. Sequence analysis of the coding regions of *CTNNB1* and *TP53* in DNA from this carcinoma was also normal, which suggests that genes in an alternative tumorigenic pathway are mutated. NA, not applicable; NI, not identified.

BER (Base Excision Repair)

Oxidative DNA damage repair

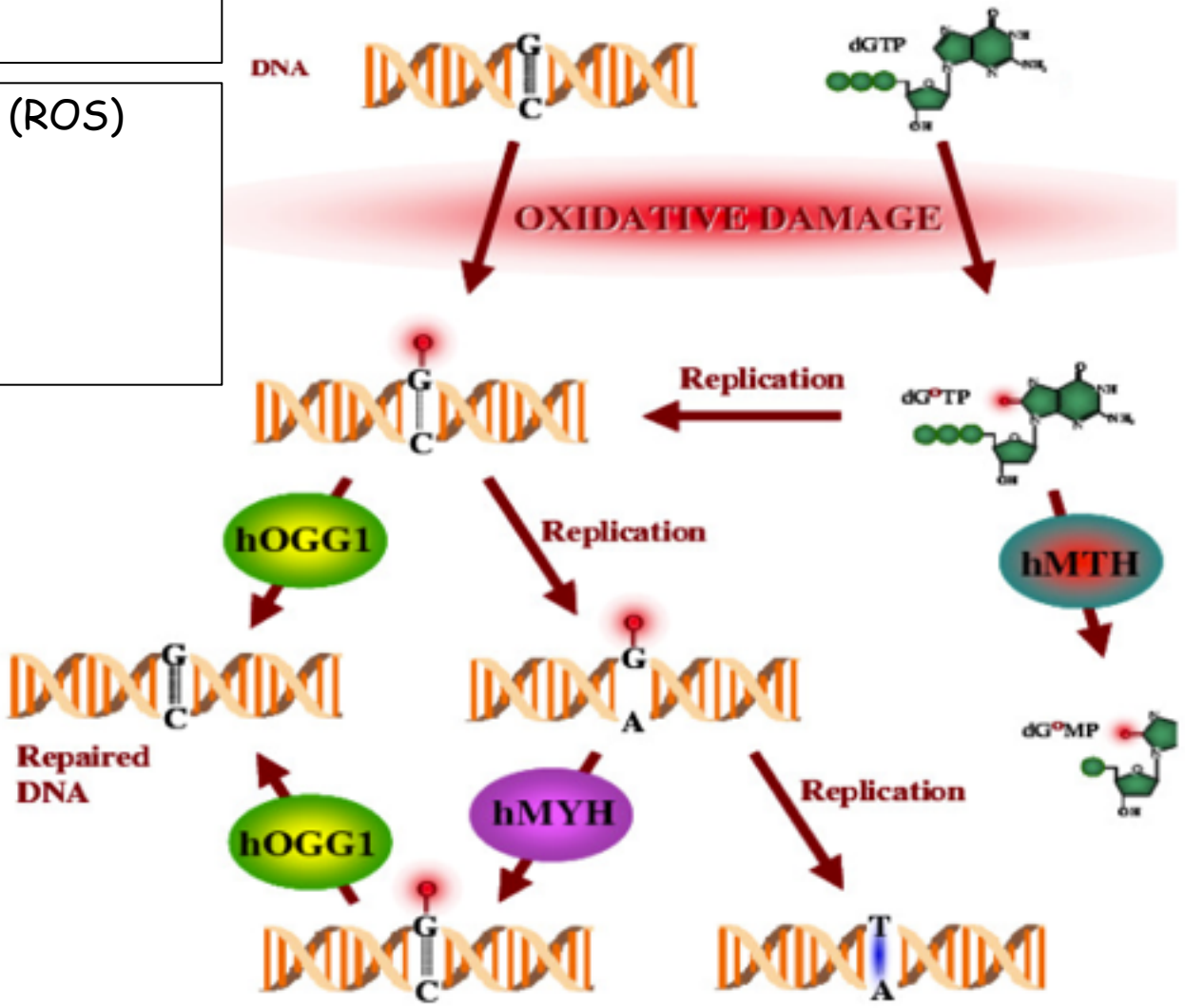
- reactive oxigene species (ROS)
- methylation
- deamination
- hydroxylation

Human homologous

OGG1 (mutM)

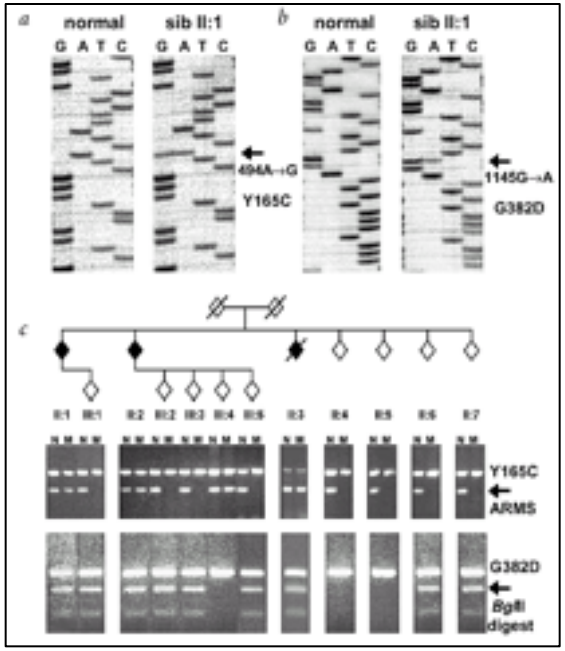
MYH (mutY)

MTH (mutT)



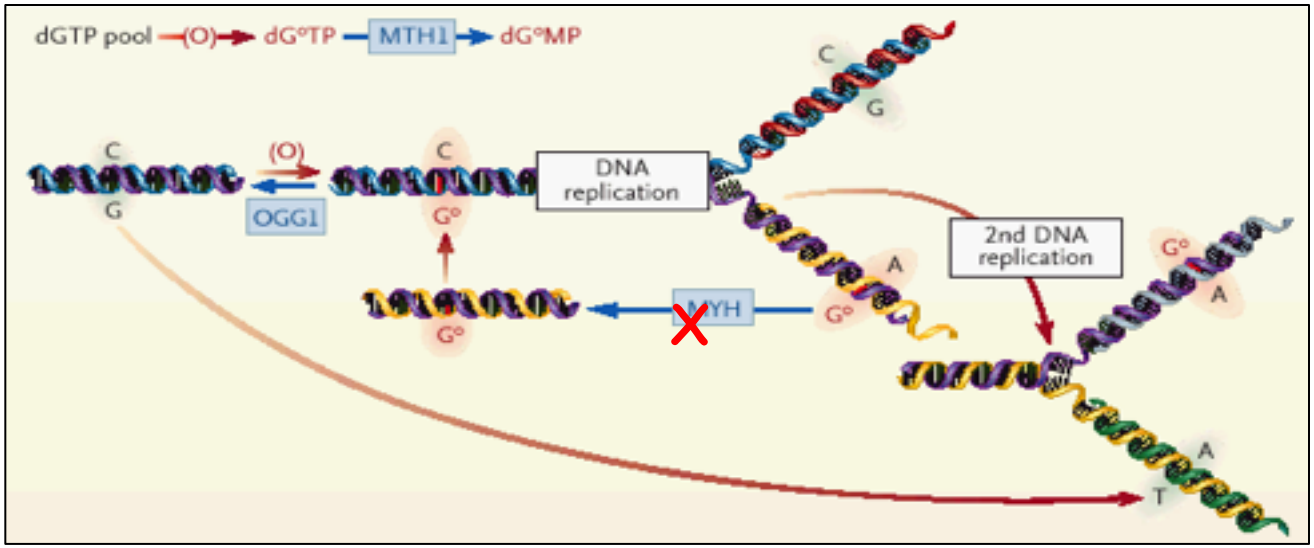
- **Constitutional analysis of** BER genes in the affected individuals of N family

- Identified biallelic mutations in MUTYH gene



exon 7 : Y165C

exon 13 : G382D



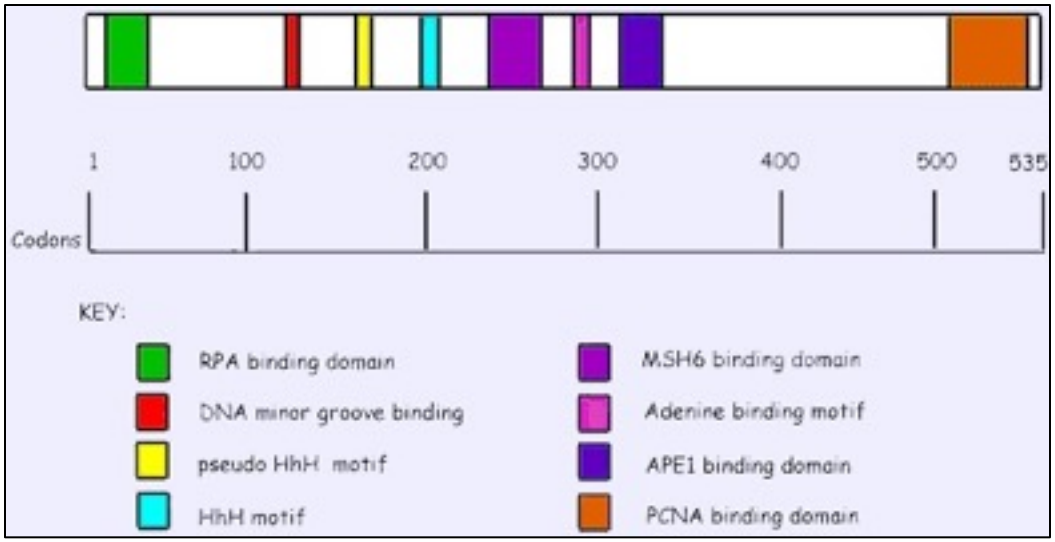
Oxidation causes the formation of 8-oxoG o GO, which mispairs with adenine. At the division A is paired with G (G>T transversion)

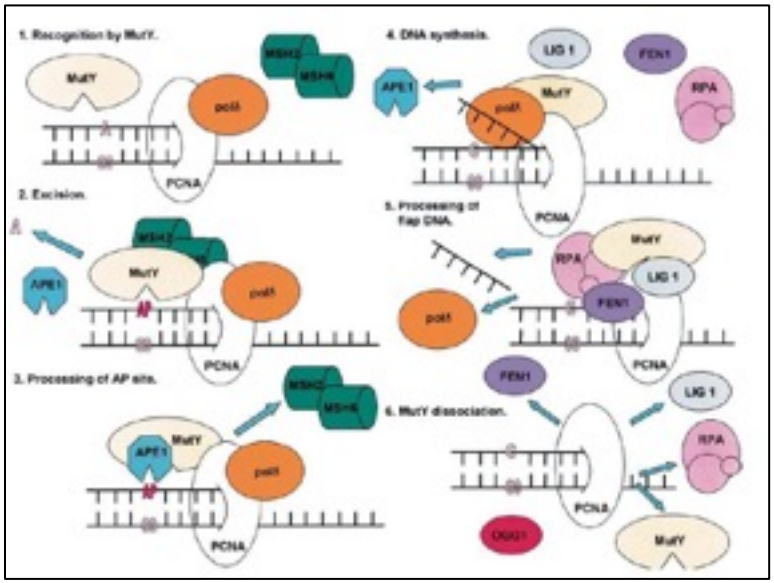
MUTYH is a 7.1 Kb gene, mapping on 1p34.3-1p32.1

It codes for a 535 aa protein

conserved domains involved in:

