How the tumor is initiated ?

- 1. **Chemical** carcinogenesis

TEST

- 2. **Hormonal** carcinogenesis
- 3. **Viral** carcinogenesis
- 4. **Bacterial/parasitic** carcinogenesis
- 5. **Chronic inflammation** carcinogenesis
- 6. **Spontaneous** carcinogenesis

  as a sum of all things above

1. **Chemical** carcinogenesis

Carcinogen

Any agent that produces cancer, e.g.
tobacco smoke,
certain industrial chemicals,
ionizing radiation
(such as X-rays and ultraviolet rays).

Carcinogens

1. Genotoxic

(direct DNA damaging carcinogens)

produce DNA adducts;

one application is enough for tumor initiation

2. Non-Genotoxic

(damage DNA as result of secondary interactions,

e.g. increase of oxidative stress, inflammation)

Genotoxic Carcinogens – mutators

Direct carcinogens
(no modification needed)

Anti-tumoral chemotherapeutic drugs
(cyclophosphamide, busulfan, chlorambucil),

beta-propiolactone

Acetylating and alkylating agents

Pro-carcinogens
that have to be modified by intracellular enzymes

benzanthracene
(first pure carcinogen)

3,4-benzpyrene
(isolated from coal tar)

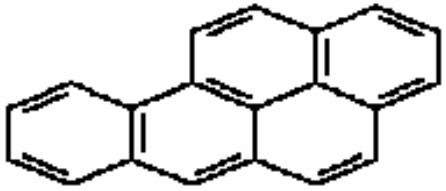
7,12-dimethylbenzanthracene
(most potent carcinogen)

Aflatoxin B1

Aromatic Amines and Azo Dyes

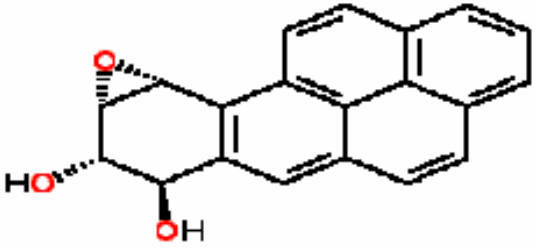
polycyclic aromatic hydrocarbons (PAHs)

Benzo[a]pyrene

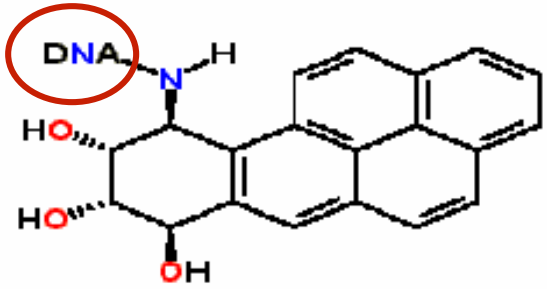


cytochrome P-450
Enzyme

arene oxide



DNA · NH_2



Benzo[a]pyrene

is initially oxidized, primarily by the microsomal NADPH- dependent cytochrome P-450, to several arene oxides.

Benzo[a]pyrene derivates can bind and damage DNA.

Benzo[a]pyrene itself can bind AR (Aryl hydrocarbon receptor) and activate gene expression:
cytochromes,
MAP kinases,
IGF-1 (insulin-like growth factor)

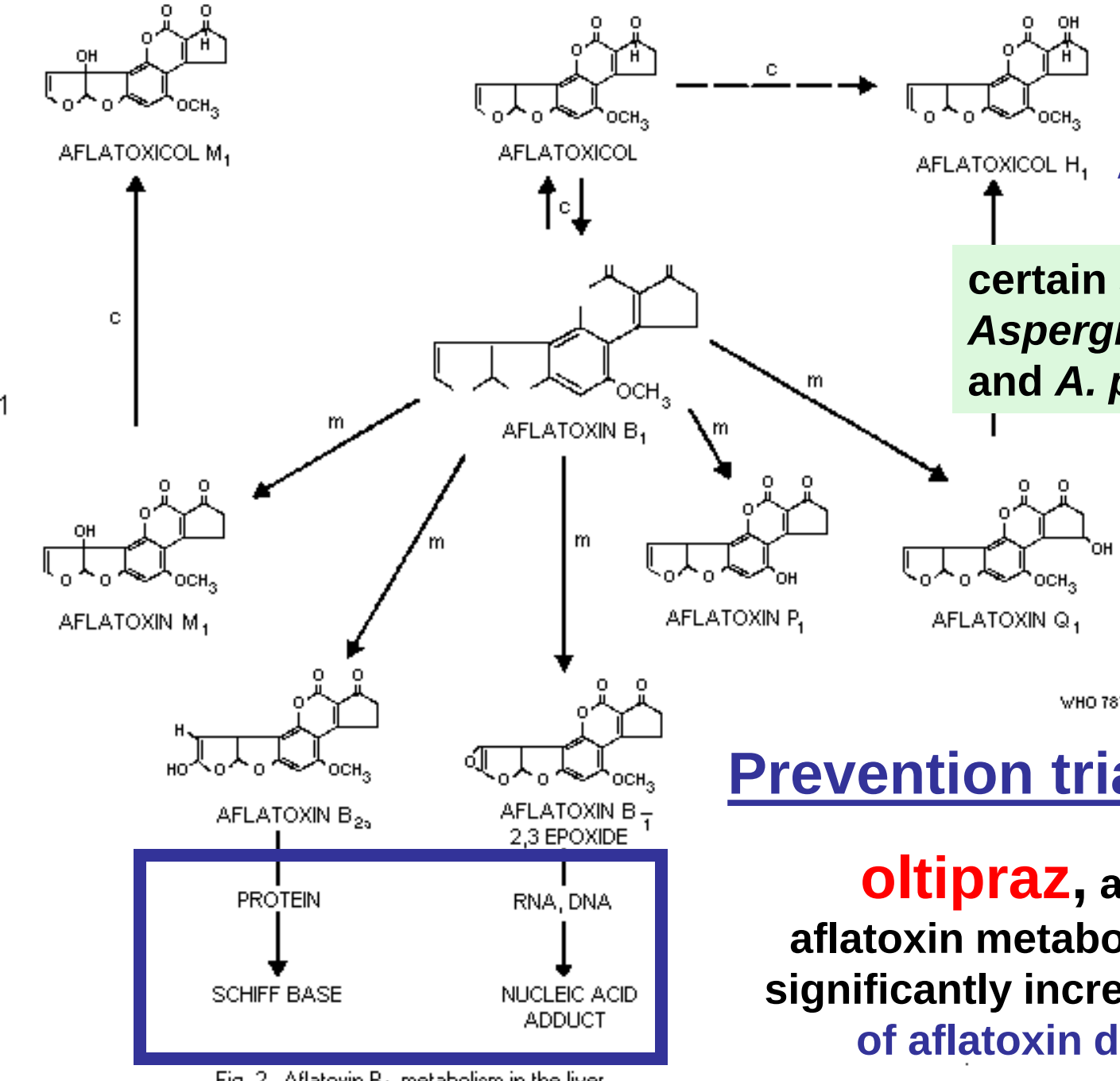


Fig. 2. Aflatoxin B_1 metabolism in the liver.

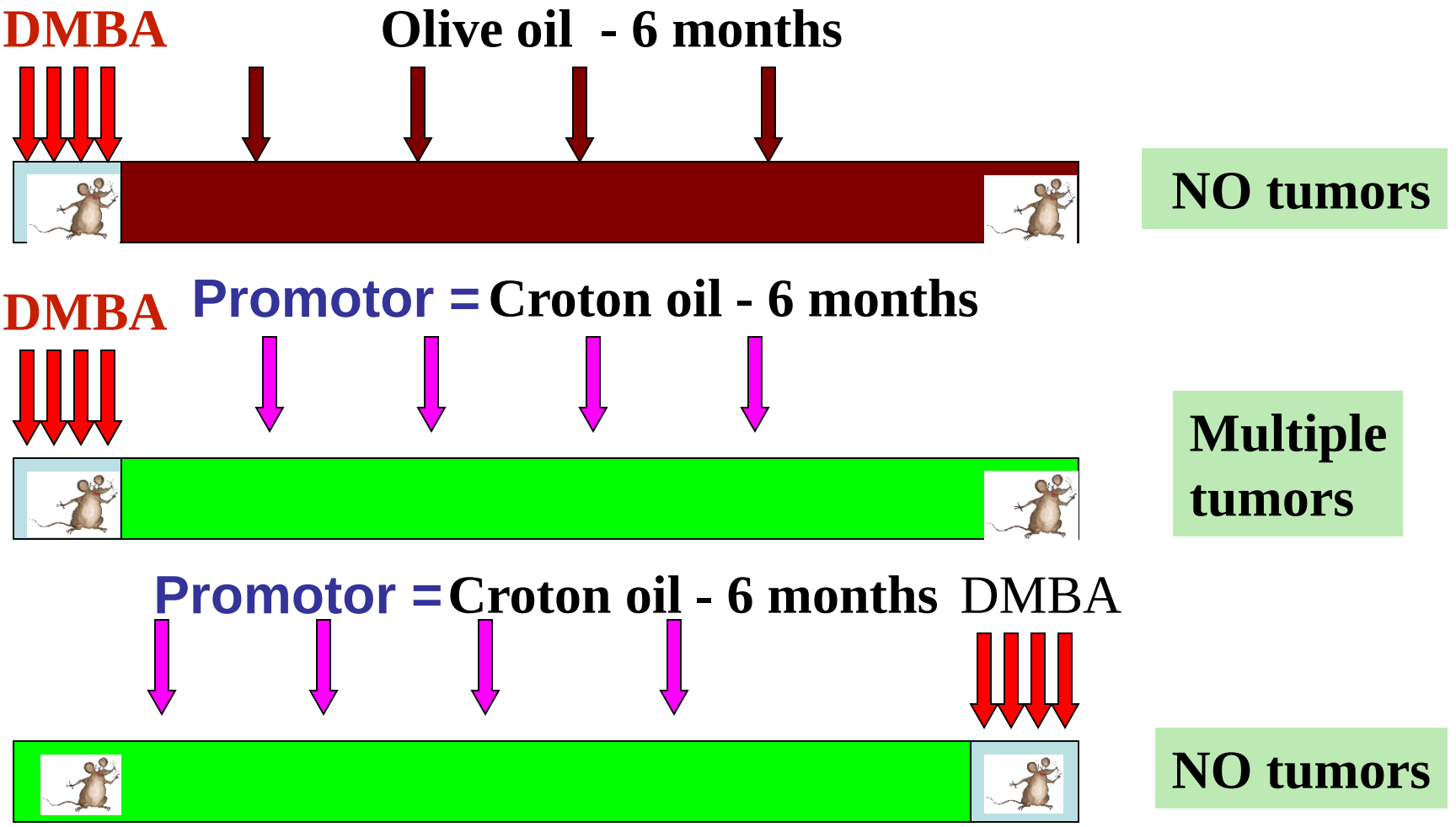
Aflatoxin B1

certain strains of the fungi *Aspergillus flavus* and *A. parasiticus*

Prevention trial in China

oltipraz, an inducer of aflatoxin metabolizing enzymes, significantly increased biomarkers of aflatoxin detoxification

Non-Genotoxic Carcinogenes: Tumor Promotors



Two-phase carcinogenesis of mouse skin

TUMOR PROMOTERS

1. **Promoting agents are not carcinogenic per se**

2. **Can promote cancer after very small doses of initiating (true carcinogenic) agents**

3. **Promoting agents can wake tumors up long time after administration of initiating agent**

Phorbol esters

Aplysiatoxin (algal toxin)

Teleocidin (fungal toxin)

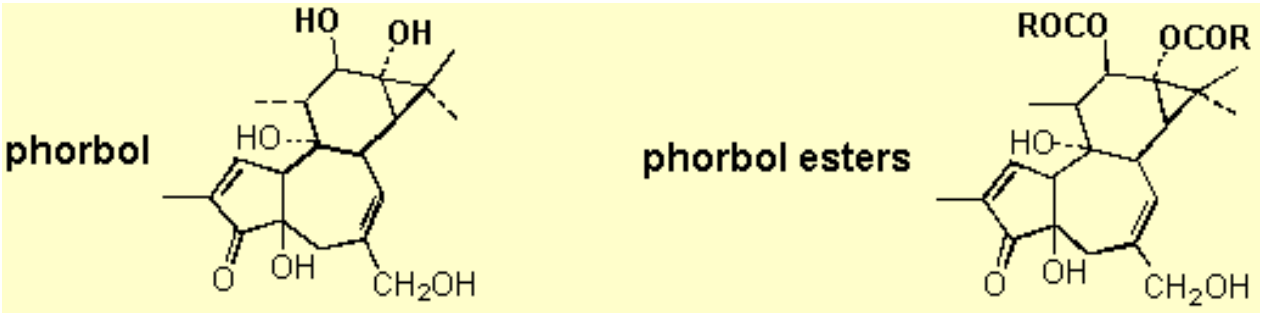
Hormones (estrogen)

Growth factors

Those substances promote growth of existing tumor clones evolved after mutation events

Phorbol ester (from croton oil)

12-O-tetradecanoylphorbol-13-acetate (TPA)



TPA activates protein kinase C

TPA is cell-toxic, pro-inflammatory agent that increase vascular permeability

TPA induces INFLAMMATION, and pushes epithelial cells to propagate

Pre-existing Tumor cell got her chance to grow

Professions and industries associated with high risk of cancer

Aluminium industry	polycyclic aromatic hydrocarbons (PAHs)	Lung and bladder cancer
Coal industry	polycyclic aromatic hydrocarbons (PAHs)	Lung, bladder, skin, scrotum cancer
Shoemaking	Benzene	Lymphomas, leukemias
Furniture making	Wood dust	Nasopharyngeal cancer
Fuchsin dye production	Fuchsin, ortho-toluidine	Bladder cencer
Rubber industry	Aromatic amines, solvents	Lung, colon, stomach, bladder, prostatic cancer, leukemia

How to block carcinogen-dependent tumorigenesis

- 1. block carcinogen uptake into body/cells
- 2. Inhibition of carcinogen formation/activation by blocking Cyp450
- 3. Stimulate carcinogen deactivation by conjugation (NAT2, GST, UGT…)
- 4. Inhibition of DNA adduct formation (antioxidants)
- 5. Stimulation of DNA repair

2. **Hormonal** carcinogenesis

- **1. Estrogens**

  - stimulate proliferation of epithelial cells;

  - estrogen metabolites are genotoxic;

- **2. Xeno estrogens**

  - DDE and other insecticides structurally similar to diethylstibestron;

  - Genistein from Soybean and other phytoestrogenes;

  - Digitalis, Sulfonamide antimicrobials,

  - Oral contraception, Hormone Replacement Therapy (estrogen should be counterbalanced by progesteron)

Cancer site	Hormones	Potentially important genes
Breast	Estrogen, progesterone	*CYP17*, *CYP19*, *HSD17B1*, *ER*, *PR*
Prostate	**Dihydrotestosterone**	*CYP17*, *HSD17B3*, *SRD5A2*, *AR*
Ovary	FSH, progesterone	*FSH*, *FSHR*, *PR*
Endometrium	**Estrogen**	*CYP17*, *HSD17B1*, *HSD17B2*, ER
Testis	*In utero* estrogen	*CYP17*, *HSD17B1*
Thyroid	TSH, estrogen	*TSH*, *CYP17*, *HSD17B1*

3. **Viral** carcinogenesis

- **1. DNA containing oncoviruses**

-- Polyomaviridae

SV40 (monkey, hamster)

Polyoma (mouse)

JC and BK viruses (hamster)

-- Papillomaviridae

Human papillomavirus (HPV 16, 18)

cervical carcinoma

-- Adenoviridae

types 12, 18, 31, 3, 7,14 (hamster)

-- Poxviridae

myxomavirus (rabbit)

Jabavirus, tanapoxvirus (benign skin histiocytoma),

contagiuos mollusc virus (benign pearl-like skin tumors, 5% of Pacific population)

3. **Viral** carcinogenesis (cont).

- **1. DNA containing oncoviruses**

  -- Herpesviridae

  Epstein-Barr virus (EBV)

  Hodgkin disease, nasopharyngeal carcinoma

  Kaposi' sarcoma virus (KHSV)

  Marek disease virus (chicken)

  American rabbit virus

  -- Hepadnaviridae

  Hepatitis B virus (liver tumors)

  Woodchuck hepatitis virus

  Duck hepatitis virus

3. **Viral** carcinogenesis

- **1. RNA containing oncoviruses**

-- Alpharetrovirus

Rous sarcoma virus (RSV),

Chicken lympholeukosis

-- Betaretrovirus

Mouse mammary tumor virus (MMTV)

-- Gammaretrovirus

Moloney sarcoma virus (mouse)

Feline sarcoma (FSV)

--Deltaretrovirus

Bovine leukosis;

Human adult T-cell leukemia virus (HTLV)

Retroviruses
(best understood oncogenic viruses)

Rous sarcoma virus

1909 Rockefeller Institute

Chicken sarcoma could be "transferred" into a healthy chicken by grafting tumor cells.

Cell-free filtrates from the tumor also led to sarcomas in healthy chickens. By 1914, Rous's laboratory had discovered three distinct types of avian sarcomas.

Virus = "filterable agent"

1966 –Nobel Prize "for his discovery of tumour-inducing viruses"

Peyton Rous

Born Baltimore (Maryland)
1866-1970

Scheme of retrovirus:

