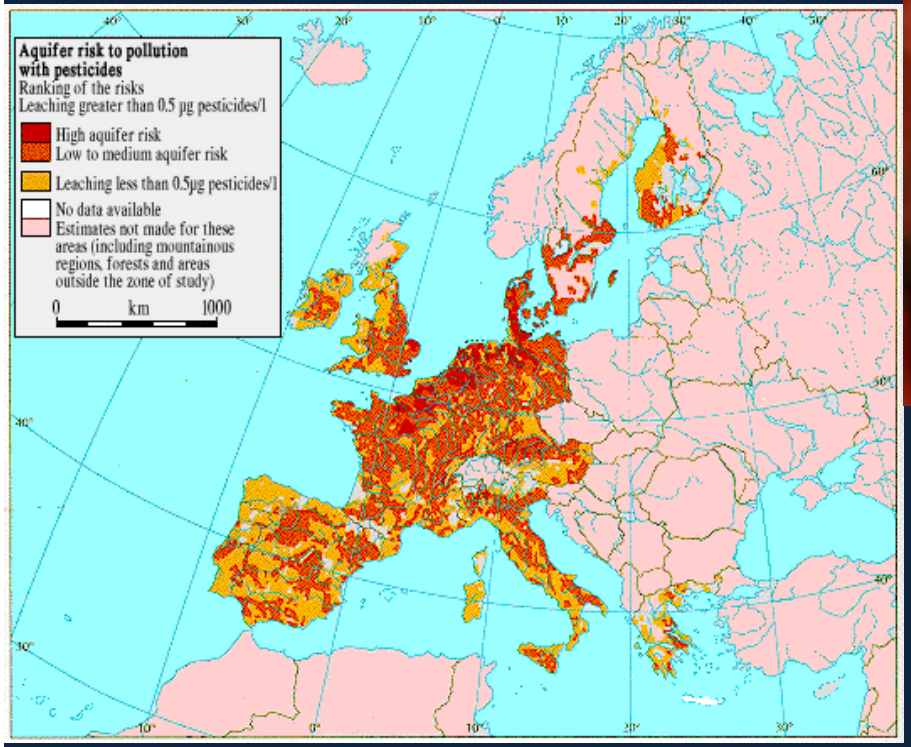
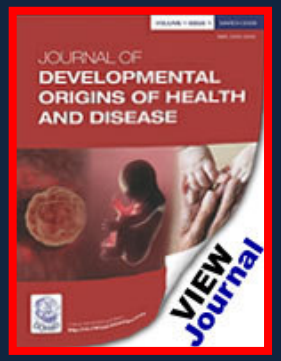
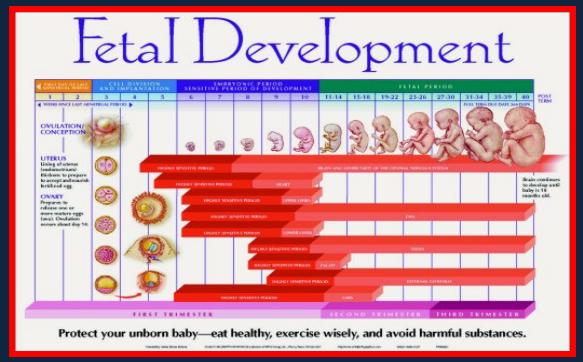
Azienda ULSS 22 Regione Veneto

EFFETTI DEI FITOFARMACI SUGLI OPERATORI AGRICOLI E SULLA POPOLAZIONE



PESTICIDI: Riflessioni sui Meccanismi Patogenetici (il problema degli effetti transgenerazionali)





Ernesto Burgio
ISDE Scientific Office

doctors for the environment

A Case–Control Study of Non-Hodgkin Lymphoma and Exposure to Pesticides

Lennart Hardell, M.D., PhD.[1]
Mikael Eriksson, M.D., Ph.D.[2]

[1] Department of Oncology, Örebro Medical Center, Örebro, Sweden.

[2] Department of Oncology, University Hospital Lund, Sweden.

BACKGROUND. The incidence of non-Hodgkin lymphoma (NHL) has increased in most Western countries during the last few decades. Immunodefective conditions are established risk factors. In 1981, the authors reported an increased risk for NHL following exposure to certain pesticides. The current study was designed to further elucidate the importance of phenoxyacetic acids and other pesticides in the etiology of NHL.

RESULTS. Increased risk for NHL was found for subjects exposed to herbicides (odds ratio [OR], 1.6; 95% confidence interval [CI], 1.0–2.5) and fungicides (OR, 3.7; 95% CI, 1.1–13.0). Among herbicides, the phenoxyacetic acids dominated (OR, 1.5; 95% CI, 0.9–2.4); and, when subclassified, one of these, 4-chloro-2-methyl phenoxyacetic acid (MCPA), turned out to be significantly associated with NHL (OR, 2.7; 95% CI, 1.0–6.9). For several categories of herbicides, it was noted that only exposure during the most recent decades before diagnosis of NHL was associated with an increased risk of NHL. Exposure to impregnating agents and insecticides was, at most, only weakly related to NHL.

CONCLUSIONS. Exposure to herbicides in total, including phenoxyacetic acids, during the decades before NHL diagnosis resulted in increased risk for NHL. Thus, the risk following exposure was related to the latency period. Fungicides also increased the risk for NHL when combined, but this group consisted of several different agents, and few subjects were exposed to each type of fungicide. ***Cancer* 1999;85:1353–60.** *© 1999 American Cancer Society.*

Is the Decline of the Increasing Incidence of Non-Hodgkin Lymphoma in Sweden and Other Countries a Result of Cancer Preventive Measures?

Lennart Hardell[1,2] and Mikael Eriksson[3]

Is the decline of the increasing incidence of non-Hodgkin lymphoma (NHL) in Sweden and other countries a result of cancer preventive measures? The yearly age-standardized incidence of NHL increased significantly in Sweden during 1971–1990, for men an average of 3.2% and for women 3.1%. The corresponding figures for 1991–2000 were –0.8% and –0.2%, respectively. A decline of the increasing incidence has also been seen in other countries, such as the United States, Finland, and Denmark. Immunosuppression is one established risk factor for NHL, possibly with interaction with Epstein-Barr virus. Phenoxyacetic acids and chlorophenols, both pesticides, have been associated with NHL. Use of these chemicals was banned in Sweden in 1977 and 1978, respectively. Also, persistent organic pollutants such as polychlorinated biphenyls, hexachlorobenzene, chlordanes, and dioxins have been shown to increase the risk. Exposure of the whole population occurs predominantly through the food chain. Exposure to such chemicals was highest in the 1960s and 1970s. Because of regulation in the 1970s, exposure has declined substantially in the population. The change in incidence of NHL in Sweden and other countries may serve as a good example of how prohibition and limitation of exposure may be reflected in cancer statistics some decades later. *Key words:* incidence, non-Hodgkin lymphoma, persistent organic pollutants, pesticides, prevention. *Environ Health Perspect* 111:1704–1706 (2003). doi:10.1289/ehp.6270 available via *http://dx.doi.org/* [Online 2 July 2003]

Acta Oncologica, 2008; 47: 347–354

INVITED ARTICLE

Pesticides, soft-tissue sarcoma and non-Hodgkin lymphoma – historical aspects on the precautionary principle in cancer prevention

LENNART HARDELL

Department of Oncology, University Hospital, SE-701 85 Örebro and Department of Natural Sciences, Örebro University, SE-701 82 Örebro, Sweden

Background. After the 2nd World War a long range of chemical agents have been introduced on the market, both in Sweden and most other countries. From the 1950's several pesticides gained increasing use in agriculture and forestry. In the 1970's public concern increased in Sweden especially regarding use of phenoxy herbicides to combat deciduous wood, although statements from different authorities were reassuring of the safety. *Materials and methods.* At the end of the 1970's the author and his colleagues published the first scientific evidence of an association between exposure to phenoxyacetic acids, chlorophenols and certain malignant tumours, i.e., soft-tissue sarcoma and malignant lymphoma. The study subjects were also exposed to contaminating dioxins such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Later studies showed also an association between certain persistent organic pollutants such as polychlorinated biphenyls and non-Hodgkin lymphoma (NHL) with an interaction with titers of antibodies to Epstein-Barr virus early antigen. These results have been corroborated in other studies. *Discussion.* Over the years industry and its allied experts have attacked our studies, but in 1997 IARC classified TCDD as a human carcinogen, Group I. The increasing incidence of NHL in Sweden levelled off about 1990. The author postulated that the regulation or ban of the use of chlorophenols, certain phenoxy herbicides and some persistent organic pollutants in Sweden back in the 1970s has contributed to the now decreasing incidence of NHL. Unfounded criticism from industry experts may prohibit the precautionary principle and early warnings of cancer risk can be ignored. Cancer risks by certain chlorinated phenols may serve as a model of how the precautionary principle should be used by taking early warnings seriously.

Agricultural pesticide exposure and the molecular connection to lymphomagenesis

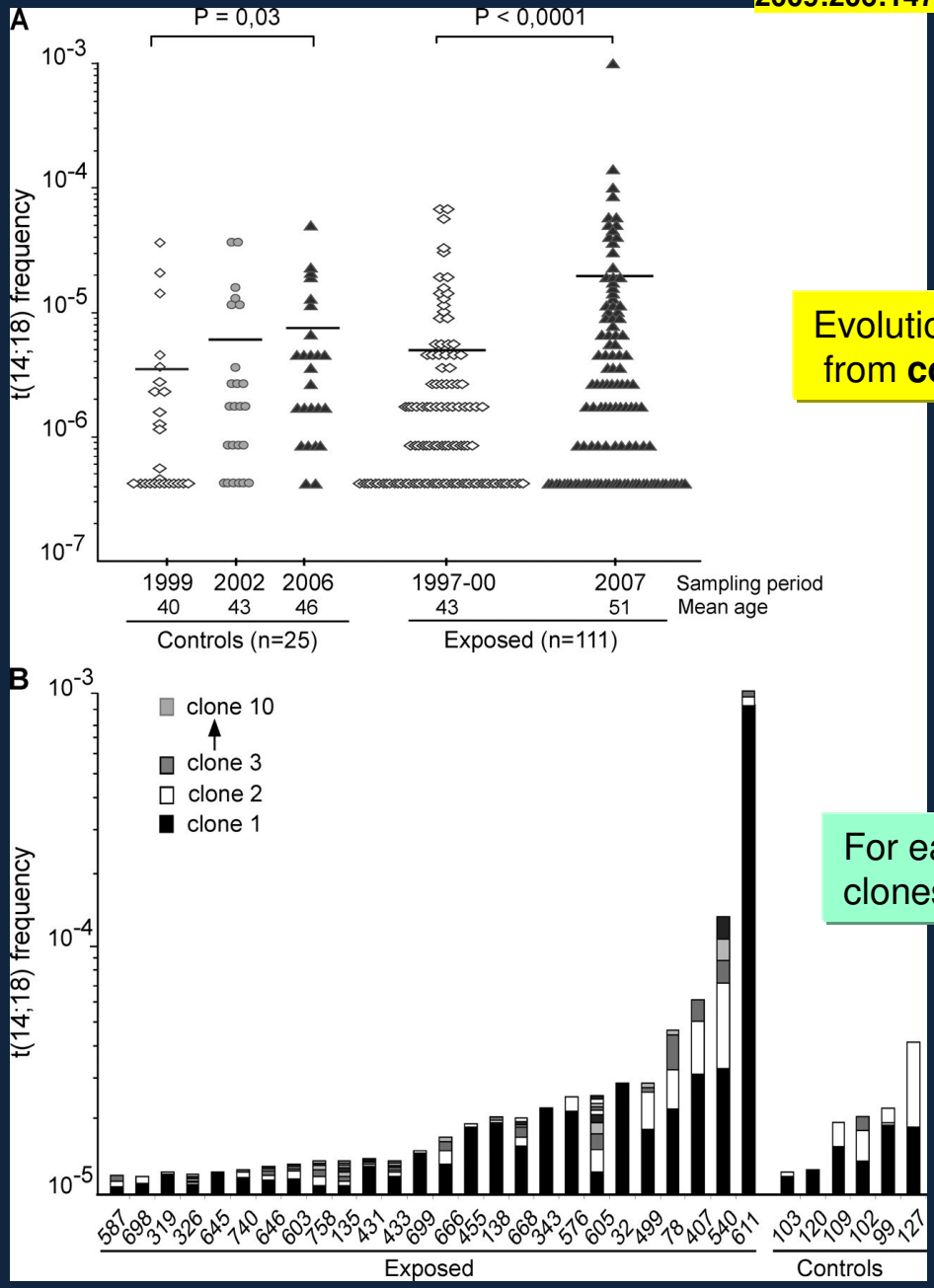
Julie Agopian,[1] Jean-Marc Navarro,[1] Anne-Claire Gac,[2] Yannick Lecluse,[2] Mélanie Briand,[2] Pierre Grenot,[1] Pascal Gauduchon,[2] Philippe Ruminy,[3] Pierre Lebailly,[2] Bertrand Nadel,[1] and Sandrine Roulland[1]

The t(14;18) translocation constitutes the initiating event of a causative cascade leading to follicular lymphoma (FL). t(14;18) translocations are present in blood from healthy individuals, but there is a trend of increased prevalence in farmers exposed to pesticides, a group recently associated with higher risk of t(14;18)+ non-Hodgkin's lymphoma development. A direct connection between agricultural pesticide use, t(14;18) in blood, and malignant progression, however, has not yet been demonstrated. We followed t(14;18) clonal evolution over 9 yr in a cohort of farmers exposed to pesticides. We show that exposed individuals bear particularly high t(14;18) frequencies in blood because of a dramatic clonal expansion of activated t(14;18)+ B cells. We further demonstrate that such t(14;18)+ clones recapitulate the hallmark features of developmentally blocked FL cells, with some displaying aberrant activation-induced cytidine deaminase activity linked to malignant progression. Collectively, our data establish that expanded t(14;18)+ clones constitute bona fide precursors at various stages of FL development, and provide a molecular connection between agricultural pesticide exposure, t(14;18) frequency in blood, and clonal progression.

J Exp Med 2009 206:1473-1483. Published June 8, 2009, doi:10.1084/jem.20082842

Figure 1. Agricultural exposure drives a dramatic increase of t(14;18)+ clones in blood

Agopian et al. Journal of Experimental Medicine
2009:206:1473-1483



Evolution of t(14;18) frequency in PBMCs from **control** and **exposed** populations

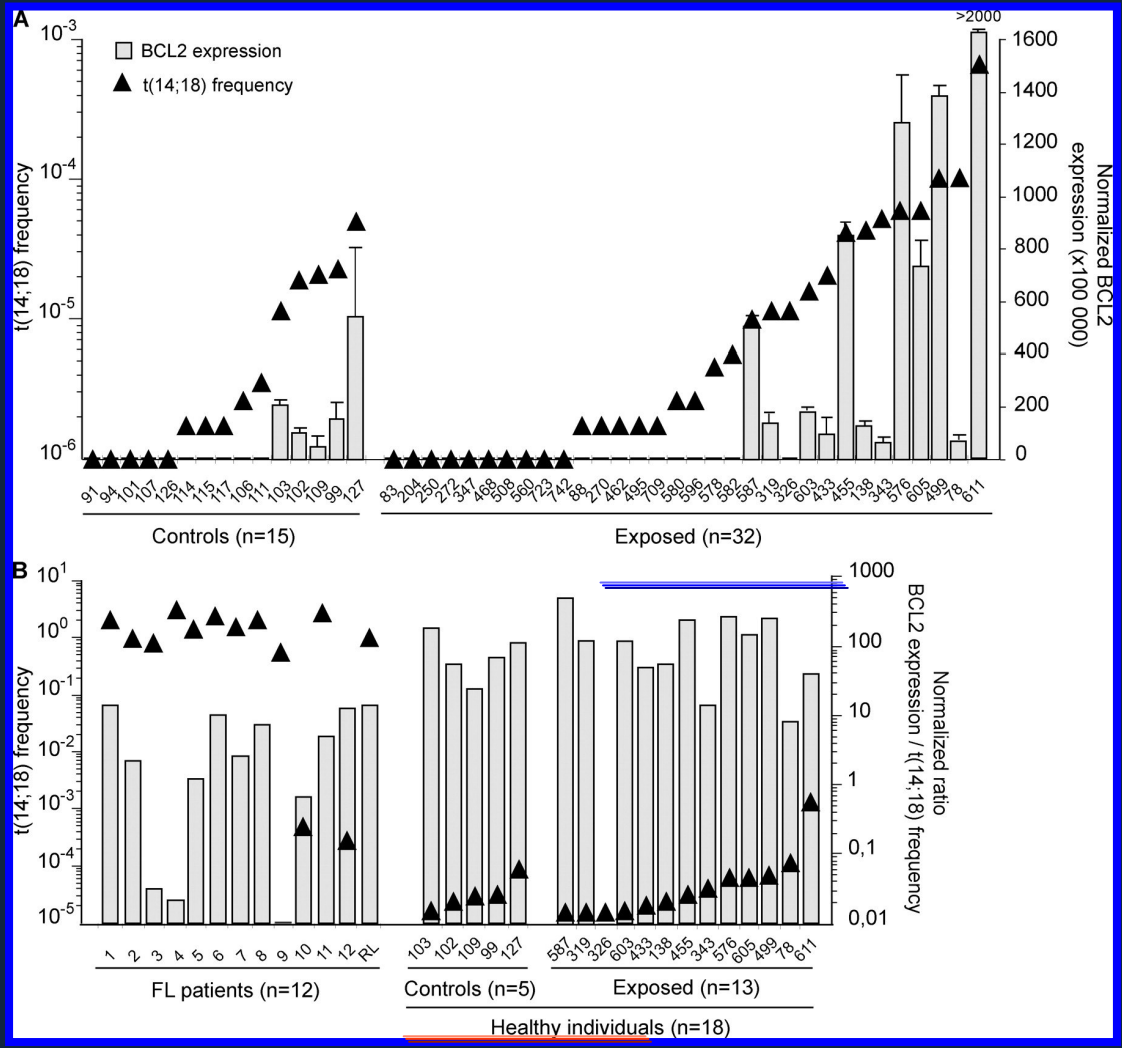
Clonality analysis

For each individual, independent **BCL2/JH** clones are pictured in distinct shades

JEM

Agopian et al. Journal of Experimental Medicine
2009:206:1473-1483

Figure 2. t(14;18)+ cells in HI are actively transcribing BCL2 from the translocated allele



... il **gene bcl 2** è espresso nella zona follicolare .. ha un **ruolo fisiologico** importante: **blocca l'apoptosi** nei cloni linfocitari destinati a diventare **cellule di memoria,** ma può **essere attivato** anche in cellule della corticale mediante diversi meccanismi: il principale è la **traslocazione t14:18..** che può porre il gene sotto il **controllo del *promoter* delle Ig** o del **TCR**

in altri casi di *linfoma follicolare* il gene espresso in modo eccessivo (→ anti-apoptosi) è il Bcl 6..

JEM

N Engl J Med 2008;359:722-34.

REVIEW ARTICLE

MOLECULAR ORIGINS OF CANCER

Chromosomal Abnormalities in Cancer

Stefan Fröhling, M.D., and Hartmut Döhner, M.D.

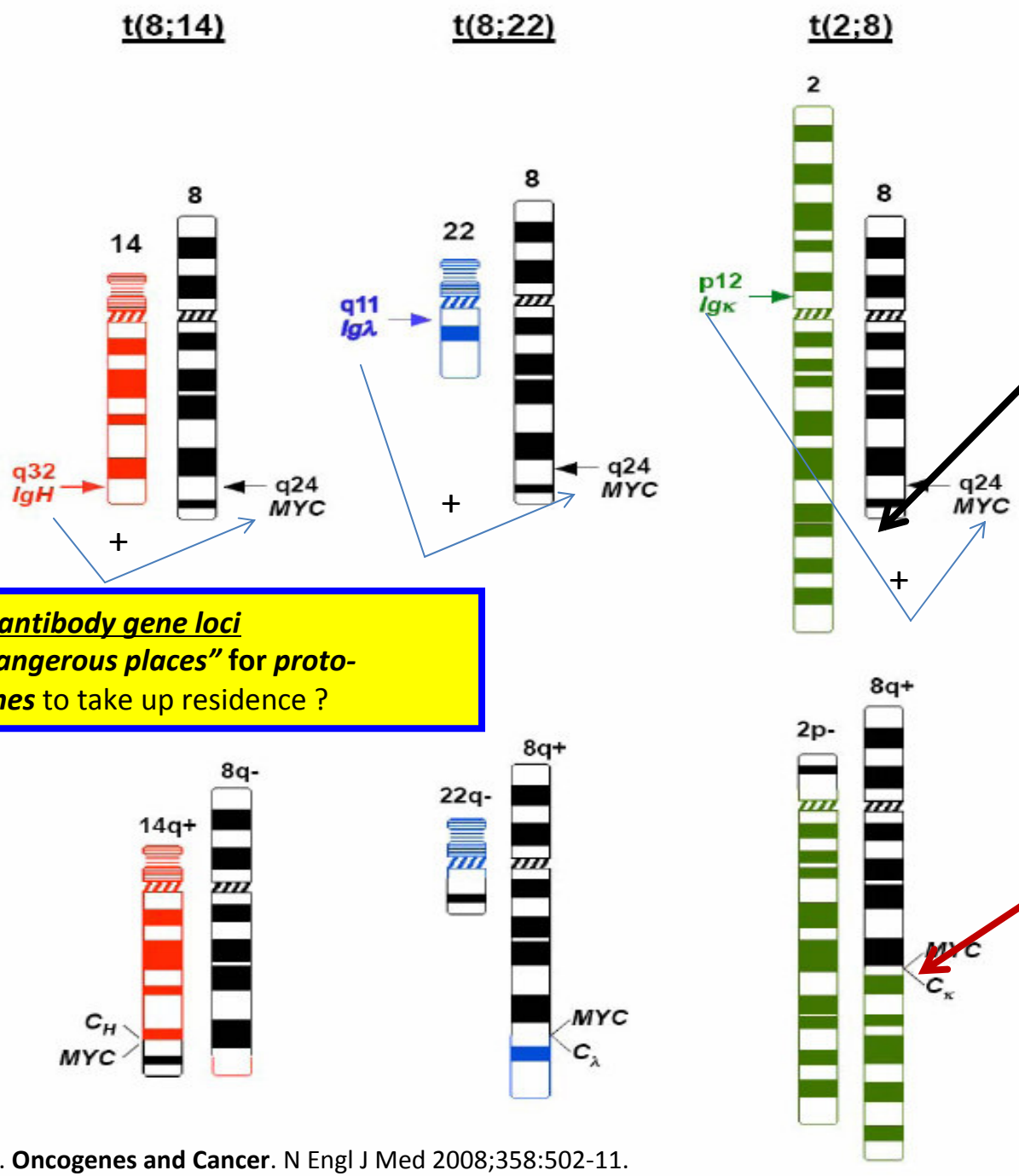
CYTOGENETIC ABNORMALITIES ARE A CHARACTERISTIC ATTRIBUTE cancer cells. To date, clonal chromosome aberrations have been found in major tumor types from more than 54,000 patients (http://cgap.nci.nih.g Chromosomes/Mitelman), and their identification continues as a result of techni improvements in conventional and molecular cytogenetics. The World Health ganization Classification of Tumours recognizes a growing number of such gene changes and uses them to define specific disease entities. Many of these aberratio have emerged as prognostic and predictive markers in hematologic cancers a certain types of solid tumors. Furthermore, the molecular characterization of cy genetic abnormalities has provided insights into the mechanisms of tumorigene and has, in a few instances, led to treatment that targets a specific genetic abn mality. This article discusses examples of two main classes of chromosomal abn malities — balanced chromosomal rearrangements and chromosomal imbalan (Fig. 1 and 2) — with particular focus on their functional consequences and their i plications (actual or potential) for the development of effective anticancer therapi

THE CAUSE OF **CHROMOSOMAL ABNORMALITIES** REMAINS POORLY UNDERSTOOD.
STUDIES OF VARIOUS TYPES OF **LEUKEMIA** HAVE SHOWN THAT **CERTAIN ENVIRONMENTAL AND OCCUPATIONAL EXPOSURES AND THERAPY WITH CYTOTOXIC DRUGS CAN INDUCE CHROMOSOMAL ABERRATIONS**. FOR EXAMPLE, CASES OF **MYELODYSPLASTIC SYNDROME** OR **AML** THAT ARISE AFTER TREATMENT WITH ***ALKYLATING AGENTS*** ARE FREQUENTLY ASSOCIATED WITH **UNBALANCED ABNORMALITIES**, PRIMARILY **DELETION** OR **LOSS OF CHROMOSOME 5 OR 7** (OR BOTH),

...WHEREAS THERAPY WITH **TOPOISOMERASE II INHIBITORS** IS TYPICALLY ASSOCIATED WITH **BALANCED ABNORMALITIES**, MOST COMMONLY **TRANSLOCATIONS INVOLVING THE *MLL GENE ON CHROMOSOME* BAND 11Q23.1**

Are ***TRANSLOCATIONS*** chromosomal aberrations
or **reactive/positive rearrangements** ??

Variant Translocations



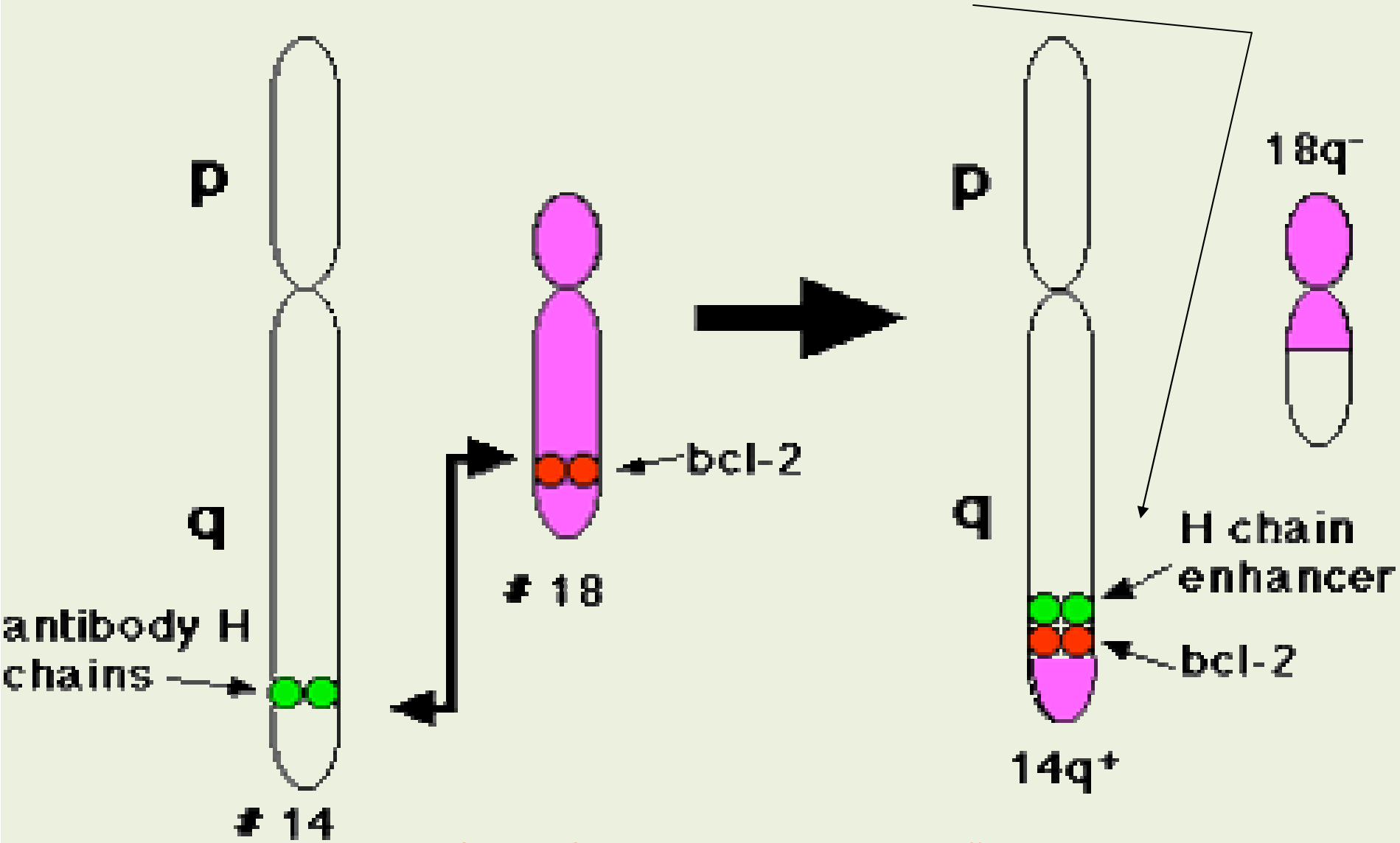
THE FIRST EVIDENCE THAT CANCER ARISES FROM SOMATIC GENETIC ALTERATIONS CAME FROM STUDIES OF **BURKITT'S LYMPHOMA**, IN WHICH ONE OF **THREE DIFFERENT TRANSLOCATIONS** JUXTAPOSES AN **ONCOGENE, *MYC*, ON CHROMOSOME 8q24** TO ONE OF THE **LOCI FOR IMMUNOGLOBULIN GENES**. CHROMOSOMES **14q, 22q, AND 2q — THE TRANSLOCATION PARTNERS — EACH CARRIES ENHANCER ELEMENTS IN THE IMMUNOGLOBULIN LOCI, THEREBY ACTIVATING THE JUXTAPOSED *C-MYC ONCOGENE***

Are the ***antibody gene loci*** **quite *"dangerous places"* for *proto-oncogenes*** to take up residence ?