- DNA binding
- protein-protein interaction
- nuclear and mitochondria signals

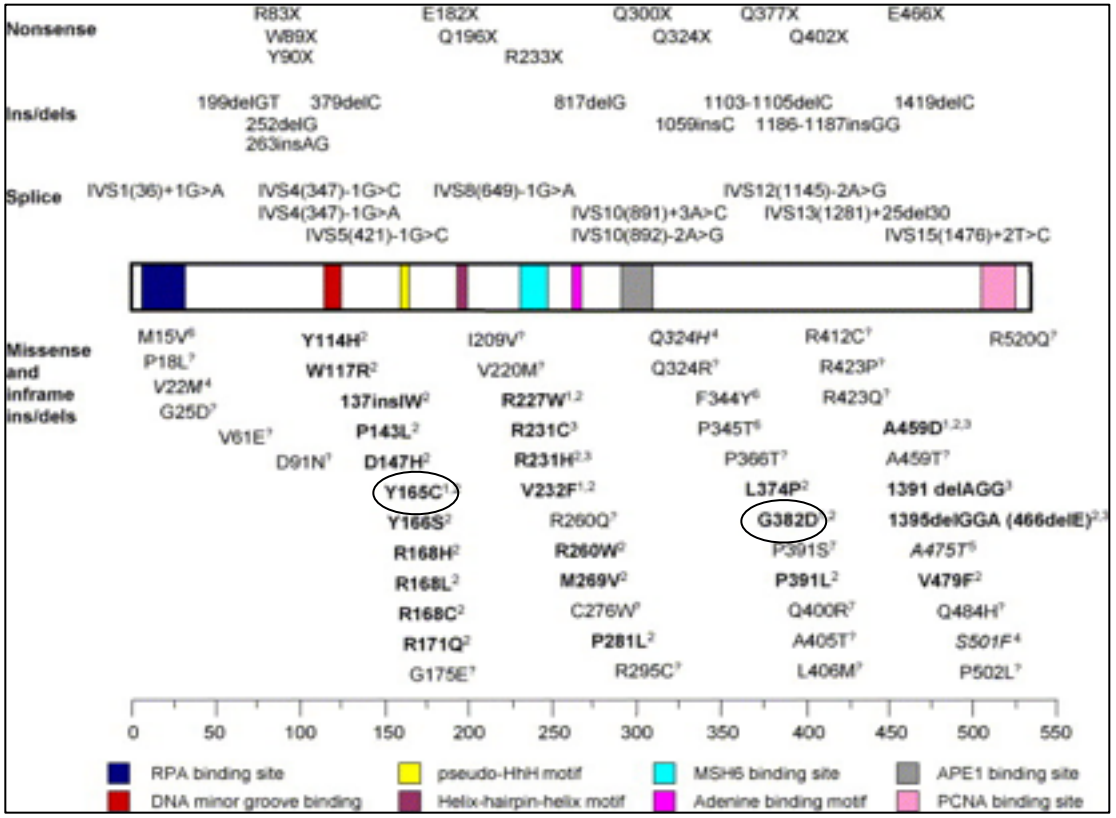




Involvement of other DNA repair systems by interacting with MSH6 (MMR), PCNA e APE1 (recombination)

- recessive autosomic inheritance
- AFAP with >15 adenomas
- most of the mutations are p.Y165C and p. G382D

Cheadle and Sampson,DNA repair, 2007

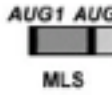


•20% of patients carry other mutations involving most of the exons

- mutations can involve also splicing and introns

- No genotype-phenotype correlation

Parker and Eshleman,Cell Mol Life Sci, 2003

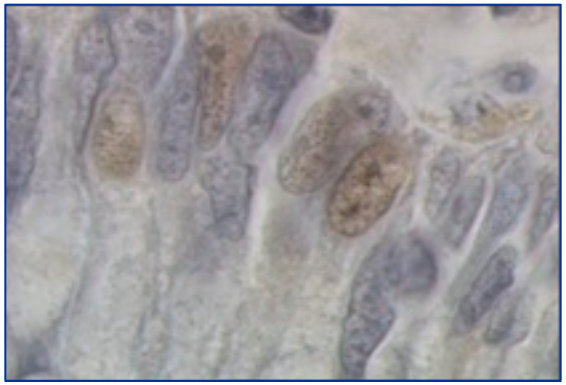


	Isoform	Amino acids	AUG 1, 2 or 3	Probable cellular location	Comments
Type 1	MutYα1	546 aa	1	mitochondira	33bp insert
	MutYα2	536 aa	1	mitochondira	3bp CAG insert
	MutYα3*	535 aa	1	mitochondira	same as Slupska et al. [17]
Type 2	MutYα4	429 aa	3	nucleus	similar to MutYy4 isoform
	MutYβ1	532 aa	2	nucleus	same 33-bp insert as MutYα1
	MutYβ3	521 aa	2	nucleus	similar to MutYy3 isoform
	MutYβ5	521 aa	2	nucleus	
	MutYy2	522 aa	2	nucleus	
	MutYy3	521 aa	2	nucleus	similar to MutYβ3 isoform
	MutYy4	429 aa	3	nucleus	similar to MutYα4 isoform

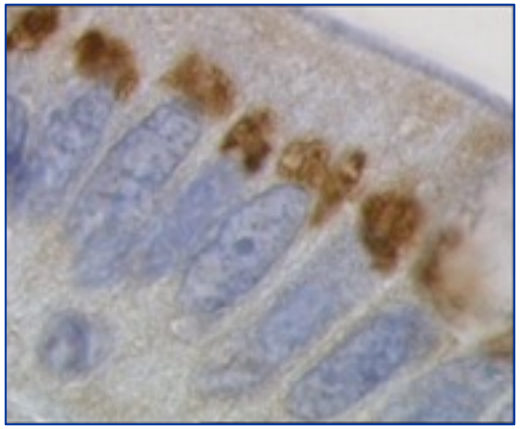
MUTYH gives rise to 10 alternative isoforms

MUTYH is expressed in both nucleus and mitochondria

MAP patients carrying biallelic germline MUTYH mutations show a characteristics perinuclear MUTYH expression in the colonic mucosa and adenomas cells



Control



MAP patient

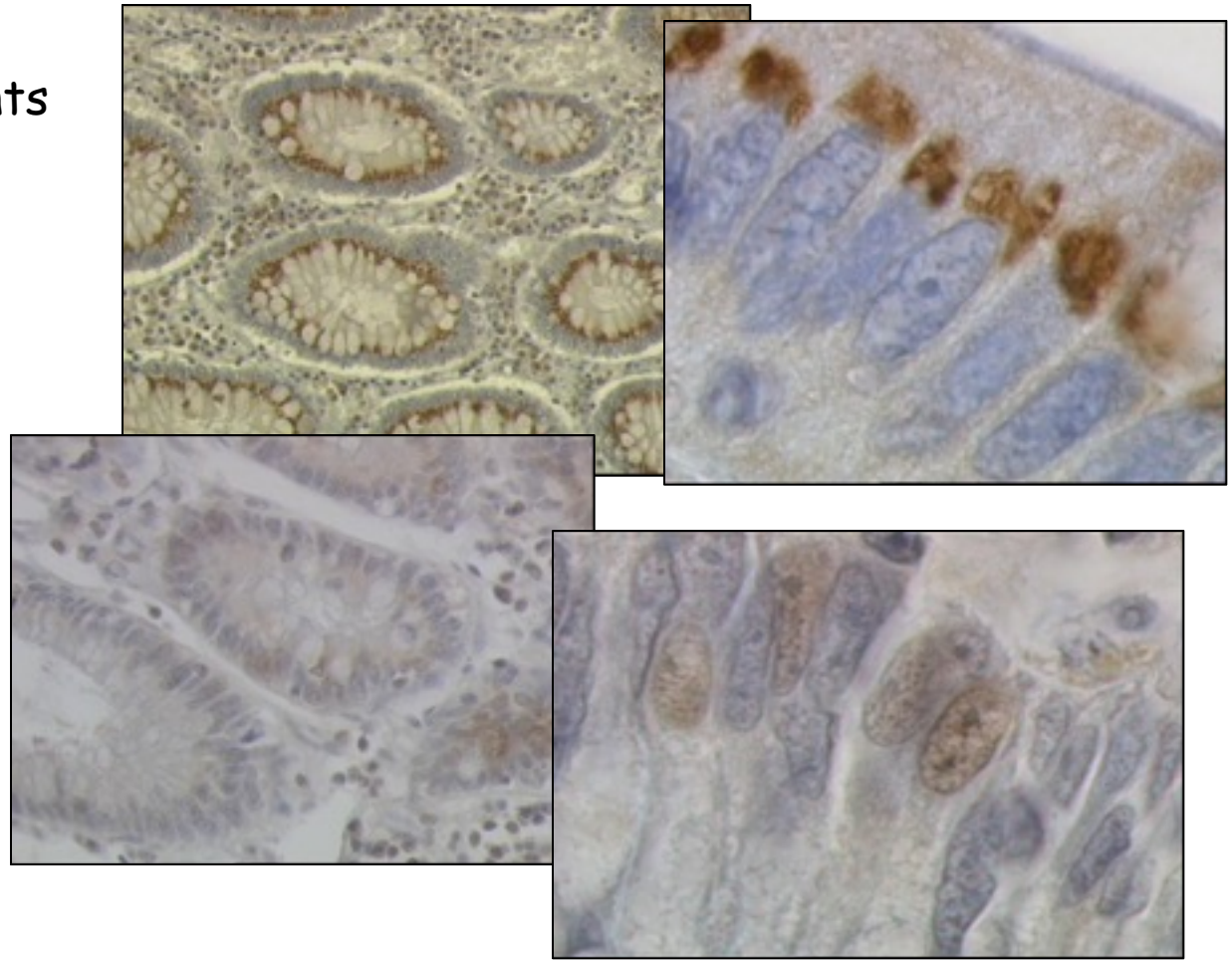
MUTYH expression in normal colonic mucosa and adenomas of MAP patients

Rabbit Polyclonal Ab vs. the region included between aa 531 - 546

Region involved in binding PCNA and the signal for nucleus recognition

MAP patients

Controls



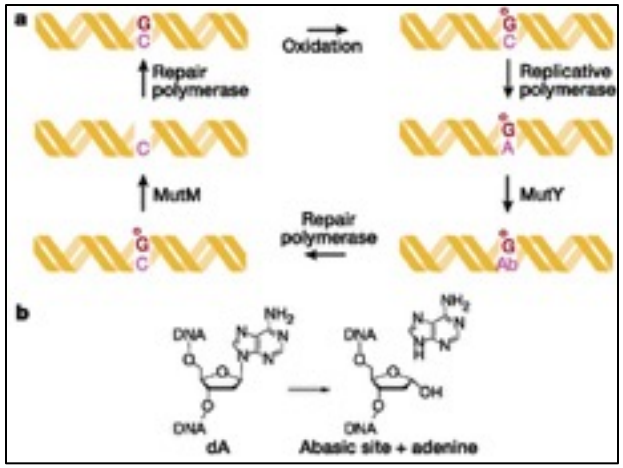
MUTYH carcinogenesis

Oxidative damage

constitutive impaired MUTYH

onset of 8-oxoG mispairing with adenine

- **G>T transversions**



somatic G>T transversions in target genes

APC and KRAS are somatic target genes

- Few mutations in p53, BRAF, SMAD4, TGFßRII

MAP partly follow adenoma-carcinoma sequence

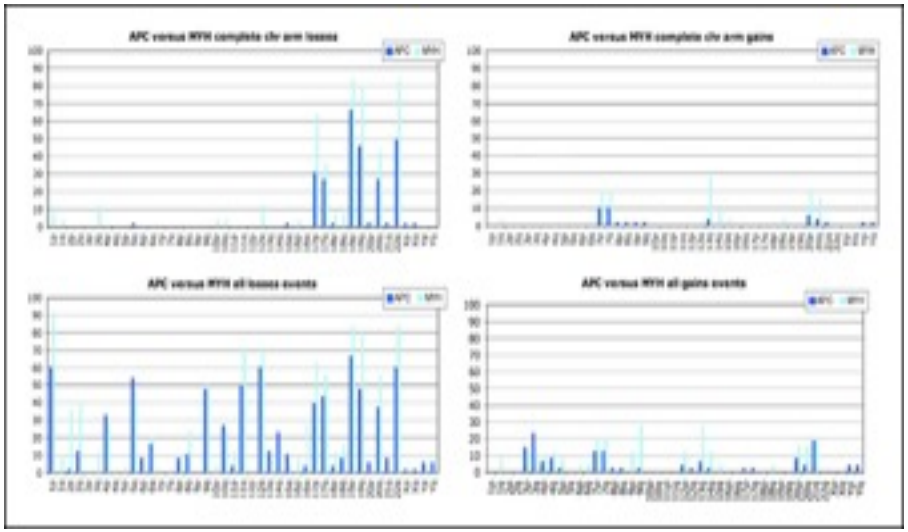
Progression can be rapid (carcinomas in 50% of patients at presentation)

Other factors ?