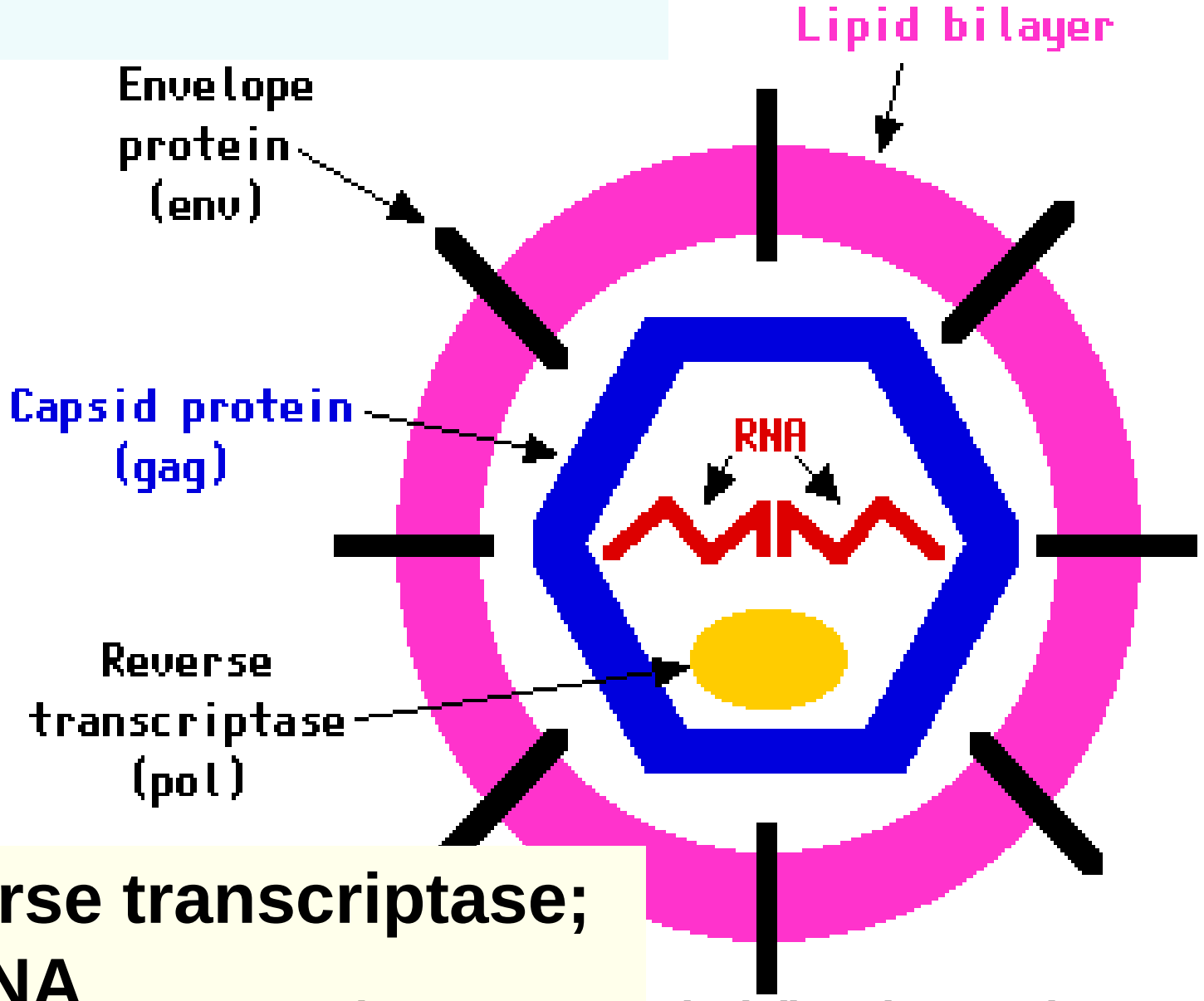
"minimal" retrovirus

contains

Env

Gag

Pol

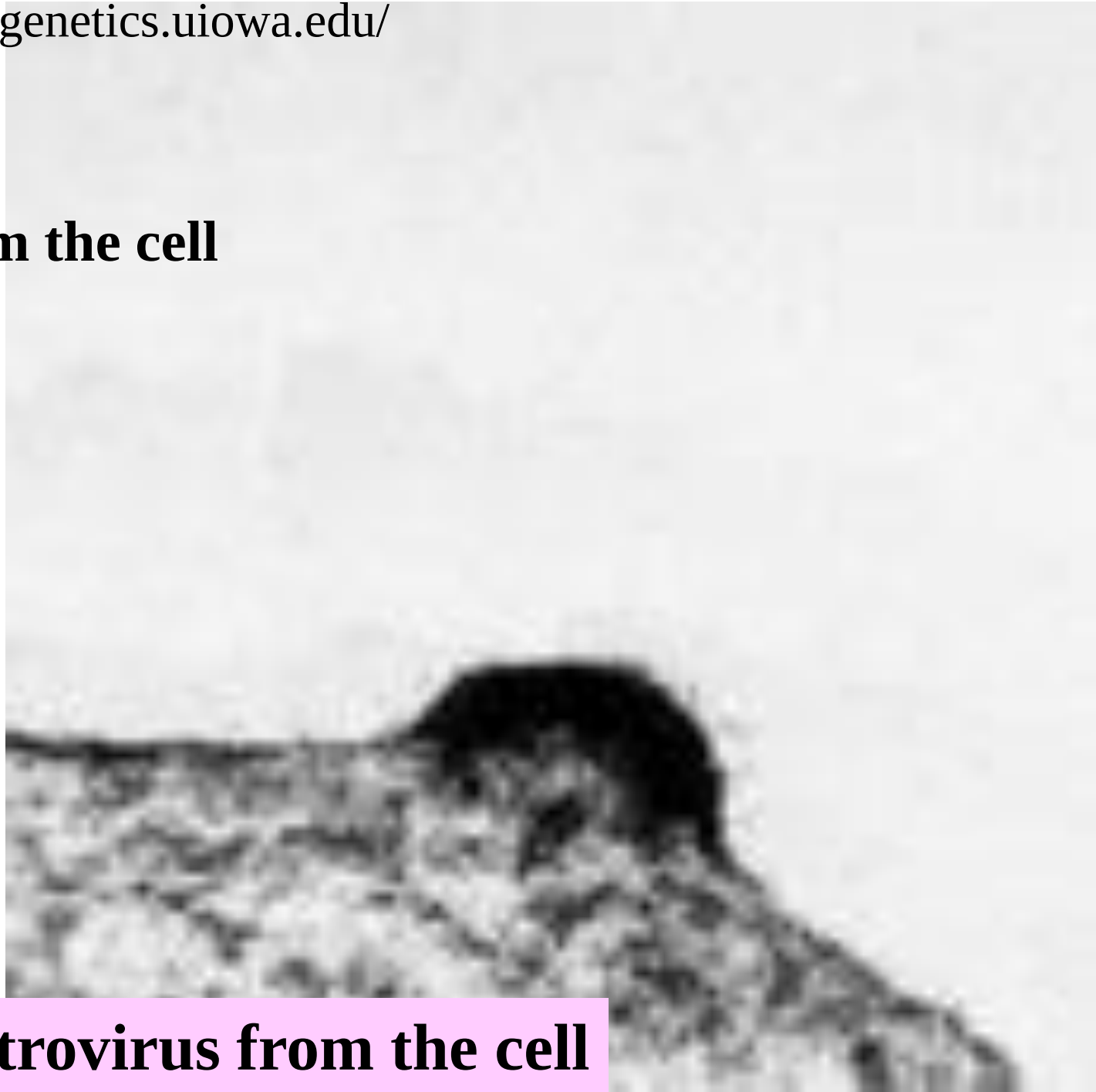


POL = reverse transcriptase;
RNA→DNA

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/

http://genetherapy.genetics.uiowa.edu/

Lipid envelope
is hijacked form the cell



Budding a retrovirus from the cell

RETROVIRUS LIFECYCLE

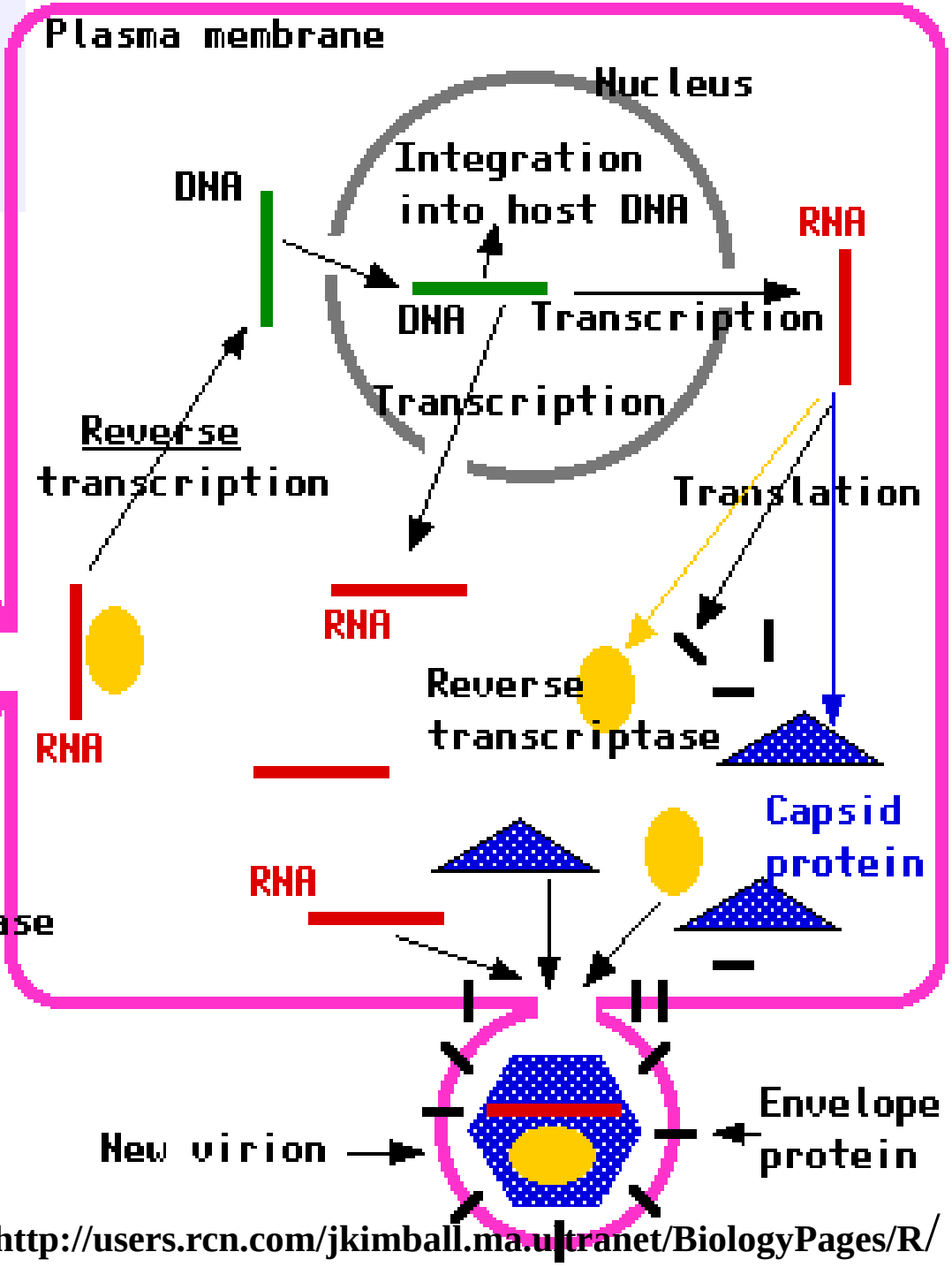
RNA-pol (RT) attached to viral genomic RNA

DNA copies

Random insertions to host genome

Producing of fresh RNAs

Translation → Packaging



http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/R/

Most natural oncogenic viruses are defective: not able to propagate without helper virus that provides necessary gene products

Rous sarcoma virus

(non defective)

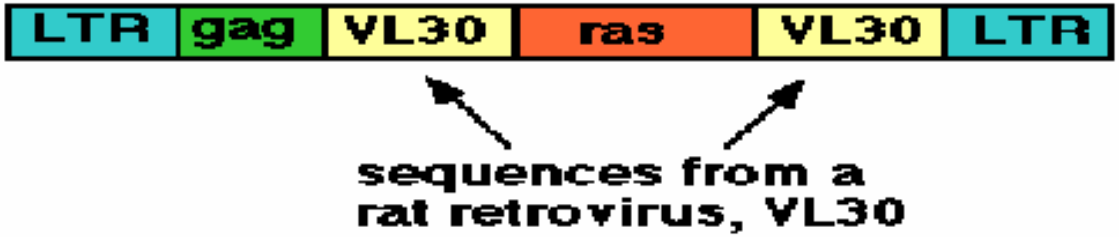
Abelson murine Leukemia virus

(defective, lack Pol and Env genes, added Abl oncogene)

Harvey sarcoma virus

(defective, lack Pol and Env genes, added Ras oncogene)

(mouse)



http://www.blc.arizona.edu/marty/411/Modules/Lectures/Figures/Trans_Retros.GIF

Oncogenes (Viral Onc- genes)

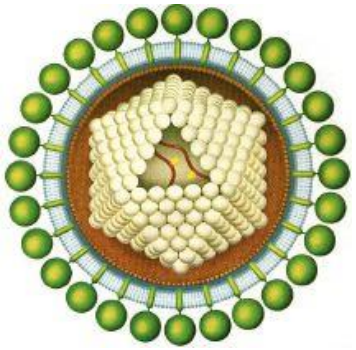
- Oncogenes were discovered in “classic” oncogenic viruses (retroviruses)
- (Sarcoma Rous virus)→ Src
- (Abelson murine leukemia → Abl

Viral oncogenes have normal cellular homologues, that also could be tumorigenic if mutated

v-SRC, v-ABL, v-HA-RAS are derived from host c-ONC sequences
(probably picked up as processed transcripts)

Human Adult T-cell leukemia viruses HTLV-1 and HTLV-2

- **HTLV-1** (human T-cell lymphotropic virus)



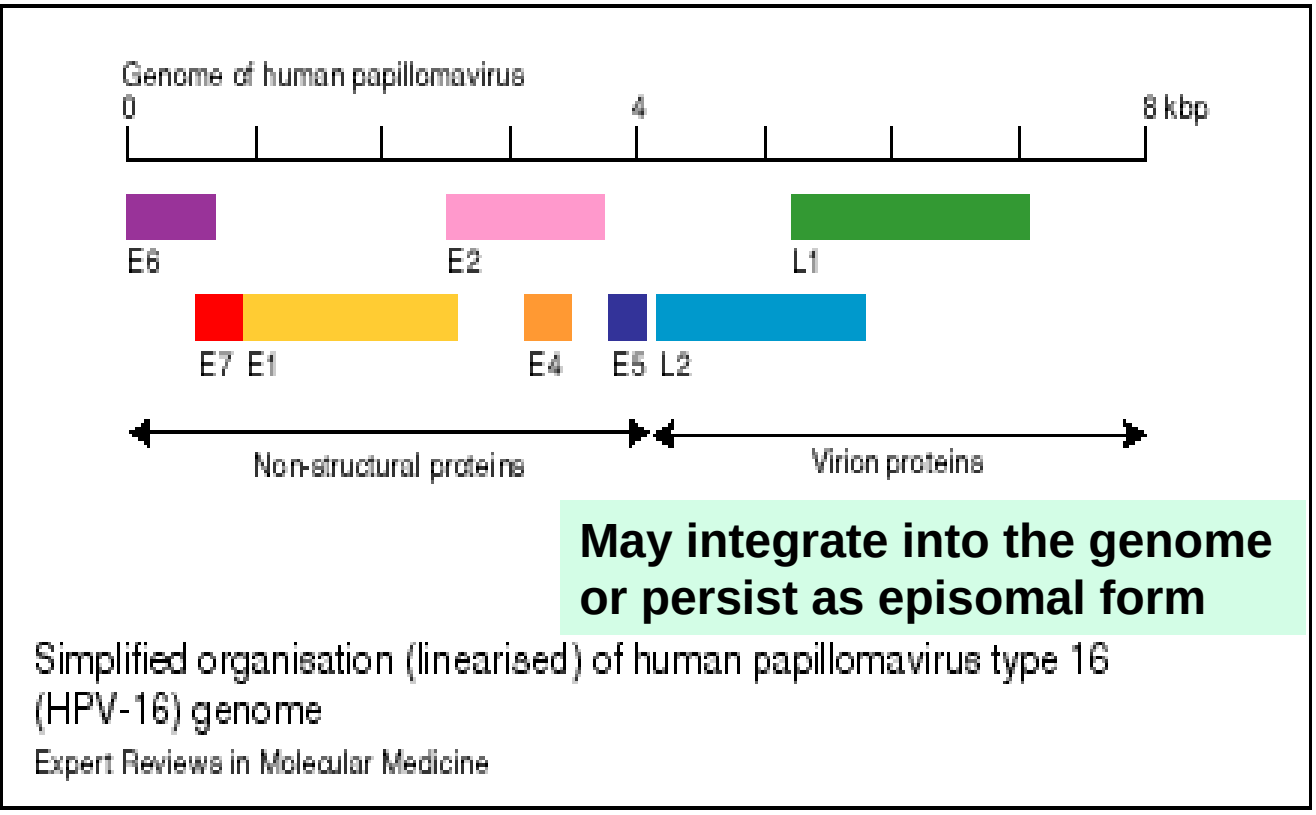
-- is sexually transmitted;
-- endemic to Japan, Caribbean
-- causes Adult T-cell leukemia (Sezary T-cell leukemia);

HTLV-2

-- T variant of hairy cell leukemia;
-- Native Amerindian populations seroprevalence is over 50%.

www.showa.gunma-u.ac.jp/. ../bp1-4/tsld014.htm

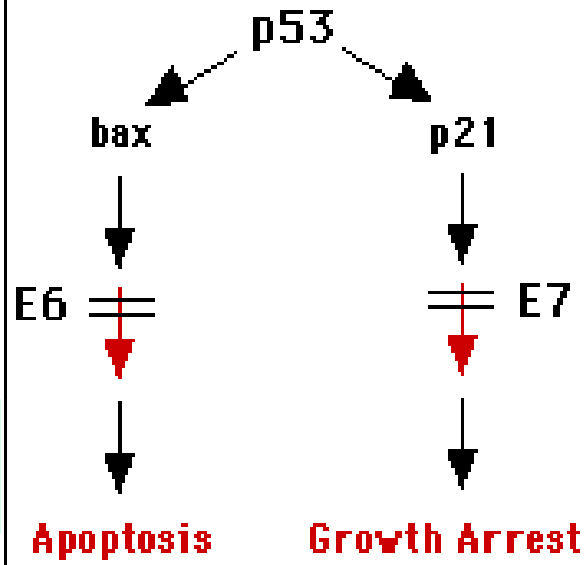
Human papillomaviruses HPV6 and HPV18



May integrate into the genome or persist as episomal form

Simplified organisation (linearised) of human papillomavirus type 16 (HPV-16) genome

Expert Reviews in Molecular Medicine



www.tulane.edu

Causative agents for female cervical carcinomas, as well as for genital and regular warts

Human papillomaviruses HPV6 and HPV18

Gene:	Function:
E1	Initiation of DNA replication (helicase)
E2	Transcriptional regulation/DNA replication
E3	???
E4	Late NS protein; Disrupts cytoskeleton?
E5	Transforming protein, interacts with growth factor receptors, e.g. PDGF
E6	Transforming protein, binds to p53 leading to degradation
E7	Transforming protein, binds to pRB
E8	???
L1	Major capsid protein
L2	Minor capsid protein

Human hepatitis B virus

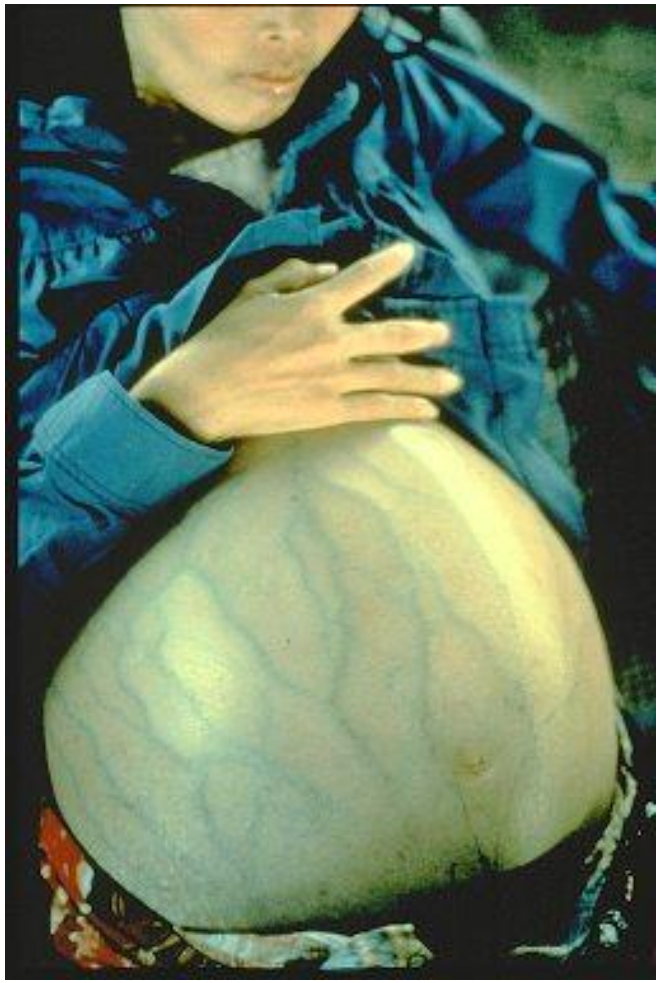
- **Cause liver cancer (30-50 yrs after infection);**
- **Average life expectancy after diagnosis of liver cancer is 6 months;**
- **DNA virus that encodes 4 genes**

  Pol – DNA polymerase

  Env -- envelope

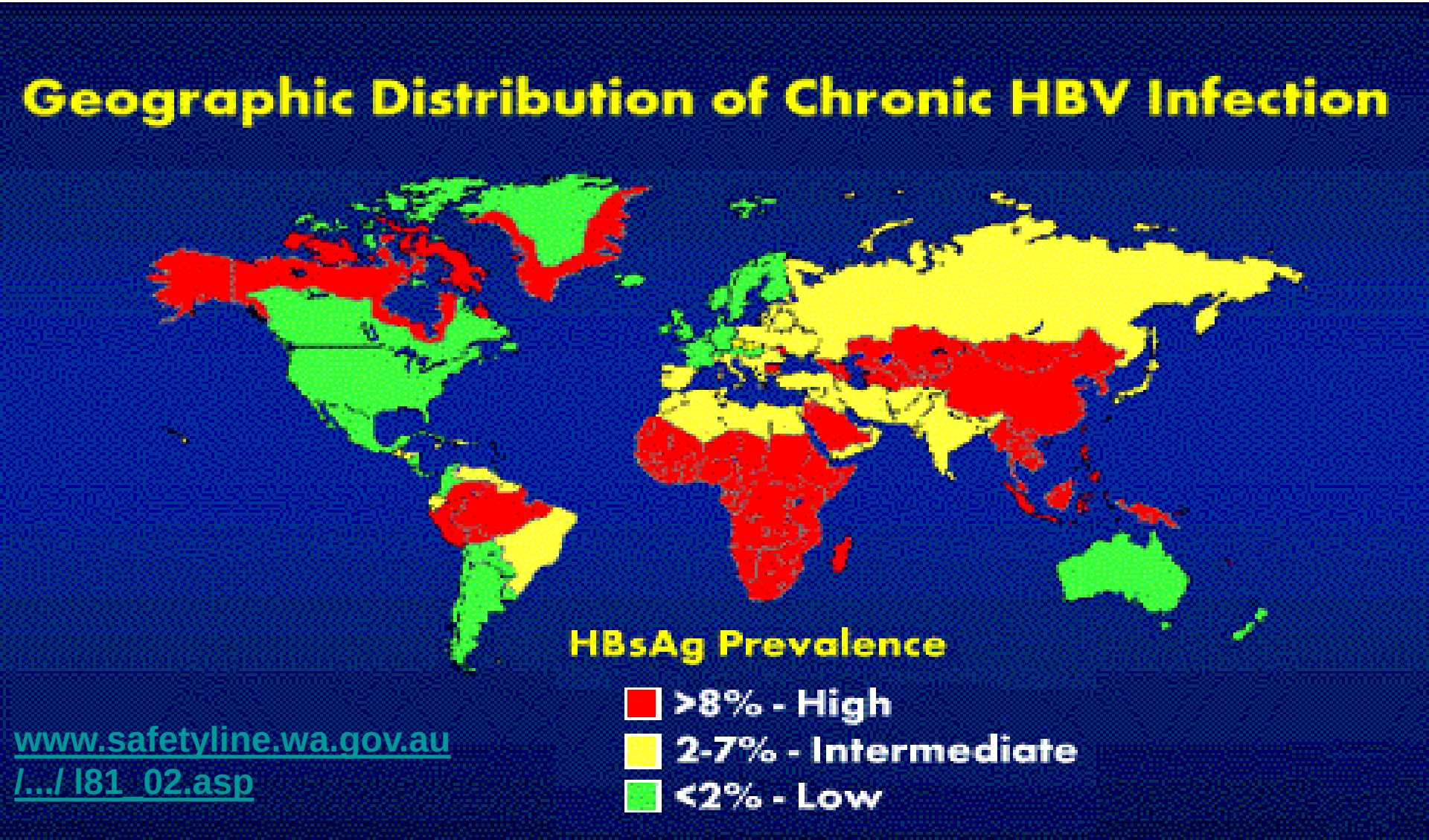
  pre-core – viral capsid

  X - activation of host cell genes and the development of cancer.



www.immunize.org/images/ca.d/ipcd1861/img0022.htm

Human hepatitis B virus



Epstein-Barr virus

Cause infectious mononucleosis;
associated with:

-- lymphomas in immunosuppressed persons,

-- nasopharyngeal carcinomas

-- Burkitt lymphoma (endemic; 8 in every 100,000 children in parts of Africa and Papua New Guinea);

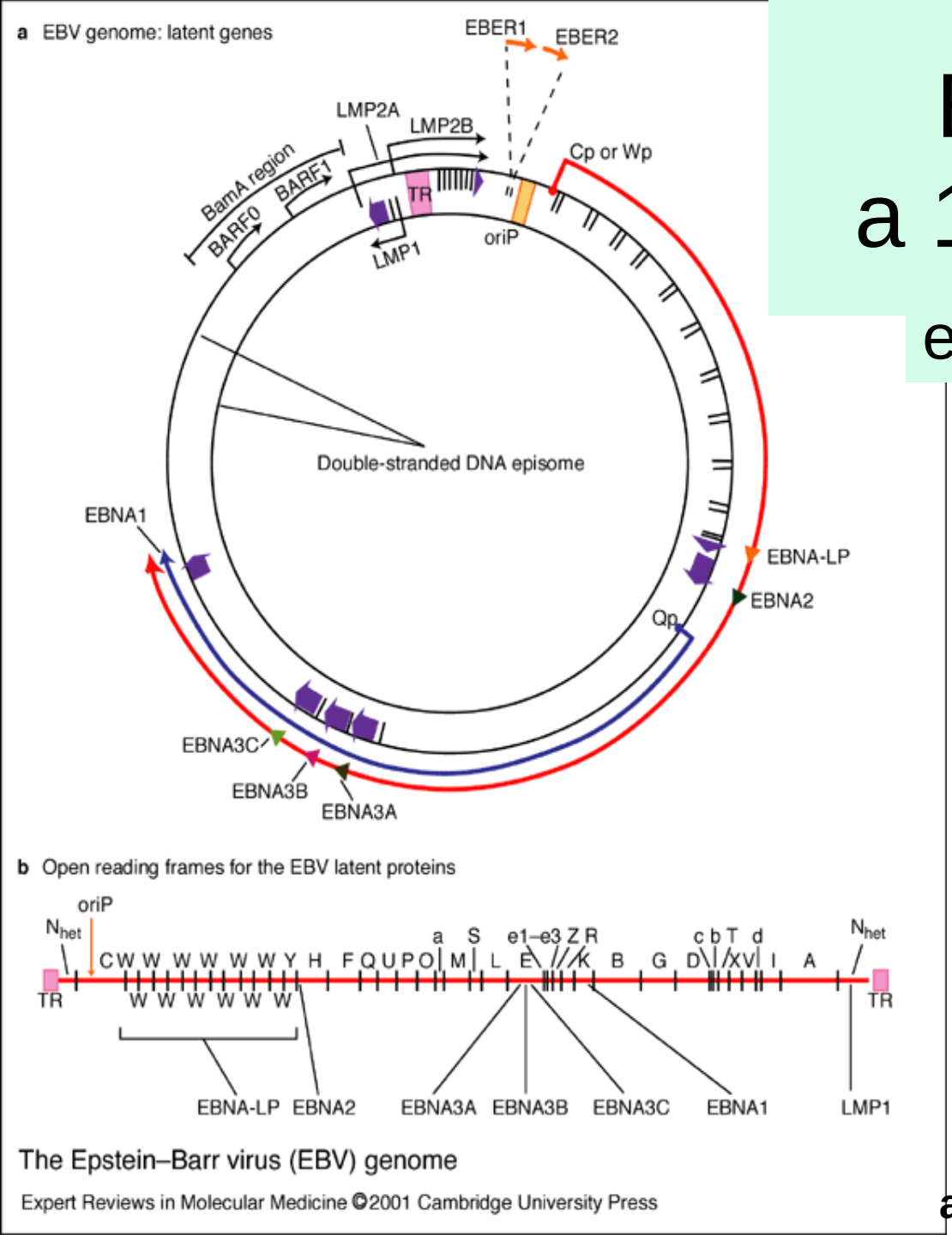
-- cofactor for Hodgkin disease (30 yrs after infection);

In Third World nations, most children are infected with EBV;

In most industrialized nations, about 50% of the people are infected.

