Epigenetic Changes and Childhood Cancer

(redacted slides, full set to be posted later)

Joseph Wiemels

Dept. of Epidemiology & Biostatics

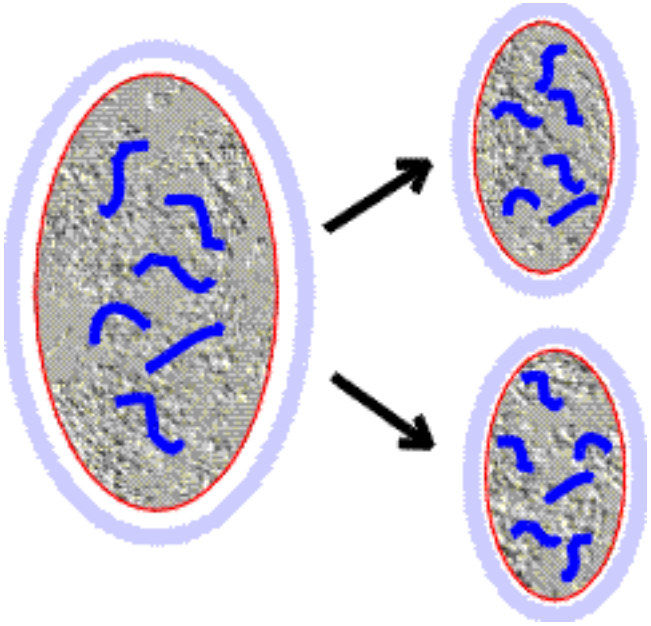
University of California, San Francisco

Epigenetics

- The processing of genomic DNA in the unfolding development of an organism.

- The study of *heritable* changes in gene function that occur without a change in the sequence of nuclear DNA.
This includes the study of how environmental factors affecting a parent can result in changes in the way genes are expressed in the offspring

"*heritable*"

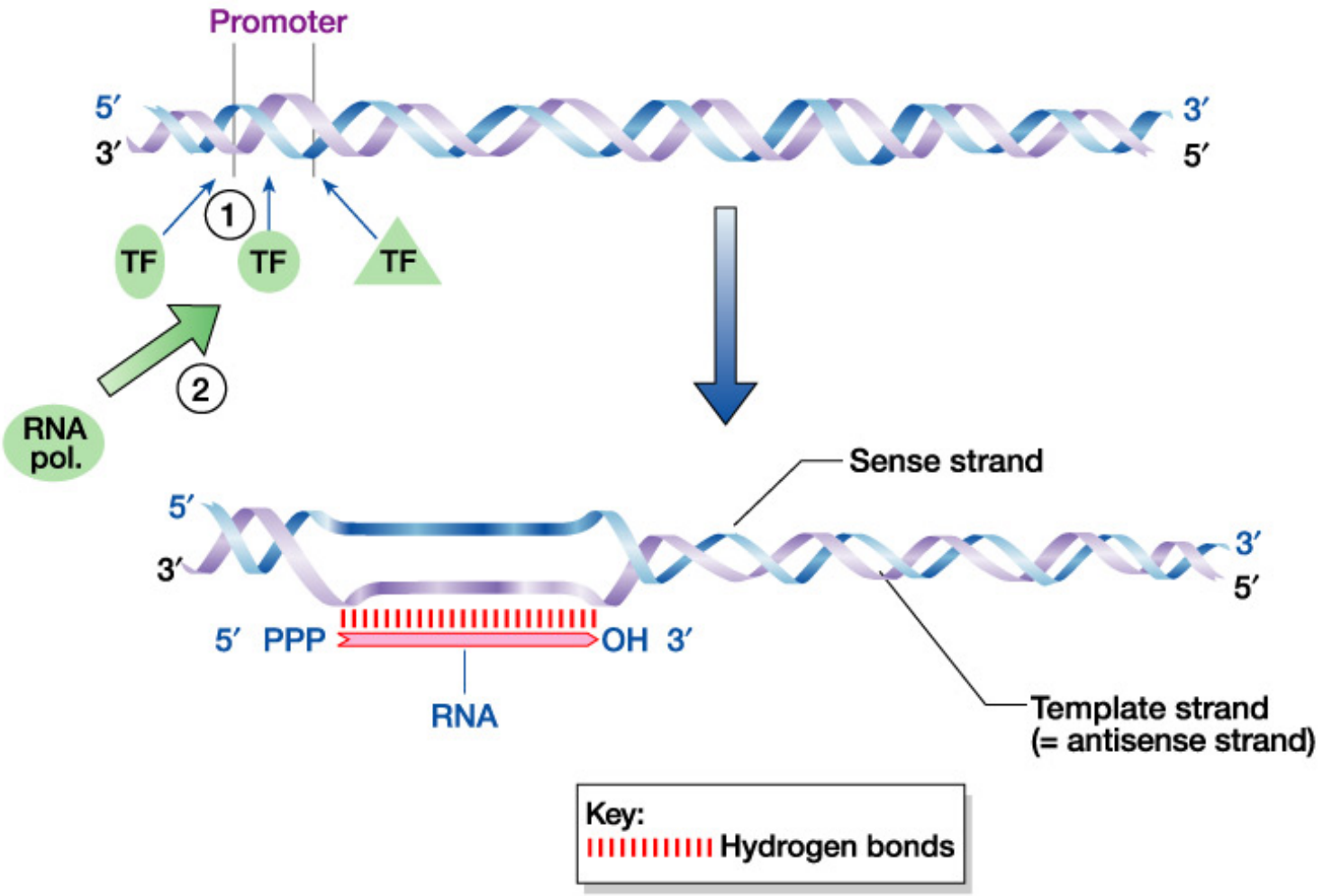


Epigenetic status is passed from the mother cell to the daughter cells. DNA of all cells is the same, but epigenetic status varies by tissue type.

