# CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2015-2016 :
"Epigénétique et Cancer"

29 Février, 2016

Cours I

**"Une brève histoire du cancer : génétique et épigénétique"**

"*A Brief History of Cancer and Epigenetics*"

# Introduction: an epigenetic perspective of cancer

## Why discuss cancer from an epigenetics perspective?

**(1) The parallels with epigenesis and development are striking - understanding the differences is the challenge!**

**(2) Increasing connections between cancer and epigenetics in the last 50 years or so**
**Epigenetic therapies**

**(3) Recent new insights, thanks to genetics in model organisms, as well as genomics and single cell approaches, have led to exciting breakthroughs into our understanding of key roles that epigenetic modifiers play in cancer**

Even Wikipedia today defines cancer as a "process characterized by a progression of changes at the cellular, genetic, and epigenetic level that ultimately reprogram a cell to undergo uncontrolled cell division, thereby forming a malignant mass…"

# Introduction: an epigenetic perspective of cancer

**Cours 1 – Lundi 16h à 17h30, 29/02/2016:**
"Une brève histoire du cancer : génétique et épigénétique »
“A brief History of Cancer and Epigenetics"

**Cours 2 – Lundi 16h à 17h30, 07/03/2016 :**
"La génomique et l'épigénomique des cancers : de la description aux mécanismes »
"Cancer Genomes and Epigenomics: from maps to mechanisms"

**Cours 3 – Lundi 16h à 17h30, 14/03/2016 :**
"Contrôle épigénétique des gènes et des génomes dans le cancer »
"Epigenetic control of genes and genomes in cancer"

**Cours 4 – Lundi 16h à 17h30, 21/03/2016 :**
"Voies épigénétiques du cancer - I »
"Epigenetic pathways in cancer - I”

**Cours 5 – Lundi 16h à 17h30, 04/04/2016 :**
"Voies épigénétiques du cancer - II »
"Epigenetic pathways in cancer - II"

**Cours 6 – Mercredi 16h à 17h30, 06/04/2106 :**
"Perspectives: Marqueurs et thérapies épigénétiques »
"Perspectives: Epigenetic Biomarkers and Therapies”
Cours suivi à 17h30-18h30 par un **Seminaire**
**Prof Kristian Helin** (Directeur du BRIC, Copenhagen, Denmark)

**Le 9 et 10 Mai 2016**
**Colloque "Epigénétique et Cancer”`**
co-organisé avec le Prof. Hugues de Thé



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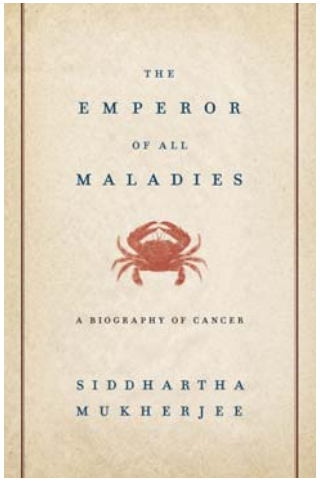
# A Brief History of Cancer




Hippocrates (460-370 BC) : “Carcinos” - crab
Galen (130-200 AD) : “oncos” – swelling (gonflement)

*Metastatic breast cancer in Siberian mummifed woman (>2500 BC)*

*'Princess Ukok' mummy in Anokhin museum, Gorno-Altaisk [Credit: Alexander Tyryshkin]*

*1500-3000 BC. Edwin Smith Papyrus, ancient Egyptian medical textbook*

*“Night”- 1526–1531, Michelangelo San Lorenzo, Florence.*

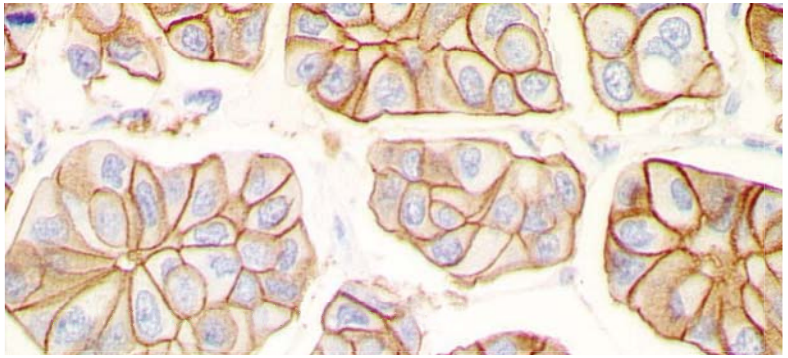
# What is Cancer?

- Pathologists have tried to define cancer for more than a century
- Difficult to define cancer cells as they originate *within* tissues; and share many properties of normal cells
- We now know that cancer is not one disease but hundreds of different diseases
- One common characteristic of tumors, is uncontrolled increase in size - due to excess or autonomous cell division?

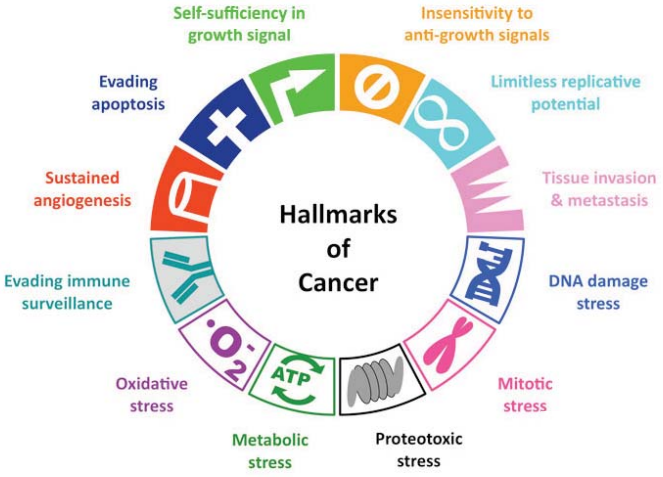
***All tumors consist of abnormal cells in which processes regulating normal cell division (and cell death) are disrupted***

- Cancer cells seem to become less dependent on signals from other cells; show altered tissue organization
- Malignant tumors can interact with their microenvironment, and can invade surrounding tissues
- Cancer cells can travel (metastasise) to other sites – and can sometimes (though rarely!) proliferate in new environments

***Tumors must be considered as organs: they are not just a collection of homogeneous cancer cells, whose entire biology can be understood by elucidating cell autonomous properties***



Cells from invasive micropapillary carcinomas (IMPCs) of the breast are characterized by a striking cell polarity inversion.
*Gruel et al, Breast Cancer Res. 2016 (Dr. A. Vincent –Salomon, Institut Curie)*




*Hanahan and Weinberg - 2011*

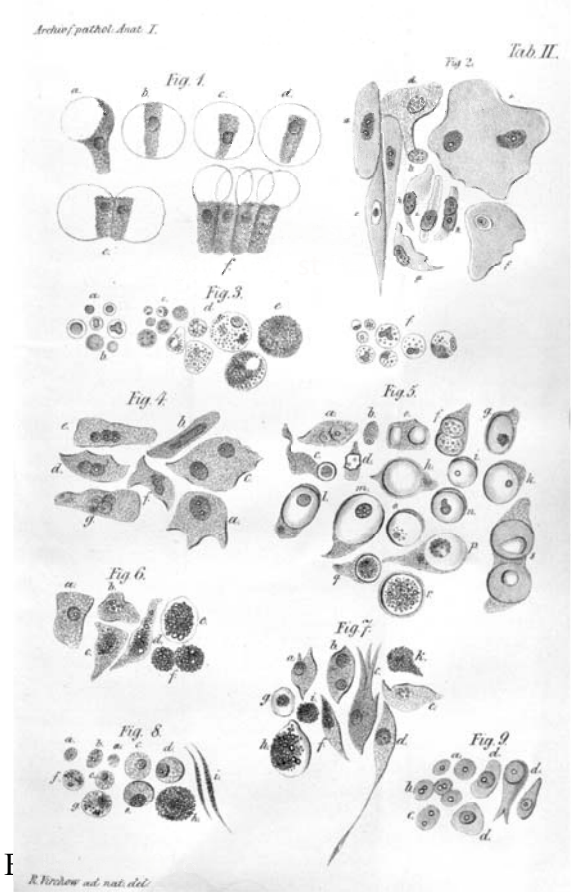
# Hypotheses to explain Cancer since the 19th Century

**Multiple Hypotheses**

- A problem of cell proliferation
- A problem of chromosome alterations
- An epigenetic process
- A problem of cell differentiation
- A problem of tissue organisation
- Tumor viruses
- Exogenous chemicals
- Altered chromosomes (and genes)
- Epigenetic alterations

# Hypotheses to explain Cancer: uncontrolled cell growth

## Virchow: Cancer is a problem of cell proliferation – unlimited cell division




- In 1845, first described and named (1847) *leukemia*
- Defined cancer using microscopy on specimens from autopsies
- In 1855, he proposed cancers arises by *activation* of dormant cells due to severe chronic irritation (inflammation?)
- Based on work of Remak, who had found convincing evidence that cells form by division, Virchow proposed (in *Cellular Pathology* published in 1858) that *Omnis cellula e cellula* ("All cells come from cells")
- Defined cancer as a disease involving uncontrolled cell growth

⇒ **All cells, including cancer cells, are derived from other cells and cancer arises from uncontrolled cell growth**



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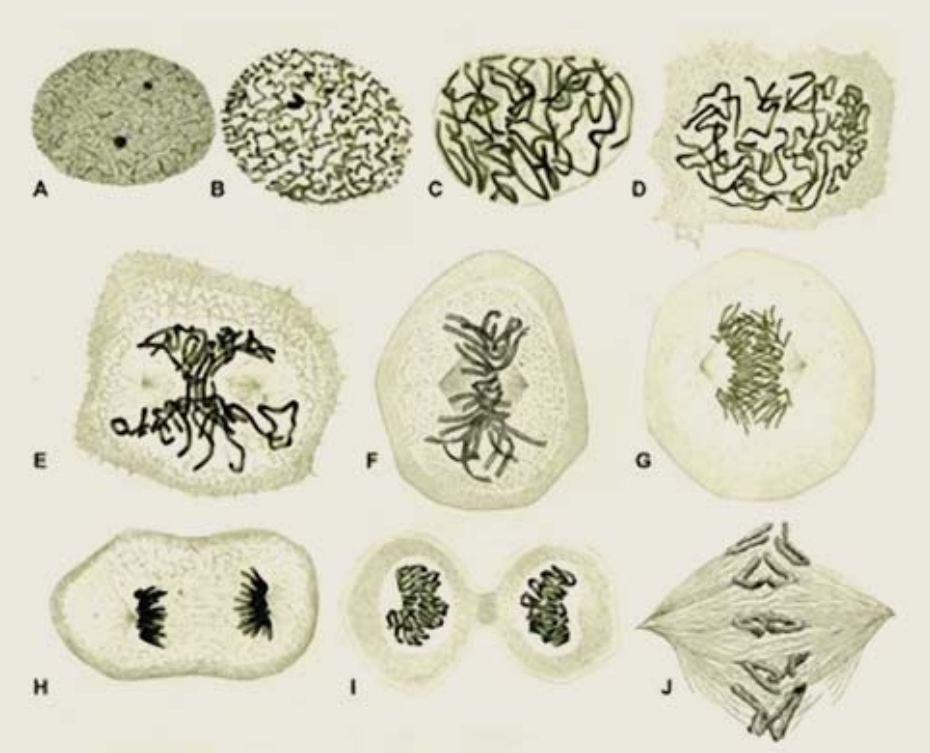
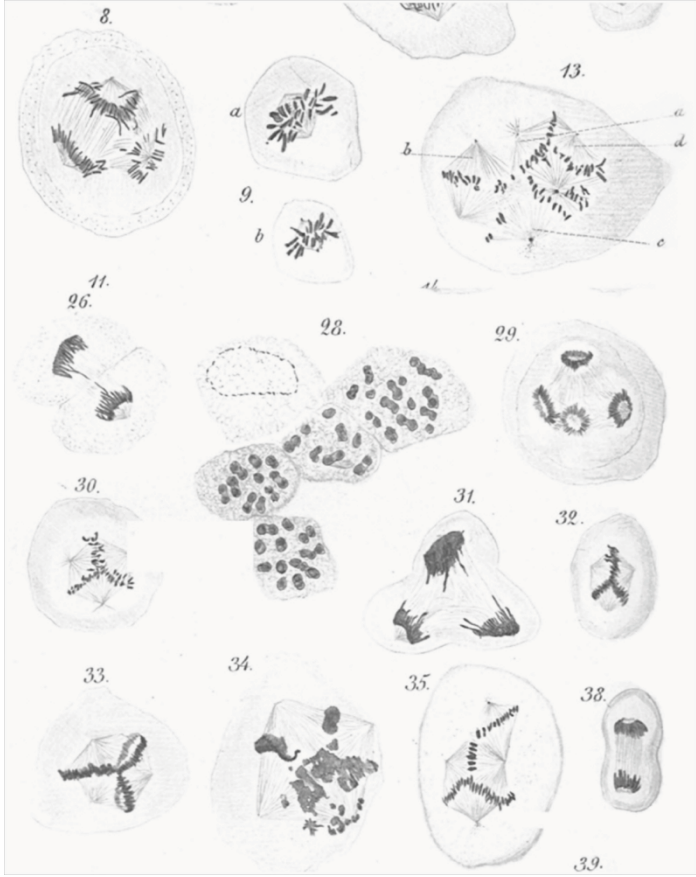
# Hypotheses to explain Cancer: Abnormal chromosome constitution

Chromosomes were discovered by Walther Flemming in 1877




Leo Hansemann in 1890 proposed that irregularities of the mitotic process are responsible for disordered growth…..