C-Myc influences the transcription of a variety of **proteins involved in cell cycle regulation**, **apoptosis**, **cell growth, cell adhesion,** and **differentiation**

Croce CM. **Oncogenes and Cancer**. N Engl J Med 2008;358:502-11.

IN THE CANCEROUS B CELLS, THE PORTION OF CHROMOSOME 18 CONTAINING THE *BCL-2* LOCUS HAS UNDERGONE A **RECIPROCAL TRANSLOCATION WITH THE PORTION OF CHROMOSOME 14 CONTAINING THE ANTIBODY HEAVY CHAIN LOCUS**. THIS **T(14;18) TRANSLOCATION PLACES THE *BCL-2* GENE CLOSE TO THE HEAVY CHAIN GENE *ENHANCER*.**



H Chain-enhancer is very active in B cells...

blood 1995 85: 2528-2536

Lymphoma-associated translocation t(14;18) in blood B cells of normal individuals

J Limpens, R Stad, C Vos, C de Vlaam, D de Jong, GJ van Ommen, E Schuuring and PM Kluin

Successive oncogenic steps are necessary to generate cancer. In many B-cell lymphomas, chromosomal translocations are considered to be an early oncogenic hit. We investigated whether the lymphoma-associated t(14;18) involving the *BCL2* oncogene can occur outside the context of malignancy. To this end, we extensively screened blood cells from healthy blood donors by a very sensitive seminested polymerase chain reaction (PCR) for breakpoint junctions at JH1-5 on 14q32 and the major breakpoint region of *BCL2* on 18q21. In each individual, mononuclear cells, granulocytes, flow-sorted B cells, and T cells were separately tested in five to seven independently performed PCRs (in total, 0.5×10^6 to 1.0×10^6 cells per fraction per individual). Amplification products that hybridized with an internal *BCL2* probe and a JH probe were sequenced. Six of nine individuals harbored t(14;18) breakpoints. Translocations were restricted to B cells, with an estimated frequency of 1 in 10^5 or less circulating B cells. In total, 23 of 51 experiments on B cells were positive in contrast to 1 of 48 on T cells and 2 of 47 experiments on granulocytes. Consistent with the presence of 4.7% to 13.0% B cells in the mononuclear cell fractions, only very few (4 of 47) tests were positive in these fractions. Sequence analysis showed that four of six individuals harbored two to five unrelated t(14;18)-carrying B-cell clones. All breakpoints had a structure similar to that in follicular lymphoma. We propose that B cells with the t(14;18) translocation are regularly generated in normal individuals, but that only very few cells with the translocation will acquire the additional oncogenic hits necessary to establish the malignant phenotype.

Carcinogenesis

Exposure to **NHL-associated carcinogens**, such as **dioxin or pesticides**, may cause **expansion of t(14;18)-positive clones.**

Carcinogenesis Advance Access first published online on March 16, 2006
This version published online on April 25, 2006
Carcinogenesis, doi:10.1093/carcin/bgl011

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Received November 28, 2005
Revised February 11, 2006
Accepted March 7, 2006

MOLECULAR EPIDEMIOLOGY AND CANCER PREVENTION

t(14;18) translocations in lymphocytes of healthy dioxin-exposed individuals from Seveso, Italy

Andrea Baccarelli [1], Carsten Hirt [2], Angela C. Pesatori [1], Dario Consonni [1], Donald G. Patterson Jr. [3], Pier Alberto Bertazzi [1], Gottfried Dölken [4], and Maria Teresa Landi [5] *

Carcinogenesis vol.27 no.10 pp.2001–2007, 2006
doi:10.1093/carcin/bgl011
Advance Access publication March 16, 2006

t(14;18) translocations in lymphocytes of healthy dioxin-exposed individuals from Seveso, Italy

Table III. Prevalence and frequency of t(14;18) translocations by plasma TCDD levels, zone of residence and diagnosis of chloracne

	t(14;18)-positive subjects		t(14;18) frequency[a]	
	%	(Positive/total)	Mean	(95% CI)
Plasma TCDD				
<10 p.p.t.	34.7	(25/72)	4.2[b]	(2.9–6.2)
10.0–475.0 p.p.t.	34.7	(25/72)	9.9[b]	(6.8–14.5)
Zone of residence at the time of the accident				
Reference	42.4	(14/33)	4.3[c]	(2.3–8.0)
R	26.9	(7/26)	4.9[c]	(2.2–10.7)
B	29.4	(10/34)	7.2[c]	(3.8–13.6)
A	37.3	(19/51)	9.3[c]	(5.8–14.8)
Chloracne after the accident				
No	35.2	(32/91)	6.2	(3.7–10.6)
Yes	34.0	(18/53)	6.7	(4.7–9.6)

[a]Geometric means and 95% CIs of the number of t(14;18) translocations/10^6 lymphocytes among t(14;18)-positive subjects, adjusted for age, smoking status (never, ex or current smoker) and smoking duration in multivariable analysis.
[b]$P = 0.006$, test for difference in mean t(14;18) frequency between plasma TCDD categories.
[c]$P = 0.04$, test for trend in mean t(14;18) frequency across residence zones.

Research

ENVIRONMENT

Pesticide Use and Cutaneous Melanoma in Pesticide Applicators in the Agricultural Heath Study

Leslie K. Dennis,[1] Charles F. Lynch,[1] Dale P. Sandler,[2] and Michael C.R. Alavanja[3]

[1]Department of Epidemiology, College of Public Health, University of Iowa, Iowa City, Iowa, USA; [2]Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, North Carolina, USA; [3]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, Maryland, USA

BACKGROUND: Melanoma rates continue to increase; however, few risk factors other than sun sensitivity and ultraviolet radiation (including sun exposure) have been identified. Although studies of farmers have shown an excess risk of melanoma and other skin cancers, it is unclear how much of this is related to sun exposure compared with other agricultural exposures.

METHODS: We examined dose–response relationships for 50 agricultural pesticides and cutaneous melanoma incidence in the Agricultural Health Study cohort of licensed pesticide applicators, along with ever use of older pesticides that contain arsenic. Logistic regression was used to examine odds ratios (ORs) and 95% confidence i
sex, and other potential confounder

RESULTS: We found significant as
(≥ 63 exposure days: OR = 2.4; 95
OR = 2.4; 95% CI, 1.3–4.4; trend
1.1–2.5; trend p = 0.013). Other as
also suggested.

We found **significant associations between cutaneous melanoma** and ***maneb/mancozeb*** (≥ 63 exposure days: OR = 2.4; 95% CI, 1.2–4.9; trend *p = 0.006),* **parathion** *(≥ 56 exposure days: OR = 2.4; 95% CI, 1.3–4.4; trend p = 0.003), and* **carbaryl** *(≥ 56 exposure days: OR = 1.7; 95% CI, 1.1–2.5; trend p = 0.013).*

CONCLUSIONS: Most previous melanoma literature has focused on host factors and sun exposure. Our research shows an association between several pesticides and melanoma, providing support for the hypotheses that agricultural chemicals may be another important source of melanoma risk.

KEY WORDS: arsenic, farmers, melanoma, pesticides. *Environ Health Perspect* 118:812–817 (2010). doi:10.1289/ehp.0901518 [Online 17 February 2010]

EPIGENETICS

Table 4. Interactions of lead arsenate and specific pesticides on risk of cutaneous melanoma among pesticide applicators completing the take-home questionnaire in the Agricultural Health Study.

Pesticide/exposure	All subjects		Not exposed to lead arsenate		Exposed to lead arsenate		
	Cases/noncases[a]	OR (95% CI)[b]	Cases/noncases[a]	OR (95% CI)[b]	Cases/noncases[a]	OR (95% CI)[b]	*p*-Value for interaction[c]
Benomyl[d] (fungicide)							
No exposure	131/21,699	1.0 (reference)	128/21,110	1.0 (reference)	3/589	1.0 (reference)	
Any exposure	13/1,613	1.2 (0.7–2.1)	7/1,440	0.7 (0.3–1.6)	6/173	6.7 (1.6–27.0)	$p = 0.006$
Carbaryl[d] (insecticide)							
No exposure	64/13,570	1.0 (reference)	63/13,444	1.0 (reference)	1/126	1.0 (reference)	
Any exposure	76/8,940	1.5 (1.0–2.0)	67/8,309	1.4 (1.0–2.0)	9/631	1.8 (0.2–14.4)	$p = 0.835$
Maneb/mancozeb[e] (fungicide)							
No exposure	127/21,793	1.0 (reference)	125/21,235	1.0 (reference)	2/558	1.0 (reference)	
Any exposure	17/1,660	1.5 (0.09–2.5)	9/1,457	0.9 (0.5–1.8)	8/203	10.8 (2.3–51.3)	$p = 0.005$
Parathion (insecticide)							
No exposure	122/21,730	1.0 (reference)	120/21,238	1.0 (reference)	2/492	1.0 (reference)	
Any exposure	21/1,608	1.9 (1.2–3.0)	13/1,331	1.5 (0.8–2.7)	8/277	7.3 (1.5–34.6)	$p = 0.065$

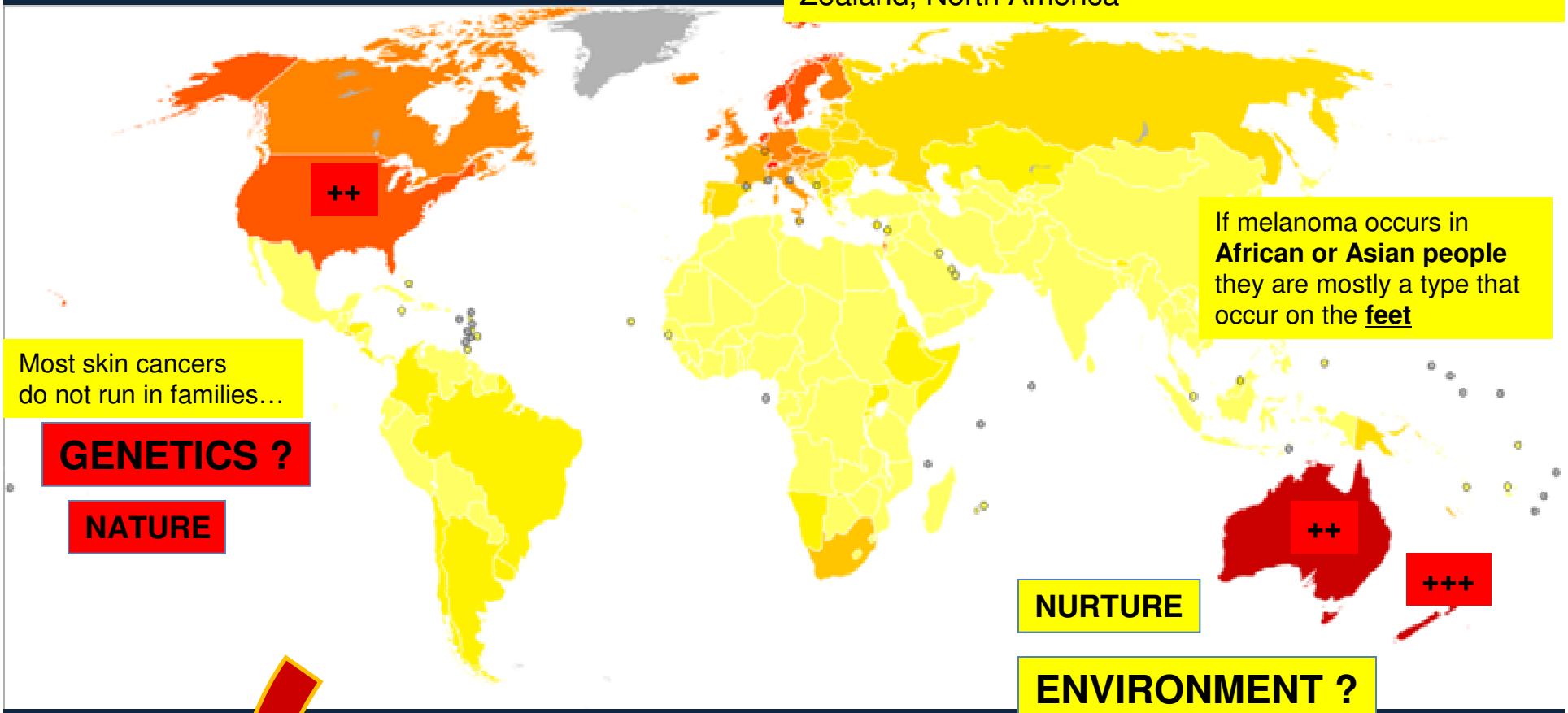
[a]Total varies based on the number of subjects with missing values for each pesticide. [b]Adjusted for age at enrollment and sex using the intensity-weighted lifetime exposure days. [c]*p*-Value for a multiplicative interaction term. [d]Carbamate pesticide. [e]Dithiocarbamate fungicide.

Increased cutaneous melanoma risk was seen among applicators who had used/applied maneb/mancozeb and parathion, and potentially benomyl as well as lead arsenate, compared with never users of these products. The results are consistent with prior findings of an association between melanoma and arsenic.

an association between carbaryl and melanoma was upheld when we added 2 additional years of cases. Most of the previous melanoma literature has focused on host factors and sun exposure, but our study suggests more research is needed on chemicals and other environmental factors that may increase the risk of cutaneous melanoma.

Worldwide Melanoma of Skin Cancer Incidence - 2008 Globocan.svg

M. is common among ***Caucasians*** ***living in sunny climates***, with high rates of incidence in Australia, New Zealand, North America



If melanoma occurs in **African or Asian people** they are mostly a type that occur on the **feet**

Most skin cancers do not run in families…

GENETICS ?

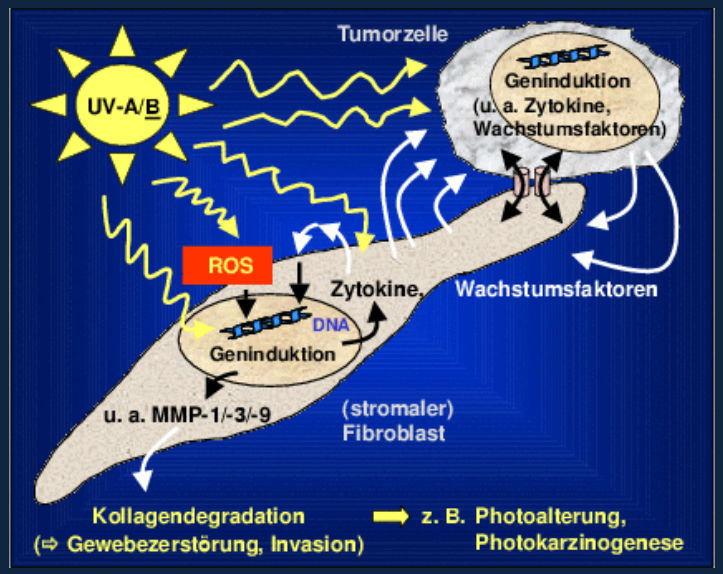
NATURE

NURTURE

ENVIRONMENT ?

EPIGENETICS ?

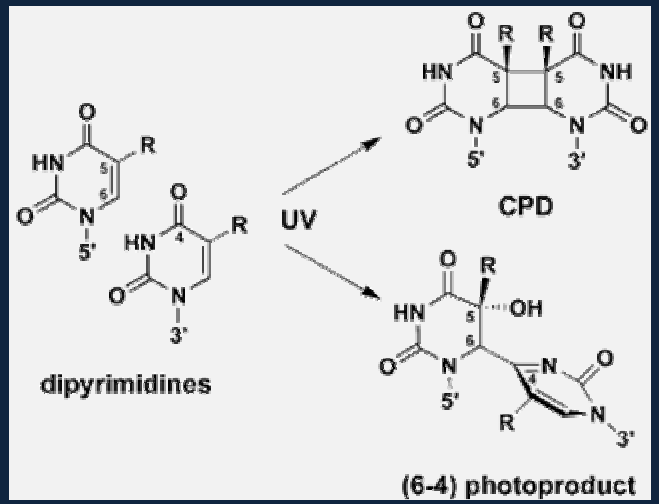
Melanogenesis **prevents the indirect DNA damage that is responsible for the formation of malignant melanoma and other skin cancers**

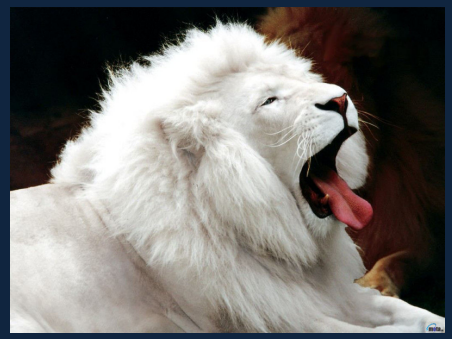


Although, in general, **human beings possess a similar concentration of melanocytes** in their skin, **the melanocytes in some individuals and ethnic groups more frequently or less frequently express the melanin-producing genes**

GENETICS ?

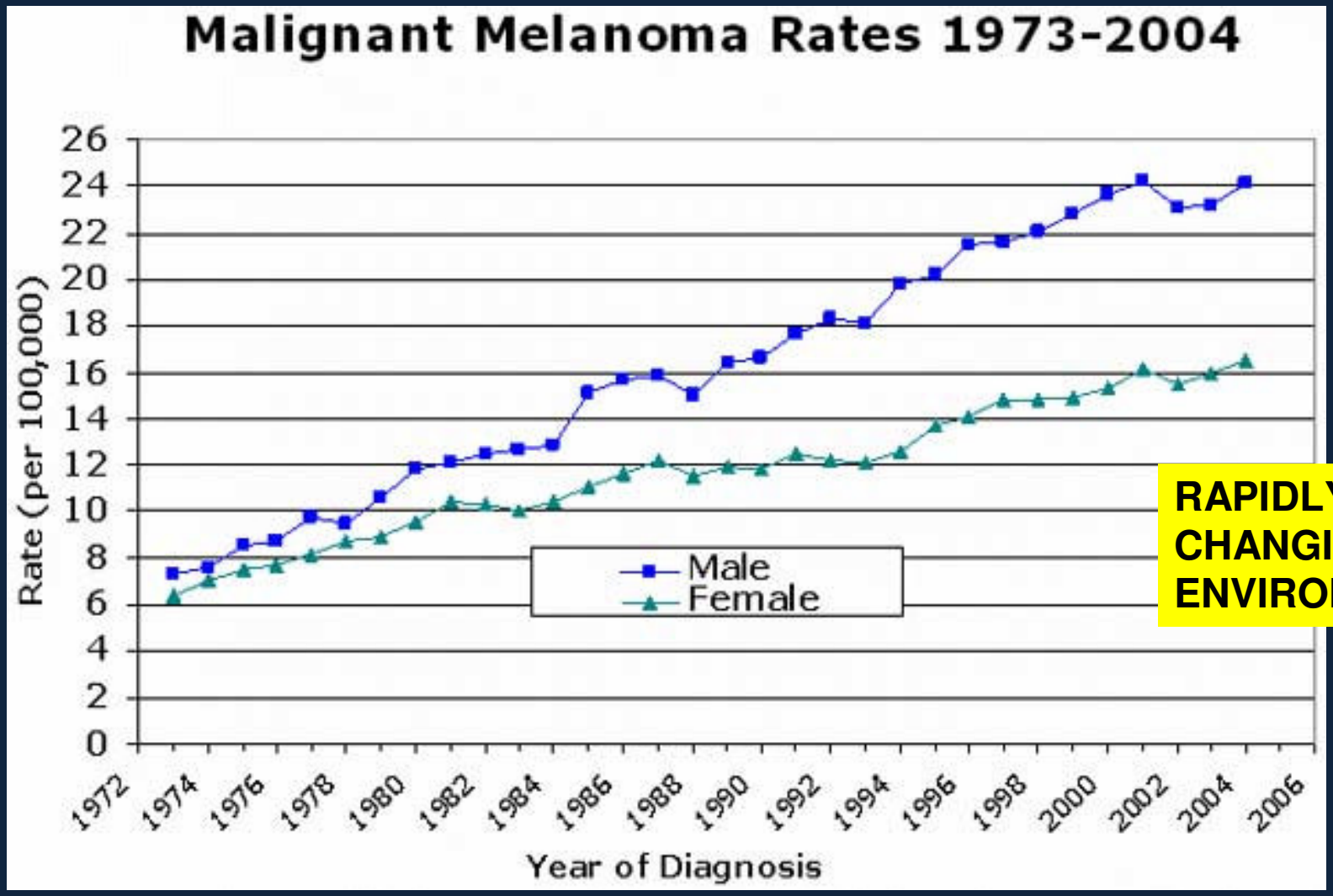
Cyclobutane pyrimidine dimer





Albinos lack *tyrosinase*

EPIGENETICS ?



RAPIDLY CHANGING ENVIRONMENT

Since **1972**, rates of malignant melanoma have **roughly doubled for women** and **tripled for men**.

Worldwide, doctors diagnose about **160,000 new cases of melanoma yearly**. The diagnosis is more frequent in **women**

EPIGENETICS !

ENVIRONMENT

ORIGINAL INVESTIGATION

Personal History of Endometriosis and Risk of Cutaneous Melanoma in a Large Prospective Cohort of French Women

Marina Kvaskoff, MPH; Sylvie Mesrine, MD; Agnès Fournier, MPH; Marie-Christine Boutron-Ruault, MD, PhD; Françoise Clavel-Chapelon, PhD

Background: An association between melanoma and endometriosis has been reported, but most findings relied on case-control studies or a limited number of mela-
noma cases, and therefore the av
Moreover, the effect of other b
eases on melanoma risk is unk

Methods: We prospectively stu
Epidémiologique auprès de fem
érale de l'Education Nationale coh
French women, insured by a natio
covering teachers, aged 40 to 65
on history of endometriosis and
cal diseases were regularly collect
tive risks and 95% confidence inte
Cox proportional hazards regres

Results: During 12 years of fo
cases were ascertained among 91 965 subjects. A history of endometriosis (n=5949) was significantly associated with a higher risk of melanoma (relative risk,
1.62; 95% confidence interval, 1.15-2.29). There was
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Of **91,965 women followed up for 12 years, 5949 had a history of endometriosis**, and **363 were diagnosed with melanoma during follow-up**. History of **endometriosis was a significant risk factor for development of melanoma** (RR, 1.62; 95% confidence interval [CI], 1.15 - 2.29). Women **with a history of fibroma (n = 24,375) also had a significantly increased risk for melanoma** vs those with no such history (RR, 1.33; 95% CI, 1.06 - 1.67).

?!

Arch Intern Med. 2007;167(19):2061-0

EPIGENETICS

Reproductive Toxicology 30 (2010) 365–369

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Reproductive Toxicology

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Organochlorine pesticides and endometriosis

Maureen A. Cooney[a,*], Germaine M. Buck Louis[a,1], Mary L. Hediger[a,1], Albert Vexler[b,2], Paul J. Kostyniak[c,3]

Limited study of persistent organochlorine pesticides (OCPs) and endometriosis has been conducted. One hundred women aged 18–40 years who were undergoing laparoscopy provided 20 cm^3 of blood for toxicologic analysis and surgeons completed operative reports regarding the presence of endometriosis. Gas chromatography with electron capture was used to quantify (ng/g serum) six OCPs. Logistic regression was utilized to estimate the adjusted odds ratios (aOR) and 95% confidence intervals (CI) for individual pesticides and groups based on chemical structure adjusting for current cigarette smoking and lipids. The hig
(aOR = 5.3; 95% CI,
and HCB. These ar
OCP exposure and
and confirm these

To the best of our knowledge, our findings are the first to suggest a **possible <u>association between OCPs other than DDE and endometriosis,</u> particularly the aromatic fungicides**. The findings add to the existing literature that suggests **<u>other hormonally active environmental chemicals such as PCBs and dioxin may affect</u> likelihood of <u>endometriosis</u>**.

Associations of Serum Concentrations of Organochlorine Pesticides with Breast Cancer and Prostate Cancer in U.S. Adults

Xiaohui Xu,[1] Amy B. Dailey,[1] Evelyn O. Talbott,[2] Vito A. Ilacqua,[3] Greg Kearney,[4] and Nabih R. Asal[1]

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Background: Organochlorine (OC) pesticides are a group of environmental endocrine disruptors that may be associated with an increased risk for hormone-related cancers including cancers of the breast and prostate. However, epidemiologic evidence is limited and inconsistent.

Objectives and methods: We used 1999–2004 National Health and Nutrition Examination Survey data to examine associations between serum concentrations of OC pesticides and prostate and breast cancers.

Results: After adjustment for other covariates, serum concentrations of β-hexachlorocyclohexane (HCH) (p for trend = 0.02), *trans*-nonachlor (p for trend = 0.002), and dieldrin (p for trend = 0.04) were significantly associated with the risk of prevalent prostate cancer. Adjusted odds ratios for the second and third tertiles of detectable values were 1.46 [95% confidence interval (CI), 0.52–4.13] and 3.36 (95% CI, 1.24–9.10) for β-HCH; 5.84 (95% CI, 1.06–32.2) and 14.1 (95% CI, 2.55–77.9) for *trans*-nonachlor; and 1.06 (95% CI, 0.30–3.73) and 2.74 (95% CI, 1.01–7.49) for dieldrin compared with concentrations in the lowest tertile or below the limit of detection. However, there was no positive association between serum concentrations of OC pesticides and breast cancer prevalence.

Conclusion: Although further study is necessary to confirm these findings, these results suggest that OC pesticide exposures may have a significant effect on cancer risk. Efforts to reduce worldwide OC use are warranted.

Key words: cancer, endocrine disruptors, organochlorine pesticides, pesticide, prostate cancer. *Environ Health Perspect* 118:60–66 (2010). doi:10.1289/ehp.0900919 available via *http://dx.doi.org/* [Online 3 September 2009]

After adjustment for age (< 65 years of age vs. ≥ 65 years), race/ethnicity, BMI, education, smoking, NHANES data cycle, and marital status, the **trends for prevalence of prostate cancer** remained **significant** for three of six OC pesticides including **β-HCH, *trans*-nonachlor,** and ***dieldrin***

Table 5. Lipid-adjusted serum concentration (ng/g) and prevalent prostate cancer in 1999–2004 NHANES adult participants, by tertile.

Chemical	Not detectable		Detectable		p_{trend}
	< LOD	< 33rd	33rd –67th	> 67th	
β-HCH					
Median concentration	—	6.2	16.2	53.9	
Cases/no.	5/453	9/528	23/531	21/307	
Age-adjusted OR (95% CI)	1.0	1.0	1.44 (0.55–3.78)	3.10 (1.36–7.11)	0.007
Adjusted OR (95% CI)[a]	1.0	1.0	1.46 (0.52–4.13)	3.36 (1.24–9.10)	0.02
p,p´-DDE					
Median concentration	—	113.0	386.0	1530.0	
Cases/no.	0/3	8/599	28/692	23/547	
Age-adjusted OR (95% CI)	1.0	1.0	1.89 (0.71–5.04)	2.02 (0.76–5.32)	0.16
Adjusted OR (95% CI)[a]	1.0	1.0	2.05 (0.76–5.50)	2.64 (0.92–7.57)	0.07
Oxychlordane					
Median concentration	—	8.0	17.2	37.0	
Cases/no.	0/273	5/488	19/506	29/441	
Age-adjusted OR (95% CI)	1.0	1.0	3.38 (1.02–11.3)	3.39 (1.03–11.1)	0.04
Adjusted OR (95% CI)[a]	1.0	1.0	3.54 (0.91–13.8)	3.54 (0.92–13.6)	0.06
Trans-nonachlor					
Median concentration	—	9.9	24.8	56.4	
Cases/no.	0/154	3/521	12/571	41/582	
Age-adjusted OR (95% CI)	1.0	1.0	4.76 (1.13–20.1)	9.51 (2.22–40.8)	0.002
Adjusted OR (95% CI[a]	1.0	1.0	5.84 (1.06–32.2)	14.1 (2.55–77.9)	0.002
Heptachlor epoxide					
Median concentration	—	5.0	8.9	18.0	
Cases/no.	10/681	7/339	12/360	21/311	
Age-adjusted OR (95% CI)	1.0	0.68 (0.20–2.29)	1.39 (0.57–3.34)	1.79 (0.59–5.47)	0.15
Adjusted OR (95% CI)[a]	1.0	1.01 (0.31–3.20)	1.65 (0.73–3.76)	1.91 (0.70–5.33)	0.12
Dieldrin					
Median concentration	—	4.8	7.9	14.7	
Cases/no.	0/257	6/286	6/362	25/343	
Age-adjusted OR (95% CI)	1.0	1.0	0.98 (0.31–3.08)	2.69 (1.09–6.68)	0.03
Adjusted OR (95% CI)[a]	1.0	1.0	1.06 (0.30–3.73)	2.74 (1.01–7.49)	0.04

[a]Adjusted for age, race, and ethnicity, BMI, education, smoking, data cycle, and marital status.