On a mechanistic level, epigenetics is the state of function of the genome related to gene expression which is controlled in part by the state of gene promoters

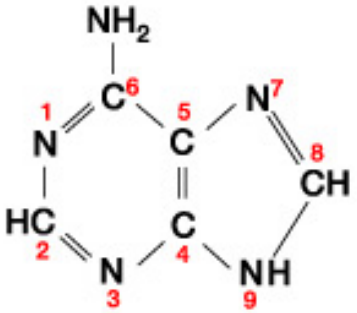
Gene expression levels are dependent on promoter function.

Epigenetics is the control of promotor accessibility to transcription factors.

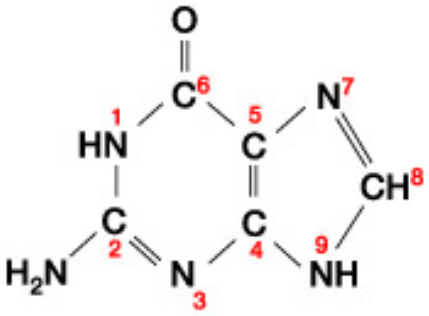


First line of epigenetic control

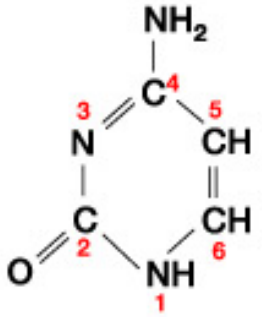
Methylation of cytosine residues on DNA



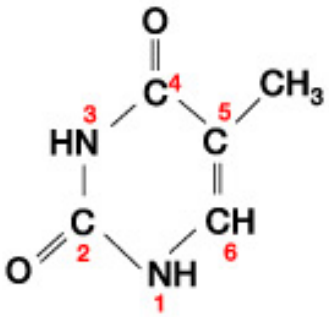
Adenine (A)



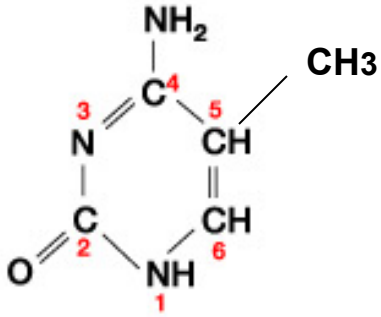
Guanine (G)



Cytosine (C)



Thymine (T)



5-methyl- Cytosine (C)

The "fifth base"

Figure 1-2 part 1 of 2 Human Molecular Genetics, 3/e. (© Garland Science 2004)

