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MicroRNAs and epigenetic in human health pathology: a paradigm for chemical induced inherited effects.

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miRNAs – What are they?

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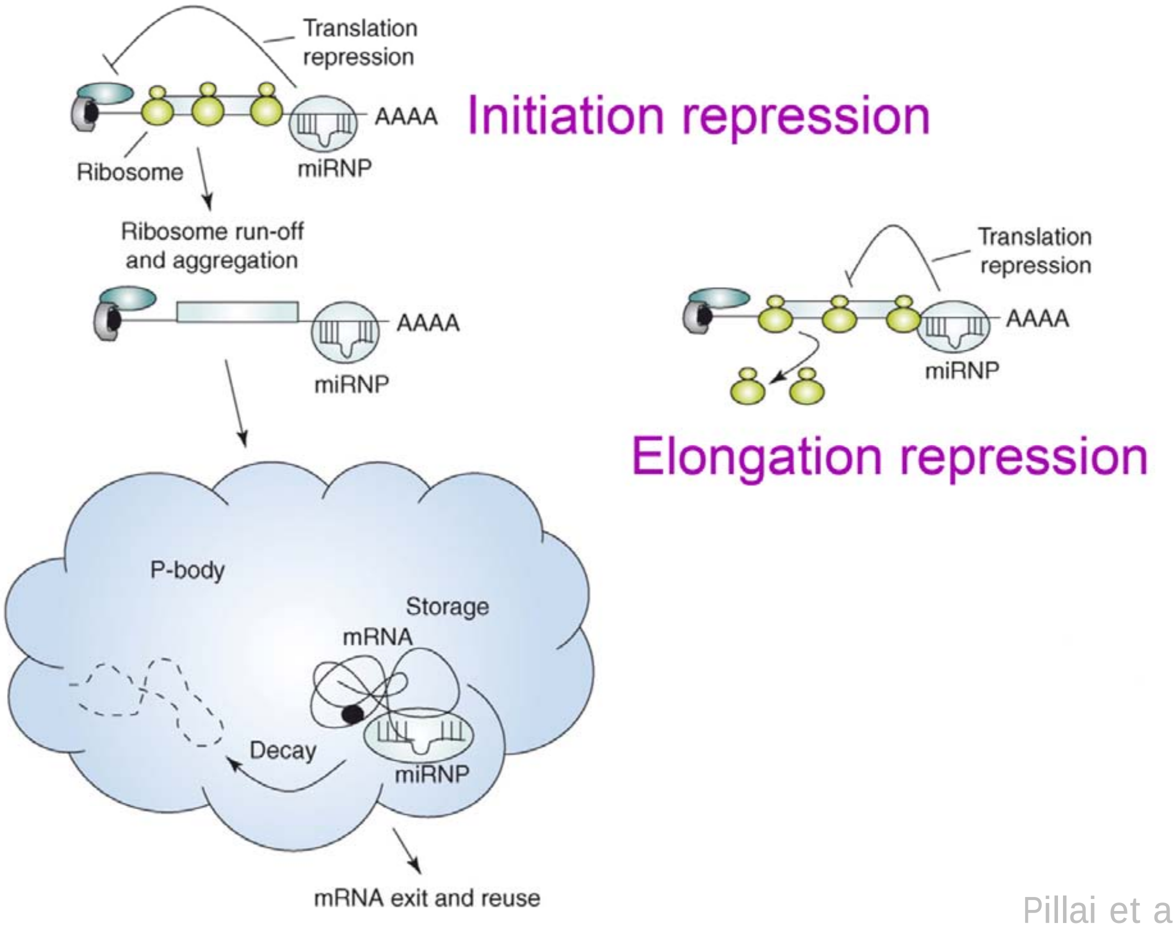
MiRNAs are 21-25nt pieces of non-coding RNA that regulate protein translation from mRNA species



Pillai et al TICB 17(3) 118 (2007)

miRNA synthesis and processing

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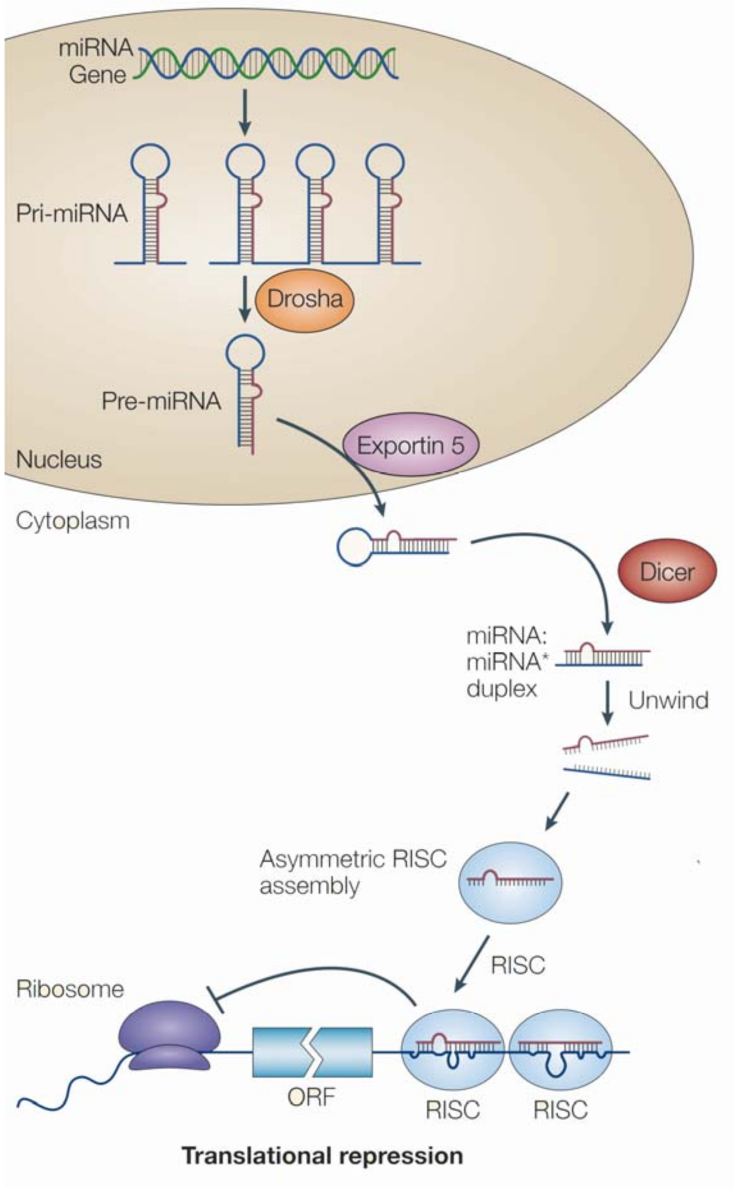