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ORIGINAL CONTRIBUTIONS

Neurodegenerative Diseases and Exposure to Pesticides in the Elderly

Isabelle Baldi[1], Pierre Lebailly[2], Brahim Mohammed-Brahim[1], Luc Letenneur[3], Jean-François Dartigues[3], and Patrick Brochard[1]

The authors investigated the hypothesis that exposure to pesticides could be related to central nervous system disorders in a prospective cohort study of 1,507 French elderly (1992–1998). Lower cognitive performance was observed in subjects who had been occupationally exposed to pesticides. In men, the relative risks of developing Parkinson's disease and Alzheimer's disease for occupational exposure assessed by a job exposure matrix were 5.63 (95% confidence interval: 1.47, 21.58) and 2.39 (95% confidence interval: 1.02, 5.63), respectively, after confounding factors were taken into account. No association was found with having a primary job in agriculture or with environmental pesticide exposure, nor was an association found in women. These results suggest the presence of neurologic impairments in elderly persons who were exposed occupationally to pesticides.

The fact that **only occupational exposure was related to neurologic outcomes (MMSE score, Alzheimer's disease, and Parkinson's disease)** and that the relation appeared **exclusively in men** is consistent with our knowledge of pesticide use in vineyards

ORIGINAL ARTICLE

Environmental risk factors for Parkinson's disease and parkinsonism: the Geoparkinson study

F D Dick, G De Palma, A Ahmadi, N W Scott, G J Prescott, J Bennett, S Semple, S Dick, C Counsell, P Mozzoni, N Haites, S Bezzina Wettinger, A Mutti, M Otelea, A Seaton, P Söderkvist, A Felice, on behalf of the Geoparkinson study group

Occup Environ Med 2007;**64**:666–672. doi: 10.1136/oem.2006.027003

Objective: To investigate the associations between Parkinson's disease and other degenerative parkinsonian syndromes and environmental factors in five European countries.
Methods: A case–control study of 959 prevalent cases of parkinsonism (767 with Parkinson's disease) and
ut. Cases were defined using the
e with drug-induced or vascular
ewer-administered questionnaire
iron, copper and manganese.
tus using a job-exposure matrix
modified by subjective exposure modelling. Results were analysed using multiple logistic regression, adjusting for age, sex, country, tobacco use, ever knocked unconscious and family history of Parkinson's disease.
Results: Adjusted logistic regression analyses showed significantly increased odds ratios for Parkinson's disease/parkinsonism with an exposure–response relationship for pesticides (low vs no exposure, odds ratio (OR) = 1.13, 95% CI 0.82 to 1.57, high vs no exposure, OR = 1.41, 95% CI 1.06 to 1.88) and ever knocked unconscious (once vs never, OR = 1.35, 95% CI 1.09 to 1.68, more than once vs never, OR = 2.53, 95% CI 1.78 to 3.59). Hypnotic, anxiolytic or antidepressant drug use for more than 1 year and a family history of Parkinson's disease showed significantly increased odds ratios. Tobacco use was protective (OR = 0.50, 95% CI 0.42 to 0.60). Analyses confined to subjects with Parkinson's disease gave similar results.
Conclusions: The association of pesticide exposure with Parkinson's disease suggests a causative role. Repeated traumatic loss of consciousness is associated with increased risk.

The **association of pesticide exposure with Parkinson's disease suggests a causative role**. **Repeated traumatic loss of consciousness** is associated with increased risk

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ENVIRONMENT

Neurology®

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Meta-analysis of the relationship between Parkinson disease and melanoma

Rui Liu, PhD,
Xiang Gao, MD, PhD,
Yi Lu, MS and
Honglei Chen, MD, PhD

The **associations were surprisingly strong**.

Women with Parkinson's disease were **one-and-a-half times** more likely to also have a diagnosis of melanoma as were women without the disease.
The link was **even stronger among men**: **Men with Parkinson's were twice as likely to have been diagnosed with melanoma as were their Parkinson's-free peers**.

No clear link was found between Parkinson's and other types of skin cancer.

Liu, R., Gao, X., & Chen, H. (2011). "***Meta-analysis of the relationship between Parkinson disease and melanoma***." Neurology June 7, **2011** vol. 76 no. 23.

Environmental Health

BioMed Central

Environmental Health 2008, **7**:50 doi:10.1186/1476-069X-7-50

Review

Open Access

Potential developmental neurotoxicity of pesticides used in Europe

Marina Bjørling-Poulsen*[1], Helle Raun Andersen[1] and Philippe Grandjean[1,2]

Pesticides used in agriculture are designed to protect crops against unwanted species, such as weeds, insects, and fungus. Many compounds target the nervous system of insect pests. Because of the similarity in brain biochemistry, such pesticides may also be neurotoxic to humans. Concerns have been raised that the developing brain may be particularly vulnerable to adverse effects of neurotoxic pesticides. Current requirements for safety testing do not include developmental neurotoxicity. We therefore undertook a systematic evaluation of published evidence on neurotoxicity of pesticides in current use, with specific emphasis on risks during early development. Epidemiologic studies show associations with neurodevelopmental deficits, but mainly deal with mixed exposures to pesticides. Laboratory experimental studies using model compounds suggest that many pesticides currently used in Europe – including organophosphates, carbamates, pyrethroids, ethylenebisdithiocarbamates, and chlorophenoxy herbicides – can cause neurodevelopmental toxicity. Adverse effects on brain development can be severe and irreversible. Prevention should therefore be a public health priority. The occurrence of residues in food and other types of human exposures should be prevented with regard to the pesticide groups that are known to be neurotoxic. For other substances, given their widespread use and the unique vulnerability of the developing brain, the general lack of data on developmental neurotoxicity calls for investment in targeted research. While awaiting more definite evidence, existing uncertainties should be considered in light of the need for precautionary action to protect brain development.

Table 2: Evidence of developmental neurotoxicity caused by pesticides belonging to groups with likely common mechanisms of neurotoxicity

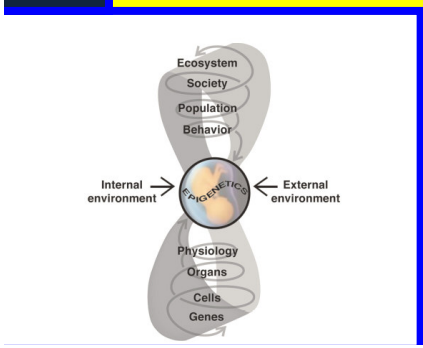
Group of pesticides (n)*	Mechanism of neurotoxicity	Developmental neurotoxicity reported in humans	References	Developmental neurotoxicity reported in animals	References
Organophosphates (8)	Inhibition of AChE (+ interference with signaling cascades at low doses)	Reflex abnormalities in neonates + affected mental development	[7,8]	Altered programming of synaptic development in rats (Chlorpyrifos)	[50,51]
		Reduced head circumference in infants + anomalies in primitive reflexes (Chlorpyrifos)	[61,9]	Behavioural abnormalities including changes in locomotor skills and cognitive performance in rats and mice (Chlorpyrifos)	[43-46]
		Reduced birth weight and length + developmental delay at 3 years of age (Chlorpyrifos)	[62,10]		
		Visuospatial deficits (prenatal exposure) + increased reaction time (current exposure in children)	[11]		
		Reduced short term memory and attention (Methyl parathion)	[12]		
Carbamates (5)	Inhibition of AChE (+ oxidative stress)	No reports were found		No reports were found	
Pyrethroids (7)	Prolongation of kinetics of voltage-gated sodium channels			Increased motor activity, lack of habituation, changes in mAChR density in mice	[85-88]
				Learning changes in rats	[89]
				Changes in motor activity in rats	[90]
				Changes in sexual behaviour and higher activity of the dopaminergic system in rats	[91]
				Changes in mAChR expression in rats	[92,93]
				Changes in blood-brain permeability in rats	[94]

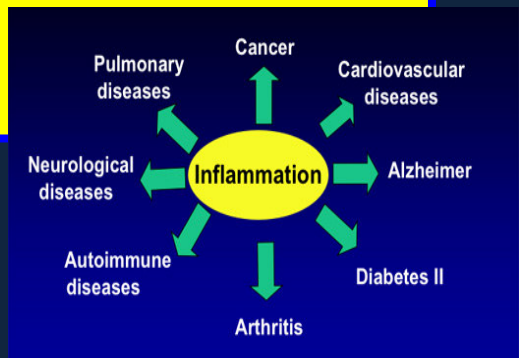
Table 2: Evidence of developmental neurotoxicity caused by pesticides belonging to groups with likely common mechanisms of neurotoxicity *(Continued)*

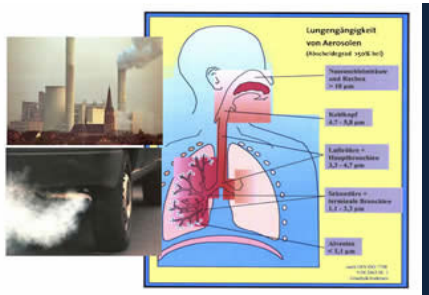
				Affected development of reflexes, swimming ability, open field activity in mice (parental exposure prior to mating)	[96]
Dithiocarbamates (EBDCs) (6)	Generation of ROS (metal chelating capacity, uncoupling of mitochondrial electron transport chain) The EBDC metabolite, ETU, inhibits thyroid peroxidase (synthesis of thyroid hormones)			Maneb (in combination with paraquat) induces loss of dopaminergic neurons in substantia nigra pars compacta in mice	[132]
				The metabolite of EBDCs, ETU, induces malformations of the nervous system (corresponding to thyroid insufficiency) in rats	Reviewed in [126]
Chlorophenoxy herbicides (11)	Not completely known: includes membrane damage, generation of free radicals, perhaps uncoupling of oxidative phosphorylation	A case of cephalic malformations and severe mental retardation in infant whose parents were heavily exposed to 2,4-D	[150]	Hypomyelination in chicks (2,4-D)	[151]
				Deficit in myelin lipid deposition in rats (2,4-D)	[152]
				Delayed CNS development in rats (2,4-D)	[153]
				Increased size and density of serotonin-reactive neuronal somata and increased fiber length in dorsal and medial raphe nuclei in rats (2,4-D)	[154]
				Oxidative stress in specific brain areas (midbrain, striatum, prefrontal cortex) in rats (2,4-D)	[155]
				Behavioural effects in rats including delay of righting reflex, negative geotaxis + motor abnormalities, excessive grooming and vertical head movements, hyperactivity (2,4-D)	[156]

What's Pollution ?

- ***Environmental Pollution*; *chemical burden***
- **Genetics/*epigenetics*; *fluid genome*; *fetal programming (Devo-Evo)***
- ***DOHAD*: *Developmental Origins of Health and Diseases***
- **The XX-XXI century *Epidemic Revolution (Barker Hypothesis, Hygiene Hypothesis; Inflammation Hypothesis).* The *Silent* Pandemic**

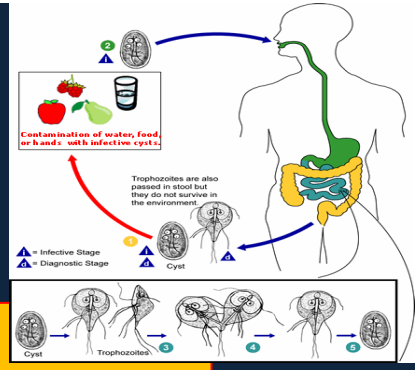






ENVIRONMENT

What's Pollution ?

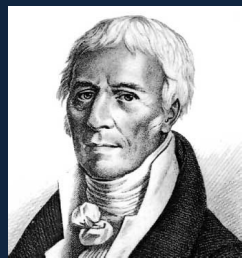


A Generalmente ci si limita a valutare il **rischio legato a singole fonti di inquinamento confrontando popolazioni più o meno direttamente esposte**,

B e trascurando il fatto che **oggi l'inquinamento è un fenomeno ubiquitario e capillare e che l'esposizione** ad **agenti epi-genotossici** (fisici, (bio)chimici, biologici..) più pericolosi concerne, e in misura sempre più rilevante, **l'intera popolazione umana, le generazioni future,** l'**intera eco/biosfera**.

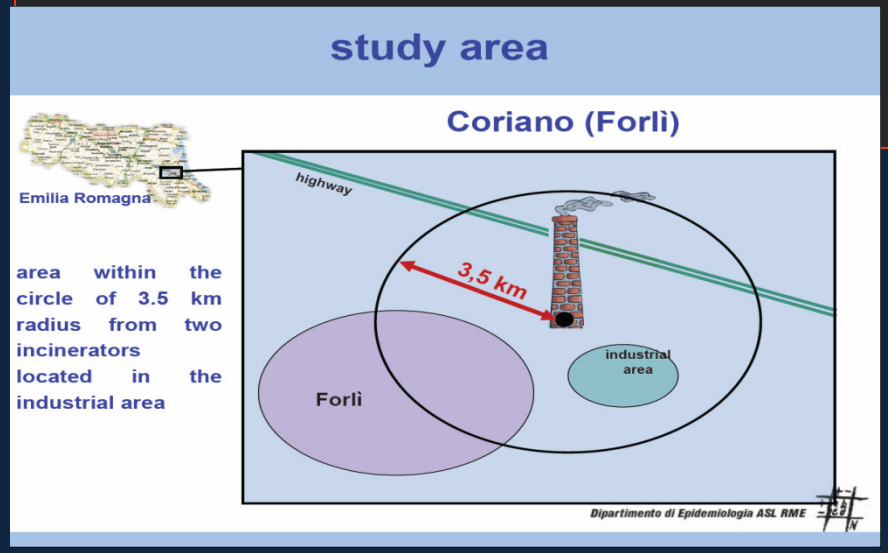
… a causa del **bio-accumulo/biomagnificazione degli *xeno-biotici*** in **ambiente, catena alimentare** e **tessuti degli organismi complessi** e della **possibile trasmissione transgenerazionale delle modifiche epigenetiche**

CHEMICAL BURDEN-Carico Chimico Globale

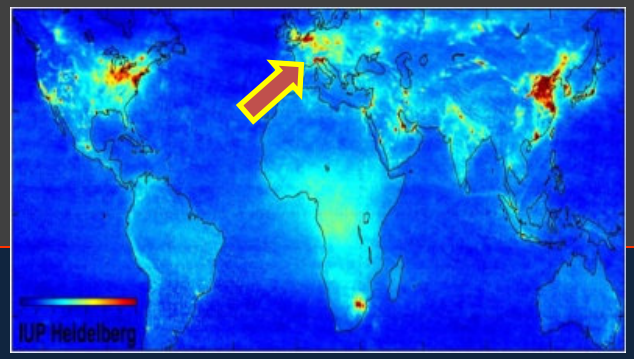


(in genere vengono paragonate **due popolazioni)**

- **una molto esposta..**
  (*id est* direttamente esposta alle emissioni dell'impianto)



- **l'altra un po' meno...**
  (*id est* più distante dall'impianto... ma comunque esposta agli stessi inquinanti (per via diretta o da fonti diffuse, *in primis* il traffico veicolare... o per via indiretta attraverso la catena alimentare)



Né il discorso cambia di molto se si paragonano
l'incidenza locale di una o più patologie (prevalentemente ***neoplastiche***)..
e le **cosiddette patologie attese**, che sono a loro volta il frutto
di una **esposizione massiccia e progressiva agli stessi inquinanti**
(e la cui incidenza aumenta nel tempo di pari passo all'inquinamento);

Endocrine Reviews 22(3): 319–341

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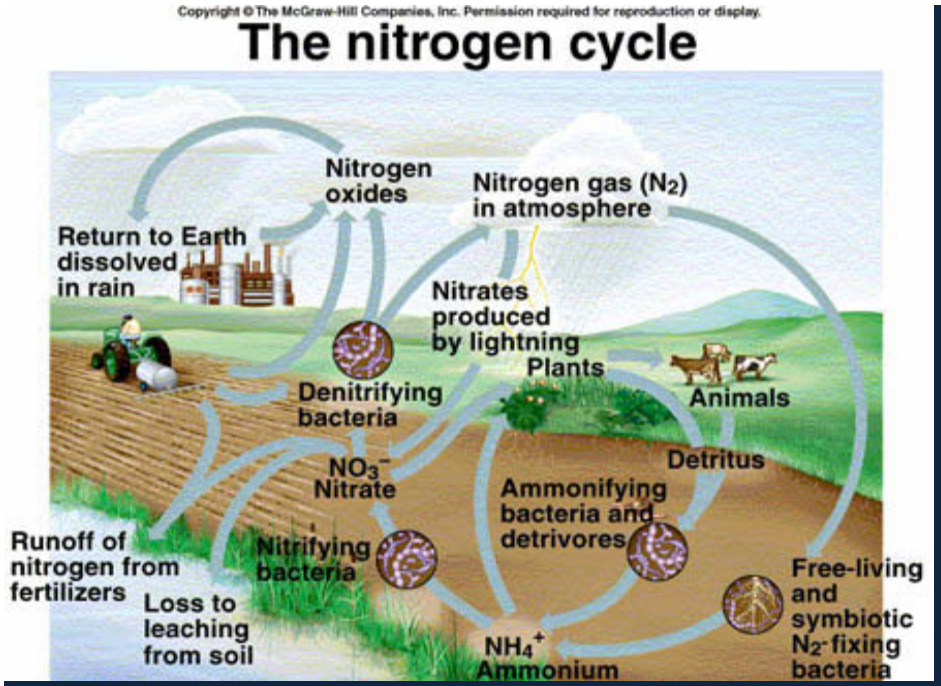
the "Steroid Cycle"…

Environmental Signaling: What Embryos and Evolution Teach Us About Endocrine Disrupting Chemicals

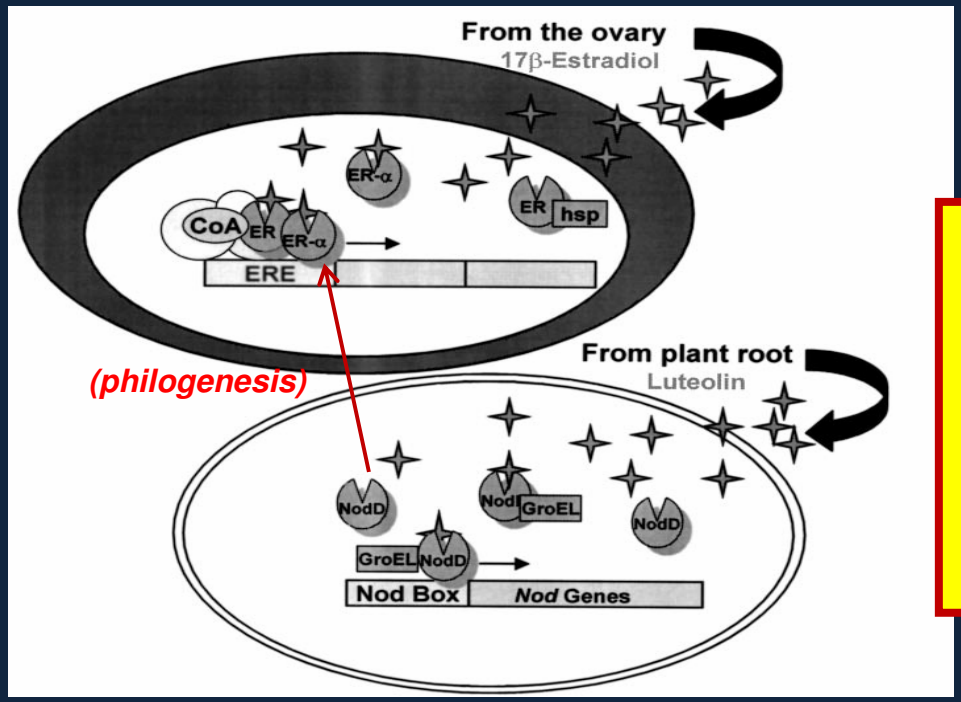
JOHN A. McLACHLAN

IN 1958, Dr. Roy Hertz described the **"steroid cycle,"** anticipating what we now call endocrine disrupter research, as follows: ". . . we have to consider that the **introduction of . . . [hormones into cattle feed lots] leads to the exposure . . . of individuals who might otherwise not ever in their lives come in contact with such materials** . . . . This is not a theoretical consideration because we . . . now have encountered two families, each with two children, who presented with **simultaneously developing gynecomastia** attributable to the accidental contamination of vitamin capsules by estrogens during manufacture. If such estrogens can, by stray handling, get into such pharmaceutical preparations, can they not very readily get where they are not wanted on the farm? There is one additional consideration in this regard . . . . **The fecal excretion of these materials . . . will be dropped on the soil and . . . over generations there will be constant replenishment of the soil surface with steroidal substances** of this kind.
This in turn has its effect potentially on surface water-supply contamination and also potentially on the **vegetable content of steroids in crops raised on such soil** . . . . I think that we are now actually **setting up a steroid cycle in our environment**, and **we have to give very serious consideration to its implications for our subsequent development and growth and possibly reproductive functions"** (taken from the discussion following Ref. 1).

1. **Gassner FX, Reifenstein Jr EC, Algeo JW, Mattox WE** 1958 Effects of hormones on growth, fattening, and meat production potential of livestock. Recent Prog Horm Res 14:183–217



Molecular nitrogen (N_2) is the most abundant element in the air we breathe. **Yet, large quantities of this nitrogen are not available to organisms.** Why is this the case? One reason molecular nitrogen is in short supply is because **it takes a large amount of energy to break the triple bond** that holds the two nitrogen elements together. **Nitrogen fixation is accomplished by nitrifying bacteria in soil and water.** These organisms are capable of breaking this bond and converting the nitrogen into **ammonia** (NH_3) and **nitrate** (NO_3). The resulting nitrogen is termed **fixed nitrogen**. Such a scenario allows **nitrifying bacteria living in conjunction with certain plants to convert atmospheric nitrogen into a form that plants can use** which is ultimately passed up the food chain. When organisms break down proteins during respiration, they release their nitrogen as **ammonia** (fish), **urea** (mammals), or **uric acid** (birds, reptiles and insects). **Decomposers** obtain nourishment from these products by converting them back into **ammonia**, which **plants** can then use again, completing the cycle. Finally, **denitrification**, accomplished again by **bacteria**, converts nitrate back into nitrogen gas



(POPs)

The Steroid Cycle

In light of the **genetic and functional similarities between ERa and NodD,** ***EDCs*** that are able to bind ***ERs*** and ***modulate*** signaling may employ the same mechanism to ***modulate*** the ability of *S. Meliloti NodD to respond to the phytoestrogen* signal, ***luteolin*** …

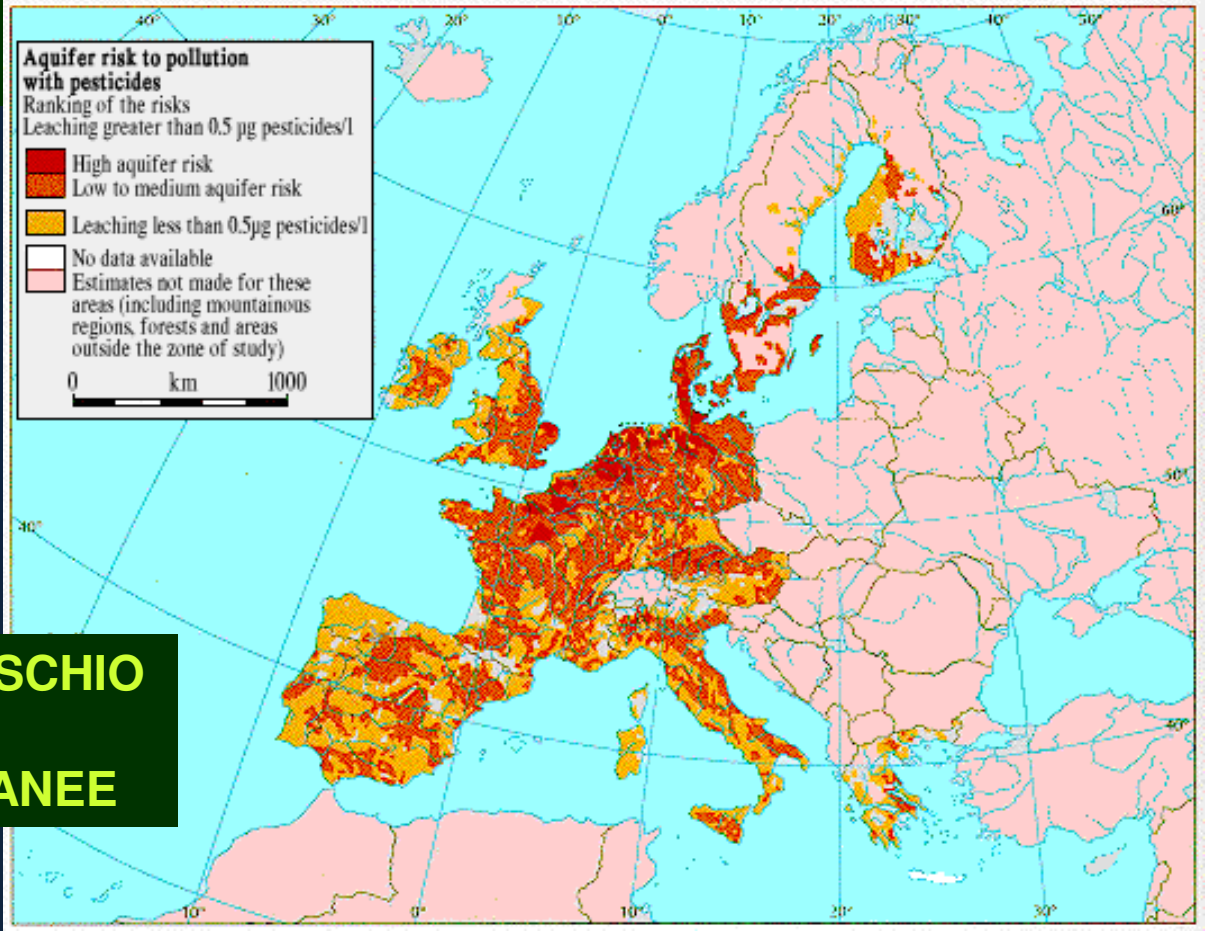
These results raise the possibility that **endocrine disruption may be seen in symbiotic environmental signaling systems that exist between organisms rather than within them.**

http://www.citruscollege.edu/apps/pub.asp?Q=847

ENDOCRINE DISRUPTORS

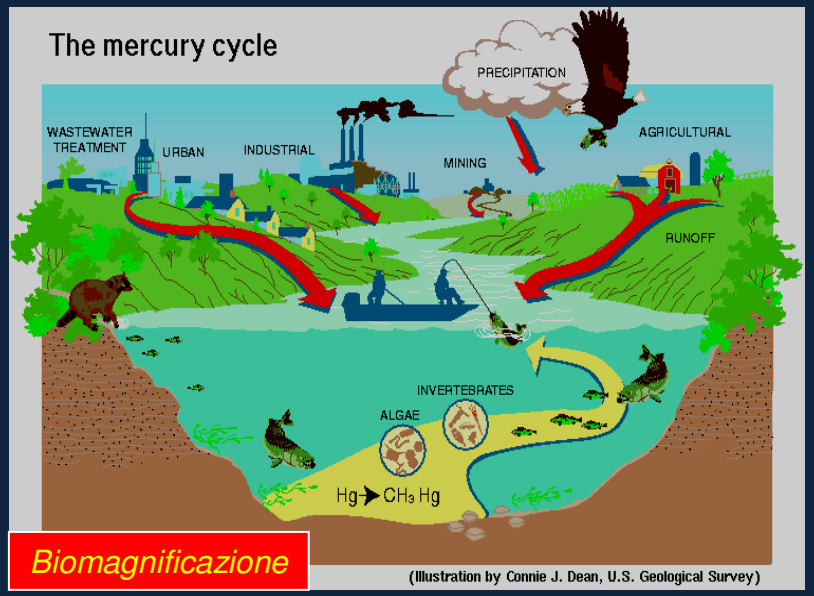
- Erbicidi, Pesticidi (Atrazina..)
- Idrocarburi Policiclici Aromatici (IPA)
- Policlorobifenili (PCBs)
- Policlorodibenzofurani (PCDFs)
- Policlorodibenenzodiossine (PCDDs) e molti altri....