Leo Hansemann's drawings of abnormal mitoses in cancer tissue

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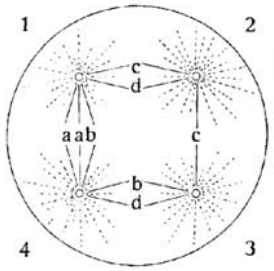
Hansemann D. Uber asymmetrische Zeilteilung in Epithelkerbsen und der biologische Bedeutung. Virch Arch [Pathol Anat] 1890;119:299-326.

# Hypotheses to explain Cancer: Abnormal chromosome constitution

Theodor Boveri, 1862-1915
(German zoologist)




Fg. A.

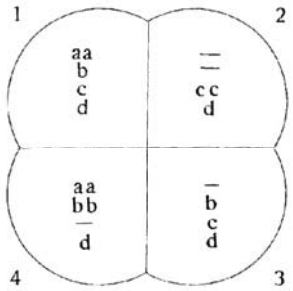



Fig. B.

| Chromosomes. | | Combinations in Gametes. | Combinations in Zygotes. |
|---|---|---|---|
| Somatic Series. | Reduced Series. | | |
| 2 | 1 | 2 | 4 |
| 4 | 2 | 4 | 16 |
| 6 | 3 | 8 | 64 |
| 8 | 4 | 16 | 256 |
| 10 | 5 | 32 | 1,024 |
| 12 | 6 | 64 | 4,096 |
| 14 | 7 | 128 | 16,384 |
| 16 | 8 | 256 | 65,536 |
| 18 | 9 | 512 | 262,144 |
| 20 | 10 | 1,024 | 1,048,576 |
| 22 | 11 | 2,048 | 4,194,304 |
| 24 | 12 | 4,096 | 16,777,216 |
| 26 | 13 | 8,192 | 67,108,864 |
| 28 | 14 | 16,384 | 268,435,456 |
| 30 | 15 | 32,768 | 1,073,741,824 |
| 32 | 16 | 65,536 | 4,294,967,296 |
| 34 | 17 | 131,072 | 17,179,869,184 |
| 36 | 18 | 262,144 | 68,719,476,736 |

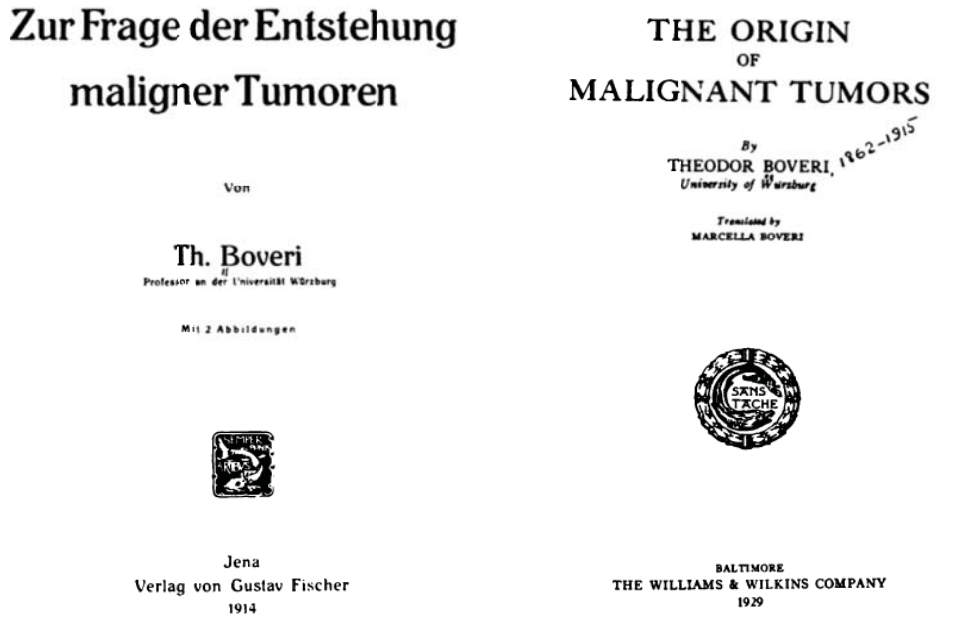
In 1902, Boveri found that only sea urchin embryos possessing the full set of 36 chromosomes could develop normally. A "***specific assortment of chromosomes is responsible for normal development and this can mean only that the individual chromosomes possess different qualities***."

Boveri also realised that the Mendelian concepts of segregation and assortment could be interpreted to operate on a cellular level, with chromosomes containing Mendel's so-called hereditary "factors". In 1903 he wrote that "*the characters dealt with in Mendelian experiments are truly connected to specific chromosomes*."

Drawing on von Hansemann's observations of abnormal mitotic figures in tumor cells and his own studies on sea urchin embryos undergoing abnormal mitotic divisions, Boveri proposed **that an abnormal chromosome constitution may promote cancer.**

# Boveri's theory for Cancer

Theodor Boveri, 1862-1915
(German zoologist)



Zur Frage der Entstehung maligner Tumoren

Von

Th. Boveri

Professor an der Universität Würzburg

Mit 2 Abbildungen

Jena
Verlag von Gustav Fischer
1914

THE ORIGIN OF MALIGNANT TUMORS

By
THEODOR BOVERI, 1862-1915
University of Würzburg

Translated by
MARCELLA BOVERI

SANS TACHE

BALTIMORE
THE WILLIAMS & WILKINS COMPANY
1929

FIG 3 *Boveri's monograph expounding the chromosome theory of neoplasia. Left: title page of German edition; right: title page of English translation.*

**Boveri's theory (1902, and translated in 1915):**

**(1) cancer is a cellular problem**
**(2) cancers originate from a single cell (…clonal evolution)**
**(3) this primordial cell has, as a result of an abnormal process, a wrongly combined "chromosome complex"**
**(4) the chromosomal abnormality which is passed on to all the descendants of the cell of origin is the cause of rapid cell proliferation.**

# Boveri's predictions for the causes of Cancer

FIG 2 *Portrait of Theodor Boveri, about 1909. (Courtesy of Dr Ulrich Wolf, Freiburg.[26])*

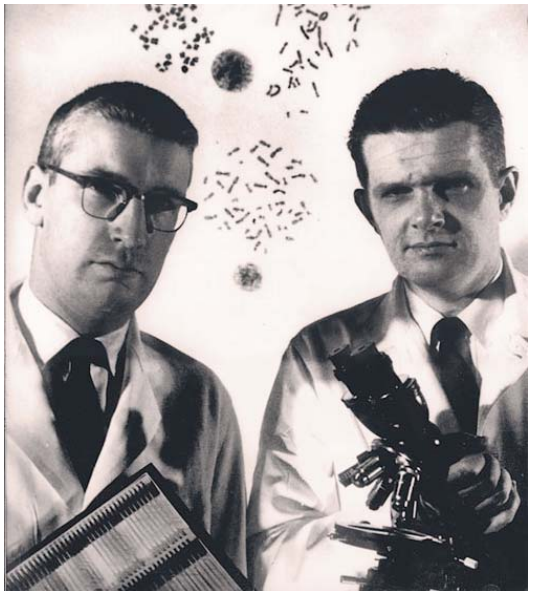
## Boveri's visionary predictions from his 1902 monograph:

- Cell-cycle checkpoints (*Hemmungseinrichtung*: inhibitory mechanism) that would allow cell division only when a specific external stimulus is experienced by the cell.
- Tumour-suppressor genes (*Teilungshemmende Chromosomen*), the effects of which can be overcome by external signals, and which are physically lost in progressively growing tumours.
- Oncogenes (*Teilungsfoerdernde Chromosomen*) that become amplified (*im permanenten Übergewicht*) during tumour development.
- Tumour progression from benign to malignant, involving sequential changes of increased growth-stimulatory chromosomes and loss of growth-inhibitory chromosomes.
- The clonal origin of tumours.
- Genetic mosaicism.
- Cancer predisposition through inheritance of chromosomes (genes) that are less able to suppress malignancy.
- Cancer predisposition through inheritance of genes that cause aberrant mitoses.
- Inheritance of the same 'weak chromosome' from both parents leads to homozygosity for the defective chromosome and, consequently, to high-penetrance cancer syndromes — for example, xeroderma pigmentosum.
- The role of wounding and inflammation in tumour promotion.
- Loss of cell adhesion in metastasis.
- Sensitivity of malignant cells to radiation therapy.

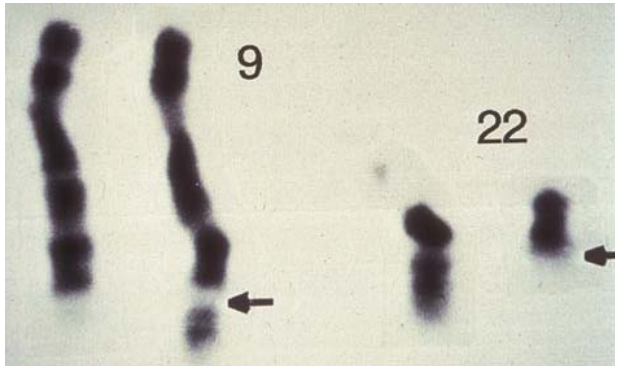
*From A. Balmain, Nat. Reviews Cancer (2001)*

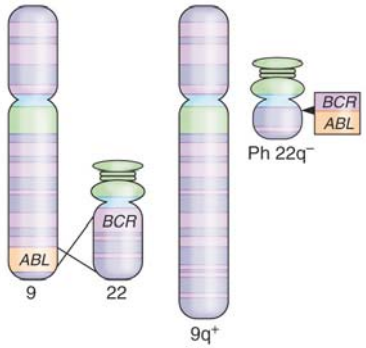
# Proof of Boveri's theory: The Philadelphia translocation

**Nowell and Hungerford (1960)** discovered strong evidence to support Boveri's hypothesis that a critical genetic alteration in a single cell, which provided the cell with a growth advantage, could give rise to a tumor (chronic myeloid leukemia CML – accounts for about 10% of all leukemias in US – *6600 new cases in 2015*).



Peter Nowell and
David Hungerford, 1960




First proof of a chromosome abnormality as a cause of cancer

Still not clear what causes it (exposure to high doses of radiation?)

Translocation leads to BCR-ABL fusion: the product of which is an abnormal kinase, stimulates proliferation of myeloid cells to produce CML

50 years later (2001) development of Gleevec, that can block the effects of this oncogene and stop progression of CML in 95% of patients.

# Epigenetic Hypotheses to explain Cancer

Since the time of Laennec, pathologists viewed cancer as acquiring properties of cells at **different developmental stages**, but **appearing inappropriately** in tumors.

**Embryonic cell remnants remain in developing organs following embryogenesis cause cancer** (Recamier, 1829, Remak, 1854, Cohnheim, 1875)

**An epigenetic process** (Waddington, 1935; Pierce DG, 1978; Harris H. 1988; Prehn RT. 1994; )

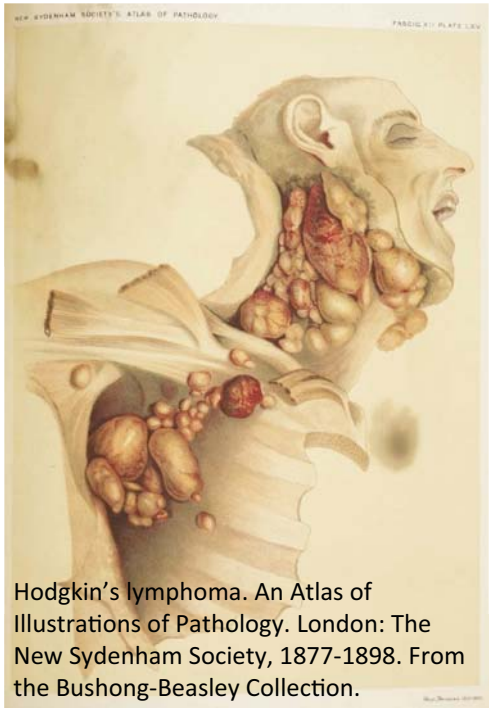
*Pierce GB et al. 1978. Cancer: A Problem of Developmental Biology*

Cancer and the Theory of Organisers

By C. H. Waddington, Christ's College, Cambridge

THE fundamental fact about cancerous tissue is that it has escaped from the normal growth-controlling agents of the body. The escape often involves a change in histological type. The problems which are raised are clearly connected with those studied in experimental embryology, where again it is the causal mechanism underlying growth and histological change which is under investigation. Experimental embryology has recently made important advances, and the time has perhaps come when it would be profitable to consider the way in which the new embryological theories would formulate the well-known problems of cancer research.

The illuminating researches of Spemann[1] provided the beginning of an answer to the outstanding embryological problem of why one part of an egg develops into one organ and another part into a different organ. Spemann showed that in the amphibian gastrula the developmental path followed by any given piece of tissue is defined by its relation to the blastopore region, which was therefore termed the organisation centre. Further research has shown that one facet of the activity



Hodgkin's lymphoma. An Atlas of Illustrations of Pathology. London: The New Sydenham Society, 1877-1898. From the Bushong-Beasley Collection.

cell to migrate or metastasize

"Normal gene activity is misprogrammed by epigenetic mechanisms to produce a neoplastic pattern of metabolism in which all of the individual components are normal."