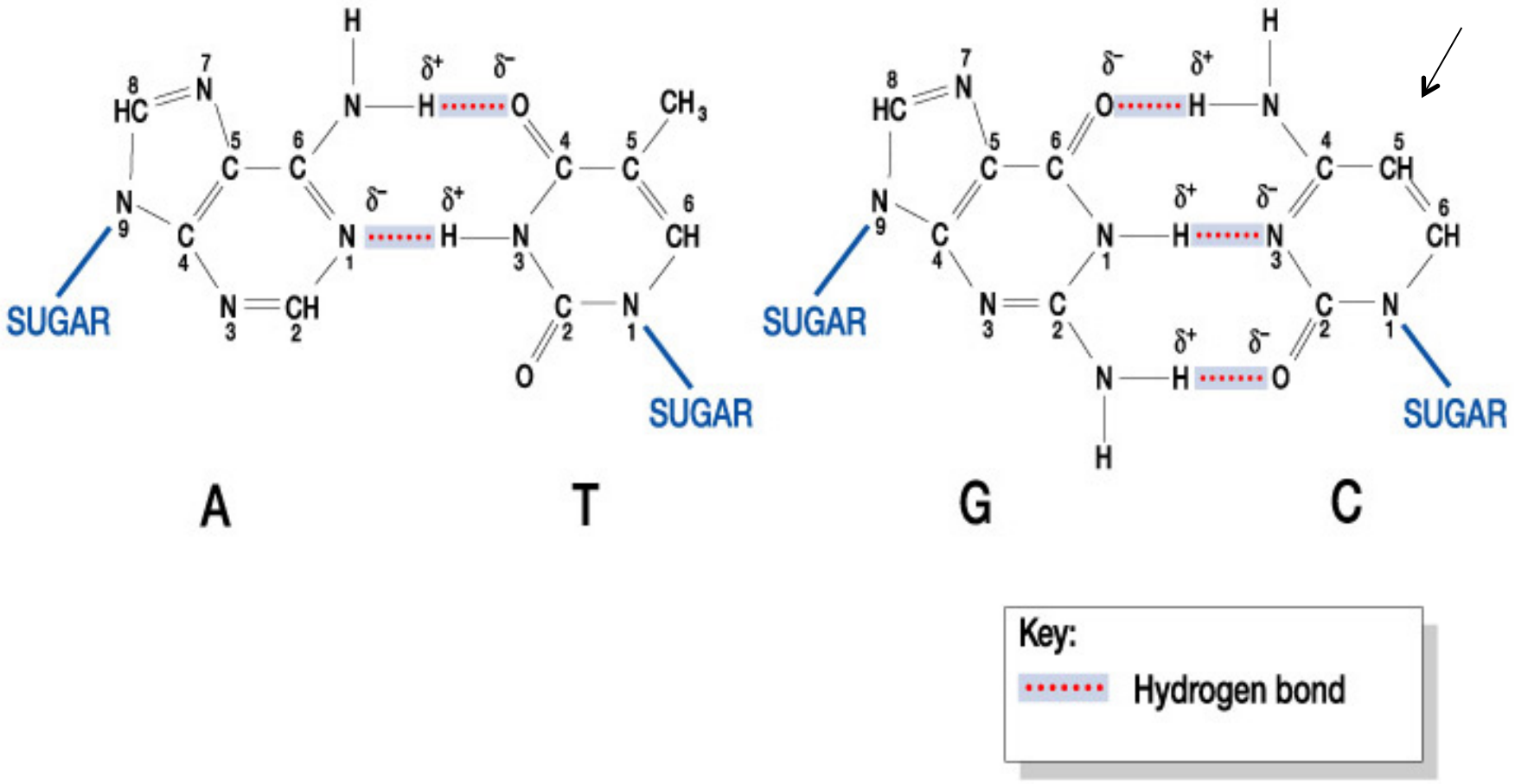


Figure 1-5 Human Molecular Genetics, 3/e. (© Garland Science 2004)

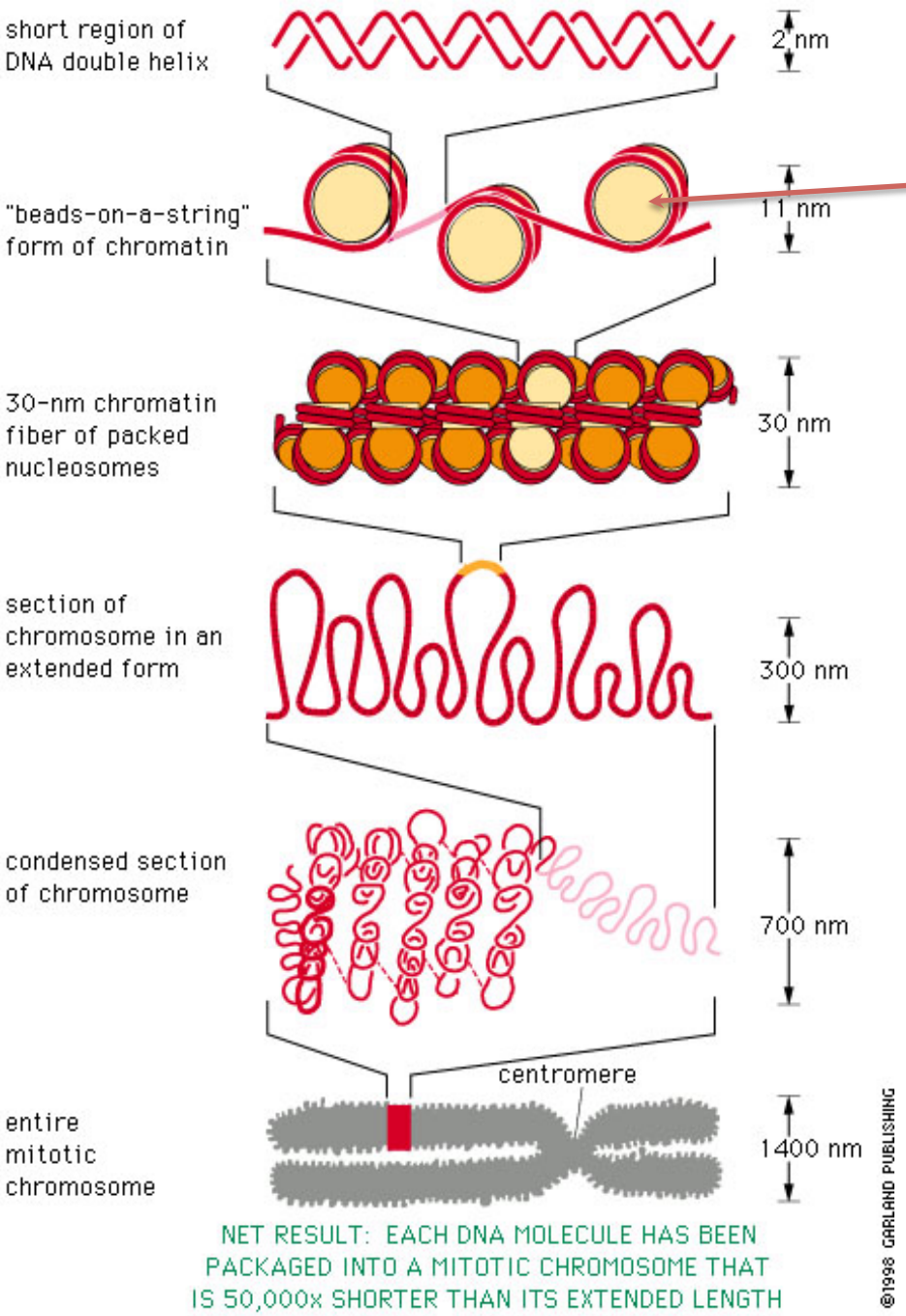
5-methyl C facts

Only occur at CpG sites 5’ -GT**CG**TAACAT**CG**ATGGCA-3’

Most CpGs are methylated in the genome, and associated with interspersed repeat sequences (“parasitic” DNA) and heterochromatin

CpGs that occur in high density are typically unmethylated. 45,000 such “CpG islands” exist.