EBV possess a 172 kb genome

encoding 100 genes.



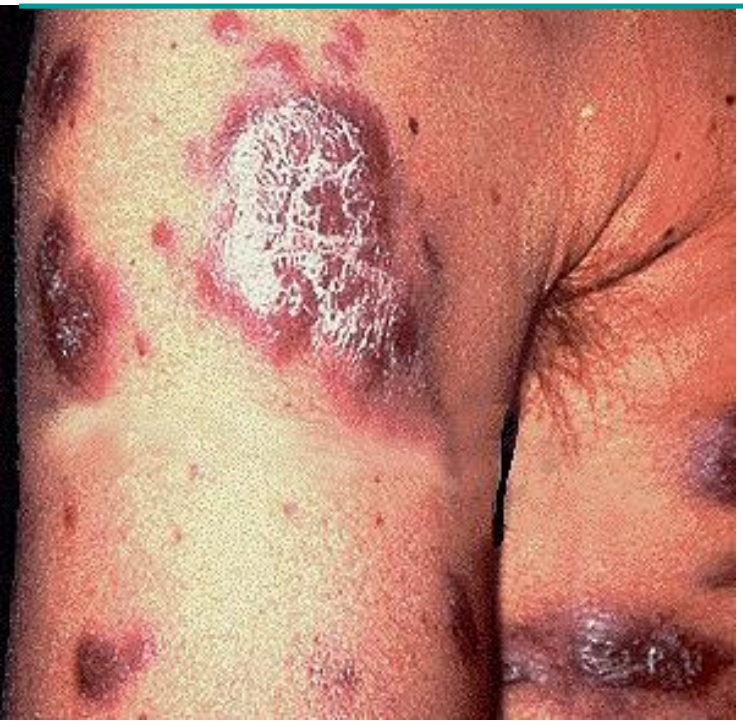
The Epstein–Barr virus (EBV) genome

EBNAs
1, 2, 3A, 3B and 3C,
and EBNA-LP
= nuclear antigenes

<u>EBNA-1</u> is involved in promoting viral DNA replication
<u>EBNA-2</u> is a transcription factor with viral and host cell targets
<u>LMP1</u> expression in rodent cell lines results in transformation
<u>LMP2</u> associates with src and several other tyrosine kinases

Kaposi's sarcoma virus

www.kcom.edu/.../ lectures/lecture/aids.htm



HHV-8 virus,
common in AIDS patients and in transplants recipients

Agent co-infecting homosexual men along with HIV

KSHV incidence:

HIV+ men 25-30%
HIV+ women 3-4%
HIV+ haemophiliacs 2-3%

Virus contains 81 genes; including chemokines vMIP1 and vMIP11, as well as a chemokine receptor, oncogene GPCR and VEGF

4. Bacterial and parasitic carcinogenesis

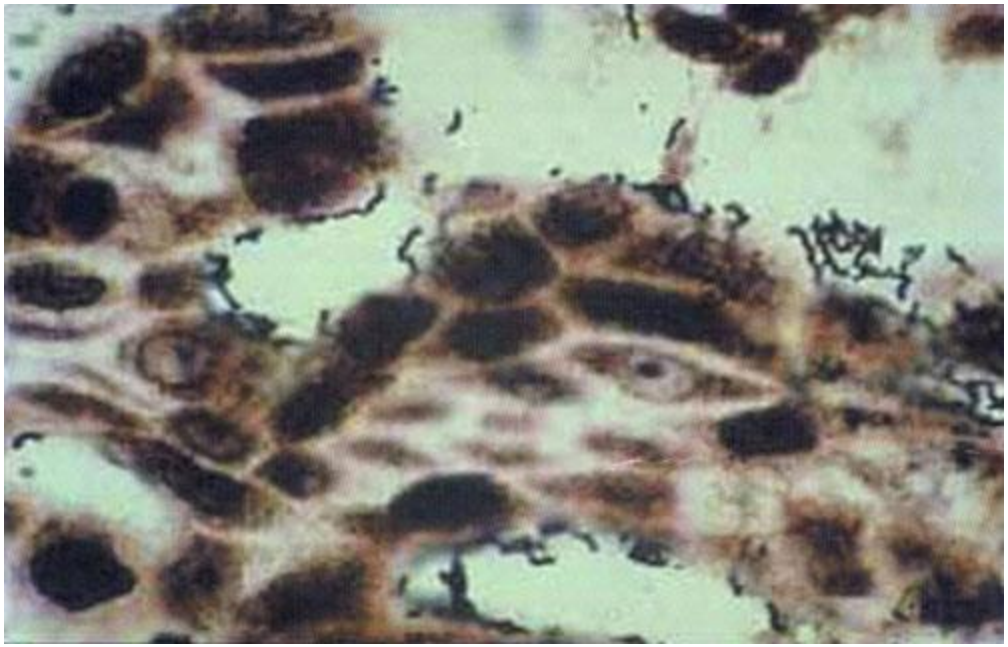
- 1. Helicobacter pilori

  and stomach lymphomas

- 2. Schistosomiasis

  and bladder carcinoma

Helicobacter pylori is linked to MALT lymphoma and gastric carcinoma



H. pylori
overlying
the gastric epithelial cells.

***H. pylori* causes**
more than 90% of duodenal ulcers
and up to 80% of gastric ulcers.

Both cellular and humoral immune responses are activated but the bacteria still manage to persist lifelong unless eradicated with antibiotics.

From Emad M El-Omar on-line lectures

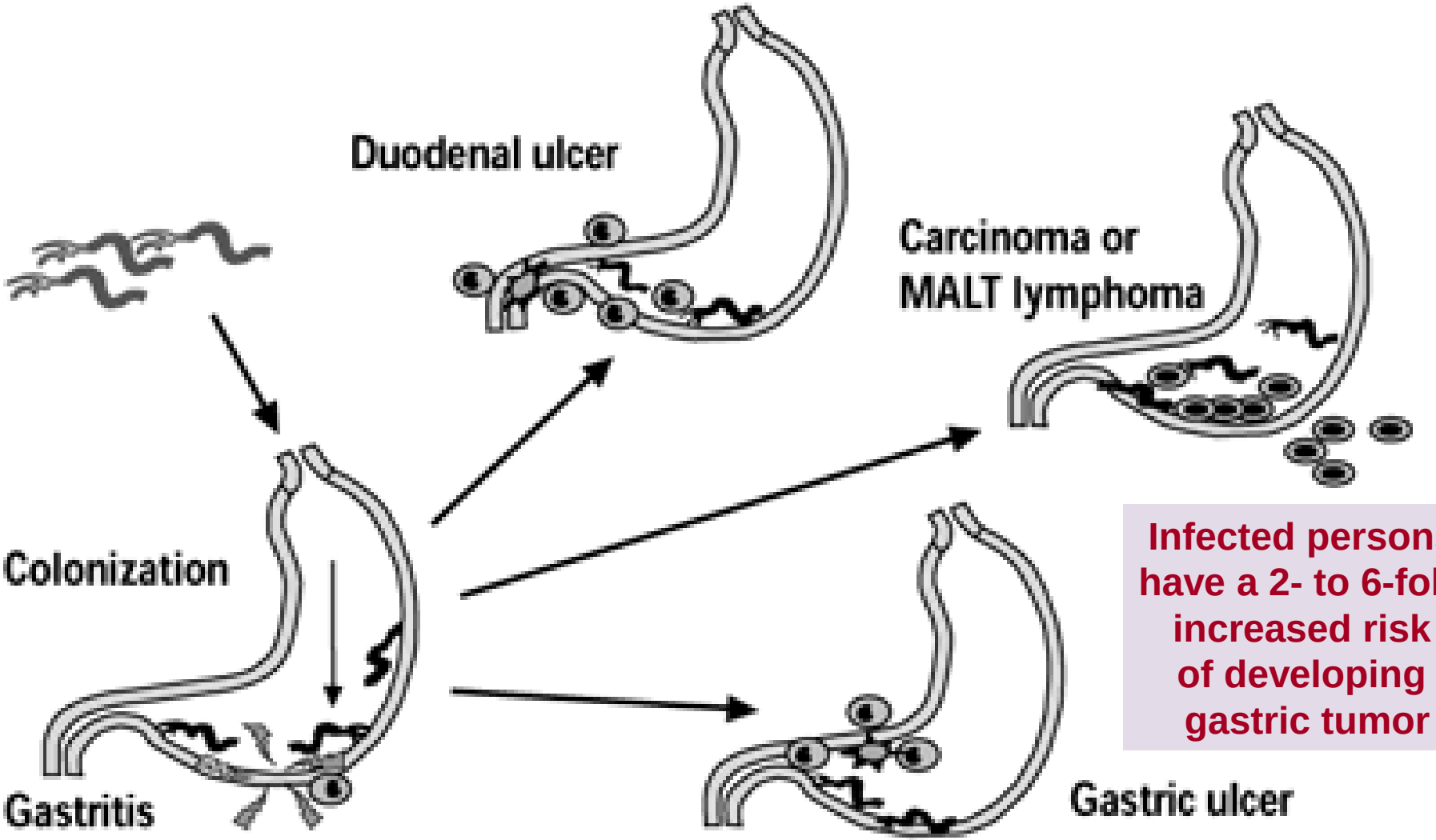
Treatment of gastric and duodenal ulcers

1. Lowering of gastric acidity:
(life-long, relapse after cease of therapy)
– H2 blockers
– proton pump inhibitors.

2. 10 days to 2 weeks :
Amoxicillin or metronidazole, or clarithromycin,
plus either:
ranitidine bismuth citrate or bismuth subsalicylate

Eradication rates of the range from 61% to 94% depending on the regimen used.

Outcomes of H.pylori infection



Infected persons have a 2- to 6-fold increased risk of developing gastric tumor

Source: Am J Clin Pathol © 2003 American Society of Clinical Pathologists, Inc.

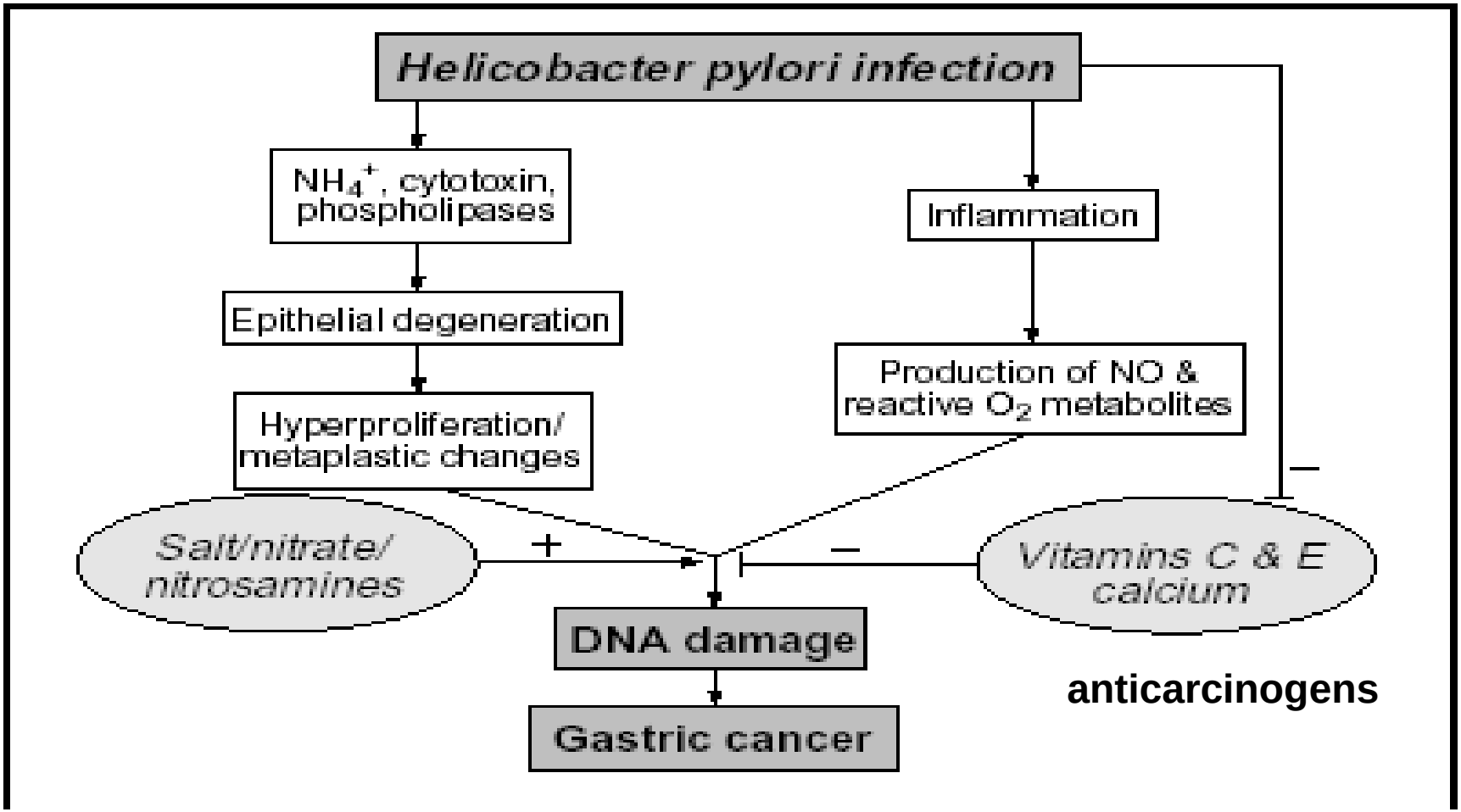
Odds ratios for gastric carcinoma

Table 1. *H. pylori* and gastric cancer risk/odds ratio

Group	Risk of gastric cancer
Patients infected with *H. pylori*	3- to 6-fold risk increase
Patients infected with *H. pylori* and randomized for prospective serological case-control studies with a follow-up period of at least 14 years	9-fold risk increase
Only patients below age 40	13.3 odds ratio

MANFRED STOLTE, ALEXANDER MEINING " The Oncologist"

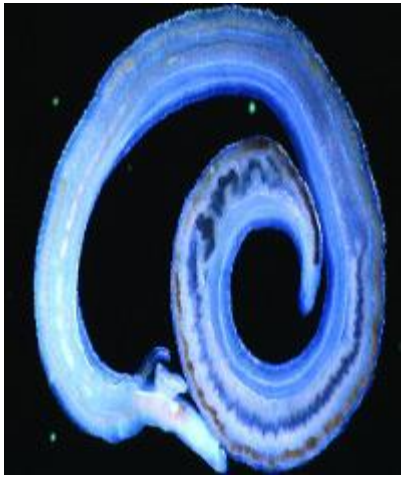
Mechanisms of gastric carcinoma induction by H.pylori



MANFRED STOLTE, ALEXANDER MEINING " The Oncologist"

Schistosomiasis

ehp.niehs.nih.gov/docs/ 2004/112-2/



- *Schistosoma mansoni (intestinal)*
- *S haematobium (urinary)*
- *S. japonicum (intestinal)*
- *S. mekongi (intestinal)*
- *S. intercalatum (intestinal)*

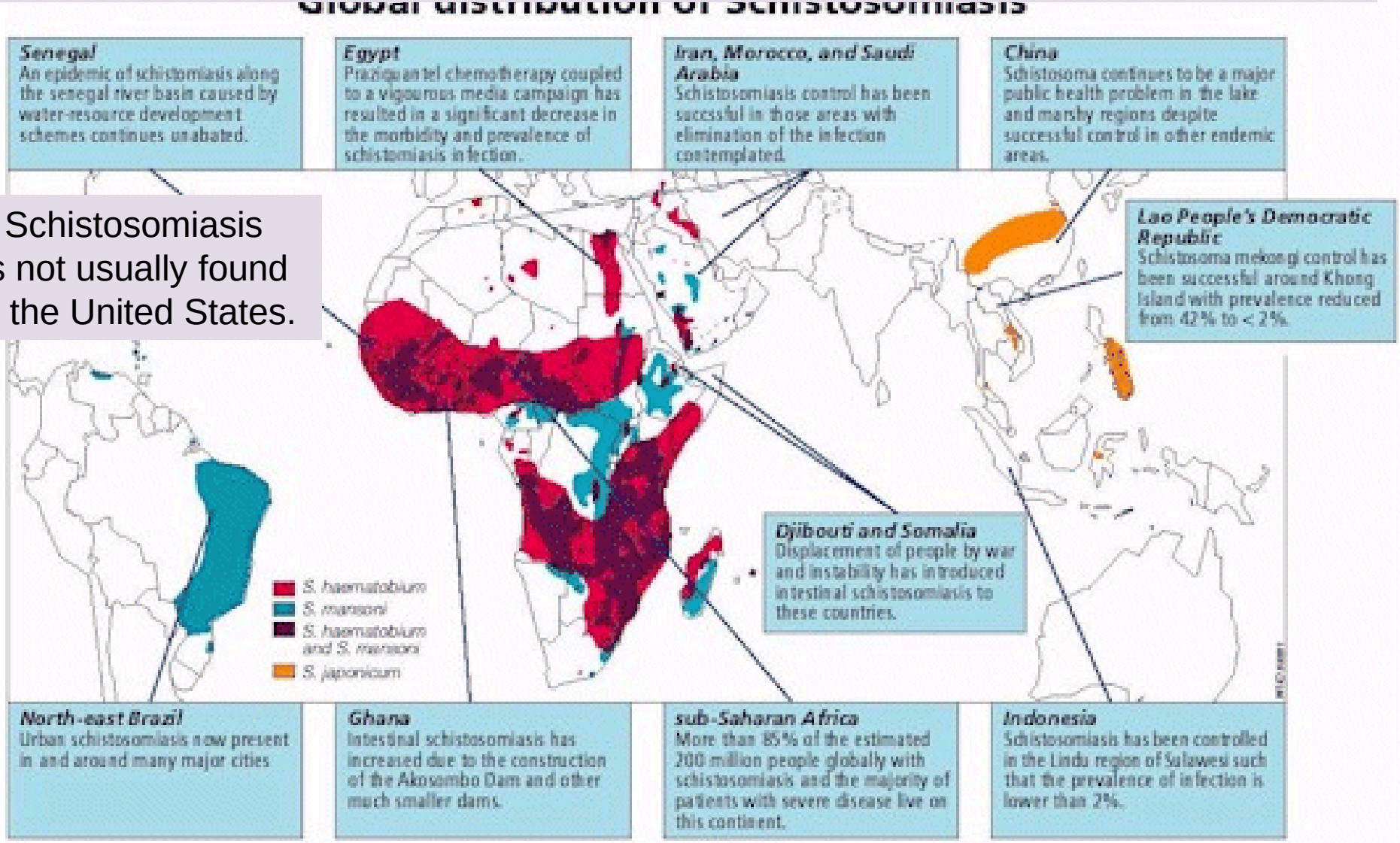
Fresh water snail is an intermediate host.

On contact with humans, the parasite burrows into the skin, matures into another larval stage (schistosomula), then migrates to the lungs and liver (where it matures into the adult form).