**Cancer is a special expression of abnormal programming of gene function during cell differentiation**

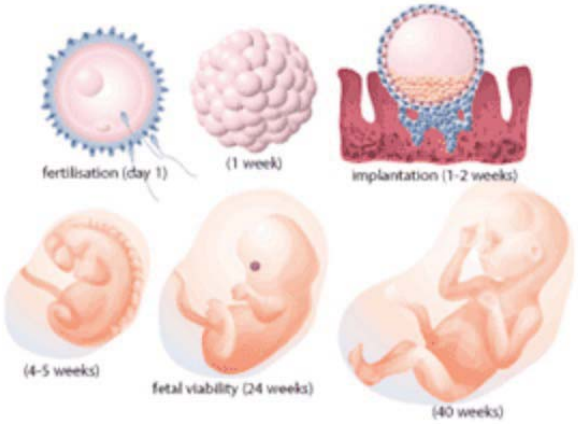
E.

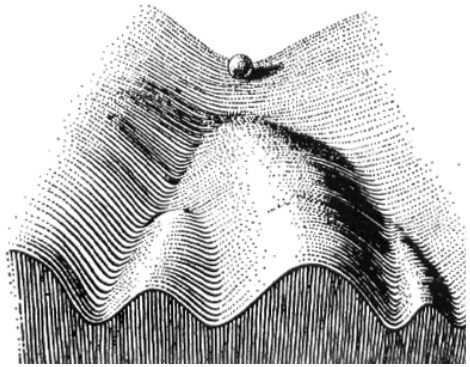
# Epigenetics

**Conrad Waddington (1942)**

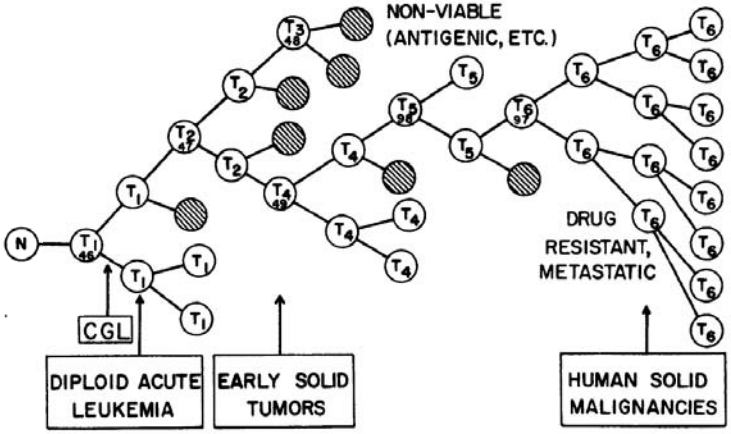
The study of the mechanisms of development through which genes bring about phenotypic effects.

*Epigenesis* (study of development) combined with *genetics* (the study of heredity)



*C.H. Waddington*




Tumor growth and "morphogenesis" (however disorganised) can be considered as a form of "epigenesis": ie growing complexity from a single cell, or clone of cells, to a complex "organism". (*Indeed - the same molecules and signaling pathways are exploited in cancer*)
However, in cancer, in addition to the changing phenotype there is also a changing genotype.

PC Nowell (1976) proposed that cancers evolve through branched evolutionary trajectories fuelled by genomic changes – as predicted by Boveri… => akin to a Darwinian process, tumor is an ecosystem and cells fight to survive… and proliferate.

# Epigenetics

**Conrad Waddington (1942)**

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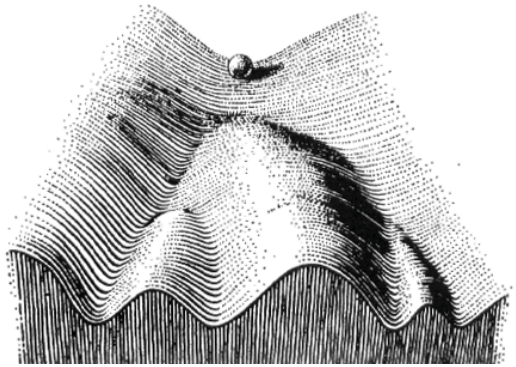


*C.H. Waddington*

**R. Holliday, A. Riggs and others (1970's onwards)**

Stable but reversible changes in gene activity, not due to DNA sequence differences.

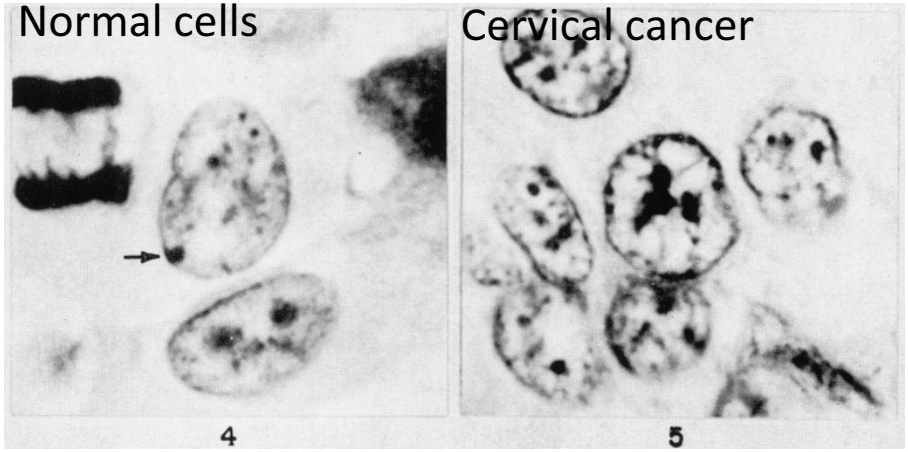
(i) constitutes an observable difference between 2 cells with the same genotype (DNA)

(ii) this difference must persist in the absence of the initial signal

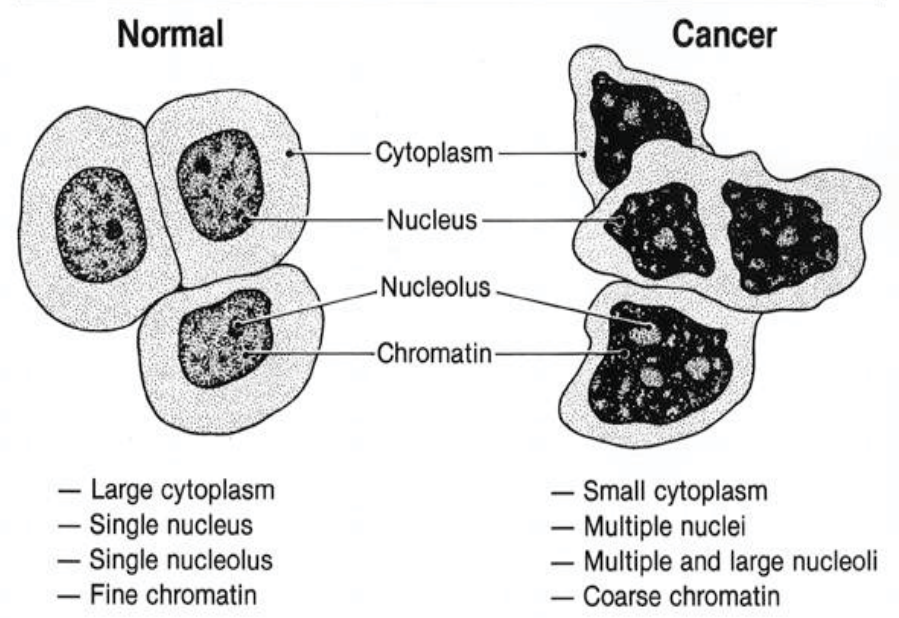
(iii) the difference must be heritable through mitotic division (or even meiosis).

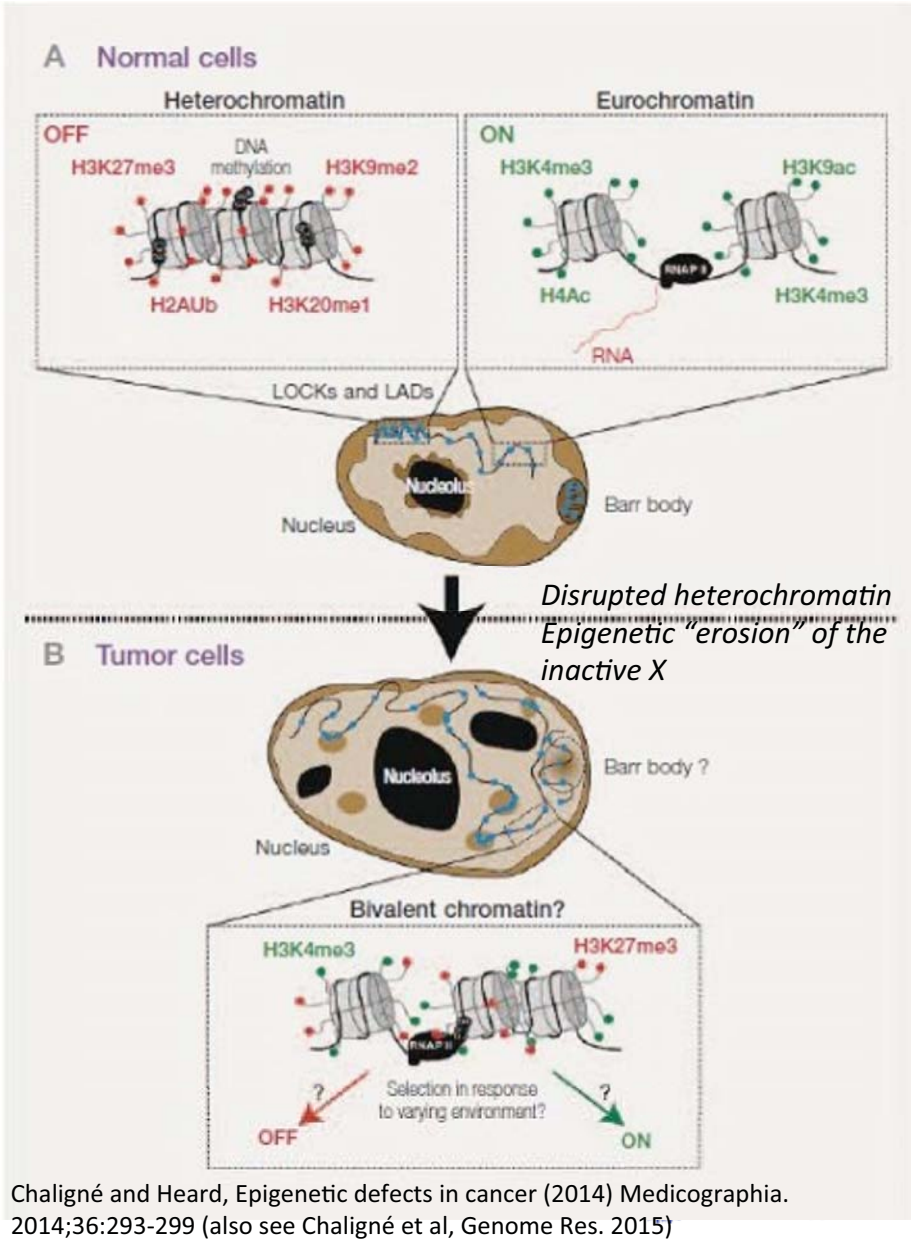
Studies on processes such as X inactivation, imprinting, paramutation, position effect variegation made the link between epigenetics and DNA methylation, chromatin and non-coding RNAs **(See COURS 2013+2015)**

# Disrupted chromatin organisation and aberrant epigenetic features in cancer cells – causes or consequences?




**The Sex Chromatin in Human Malignant Tissues**
K. L. Moore and M. L. Barr, **1957**

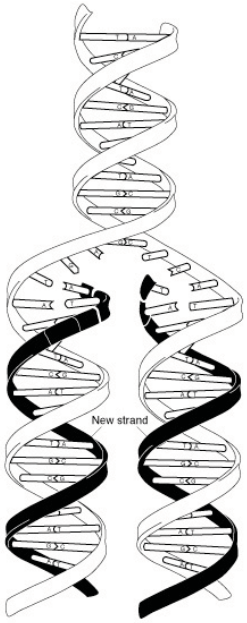



Chaligné and Heard, Epigenetic defects in cancer (2014) Medicographia. 2014;36:293-299 (also see Chaligné et al, Genome Res. 2015)

# Discovery of the Genetic Material and the Genetic Code

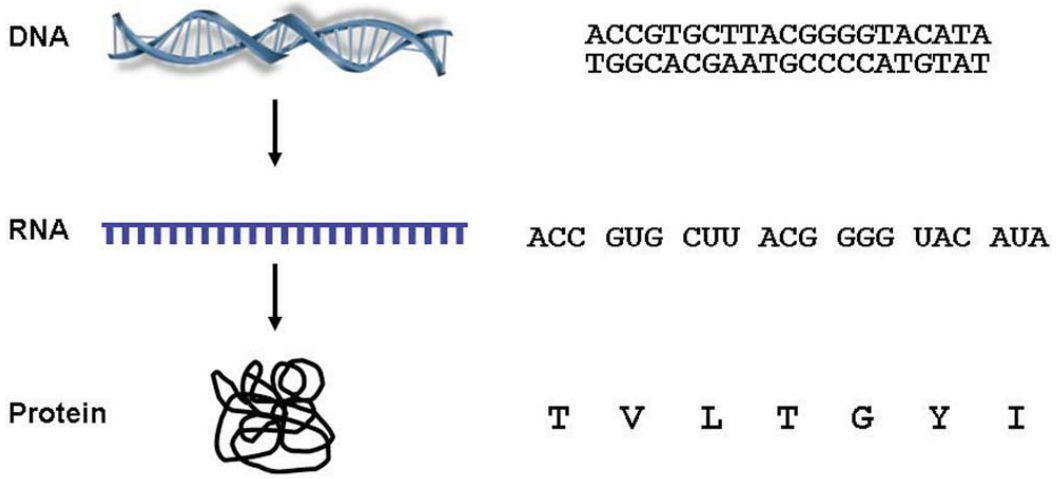
Oswald T. Avery, Colin MacLeod, and Maclyn McCarty (1944) demonstrated that **DNA** could function as the **genetic material**




**The structure of the double helix**

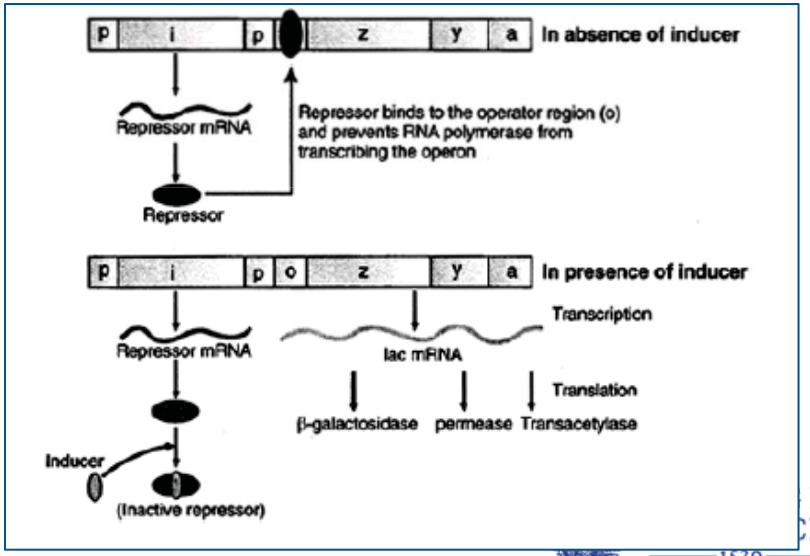
J. Watson and F. Crick (1953)

"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible **copying mechanism for the genetic material**."




