EPIGENETICS: an introduction

Epigenetics

In the 1940's (Waddington et al.)

- **The sum of the genes and their products and how they define a phenotype (dividing kidney and skin cells are identical in their DNA but give rise to cells of different phenotype).**

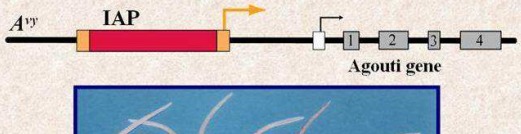
Today

- **Changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence.**

What does this mean?

Agouti Genes in Mice

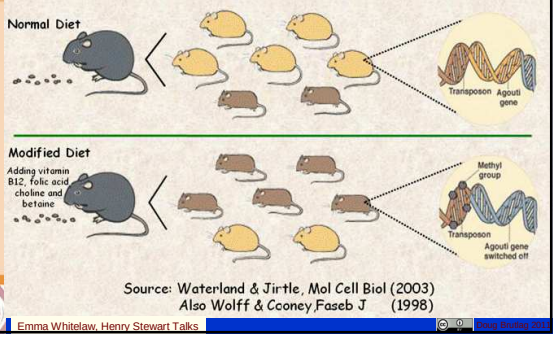
Agouti viable yellow (A^{vy})



Environment can Influence Epigenetic Changes

Can environment influence these processes?

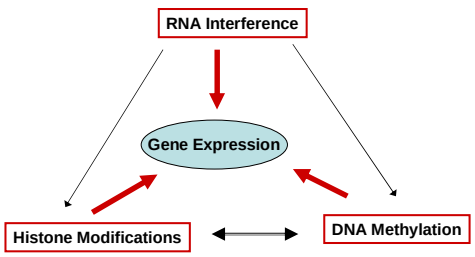
They are what she ate...

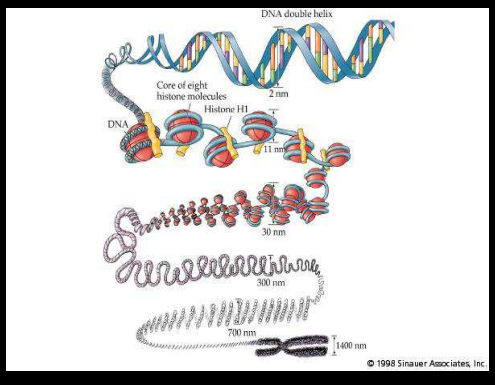


Source: Waterland & Jirtle, Mol Cell Biol (2003)
Also Wolff & Cooney,Faseb J (1998)

Emma Whitelaw, Henry Stewart Talks

Epigenetics Mechanisms

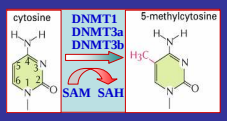




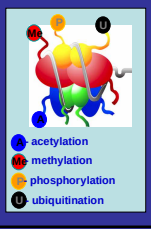
EPIGENETICS

Epigenetic alterations – changes induced in cells that alter the expression of the information on transcriptional, translational, or post-translational levels without changes in DNA sequence

Methylation of DNA



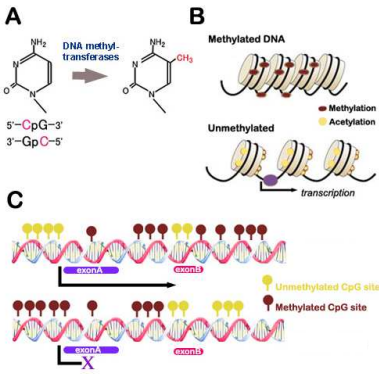
Modifications of histones



RNA-mediated modifications

- RNA-directed DNA methylation
- RNA-mediated chromatin remodeling
- RNAi, siRNA, miRNA
- microRNA