1. **Aneuploidy**: 80 % MAP vs 60% FAP

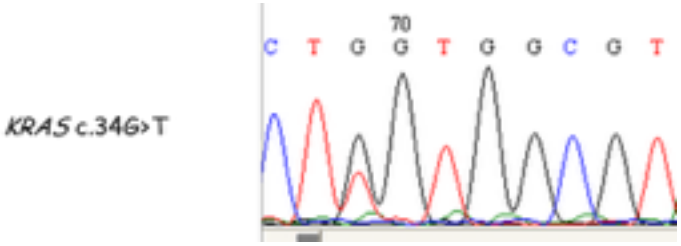
Deletions of chromosome 1p, 17, 19 and 22;duplications of chromosomes 7 and 13

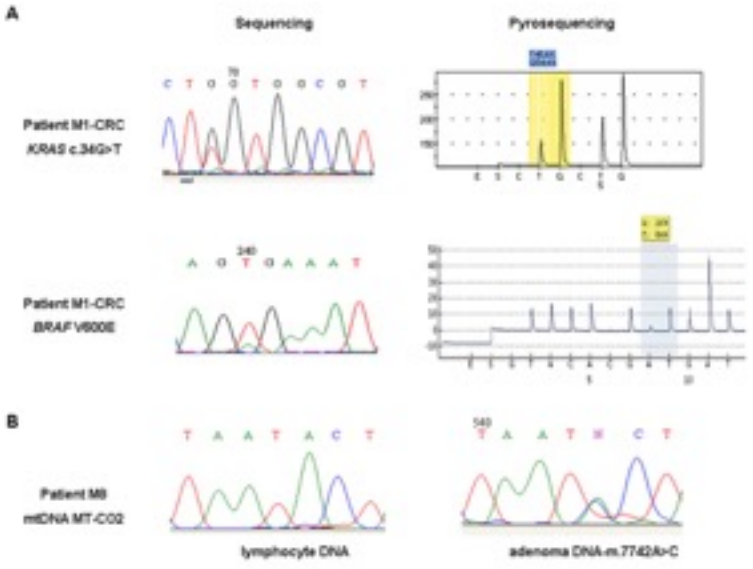
Cardoso et al, Cancer Res, 2006



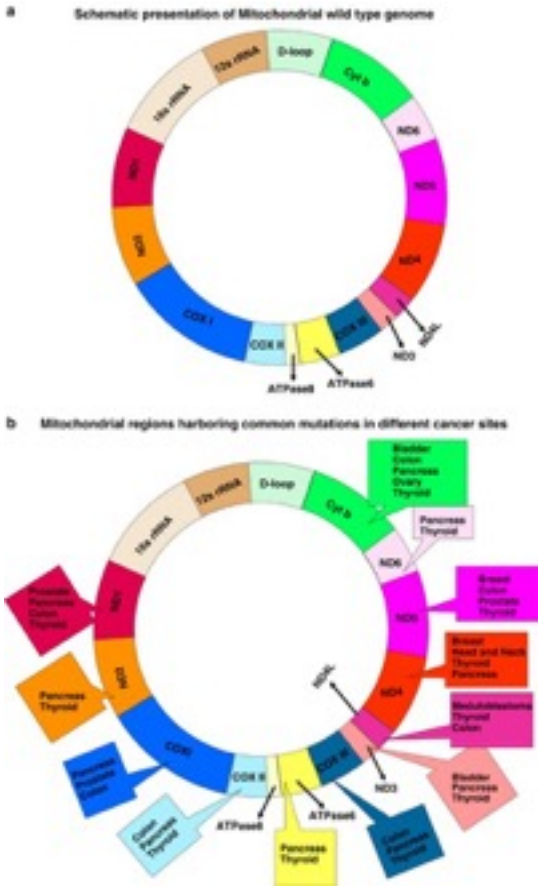
2. **Somatic G>T transversions** in "target" KRAS gene of MAP adenomas and carcinomas

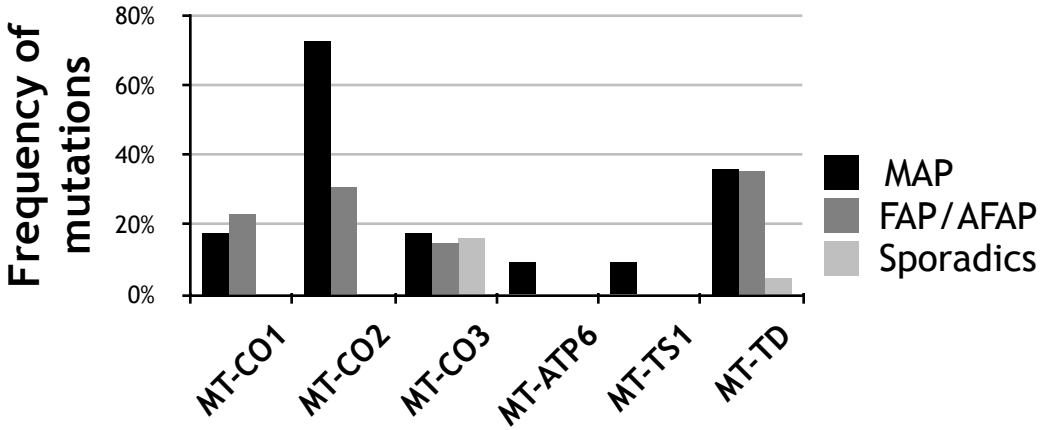
Venesio et al., 2014





2. Specific mutations in **"target" mitochondrial genes** of MAP adenomas and carcinomas

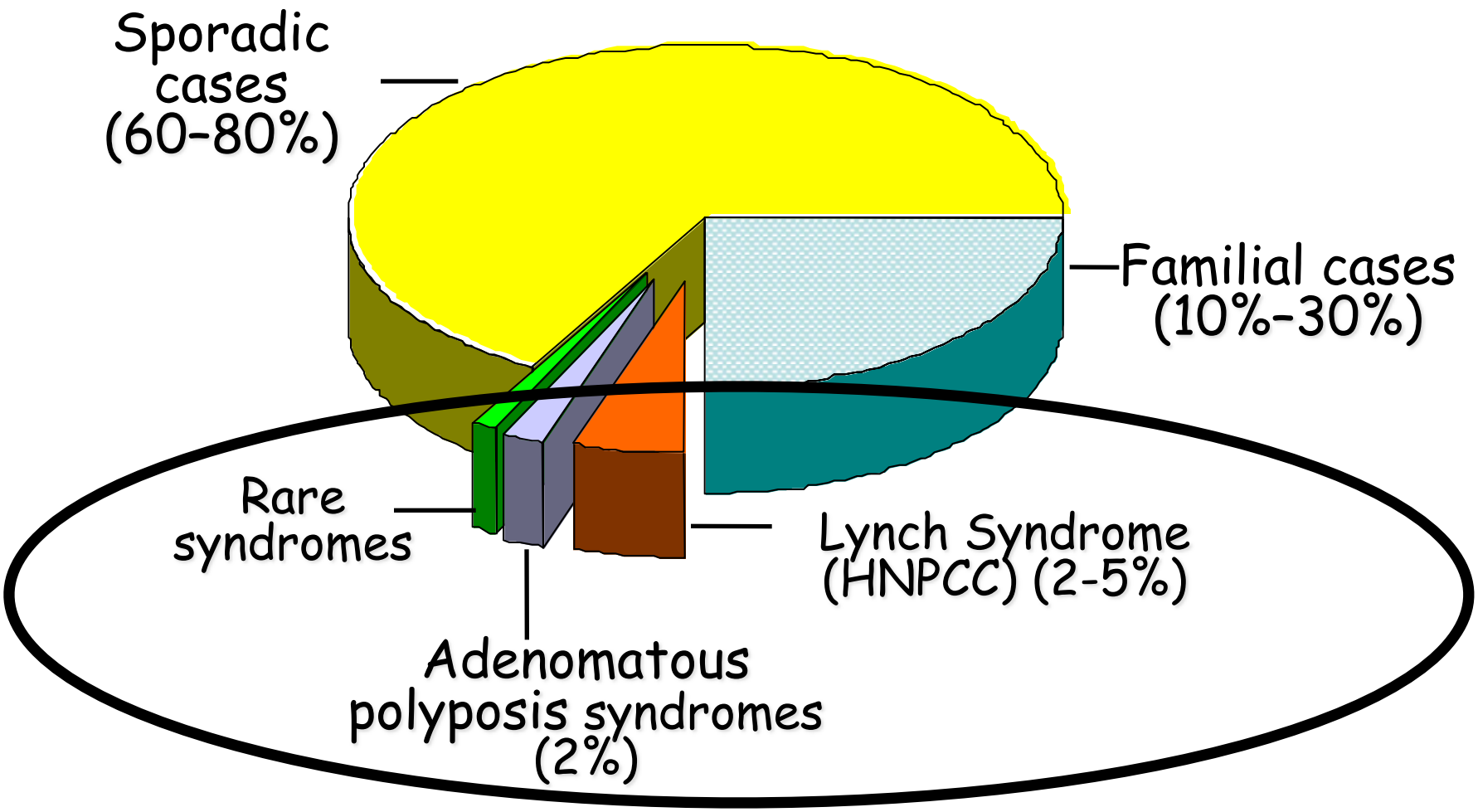




Errichiello et al., 2015

…The sequence analysis revealed **17 different variants**, in the **MT-CO2** gene of MAP patients (P<0.0001) who frequently carried the hotspot m.7763 G>A mutation…**D-loop instability** was also significantly associated with variants grouped inside the MT-CO2 gene (P=0.006)…

Colorectal cancer



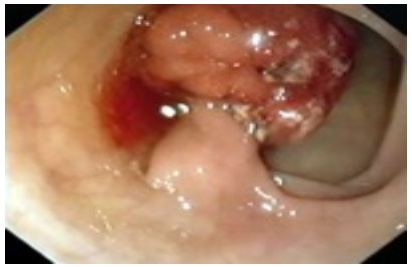
Lynch syndrome or HNPCC

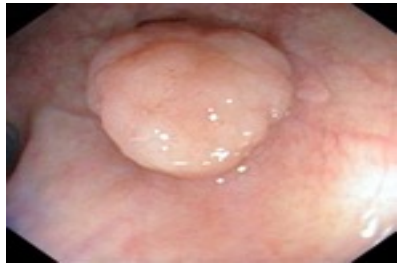
Most common form of hereditary CRC

Vertical transmission, CCR develops at a young age (<50 years), mainly in right colon

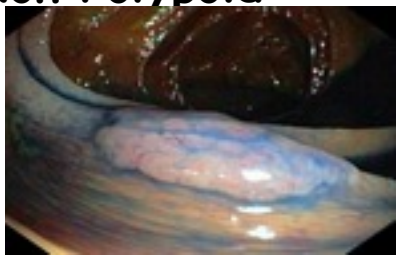
Several extra-colonic tumours (endometrial, gastric, urinary and biliopancreatic carcinomas)