.

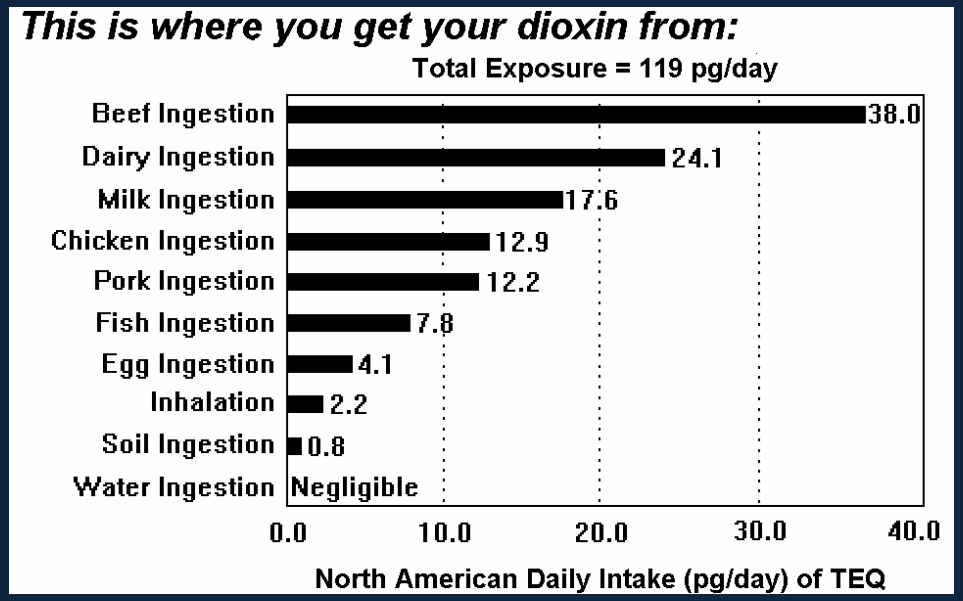


REGIONI EUROPEE A RISCHIO PER PESTICIDI NELLE ACQUE SOTTERANEE

E' vero che **metalli pesanti, diossine** e altri agenti cancerogeni immessi in ecosfera e così veicolati all'interno degli organismi viventi, si **bio-accumulano** nei tessuti (osseo e adiposo) e si **bio-magnificano** nelle catene alimentari ? E che dai tessuti in cui si sono accumulati (a volte per decenni) il loro rilascio è generalmente lento e continuo?



Biomagnificazione

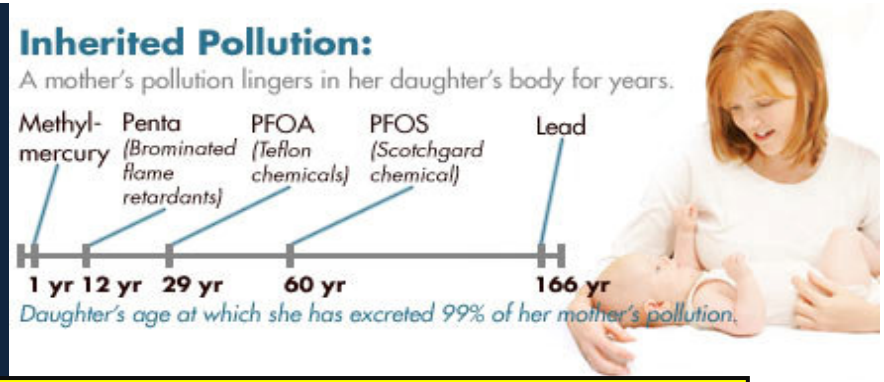


Is it 'true that heavy **metals, dioxins and other carcinogens** released into ecosphere, and conveyed in living organisms, may **bio-accumulate in *tissues*** (*bones* and *fat*) and **bio-magnify** in ***food chains***? And that from tissues where they accumulated (sometimes for decades), their **release** is generally slow and continuous?

3

What is the Global Chemical Burden..

Industrial chemicals in mothers and daughters: the pollution we share and inherit



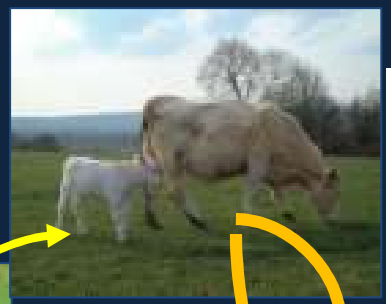
E' vero che **nel sangue e nei tessuti** di **tutti** gli uomini e le donne che vivono in ambienti urbani e/o industriali e persino nel **sangue cordonale e placentare** e nei **tessuti fetali** sono presenti questi stessi inquinanti in quantità di anno in anno, di decennio in decennio maggiori ?

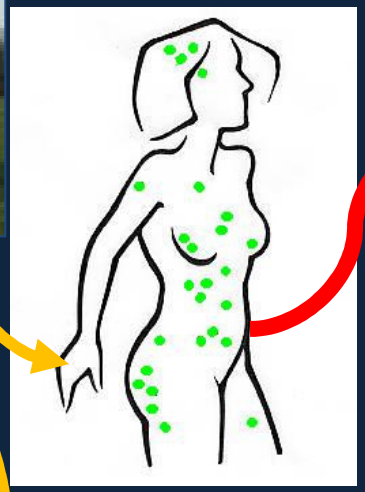
Table 1. Chronology of human exposure.

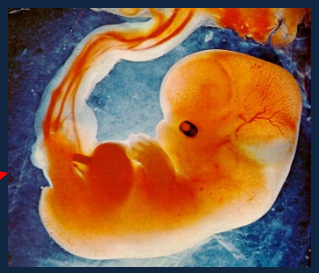
Years	Exposure scenario
1920s–1930s	BPA, PCBs, and DDT commercially introduced. Chlorine industry expanding. Discrete postnatal and prenatal exposure.
1940s–WWII	First wide-scale production and exposure to the above and other chemicals including plastics and chlorinated compounds as technology advanced.
1940s–1950s	First generation widely exposed postnatally and some who may have been exposed prenatally.
1950s–1970s	First generation born that was widely exposed prenatally.
1970s–1990s	First generation that was widely exposed prenatally reached reproductive age.
1980s–present	Second generation born that was exposed in the womb and beginning to produce the third generation. Production volume and exposure still increasing.

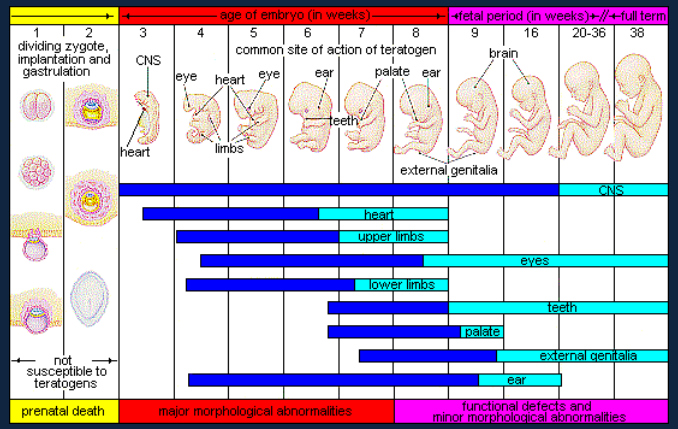
Is it true that these ***pollutants*** are present **in blood and tissues** of all men and women living in **urban** and **industrial environments** and even in the **cord blood and placental and fetal tissues** in more and more significant amounts year after year ?

E' vero, in particolare, che **metalli, diossine e altri inquinati lipofili** **accumulati nei tessuti materni** possono passare, anche **a distanza di anni dal loro assorbimento, nel sangue** e raggiungere il **feto** ?









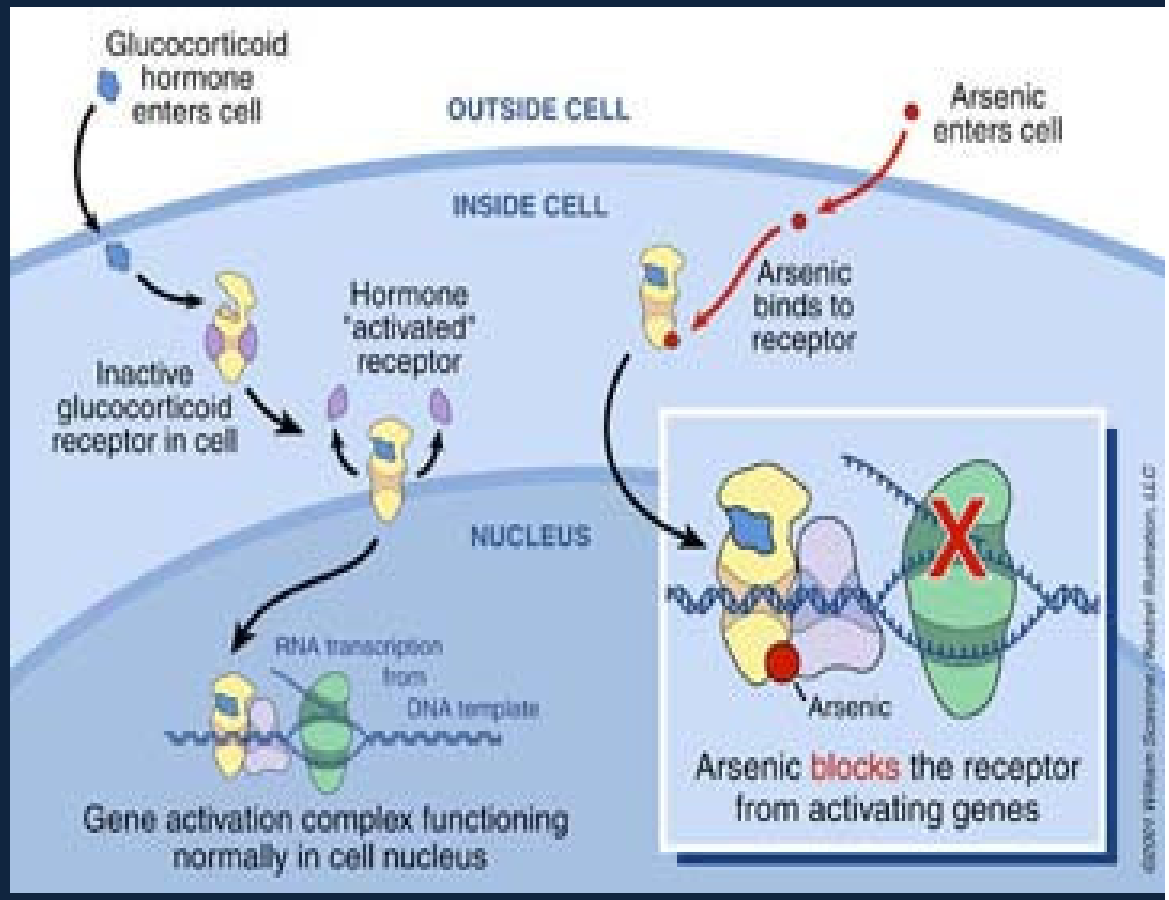
Is it true that **metals, dioxins** and other **lipophilic pollutants,** accumulated in maternal tissue, may pass, even **many years** after their absorption, into the blood and **reach the fetus**?

Everyday levels matter

At high levels… arsenic kills people

At moderately low levels… it causes a range of diseases

At truly low levels … it interferes with gene activation

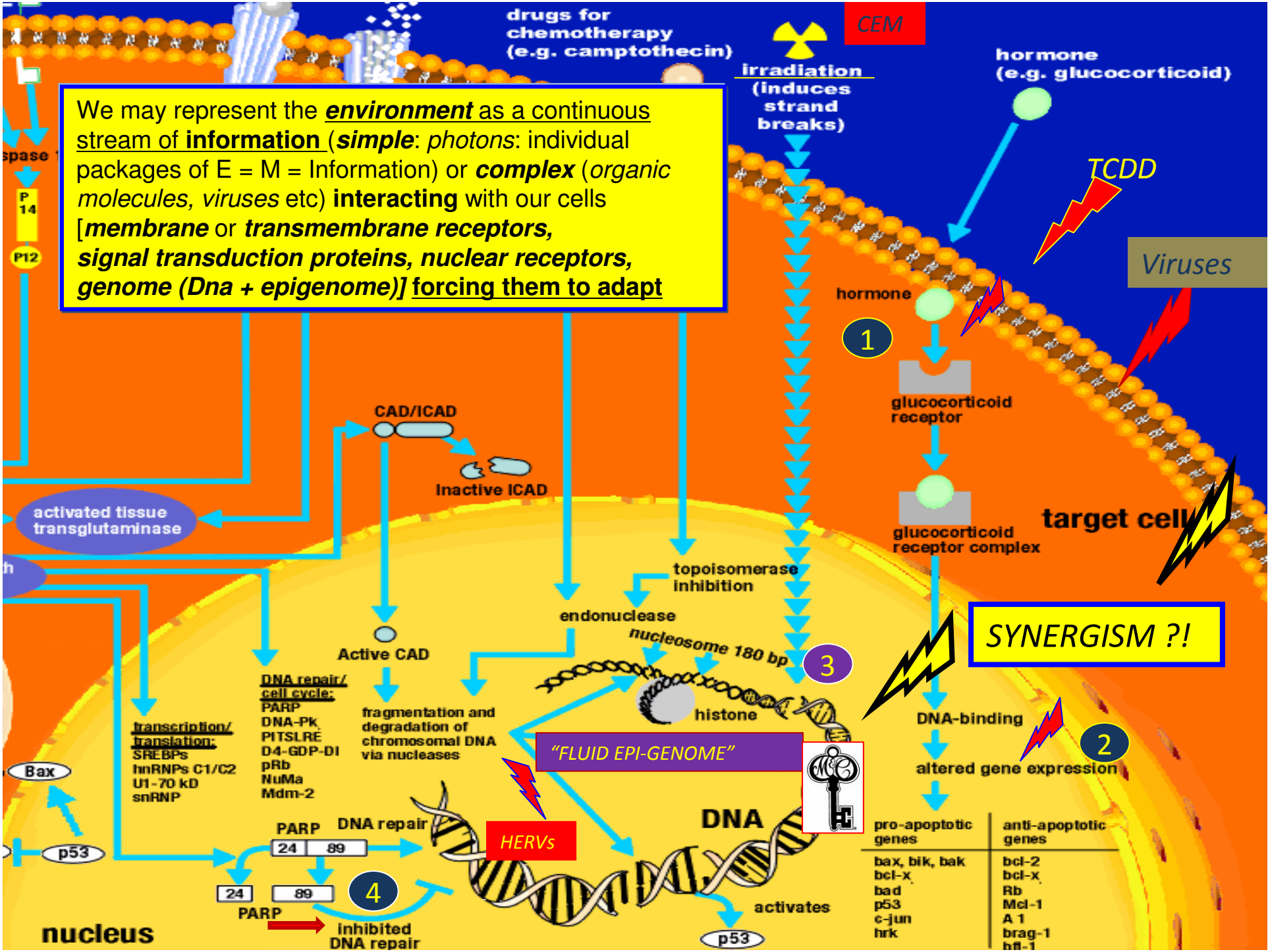


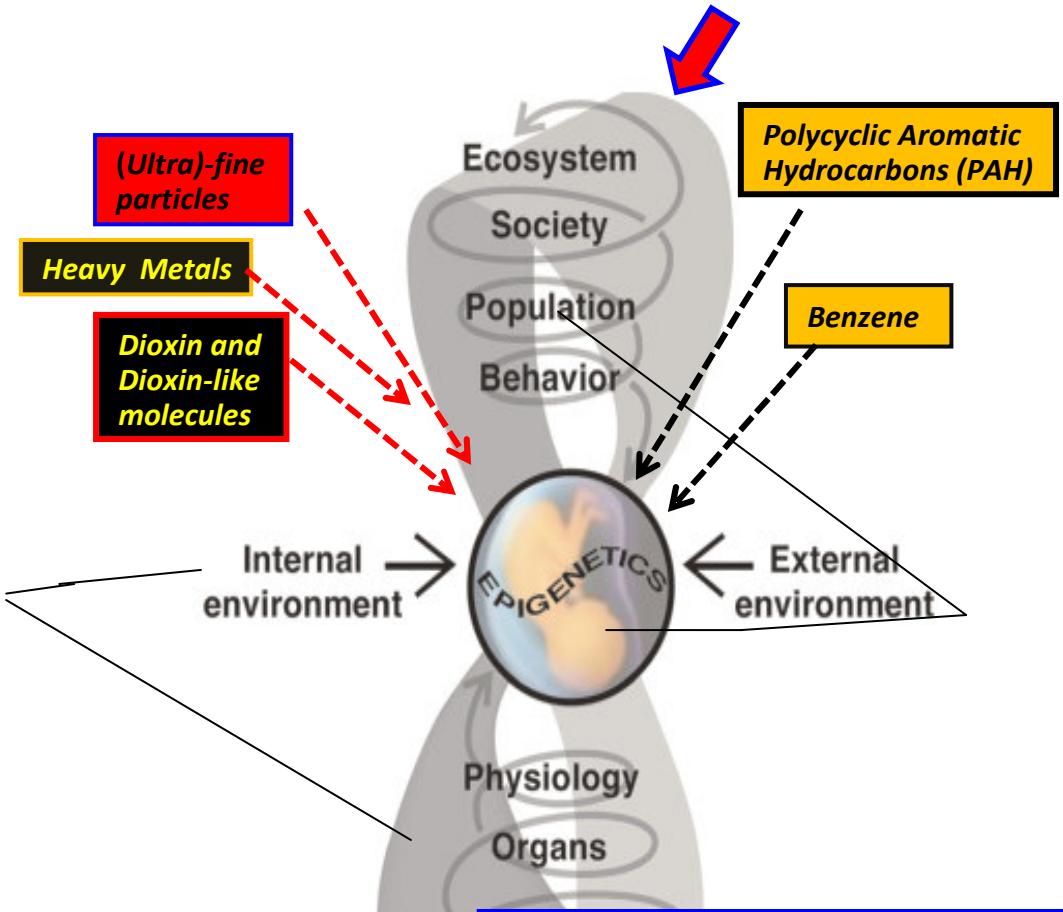
Many of these pollutants (***EDCs, Heavy Metals, Polycyclic aromatic hydrocarbons (PAHs***)) are **mutagens or epi-mutagens, carcinogens or co-carcinogens** at **infinitesimal doses**,

It is universally known that their **dangerousness** is linked to **daily exposure to very-small doses** rather than to **massive exposure**

Kaltreider *et al.* 2002

We may represent the ***environment*** as a continuous stream of **information** (***simple***: *photons*: individual packages of E = M = Information) or ***complex*** (*organic molecules, viruses* etc) **interacting** with our cells [***membrane*** or ***transmembrane receptors, signal transduction proteins, nuclear receptors, genome (Dna + epigenome)]*** **forcing them to adapt**



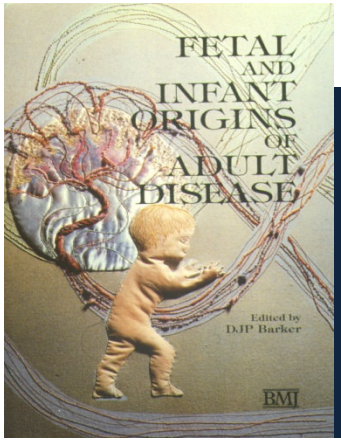


1

….particular awareness has aroused a study that documented the presence of **(geno) toxic and mutagenic substances in all the umbilical cords tested, demonstrating the ubiquity of embryo-fetal exposure**

Mothers Milk: Record levels of toxic fire retardants found in American mothers' breast milk. Washington, DC. Available at http://www.ewg.org/reports/mothersmilk/ NAS (National Academy of Sciences). 2000. ***Scientific Frontiers in Developmental Toxicology and Risk Assessment. Committee on Developmental Toxicology***. Washington, DC: National Academies Press; EPA (U.S. Environmental Protection Agency). 2003a. ***America's children and the environment. Measures of contaminants, body burdens, and illnesses***. Available online at http://www.epa.gov/envirohealth/children.

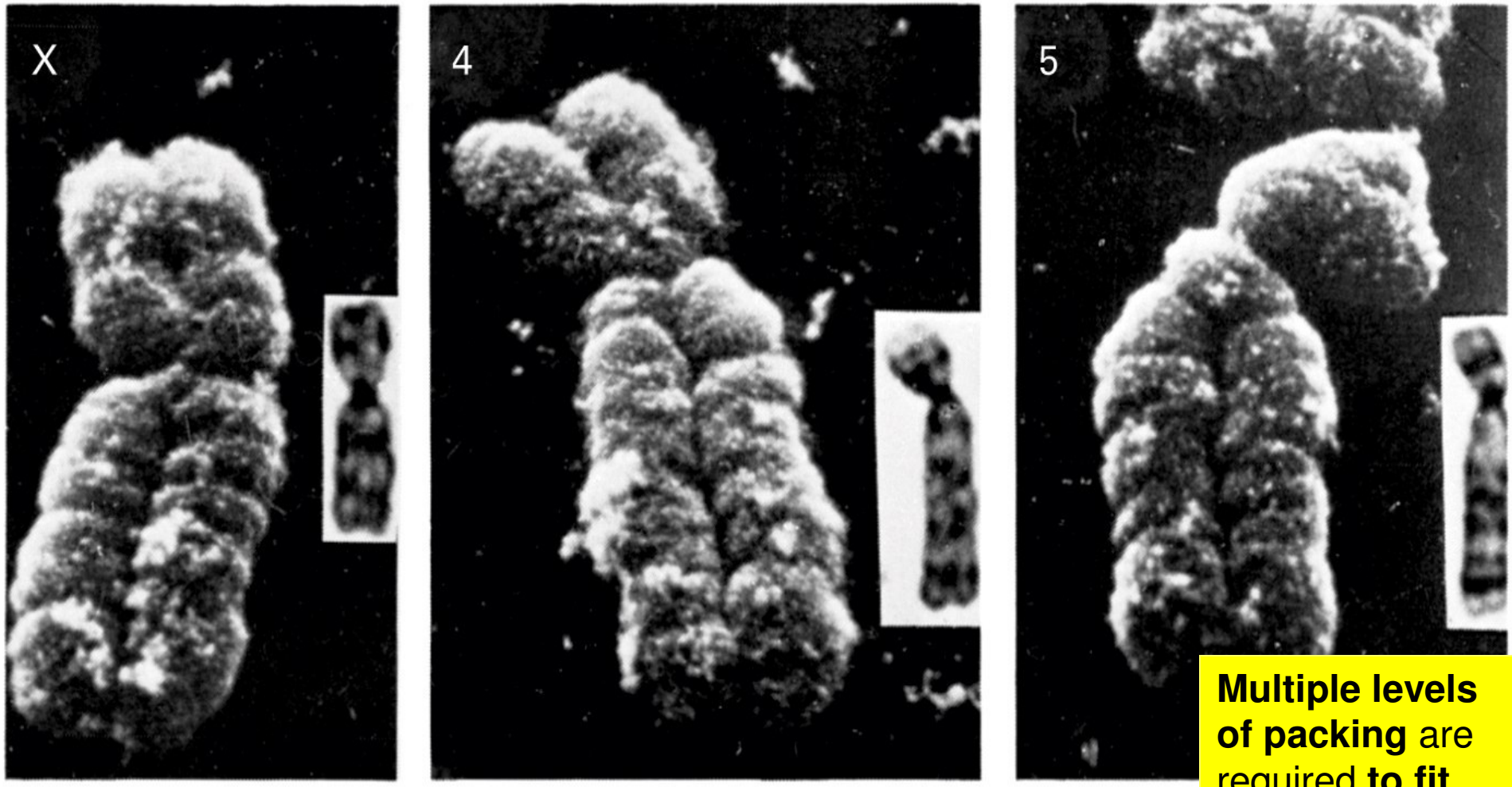
2



3

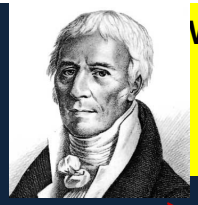
FIG. 1. The fetus is particularly vulnerable to changes in the external and internal environments, which interact to influence fetal development and have both immediate and life-long consequences. Such environmentally induced changes can occur at all levels of biological organization, from the molecular to the organism's behavior and place in society, and tend to be amplified in their consequences as they ascend through these levels. Ultimately, these influences may be epigenetic in nature, inducing mitotically heritable alterations in gene expression without changing the DNA.

2 What's ***Epi-Genetics*** ?



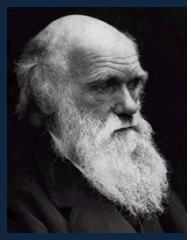
Multiple levels of packing are required **to fit the DNA** into the cell **nucleus**

The chimpanzee Dna is for 98.77% identical to the human .
On average, a gene encoding a protein in a man differs from its chimpanzee ortholog by only two aa substitutions
.. almost one third of human genes
has exactly the **same protein translation** as their orthologs
in chimpanzee



We are quite stable (for millions of years) both genetically and phenotypically

Species *phylogeny*

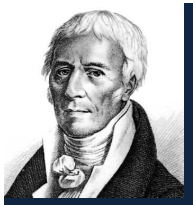


Evo

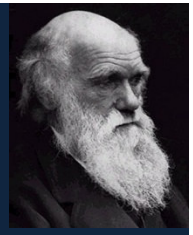
From the Tree of the Life Website, University of Arizona

Orangutan | *Gorilla* | ***Chimpanzee*** | ***Human***

Sanger Institute



Chimpanzee-human divergence



Evo

Hominids or hominins

Brain:
a *rapidly evolving Organ ?*

Broad phenotypic variation in humans

Their DNA sequences differ by 0.1%

(1 in 1,000 base pairs are different)

Epigenetic modifications : a molecular environmental effect

Nature

Genetics

Critical determinants of the epigenome

Nurture

Environment

Devo

Acute enviroemental events

Daily (low intensity) enviromental events

Celiac Disease

Why this disease is concordant in only **60%** to **70%** of **identical twins.**

Genetic Mutations etc.

Epi-Mutations

CHANGING EPIGENOME

And Alzheimer Disease ?

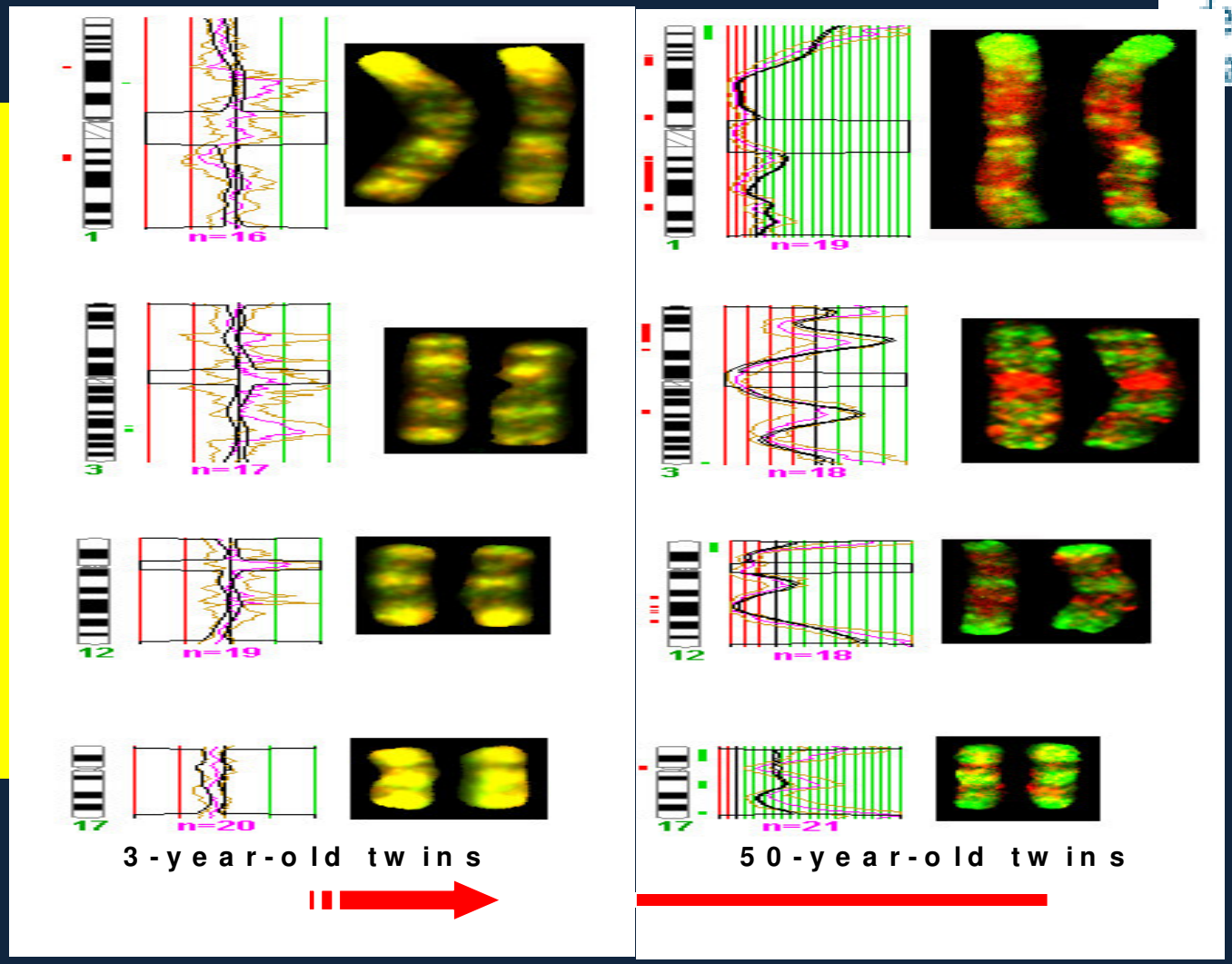
Epigenetic differences arise during the lifetime of monozygotic twins

Fraga et al., PNAS. 2005.