- miRNAs are located either in defined genome regions or within gene introns. For both transcription is regulated controlled by normal gene transcription mechanisms.

- After initial processing by Drosha to a ~70bp loop form they are exported to the cytoplasm

- In the cytoplasm Dicer removes the loop and unwinds the double strand, the antisense of which goes to the RISC complex

- Once activated by the mature miRNA the RISC complex suppresses translation from target genes.

miRNA abundance and accessibility

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Sample (Hu)	Median total RNA µg/L	Number of detectable miRNAs
Amniotic fluid	570	359
Breast milk	47240	429
Bronchial lavage	1128	260
CSF	111	212
Colostrum	585	386
Peritoneal fluid	775	397
Plasma	308	349
Pleural fluid	470	210
Saliva	1945	458
Seminal fluid	17770	436
Tears	564	320
Urine	94	204

miRNAs: Tissue specificity

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miRNA	Brain	Heart	Kidney	Liver	Lung	Spleen
miR-122	0.5	0.6	3.0	49,000	9.7	1.9
miR-124	120,000	19	17	5.0	18	14
miR-133a	220	170,000	55	14	1700	49

miRNA copies per cell *(estimated by copies per 20pg total RNA)*

Laterza et al. 2009; Molecular Diagnostics and Genetics

Physiological roles: Insulin secretion

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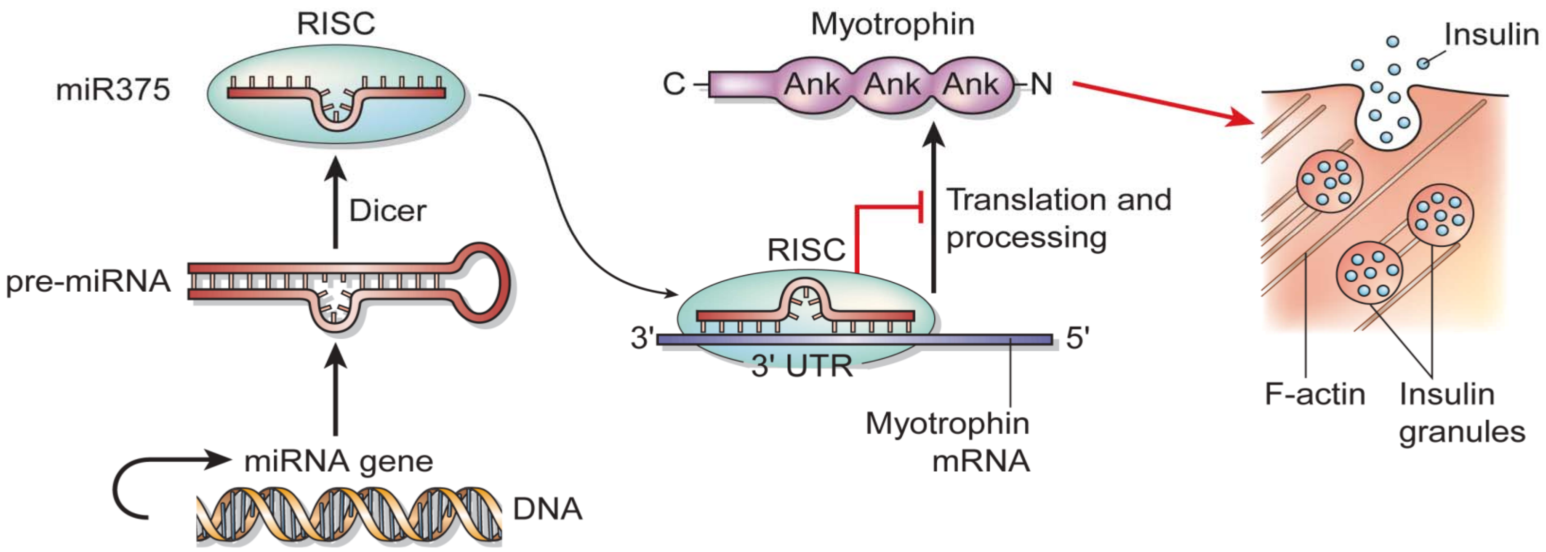
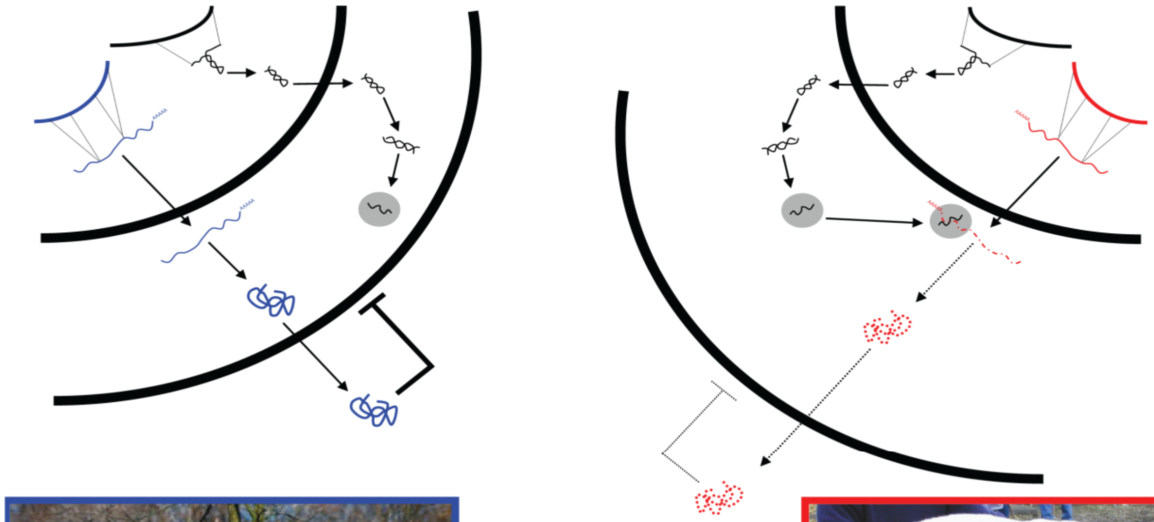