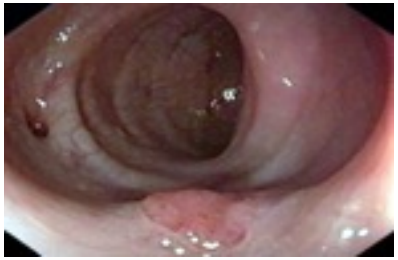
Polypoid

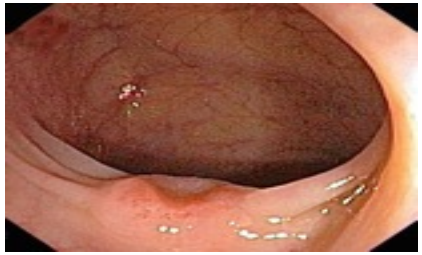




Non Polypoid

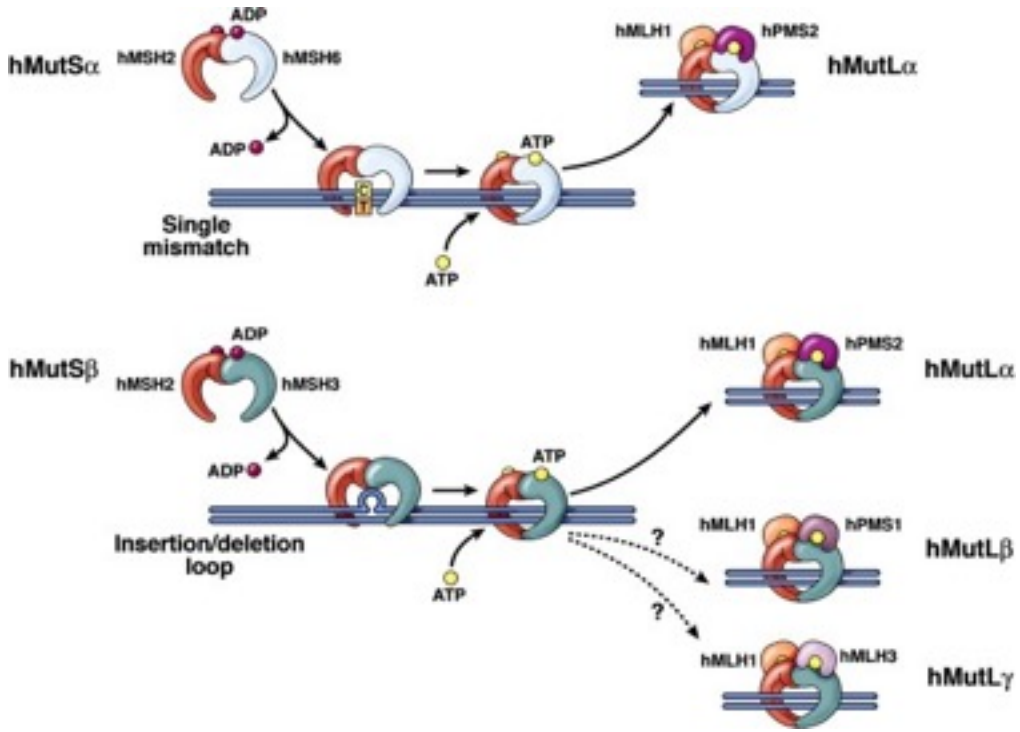






Lynch syndrome or HNPCC

- Autosomic dominant transmission
- This syndrome is in likage with mutations in "DNA mismatch repair" (MMR) genes



MMR gene	chrom.	%.
MSH2	2p21	38%
MLH1	3p21-23	49%
PMS1	2q31-33	0.3%
PMS2	7p22	2%
MSH6	2p21	9%
MLH3	14q24.3	2%

during DNA replication….

base misincorporation ⟶ Mismatches

purine-purine (G/G, A/A, G/A)
purine-pyrimidine (G/T, A/C)
pyrimidine-pyrimidine (C/C, T/T, T/C)

Fidelity in DNA replication

Single-base substitutions arise once in every 10^4 - 10^6 nucleotides incorporated

The proofreading activity of replicative DNA polymerases increases the fidelity to one error in 10^7 to 10^8

DNA Mismatch Repair (MMR) reduces the error rate to one error in 10^9 to 10^{10}

The Mismatch Repair (MMR) is an evolutionary well conserved pathway which greatly improve **fidelity** during **DNA replication** by repairing errors of the DNA polymerase:

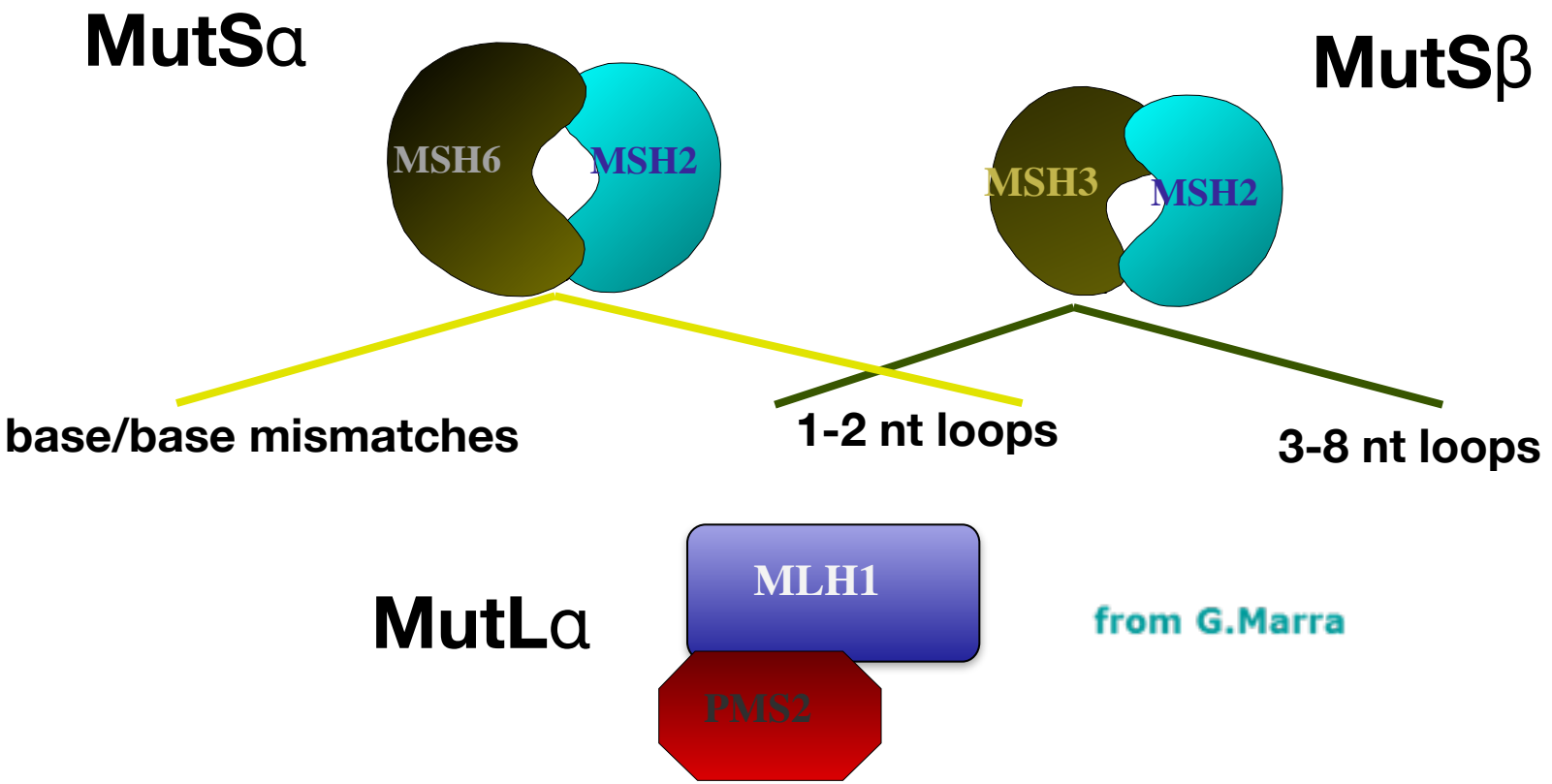
i) base-base mismatches due to insertion of an incorrect nucleotide in the newly synthesized strand

ii) insertion/deletion loops (IDL) caused by strand slippage during DNA replication

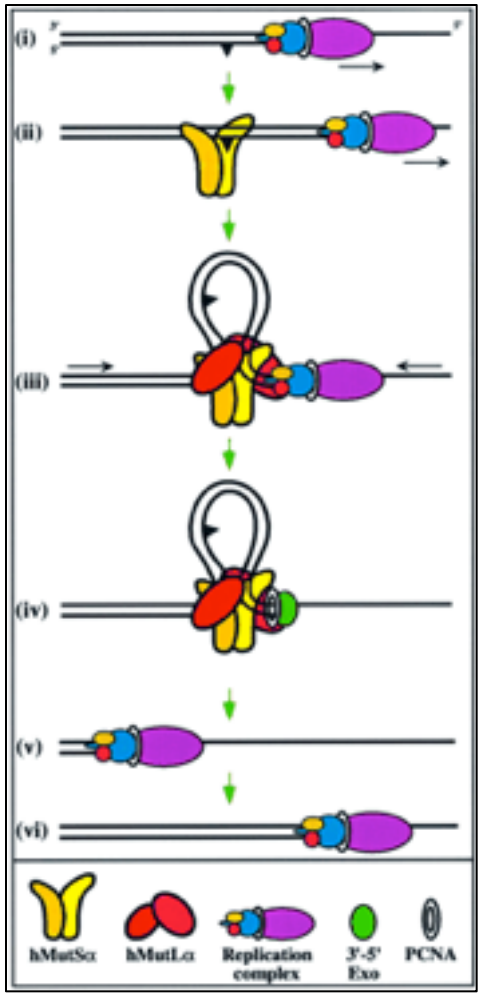
MMR also affects:

- Meiotic and mitotic recombination
- Processing and signaling of DNA-damage by methylating agents
- Processing of oxidative DNA damage
- Triplet Repeat stability
- Immunoglobulin diversity
- Bacterial adaptation and evasion of host immunity
- Genetic diversity in plants

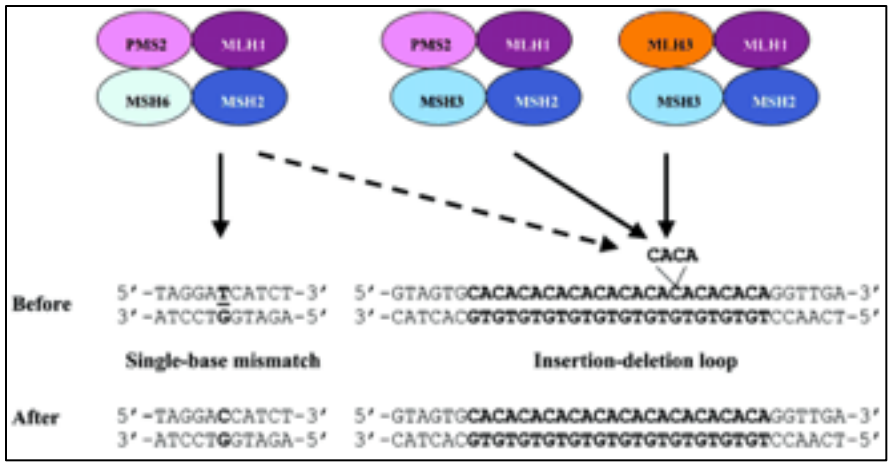
DNA errors are detected by
MSH2/MSH6 and **MSH2/MSH3** heterodimers



MLH1/PMS2 heterodimers
are recruited for excision and synthesis of the new repaired strand

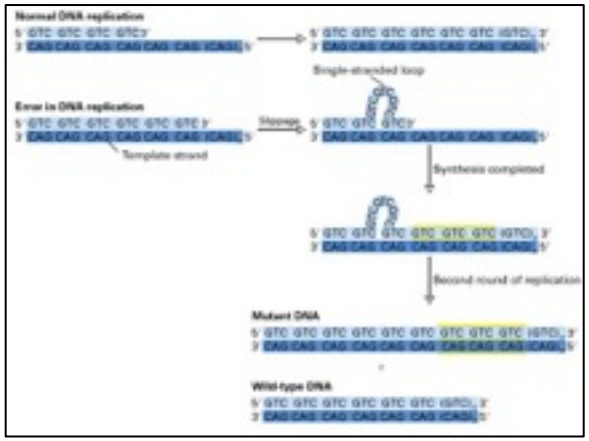


MMR genes form multiproteic complexes that jointly work to repair mispairing during DNA replication