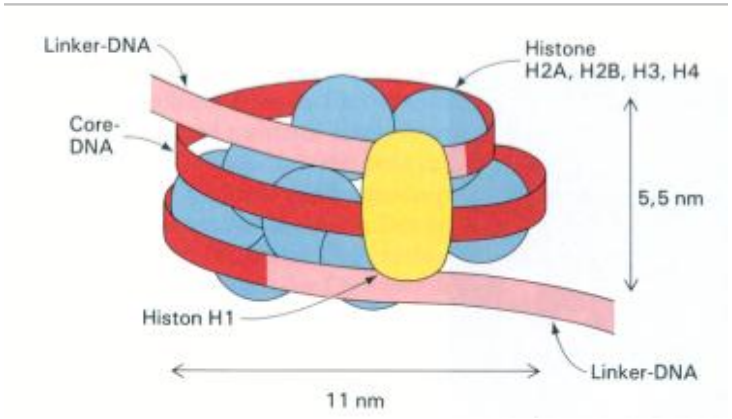
Acts as a tag for repression of gene promoters.

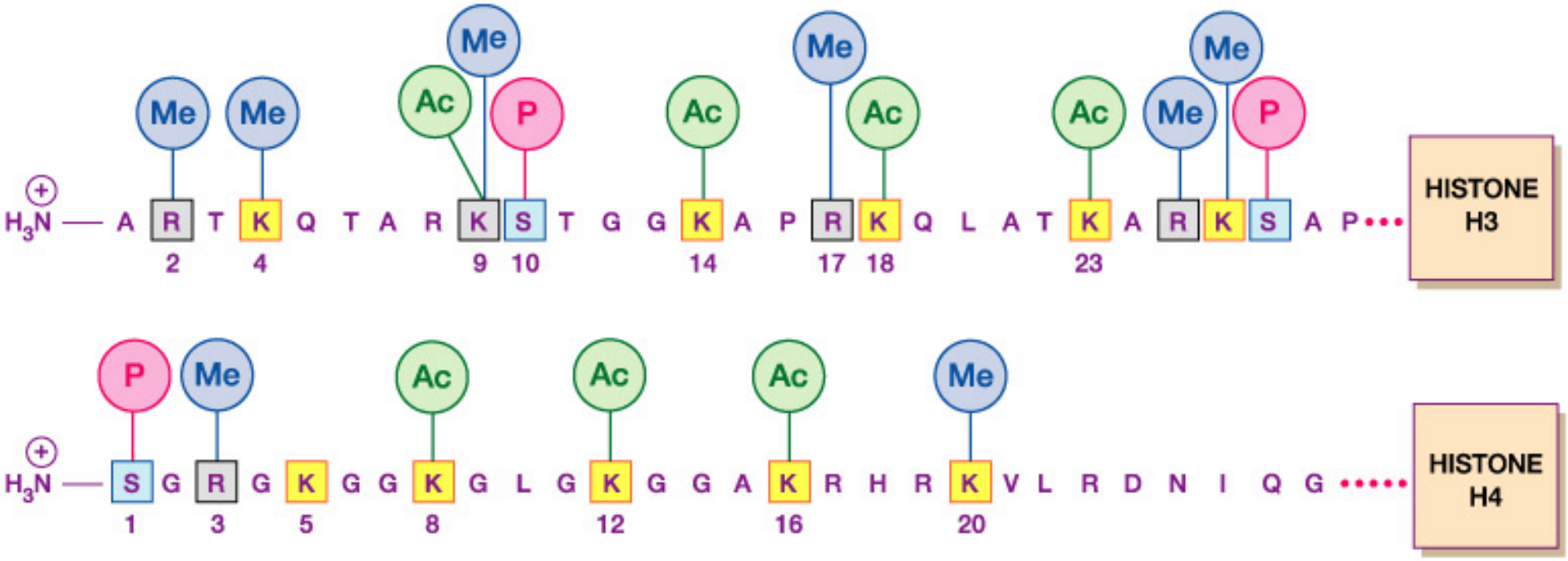


histones

Epigenetics beyond DNA methylation:

Chromatin structure





Histone modification: controls the status of chromatin and gene promoters

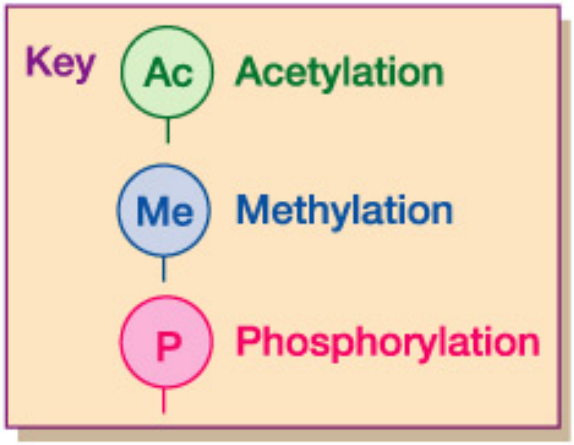
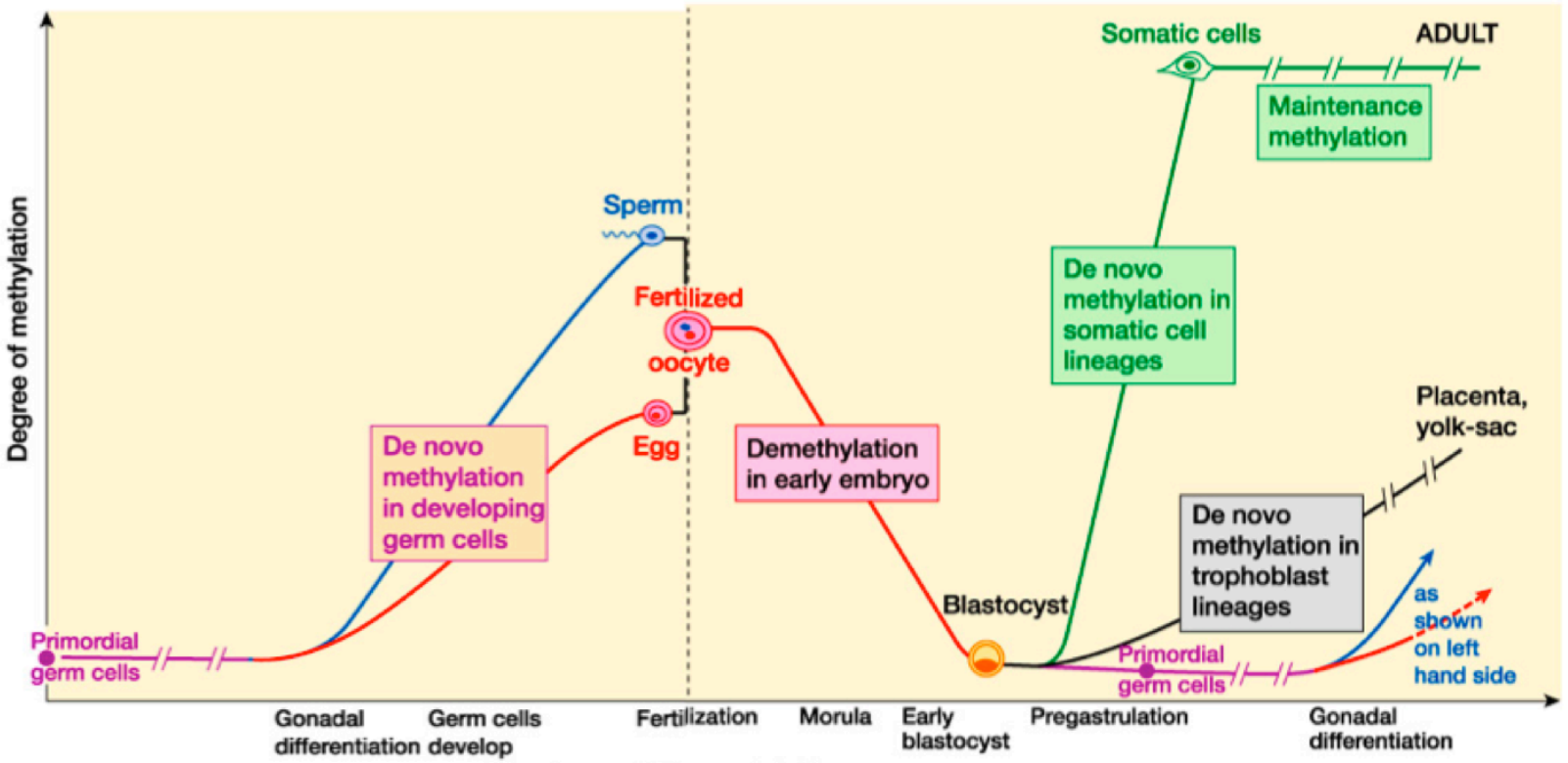


Figure 10-1 Human Molecular Genetics, 3/e. (© Garland Science 2004)

Why do epigenetic researchers concentrate on DNA methylation?

- Most accessible epigenetic mark to epidemiologists
- Only need to isolate and evaluate DNA.
- Other markers require large amounts of intact cells.

DNA methylation in development



Crucial DNA methylation changes during development

Childhood acute lymphoblastic leukemia (ALL)

- A form of leukemia with excess lymphoblasts
- Mostly B-ALLs, of which tumor cells originate from precursor B-cells
- Three major cytogenetics categories
  - Hyperdiploid : >47 chromosomes
  - *ETV6/RUNX1* (TEL/AML1) : t(12;21)(p13;q22)
  - Others (including normal karyotype)
- Evidence that leukemic clones originate from fetal life (before birth).
- Increased risks of childhood B-ALLs in parental exposure to environmental factors including pesticide and cigarette smoking.

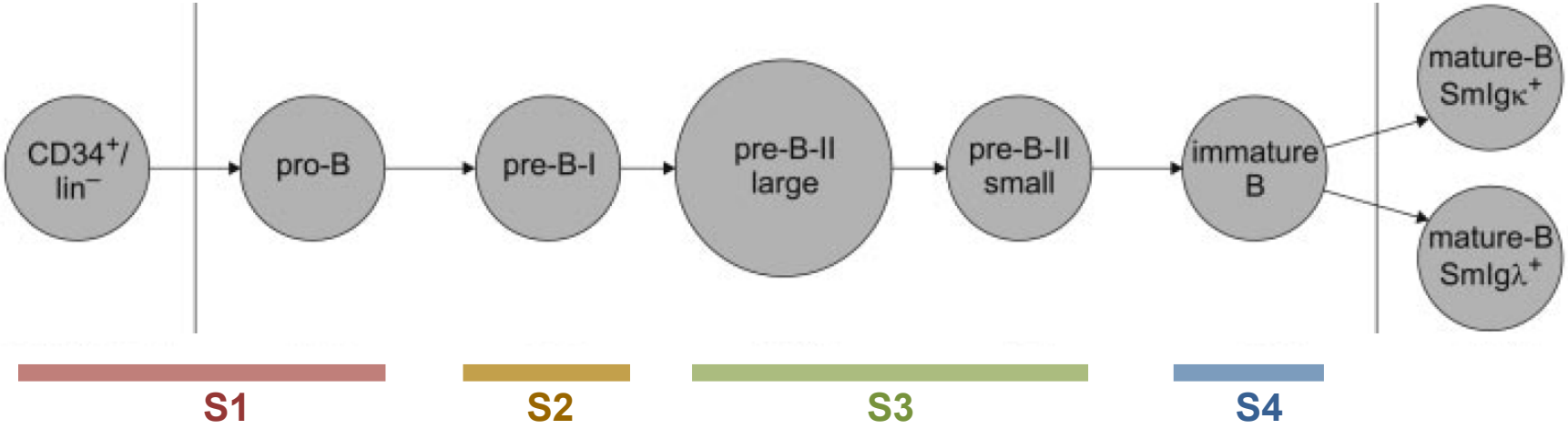
Current Research Goals

- Establish methylation patterns during normal B-cell development
- Identify methylation patterns in childhood acute leukemias in comparison with normal precursor B-cells
- Discover methylation patterns associated with environmental factors
  - Parental exposure to pesticides, herbicides, insecticides, paint and solvent
  - Parental smoking
  - Maternal folate intake
- Trace back to birth
  - Methylation profiles in Guthrie cards