MIR375 binds to myotrophin which is inhibited in translation. Myotrophin controls the exocytosis of insulin in the pancreatic beta cells.

miRNA polymorphisms



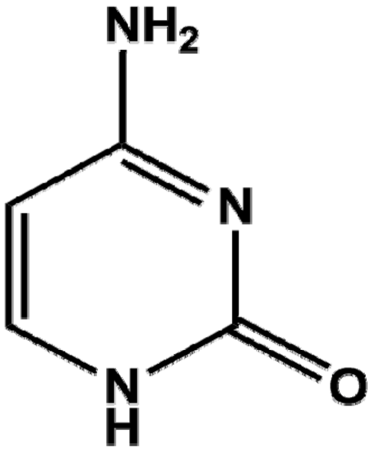
Normal sheep where muscle growth is controlled by GDF8

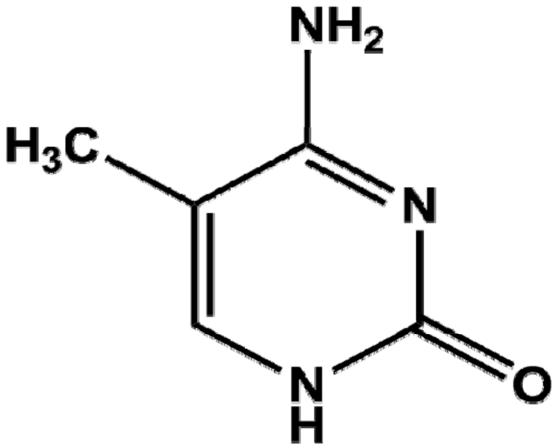
Georges M et al *Cold Spring Harbor Symposia on Quantitative Biology*

Texel sheep where a SNP in GDF8 created a miRNA binding site for miRNA-1 and 206 leading to reduced translation of GDF8 mRNA and less control over muscle formation

Epigenetic modification

- There are a number of forms of DNA and DNA histone modification
- For miRNAs the epigenetic modification that we are primarily interested in is that of cytosine modification, primarily methylation