Instituto de Salud Carlos III

Epigenetic differences in homozygotic twins

… although **twins are epigenetically indistinguishable during the early years** of life,
… **older monozygous** twins exhibited remarkable differences in their **overall content and genomic distribution** of 5-methylcytosine DNA and histone acetylation, affecting their gene-expression portrait.



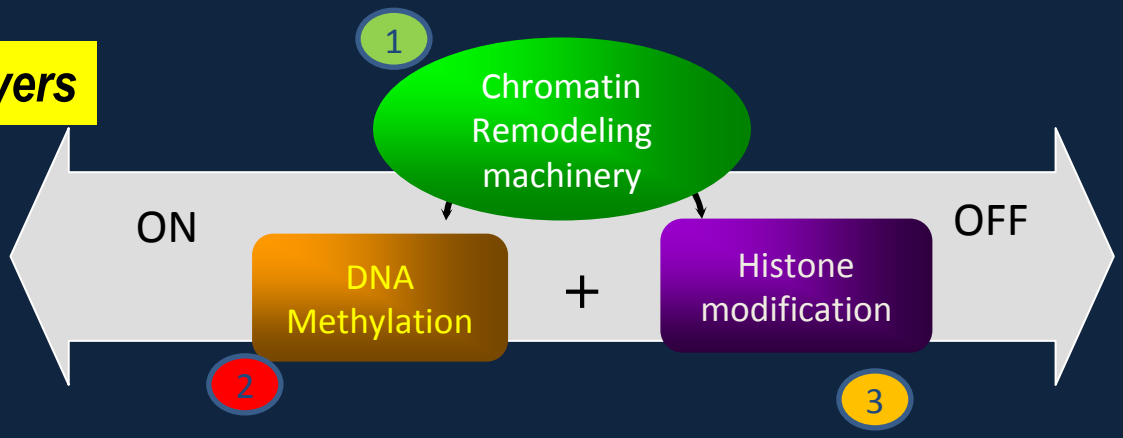
Epigenetic differences arise during the lifetime of monozygotic twins

Fraga et al., PNAS. Jul 26 (2005);102(30):10604-9..

What is ***Epi-Genetics ?***

Instituto de Salud Carlos III

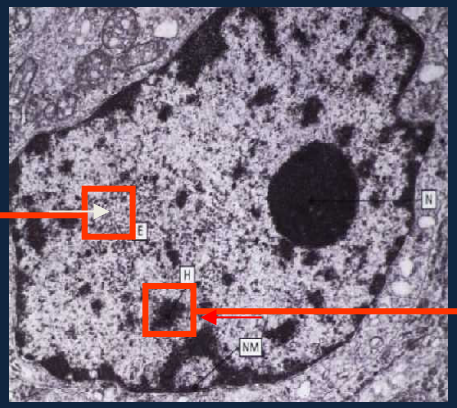
The Epigenetic Players



4

MicroRNAs

Euchromatin

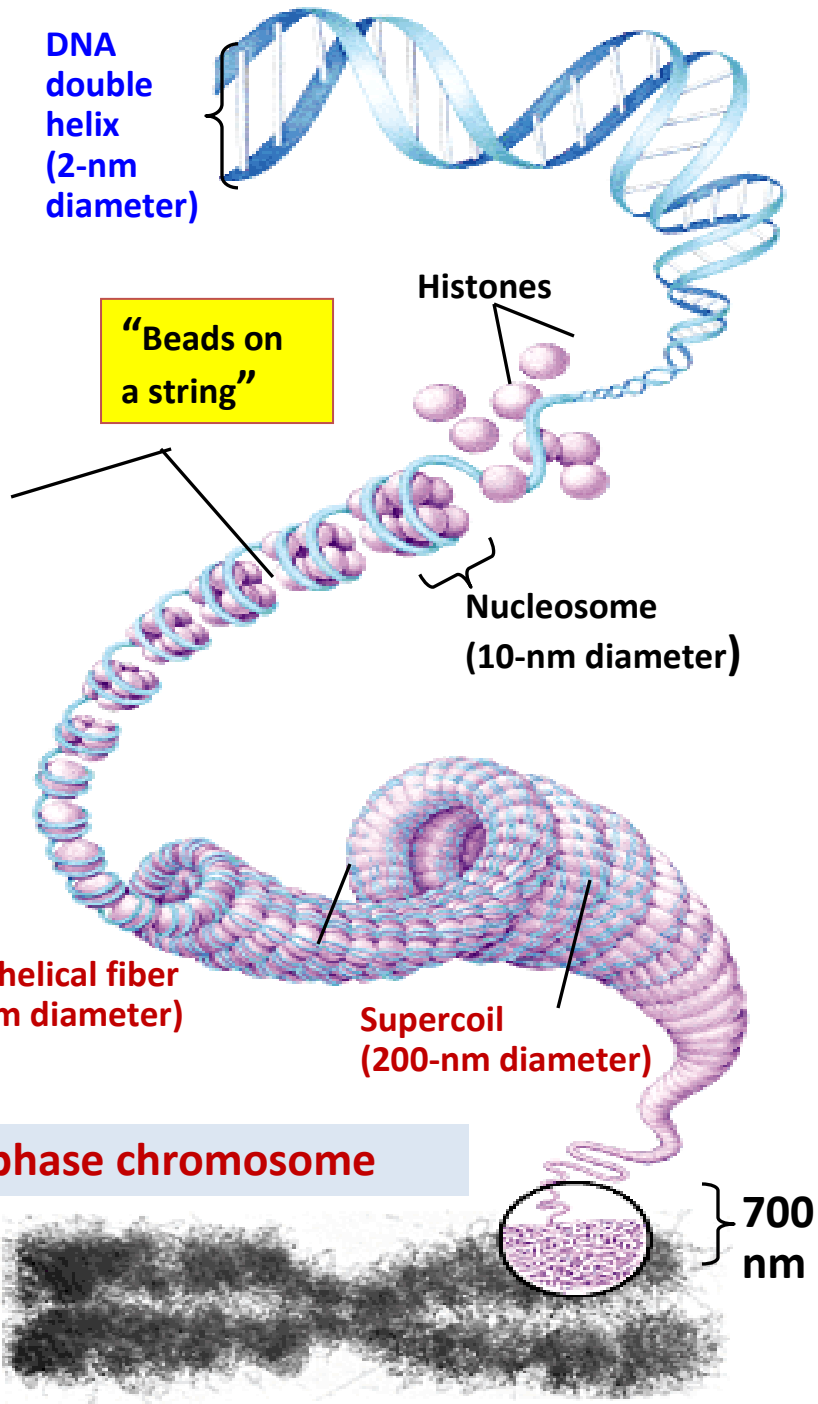


Heterochromatin

Epigenetics is defined as <u>mitotically and meiotically heritable changes in gene expression that do not involve a change in the DNA sequence</u>…. <u>DNA methylation</u> and <u>histone modifications</u> are known to have profound effects on controlling gene expression.. <u>MicroRNAs (miRNAs)</u> are small RNA molecules, _22 nucleotides long that can <u>negatively control their target gene expression posttranscriptionally</u>

Chuang JC, Jones PA ***Epigenetics and MicroRNAs*** Pediatr Res 2007; 61: 24R–29R

Nuclear DNA is **normally tightly wrapped around histones**



Metaphase chromosome

Euchromatin

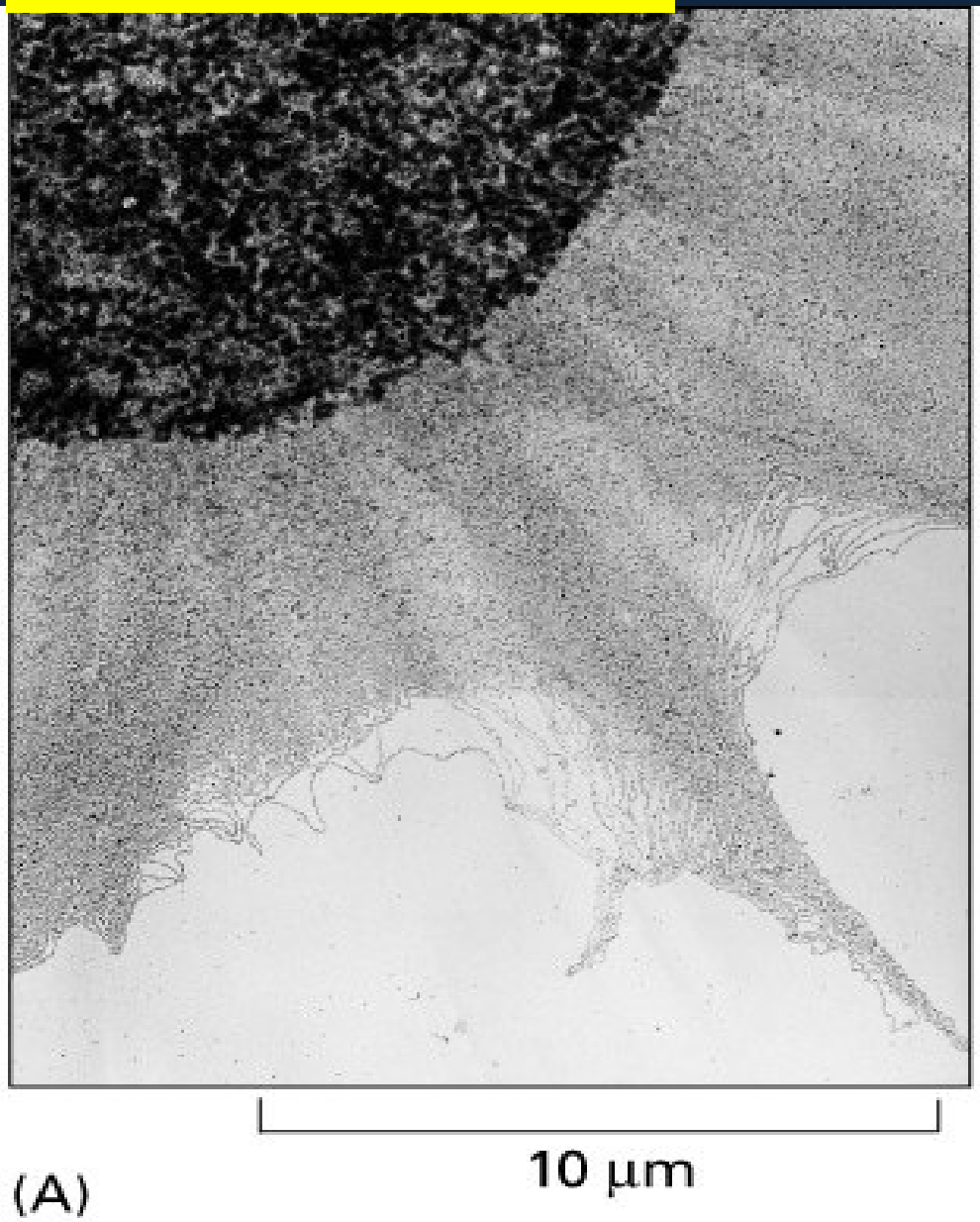
Multiple levels of packing are required **to fit the DNA** into the cell **nucleus**

Heterochromatin

Campbell NE et al (Eds):
Biology: Concepts & Connections
4th Edition, 2003

Interphase chromosomes

Mitotic chromosome



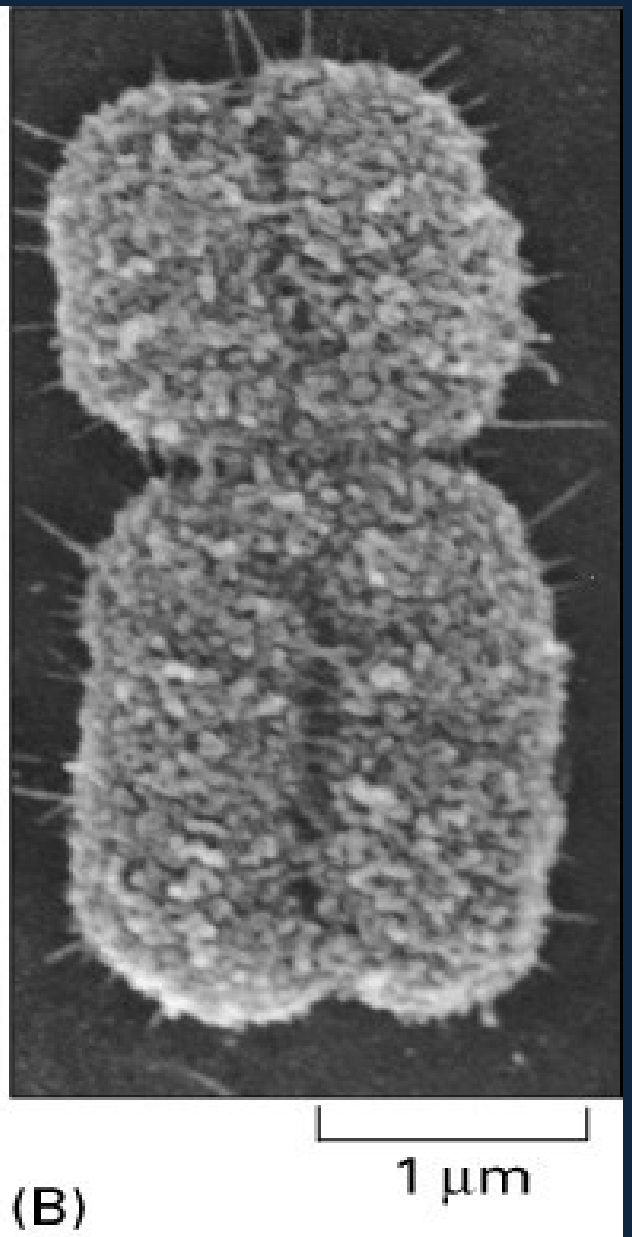
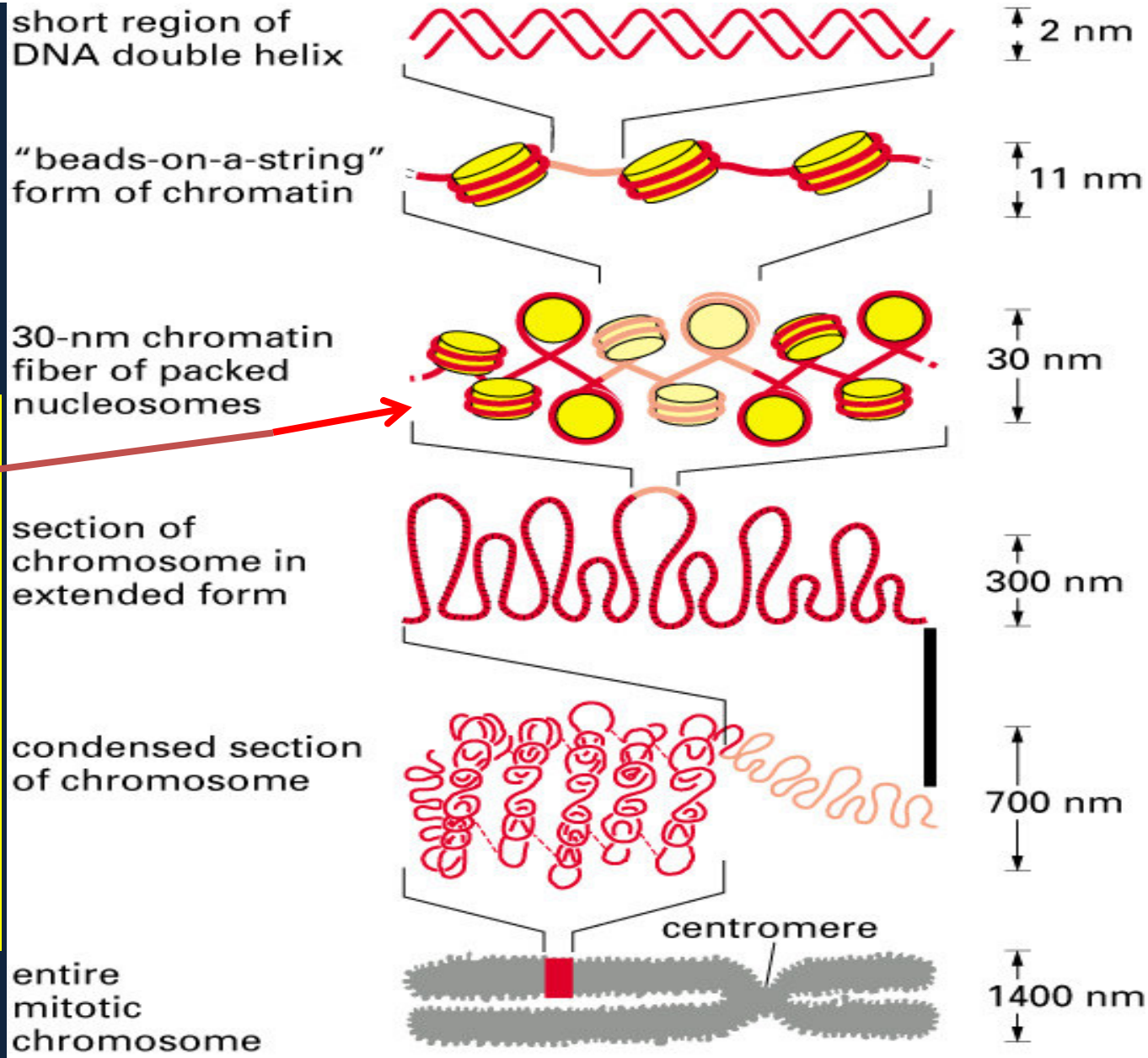


Figure 4–21. Molecular Biology of the Cell, 4th Edition.

Nuclear DNA is **normally tightly wrapped around histones rendering the DNA inaccessible to the general transcription machinery** and hence this **tight** association **prevents transcription of DNA**

Eterochromatin



NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 10,000-FOLD SHORTER THAN ITS EXTENDED LENGTH

Figure 4–55. Molecular Biology of the Cell, 4th Edition.

The
Histone tails
are a **critical**
determinant
of chromatin structure

1

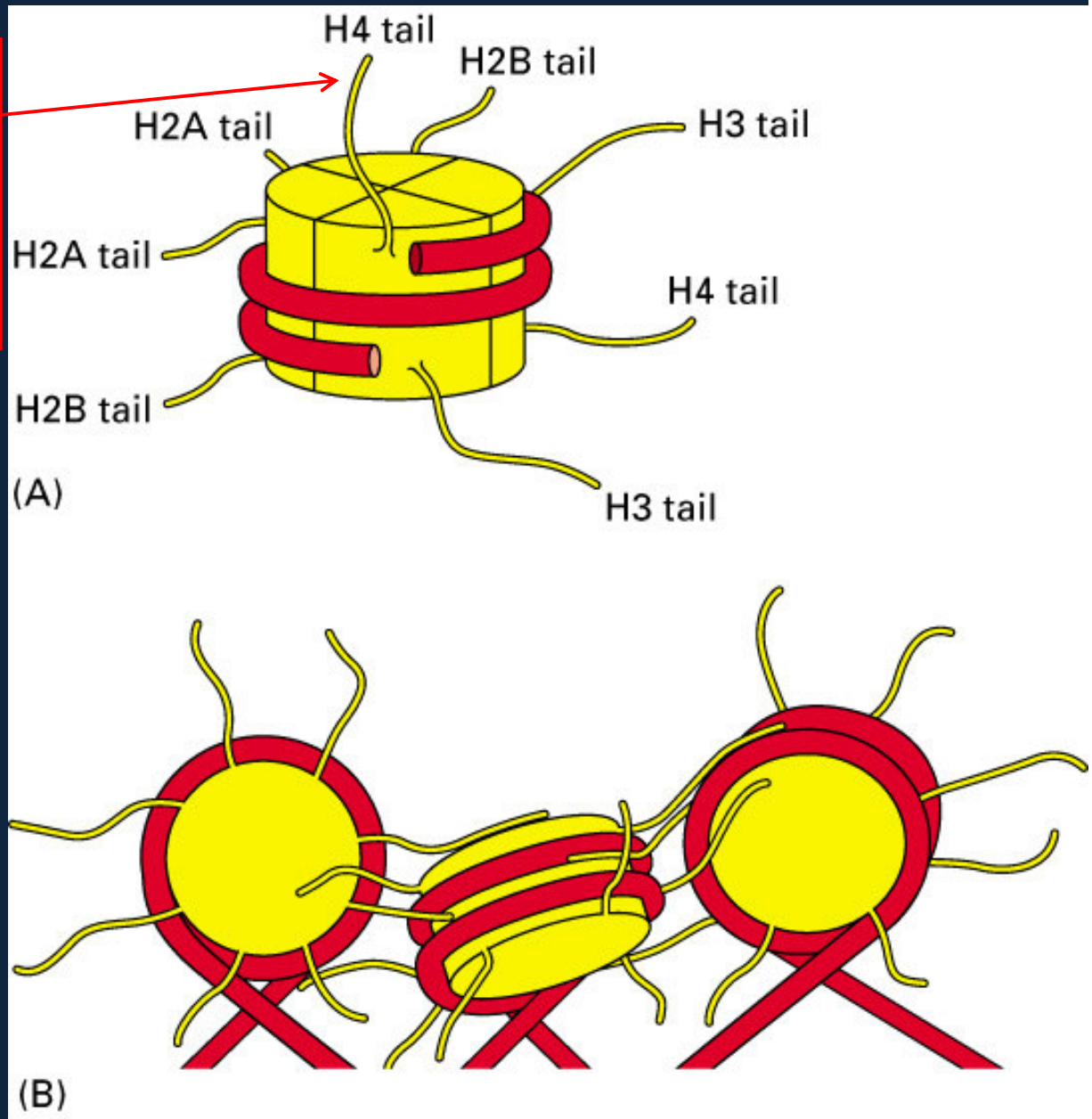


Figure 4–32. Molecular Biology of the Cell, 4th Edition.

Histone Tails are subject to a variety of **covalent modifications**

Histone Code" hypothesis: **modifications of the Histone tails** act as marks **read by other proteins** to control the **expression** or **replication** of chromosomal regions

E.g. generally,
Histone Acetylation *is associated with* ***transcriptionally active genes***
Deacetylation is associated with **inactive genes (= gene silencing)**

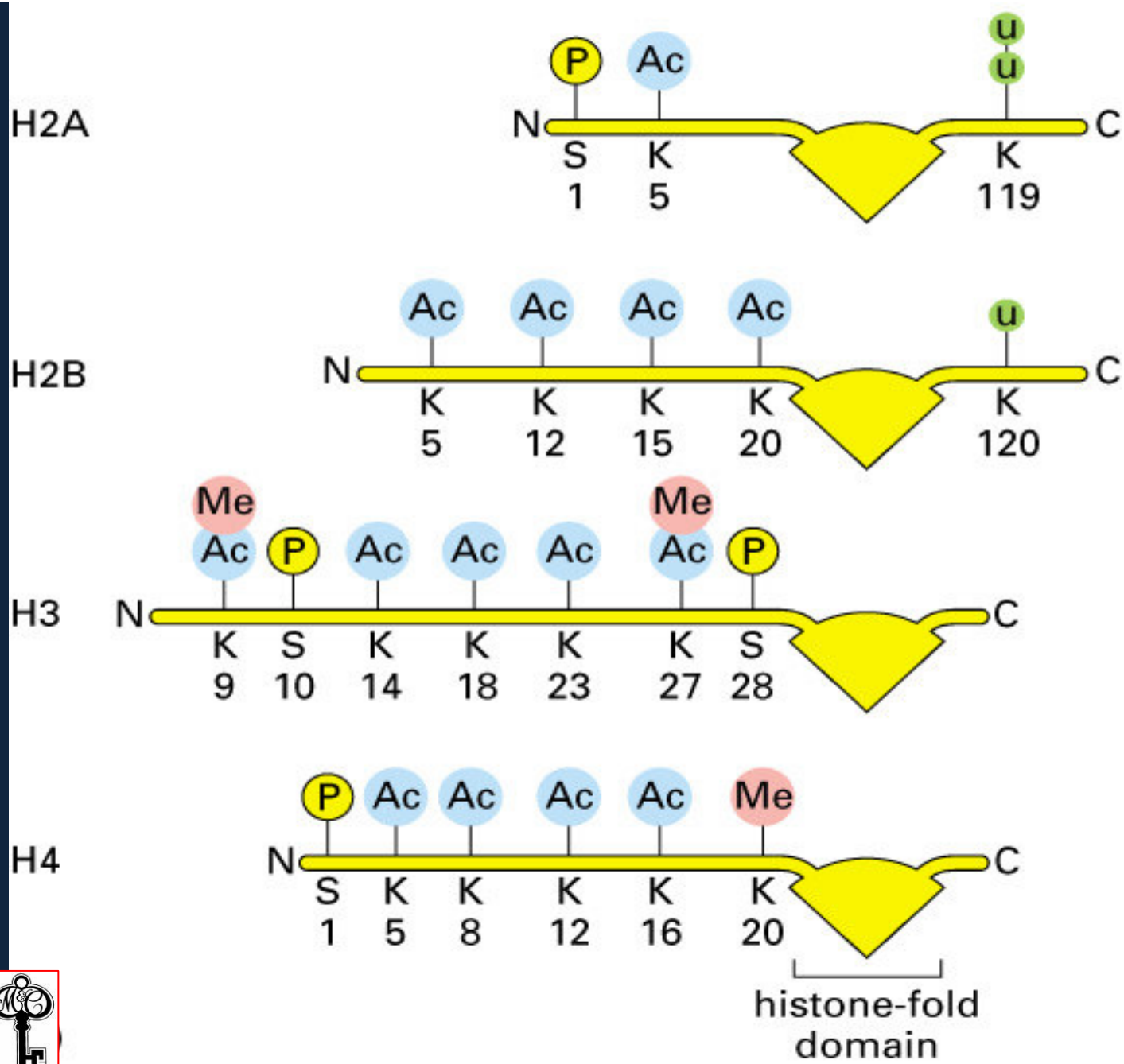


Figure 4–35 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

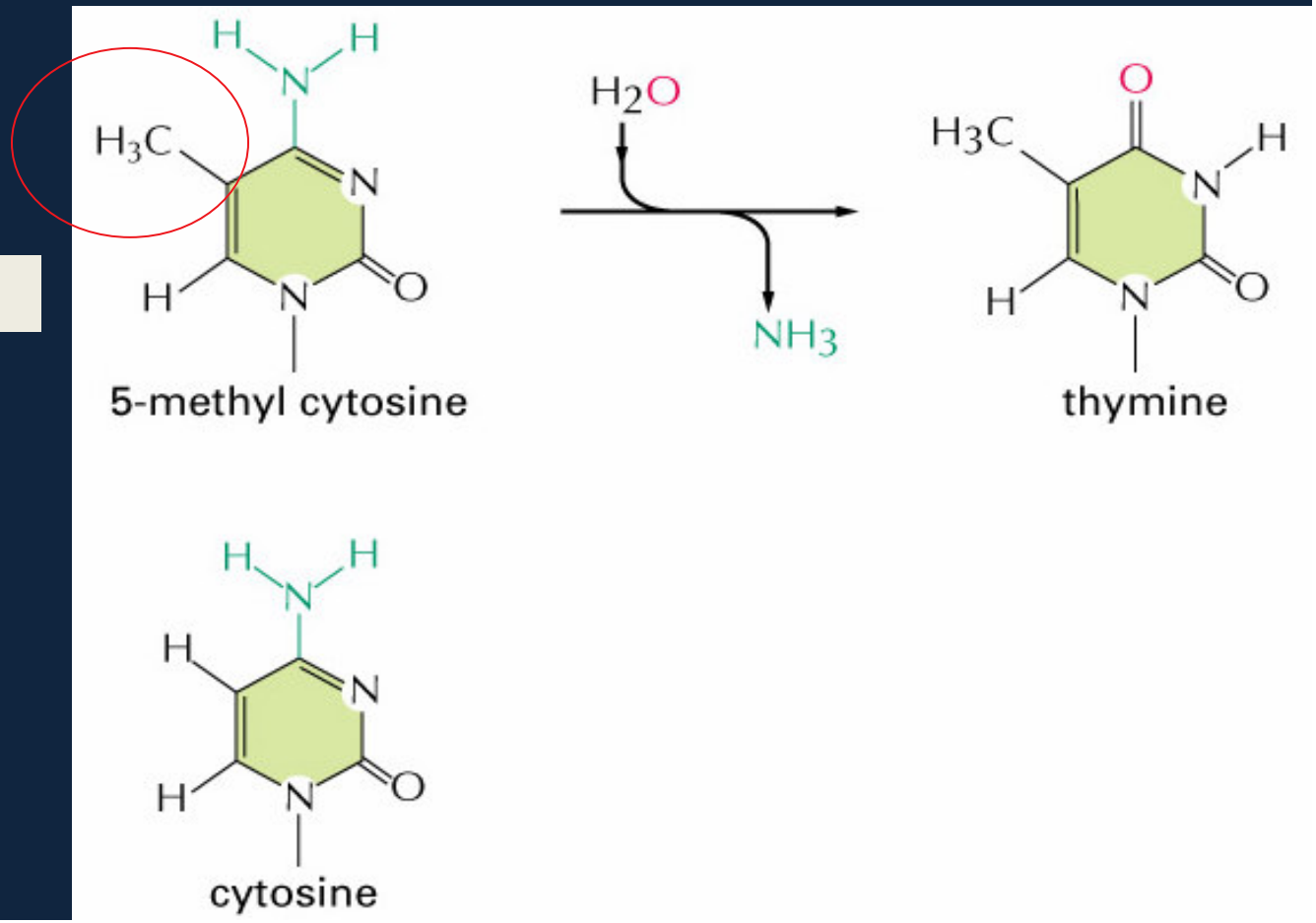
DNA methylation

→ **Covalent modification of the DNA** is also important for gene silencing human cells.