The adult worm then migrates to the intestine, liver or bladder

It affects 200 million people worldwide, mostly in sub-Saharan Africa

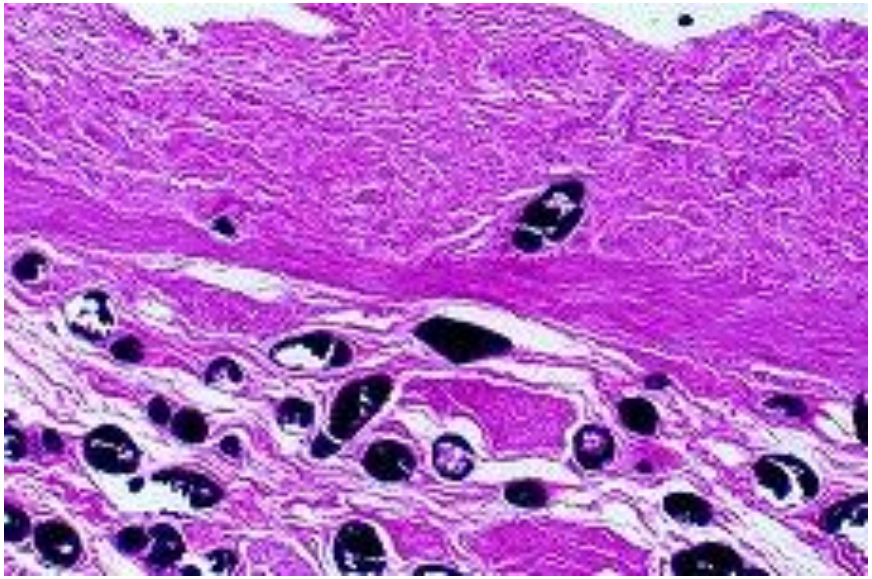
Schistosomiasis is not usually found in the United States.

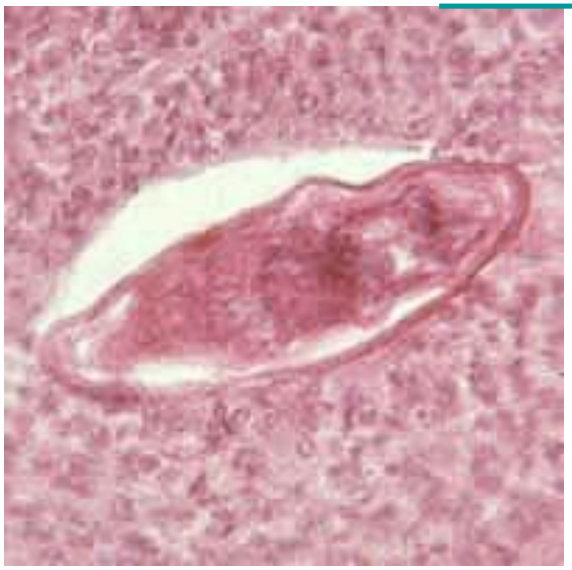


web de l'Organisation Mondiale de la Santé

In Egypt, **schistosomiasis** linked with **cancer**
is the primary cause of death among men aged 20 - 44.

www.dpd.cdc.gov/





Cross-section of different human tissues showing *Schistosoma* sp. eggs.
***Schistosoma* sp. in bladder and liver, respectively**

http://www-micro.msb.le.ac.uk/224/Schisto.html

5. Inflammatory carcinogenesis

ALL Pro-inflammatory agents are tumor promoters

Prostaglandins PGE2 and PGF2alpha

Phenobarbital that makes a foci in liver

Any type of Chronic tissue wounding – tumor can arise on chronic ulcer, burn, trauma site…

www.eatonhand.com/handbase/ 1497905.jpg

Anti-inflammatory agents can reverse action of tumor promoters

anti-inflammatory steroids (dexamethasone)

COX inhibitors such as indomethacin, piroxicam and sulindac

How the tumor is initiated ?

- 1. Sporadic tumors

  (occasional cancers in pedigree, various types of tumors, late onset)

  Grandmother (mother line): breast cancer at 83.

  Father: prostatic carcinoma at 78. No other cancers in pedigree.

- 2. Inherited cancer syndromes

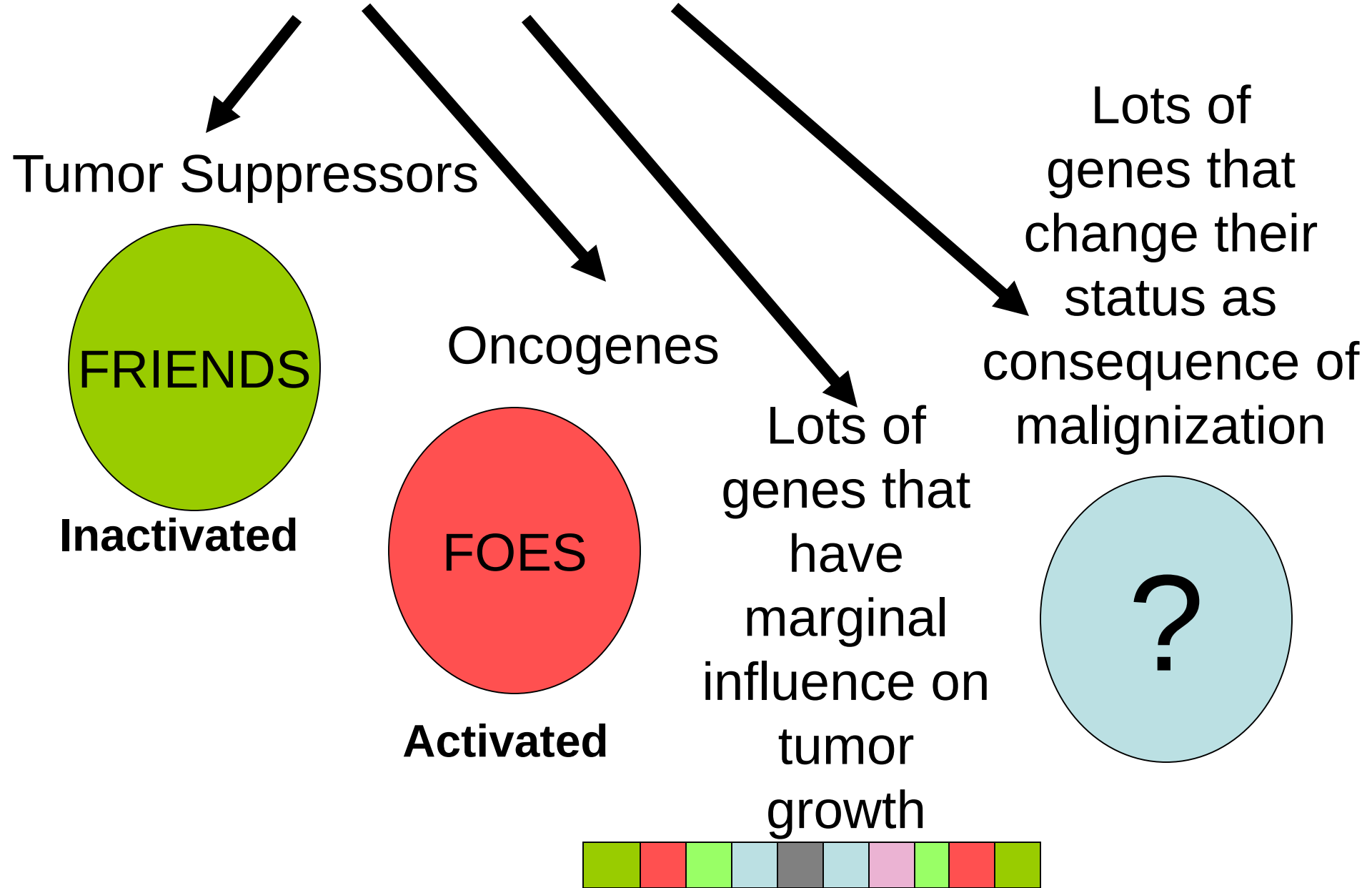
  (same type of cancer in many relatives, early onset)

Grandmother: renal carcinoma at 37.

Father: renal carcinoma at 29.

Son: angioblastoma at 8.

GENES INVOLVED IN TUMOR DEVELOPMENT



Classical point of view

Oncogenes

-- Accelerators of cell division

-- STOP for apoptosis

-- Dominantly inherited

(one defective allele can predispose the cell to tumor formation)

Tumor suppressors

-- Inhibitors of cell division

-- HELP for apoptosis

-- Recessive

(Mutation in one allele predispose human to cancer, but do not cause it)

WAYS of STATUS CHANGING

Oncogenes

-- Activating point mutations

-- Translocation under strong promoter

-- Amplification

-- Overexpression

Tumor suppressors

-- Inactivating point mutations

-- Promotor methylation

-- Gross chromosomal deletions

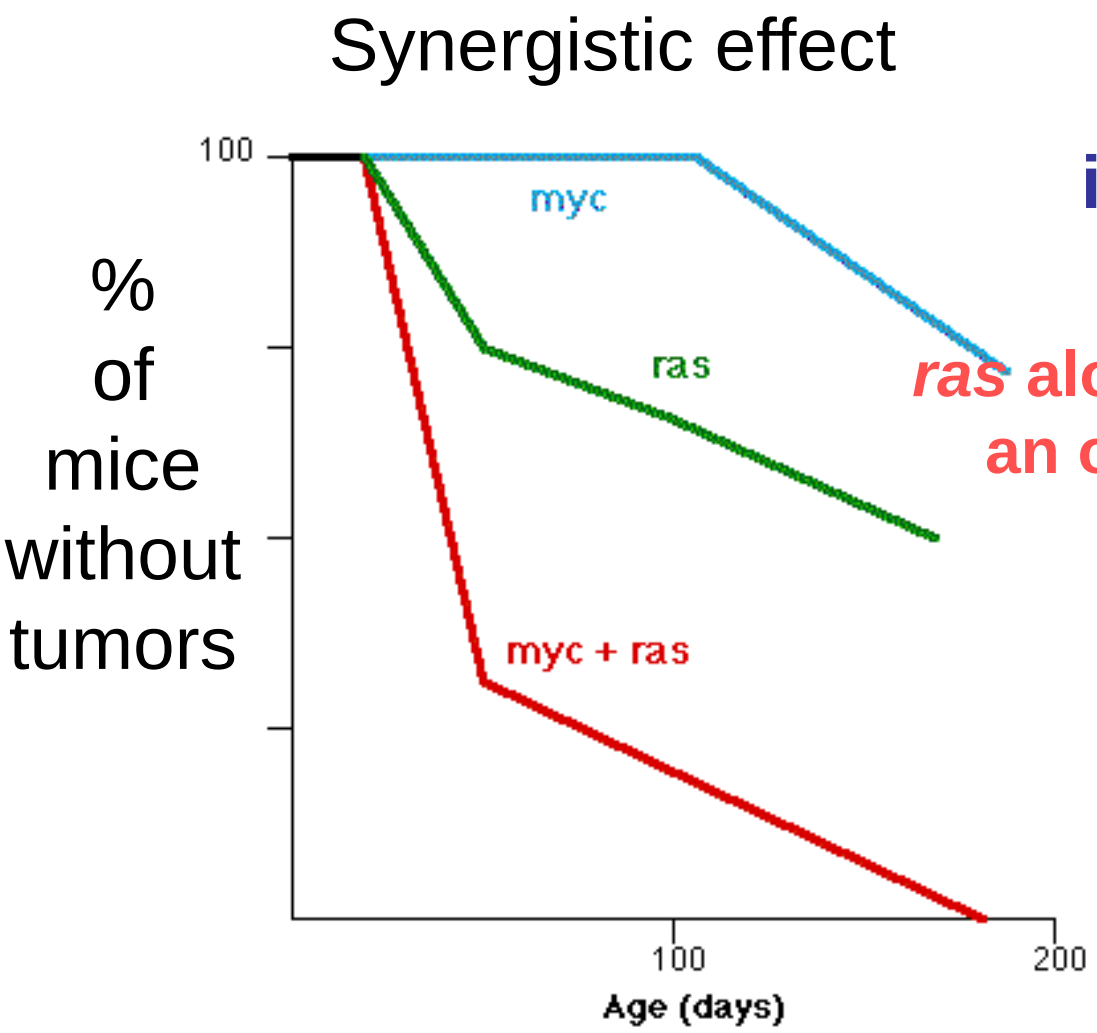
-- Underexpression

MULTISTEP MODEL
of the human cancer development

MALIGNANCY	MOLECULAR EVENTS
LEUKEMIA, chronic	2-3
LEUKEMIA, acute	3-4
CARCINOMA, in situ	3-4
CARCINOMA, metastatic	5-12

One molecular event
(activation of just one oncogene)
is never enough

Experiments on oncogene cooperation



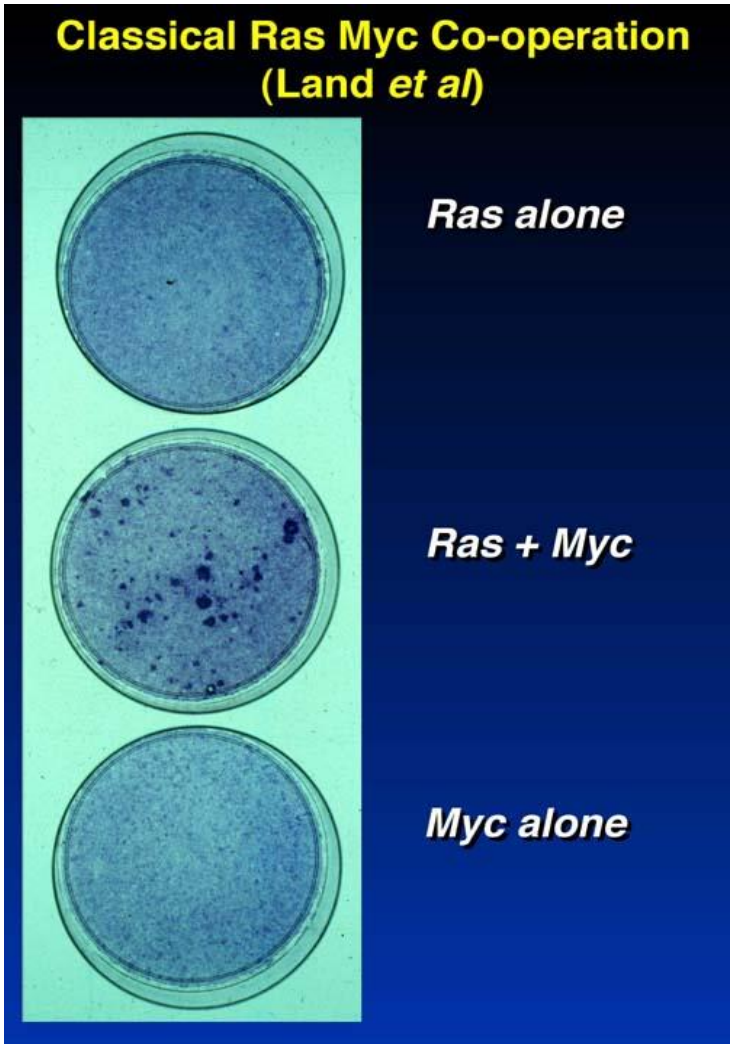
***myc* alone is a weak oncogene,**

***ras* alone is somewhat stronger an oncogene but still weak,**

***myc* and *ras* together show dramatic synergy.**

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/M/myc_ras(Sinn).gif

IN VITRO WE SEE THE SAME EFFECT



Fibroblasts with activated ras (top) or activated *myc* (bottom) alone do not undergo transformation.

However, co-expression of activated *ras* and *myc* (middle) does lead to foci of transformed cells

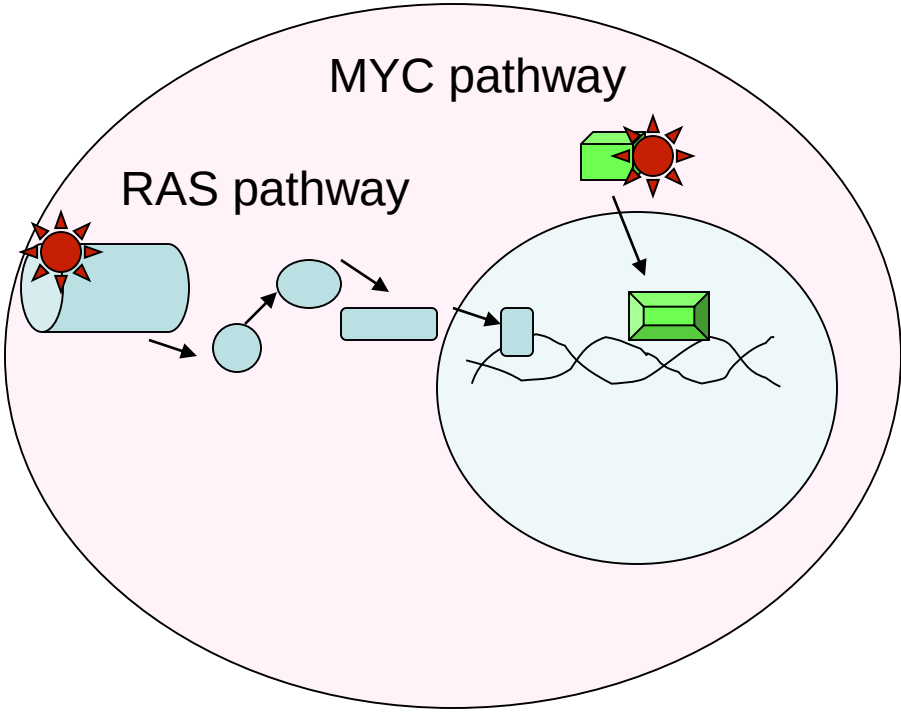
Ras and Myc cooperate as they belong to different signaling cascades

Cells are “durable”.

Most systems
have double and triple controls.