## The Gene Regulation Paradigm in Prokaryotes

**The Lac operon (1960)**: Gene control relies on specific repressors and activators and the DNA sequence elements they recognize.



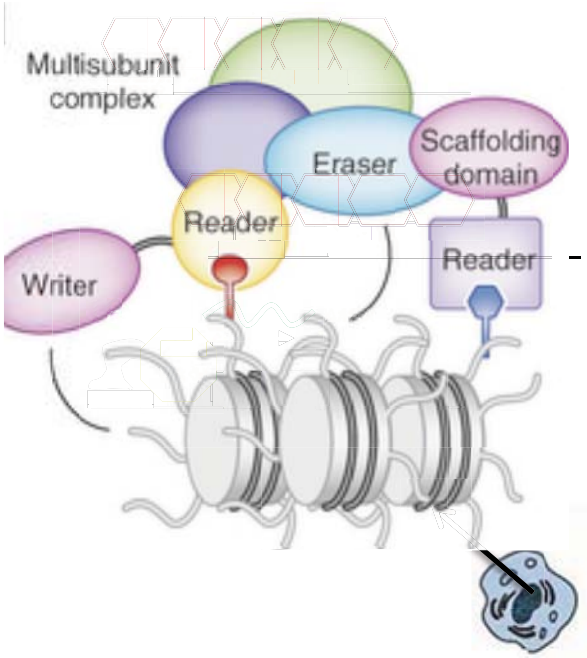
# Gene Regulation in Eukaryotes

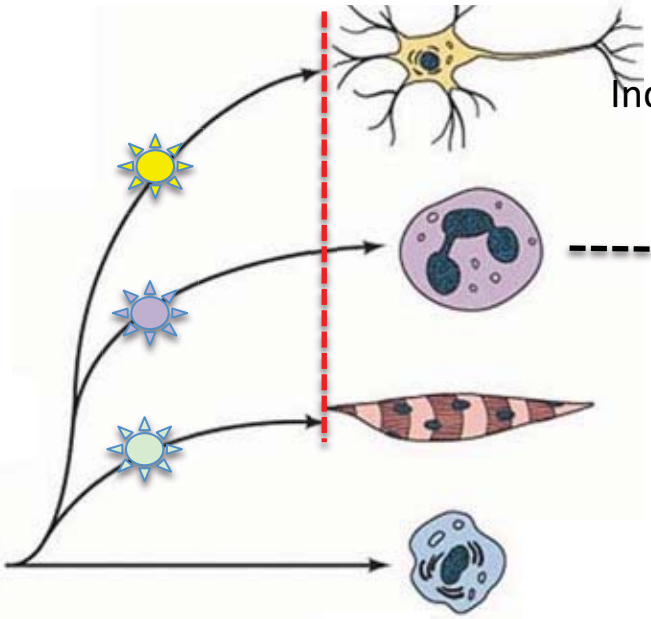
## Chromatin as a barrier and facilitator (COURS 2014, 2015)

## Epigenetics: memorisation of gene activity states

Heritable (somatic) states

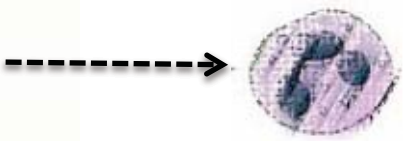
**(Epigenetic "Barriers")**



**Altered Epigenome**

Induced (eg environment, stress, damage)
or accidentally altered (eg aging)



**Phenotypic consequences?**

**In the context of cancer:**

**Genome instability**
**Loss or change in identity?**
**Uncontrolled cell growth?**
**Invasion, Metastasis?**

**One Genome**

**Multiple Epigenomes**

gene expression & chromatin states

**Different Cell Identities**

DNA sequence-specific Transcription Factors
& Signalling pathways

(positional information, cell-cell contacts, growth factors, etc
(to establish cell type, patterning, morphogenesis)

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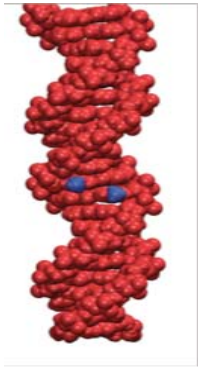
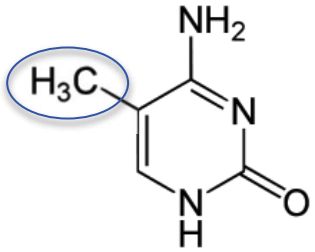
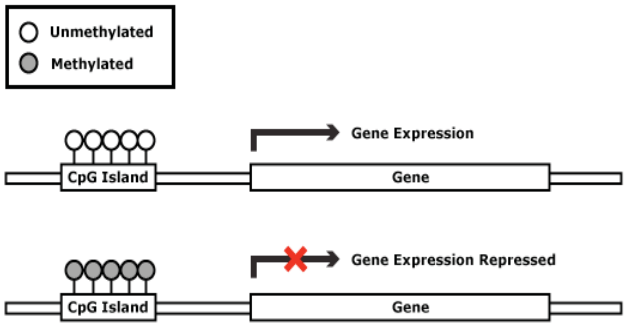
# DNA Methylation: the first "epigenetic modification" to be explored

Work of Bestor, Bird, Jones and others in 1990's discovered the enzymes that lay down and propagate DNA methylation; and the proteins that bind to methylated DNA **(COURS 2013)**






**In 1975, Robin Holliday and Art Riggs independently postulated that:**

**1.** DNA methylation might affect gene expression

**2.** Changes in DNA methylation could explain switching on & off of genes in development.

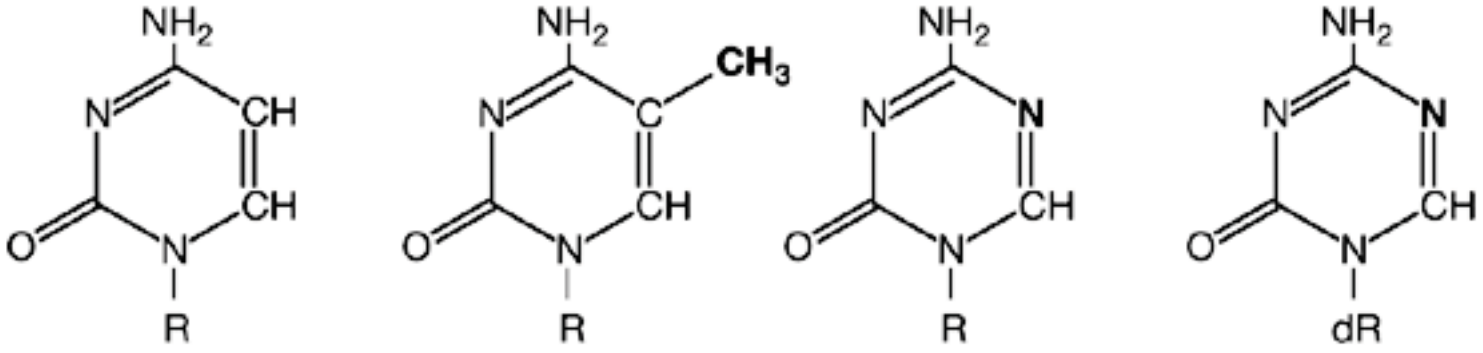
**3.** Predicted existence of enzyme(s) methylating a particular region of DNA – either by sequence specific binding, or via interaction with other sequence specific bound proteins

**4.** DNA methylation patterns could be heritable, if maintenance methylases existed that recognize hemi-methylated DNA soon after replication, but do not act on unmeth DNA

⇒ mechanism for heritability of the methylated and non-methylated DNA

⇒ heritability of a given pattern of gene activities

# Inhibition of DNA Methylation could affect gene expression




Advent of the DNA methylation inhibitor 5-azacytidine (Jones, 1984), one of the first drugs to be used to treat cancer

Data on cultured mammalian cells showed that gene expression could be affected by methylation, and loss of DNA methylation could lead to gene reactivation and a change in cell identity

⇒ The inactive expression state of a gene could be stably maintained by DNA methylation (Razin and Riggs,1980; Lock et al., 1987)

⇒ Robin Holliday went on to propose in 1987, that aberrant DNA methylation could sometimes lead to ***epimutations***, or event ***mutations***, for example in cancer…

⇒ **NEXT LECTURE**

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# Hypotheses to explain Cancer since the 19th Century

**Multiple Hypotheses**

- A problem of cell proliferation
- A problem of chromosome alterations
- An epigenetic process
- A problem of cell differentiation
- A problem of tissue organisation
- Exogenous chemicals
- Tumor viruses
- Altered chromosomes (and genes)
- Epigenetic alterations

# Chemical induction of Cancer

Links between environmental agents and cancer had been made for centuries (tobacco and snuff, observations by Percivall Pott (1714-1788) chimney sweeps (ramoneur) and cancer of the scrotum, smoking…)

In 1950 Wynders and Graham conducted one of the first case-control studies suggesting a link between tobacco smoking and lung cancer. Similar conclusion were reached by Richard Doll and Bradford Hill: concluded that a chemical in tobacco smoke caused lung cancer, but were unable to explain the mechanism.



Other carcinogenic agents included X-rays, UV light, viruses, pollutants, and many other chemicals, including benzene, other organic solvents, and arsenic.

***How* they participate in cancer was not (in some cases still not) clear!**

# Viral Hypothesis to explain Cancer

F. Peyton Rous (1879-1972)
(US pathologist & virologist)

Nobel Prize in Physiology or Medicine 1966 *"For his discovery of tumour-inducing viruses"*

In 1911, Rous had observed that a malignant tumor (a sarcoma) growing on a domestic chicken could be transferred to another fowl simply by exposing the healthy bird to a cell-free filtrate.
⇒ Cancer could be virally transmitted (Rous sarcoma virus, retrovirus),

Rous, Peyton (1910). "A Transmissible Avian Neoplasm (Sarcoma of the Common Fowl)". *Journal of Experimental Medicine* 12 (5): 696–705.

Rous, Peyton (1911) "A sarcoma of the FowL transmissible by an agent separable from the tumor cells". *Journal of Experimental Medicine* **13** (4): 397–411.

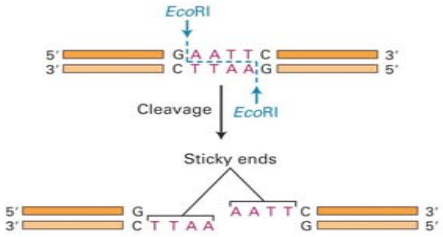
New field of tumor virology; led to discovery of further tumor viruses
Laid the foundations of **molecular mechanisms of carcinogenesis**.

In his Nobel lecture, Rous stated *"Despite protracted search, aided by the electron microscope, no tumor virus has ever been found in human milk, and family histories definitely rule it out."*

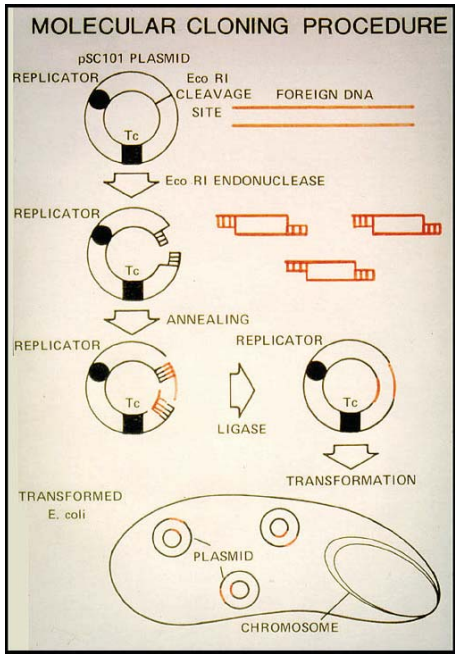
In humans, some tumor viruses exist - eg papilloma viruses discovered a couple of decades later.