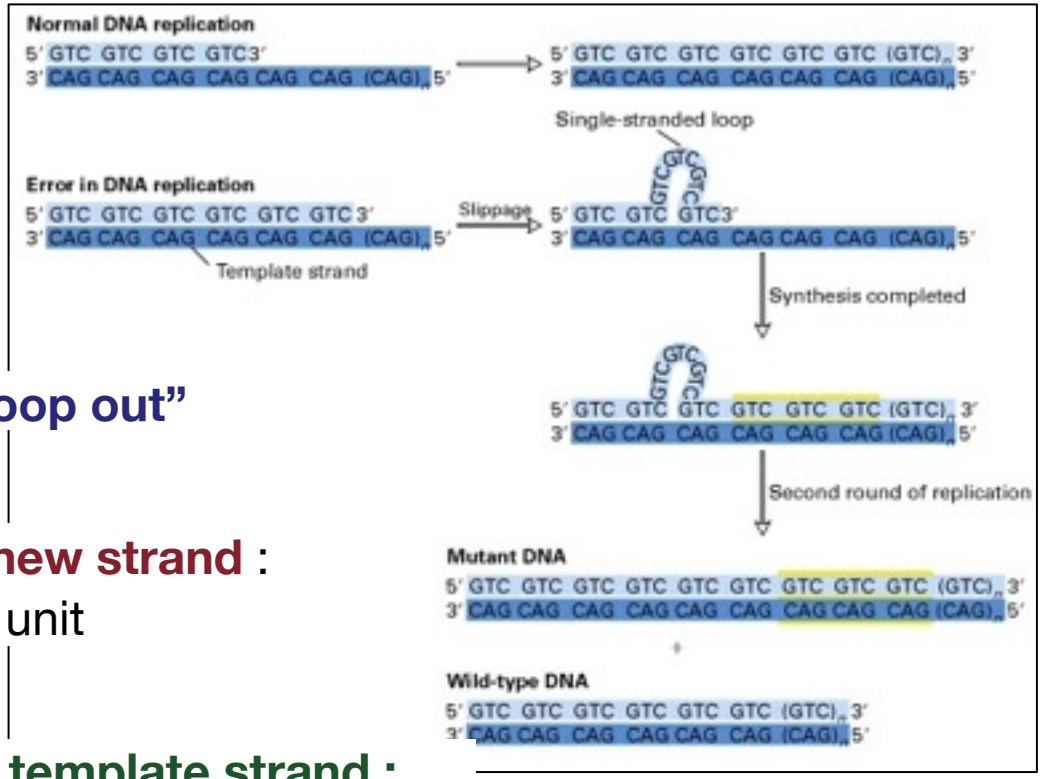
When MMR is impaired, short repeated sequences (microsatellites) are prone to insert errors during replication

Microsatellite instability (MSI)



Microsatellites are prone to "slippage" during DNA replication

Mispairing *occurs between the template and the new synthesised strand*



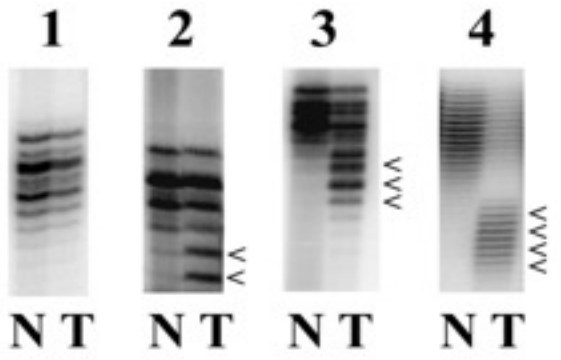
Unpaired DNA is forced to **"loop out"**

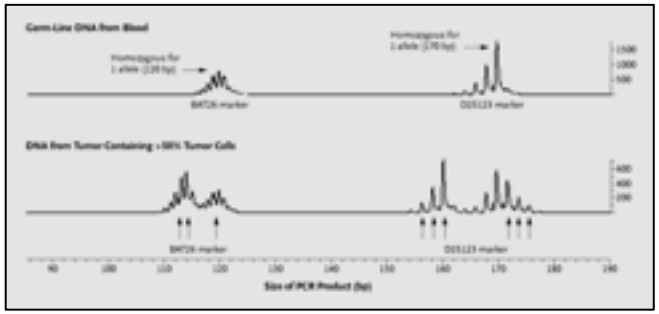
- The"loop" is on the **new strand** : **addition** of a repeat unit
- The "loop" is on the **template strand : loss** of a repeat unit

Microsatellite mutation rate is about 10,000 times that for a single base change

MMR protein inactivation causes the huge accumulation of mutations at the microsatellites…..

Microsatellite Instability - MSI





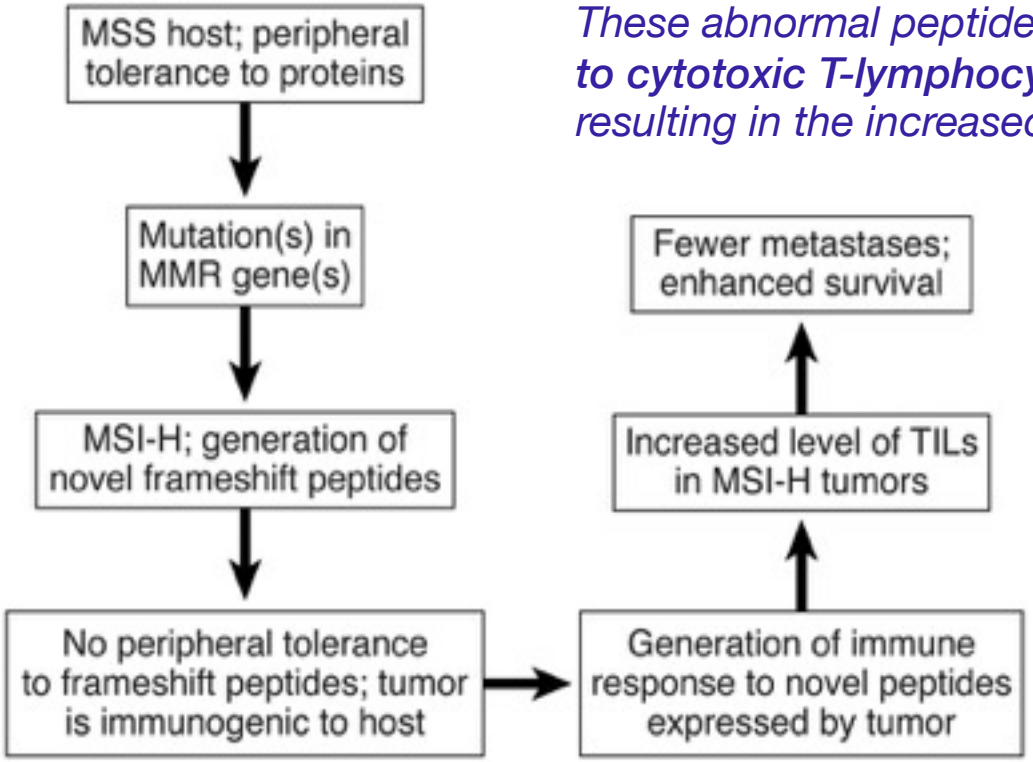
DNA alterations inactivate several proteins facilitating carcinogenesis…

TGFβ Receptor II AAG TGC ATT ATG AAG G**AA AAA AAA AA**G CCT GGT GAG

Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu

BAX, MSH3, TCF4, MSH6, Axin, MBD4, IGF Receptor II, β2-microglobulin, POLD3 ……

MSI tumors result in frameshifting mutations within coding sequences, functionally inactivating the corresponding proteins



*These abnormal peptides are presented through the **MHC I to cytotoxic T-lymphocytes (CTLs) as neoantigens**, resulting in the increased TIL density*

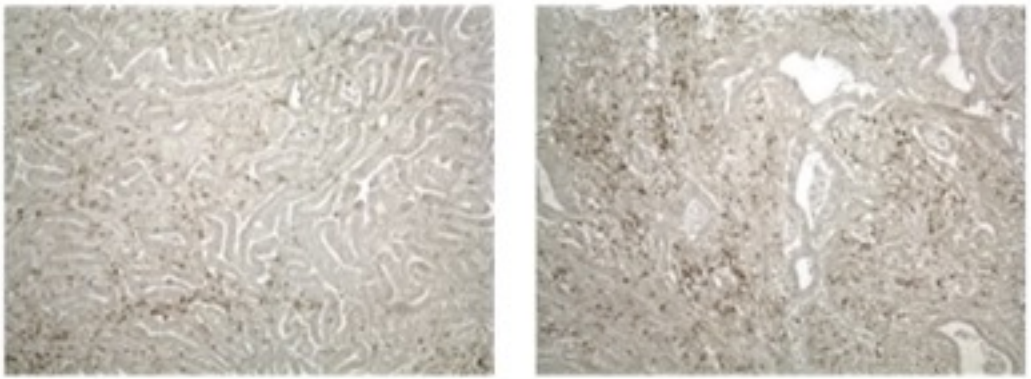
MSI tumors show a better prognosis than MSS ones

1. MSI tumours loss β2 microglobuline and HLA I expression

Cells are not able to present the new proteins to the antigens

Immunosurveillance decreases with tumour increase

2. MSI tumours draw cytotoxic T lymphocites (TIL)



(a) (b)

MSI patients show an increased level of CD3+ lymphocytes (b) in respect to non-MSI (a)

New epitopes are produced

Immunosurveillance increases with tumour shrinkage

How does MMR altered genes-associated carcinogenesis work ?

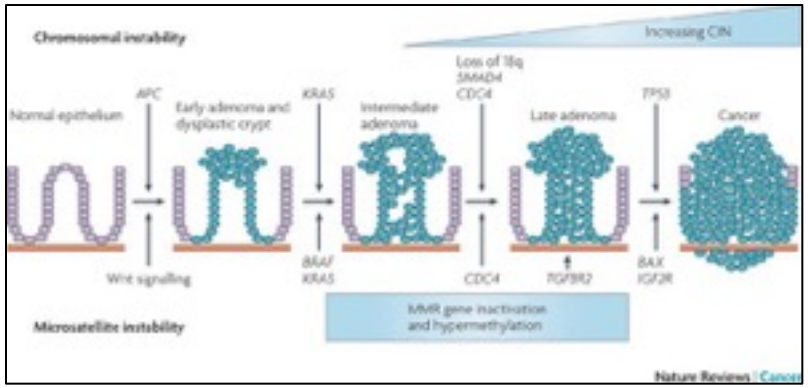
MMR inactive gene → High number of mutations in repeated sequences of target genes → Selective advantage of a cell clone

Target genes			
	•BAX •IGFIIR •TGFβRII	•TCF4 •AXIN2 •PTEN	•hMSH3 •hMSH6 •MBD4

- HNPCC show an accelerated carcinogenesis
  - Poor information concerning the early lesions
    - In advanced stages both CIN and MSI features

LOH of MMR genes

Microsatellite instability of adenoma - carcinoma sequence genes



MSI tumors typically harbor more than 1,000 coding somatic mutations per tumor cell genome compared with the 50 to 100 somatic mutations found in microsatellite stable (MSS) tumors