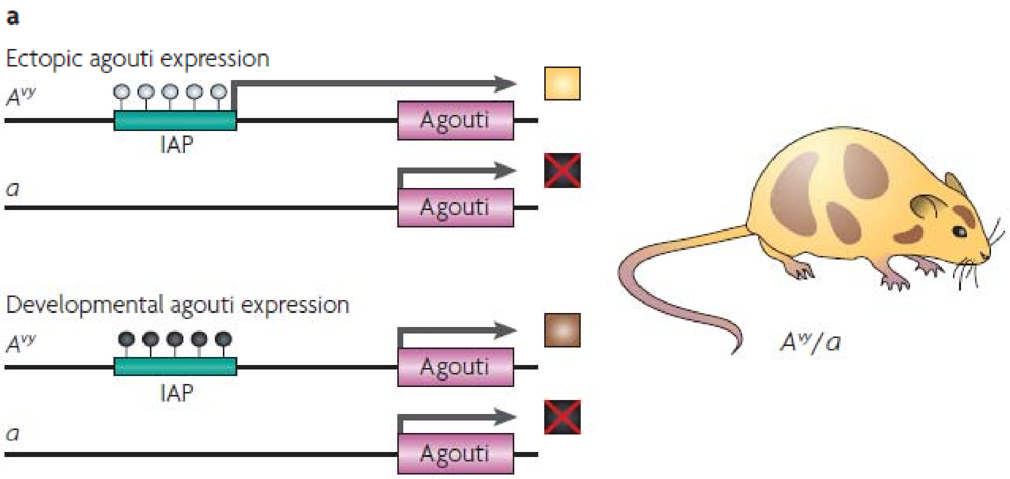




Methylation of gene promoter regions represses transcription

Chemical exposures, epigenetic change and phenotype

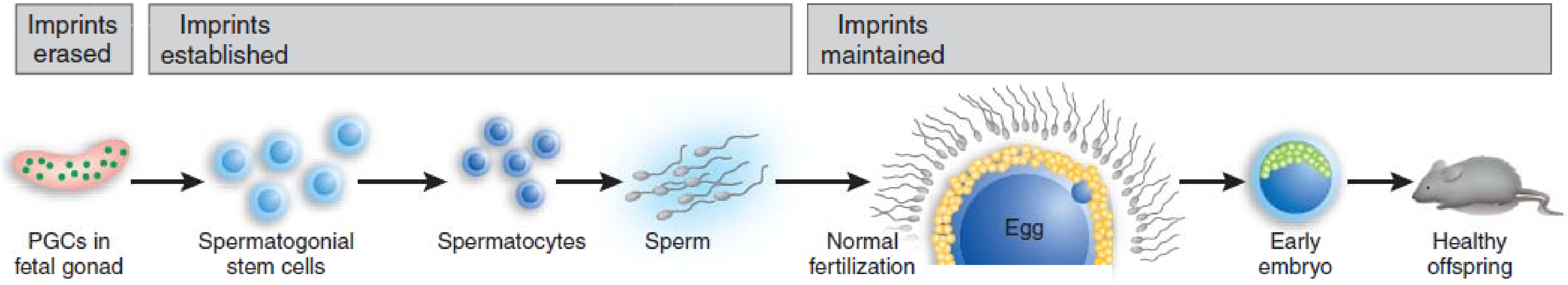
In utero exposure



Other chemical modifiers of epigenetic marks: PAHs, Traffic particulates, Nickel, Genestein, Diet, Ethanol, Vinclozolin, methoxychlor, phthalates

Jirtle and Skinner Nature Reviews

Imprint cycles in sperm formation

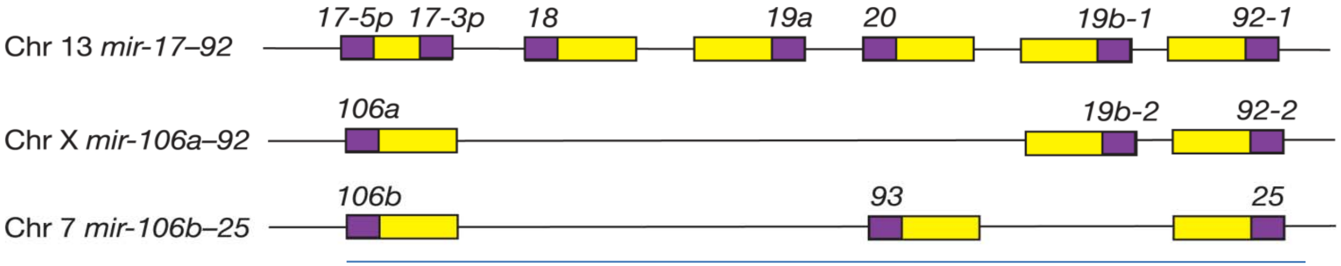


Changes in imprints in Somatic cells will only likely affect those cells, or if in a stem cell that lineage. Changes in germ cells though will affect all cells in the offspring and potentially be passed through generations after that.

From Lucifer and Reik Nature Biotechnology 2006

Genomic location of miRNA genes

Polycistronic



Approx 1Mb

He *et al* Nature 435, 828 (2005)

Genomic location	Human	Mouse	Rat	Dog
Intergenic	396 (52%)	405 (62%)	359 (76%)	225 (64%)
Intronic	308 (40%)	235 (24%)	112 (24%)	107 (31%)
Exonic	58 (8%)	12 (2%)	0 (0%)	18 (5%)
Total	762	652	471	350

From miRNAMap: Genomic maps of microRNA genes and their target genes in mammalian genomes NAR 34 D135-D139 (2006)

miRNAs associated with a CpG island sites

Species	Human	Mouse	Rat	Chicken
Total miRNA	462	373	234	149
CpG associated	74	37	25	25
Percentage	16%	10%	10.7%	16.8%

Tsai, Huang and Ng. Mammalian microRNA genes and CpG island sites.
http://bioinfo.asia.edu.tw/english/faculty/pdf/ng_kl.pd