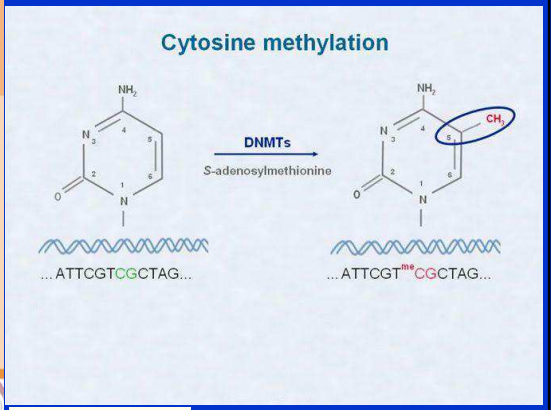
DNA Methylation



Hypomethylation
Hypermethylation

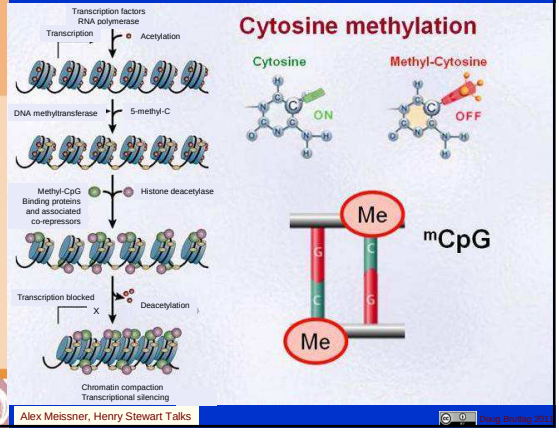
http://www.cellscience.com/reviews7/Taylor1.jpg

Methylation of Cytosine in DNA



Paula Vertino, Henry Stewart Talks

Cytosine Methylation Maintains Inactive-Condensed Chromatin State



Alex Meissner, Henry Stewart Talks

DNA Methylation

- In mammals, ~1% of DNA bases methylated on carbon-5 of cytosine pyrimidine ring (5-methylcytosine).
- Most frequent at 5'-CpG-3' dinucleotides (~70% of all CpGs).
- In general, CpGs are under-represented (suppressed)

CpG islands

- Regions (500-1 kb) of higher G+C than genome average.
- Relatively devoid of methylation.
- ~60% mammalian RNA Pol II promoters found in CpG islands.

DNA Methylation and Gene Expression

•• Cytosine residues in 5'CpG are often postsynthetically methylated

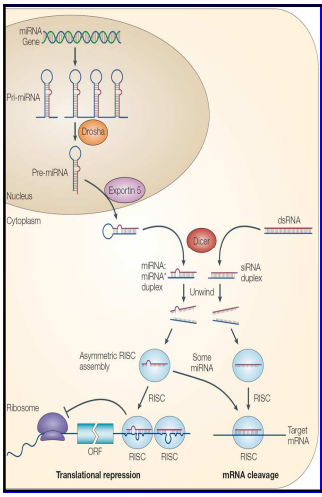
•• CpG methylation is involved in long-term silencing of certain gene during development

•• The methyl-CpG-binding proteins MeCP1 and MeCP2 interact specifically with methylated DNA and mediate transcriptional repression.

Table 2 | **Characteristics of histone deacetylases**

HDAC group	Yeast HDAC*	Inhibitor sensitivity‡	Human HDAC	Inhibitor sensitivity‡
Class I	Rpd3	S	HDAC1	S
			HDAC2	S
			HDAC3	S
			HDAC8	S
Class II§	Hda1	S	HDAC4	S
			HDAC5	S
			HDAC6	S
			HDAC7	S
			HDAC9	S
Class III	Sir2	NS	SIRT1	ND
	Hst1	ND	SIRT2	ND
	Hst2	ND	SIRT3	ND
	Hst3	ND	SIRT4	ND
	Hst4	ND	SIRT5	ND
			SIRT6	ND
			SIRT7	ND

*Another group of histone deacetylases (HDACs) — designated Hos1, Hos2 and Hos3 — have been found in yeast, but homologues have not been identified in mammalian tissues so far[13]. Of these, only Hos3 has been characterized with regard to sensitivity to inhibitors, and was found to be relatively insensitive to trichostatin A (TSA). ‡HDAC inhibited by TSA, SAHA (suberoylanilide hydroxamic acid) and/or related compounds. §Another mammalian HDAC, HDAC10, that is related to class II has been identified and characterized, but no data are reported with respect to inhibitor sensitivity[94]. HDAC, histone deacetylase; ND, Not determined; NS, HDAC not sensitive to inhibition by TSA or SAHA; S, sensitive to inhibition by TSA or SAHA.