To break control of proliferation,

fatal errors should occur
in two or more signaling cascades



Tumor suppressor genes

Have been

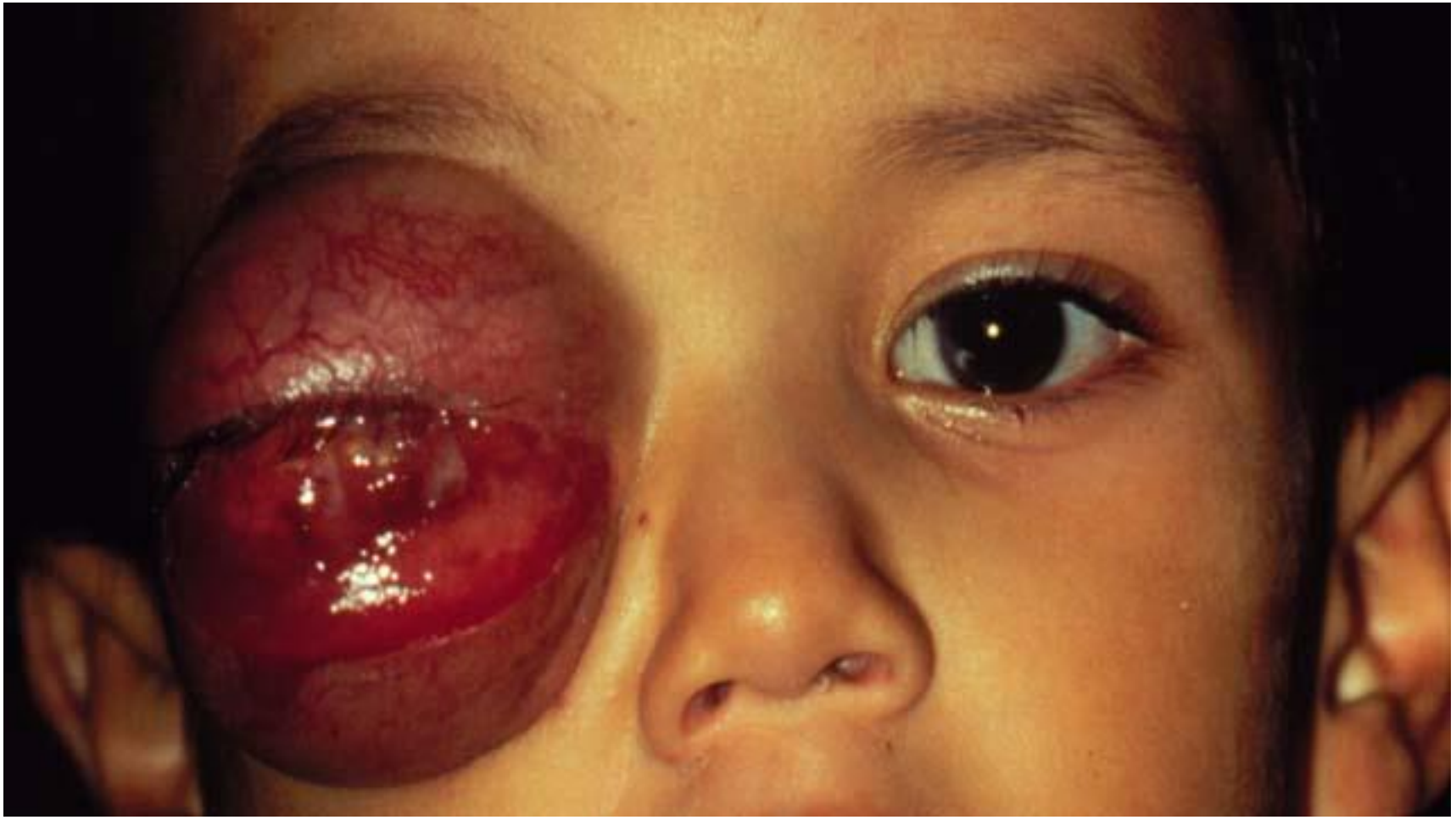
theoretically predicted

by Alfred Knudson

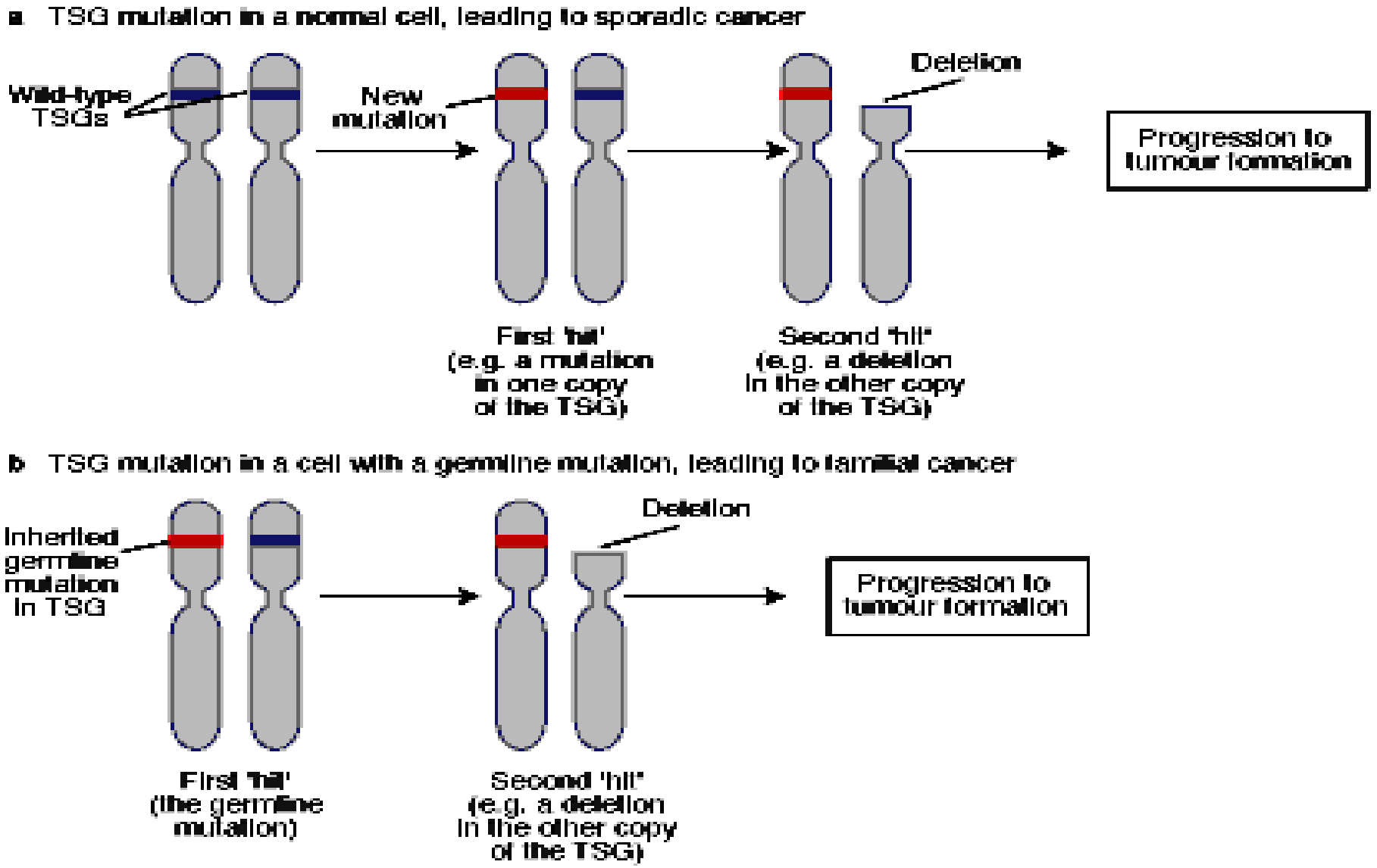
in 1971

(Two-hit hypothesis)

Familial retinoblastoma



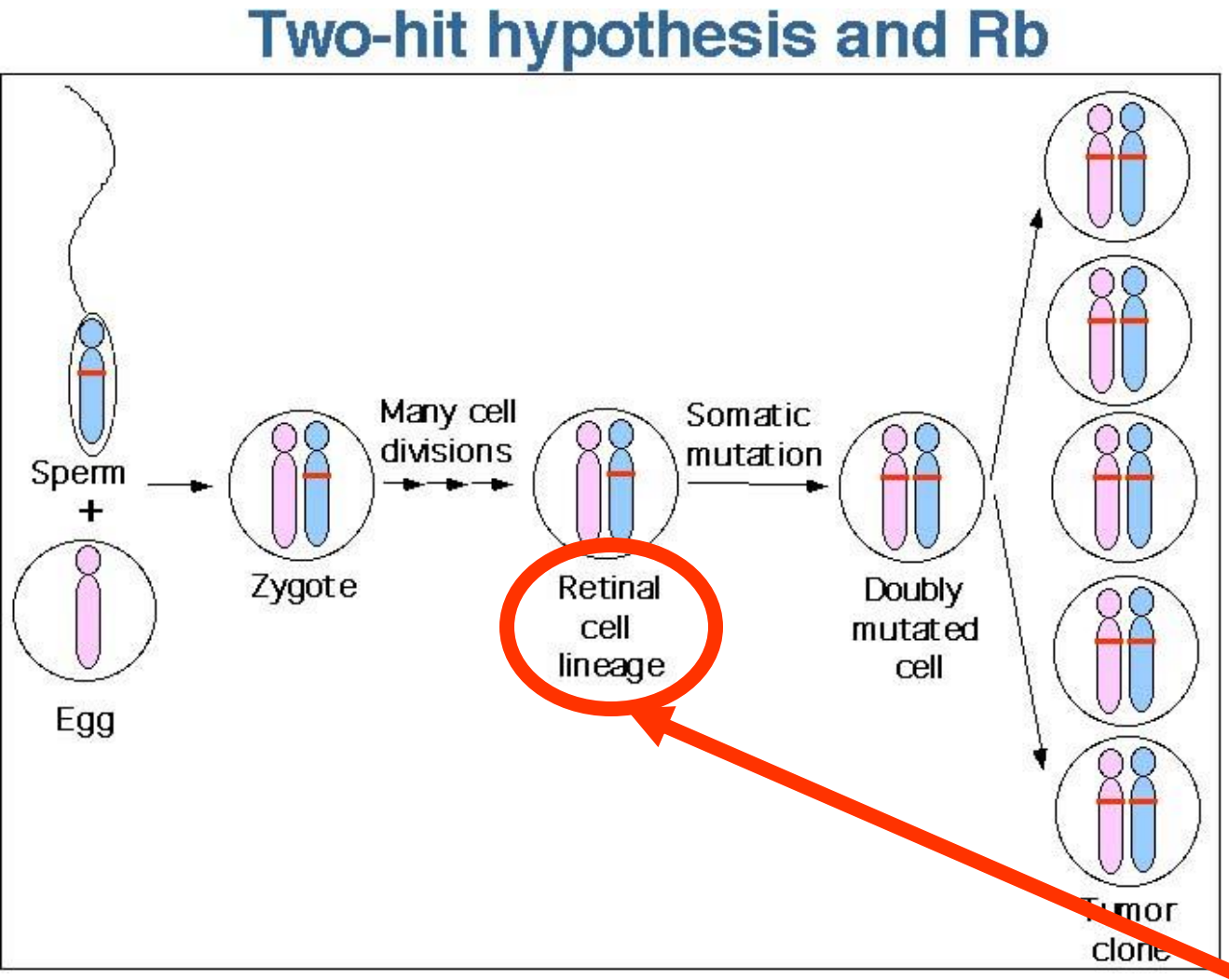
http://www.djo.harvard.edu/meei/OA



Knudson's two-hit hypothesis for tumourigenesis involving a tumour suppressor gene (TSG)

http://www-ermm.cbcu.cam.ac.uk/fig002frb.gif

Mutation should be **in retinal cell**



If 2nd mutation arises in connective tissue

Osteosarcoma

Retinoblastoma

•http://pathology.uthscsa.edu/MSII/image1/Figure10.13.jpeg

Two-hit hypothesis relates to TSGs only

Oncogenes – activating mutation – damage of one allele is enough.

Gain-of-activity mutation.

One mutation = disease.

Tumor Suppressor Genes – inactivating mutation – when one allele is damaged, second allele stays functioning.

Loss-of-activity mutation.

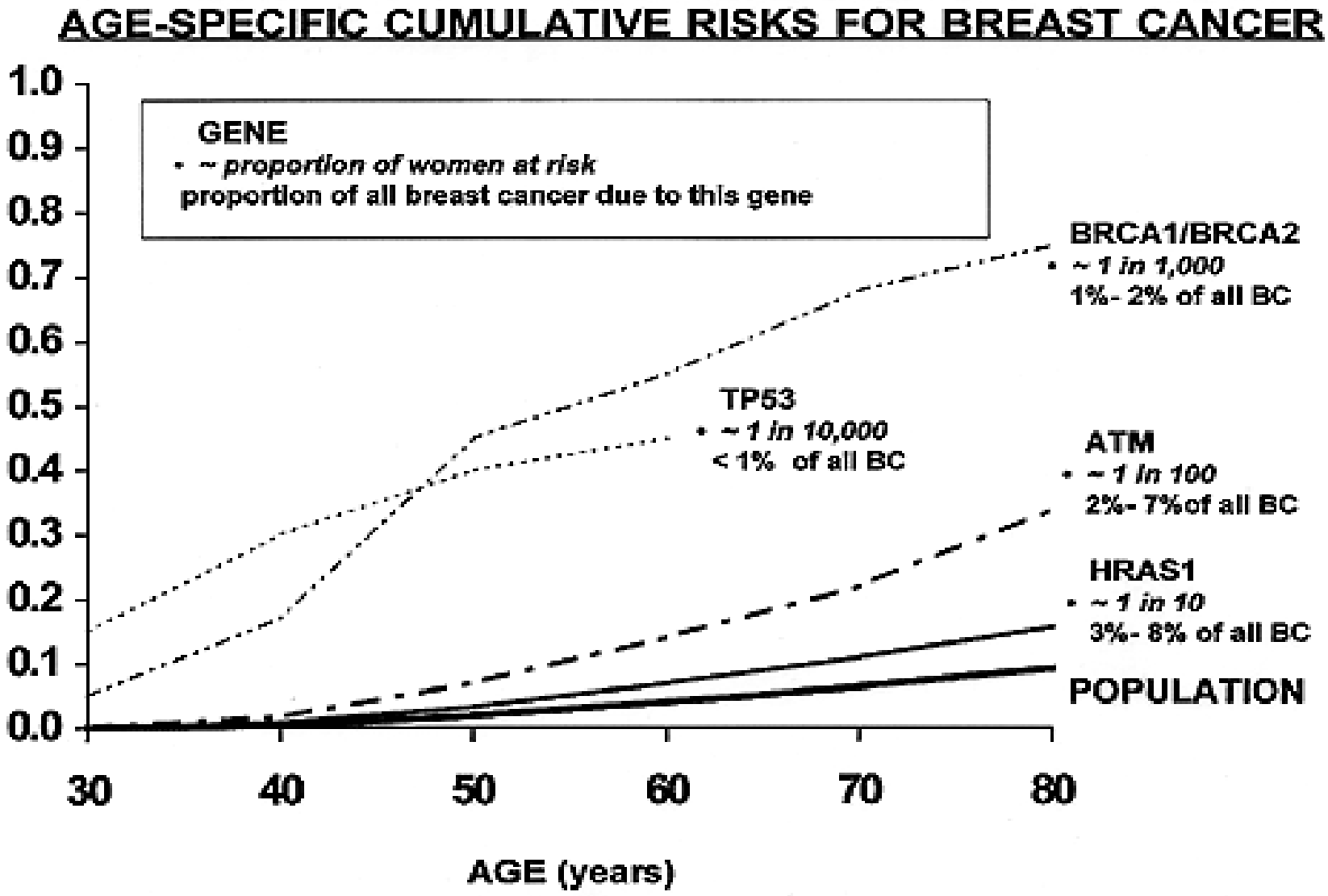
One mutation = predisposition. Two mutations = disease.

Most cancer syndromes result from inherited TSG mutations

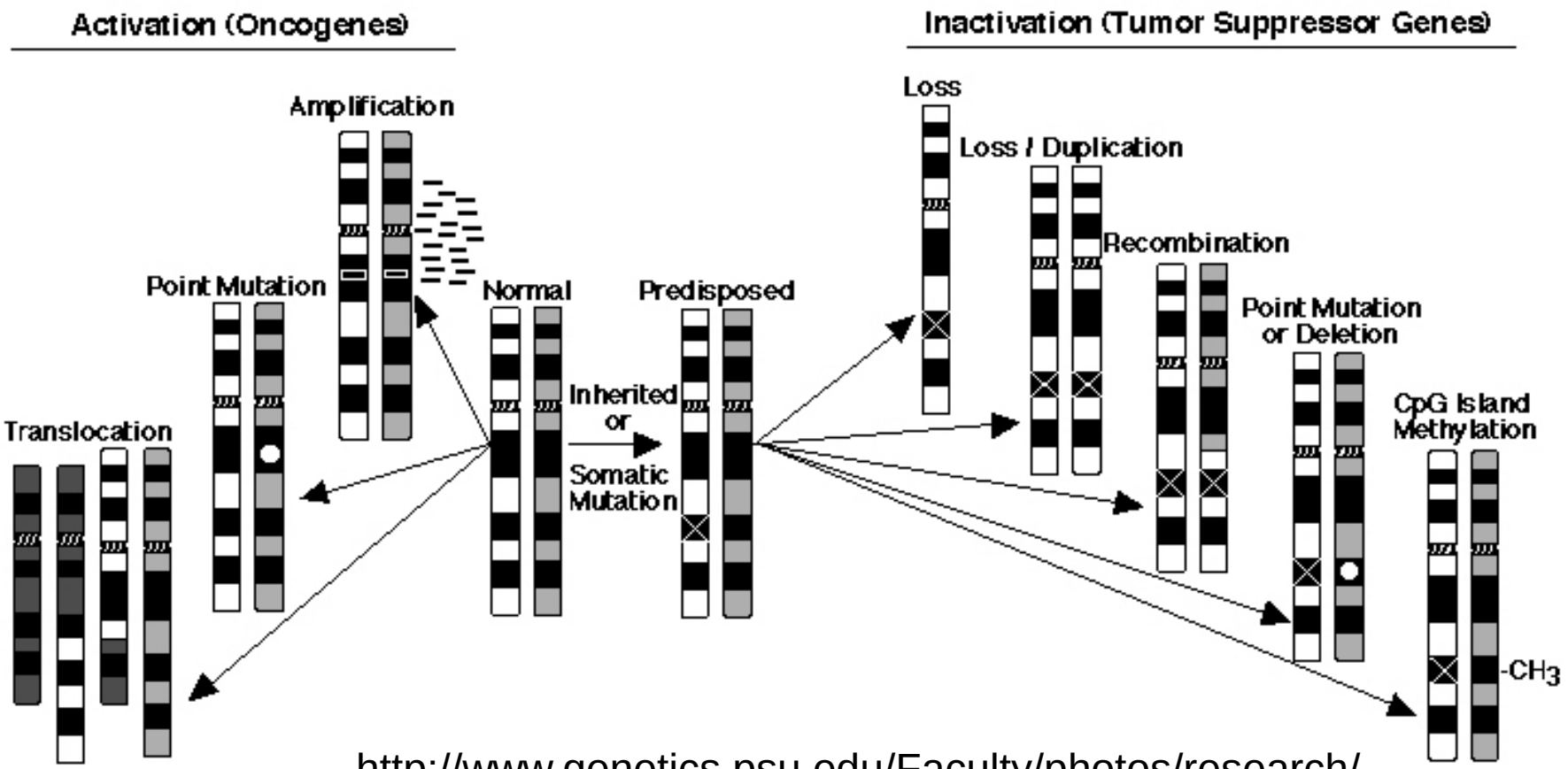
Inherited conditions that increase risk for certain cancers

Name of Condition	Type of Cancer
Hereditary retinoblastoma	Retinoblastoma
Xeroderma pigmentosum	Skin
Wilms' tumor	Kidney
Li-Fraumeni syndrome	Sarcomas, brain, breast, leukemia
Familial adenomatous polyposis	Colon, rectum
Paget's disease of bone	Bone
Fanconi's aplastic anemia	Leukemia, liver, skin

Strong or weak predisposition to cancer development



INSTABILITY OF GENOME as a fundamental feature of a cancer cell



http://www.genetics.psu.edu/Faculty/photos/research/

Natural diversity of tumors

Tumors developed in the same organ and presented with the same histology

Have the same name

(e.g. Infiltrating Moderately Differentiated Squamous Cell Carcinoma of Lung)

But molecular picture of mutations in this tumors can be totally different

The same effect can be obtained by means of any genomic event.

Nature of event **(type of change) is random;**

Choice of event **(particular gene) is random;**

Oncogenes

- **Activating point mutations**
- **Translocation under strong promoter**
- **Amplification**
- **Overexpression**

Tumor suppressors

- **Inactivating point mutations**
- **Promotor methylation**
- **Gross chromosomal deletions**
- **Underexpression**

RATES of molecular events in cancer cells

Random gain of mutations

(low-level; natural cause)

Early stages of natural cancer in elderly

Forced gain of mutations

(median level; X-ray, chemical carcinogens)

Early stages of cancer in exposed people

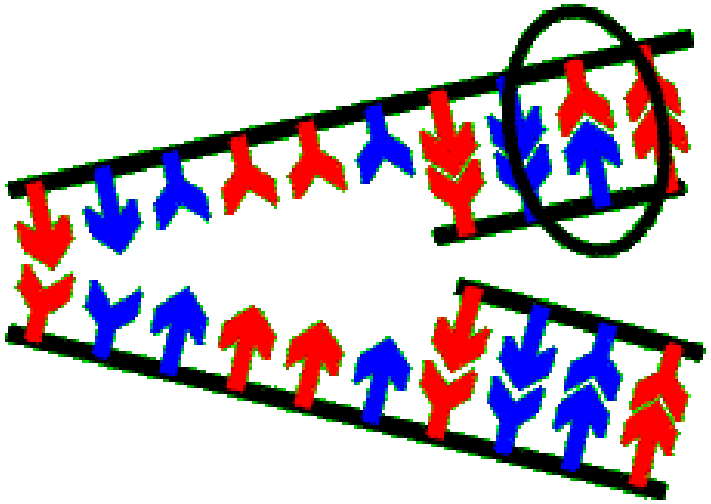
Very high rate of mutations

(cell lost one or more major mechanisms of DNA repair)

Early stages of cancer in certain syndromes; late stages of almost any cancer

Naturally occurring mutations

How we can count mutations?



Human HPRT gene

-- Located on chromosome X

-- Encodes the enzyme hypoxanthine-guanine phosphoribosyltransferase

-- Normal function of HPRT is metabolic salvage of the purine bases hypoxanthine and guanine into nucleotides, inosinic acid, and guanylic acid

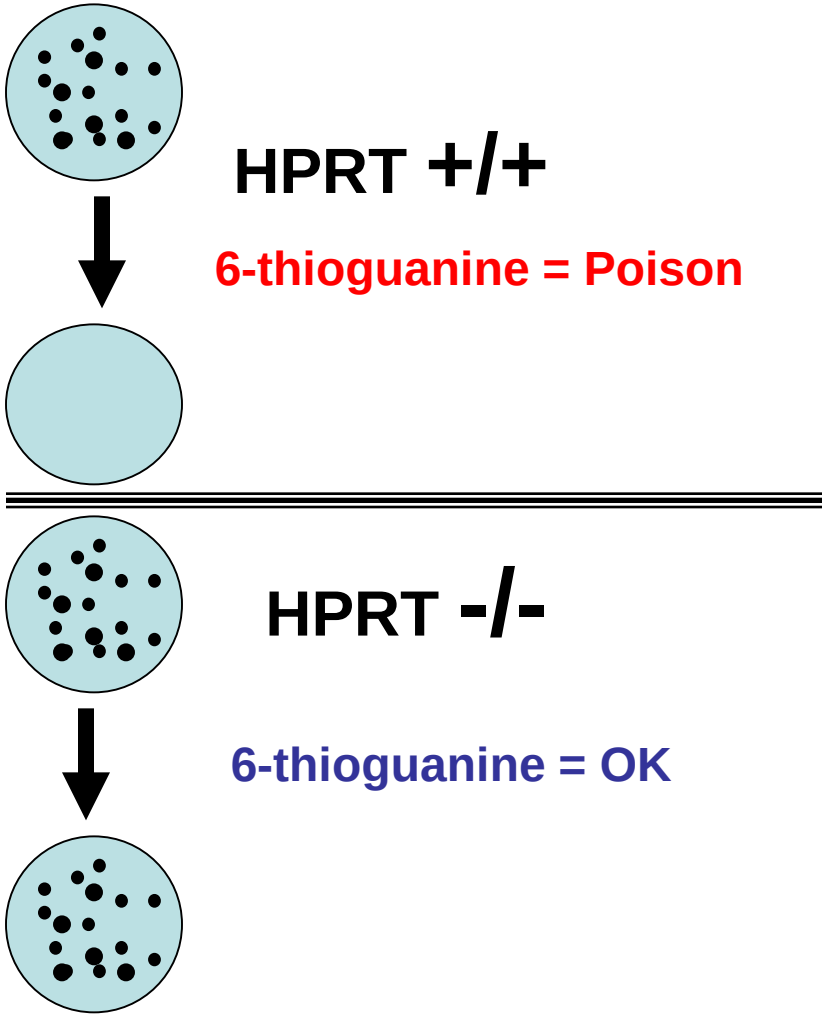
IN VIVO complete deficiency of HPRT activity = too much purunes is Lesch-Nyhan Syndrome (urate crystals + self-Injuring)

Partial deficiency - nephrolithiasis, gouty arthritis, & some neurological manifestations

Cells can survive without HPRT.
Cells are resistant to 6-thioguanine poison
ONLY if HPRT gene is mutated

HPRT ASSAY

The Hypoxanthine Phosphoribsyltransferase Assay.