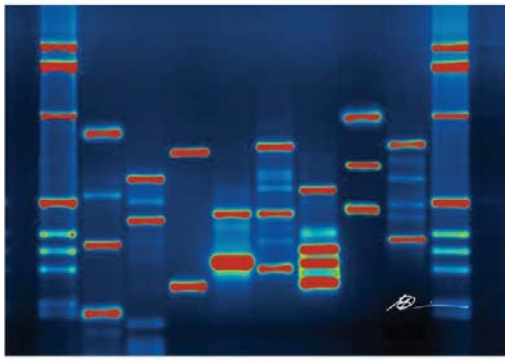
# The Golden Years of Molecular Biology…1960's, 70's, 80's,

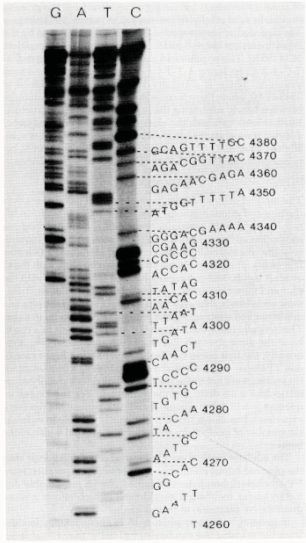
**A convergence of work by geneticists, physicists, and structural chemists tackling a common problem: the nature of inheritance.**




Fred Sanger (1918-2013)
British biochemist
Two Nobel Prizes for Chemistry
For protein (1958) & DNA (1980) sequencing



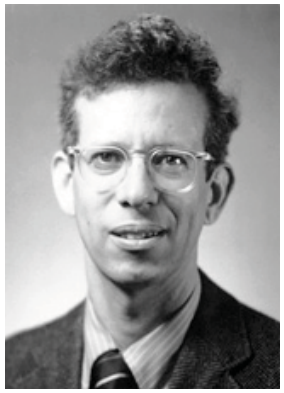






**Approaches to clone DNA, to analyse it (restriction enzymes, southern blots) and eventually to sequence it, rapidly progressed**

# From Tumor Viruses to the discovery of Reverse Transcriptase

David Baltimore



Howard M.Temin

Nobel Prize in Physiology or Medicine 1975 to Baltimore, Temin and Dulbecco: *"for their discoveries concerning the interaction between tumour viruses and the genetic material of the cell"*.

In 1958, Howard Temin and Harry Rubin developed a quantitative "plaque" assay for studying Rous sarcoma virus in tissue culture, taking advantage of the rapid and reproducible way in which RSV transformed cells.

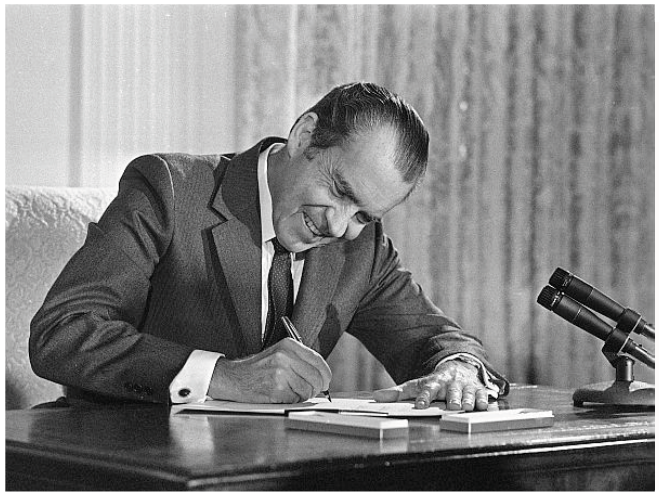
By the 1960s, the study of RNA tumor viruses had become a mature field encompassing both in vivo animal studies and tissue culture-based, bench-top experiments.

Temin went on to show that **RNA tumor viruses replicated through a DNA intermediate**, or *provirus*.

Temin and David Baltimore independently proved that virions of RNA tumor viruses contained **RNA-dependent DNA polymerase activity**: **Reverse-Transcriptase (RT)**
**=> RNA could make DNA ("challenge" to central dogma!)**

The discovery of RT was a watershed event in molecular biology, providing the means for generating cDNA and the key to reverse transcription–PCR (RT-PCR).
In recognition of their work, Temin and Baltimore received the Nobel Prize in 1975.

# U.S National Cancer Act in 1971



Nixon Signs $1.6 Billion Cancer Bill, Names Man to Head Fight

WASHINGTON (UPI)—President Nixon today signed into law a $1.6 billion program to find a cure for

the act was "a milestone in the long and difficult effort

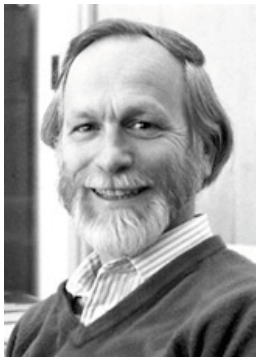
and cures of can

"This law is

On December 23, 1971, President Richard Nixon signed the National Cancer Act and declared "war on cancer." The act was meant to strengthen the National Cancer Institute, which was established in 1937.

*24 mars 2003 : Jacques Chirac lance le premier Plan de mobilisation nationale contre le cancer et en présente les 70 mesures….*

# Discovery of Oncogenes and Proto-oncogenes

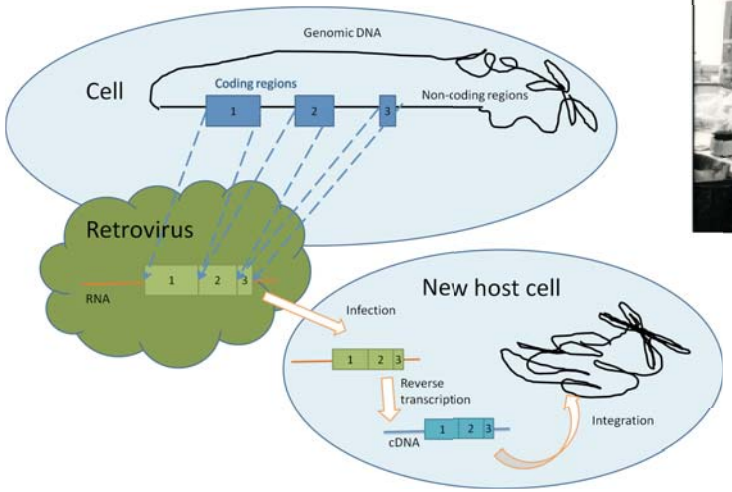


J. Michael Bishop

Harold E. Varmus

Nobel Prize in Physiology or Medicine 1989
*"for their discovery of the cellular origin of retroviral oncogenes"*

**DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA**
*Nature Vol. 260 March 11 1976*




Dominic Stehelin

In 1970, Duesberg and Vogt reported that the Rous Sarcoma Virus genome contained DNA sequences **not found** in the genomes of very closely related, **non-transforming** viruses

In 1976, Stehelin, Bishop and Varmus demonstrated that this gene (Src) existed in the untransformed genomes of many organisms (including humans).

⇒ The **cellular** gene they discovered (c-src) was highly similar to RSV v-src

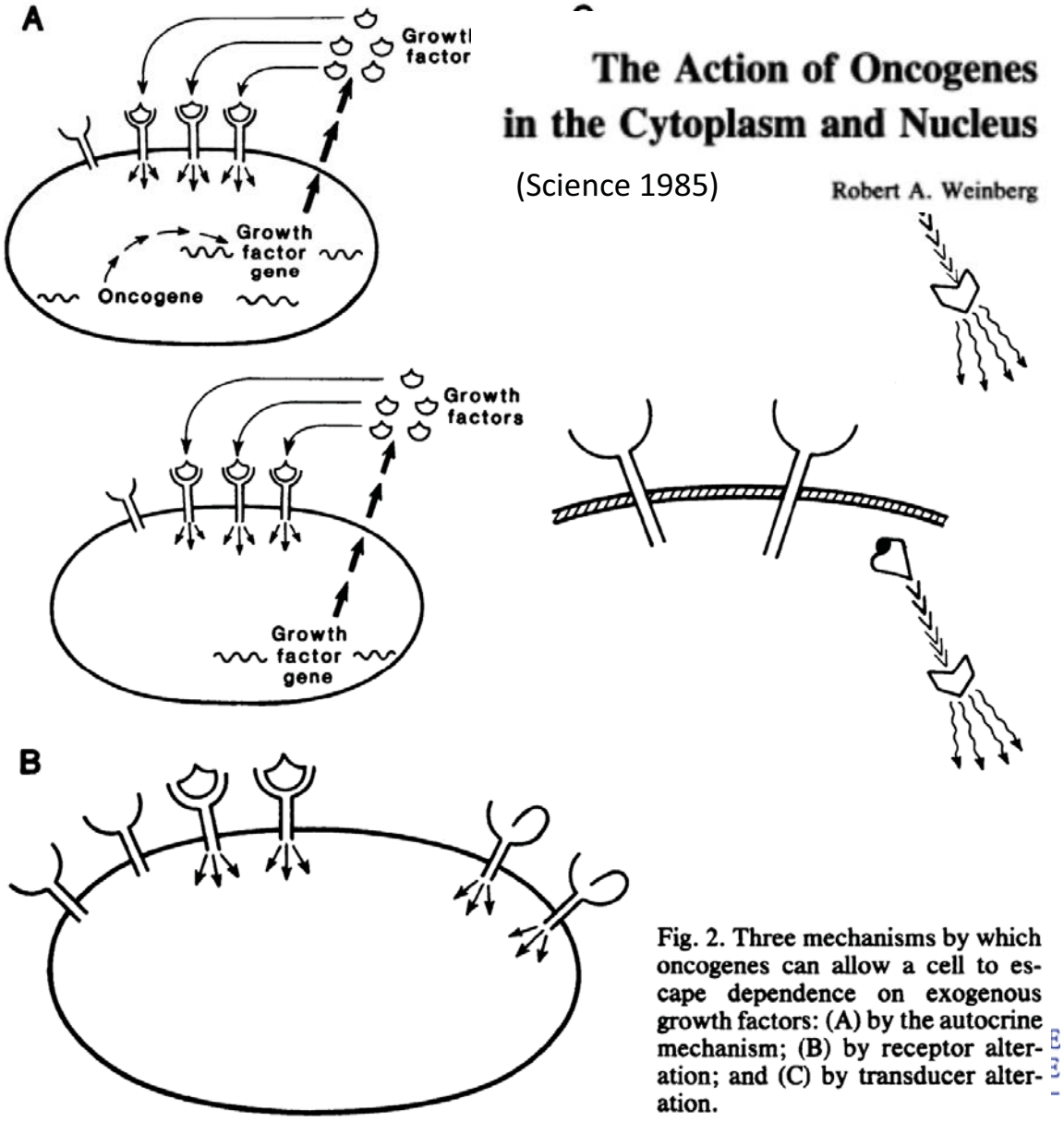
⇒ Thus the virus had obtained the gene sometime during replication in a different host to give rise to the *v*-src oncogene

⇒ Src is a **proto-oncogene**, which normally serves to activate cell division when the cell receives an appropriate signal; the mutant form (the **oncogene**) causes unrestrained activation of cell division...

E.

# Proto-oncogenes have a diversity of roles, controlling normal cell growth and division

| Nuclear | Cytoplasmic |
|---|---|
| *Viral oncogenes* | |
| SV40 large T | Polyoma middle T |
| Polyoma large T | |
| Adenovirus E1a | |
| *Cellular oncogenes* | |
| *myc, myb,* N-*myc* | *ras, src, erb*B, *neu,* |
| *p53, ski, fos* | *ros, fms, fes/fps, yes,* |
| | *mil/raf, mos, abl* |




The Action of Oncogenes in the Cytoplasm and Nucleus

(Science 1985)

Robert A. Weinberg

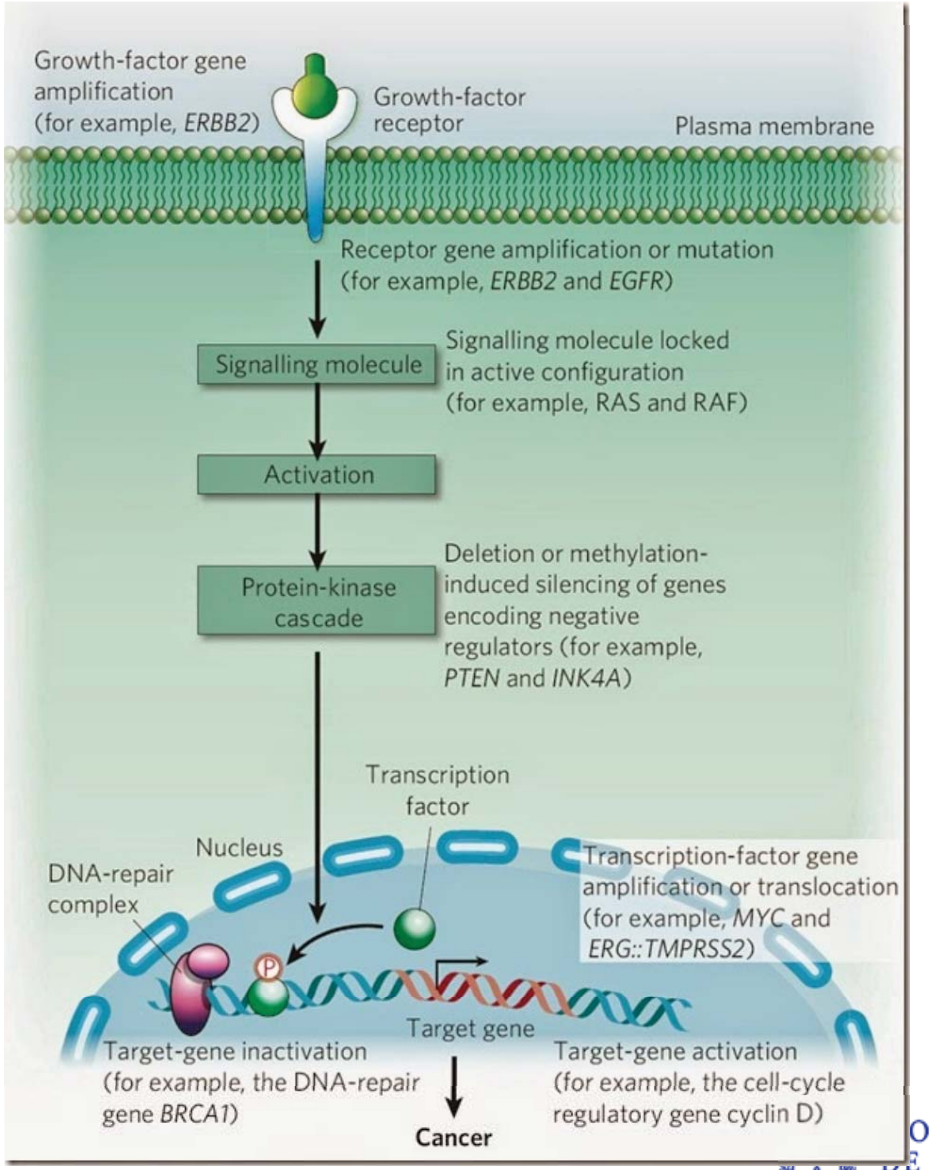
Fig. 2. Three mechanisms by which oncogenes can allow a cell to escape dependence on exogenous growth factors: (A) by the autocrine mechanism; (B) by receptor alteration; and (C) by transducer alteration.