MicroRNAs: After the transcription of a miRNA gene follows the nuclear cleavage of the pri-miRNA performed by the Drosha RNase III endonuclease. This enzyme cuts both strands of the pri-miRNA near the stem loop and generates ~60–70 nt stem loop miRNA precursor (pre-miRNA). This pre-miRNA is transported to the cytoplasm by the export receptor Exportin-5. The nuclear cut by Drosha defines one end of the mature miRNA and cytoplasmic cut by Dicer, also RNase III endonuclease, defines the opposite one. Dicer recognizes the pre-miRNA and cuts both of its strands at about two helical turns away from the base of the stem loop. Then one of this ~22 nucleotide miRNA duplex arms is chosen and mature miRNA is associated with RNA-induced silencing complex RISC. RISC acts to repress the translation of target mRNA by mechanisms of translational repression or mRNA cleavage.

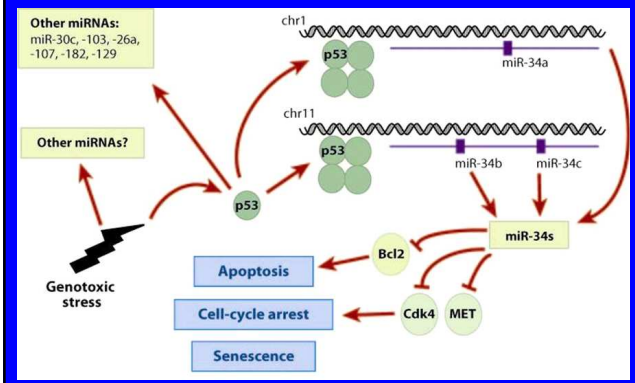
ROLE IN CANCER

- Recent studies show that some microRNAs regulate cell proliferation and apoptosis, processes that are important in cancer formation.

- In addition, some miRNAs may function as oncogenes or tumor suppressors.

-More than 50% of miRNA genes are located in cancer associated genomic regions or in fragile sites.

ROLE OF miRNA in cell growth and cell death



miRNA and CANCER: what is the link ?

- Global KD of miRNAs (obtained through RNA-interference silencing of the miRNA processing enzymes Drosha, DGCR8 and Dicer) enhances tumorigenicity and malignant behaviour (MTX, growth) of cancer cells (Kumar et al. 2007). BUT, miRNAs deletion is NOT sufficient to promote cancerogenesis !
- Hemizigous deletion of Dicer is found in 27% of cancers (haploinsufficient oncosuppressor)

CANCER miRNOMA

- A large microarray analysis (363 solid tumours and 177 normal tissues) identified 36 miRNA over-expressed and 21 down-regulated miRNAs, suggesting both oncosuppressive and oncogenic functions of miRNAs. target

- ONCOMIRNAs: target and down-regulate oncosuppressors
- TUMOR SUPPRESSIVE MIRNAs: target and down-regulate oncogenes

miRNA double-face

- EXAMPLE:
- high expression of the cluster miR-17-92 only induces lympho-proliferative disorders, but in a MYC-driven B-cell lymphoma it accelerates *in vivo* tumorigenicity,
- whereas homozygous deletion of the miR-17-92 locus causes pre-B cell death and lymphopenia.

IMPORTANCE OF THE CELLULAR CONTEXT:

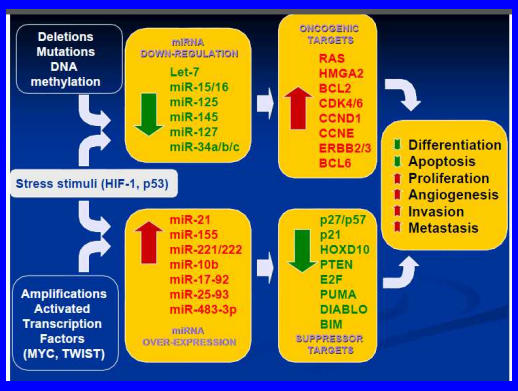
MiR-221 and MiR-222 inhibit erithropoiesis by inhibiting the expression of the oncogene c-Kit

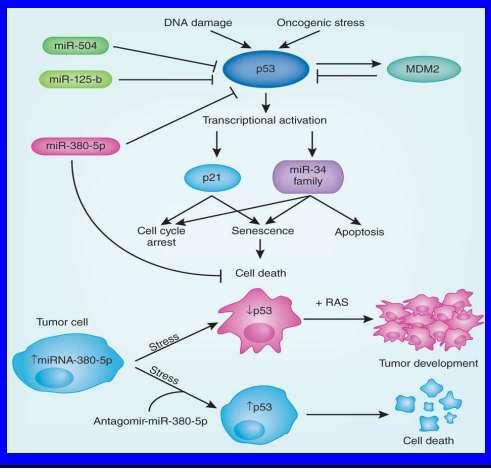
However, in liver, these miRNAs stimulate the growth of transplanted cancer cells: in this case, miRNA-221 and 222 down-regulate PTEN and activate the Akt-mTOR pathway !