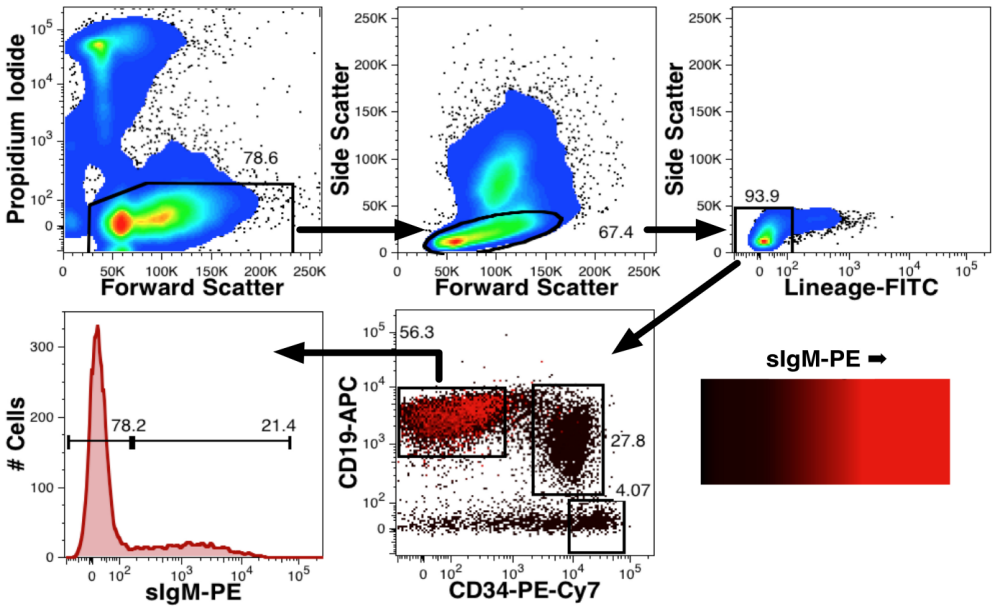
Methylation changes during normal development

Sorting of cells according to different developmental stages in early B-cell precursors

(6 fetal blood samples x 4 stages)



Isolation of fetal B-cells and their progenitors. The gating strategy used to sort FBM cells employed a PI$^-$ live cell gate, a light-scatter gate that encompassed lymphoid and progenitor cells followed by a gate to eliminate any lineage$^+$ cells that have not removed by the immunomagnetic bead depletion. CD34^{++}CD19$^-$ early progenitors and CD19$^+$CD34$^+$ B-cell progenitors were gated as shown in the dot plot. Additionally, CD19$^+$CD34$^-$ B-cells were gated and sorted based on sIgM expression (red color in dot plot) using the gates shown in the histogram. Numbers refer to the percentage of events found in the corresponding gates.



Methylation changes in childhood ALLs

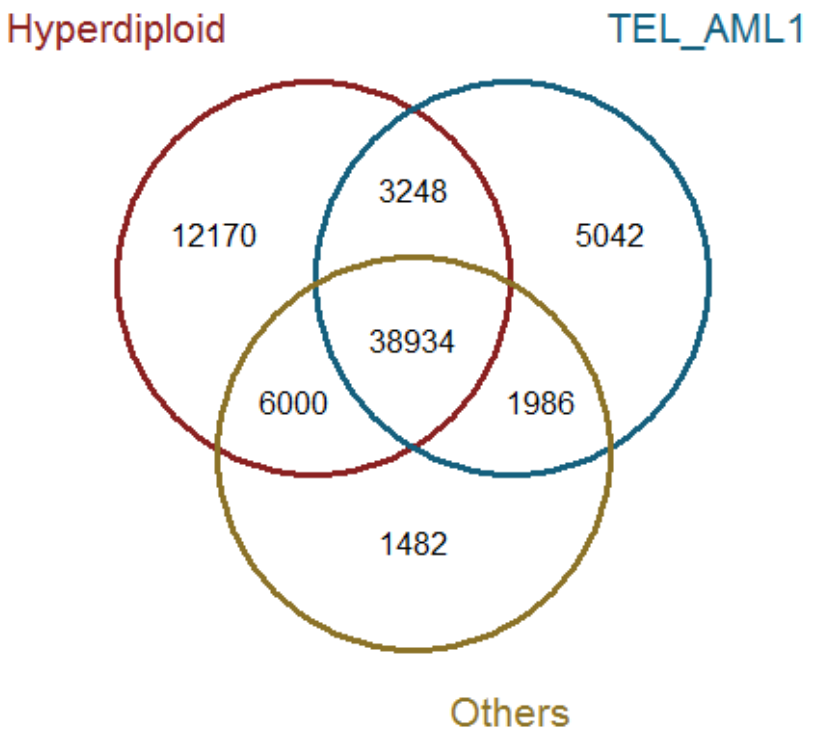
Childhood B-ALL samples
(Illumina Infinium 450K methylation chip)

- 231 childhood B-ALL patients (after excluding 10 patients with bad quality)