Genome Medicine

RESEARCH Open Access

Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden

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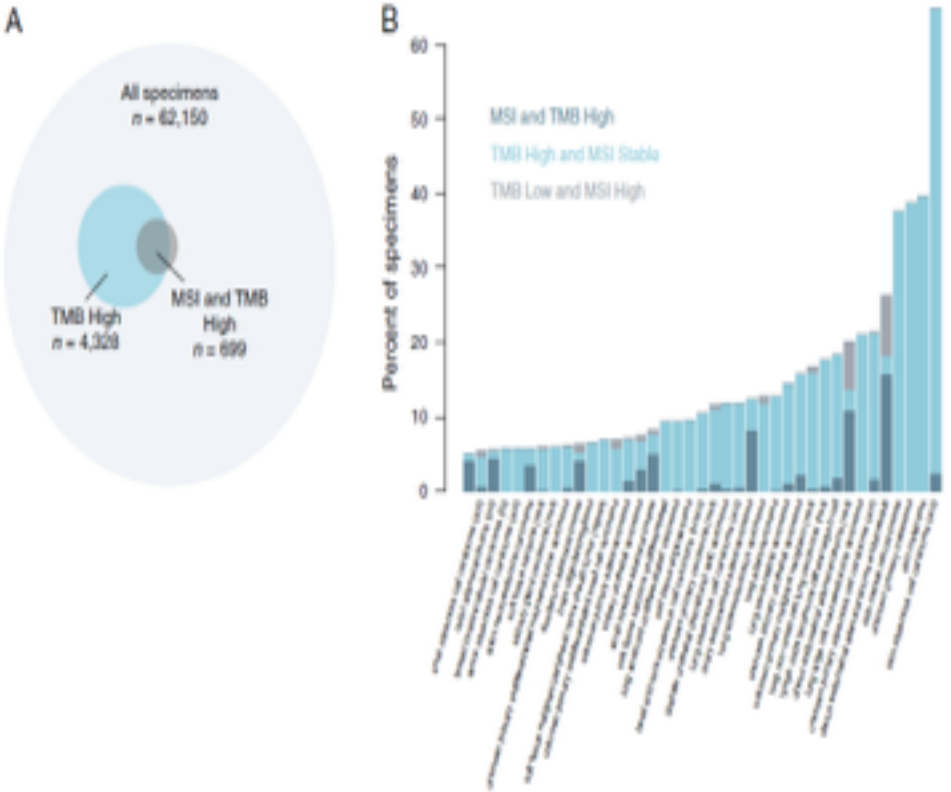


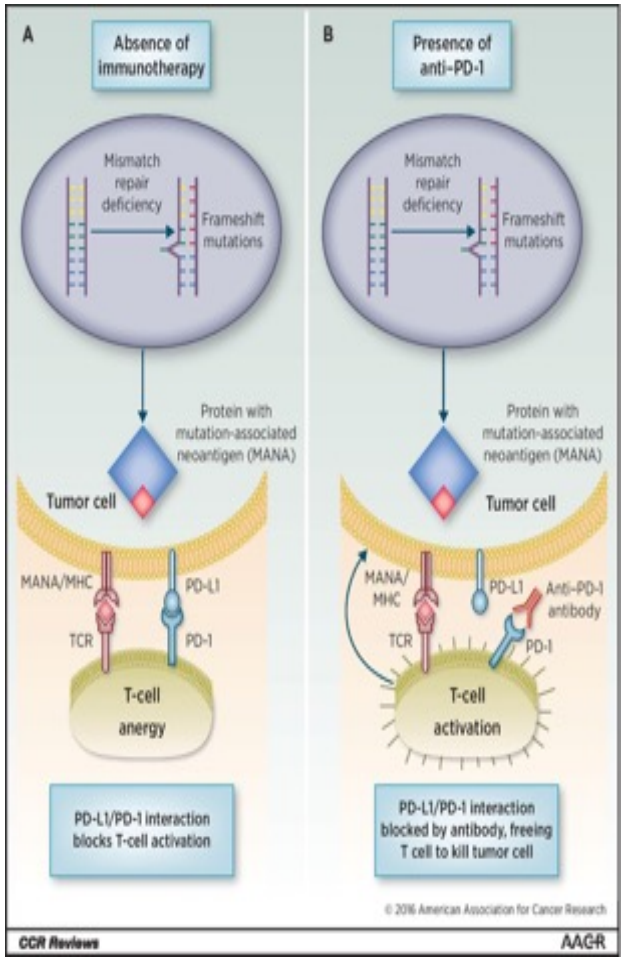
The majority of MSI samples show high tumoral mutational burden (TMB)

However, the converse is not true

Tumor cells interact with the immune system in the tumor microenvironment

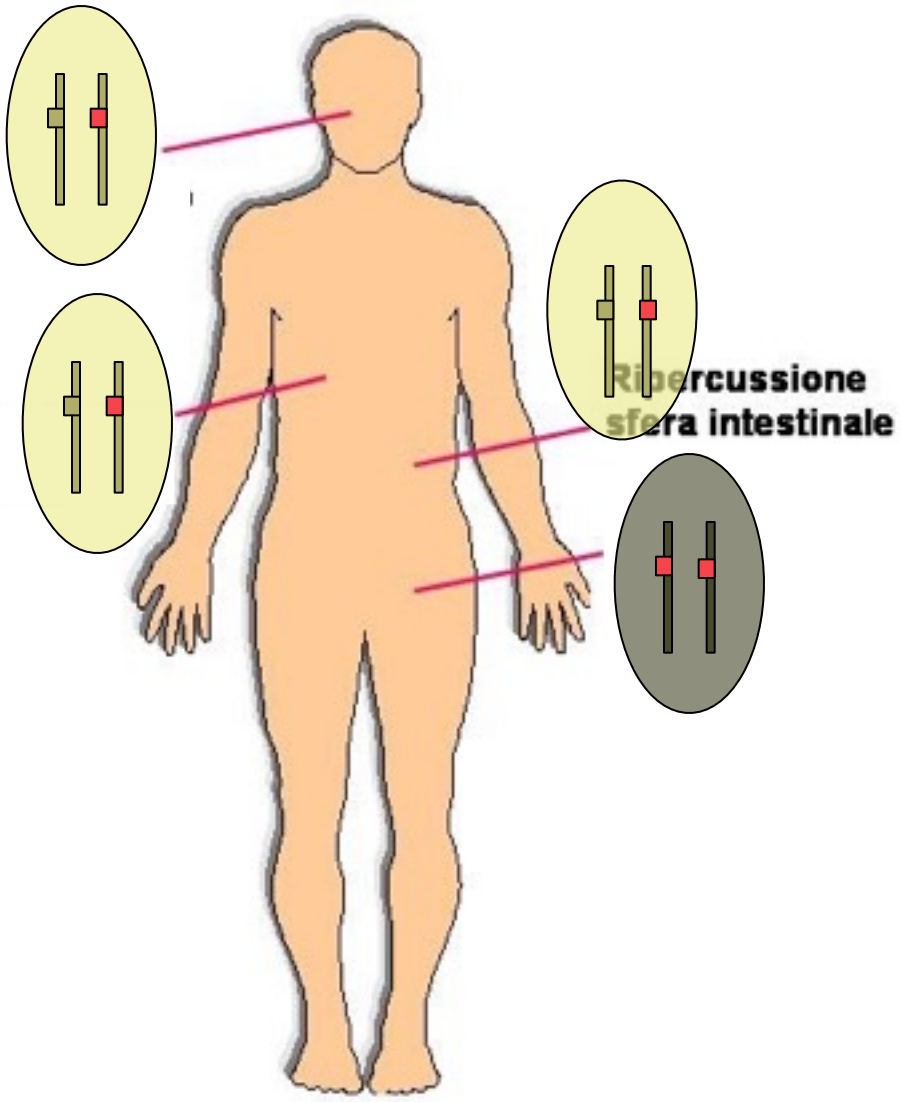
Three steps:
- *elimination of tumor cells by T lymphocytes*
- *equilibrium*
- *immune escape/ immune tolerance*

***Immunoediting** process regulated through **checkpoint receptors**, including programmed death 1 pathway (PD-1 and its ligand PD-L1)*



Dudley et al, Clinical Cancer Res, 2016

Inhibitors of these pathways lead to stimulation of activated T-cells and antitumor immunity



Lynch syndrome or HNPCC

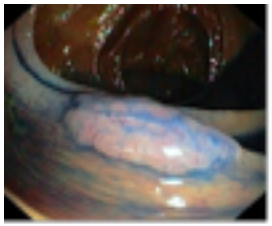
HNPCC:
two "hits" model,
first costitutive,
second somatic

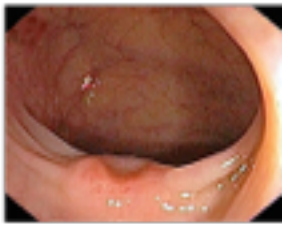
The syndrome is associated with a germline mutations in one of the MMR genes

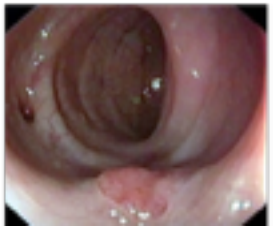
Heterozygous status causes susceptibility

The second "hit" is associated with MSI in tumours

Lynch syndrome or HNPCC





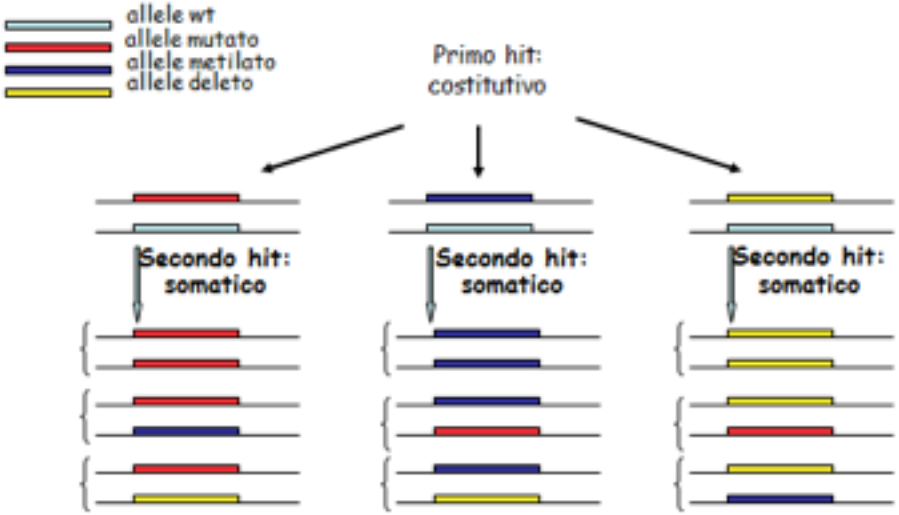


- Most common hereditary CCR(3-4%)
- Vertical transmission ; CCR may onset at young age (<50 aa), mainly involving right colon
- Extra-colonic tumours (endometrial, gastric, urinary and e biliopancreatic cancer)
- In linkage with germinal mutations of MMR genes

1.Germline mutations

49% MLH1 mutations
39% MSH2 mutations
10% MSH6 mutations
1-2% PMS2 mutations

2.Somatic mutations



1998 BETHESDA CONSENSUS CONFERENCE

[CANCER RESEARCH 58, 5248–5257, November 15, 1998]

Meeting Report

A National Cancer Institute Workshop on Microsatellite Instability for Cancer Detection and Familial Predisposition: Development of International Criteria for the Determination of Microsatellite Instability in Colorectal Cancer

C. Richard Boland,[1] Stephen N. Thibodeau, Stanley R. Hamilton, David Sidransky, James R. Eshleman, Randall W. Burt, Stephen J. Meltzer, Miguel A. Rodriguez-Bigas, Riccardo Fodde, G. Nadia Ranzani, and Sudhir Srivastava[2]

Reference panel

Marker	Repeating unit	GenBank accession no.
BAT25	Mononucleotide	9834508
BAT26	Mononucleotide	9834505
D5S346	Dinucleotide	181171
D2S123	Dinucleotide	187953
D17S250	Dinucleotide	177030