Oncogenic activity of miR-17-92

- Cluster arising from a polycistronic RNA that gives rise to 7 miRNAs: miR-17-5p, miR-17-3p, miR-18a, miR-19a, miR-20a, mi-R-19b, miR-92-1.
- miR-19a and miR-19b target PTEN mRNA and activate the Akt-mTOR pathway.
- Other targets: pro-apoptotic Bim (in B-CLL)

miRNA down-regulation or over-expression IN CANCER





Roles of small RNAs in tumor formation

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Table 1. MiRNA signatures in human cancers. Representative examples of miRNA expression signatures in the most common human cancers.

Tumor	Results	References
Chronic lymphocytic leukemia (CLL)	CLL cells show a unique signature that associates with the presence or absence of disease progression and cytogenetic abnormalities.	[18,83,92]
Acute myeloid leukemia (AML)	CD34(+) cells and AML cases differentially express miRNAs that associate with several cytogenetic groups (11q23 translocations and isolated trisomy 8).	[19]
Acute promyelocytic leukemia (APL)	Identified 12 miRNAs in APL blasts compared with normal promyelocytes; miR-342 and let-7c are modulated by retinoic acid.	[100]
Multiple myeloma (MM)	First identification of miRNA signature in myeloma cell lines and patients.	[23]
Acute lymphoblastic leukemia (ALL)	Identification of miRNA signature in ALL patients.	[101]
B-cell lymphomas (DLBCL) and follicular lymphomas (FL)	DLBCL- and FL-specific miRNA signatures. A total of 98% of all 111 cases were correctly identified based on the expression of four miRNAs (miR-210/106a/17-5p/330).	[103]
Barrett's esophagus and esophageal adenocarcinoma	Identification of several miRNAs differentially expressed in the progression from low grade-dysplasia Barrett's esophagus to adenocarcinoma.	[105]
Breast cancer	Reported miRNomes of normal and breast cancer samples. Dysregulated miRNAs associate with invasive breast cancer pathologic features, such as ER status.	[22]
Glioblastoma	Reported miRNA profiles from case-matching pairs of tumor and control samples. Nine miRNAs are overexpressed and four are underexpressed in tumors compared with normal.	[21]
Hepatocellular carcinoma (HCC)	Reported miRNA profiles in HCCs compared with adjacent normal tissue and additional chronic hepatitis specimens. Identified a new diagnostic tool for HCCs.	[17]
Ovarian cancer	Identification of miRNAs whose expression correlates with specific ovarian cancer pathologies, such as histotype, lymphovascular and organ invasion and involvement of ovarian surface.	[93]
Lung adenocarcinoma	Reported molecular signatures that differ across tumor histology; miR-155 and let-7 correlate with survival.	[84]
Papillary thyroid carcinoma	Reported upregulation of miRNA-221/222 in tumor cells and adjacent normal cells compared with normal thyroid.	[85]
Endocrine pancreatic cancer	Reported miRNA signatures from endocrine and acinar tumors; miR-21 associates with proliferation index and liver metastasis.	[86]
Colon cancer	Reported differential expression levels for 39 miRNAs between colon tumors and normal tissue. Colon tumors differentially express miRNAs according to their mismatch repair status.	[20]
Gastric cancer	Identified 22 miRNAs upregulated and 13 downregulated in gastric cancer by comparing non-tumor mucosa to cancerous tissue.	[87]
Cervical cancer	Identification of miRNA signature in 102 cervical cancers by PCR-based miRNA assay. miR-200a and miR-9 predict patient survival.	[88]
Clear-cell kidney cancer (ccRCC)	Identified 26 miRNA downregulated and 9 upregulated in 28 ccRCC patient-matched specimens. Downregulated microRNAs are correlated with common chromosome deletion in ccRCC.	[89]
Sarcoma	MicroRNA expression is able to classify different histological types of sarcoma reflecting differentiation status and apparent lineage of the tumors.	[102]
Bladder cancer	Identification of miRNA differentially expressed between normal urothelium/cancer and different disease stages. miR-128 predicts disease progression.	[104]