Cytogenetics	n	Female/Male	Age at diagnosis
Hyperdiploid	74	35/39	4.64 ± 2.63
***ETV6/RUNX1* - t(12;21)(p13;q22)**	62	25/37	4.26 ± 2.19
***TCF3/PBX1* - t(1;19)(q23;p13)**	5	3/2	5.62 ± 5.38
MLL translocations	2	2/0	0.55 ± 0.35
Others	80	35/45	5.95 ± 3.64
Unknown	8	5/3	4.92 ± 2.85
Total	231	105/126	4.99 ± 3.07

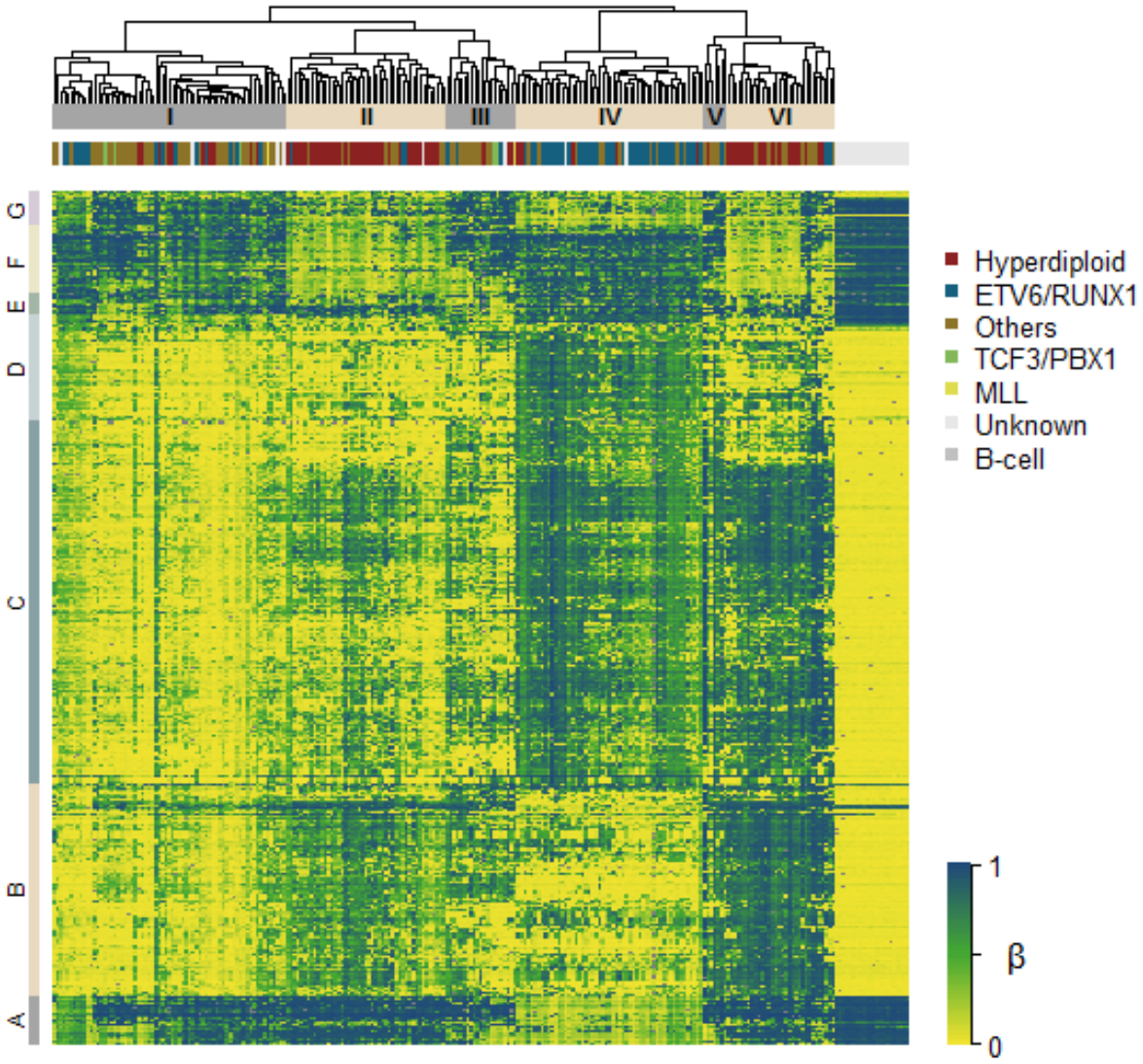
- 24 controls (normal precursor B-cells)

DMRs according to cytogenetics



Differentially methylated regions (DMRs; *vs* normal B-cells) according to cytogenetics. Comparative analyses of methylation levels between different leukemia subgroups and normal B-cells identifies a large set of common DMRs and minor sets of DMRs specific to each cytogenetics groups.

Within-case comparison and clustering using 500 hypervariable loci



Within-case comparison and clustering. A hierachial clustering and heatmap generation using 500 hypervariable CpGs across all leukemia samples identifies six distinct clusters, mostly correlated with cytogentic abnormalities. Hyperdiploid group can be divided into two clusters that have far different methylation patterns.