# Proto-oncogenes have a diversity of roles, controlling normal cell growth and division

**Oncogenes can act to:**

- prevent cell cycle inhibition or overstimulate the cell cycle
- avoid cell death (apoptosis)
- prevent cell contact inhibition
- alter metabolism
- promote invasion

NB 1986 Nobel Prize for Physiology and Medicine: Discovery of Growth Factors by **Rita Levi-Montalcini** and **Stan Cohen.** Tumor secretion of diffusible factors cause growth-promoting effects, Levi-Montalcini (1946). Levi-Montalcini and Cohen identified the first growth factors and their involvement in tumour biology, paving the way for cancer therapies that target growth factor receptors.



# The era of oncogenes

**Transfected DNA** from a transformed cell could cause transformation in a normal cell (Shih et al, 1979)

**Proto-oncogenes** (point mutation in an endogenous proto-oncogene *HRAS* makes an oncogene (Tabin et al, 1982, Reddy et al; Taparowsky)

**Chemical mutagenesis** (mice) induces cancer through **mutation of Ras** proto-oncogene… (Balmain Pragnell 1983; Sukumar et al, 1983)

**Altered proto-oncogene expression or function can turn them into an oncogenes….**

**However one oncogene was not enough in mammalian cells - combinations of multiple oncogenes required to transfrom normal cells into "tumor" cells…**Land H, Parada LF, Weinberg RA. 1983. Nature 304:596–602

⇒ **Could all tumors simply be caused by a few activating mutations – dominant oncogenes?**

*Predictions that Tumor Suppressors must also exist… ie that cancer could arise through the inactivation of recessive genes, that normally block cell growth*

# Predicting the existence of tumor suppressors

## A General Theory of Carcinogenesis

**(genes/viruses)**

DAVID E. COMINGS

Department of Medical Genetics, City of Hope National Medical Center,

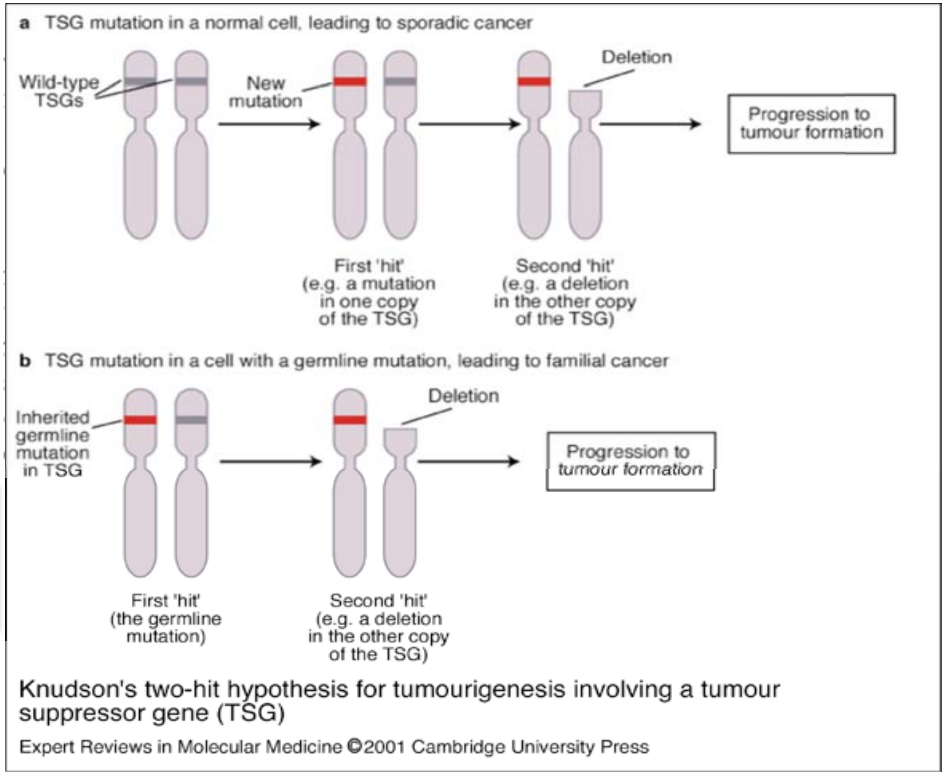
*Communicated by James V. Neel, July 26, 1973*

**ABSTRACT** **A general hypothesis of carcinogenesis is proposed consisting of the following features: (*1*) It is suggested that all cells possess multiple structural genes (*Tr*) capable of coding for transforming factors which can release the cell from its normal constraints on growth. (*2*) In adult cells they are suppressed by diploid pairs of regulatory genes and some of the transforming genes are tissue specific. (*3*) The *Tr* loci are temporarily activated at some stage of embryogenesis and possibly during some stage of the cell cycle in adult cells. (*4*) Spontaneous tumors, or tumors induced by chemicals or radiation, arise as the result of a double mutation of any set of regulatory genes releasing the suppression of the corresponding *Tr* genes and leading to transformation of the cell. (*5*) Autosomal dominant hereditary tumors, such as retinoblastoma, are the result of germ-line inheritance of one inactive regulatory gene. Subsequent somatic mutation of the other regulatory gene leads to tumor formation. (*6*) The Philadelphia chromosome produces inactivation of one regulatory gene by position effect. A somatic mutation of the other leads to chronic myelogenous leukemia. (*7*) Oncogenic viruses evolved by the extraction of host *Tr* genes with their conversion to viral transforming genes. As a result, in addition to the above mechanisms, tumors may also be produced by the reintroduction of these genes into susceptible host cells.**

## Heredity and Human Cancer

Alfred G. Knudson Jr, MD

**Based on his epidemeliogical studies** "*Mutation and cancer: statistical study of retinoblastoma*"
Knudson, PNAS 1971.




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



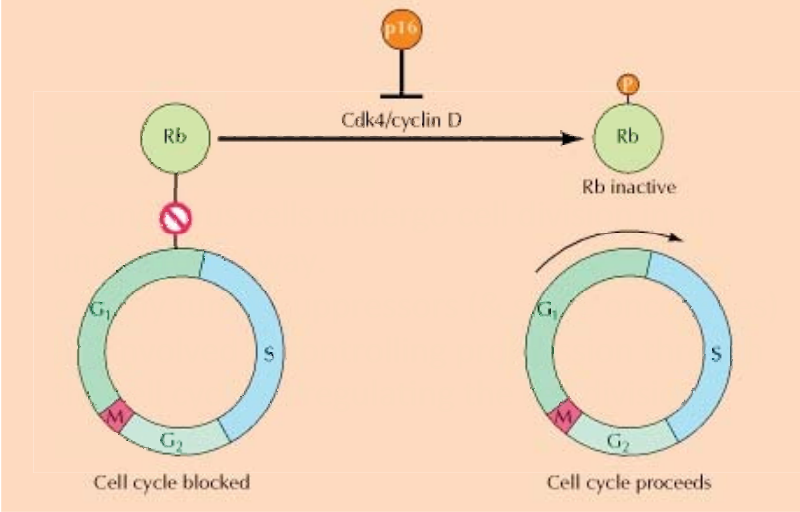
For historical perspective, see Kern et al 2002 "*Whose hypothesis? Ciphering, sectorials, D lesions, freckles and the operation of Stigler's Law*."

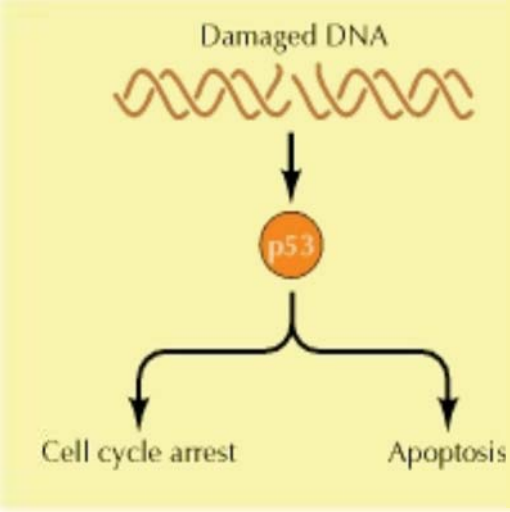
# Discovery of tumor suppressors (1980s)

• Studies of familial, **hereditary** cancers (~5% of all human cancers) revealed that the DNA defects transmitted through the germline were due to deletions in specific genes => loss of function

• Retinoblastoma gene (Rb) as 1st "tumor suppressor" gene (Friend et al, 1986) – subsequently found to be involved in regulation of cell division => *Mutations affecting cell cycle regulatory genes became a major research focus*

• p53 tumor suppressor: regulates both cell cycle progression and apoptosis. Hereditary p53 gene mutations can lead to Li-Fraumeni syndrome (LFS), increased risk of developing various types of cancers. Homozygous loss of p53 is extremely frequent in many cancers




Cells that are defective in p53 can still divide when they have DNA damage.

Cells lacking p53 fail to undergo apoptosis in response to agents that damage DNA

Loss of p53 interferes with apoptosis induced by other stimuli, such as growth factor deprivation and oxygen deprivation.

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• p53 tumor suppressor: Hereditary p53 gene mutations can lead to Li-Fraumeni syndrome (LFS), increase risk of developing various types of cancers. Homozygous loss of p53 is frequent in many cancers

• PTEN acts by opposing the action of PI3K, which is essential for anti-apoptotic, pro-tumorogenic Akt activation.

• BRCA proteins are multitasking: involved in DNA damage repair, regulation of gene expression (eg *p53*, and its target gene *p21) => Associated with genetic cancer syndromes (BRCA1 in 1994)*

Other examples of tumor suppressors include pVHL, APC, CD95, ST5, YPEL3, ST7, and ST14…

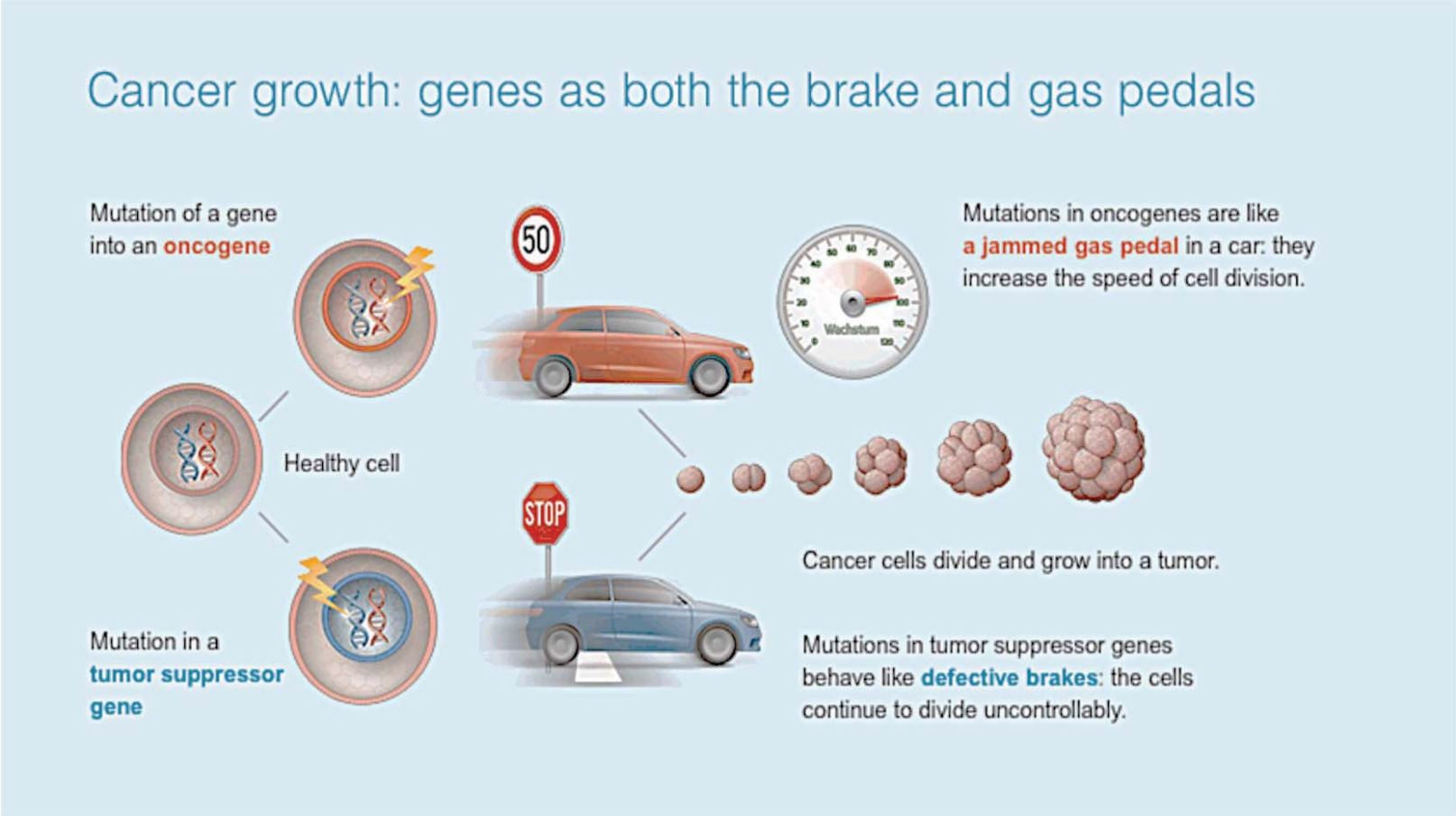
**Tumor suppressors were quickly shown to function in many key cellular processes (in tissue culture) including the regulation of transcription, DNA repair, cell-cell communication… Loss of function of these genes leads to abnormal cellular behaviour.**

**However, *in vivo* precise roles of TS genes was less easy to define**

# Can genetic alterations in proto-oncogenes & tumor suppressor genes explain cancer?

Most cancers were found to show alterations in one or more TS and oncogenes.
In normal cells, these two groups of proteins work together to regulate cell division but in cancer cells the controls are no longer functioning properly.