Altered miRNA methylation and disease

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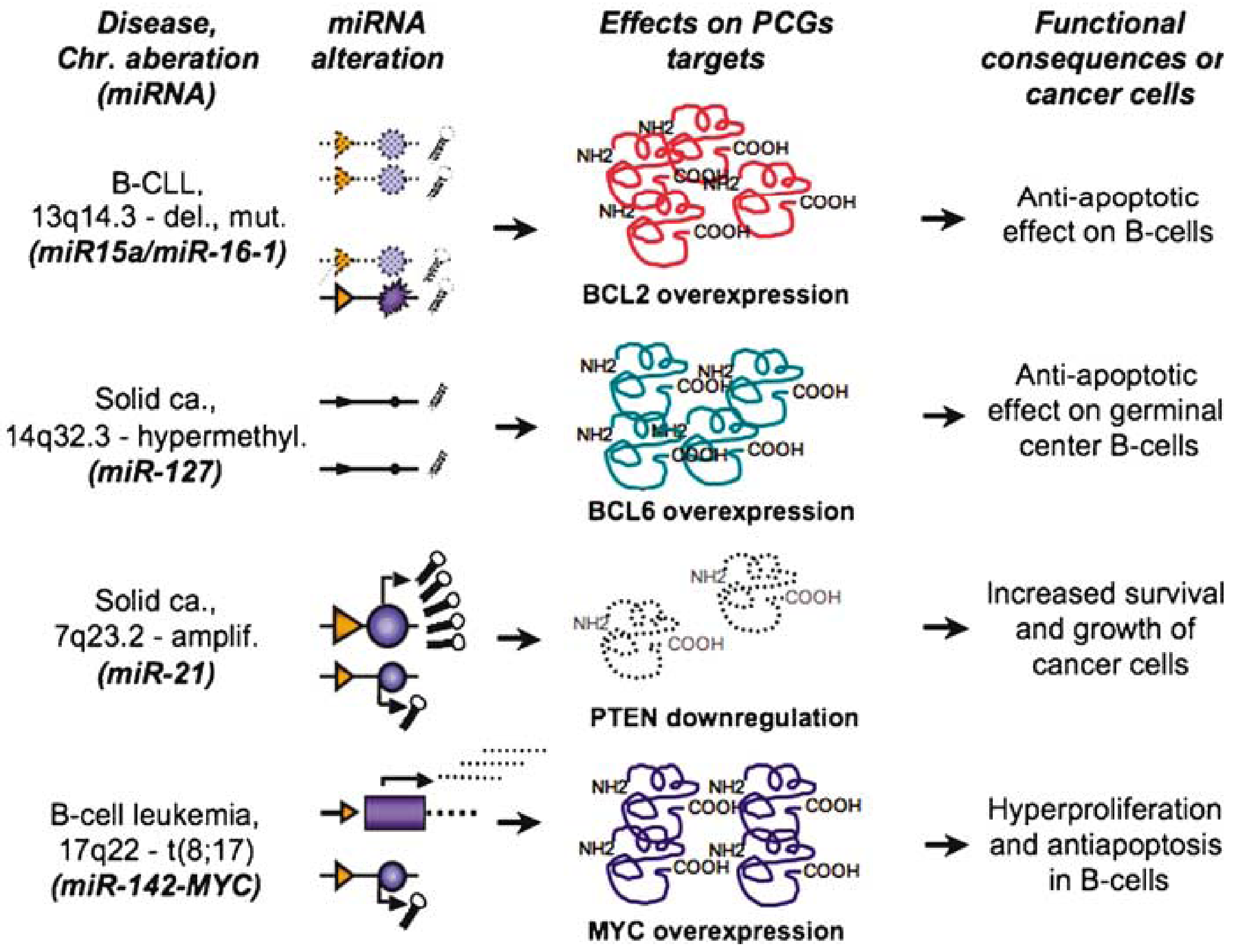

Disease, Chr. aberation (miRNA)	miRNA alteration	Effects on PCGs targets	Functional consequences or cancer cells	
B-CLL, 13q14.3 - del., mut. *(miR15a/miR-16-1)*		BCL2 overexpression	Anti-apoptotic effect on B-cells	Deletion
Solid ca., 14q32.3 - hypermethyl. *(miR-127)*		BCL6 overexpression	Anti-apoptotic effect on germinal center B-cells	Hypermethylation
Solid ca., 7q23.2 - amplif. *(miR-21)*		PTEN downregulation	Increased survival and growth of cancer cells	Amplification
B-cell leukemia, 17q22 - t(8;17) *(miR-142-MYC)*		MYC overexpression	Hyperproliferation and antiapoptosis in B-cells	Translocation