Methylation changes in association with environmental variables & tracing them back to birth

Guthrie cards: 248 case and 255 controls
Leukemias: 238 blast cell leukemias

Epidemiological variables

- Birth weight
- Gestational age
- Maternal / paternal age
- Maternal / paternal smoking
  - 3 months prior to pregnancy
  - during pregnancy
  - after birth
- Maternal folate intake
  - Food
  - supplemental
- Maternal exposure to pesticide
- Maternal exposure to insecticide
- Maternal exposure to herbicide
- Maternal exposure to paint
- Maternal exposure to solvents

Using “tail strength” to quantify predictor “strength”

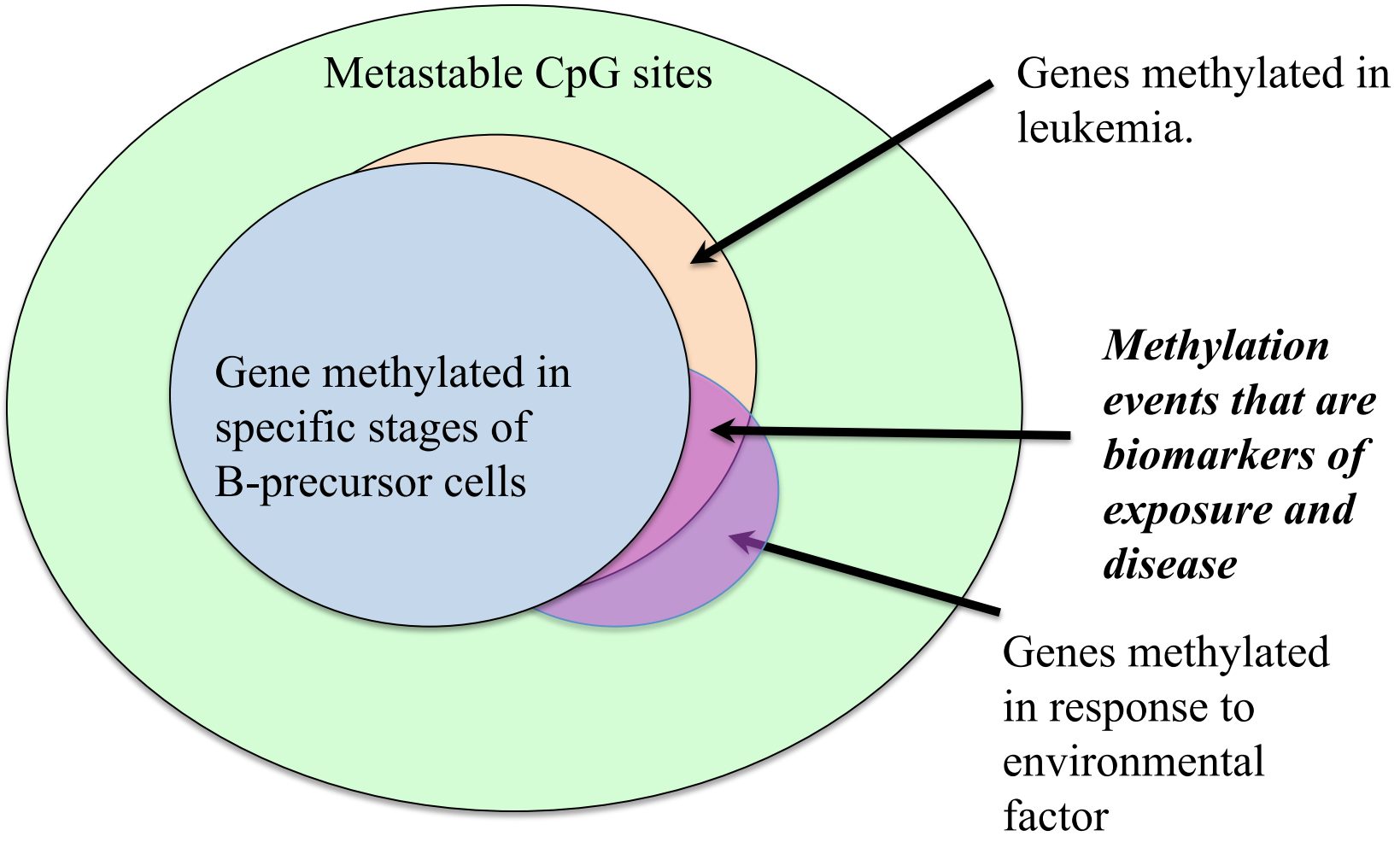
- The ‘tail strength’ is a measure of the overall statistical significance in testing the global hypothesis of no effects. It is simple function of the *p*-values computed for each of the tests. This measure is useful, for example, in assessing the overall univariate strength of a large set of features in microarray and other genomic and biomedical studies.

Taylor and Tibshirani. Biostatistics 2006 7:167

Ranks of smoking variables by tail strength

Rank	Predictor	TS	Rank	Predictor	TS
1.	mo.preg.N	0.037	7.	mo.3m.N	0.009
2.	fa.3m	0.029	8	mo.after.N	0.006
3.	mo.preg	0.027	9.	Mo.bf.N	0.004
4.	mo.bf	0.023	10.	Passive.sm.home.post	-0.006
5.	mo.3m.preg.N	0.014	11.	Mo.ever	-0.016
6.	fa.3m.N	0.014	12.	Fa.ever	-0.037

DNA methylation is directly involved in cancer but understudied in the childhood leukemias



Summary

- In early B-cell development, DNA methylation changes especially in other than promoters are associated with profound effects on gene expression.
- The changes were nonrandomly located in terms of CGIs, alternative TSSs, and TF binding sites.
- The impact of DNA methylation on gene regulation was reduced in later stages.

- In childhood ALLs, prominent down-regulation of many cancer-associated genes were noted accompanying methylation changes.
- Aberration in DNA methylation was nonrandomly located in terms of CpG islands, imprinted regions and TF binding sites. The *de novo* methylation in CpG islands seems to overrides other changes.

- Different methylation levels in specific CpGs in Guthrie cards and specific subgroups of leukemia were noted according to parental smoking, nutritional status and exposures to smoking chemicals, suggesting a possible mediating role of methylation between environmental factors and leukemogenesis (replication analysis under way).

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