Criteria for interpretation

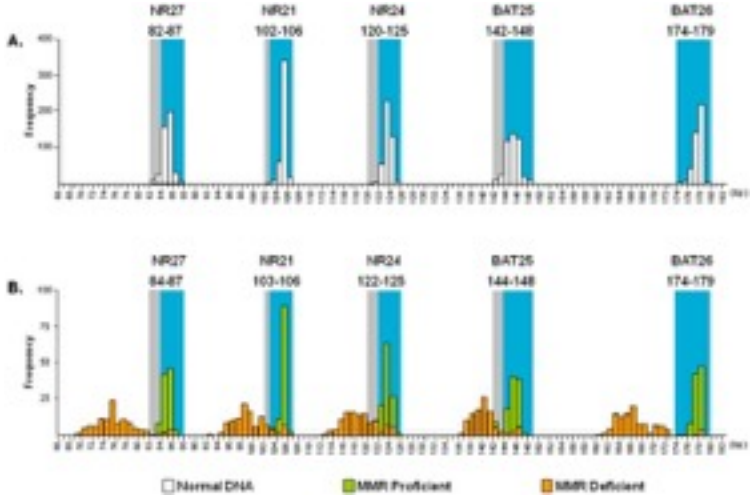
	5 loci analyzed	>5 loci analyzed	Interpretation
No. of markers	≥2	≥30–40%	MSI-H
Exhibiting instability	1	<30–40%	MSI-L
Length changes	0	0	MSS or MSI-L

- *Poorly proficient for MSH6-associated instability detection*
- *Recommended to use matched normal/tumor pairs*

VOLUME 24 · NUMBER 2 · JANUARY 10 2006
JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

2004-2006

New panel with 5 mononucleotides: the ***"pentaplex"***

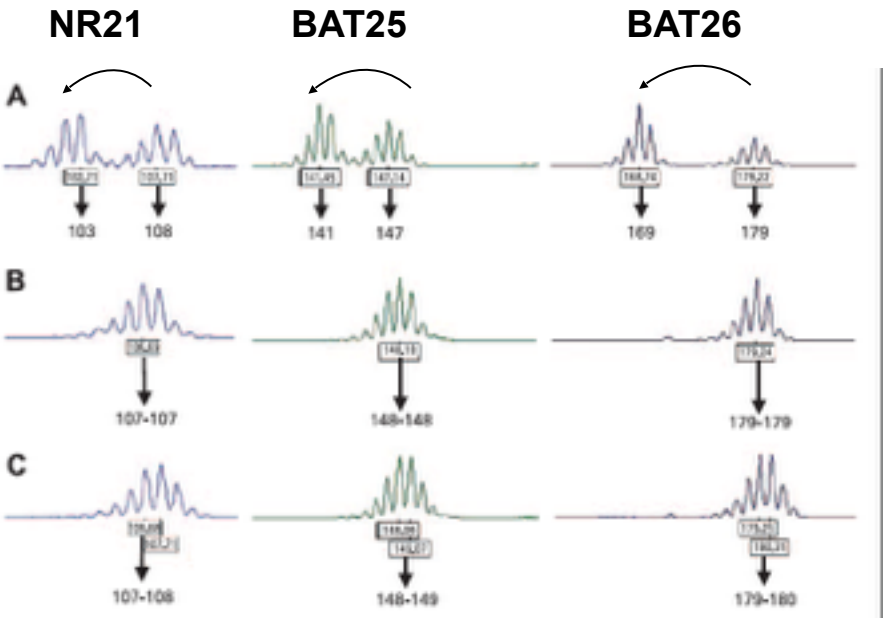


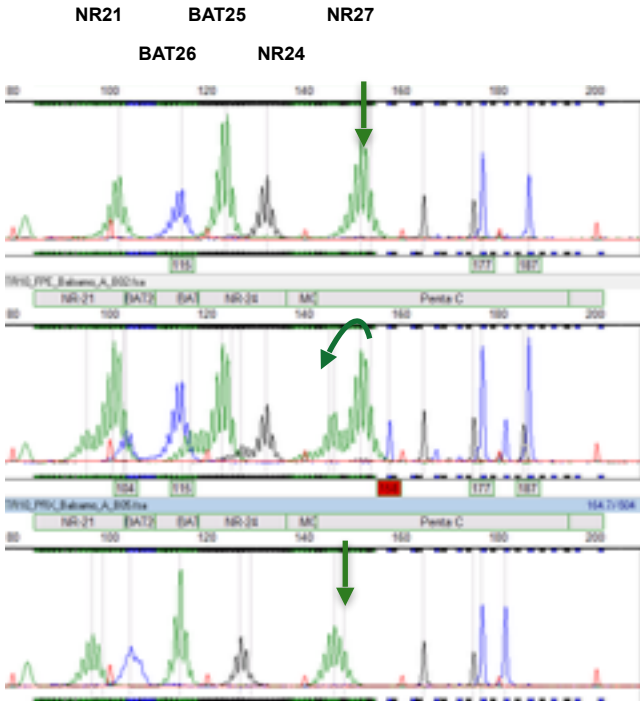
Multipopulation Analysis of Polymorphisms in Five Mononucleotide Repeats Used to Determine the Microsatellite Instability Status of Human Tumors

Olivier Buhard, Francesca Cattaneo, Yick Fu Wong, So Fan Yim, Eitan Friedman, Jean-François Flejou, Alex Duval, and Richard Hamelin

Marker	Gene	GenBank No.	Localization
NR-27	Inhibitor of apoptosis Protein-1	AF070674	5'UTR
NR-21	*SLC7A8*	XM_033393	5'UTR
NR-24	Zinc finger 2	X60152	3'UTR
BAT-25	*c-kit*	X69313	intron 16
BAT-26	*hMSH2*	AY601851	intron 5

- *Improved MSH6-associated instability detection*
- *No need of matched normal/tumor pairs*
  - Poor detection of MSI-L

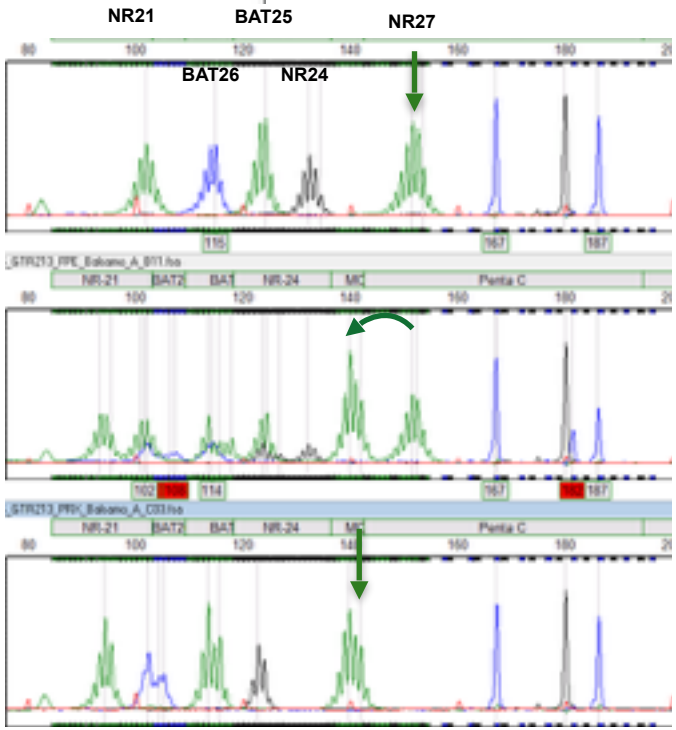




Lymphocytes

FFPE tumor

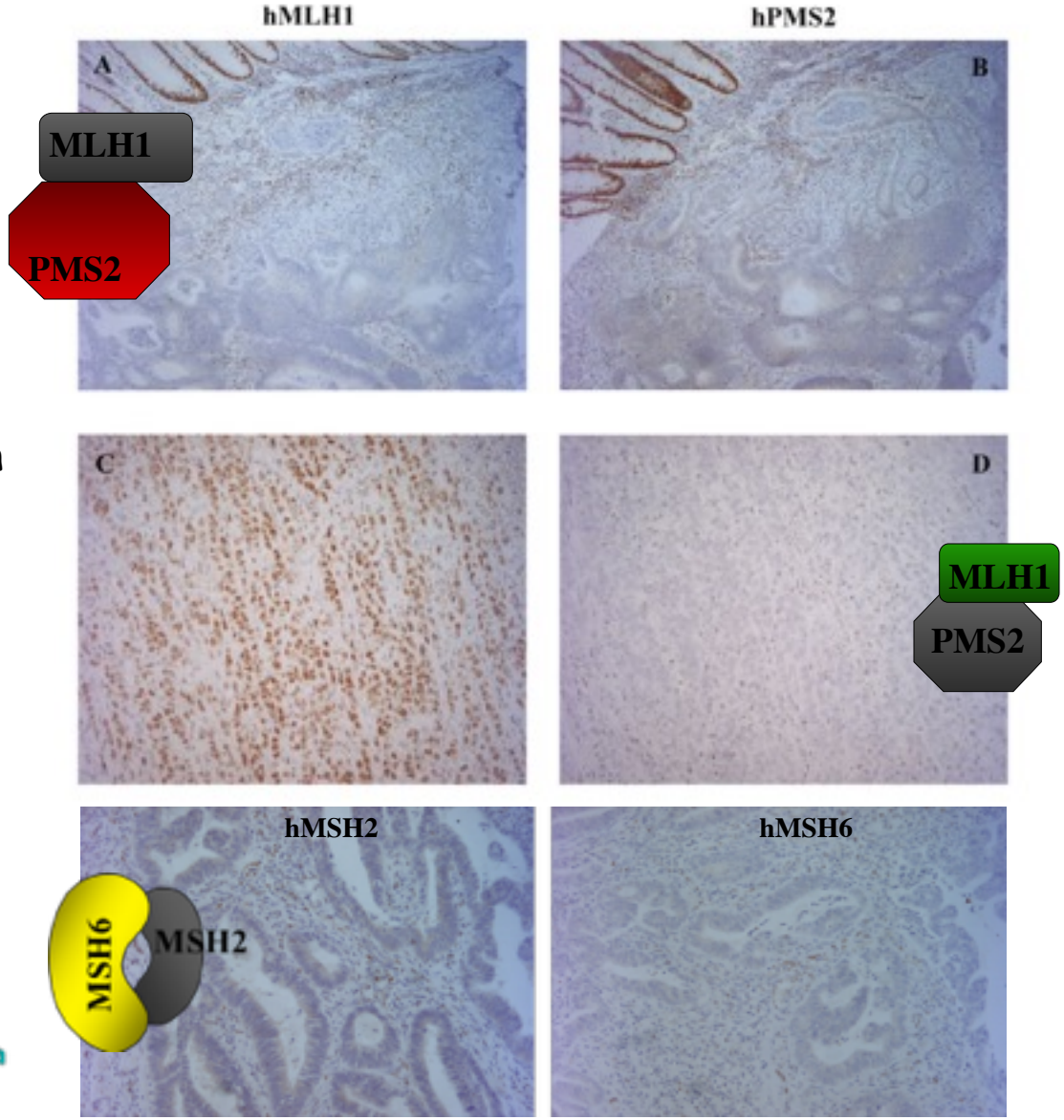
Xenopatient tumor



Protein expression of MMR genes

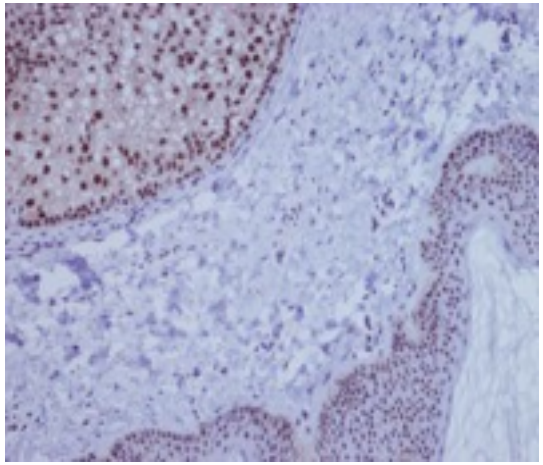
Expression loss of one of the proteins suggest the genetic analysis