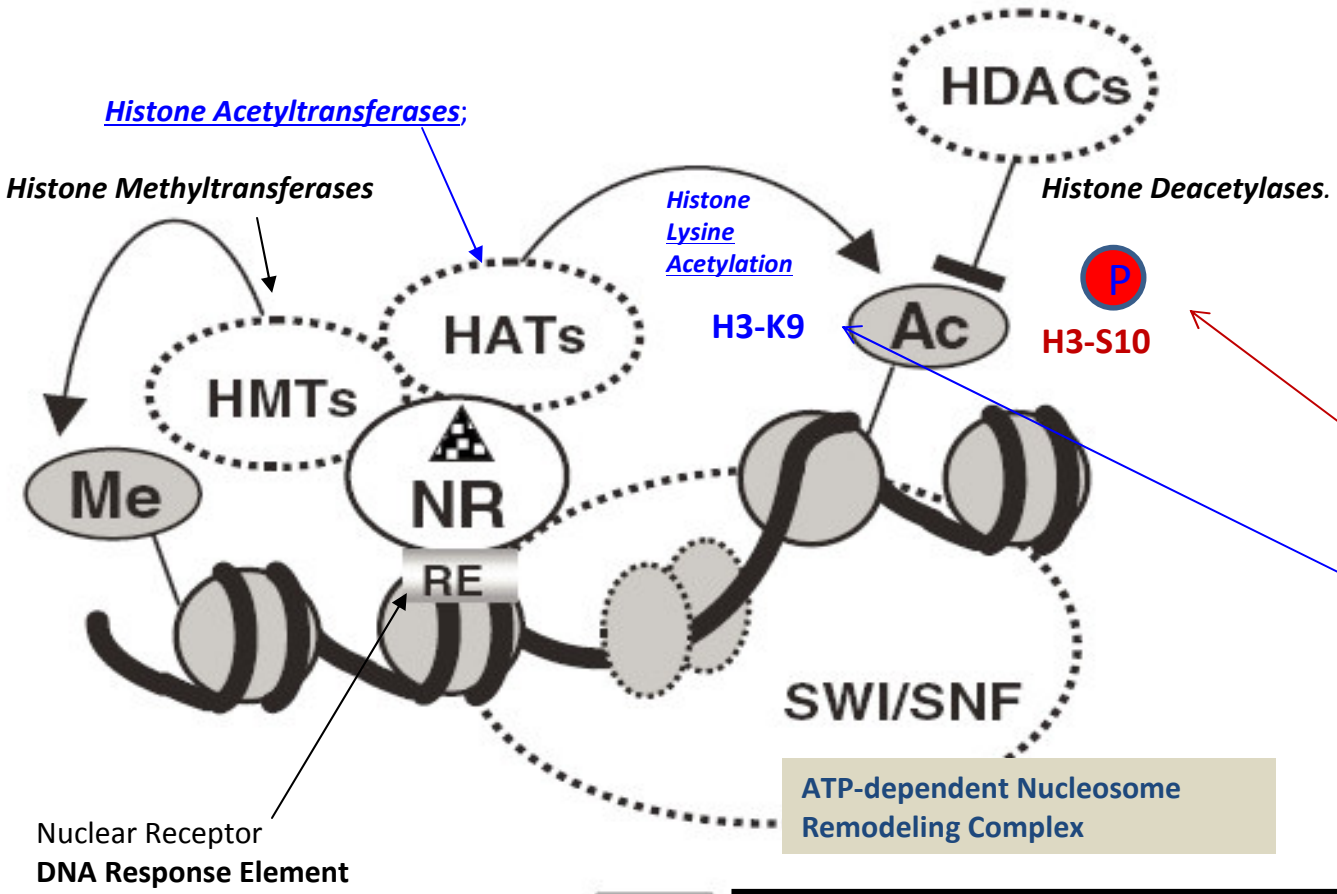
→ **Most genes have GC rich areas of DNA in their promoter regions,** referred to as **CpG islands.**
→ **Methylation of the C residues within the CpG islands leads to gene silencing**

2

(highly *unstable* base)



Modification of chromatin structure by histone modifying and nucleosome remodelling proteins



These modifications are believed to be essential for the full transcriptional response to stress-inducing chemicals

Many toxicants cause rapid alterations in gene expression by **activating protein kinase signaling cascades.**

The resulting **rapid, defensive alterations in gene activity require the transmission of a signal directly to the histones** present in the **chromatin of stress response genes**:

within minutes of exposure the **phosphorylation of serine 10 of histone H3** and the **acetylation of lysines 9 and/or 14** take place

Epigenetics and environmental chemicals

Andrea Baccarelli and Valentina Bollati

Current Opinion in Pediatrics 2009, 21:243–251

Purpose of review

Epigenetics investigates heritable changes in gene expression occurring without changes in DNA sequence. Several methylation, histone modifications, a function under exogenous influence, epigenetic alterations mediate toxic

Recent findings

In-vitro, animal, and human investiga environmental chemicals that modify arsenic, nickel, chromium, and meth (trichloroethylene, dichloroacetic aci carbon, and benzene), and endocrin (diethylstilbestrol, bisphenol A, persistent organic pollutants, and dioxin). Most studies conducted so far have been centered on DNA methylation, whereas only a few investigations have studied environmental chemicals in relation to histone modifications and microRNA.

In-vitro, animal, and human investigations have identified several classes of **environmental chemicals that modify epigenetic marks..** including

- ***metals*** (***cadmium, arsenic, nickel, chromium, CH3-mercury***),
- ***peroxisome proliferators*** (***trichloroethylene, dichloroacetic acid…***),
- ***Air Pollutants (PM 0,1/2,5/10, black carbon, benzene),***
- ***EDCs*** **- Endocrine-Disrupting/reproductive toxicants** (***DES, bisphenol A, persistent organic pollutants, dioxin***).

Summary

For several exposures, it has been proved that chem and that the same or similar epigenetic alterations c disease of concern or in diseased tissues. Future pros to determine whether exposed individuals develop epi in turn, which such alterations increase the risk of di needed to determine whether environmental epigenetic changes are transmitted transgenerationally.

Because these **epigenetic changes are small, potentially cumulative**, **and they may develop over time**, it may be difficult to establish the cause-effect relationships among **environmental factors**, **epigenetic changes**, and **diseases**.

Table 1 Effects of environmental chemicals on DNA methylation

Exposure	↑/↓*	Genes	Type	Tissue	References
Arsenic	↓	Global	Rat	Liver	Zhao *et al.* [19]
	↑	*p53*	*In vitro*	A549 cells	Mass and Wang [20]
	↑↓	Multiple genes	*In vitro*	Human kidney cells	Zhong and Mass [21]
	↑	*p16*, *p53*	Human	PBL	Chanda *et al.* [22]
	↑	Global	Human	PBL	Pilsner *et al.* [23,24]
	↑	*p16*	Human	PBL	Zhang *et al.* [25]
Cadmium	↓	Global	*In vitro*	Rat liver cells	Takiguchi *et al.* [26]
Nickel	↑	*ATF-1*, *HIF-1*, *Rb*	*In vitro*	G12 cell line	Lee *et al.* [27]
	↑	*p16*	Mouse	Histiocytomas	Govindarajan *et al.* [28]
Chromium	↑	*p16*	Human	Lung	Kondo *et al.* [29]
Methylmercury	↑	*BDNF*	Mouse	Hippocampus	Onishchenko *et al.* [30]
TCE, DCA, TCA	↑	*c-jun*, *c-myc*	Mouse	Liver	Tao *et al.* [31]
Air pollution	↓	Global (*Alu*, *LINE-1*)	Human	Buffy coat	Tarantini *et al.* [32]
	↓	*iNOS*			
Benzene	↓	Global (*Alu*, *LINE-1*)	Human	Blood	Bollati *et al.* [33]
	↑	*p15*			
	↓	*MAGE*			
Vinclozolin	↑	Gene-specific	Ray	Testis	Anway *et al.* [34]
DES	↓	Global	Mouse	Uterus	Li *et al.* [35]
BPA	↓	Agouti gene, *CabpIAP*	Mouse	Embryo	Dolinoy *et al.* [36]
POPs	↓	*Alu*, *LINE*	Human	Blood	Rusiecki *et al.* [37]

ATF-1, activating transcription factor 1; *BDNF*, brain-derived neurotrophic factor; BPA, bisphenol A; DCA, dichloroacetic acid; DES, diethylstilbestrol; *HIF-1*, hypoxia-inducible factor-1; *LINE-1*, long interspersed nuclear element-1; *MAGE*, melanoma antigen-1; PBL, peripheral blood leukocyte; *Rb*, retinoblastoma; TCE, trichloroethylene.

*Increase (↑) or decrease (↓) in DNA methylation.

Table 2 Effects of environmental chemicals on histones

Exposure	↑/↓*	Modification	Type	Tissue	References
Nickel	↓	Acetylation	*In vitro*	Liver, brain	Ke *et al.* [38]
	↑	H3K9 dimethylation			
	↑	H2A and H2B monoubiquitination			
	↓	H4K12 acetylation	*In vitro*	Yeast cells	Broday *et al.* [39]
	↓	H4K4 acetylation	*In vitro*	Mammalian cells	
	↑	H3K9 monomethylation and dimethylation	*In vitro*	G12 cell line	Chen *et al.* [40]
	↓	Acetylation of histone H2B	*In vitro*	HAE and NRK cell lines	Golebiowski and Kasprzak [41]
	↓↑	H2B ubiquitination	*In vitro*	HAEo and HPL1D cell lines	Karaczyn *et al.* [42]
Arsenic	↑	H3K9 dimethylation	*In vitro*	A549 cell line	Zhou *et al.* [43]
	↓	H3K27 trimethylation			
	↑	H3K4 trimethylation			

HAE, human airway epithelial; NRK, normal rat kidney.
*Increase (↑) or decrease (↓) in histone modification.

Table 3 Effects of environmental chemicals on microRNA

Exposure	↑/↓*	Genes	Type	Tissue	References
RDX	↓	Tumor-suppressing miRNAs	Mouse	Liver, brain	Zhang and Pan [44]
	↑	Oncogenic miRNAs			
Arsenic	↓↑	Multiple miRNAs	*In vitro*	Lymphoblastoid cells	Marsit *et al.* [18]

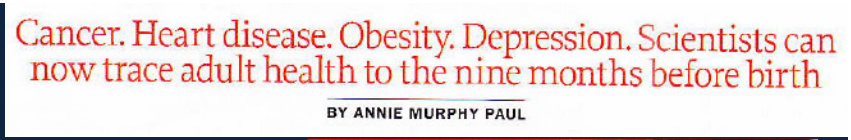
miRNAs, microRNAs; RDX, hexahydro-1,3,5-trinitro-1,3,5-triazine.
*Increase (↑) or decrease (↓) in microRNA expression.

Parole chiave
EDCs
DOHAD
Fetal Programming
Fluid (Epi-Genome)

1

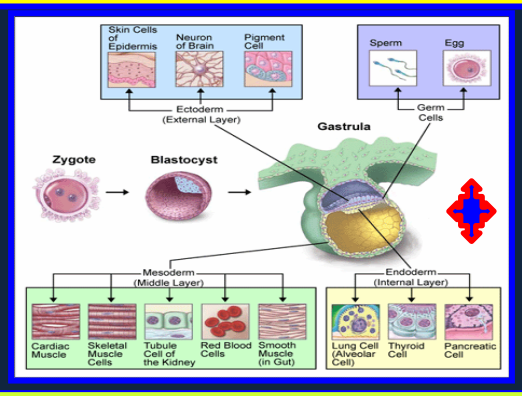
2

….**NON** si tratta di una tematica di esclusiva **pertinenza endocrinologica**…e/o**pediatrica**


Cancer. Heart disease. Obesity. Depression. Scientists can now trace adult health to the nine months before birth
BY ANNIE MURPHY PAUL

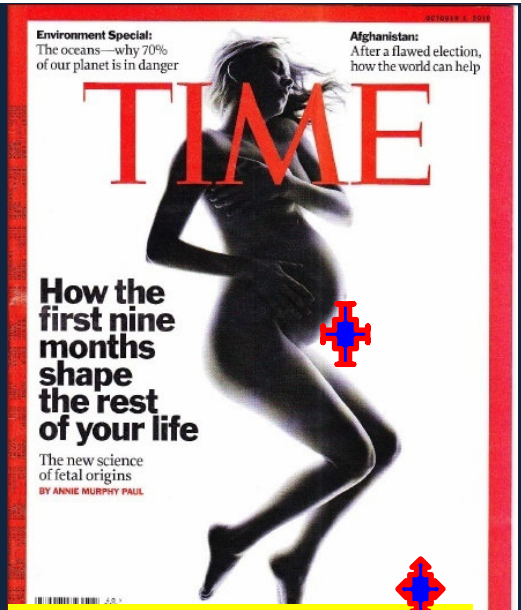
Organismo umano: **clone cellulare** derivato da un'unica cellula **totipotente (zigote) e** costituito da miliardi di cellule con **unico DNA e differente (epi)genoma**

3



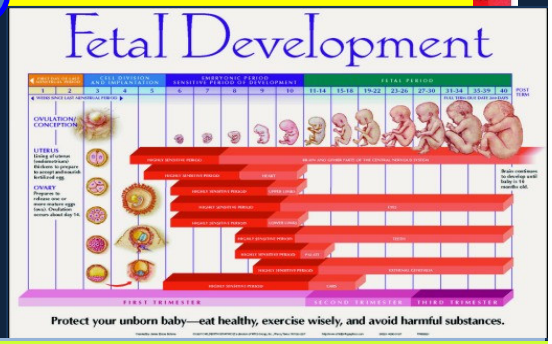
Cellule **totipotenti…multipotenti…**

Plasticità potenzialità MASSIMA → **Differenziazione cellulare**



4

Finestre di Esposizione



Gli ***interferenti endocrini*** sono sostanze mimetiche o comunque in grado di **interferire** sul *cell signaling* intercellulare e intracellulare a vario livello: comunicazione intercellulare (discorso analogo potrebbe esser fatto per gli antibiotici tra gli organismi monocellulari), sui recettori membranari e nucleari, sulle *pathways* di trasduzione dei segnali dalla membrana al nucleo, sui meccanismi di trascrizione, traduzione etc. Gli effetti più significativi sono connessi alla possibile loro **azione su cellule/tessuti di organismi in via di sviluppo,** con particolare rilievo per specifiche fasi di sviluppo di organi e tessuti (***finestre di esposizione***).
Il problema fondamentale (in ambito biomedico) connesso alla diffusione in ambiente (catene alimentari !) di molecole *xenobiotiche*, metalli pesanti ecc.. in grado di agire da interferenti endocrini (*perturbatori informatici*) può essere riassunto in una sigla: **DOHAD**

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1: Natl Cancer Inst Monogr. 1979 May;(51):159-84.

Prenatal exposure to chemical carcinogens and its effect on subsequent generations.

Tomatis L.

1

That exposure of pregnant animals to chemical carcinogens results in the occurrence of tumors in the progeny is well documented. Evidence has been accumulated on at least 38 chemicals pertaining to different chemical groups. The experimental evidence was followed by observations in humans regarding the increased risk of cancer in daughters of women who received stilbestrol during pregnancy. Additional experimental evidence is accumulating on the possibility that exposure during pregnancy results in an increased incidence of tumors for more than one generation of untreated descendants. Studies done on mice with DMBA and on rats with MNU and ENU showed that exposure to the carcinogens during pregnancy resulted in a high incidence of tumors in animals of the first generation and in an increased incidence of tumors at specific sites in untreated animals of the second and third generations.

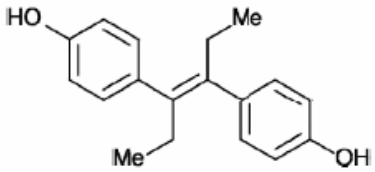
2

3

PMID: 384260 [PubMed - indexed for MEDLINE]

Diethylstilbestrol

What happened?

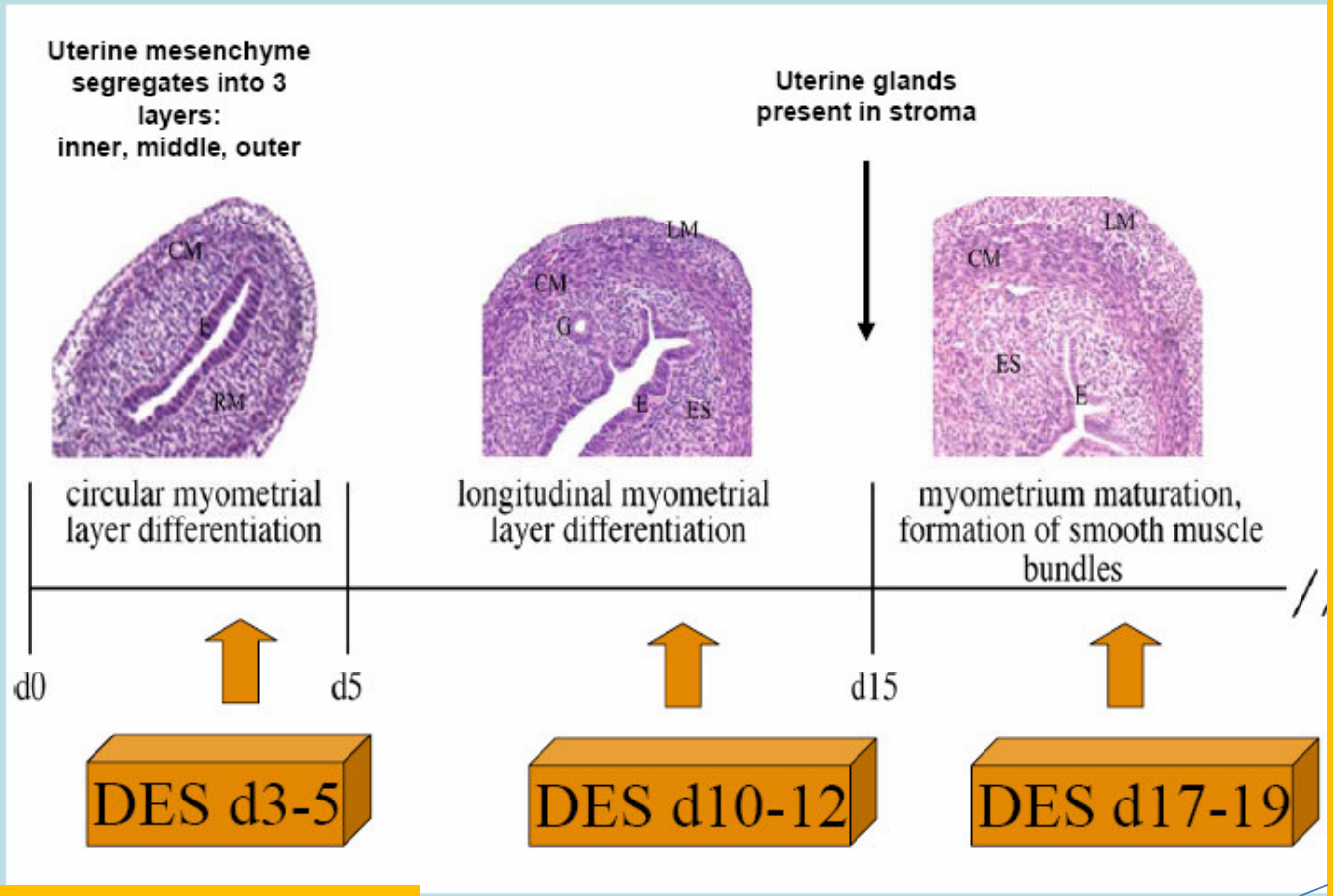


diethylstilbestrol

- *Parental generation:* DES exposure moderately elevated the risk of breast cancer later in life in mothers that took the drug
- 1st generation offspring: DES exposure caused a suite of reproductive abnormalities
  - In girls, DES exposure as a fetus led to 2.5 fold increase in breast cancer risk, greatly elevated risk of uterine cancer (during ages 20-40) and abnormal urogenital development
  - In males, DES exposure has an elevated risk of epidydimal cysts and sometimes led to abnormal testicular development and 20-fold increased chance of hypospadia

Figure 2
Medical journal advertisement for prenatal tablets with vitamins and diethylstilbestrol

Window of Susceptibility to Developmental Programming: When does it Close?



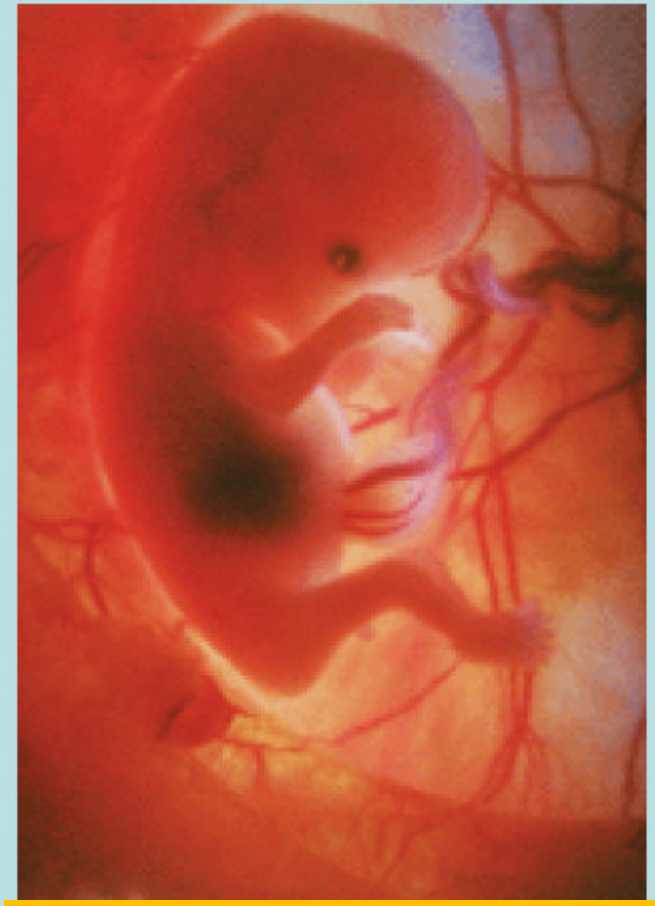
Diethylstilbestrol

J Pediatr Hematol Oncol 2003; 25:635-636

DES Daughters, are at an increased risk for clear cell **adenocarcinoma** (**CCA**) of the **vagina** and **cervix**, **breast cancer** (2.5 fold increase in after age 40) **reproductive tract structural differences**,, **pregnancy complications** **infertility** and **auto-immune disorders…**

but the effect of DES might be **transgenerational**

Developmental Programming



Exposure of developing tissues or organs to an adverse stimulus or insult during critical periods of development that can permanently reprogram normal physiological responses in such a way as to give rise to disease later in life

XXI secolo: drammatica trasformazione dell'**ambiente** → del **microambiente uterino**

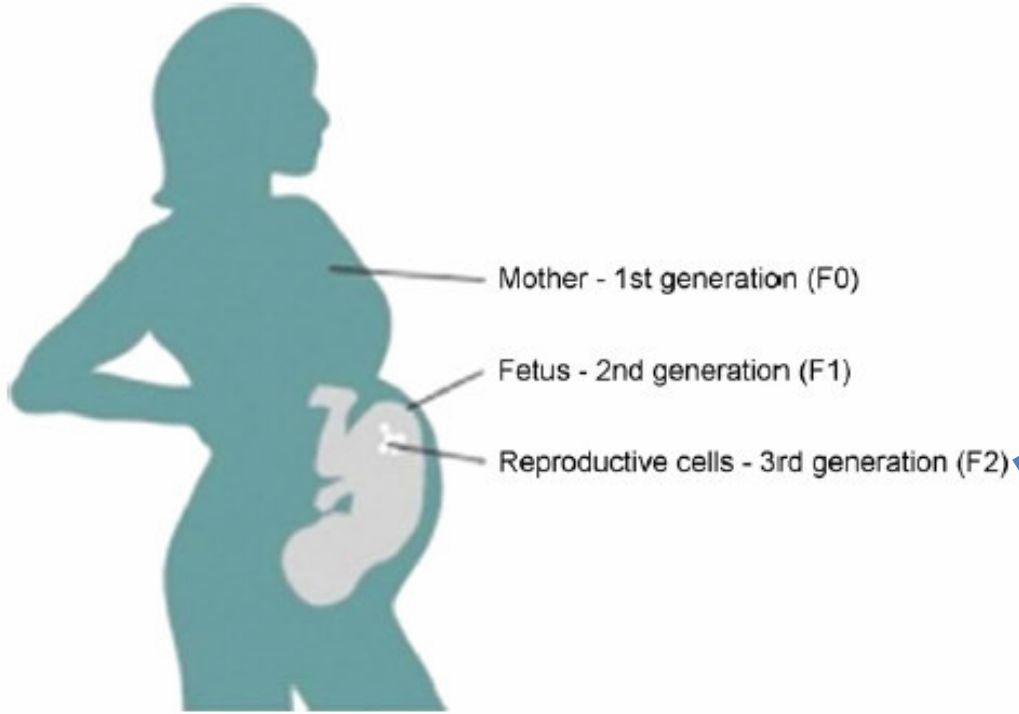


Fig. 2. Three generations at once are exposed to the some environmental conditions (diet, toxics, hormones, etc.). In order to provide a convincing case for epigenetic inheritance, an epigenetic change must be observed in the 4th generation.