**Oncogenes drive cancer; tumor suppressors prevent it…**



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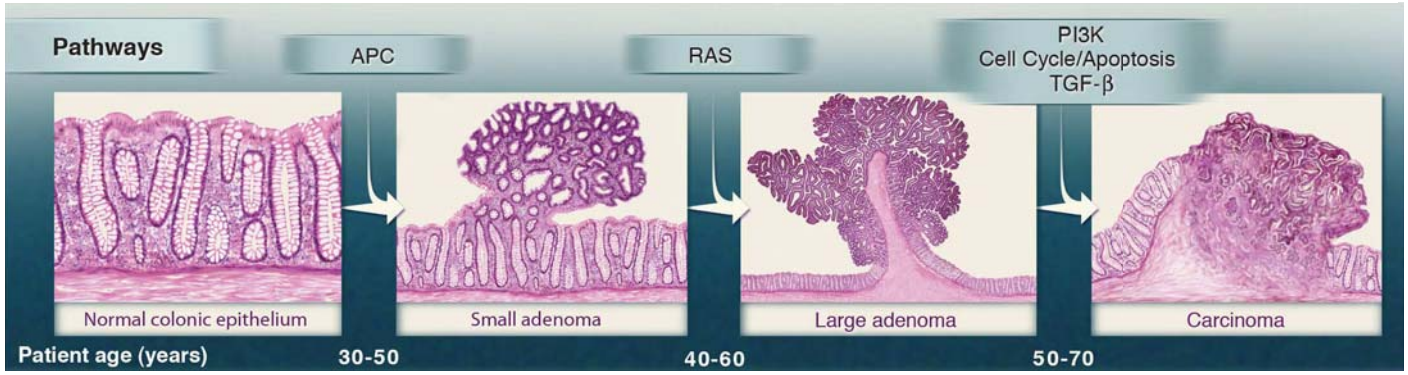
**Oncogenes drive cancer; tumor suppressors prevent it…**

Colorectal cancer: *APC* 1st mutation "flowchart" of events (Fearon,Vogelstein, 1990, 1991)
Mutations of the *APC* (adenomatous polyposis coli) gene are strongly associated with both inherited and sporadic cases of colon cancer. APC, like many tumor suppressors, functions to control the expression of genes critical in the cell division process

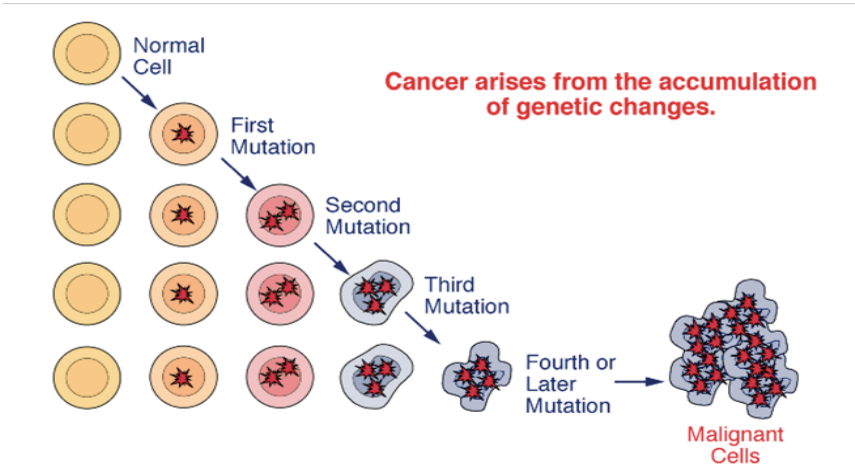



- A series of mutations in a cell causes it to proliferate more than its immediate neighbors.
- As the cluster of dividing cells grows over time, further mutations turn atypical hyperplasia into a cancer (carcinoma).
- The spreading of cancer cells to other tissues and organs (metastasis) occurs when the adhesion of these cancerous cells breaks down, and they are able to travel easily to new locations.

**Is it really that simple?**

# Somatic mutation model for the basis of cancer

The prevailing model for cancer development was that **mutations** in genes for tumor suppressors and oncogenes lead to cancer.




**In most cases:**

*"Each tumor seemed a unique experiment of nature – acquiring a unique set of mutant genes in an unpredictable chronological order…" (R. Weinberg, Cell 2014)*

**Was this view too simple?**

- Different cancers seem to involve very different sets of genes (except for specific hematological cancers)
- Somatic mutation rates could not explain the rapid evolution of many tumors
- Did not adequately explain the many chromosomal aberrations typical of cancer cells
- Failed to explain the genetic diversity among cells within a single tumor
- Does not explain frequent resistance to therapies

**Alternative Models:** Master genes controlling cell division? Chromosomal catastrophic events ("big leaps")? Epigenetic models – epimutations and/or global epigenetic changes?

*New insights from whole genome and epigenome sequencing…***Next Week!**

# Epigenetic Models for Cancer

## The Inheritance of Epigenetic Defects

ROBIN HOLLIDAY

Many rodent cell lines that lack particular enzymes or proteins are very stable and appear at first sight to have classical mutations in structural genes. However, 5-aza-CR treatments can induce massive reactivation of such genes, with as many as 10 to 30% of the survivors recovering enzyme activity, which represents about a millionfold increase over the spontaneous reversion rate. Mutagens or agents that damage chromosomes do not have these effects, and 5-aza-CR is itself only a weak mutagen in mammalian cells (*20*). Silent genes that have been reactivated by 5-aza-CR are listed in Table 1 (*21–38*). These results provide strong evidence that genes are often inactivated in permanent cell lines by methylation, that the methylation is very stably inherited, and that 5-aza-CR results in demethylation of sites important for the control of gene activity. In several cases this has been confirmed by examination of the gene in question by means of methylation-sensitive restriction enzymes (Table 1, *21–38*).

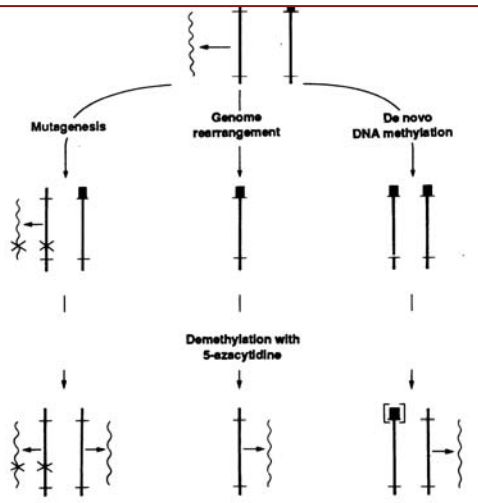
*Br. J. Cancer* (1979) **40**, 513

A NEW THEORY OF CARCINOGENESIS*

R. HOLLIDAY

*From the National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA*

Received 29 March 1979 Accepted 7 June 1979




- Holliday proposed that heritable changes in gene activity could be due to DNA Methylation and that de novo methylation and 5-azaC treatment act to produce forward and reverse “Epimutations” in cell lines
- If DNA methylation is essential for the normal controls of gene activity during development, it follows that defects in methylation may have severe phenotypic consequences in diploid somatic cells.
- DNA me could act either by “shutting off” one or both alleles - or by inducing mutation
- Holliday favored the hypothesis that global DNA hypomethylation in cell lines could lead to mutation – such as chromosome rearrangements

Quite visionary as now we know that DNA me is key for controlling repeat elements – and unleashing of repeats may underlie chromosome instability -- at centromeres and also other repeats elements

# Epigenetic Models for Cancer

## Evidence for DNA me changes in cancer (pre-genome wide technologies)

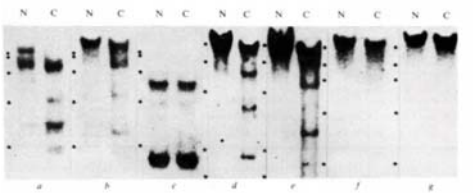
**Global DNA hypomethylation** in cancer cell lines (Dilala and Hoffman, 1982; Ehrlich, 1982)

**Local DNA hypomethylation at some oncogenes** – eg Ras (Feinberg and Vogelstein, 1983)

**DNA hypermethylation of CpG islands of tumor suppressor genes?** (Jones and Baylin, 2002)

**Hypomethylation distinguishes genes of some human cancers from their normal counterparts**

**Andrew P. Feinberg & Bert Vogelstein**
Cell Structure and Function Laboratory, The Oncology Center, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA



*Am. J. Hum. Genet. 48:880–888, 1991*

**Allele-specific Hypermethylation of the Retinoblastoma Tumor-suppressor Gene**

Toshiyuki Sakai, Junya Toguchida, Naoko Ohtani, David W. Yandell, Joyce M. Rapaport, and Thaddeus P. Dryja

Howe Laboratory of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston

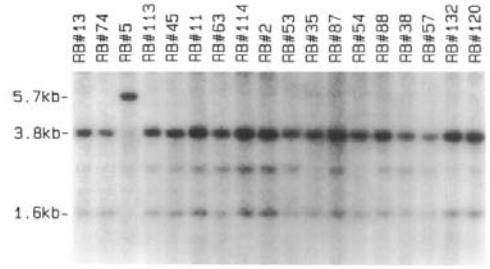



**Figure 2** Screening for hypermethylation in retinoblastomas. This figure shows the results of digesting 18 tumors with SacI and SacII and probing with p123M1.8. Tumor 5 has an aberrant 5.7-kb fragment, as expected if SacII restriction sites were methylated.



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# Epigenetic Models for Cancer