HPRT +/+

6-thioguanine = Poison

HPRT -/-

6-thioguanine = OK

Cell that acquired mutation

in HPRT

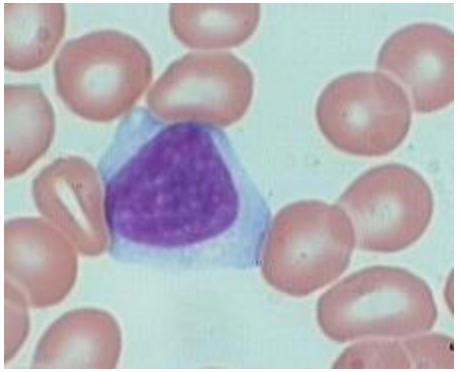
become resistant

to 6-thioguanine compound

We can directly count mutant colonies and compare this number with number of cell seeded on plate

HPRT mutation frequency

In peripheral T cells



In 49 healthy,
non-smoking adults:
rates varied 0.25 -- 9.64 x 10(-6).

What made mutation frequencies so different (>10 times)???

1. **Polymorphisms of genes metabolizing carcinogens;**
2. **Polymorphisms of genes responsible for DNA repair;**
3. **Alcohol consumption and smoking;**
4. **Exposure to envinronmental carcinogens;**
5. **Exposure to radiation**

Experimental check on hypothesis listed above

1) **CYP1A1, GSTM1 and NAT2 polymorphisms have no influence on HPRT mutation frequencies;**
2) **Mismatch repair genes was also not damaged;**
3) **Correlation between maternal alcohol consumption during pregnancy and results of HPRT assay on T-lymphocytes from newborns?**

Early pregnancy alcohol RR of high mutation status = 1.84
Through pregnancy alcohol RR of high mutation status = 2.99

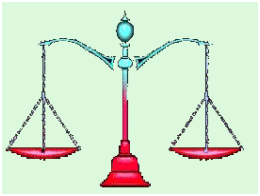
Smoking during pregnancy
have an influence on mutational spectrum,
but not on mutation frequency

Mutat Res 1999 Dec 17;431(2):279-89

Mutations in HPRT and smoking

Yes, smoking increases mutation frequency

No, smoke does not have any influence



A comparison of mutation frequencies in the K-ras, p53 and HPRT genes between the normal lung tissue of smokers and non-smokers indicates that the rate of mutation in smokers is only ~1.6 fold higher than in non-smokers

4) Exposure to envinronmental carcinogens

ethylene oxide ; 1,3-butadiene ; benzene

JUST marginal increase in HPRT mutability
when measured as in vivo exposure
(on plant workers populations)

Cell line-based or mice/ rat-based

HPRT assays

after direct addition of carcinogen

show strong increase in HPRT mutability

4) Exposure to radiation

Among Hiroshima-Nagasaki survivors (43 Rad in average)

HPRT rates : 1 mut per 10^{-8} per base pair per generation

indistinguishable from that of Japanese controls

Chernobyl clean-up workers:

40% increase in mutation rate

in the first year after accident, ….then it declined….

Conclusion: classical HPRT assay in lymphocytes

do not support any hypothesis explaining population varaiances in mutational load

Epithelial tissues
(carcinoma progenitors)

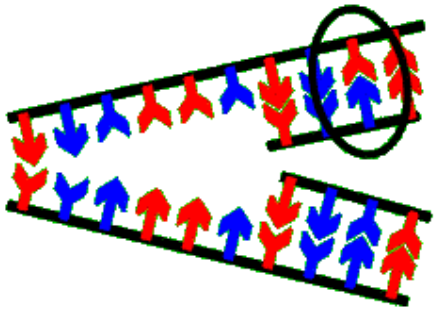
In kidney epithelium

Mut load is 0.5 -4.2 x 10(-4)
– much higher than in lymphocytes
(10- 100 times higher!)

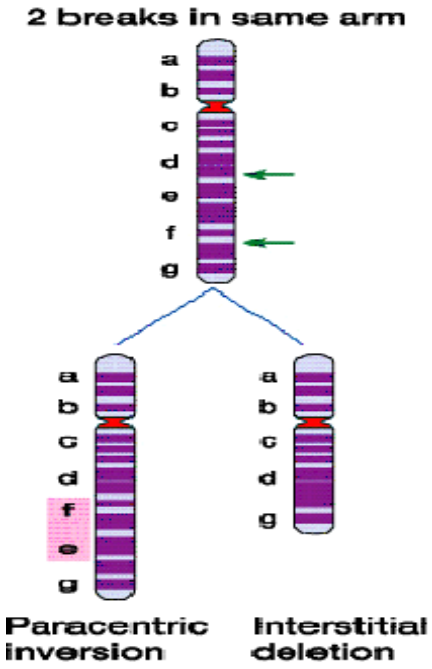
Such rate is sufficient to account
for a large proportion of human cancers
without the need of mutator phenotype

So, at least in kidney carcinogenesis can happens
without any additional environmental/genetic causes

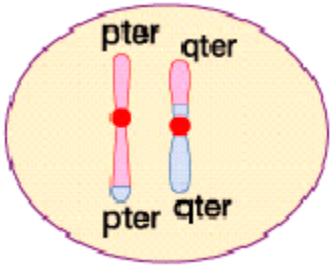
COMMON TYPES OF MUTATIONS in human cells



1. Point mutations
2. Microdeletions/microinsertions (1-3 bp)



3. Large chromosomal deletions
4. Chromosomal insertions and inversions



5. Translocations
6. Aneuploidy (extra chromosome or chromosomal loss)

Point mutations (single nucleotide changes)

A → G
G → A
C → T
T → C

} **TRANSITIONS**

MOST COMMON

PURINE→PURINE or
PYRIMIDINE→PIRIMIDINE

Pairing is possible due to tautomeric shifts or ionizing that allows mispairing

A→T T→G
T→A G→T
A→C C→G
C→A G→C

} **TRANSVERSIONS**

purine → pyrimidine or pyrimidine → purine

Pairing is energetically infavourable, but Pur-Pur pairs are possible (G-A)

When people talk about carcinogenic mutations,

most often they talk about point mutations.

Point mutations much more tolerable
for cell reparation system,
As they unlikely to awake apoptosis pathway.

On the other hand,
central pathogenetic event
in human leukemias and lymphomas
often is a specific translocation

Carcinoma – search for point mutation;
Lymphoma – search for translocation

RATES of molecular events in cancer cells

Random gain of mutations

(low-level; natural cause)

Early stages of natural cancer in elderly

Forced gain of mutations

(median level; X-ray, chemical carcinogens)

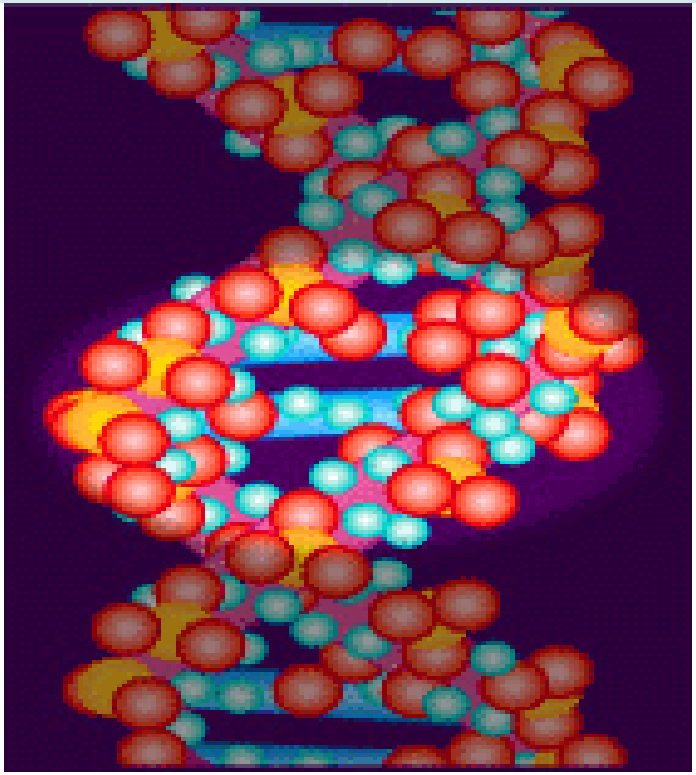
Early stages of cancer in exposed people

Very high rate of mutations

(cell lost one or more major mechanisms of DNA repair)

Early stages of cancer in certain syndromes; late stages of almost any cancer

INTRACELLULAR SYSTEMS ERRORS
producing high-rate mutations

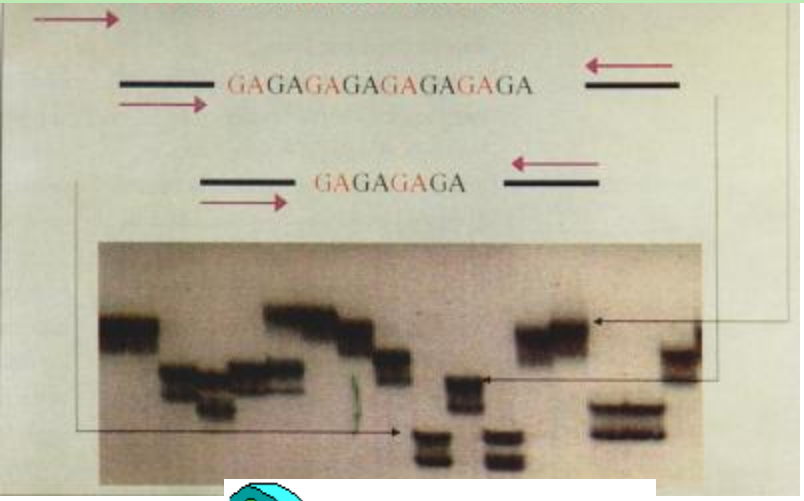


Can be classified in three major subtypes

TYPES OF INSTABILITY

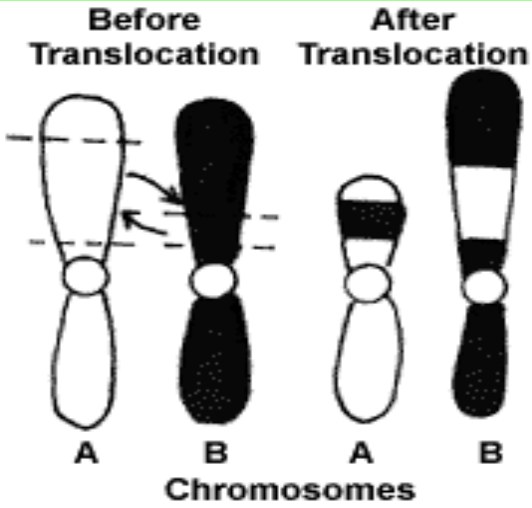
MIN

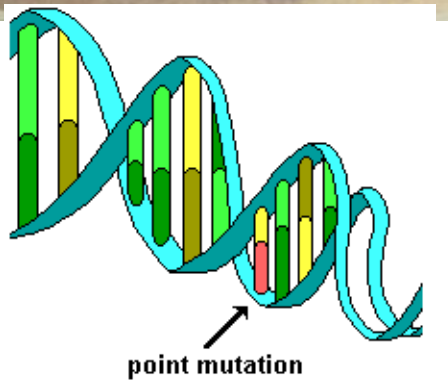
(Microsatellite instability)



CIN

(chromosomal instability)





SIN

(single nucleotide instability)

MIN phenotype (Microsatellite INstability)

progressive accumulation of frameshifts in microsatellite repeats

Poly-A and Poly-CA is especially prone; e.g. (A)6→(A)7 or (CA)5→(CA)4

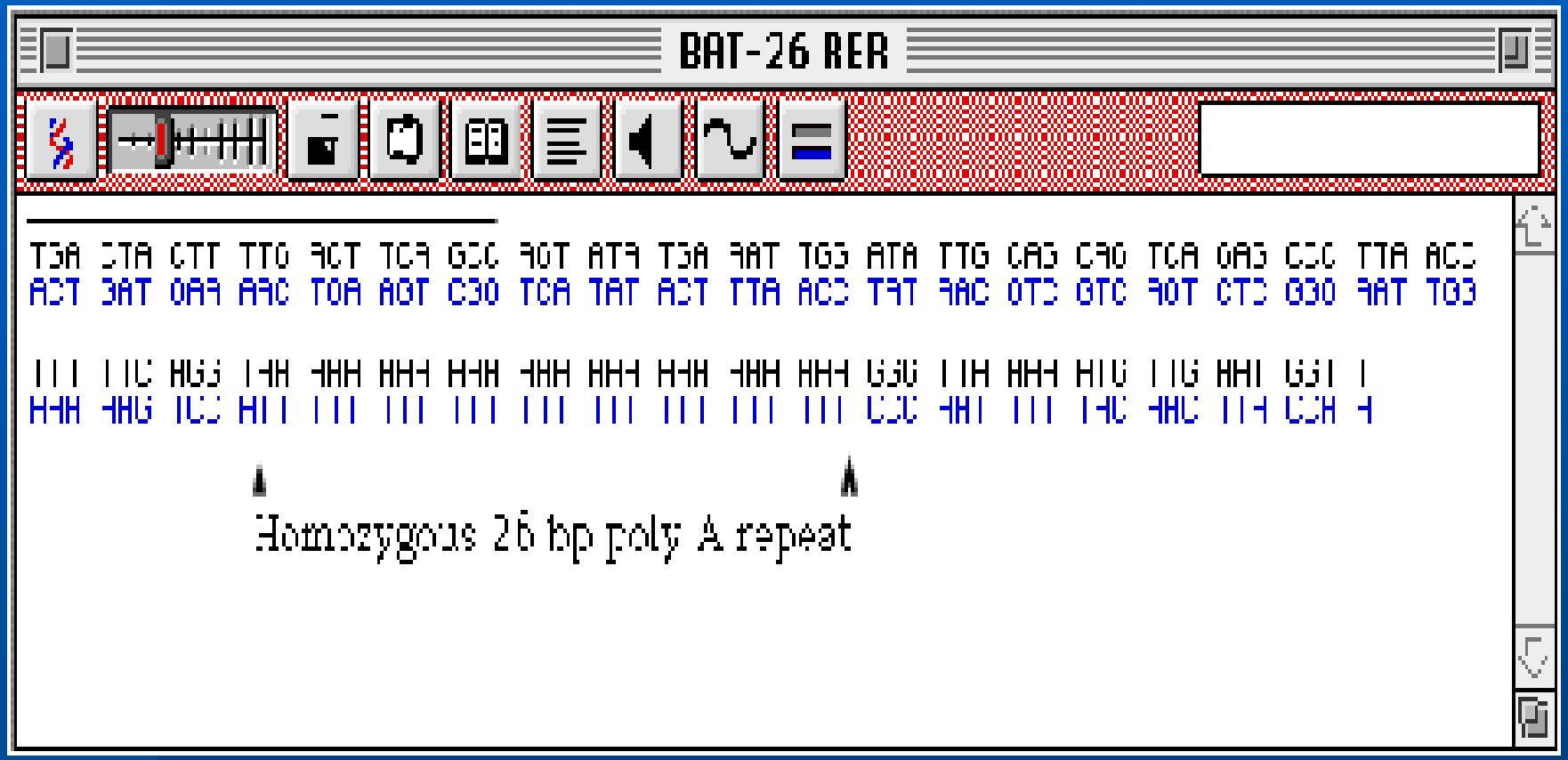
Microsatellites as a withesses (innocent bystanders)

Screening for MIN can be performed with a panel of 5 or more loci (D2S123, D5S346, D17S250, BAT25, BAT 26)

Microsatellites in coding regions of cancer-involved genes

TGFbetaRII, IGFIIR, TCF-4, BAX, hMSH3, hMSH6, CHK1, and BRCA2

MIN detection on BAT-panel



three (CA)n dinucleotide repeats (D2S123, D5S346, D17S250) and two mononucleotide tracts (BAT 25 and BAT 26).

MIN as a DIAGNOSTIC TOOL

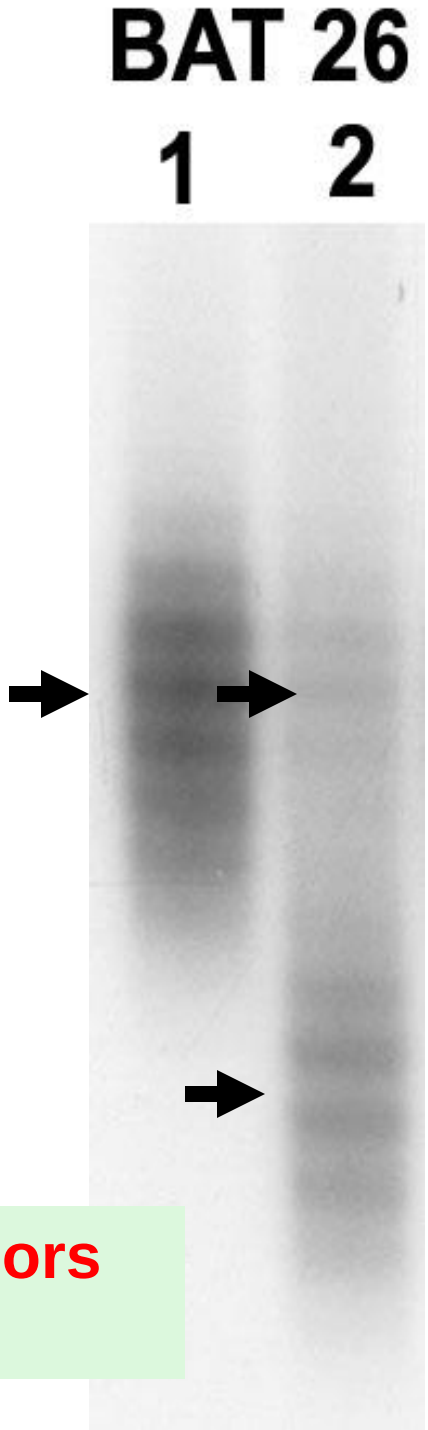
Shorthening of repeats in subset of cells could be diagnosed

1) both in somatic cells (lymphocytes) and tumor cells of HNPCC patients (colon cancer syndrome);

2) in tumor cell of sporadic cancer patients with MIN + phenotype

MIN+ → better prognosis for pancreatic and colon carcinomas (better immunoreactivity of the tumor)

MIN+ → Worse prognosis for germ cell tumors (testis is immunoprivileged organ)



MIN
(Microsatellite INstability)

Family cancer syndromes

HNPCC colon cancer

mutations

MSH2, MSH3, MSH6,
MLH1, MLH3,
PMS1, PMS2 genes

DNA mismatch repair proteins

Recognize and bind mismatches, especially in microsatellite repeats

Sporadic cancer cases

Methylation

MLH1 or MSH2 promoter

10-15% of gastric carcinomas

30% of sporadic endometrial tumors

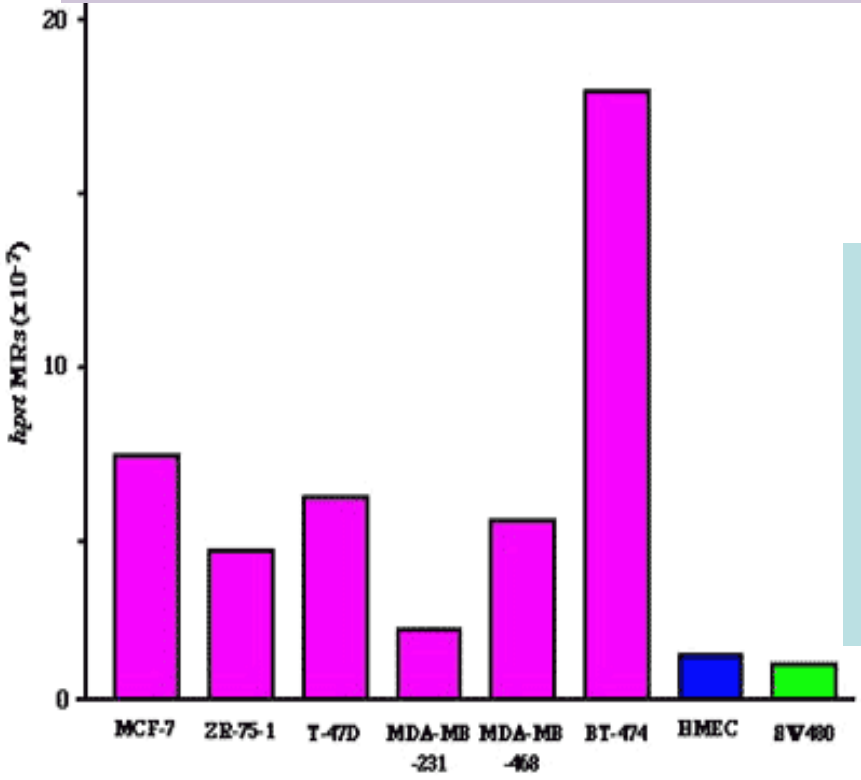
30-40% of sporadic breast carcinomas

20% of sporadic colon cancers

Loss-of MSH/MLH gene function – Primary cause of NHPCC colon tumors;
-- Secondary event in sporadic colon tumors

SIN - instability

Recent findings in NCC (Japan)



Single nucleotide instability

Spontaneous Point Mut Rates measured by HPRT system are higher in cancer cell than in normal counterpart

six human breast cancer cell lines (MCF-7, ZR-75-1, T-47D, MDA-MB-231, MDA-MB-468, BT-474), normal human mammary epithelium (HMEC) and a colon cancer cell line (SW480) without microsatellite instability.

http://www.ncc.go.jp/en/nccri/divisions/14carc/14carc05.html

CIN
(Chromosomal INstability)

Looks like

LOH
(loss of heterozygosity)

or

Aneuploidy

LOH Detected as :

Loss of one allele of polymorphic markers arranged on the same chromosome

(usually by PCR).