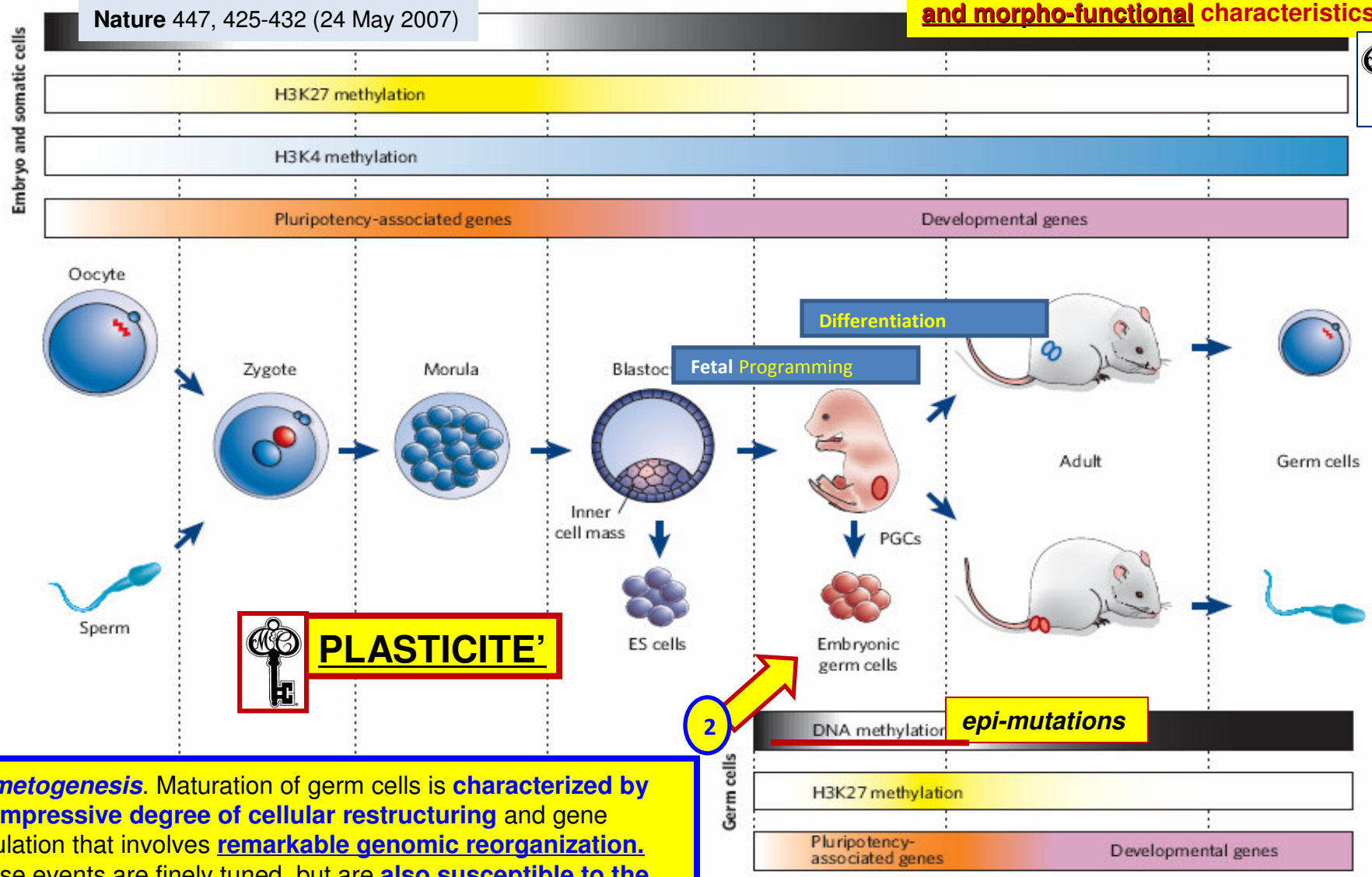
The **best developed example of transgenerational effects of environmental chemicals comes from** **the classic experiment** by **Anway** et al.. **exposing male rats to vinclozolin (an antiandrogenic fungicide) or methoxychlor (an estrogenic organochlorine insecticide) during the period of gonadal sex determination**. Exposure resulted in **reduced sperm count and viability and increased rates of infertility** in adulthood.
This **loss of fertility was perpetuated through the male germline for four generations**.
Investigation of the mechanism for the transgenerational phenotype found that **endocrine disruptors reprogrammed the male germline during development and induced heritable methylation changes that were stably transmitted through the male germline**

Cellular Differentiation: an Epigentic process

Differentiation occurs numerous times **during the development of a multicellular organism** as the organism changes **from a single zygote to a complex system** of **tissues** and **200 cell types (genetically identical.. each with its own epigenetic and morpho-functional characteristics)..** 1

Stability and flexibility of epigenetic gene regulation in mammalian development

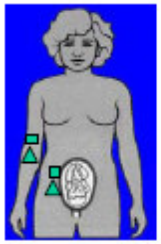
Nature 447, 425-432 (24 May 2007)

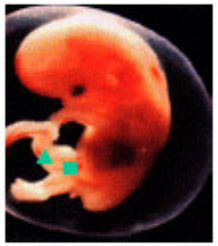


Gametogenesis. Maturation of germ cells is **characterized by an impressive degree of cellular restructuring** and gene regulation that involves **remarkable genomic reorganization.** These events are finely tuned, but are **also susceptible to the introduction of various types of error…**

methylation. During the early development of PGCs, DNA methylation and

Exposures: PAHs, diesel particulates, environmental tobacco smoke, metals, pesticides, allergens





■ ▲ Biomarkers: e.g. exposure: blood or urinary levels of pesticides, PAHs, lead, mercury, genetic damage from PAH, immune changes, *gene or protein expression*; susceptibility: genetic variants and micronutrients

Developmental disorders, Cancer risk (chromosomal abnormalities), Asthma

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PEDIATRIC RESEARCH

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Endocrine Disruptors and Epigenetic Transgenerational Disease Etiology

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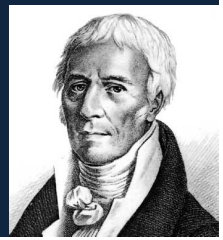
ABSTRACT: Exposure to an environmental factor (*e.g.* endocrine disruptor) during embryonic gonadal sex determination can epigenetically reprogram the male germ-line and subsequently promote transgenerational adult-onset disease. Disease phenotypes resulting from this epigenetic phenomenon include testis abnormalities, prostate disease, kidney disease, tumor development, and immune abnormalities. The epigenetic mechanism involves the induction of new imprinted-like genes/DNA sequences in the germ-line that appear to transgenerationally transmit disease phenotypes. This epigenetic transgenerational disease mechanism provides a unique perspective from which to view adult onset disease and ultimately offers new insights into novel diagnostic and therapeutic strategies. (*Pediatr Res* **61: 1–2, 2007**)

Environmental exposures have been found to promote several transgenerational disease states or **phenotypes**

The **reproducibility** and **frequency** of these disease phenotypes **suggests they are likely epigenetic rather than due to DNA sequence mutations.**



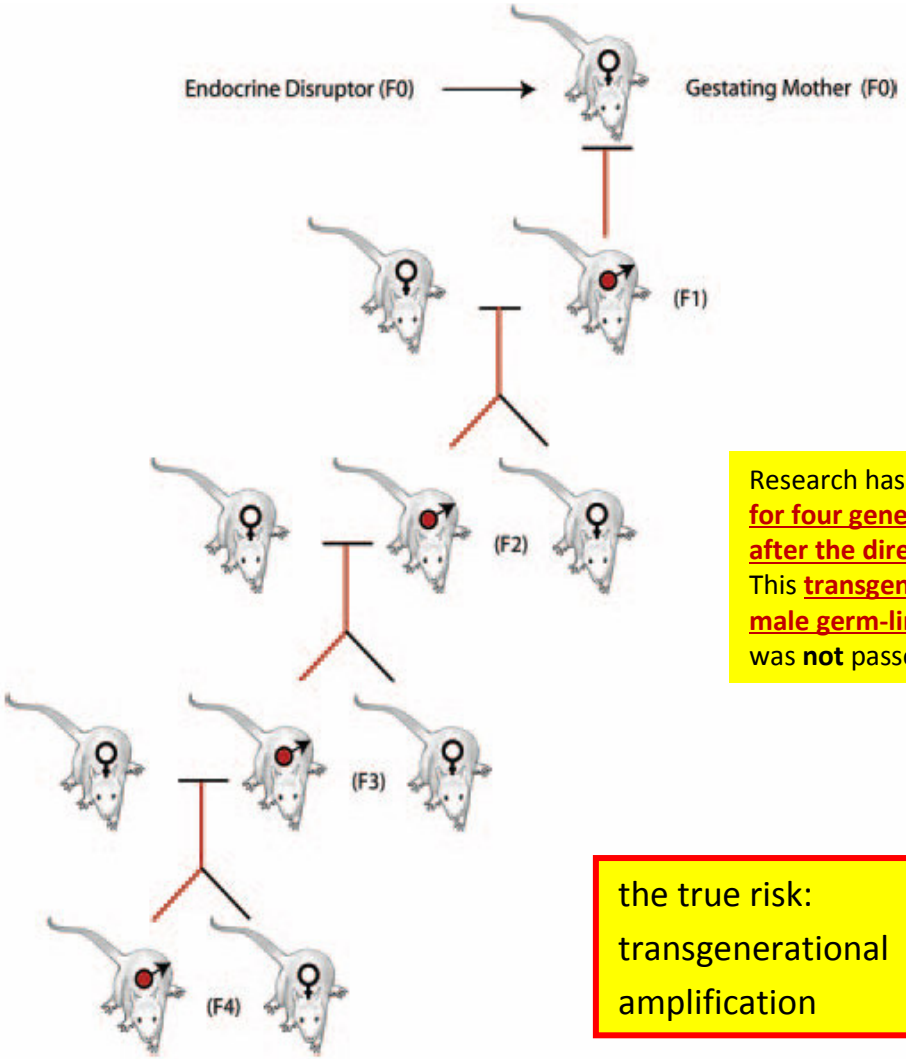


Figure 1. The postulated mechanism involved in endocrine disruptor actions during sex determination. Schematic of an F0 generation mother being transiently exposed to an endocrine disruptor, during embryonic sex determination and subsequent progeny through the male germ line, transgenerationally transmitting adult onset disease states.

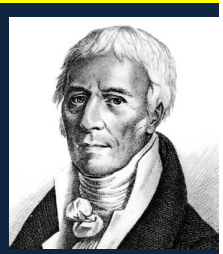
the true risk: transgenerational amplification

The **exposure** of a gestating mother exposes
the F0 generation mother,
the F1 generation embryo and
the germ-line of the F2 generation.

The F3 generation would be the **first unequivocal transgenerational generation not exposed**.

Research has demonstrated that 90% of all male progeny for four generations (F1–F4) developed these disease states after the direct exposure of the F0 gestating rat
This transgenerational phenotype was only transmitted through the male germ-line (sperm) and
was **not** passed through the **female germ-line (oocyte).**

These animals had the following disease state frequencies;
20% tumor development,
50% prostate disease, **40% kidney disease,**
30% immune abnormalities, and 30% severe infertility in males from F1 to F4 generations



4. Anway MD, Cupp AS, Uzumcu M, Skinner MK 2005 Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science 308:1466–1469
5. Anway MD, Memon MA, Uzumcu M, Skinner MK 2006 Transgenerational effect of the endocrine disruptor vinclozolin on male spermatogenesis. J Androl 27:868–879
6. Anway MD, Leathers C, Skinner MK 2006 Endocrine disruptor vinclozolin induced epigenetic transgenerational adult-onset disease. Endocrinology 147:5515–5523

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Review

Environmental Exposures and Gene Regulation in Disease Etiology

Thea M. Edwards[1,2] and John Peterson Myers[2]

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DATA SYNTHESIS: Pharmaceuticals, pesticides, air pollutants, industrial chemicals, heavy metals, hormones, nutrition, and behavior can change gene expression through a broad array of gene regulatory mechanisms. Mechanisms include regulation of gene translocation, histone modifications, DNA methylation, DNA repair, transcription, RNA stability, alternative RNA splicing, protein degradation, gene copy number, and transposon activation. Furthermore, chemically induced changes in gene regulation are associated with serious and complex human diseases, including cancer, diabetes and obesity, infertility, respiratory diseases, allergies, and neurodegenerative disorders such as Parkinson and Alzheimer diseases. One of the best-studied areas of gene regulation is epigenetics, especially DNA methylation. Our examples of environmentally induced changes in DNA methylation are presented in the context of early development, when methylation patterns are initially laid down. This approach highlights the potential role for altered DNA methylation in fetal origins of adult disease and inheritance of acquired genetic change.

1

2

3

++ epigenetic patterns

Barker Hypothesis

Developmental Origins of Adult Diseases (DOHAD)

Pharmaceuticals, pesticides, air pollutants, industrial chemicals, heavy metals, hormones, nutrition, and behavior can **change gene expression** through a **broad array of gene regulatory mechanisms..** highlighting the **potential role for altered DNA methylation in fetal origins of adult disease and inheritance of acquired genetic changes**

fluid (epi)genome

Environment

DNA

Gene translocation
Histone modifications
DNA methylation
DNA repair
Gene copy number
Transposon activation

Transcription

RNA

RNA stability
Alternative RNA splicing
Retrotransposons

mRNAs

Protein

Transcription factors
Protein degradation

ROS

Pharmaceuticals
Pesticides
PCBs, PAHs, dioxin
Air pollutants
Heavy metals
Hormones
BPA, NP, OP
Nutrition
Parental care

The Epidemic Revolution Of the XX century

Inheritance
Development
Growth
Aging

Cancer
Diabetes, obesity
Infertility
Respiratory diseases
Allergies, immune response
Neurodegenerative disorders
Stress response

HSPs Activation

Danger Theory

HSPs/petides

(Auto)Immunity

Autoinflammation

developmental plasticity

Summary of gene regulatory mechanisms affected by environmental exposures, with disease implications. Abbreviations: BPA, bisphenol A; NP, 4-nonylphenol; PAHs, polycyclic aromatic hydrocarbons, PCBs, polychlorinated biphenlys; OP, 4-*tert*-octylphenol.

WILEY InterScience

Does Breast Cancer Start in the Womb?

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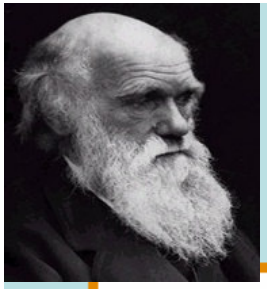
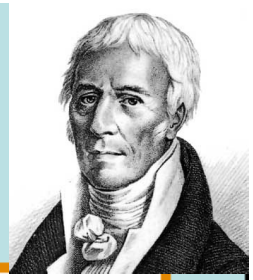
(Received June 28, 2007; Accepted September 7, 2007)

Barker Hypothesis

Abstract: Perturbations in the foetal environment predispose individuals to diseases that become apparent in adulthood. These findings prompted researchers to hypothesize that foetal exposure to environmental oestrogens may play a role in the increased incidence of breast cancer observed in European and US populations over the last 50 years. There is widespread human exposure to bisphenol A, an oestrogenic compound that leaches from dental materials and consumer products. In CD-1 mice, perinatal exposure to environmentally relevant bisphenol A levels induced alterations of the mammary gland architecture. Bisphenol A increased the number of terminal end buds at puberty and terminal ends at 6 months of age and increased ductal lateral branching at 4 months of age. Exposed mice also showed an enhanced sensitivity to oestradiol when ovariectomized prior to puberty. All these parameters are associated in human beings with an increased risk for developing breast cancer. To assess whether bisphenol A induces mammary gland neoplasia, we chose a rat model because it more closely mimics the human disease than mouse models. Examination of the mammary glands of Wistar/Furth rats during early adulthood revealed that gestational exposure to bisphenol A induced the development of pre-neoplastic lesions and carcinoma *in situ* in the absence of any additional treatment aimed at increasing tumour development. Emerging epidemiological data reveal an increased incidence of breast cancer in women exposed to diethylstilboestrol during gestation. Hence, both animal experiments and epidemiological data strengthen the hypothesis that foetal exposure to xenooestrogens may be an underlying cause of the increased incidence of breast cancer observed over the last 50 years.

In contrast to the **somatic mutation theory**, the **TOFT** postulates that:
(i) carcinogenesis represents a **problem of tissue organization**,
(ii) **proliferation is the default state** of all cells, and
(iii) carcinogenesis is a **reversible phenomenon**





Is Cancer Risk Determined by Developmental Programming Induced by Environmental Exposures?

Ce sont des **<u>quantités minimales de molécules (epi)génotoxiques</u>**, induisant des **transformations continuelles de la chromatine**, qui constituent le véritable problème. C'est un processus très lent pouvant démarrer lors des **premières étapes du développement fœtal**. Et, même dans les ***gamètes***.
Si **<u>les tissus du fœtus sont mal programmés au début et s'il y a un stress épigénétique progressif</u>**, les mutations génétiques et chromosomiques vont davantage se produire

It is generally argued that **childhood cancers** are a **rare condition**.

But **it should be reminded** that CANCER is **the main cause of death by disease** in **childhood**

that there is a **constant and significant increase** of tumors in the world for this age group

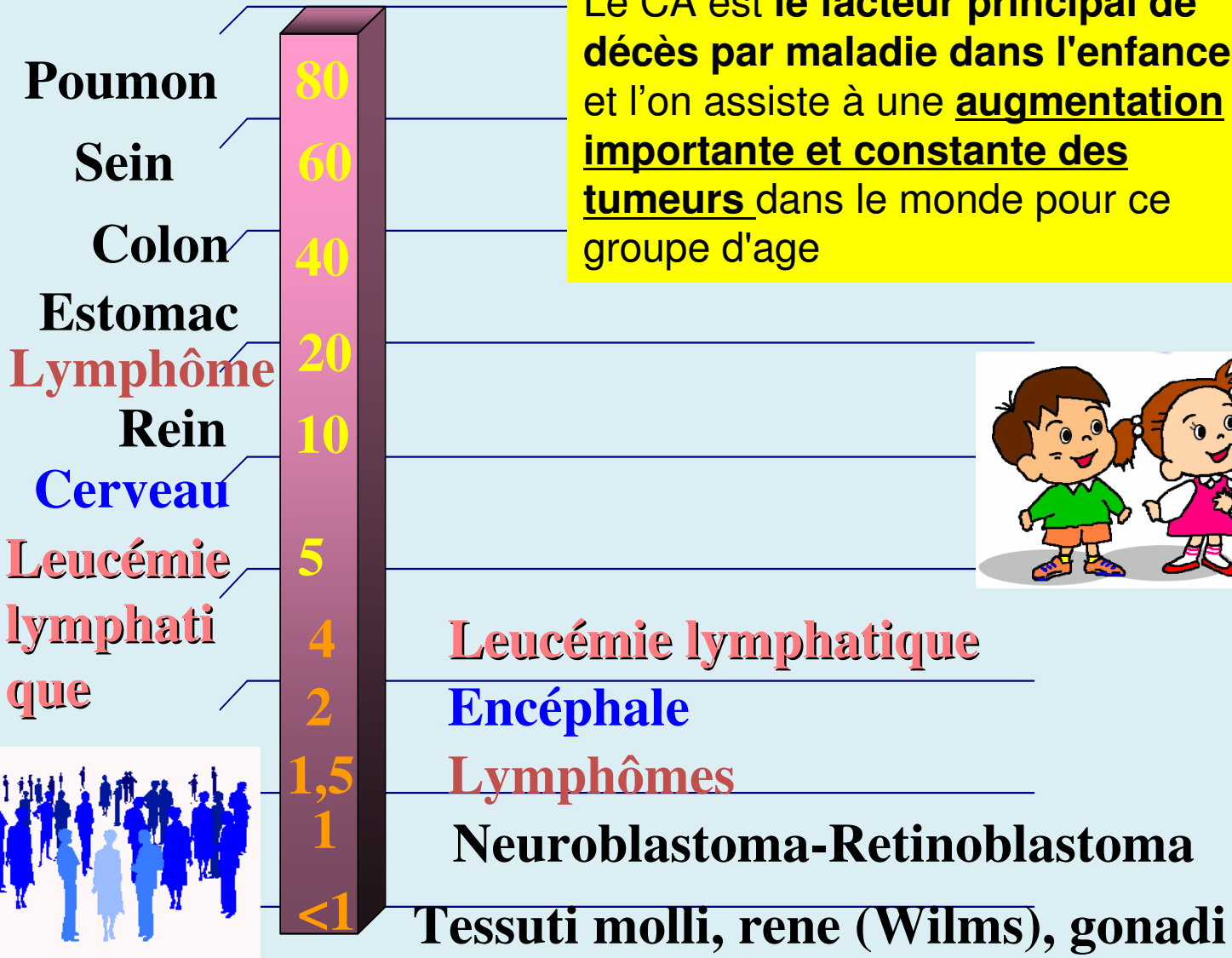
that **1 : 5-600 children falls ill with cancer**

That more than **13 000 children fall ill with cancer each year in the U.S.**

Bleyer A, O'Leary M, Barr R, Ries LA, editors. ***Cancer epidemiology in older adolescents and young adult: 15-29 ears of age, including SEER incidence and survival: 1975-2000***. NIH Pub. No. 06-5767. Bethesda (MD): National Cancer Institute; 2006. Jemal A, Siegel R, Ward E, et al. ***Cancer statistics***, 2008. CA Cancer J Clin 2008;58:71 – 6.

Incidenza di tumori (anno/100.000)

Alberto Tommasini,
Laboratorio Immunologia Pediatrica, IRCCS Burlo Garofolo

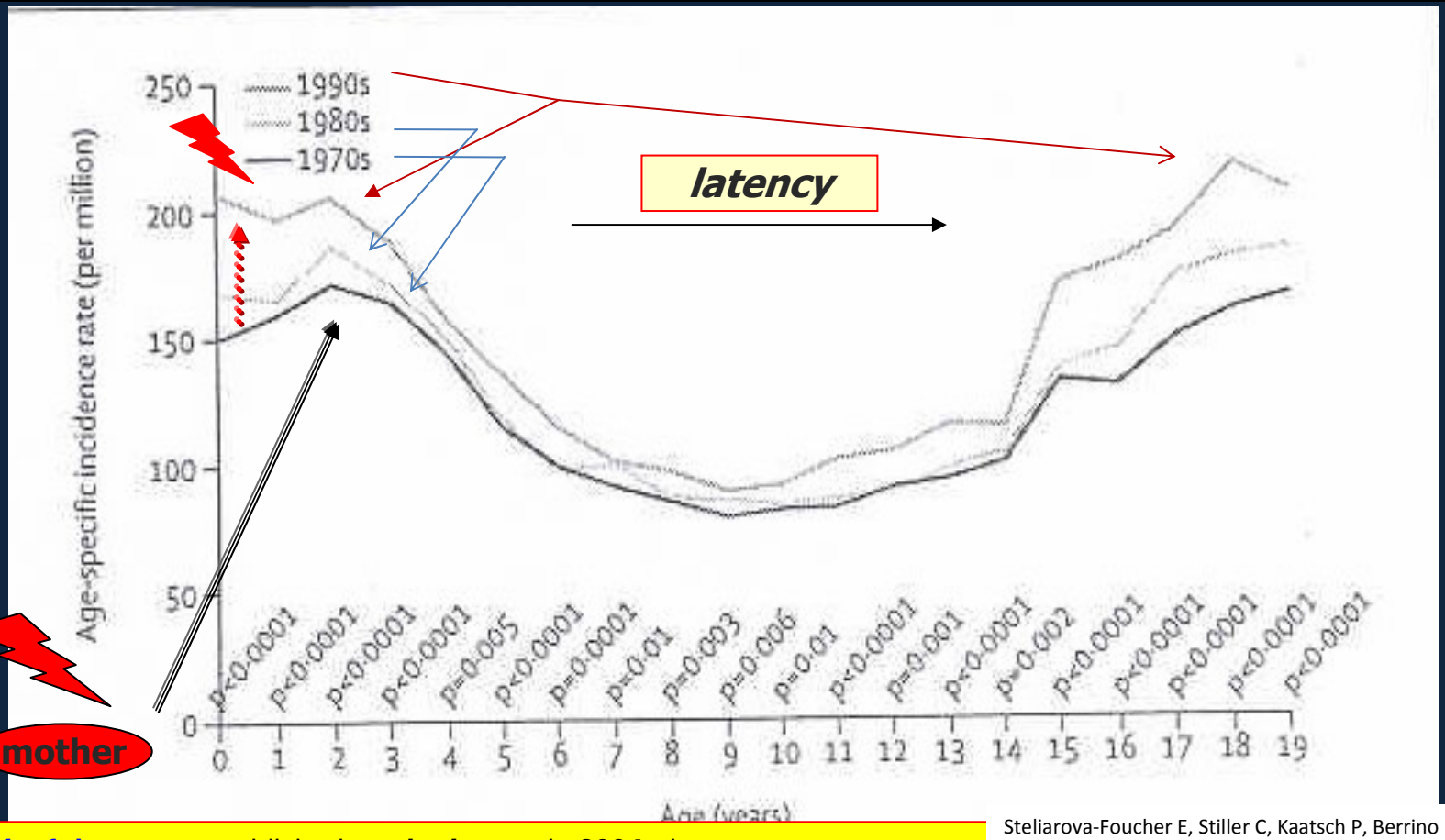


Le CA est **le facteur principal de décès par maladie dans l'enfance** et l'on assiste à une **augmentation importante et constante des tumeurs** dans le monde pour ce groupe d'age

As we may easily argue from recent project *ACCIS (Automated Childhood Cancer Information System)* - a comprehensive monitoring **conducted by a team of epidemiologists IARC on 63 cancer registries from 19 European countries**, for a total of over ***130 thousand tumors*** of all types (113 thousand children and 18 thousand teenagers)

http://www-dep.iarc.fr/accis.htm

Cancer incidence in childhood and adolescence IN EUROPE (1970-1999)



A first draft of the report, published on ***the Lancet*** in **2004**, demonstrates an **annual increase of 1-1,5% for all cancers** (with more marked increases in **lymphomas**, **soft tissue sarcomas**, **tumors of the nervous system…**) .

Steliarova-Foucher E, Stiller C, Kaatsch P, Berrino F, Coebergh JW, Lacour B, Parkin M. ***Geographical patterns and time trends of cancer incidence and survival among children and adolescents in Europe since the 1970s (the ACCISproject): an epidemiological study.*** Lancet. 2004 Dec 11-17;364(9451):2097-105

The data from the **ACCIS project** published in *the Lancet* were soon **confirmed by the next review** (the most complete to date) of the data emerging from the study (which has become the largest European database on cancer) published two years later on the ***European Journal of Cancer*** (18 items in all, containing detailed analysis of data on incidence rates and trends of prevalence and survival)..

EUROPEAN JOURNAL OF CANCER 42 (2006) 1961–1971

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journal homepage: www.ejconline.com

Time trends of cancer incidence in European children (1978–1997): Report from the Automated Childhood Cancer Information System project

Peter Kaatsch[a,*], Eva Steliarova-Foucher[b], Emanuele Crocetti[c], Corrado Magnani[d], Claudia Spix[a], Paola Zambon[e]

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[b]Descriptive Epidemiology Group, International Agency for Research on Cancer (IARC), Lyon, France

[c]Tuscany Cancer Registry, Firenze, Italy

[d]Childhood Cancer Registry of Piedmont, CPO-Piemonte, CERMS and University of East Piedmont, Novara, Italy

[e]Veneto Cancer Registry University of Padua, IOV, Italy

Within the framework of the Automated Childhood Cancer Information System (ACCIS), time trend analyses for childhood cancer were performed using data from 33 population-based cancer registries in 15 European countries for the period 1978–1997. The overall incidence rate based on 77,111 cases has increased significantly ($P < 0.0001$), with an average annual percentage change (AAPC) of 1.1%. The rising trend was observed in all five geographical regions and in the majority of the disease groups (in order of AAPC): soft tissue sarcomas (1.8%), brain tumours, tumours of the sympathetic nervous system, germ-cell tumours, carcinomas, lymphomas, renal tumours, and leukaemias (0.6%). No change was seen in incidence of bone tumours, hepatic tumours and retinoblastoma. The increased incidence can only partly be explained by changes in diagnostic methods and by registration artefacts. The patterns and magnitude of these increases suggest that other factors, e.g. changes in lifestyle and in exposure to a variety of agents, have contributed to the increase in childhood cancer in the recent decades.

..in the last 20 years (**1978** e il **1997**) the overall incidence rate has increased significantly with an **average annual percentage change** (AAPC) of **1,1%** (> **2% in the first year; 1,3 % during adolescence**).