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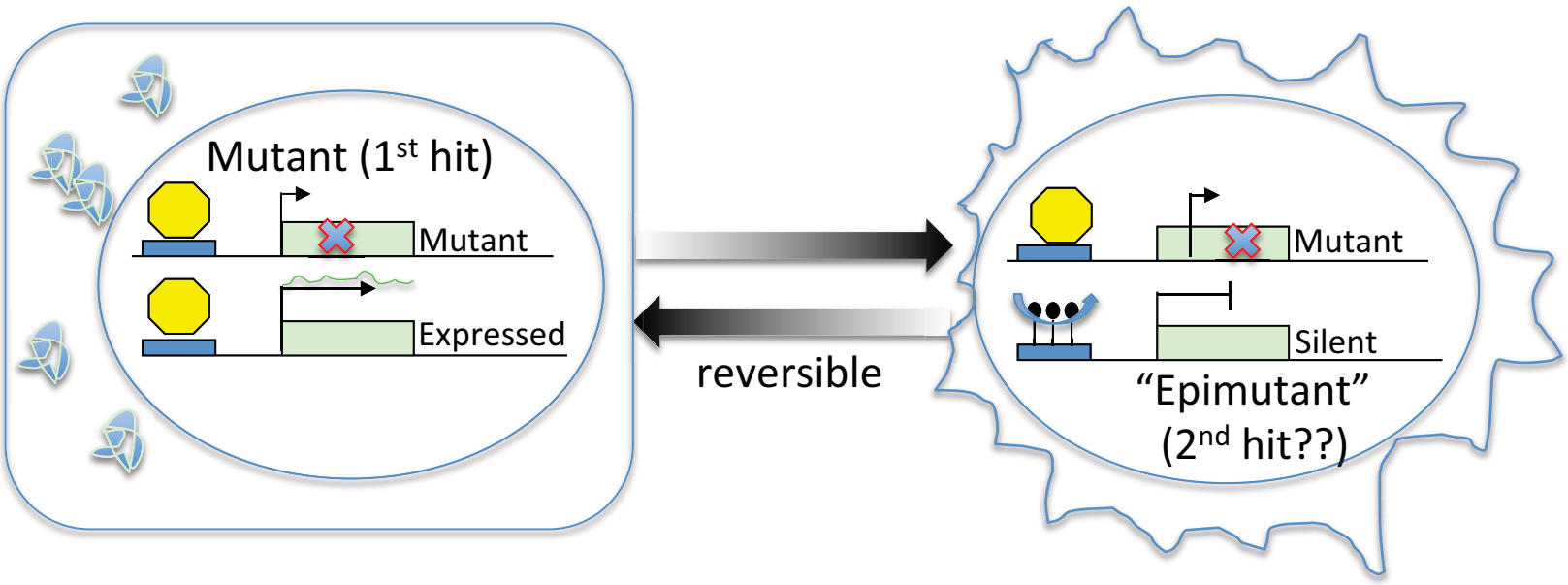
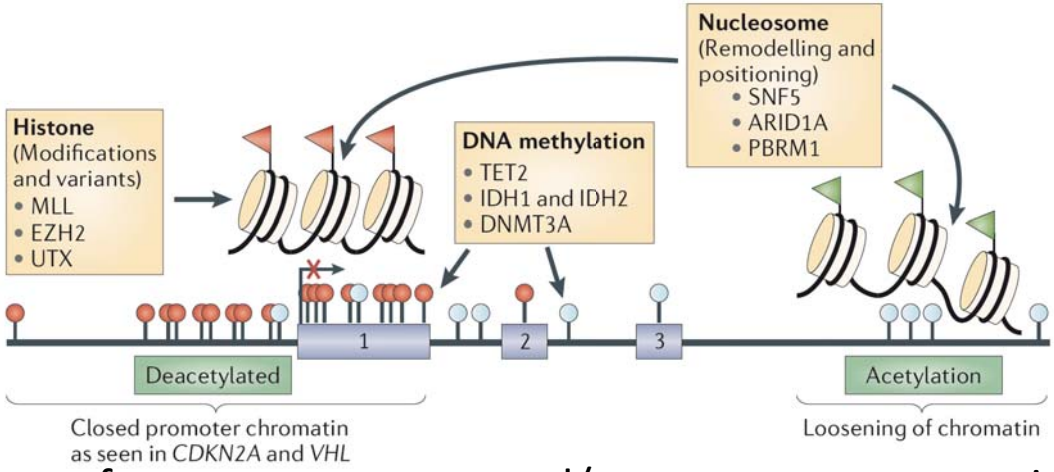
**DNA hypermethylation of CpG islands of multiple tumor suppressor genes** (Jones and Baylin, 2002)



# Epigenetic Models for Cancer

- Both genetic and epigenetic views ultimately involve ***abnormal gene expression***.
- The expression state of a gene is determined by presence of TFs and chromatin modifying enzymes, and the packaging of its DNA regulatory landscape.




- DNA mutations of tumor suppressors and/or oncogenes causes either loss or gain of function and abnormal expression.
- Epigenetic pathway to cancer is more complex – chromatin structure, DNA me, histone variants and modifications, nucleosome remodeling…Key aspect is potential ***plasticity***
- The epigenome undergoes multiple alterations in cancer: genome-wide loss of DNA methylation, frequent increases in promoter methylation of CpG islands, changes in nucleosome occupancy, and histone modification profiles.

**How much of this is involved in early events – in "stem" or "progenitor" cells – or is just a consequence, and/or is involved in later events?** **NEXT LECTURES!**

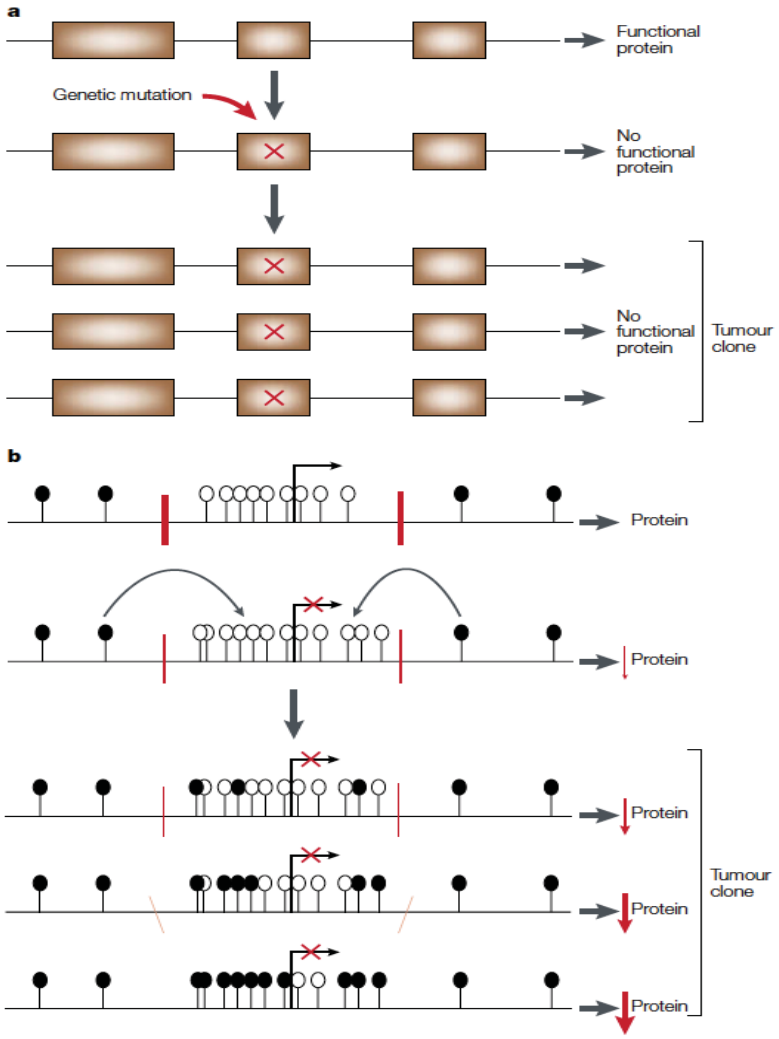
# Epigenetic Models for Cancer

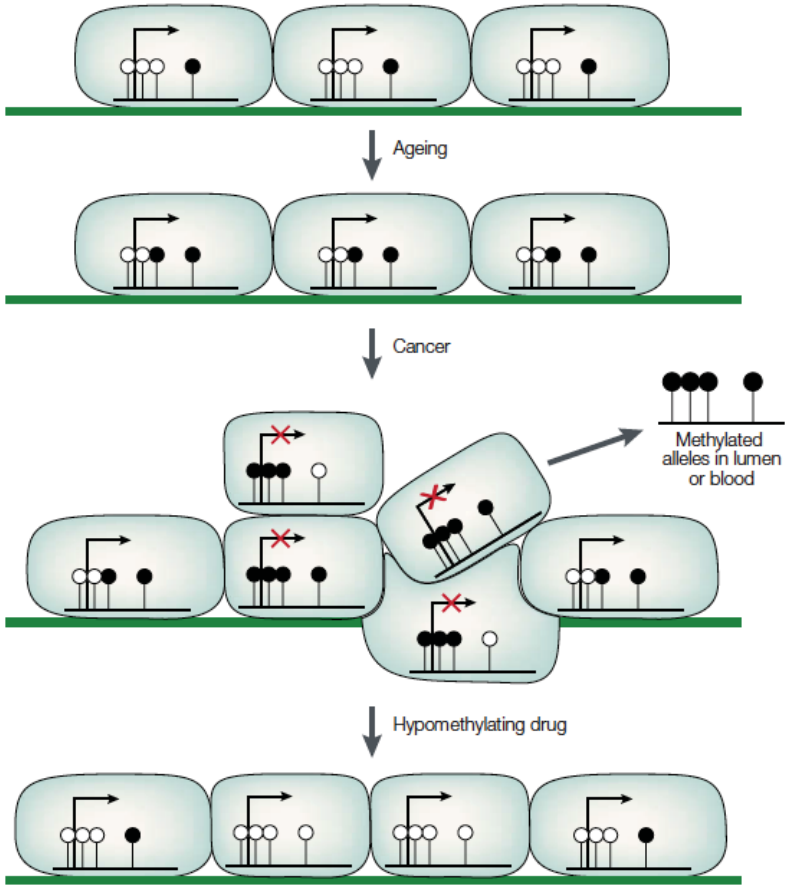
Most genetic changes lead to all/nothing gene expression change

Epigenetic changes can lead to range of expression levels: help explain tumor heterogeneity?

Dosage may also be a key aspect for some proteins in selective advantage in cancer

Epimutations also useful as biomarkers and as therapeutic targets?

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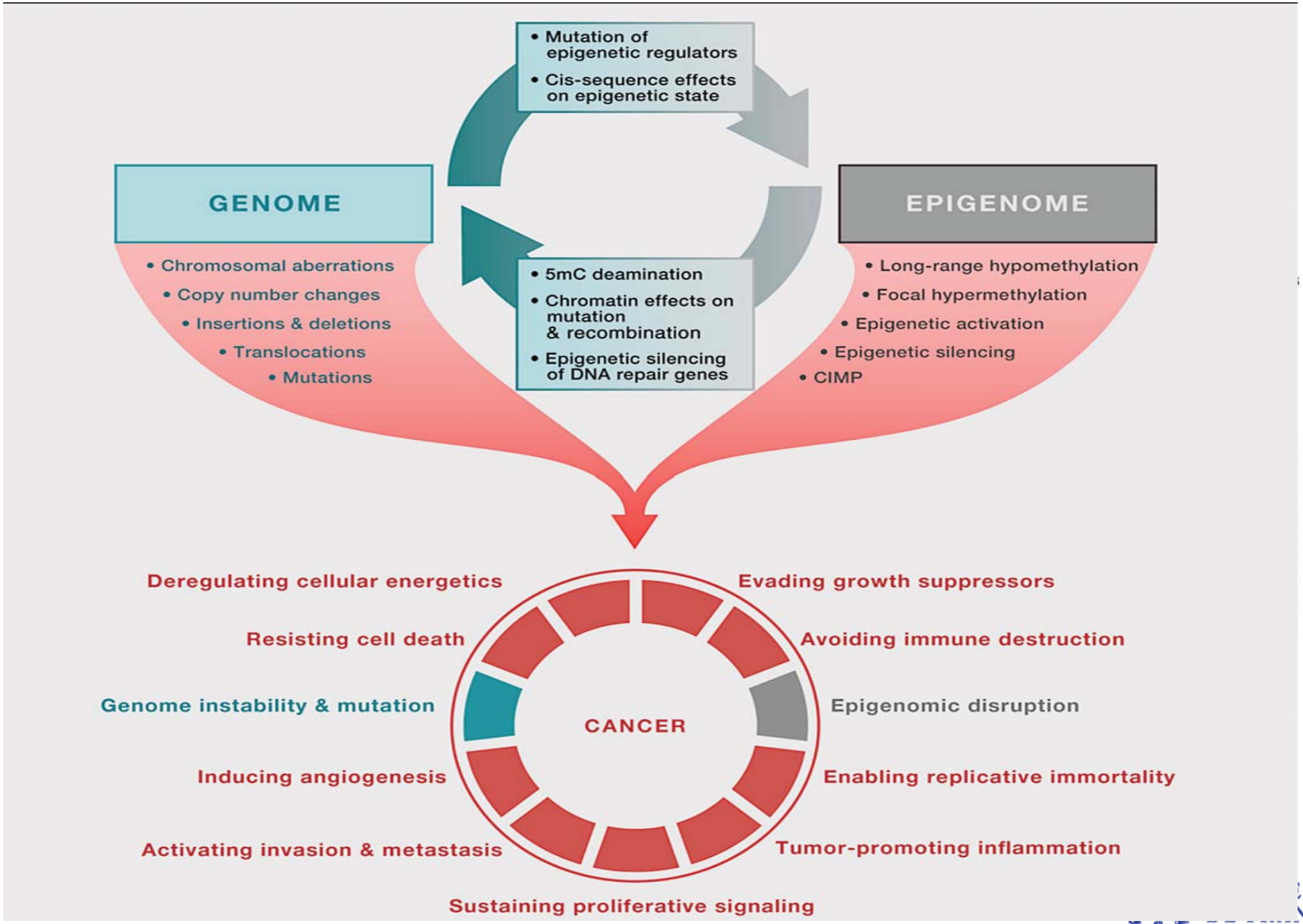
# Understanding Cancer: from an Epigenetics perspective

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# Epigenetics and Cancer

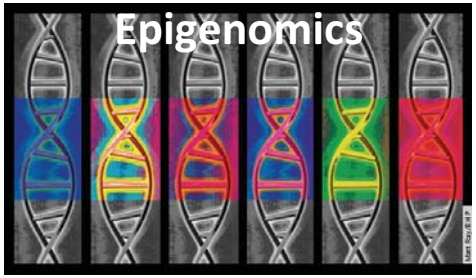
1. Are epigenetic changes simply a consequence of gene expression changes due to DNA sequence mutations and genomic instability in cancer?

2. Or might epigenetic changes *contribute* to cancer, by causing stable (potentially reversible) alterations in gene expression? (somatic – or even germ line?)

3. Can epigenetic changes induce mutations in cancer – cytosine deamination, or loss of repetitive element control, or aberrant silencing of DNA repair genes.

4. Can epigenetic changes contribute to tumor cell heterogeneity, and to the plasticity underlying phenotypic changes eg during invasion or metastasis

5. How can a global knowledge of the epigenetic characteristics of cancer cells be used for translational purposes (diagnostic, prognostic, therapeutic…)?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# Cours II

Lundi, 7 mars, 2016
16h à 17h30

## La génomique et l'épigénomique des cancers : de la description aux mécanismes

"Cancer Genomes and Epigenomics: from maps to mechanisms"