Calin GA and Croce CM. Oncogene (2006) 25, 6202–6210

Evidence for the role of miRNAs in transgenerational epigenetics

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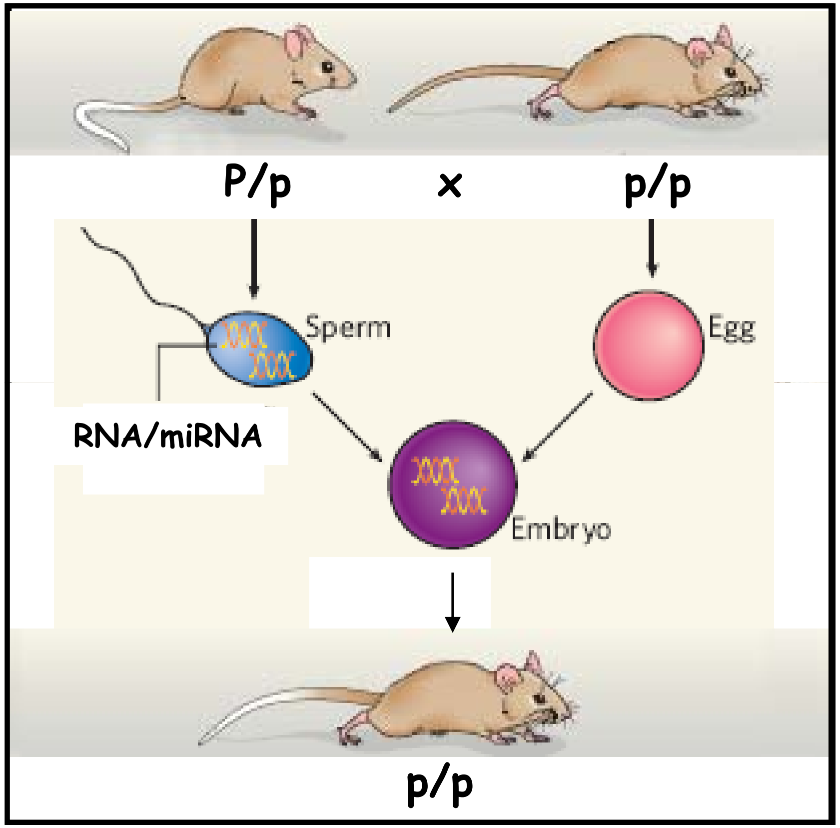
Paramutation (silencing) at the Kit locus:



P = paramutagenic allele (white tail)
p = susceptible wild type allele (brown tail)

miR-221 & 222 implicated

Ashe & Whitelaw, 2007, Trends Genet, 23:8
Soloway, 2006, Nature, 5:522

miRNAs and smoking in human sperm

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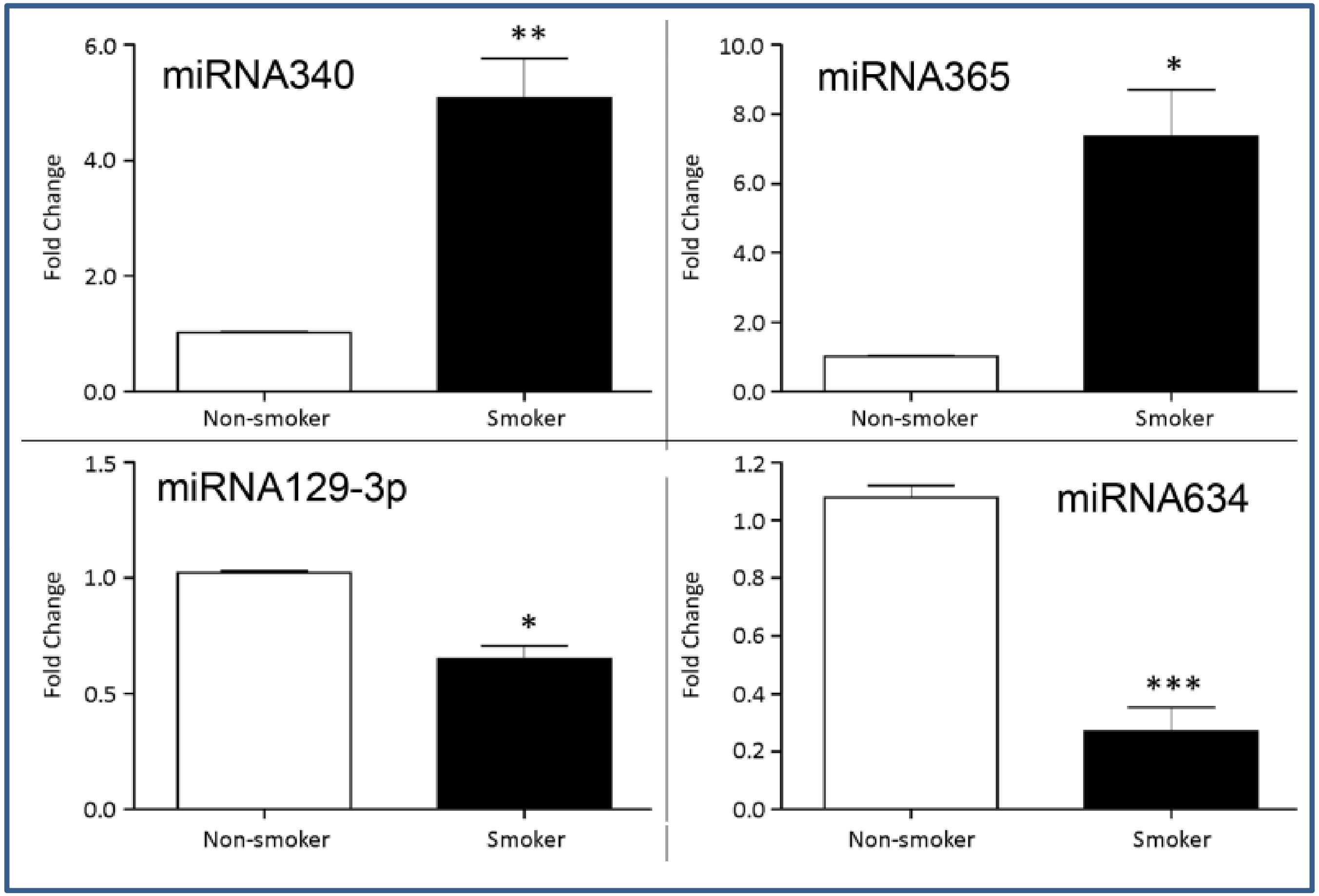