Table 4 – Average annual percent of change (AAPC) and result of trend test for childhood cancer (age 0–14 years) in Europe by age groups and sex for total cancer and main diagnostic groups (*P < 0.05; ** P < 0.01; *** P < 0.0001) (1978–1997) (Source: ACCIS)

	AAPC for diagnostic groups												AAPC for total (%)
	Leu (%)	Ly (%)	CNS (%)	Symp (%)	Ret (%)	Ren (%)	Hep (%)	Bone (%)	Soft (%)	Germ (%)	Ca (%)	Oth (%)	
Age 0	0.6	−1.6	2.4***	2.2***	0.9	1.9*	1.5	−7.4	1.3	3.9***	−0.4	3.2	2.1***
Age 1–4 years	0.7***	0.6	1.8***	1.7***	0.4	0.8*	1.2	−0.5	1.9***	−0.1	0.6	−0.2	1.1***
Age 5–9 years	0.5*	0.7	1.6***	0.1	−0.6	0.5	−1.8	−1.2	1.3*	0.90	−0.9	1.0	0.8***
Age 10–14 years	0.5*	1.3***	1.7***	1.9	−6.0	0.5	0.3	0.2	2.6***	2.5***	2.2***	1.7	1.3***
Male	0.7***	0.5*	1.5***	1.5***	0.3	0.4	0.9	−0.3	1.7***	1.2*	1.2	0.2	0.9***
Female	0.6**	1.7***	2.0***	2.0***	0.7	1.3**	0.6	−0.2	2.0***	2.0***	1.3*	2.0	1.4***

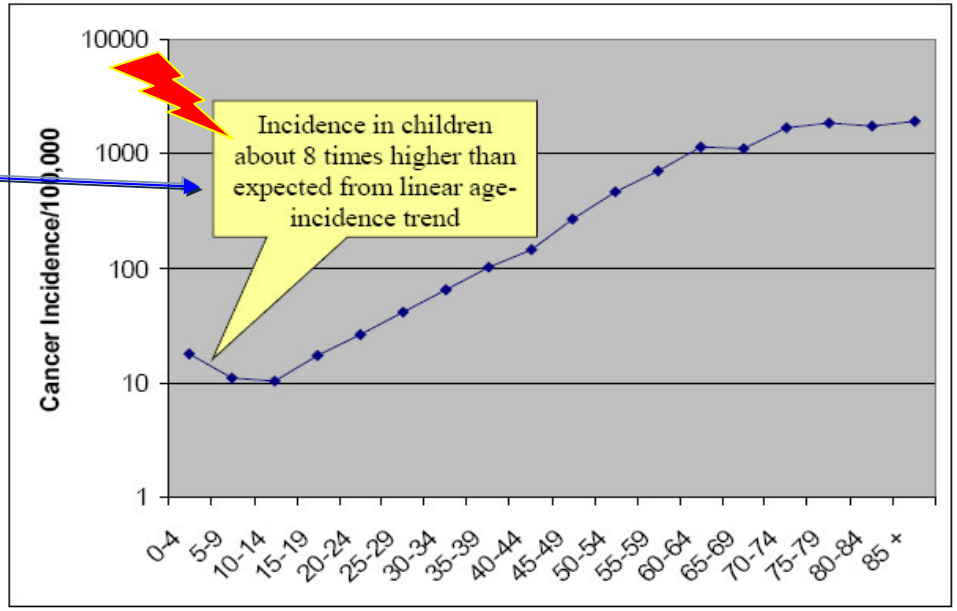
Leu, leukaemias; Ly, lymphomas; CNS, CNS tumours; Symp, tumours of the sympathic nervous system; Ret, retinoblastoma; Ren, renal tumours; Hep, hepatic tumours; Bone, malignant bone tumours; Soft, soft tissue sarcomas; Germ, germ-cell tumours; Ca, carcinomas; Oth, other and unspecified malignant neoplasms.

Cancers in *adults* predominantly arise in (epithelial) tissues chronically exposed to *environmental stress*
and **in cells and tissues continually urged to respond/react to it**

While almost all ***childhood cancers*** belong to three major groups:
45% oncohaematologic tumors (leukemias and lymphomas)
25% **brain** tumors
25% neoplastic degeneration of ***embryonal residuals***

The **increase** particularly affects children in their **first life year (the incidence rate increased by> 2%)**

Cancer Incidence by Age



Austria, 2003

Early Human Development (2005) 81, 123–129

ELSEVIER

Early Human Development

www.elsevier.com/locate/earlhumdev

In utero origins of childhood leukaemia

Mel Greaves*

Abstract Chimaeric fusion genes derived by chromosome translocation are common molecular abnormalities in paediatric leukaemia and provide unique markers for the malignant clone. They have been especially informative in studies with twins concordant for leukaemia and in retrospective scrutiny of archived neonatal blood spots. These data have indicated that, in paediatric leukaemia, the majority of chromosome translocations arise in utero during foetal haemopoiesis. Chromosomal translocations and preleukaemic clones arise at a substantially higher frequency (~100×) before birth than the cumulative incidence or risk of disease, reflecting the requirement for complementary and secondary genetic events that occur postnatally. A consequence of the latter is a very variable and occasionally protracted postnatal latency of disease (1–15 years). These natural histories provide an important framework for consideration of key aetiological events in paediatric leukaemia.

The first unambiguous evidence for a **prenatal origin of leukaemia** was derived **from studies in identical twins with leukaemia**. A case of **identical (monozygotic) infant twins with leukaemia was recorded in 1882,** and, since that time, more than 70 pairs have been published albeit in variable detail ...

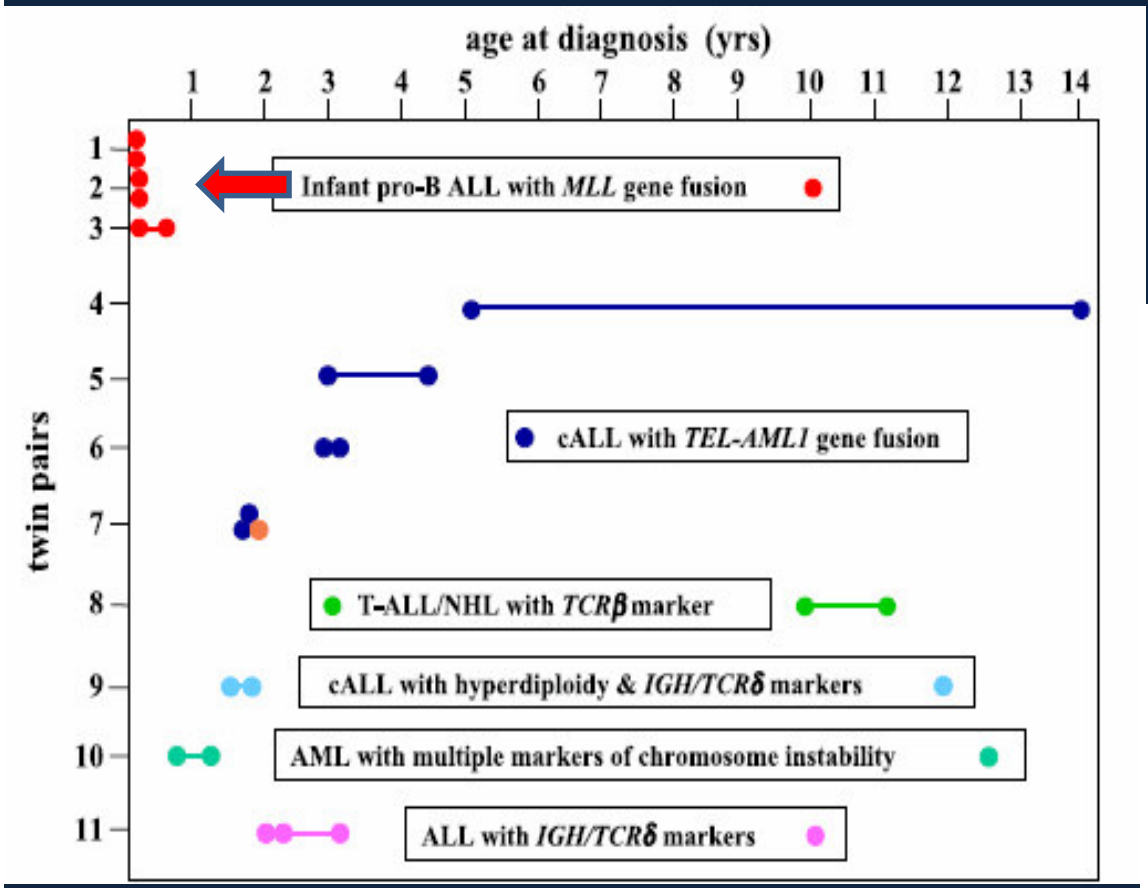
The **concordance** rate of leukaemia varies according to subtype and age. **For infants with ALL, the rate is exceedingly high (> 50%), for "COMMON" child-ALL, is ~10%.** 1

Adult leukaemia (ALL/ AML), in contrast, has a **very low rate of concordance (< 1%).**

Chromosomal translocations and preleukaemic clones arise at a substantially **higher frequency (~100 X) before birth than the cumulative incidence or risk of disease**, reflecting the requirement for complementary and **secondary genetic events** that occur postnatally. A consequence of the latter is a very variable and occasionally protracted **postnatal latency** of disease (1—15 years). 2

~1% of newborns had TEL-AML1 positive B lineage clones…
This represents **100 times** the incidence of **TEL-AML1 positive ALL (~1 in 12,000).**

Figure 1 Concordant leukaemia in identical twins: the LRF Series (1993–2003). Figure illustrates age at diagnosis for each twin in the 11 pairs studied, the biological subtype of leukaemia and the molecular markers of clonality used.



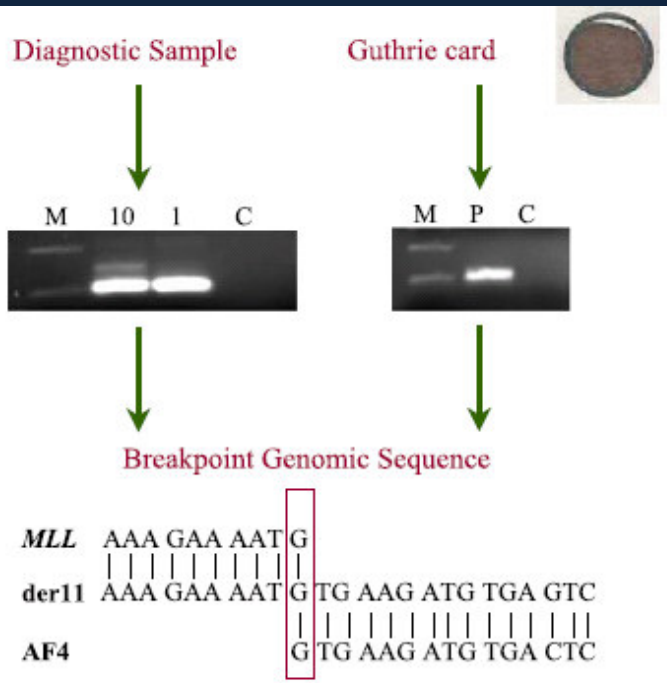


Figure 3 Detection of clonotypic fusion gene sequences (*MLL-AF4*) in neonatal blood spots (Guthrie card). 10, 1 μg DNA; C, control DNA; M, marker. Diagnostic DNA amplified by long-range PCR or long-distance inverse PCR [21]. Guthrie card DNA amplified by short-range (conventional) PCR using primers based on diagnostic DNA-derived genomic *MLL-AF4* sequence. Note diagnostic (leukaemic) DNA and Guthrie card contain the same unique *MLL-AF4* sequence as shown here for one case.

Even if **leukaemia fusion gene** formation is spontaneous, the risk of this occurring may be **modified by other factors, including folate availability**. There is dietary and genetic evidence that **folate has an impact on the risk of infant and childhood leukaemia ..**

MLL (myeloid/lymphoid or mixed lineage leukemia)

IN ALL AND AML, THE *ALL1* (ALSO NAMED *MLL*) GENE CAN FUSE WITH 1 OF MORE THAN 50 GENES. **ALL1 IS PART OF A MULTIPROTEIN COMPLEX**. MOST OF THE PROTEINS IN THE COMPLEX ARE COMPONENTS OF **TRANSCRIPTION COMPLEXES**; OTHERS ARE INVOLVED IN **HISTONE METHYLATION AND RNA PROCESSING**. THE ENTIRE **COMPLEX REMODELS, ACETYLATES, DEACETYLATES, AND METHYLATES NUCLEOSOMES** AND **HISTONES**.

ALL1 (MLL) FUSION PROTEINS DEREGULATE HOMEOBOX GENES (WHICH ENCODE TRANSCRIPTIONS FACTORS) AND **MICRORNA GENES SUCH AS *MIR191*.**

The first and most striking property of MLL fusion proteins is their incredible div
MLL has been found in **73 different translocations** and **54 partner genes** ha
cloned (*http://atlasgeneticsoncology.org/Genes/MLL.html).*

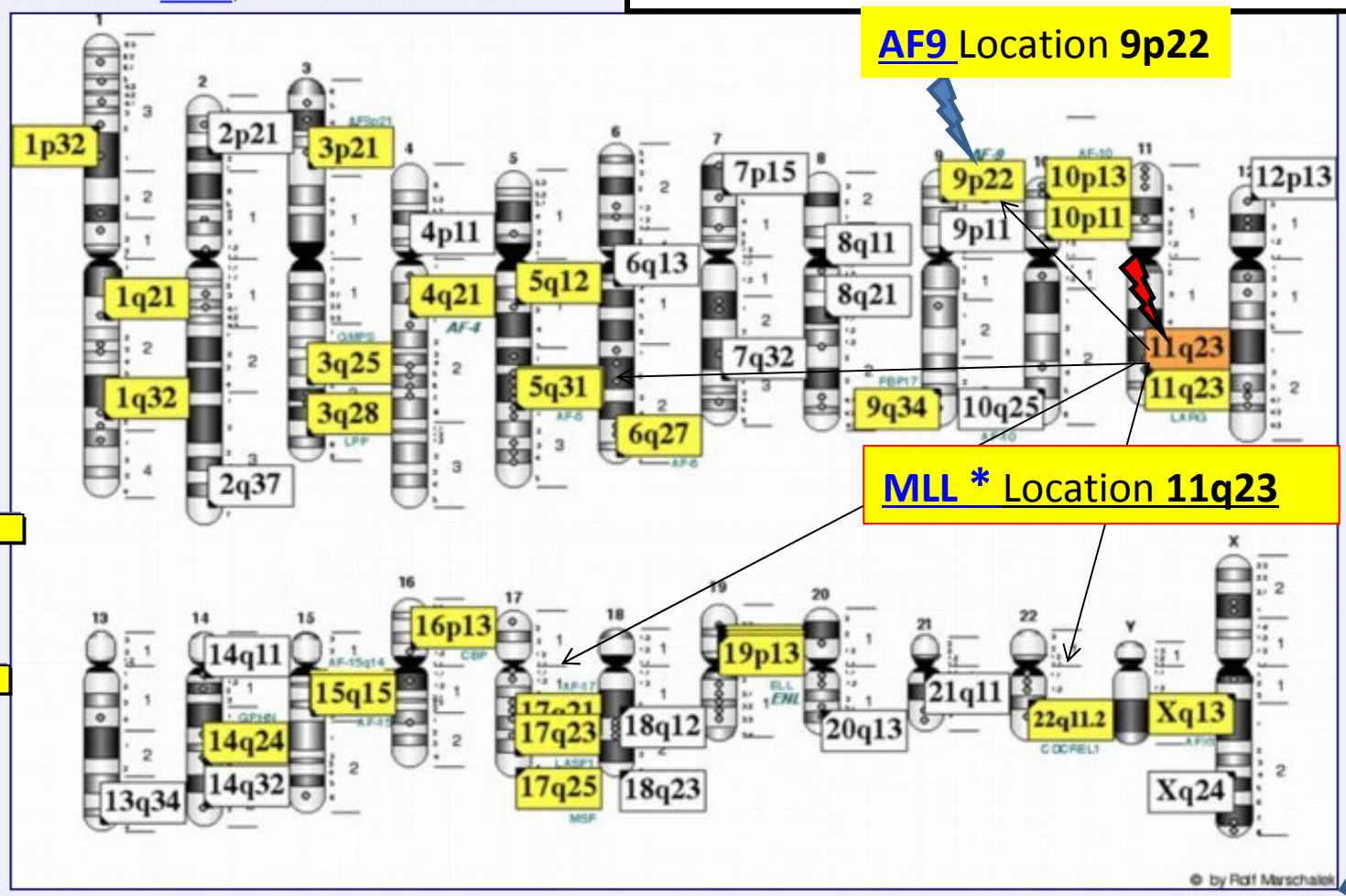
ALL1, HRX, Htrx (human trithorax), TRX1

MLL

11q23

telomeric to PLZF, centromeric from RCK

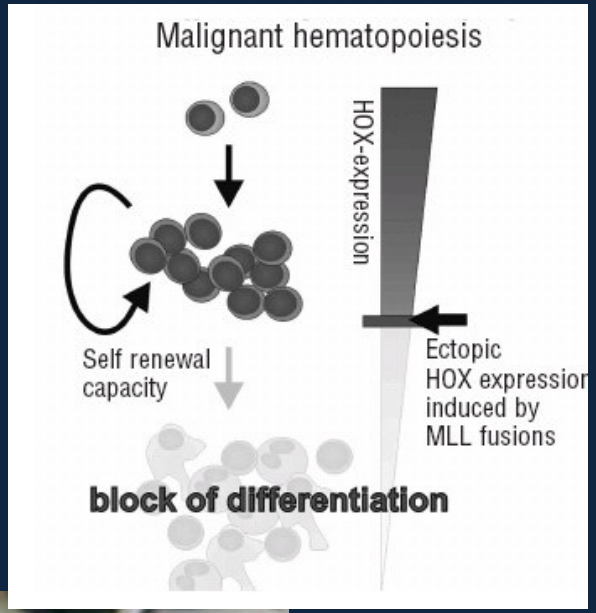
Nakamura T, Mori T, Tada S, et al. ***ALL-1 is a histone methyltransfe that assembles a supercomplex of proteins involved in tran regulation.*** Mol Cell 2002;10:1119-1128.

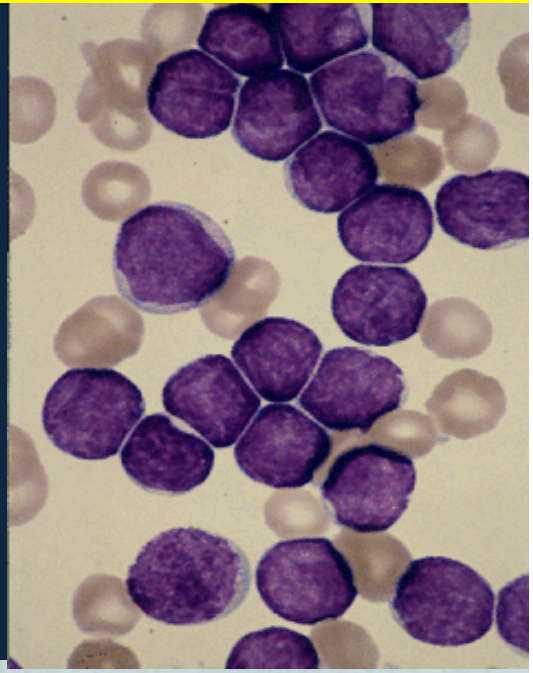


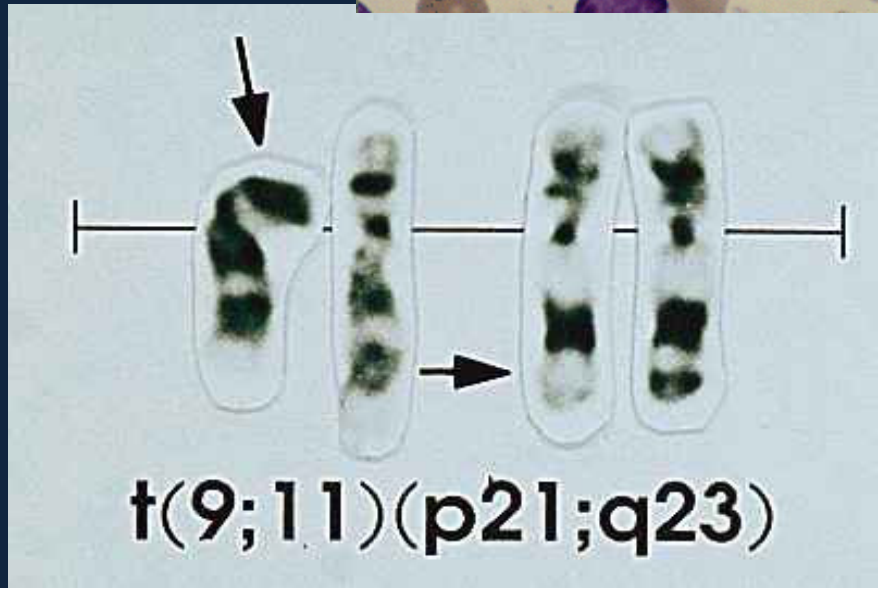
Several lines of evidence point to a mishap in non-homologous end joining of double strand breaks as the most likely reason for 11q23 translocations.

Translocations typical of myeloid leukaemia, probably due to maternal exposure to some toxic compound, were shown to be **present at birth in children who developed the disease years later** (while not sufficient per se to cause the disease, they might increase the risk for leukaemia by inducing genomic instability) Tomatis L. ***Identification of carcinogenic agents and primary prevention of cancer.*** Ann N Y Acad Sci. 2006 Sep;1076:1-14

Translocation **involving band 11q23 in AML** may occur as a result of a **deletion or trans-locations**
with a number of other chromosomes and is usually associatedwith **M4** or **M5** and a poor prognosis







[CANCER RESEARCH 61, 2542–2546, March 15, 2001]

Transplacental Chemical Exposure and Risk of Infant Leukemia with *MLL* Gene Fusion[1]

Freda E. Alexander,[2] Sherry L. Patheal, Andrea Biondi, Silvia Brandalise, Maria-Elena Cabrera, Li C. Chan, Zhu Chen, Giuseppe Cimino, Jose-Carlos Cordoba, Long-Jun Gu, Hany Hussein, Eiichi Ishii, Azza M. Kamel, Silvia Labra, Isis Q. Magalhães, Shuki Mizutani, Eleni Petridou, Maria Pombo de Oliveira, Patrick Yuen, Joseph L. Wiemels, and Mel F. Greaves

Infant acute leukemia (IAL) frequently involves breakage and recombination of the *MLL* gene with one of several potential partner genes. These gene fusions arise *in utero* and are similar to those found in leukemias secondary to chemotherapy with inhibitors of topoisomerase II (topo-II). This has led to the hypothesis that *in utero* exposures to chemicals may cause IAL via an effect on topo-II. We report a pilot case-control study of IAL across different countries and ethnic groups. Cases ($n = 136$) were population-based in most centers. Controls ($n = 266$) were selected from inpatients and outpatients at hospitals serving the same populations.

ing Baygon). Elevated odds ratios were observed for MLL^{+ve} (but not MLL^{-ve}) leukemias (2.31 for DNA-damaging drugs, $P = 0.03$; 5.84 for dipyrone, $P = 0.001$; and 9.68 for mosquitocidals, $P = 0.003$). Although it is unclear at present whether these particular exposures operate via an effect on topo-II, the data suggest that specific chemical exposures of the fetus during pregnancy may cause *MLL* gene fusions. Given the widespread use of dipyrone, Baygon, and other carbamate-based insecticides in certain settings, confirmation of these apparent associations is urgently required.

Our study has supported the hypothesis that ***in utero* exposure to chemicals causes MLL*** infant leukemia and has generated specific hypotheses that require further testing.

Exposure to ***dipyrone*** is widespread, particularly in Central and South America where it is available as an inexpensive, nonprescription drug. *Mosquitocidals* are similarly in general use in these same settings. ***Propoxur*** (***Baygon***°) is also widely used against cockroaches, fleas, and similar pests.

Therefore, it is important that the associations observed in this study are reevaluated in an extended case-control study

Are There Solutions? Yes!

- The "Swedish Solution"
  - The Swedish Environment Committee has proposed that **if a chemical is bioaccumulative or persistent, it should not be used in products, or released into the environment**,
  - thus **bypassing the long and tedious scientific and political arguments** about **toxicity**

!

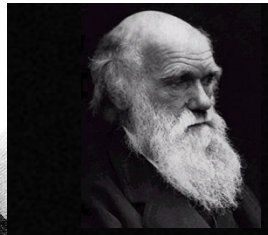
1: Ann N Y Acad Sci. 2002 Dec;982:190-7.

Primary prevention protects public health.

Tomatis L.

Cave 25/r, 34011 Aurisina (Trieste), Italy. ltomatis@hotmail.com

It is widely accepted that epidemiological data provide the only reliable evidence of a carcinogenic effect in humans, but epidemiology is unable to provide early warning of a cancer risk. The experimental approach to carcinogenicity can ascertain and predict potential cancer risks to humans in time for primary prevention to be successful. Unfortunately, only in rare instances were experimental data considered sufficiently convincing per se to stimulate the adoption of preventive measures. The experimental testing of environmental agents is the second line of defense against potential human carcinogens. The first line is the testing of synthesized agents, be these pesticides, medical drugs, or industrial chemical/physical agents, at the time of their development. We do not know, however, how many substances have been prevented from entering the environment because most tests are carried out by commercial or private laboratories and results are rarely released. A better understanding of the mechanisms underlying the sequence of events of the carcinogenesis process will eventually lead to a m accurate characterization and quantification of risks. However, the ways that mechanistic d have been used lately for evaluating evidence of carcinogenicity have not necessarily meant that the evaluations were more closely oriented toward public health. A tendency has surfaced to dismiss the relevance of long-term carcinogenicity studies. In the absence of absolute certainty, rarely if ever reached in biology, it is essential to adopt an attitude of responsible caution, in line with the principles of primary prevention, the only one that may prevent unlimited experimentation on the entire human species.



"Everything should be made as simple as possible, but not simpler."

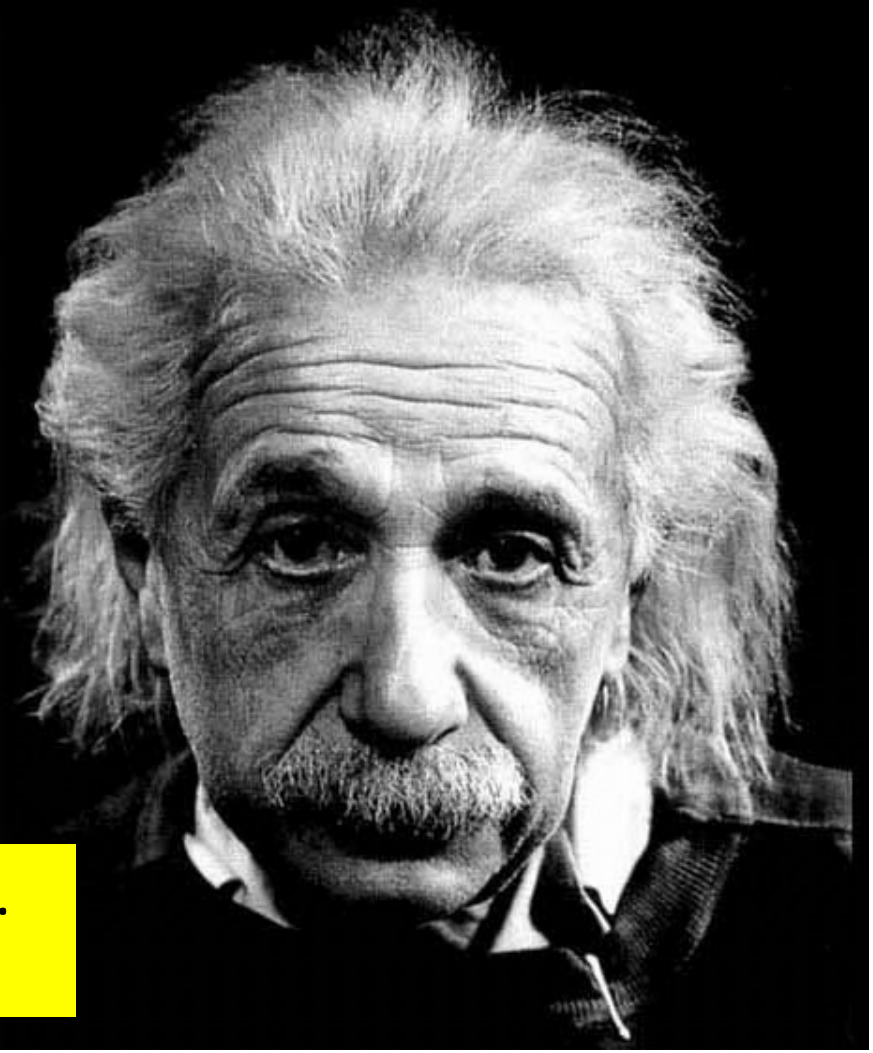
Albert Einstein

God is **subtle** but not malicious

God does **not** play dice

È la celebre affermazione che suggella l'acceso dibattito tra Einstein e i sostenitori di una certa interpretazione della fisica quantistica…

I believe in Spinoza's God who reveals himself in the orderly harmony of what exists



''We ***can't solve*** problems by using the ***same kind of thinking*** we used when we ***created*** them''

"A clever man ***solves*** a problem… a wise man ***avoids*** it"