ANEUPLOIDY Detected as:

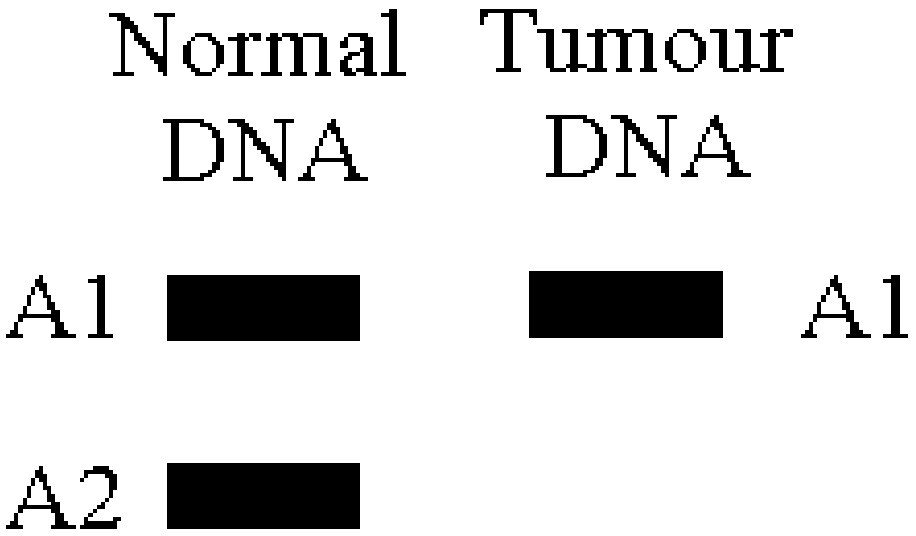
Loss or addition of extra copy of chromosome

(usually by FISH or fluocytometry)

DETECTION of LOH

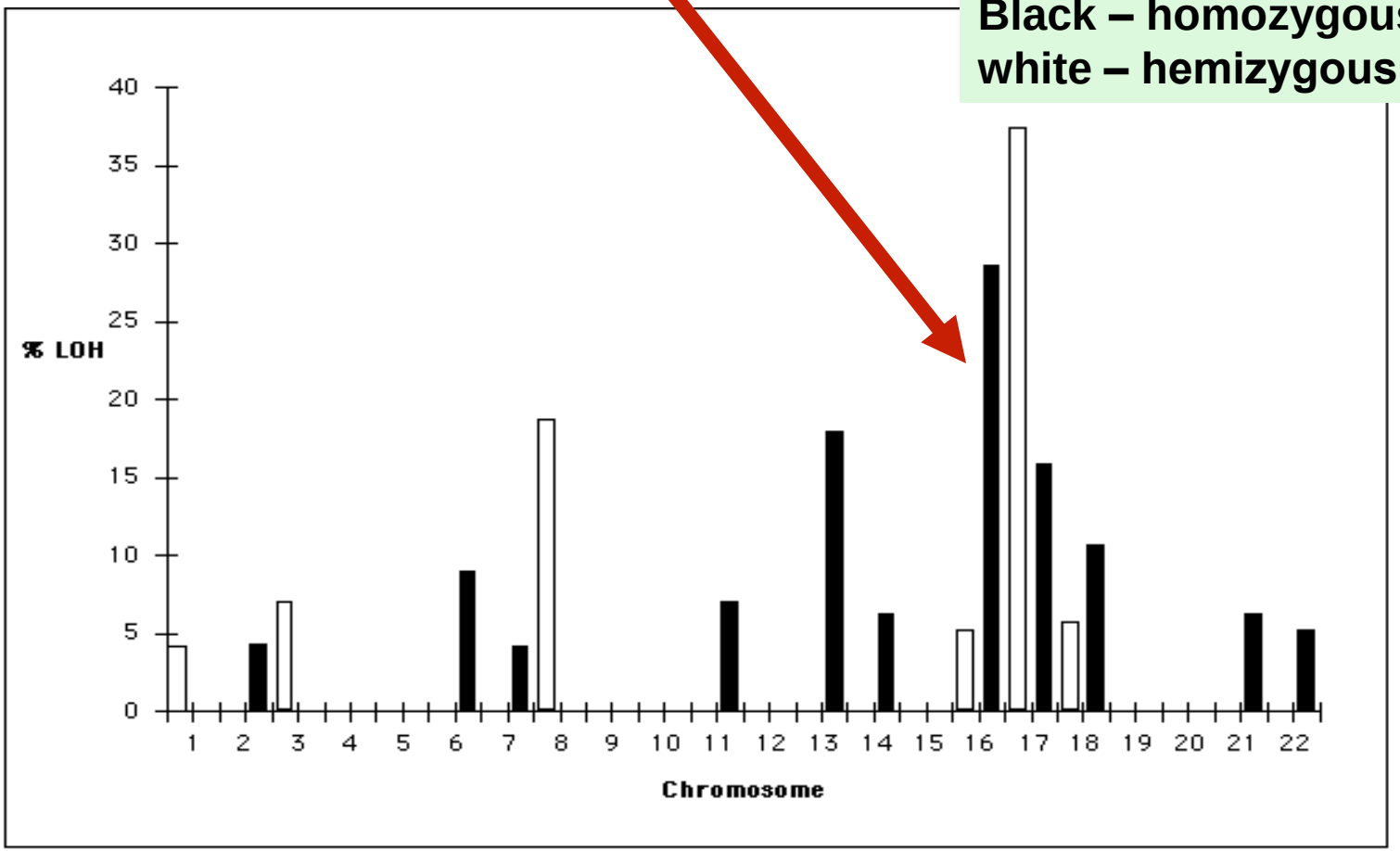
Informative microsatellite
(polymorphic in this particular normal sample)

TWO alleles in normal tissue
versus ONE allele in tumor tissue



Pattern of LOH can be different in clinically similar tumors from different patients (but some loci have LOH more often than others)

Black – homozygous losses; white – hemizygous….



www.genlink.wustl.edu/.../image/ figure1_radford1.gif

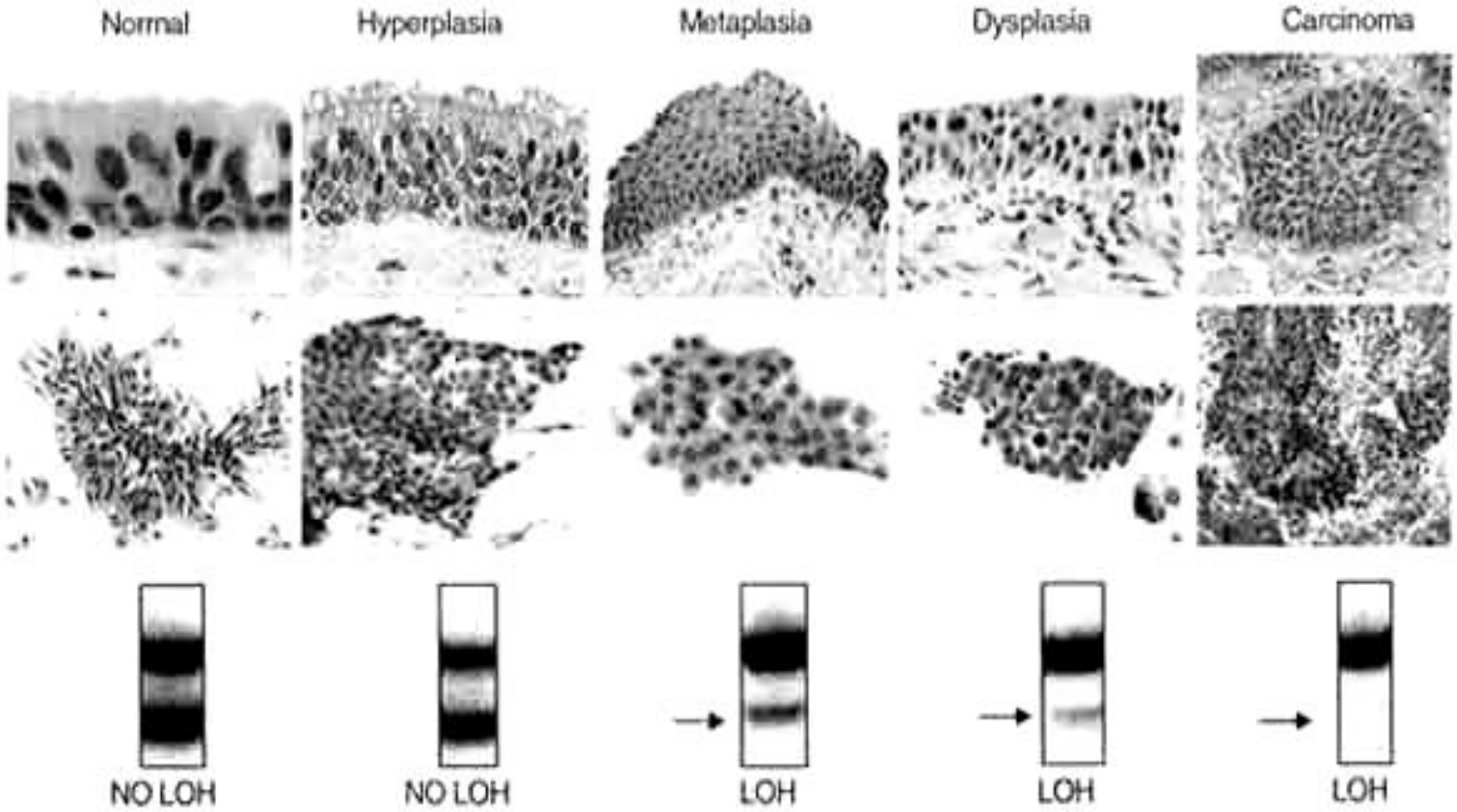
Overlaps of deletions in breast carcinomas

Two different regions of LOH on the same chromosome are

indicative of two different frequently deleted areas

www.medfac.leidenuniv.nl/lab-devilee/ Projects/16lohsro.gif

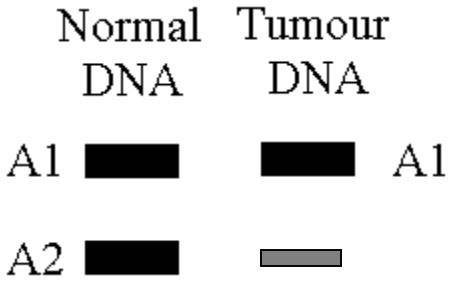
LOH appears on a certain stage of tumor development



http://www.bentham.org/cmm1-1/miatra/Miatra-fig3-pg159.jpg

Natural difficulties with LOH technics

1. **Every tumor contains normal cells (stromal cells, vasculature, lymphocytes etc…)**

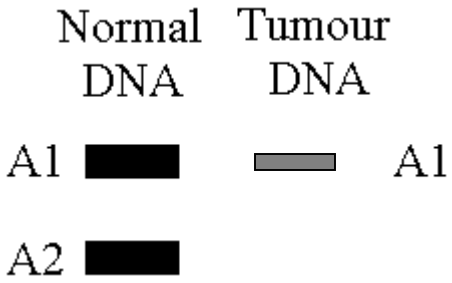


Normal cell have normal DNA without LOH.

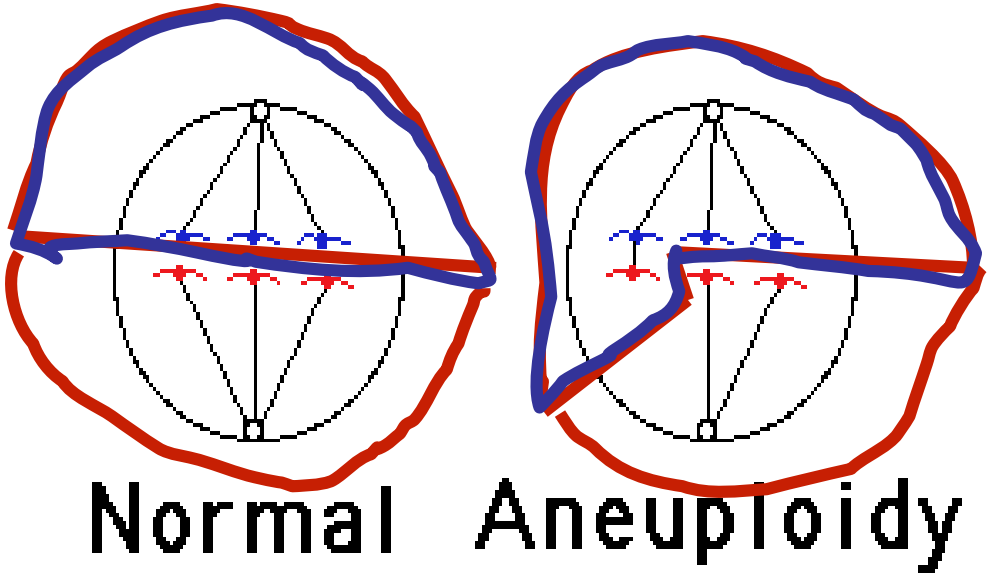
They contaminate population of tumor cell, and make results unclear.

2. **Homozygous deletions produce no PCR product,**
that is a situation indistinguishable from failure of PCR reaction

Deletions can be homozygous or hemizygous.
Hemizygous deletions produce LOH.



ANEUPLOIDY

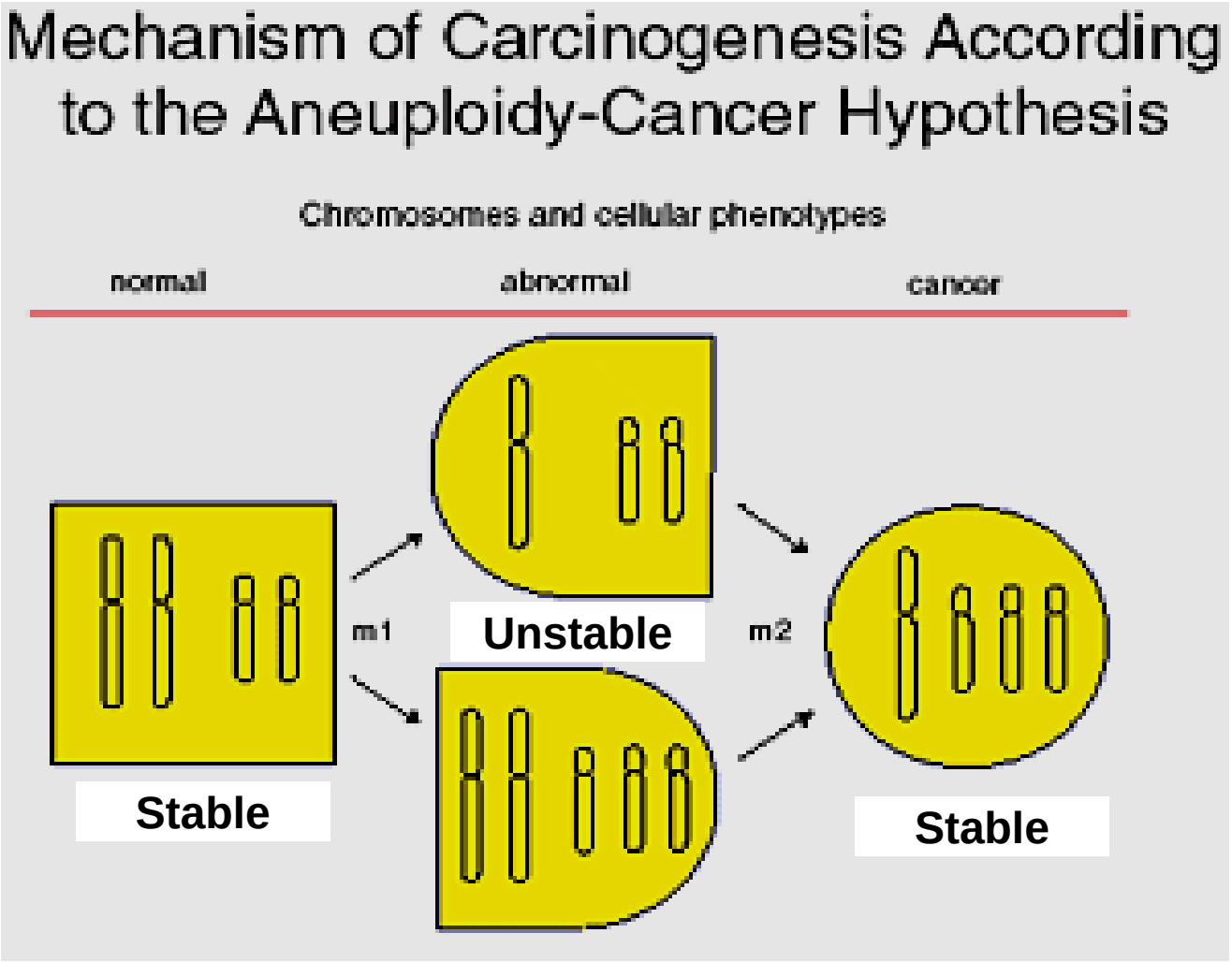


Loss or addition

of extra copy
of chromosome.

Aneuploidy alters the dosages and expression of thousands of normal genes
(most of them are bystanders, not a cause of cancer)

Cancer-Aneuploidy hypothesis



P. Duesberg

Carcinogen produce aneuploidy

Aneuploidy produce cancer cell

Details of Duesberg theory

carcinogens initiate carcinogenesis with a random aneuploidy

Aneuploid cells are error prone

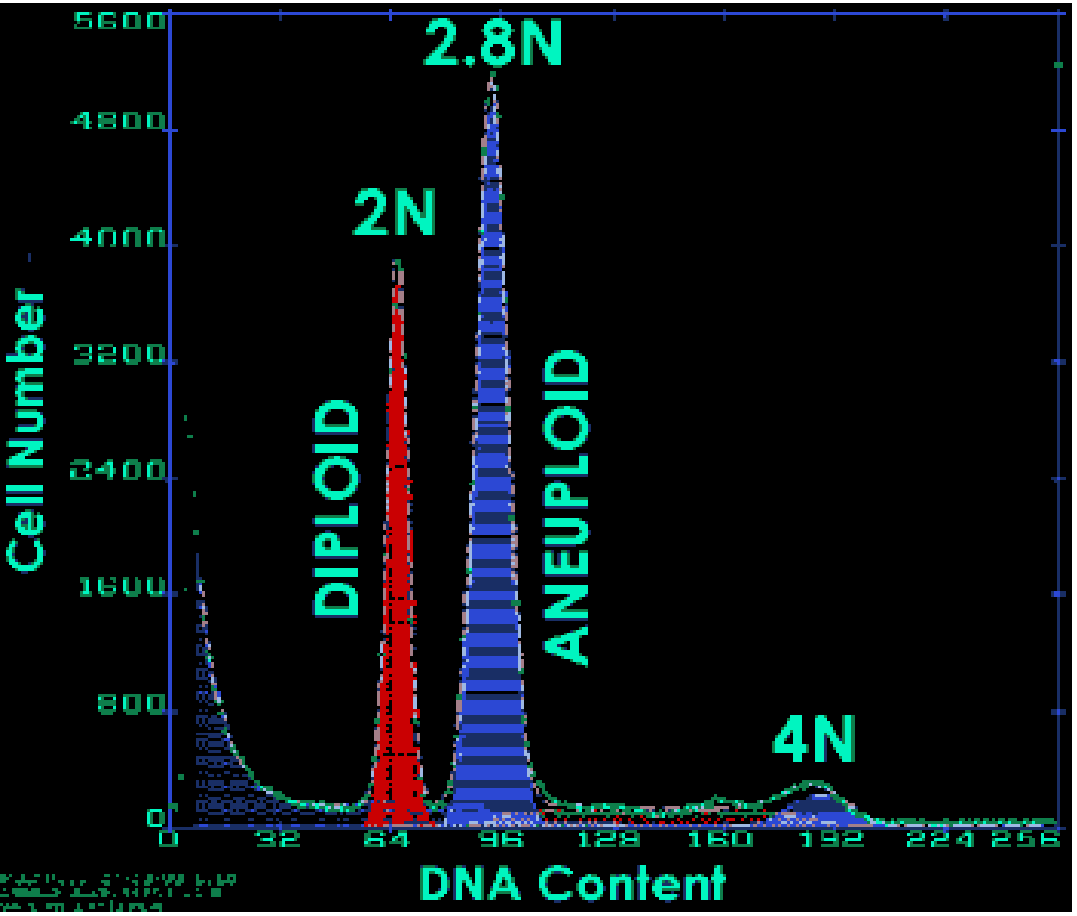
as chromosome segregation and maintenance systems

are disbalanced as a result of **unbalancing of spindle proteins, repair enzymes, and centrosome numbers.**

P.Duesberg, Nobel prize winner, Does not believe in HIV virus

did, after all, isolate the first oncogene by age 33

How to measure aneuploidy



Flow Cytometry

DNA content flow cytometry

frozen biopsy is thawed and placed in a solution that ruptures the cells leaving only the nuclei

The nuclei are stained with a fluorescent dye that binds to the DNA

The fluorescent dye bound to the nuclear DNA is excited by the light and fluoresces.

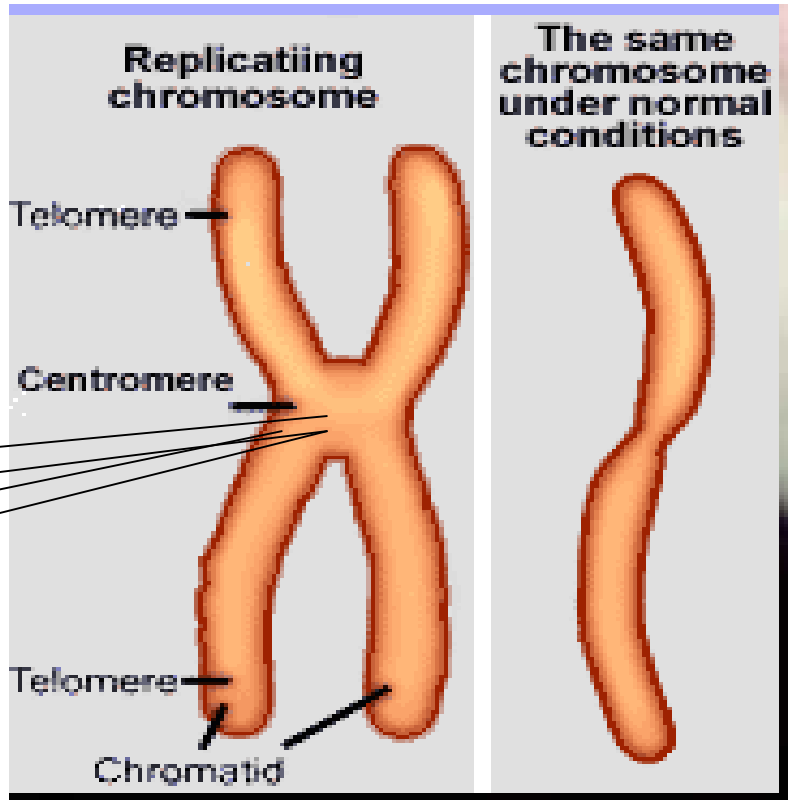
intensity or brightness of the cell's fluorescence Is proportional to the amount of DNA in the cell

Telomeres and telomerase: story of extra stability

Reminder: Chromosomes are comprised of a single, uninterrupted DNA molecule complexed with proteins (histones and others).