Marczylo *et al* Epigenetics 7, 1 (2012)

Methylation of sperm loci with methoxychlor

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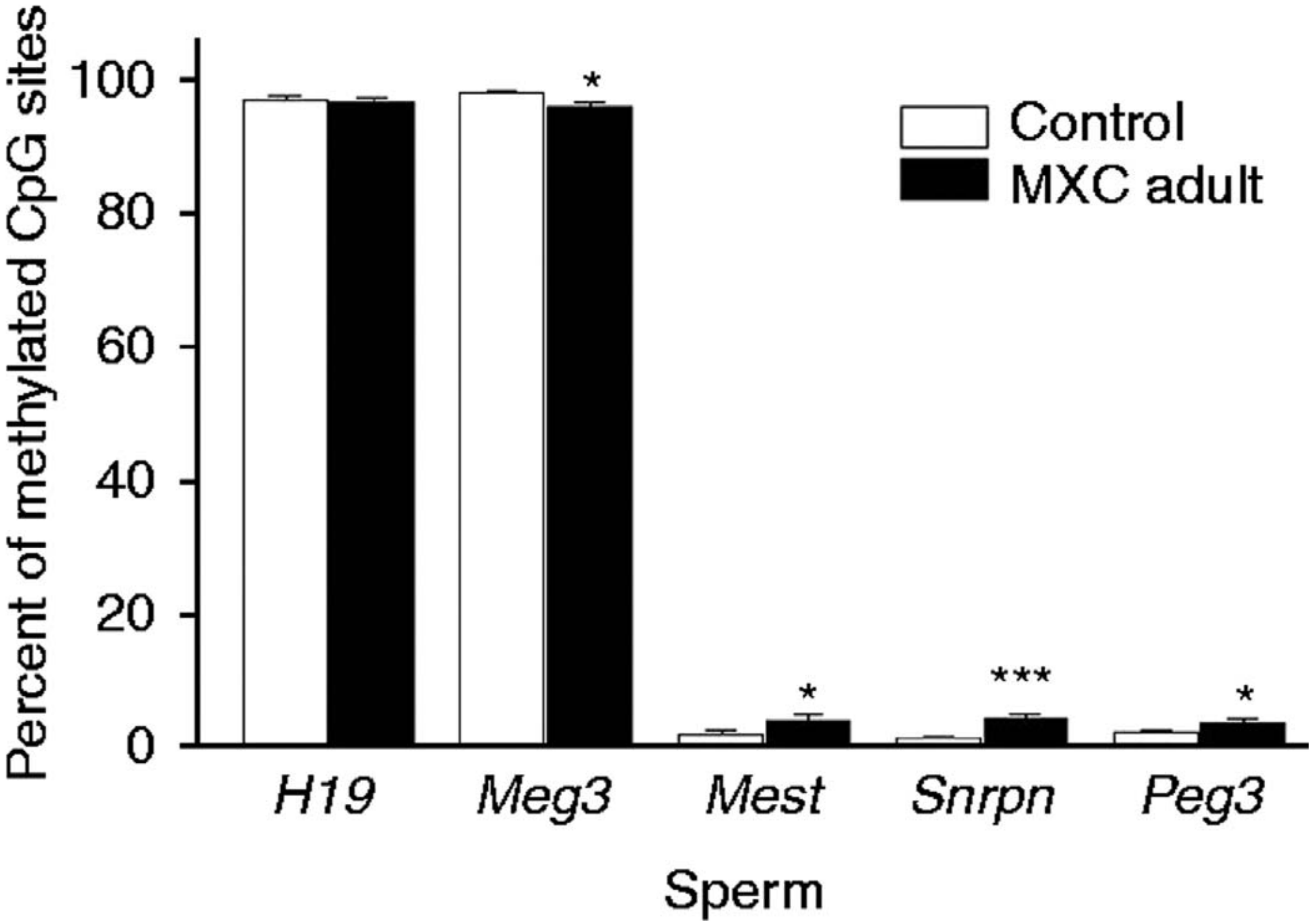
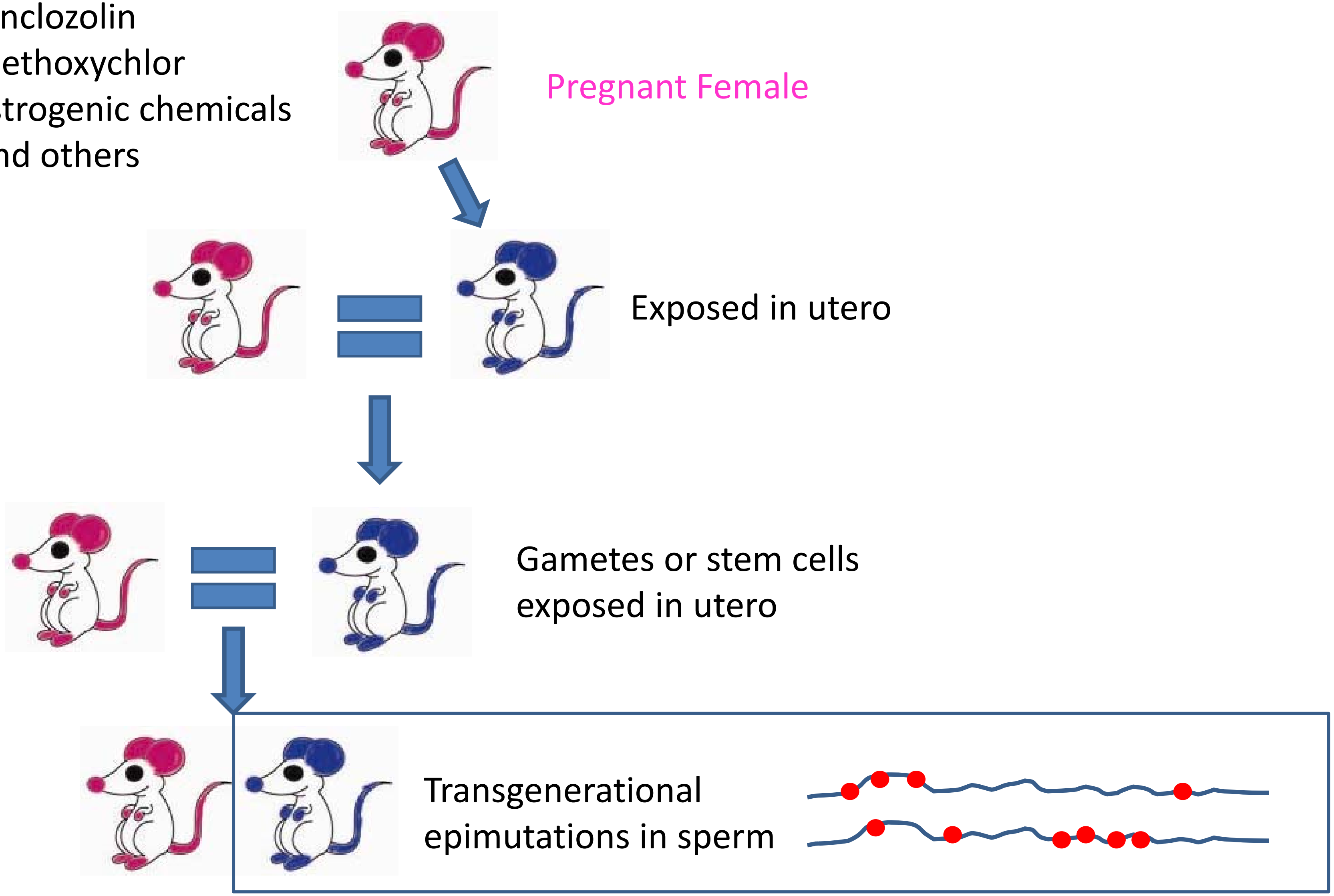