- hMLH1/hMSH2 are stable when hPMS2/hMSH6 are lost



from G.Marra

Normal nuclear MLH1 expression in sebaceous adenoma and epidermis



from C. Digregorio

*IHC is **inexpensive** and readily **available** at most institutions*

*Lack of expression of a specific protein may indicate **a specific gene to be tested***

*Useful in the search of **MSH6 mutations***

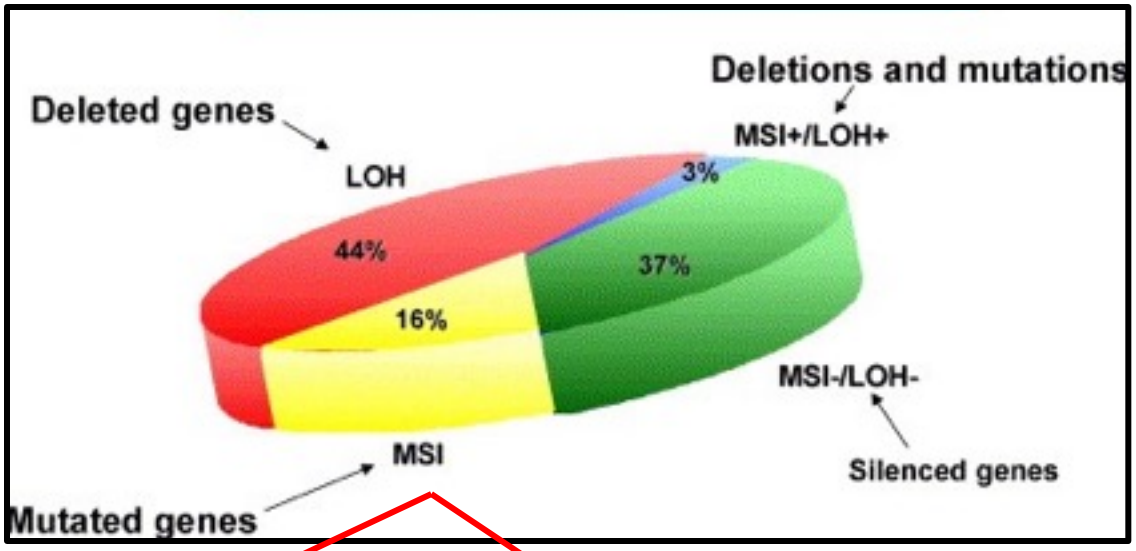
*It may miss **abnormalities** caused by untested proteins or mutations that lead to **qualitative**, but not quantitative (missense mutations)*

*Staining can be heterogeneous throughout tumor samples, and **scoring may not be readily reproducible***

IHC and PCR are sensitive and specific biomarkers for MMR and MSI
The two tests show high concordance (>95%)

Microsatellite instability is a common feature of different tumour types

It involves 15 % of CRC



Lynch syndrome 20%

Germline inactivation of one allele and following somatic inactivation of the second allele

Sporadics 80%

Somatic inactivation of both alleles, mainly for MLH1 hypermethylation

MMR altered genes-associated carcinogenesis

Hereditary vs sporadic model

Hereditary ~ 20%

2nd mutational hit

↓

Normal mucosa -> Adenoma -> Adenocarcinoma **(HNPCC o Lynch Syndrome)**

Heterozygous MMR gene mutation

~ 18% mutazioni exon 3 di *CTNBB1*

Sporadic ~ 80%

***MLH1* silencing**

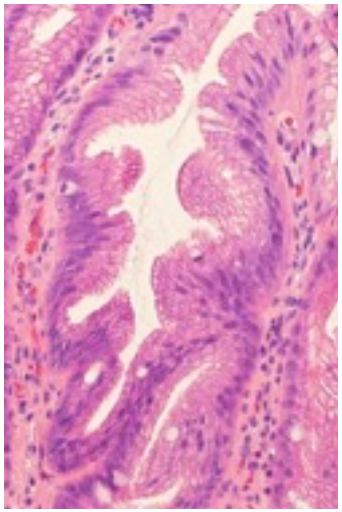
↓

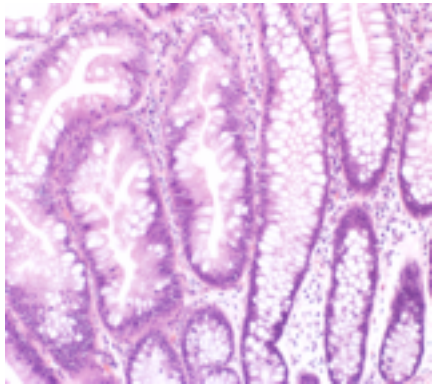
Normal mucosa -> *Serrated* Adenoma -> *Serrated* adenocarcinoma

MGMT inactivation by hypermethylation

~ 50% *BRAFV600E* mutations

MSI in sporadic CRC





From J.Jass

Hystotype : presence of **serrated adenomas**

Serrated pathway

- high frequency of BRAF p.V600E mutations
- high frequency of hypermethylated genes (CIMP)

1. Somatic mutation

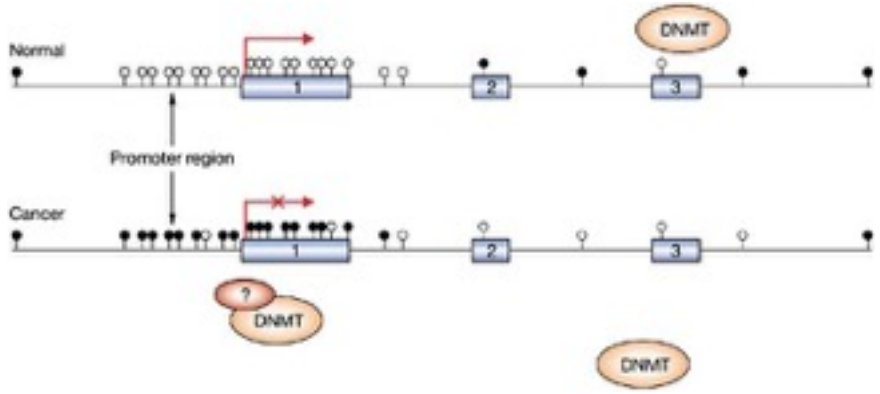
Methylation of MLHI promoter

2. Somatic mutation

Methylation of MLHI promoter

Some CRCs are not characterized by either CIN or MIN phenotype

Hypermethylation pathway



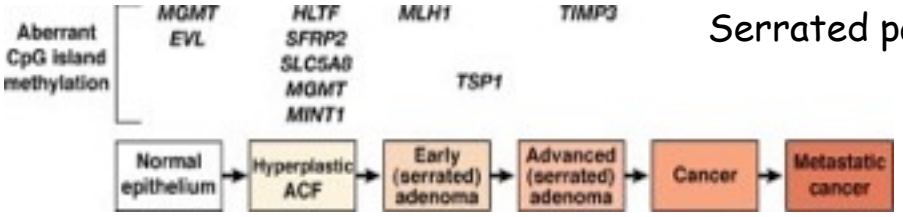
A lot of genes affected by epigenetic alterations, mainly hypermethylation

Methylation target genes

hMLH1, MGMT, p16, p14, HPP1/TPEF, COX2, APC, CDH1

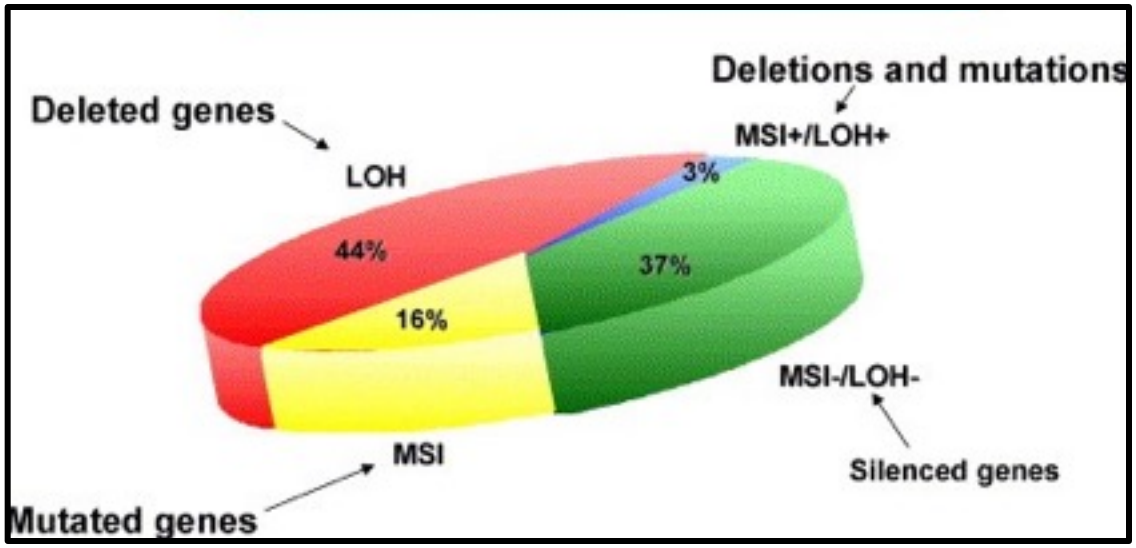
These tumors show a "metilator" phenotype

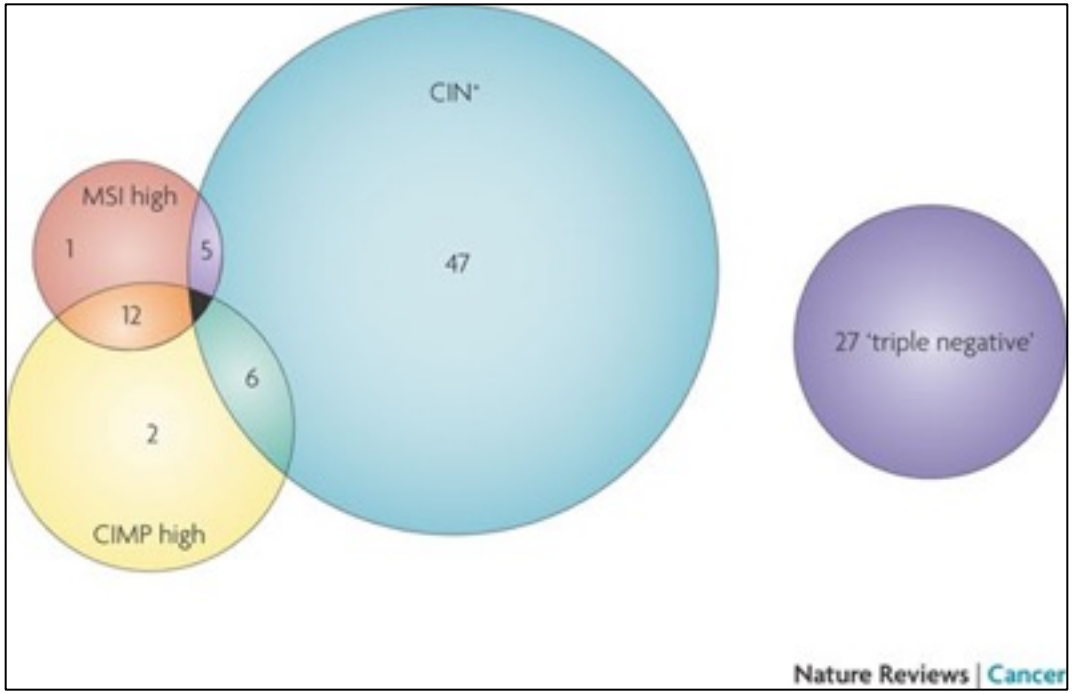
They are called CIMP tumours



Serrated pathway

hMLH1 inactivation by sporadic methylation ⟶ CIMP tumours can be MIN


Deleted genes
Deletions and mutations
MSI+/LOH+
LOH
3%
44%
37%
16%
MSI-/LOH-
MSI
Silenced genes
Mutated genes


CIN+
MSI high
1
5
47
12
6
27 'triple negative'
2
CIMP high
Nature Reviews | Cancer