Telomeres and centromere are demarcating the two “arms” (p and q).

Kinetochore microtubules



Telomeres – Ends of linear chromosomes

Repetitive DNA sequence: **TTAGGG** in vertebrates

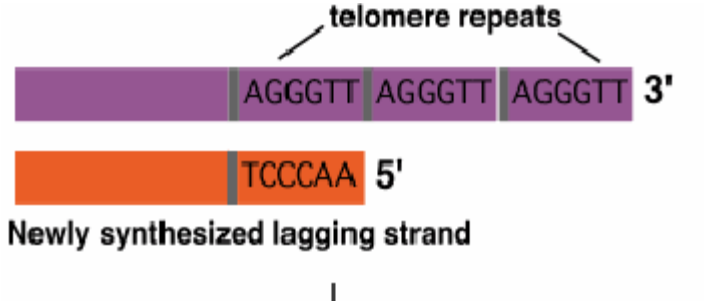
Associated with specialized proteins.

Telomeres are necessary because:

- they allow cells to distinguish chromosome ends from broken DNA and prevents chromosomal fusions by non-homologous end-joining (NHEJ) machinery;
- they provide a mechanism for "counting" cell divisions as they shorten with each cell division (to be discussed in cancer section)
- they help to establish 3D structure of the nucleus

TELOMERE – story of extra stability

During the replication the lagging strand requires new priming for every piece

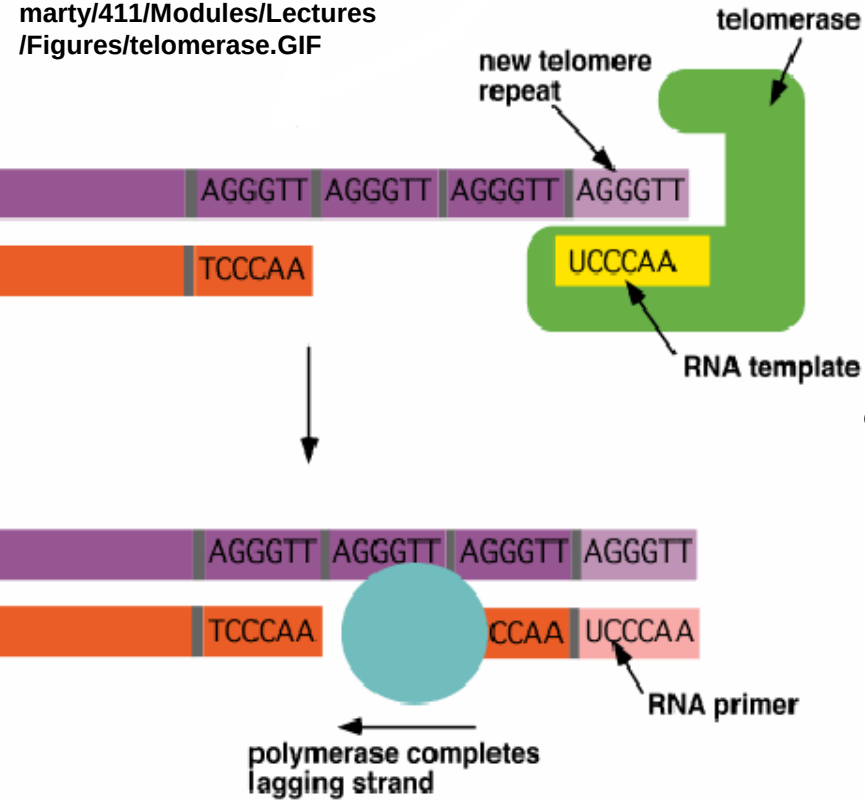


As a consequence, every telomere shorthen by an amount equal to primer length

It's OK for measuring of the cell life span, it's not OK for maintenance of germ line cells

How to solve telomere maintenance problem?

http://www.blc.arizona.edu/marty/411/Modules/Lectures/Figures/telomerase.GIF



Telomerase enzyme is related to reverse transcriptase,

as it has a small RNA template

as a part of the enzyme structure.

Telomerase is highly expressed in germ cells and stem cells

Somatic mouse cells express telomerase, that is why they are easier to immortalize; human cell - do not express telomerase.

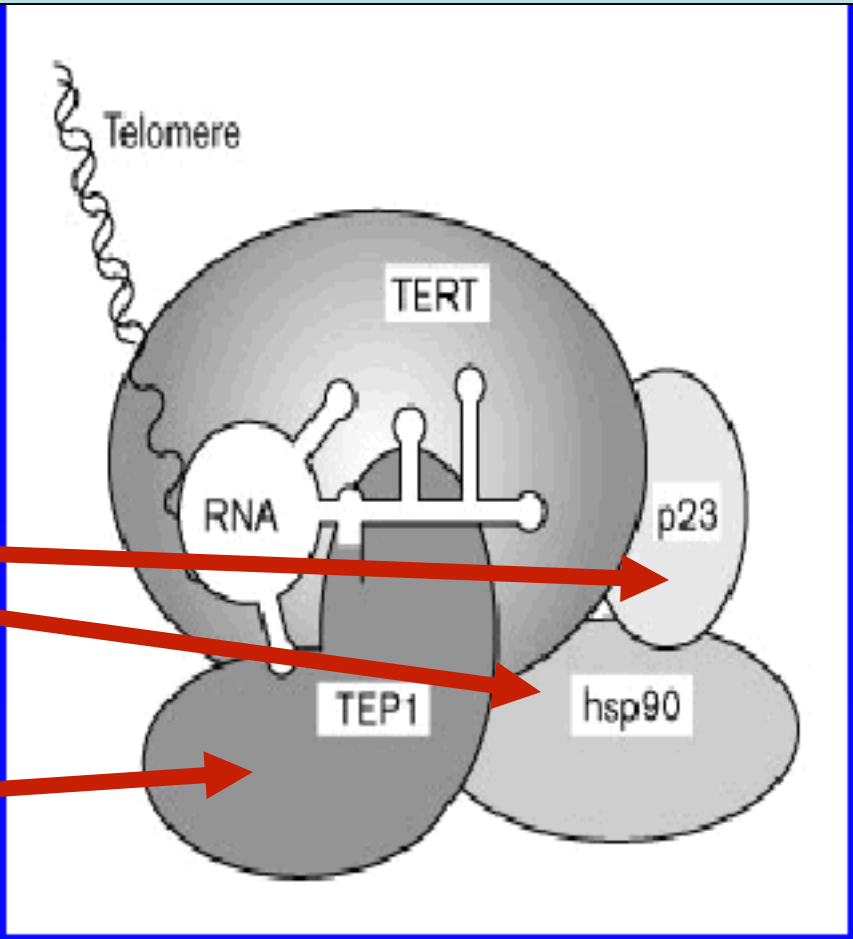
Cancer cells express telomerase !!!

Telomerase structure:

Accessory proteins TEP1, p23 and hsp90 also contribute to activity of the complex

P23 and HSP90 are involved in assembly

TEP1 may facilitate DNA binding



biochem.uwa.edu.au/.../B352/Lectures/ Telomerase/img001.gif

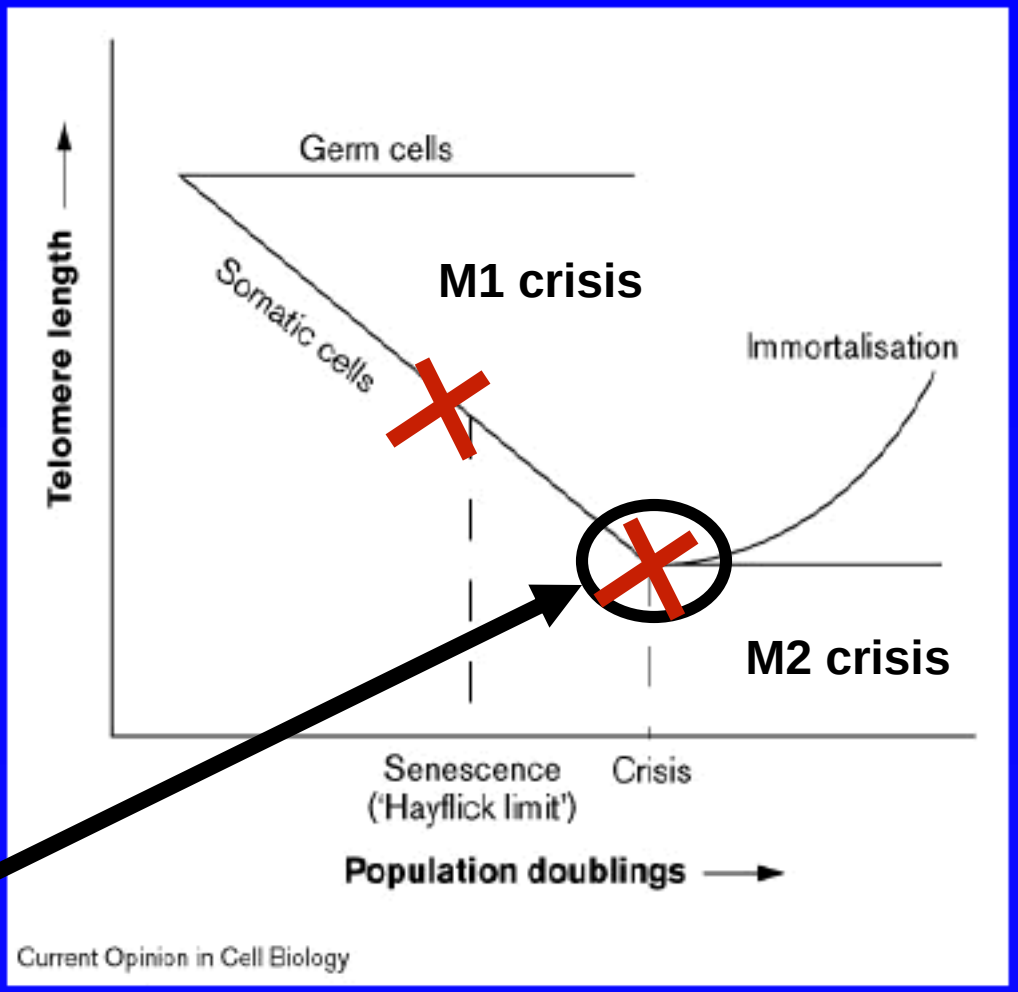
Telomerase and senescence

Germ cells maintain their telomere length.

Telomeres shorten with successive divisions in somatic cells.

At the Hayflick limit (~60 divisions) cells senesce.

Following crisis, a minor population of cells become immortalised and are able to increase their telomere lengths.



Awakening of telomerase

biochem.uwa.edu.au/.../B352/Lectures/ Telomerase/img001.gif

Different cell types have different requirements for immortalisation

Transfection of hTERT (telomerase) gene to normal human cells gave them an extra capacity to be passaged appr. 200 times without transforming them to tumor cells

TRUE for human fibroblasts

and for human retinal epithelial cells

FALSE for epithelial breast cells:

They require: both TEL and del of pRB or p16

Some cell type will age

even if telomerase is re-activated

(such cells are more tumorigenesis-proof)

So, tissue-specific differences are strong !!!

CROSS-SPECIES differences

can be even stronger!!

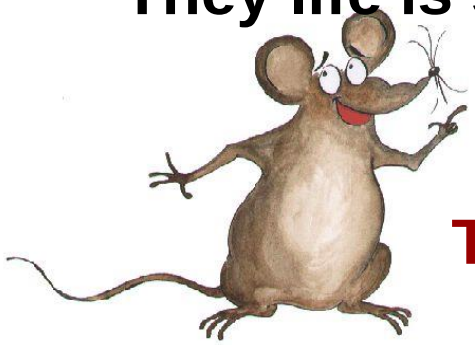
Everyone should remember it
when modeling cancer (or other diseases) in animals

Especially in mice,
as mice are very weird
in sense of their biology

MOUSE is very different from human

They are smaller

They life is shorter



BUT!!!!

They telomeres are longer!!!

They have less stringent regulation of telomerase!

mice do not have the barrier of telomerase shortening for cell proliferation

Human do have this barrier

Mice cells are easier to immortalize in culture compared to human counterparts

Mice are not so protected from tumors like human beings (they no need it….)

MOUSE without a telomerase

TERC-/- mouse lack the telomerase RNA component

Mice are viable AND FERTILE !

No telomerase activity!!!!

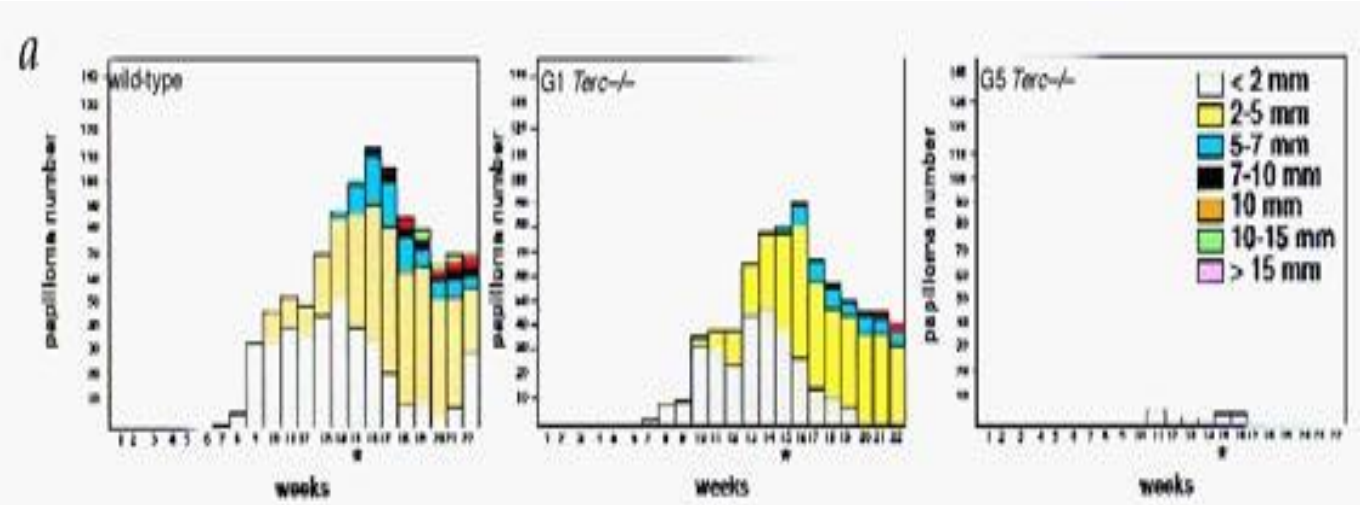
Tissues requiring constant renewing develop normally (gut, skin, blood cells)

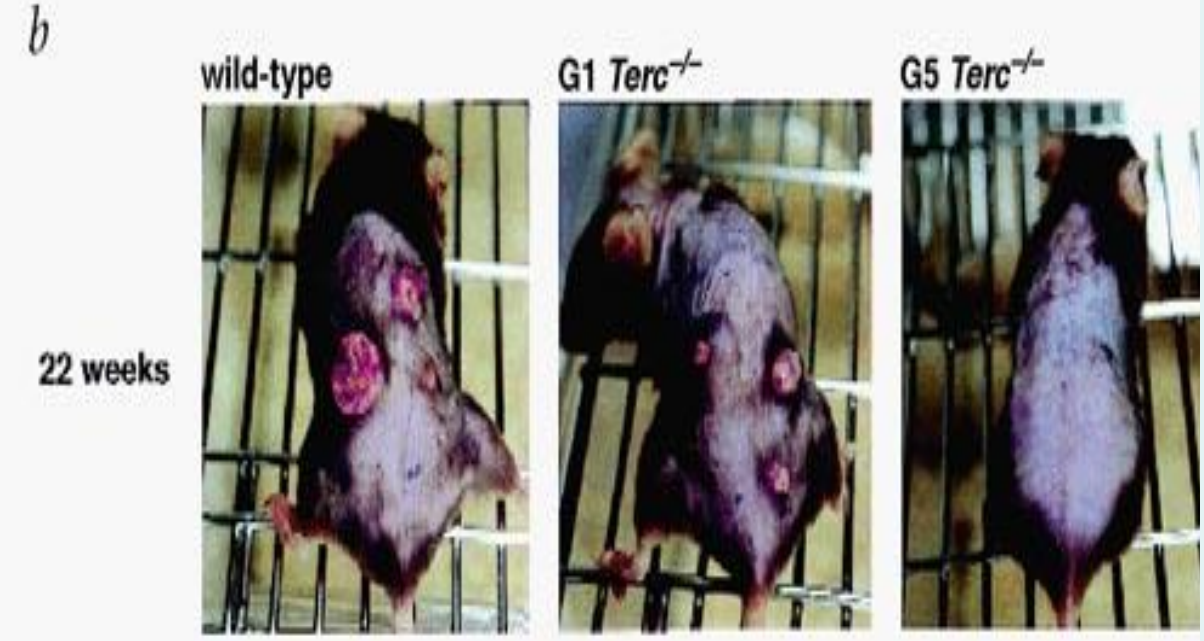
Generation number 5 (inbred)
have shorter telomeres
than those of wild-type and first-generation;

Decresead fertility and wound healing and,

as well as an increase of death *in utero* and increase in failures of neural tube closure

CHEMICAL TUMORIGENESIS IN TELOMERASE DEFICIENT MICE





TERC-/- mice develop less papillomas in multi-stage skin tumorigenesis; only 37% of mice ever develop them. Papillomas regress 1 week after stop of TPA treatment

Gonzales-Suarez et al., Nature genitics, 2000

TERT-/- mouse lack the catalytic telomerase component

Looks the same.

After some generations offsprings become prone to pre-mature aging and tumors!!!

Contradiction!!!

TERC-/- mice were checked in multi-stage skin tumorigenesis experiments (artificial initiation of the tumors);

In this case absence of telomerase prevents immortalization of mutated cells

TERT-/- mice were checked in spontaneous tumorigenesis which is very different from forced tumorigenesis

Why mice without telomerase are PRONE to spontaneous tumors ?

As telomeres erode,
genomic instability is increased
due to chromosome fusion/breakage.

In mice, telomeres can be extended
by alternative ways ("ALT"),
accounting for immortalization
of telomerase-null cells.

ALT (alternative lengthening of telomeres)

ALT cell lines are characterized by the combination of
no detectable telomerase activity
and the presence of telomeres
of very heterogeneous length,
ranging from very short to much longer than normal

ALT telomere lengthening can be maintained by:

1. **Another (..mutated..) type of telomerase, that can not be recognised biochemicaly** **OR**

2. **could also be caused by retrotransposition or recombination**

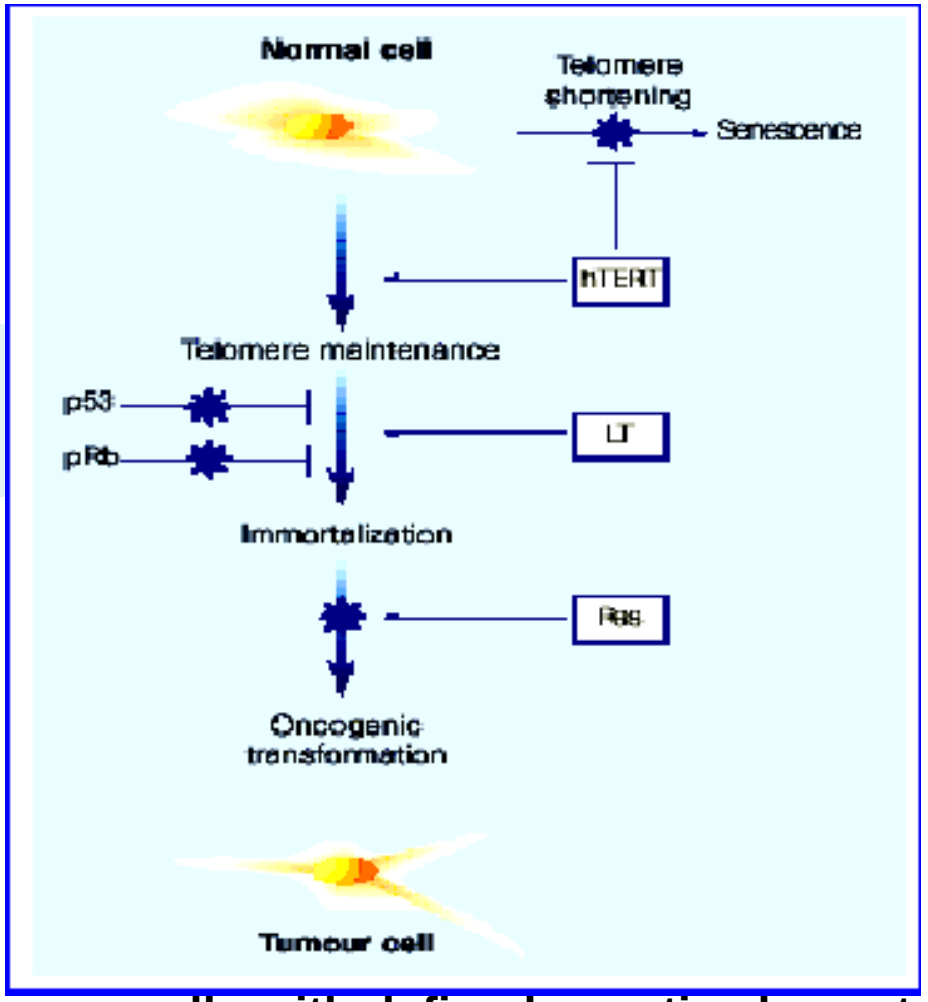
What is the minimal tumor cell? (after all)

Minimal tumor phenotype requires 3 genetic events

Expression of Large T-antigen (that inactivates RB and p53)

hTERT expression (telomerase re-activation)

Activation of RAS oncogene



Creation of human tumour cells with defined genetic elements. Hahn WC, Counter CM, …..Weinberg RA. Nature 99