Stouder C , Paoloni-Giacobino A Reproduction 2011;141:207-216

Transgenerational

- Vinclozolin
- Methoxychlor
- Estrogenic chemicals
- And others



Pregnant Female

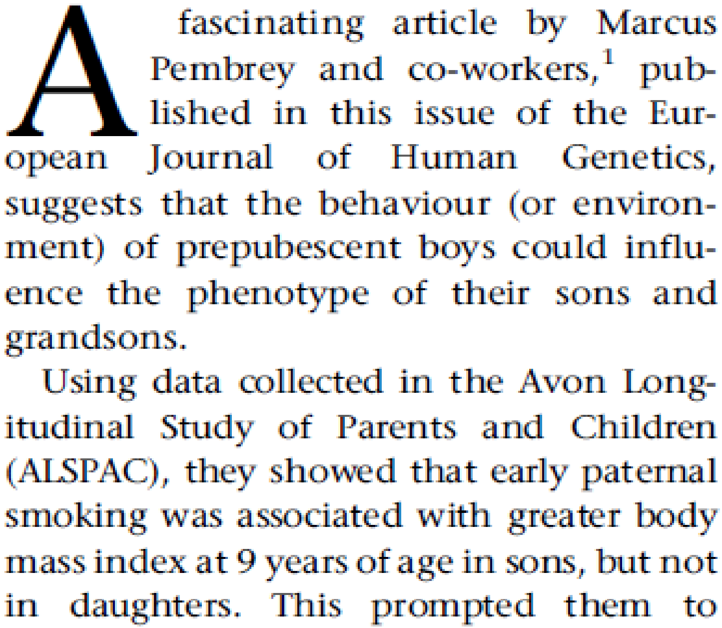
Exposed in utero

Gametes or stem cells exposed in utero

Transgenerational epimutations in sperm

Sins of the fathers

Emma Whitelaw – Sins of the fathers and their fathers. *European Journal of Human Genetics 14, 131 (2006)*



A fascinating article by Marcus Pembrey and co-workers,[1] published in this issue of the European Journal of Human Genetics, suggests that the behaviour (or environment) of prepubescent boys could influence the phenotype of their sons and grandsons.

Using data collected in the Avon Longitudinal Study of Parents and Children (ALSPAC), they showed that early paternal smoking was associated with greater body mass index at 9 years of age in sons, but not in daughters. This prompted them to

Take home messages

- Epigenetic marks on DNA are changed throughout life but particularly during gamete formation and embryogenesis and can be influenced by physical and chemical factors.
- Epigenetic marks are passed through the germ line
- miRNAs are small non-coding RNAs with important roles in physiology
- Mutations in miRNAs, or recognition sites, can give rise to inherited phenotypes.
- miRNA expression can be controlled by xenobiotics and altered epigenetic DNA marks
- miRNAs may control, or have a role in controlling, the transmission of epigenetic marks on fertilisation

Acknowledgements

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