Different Epigenetic Marks and Significance for Risk Assessment

Epigenetics and Cumulative Risk Workshop
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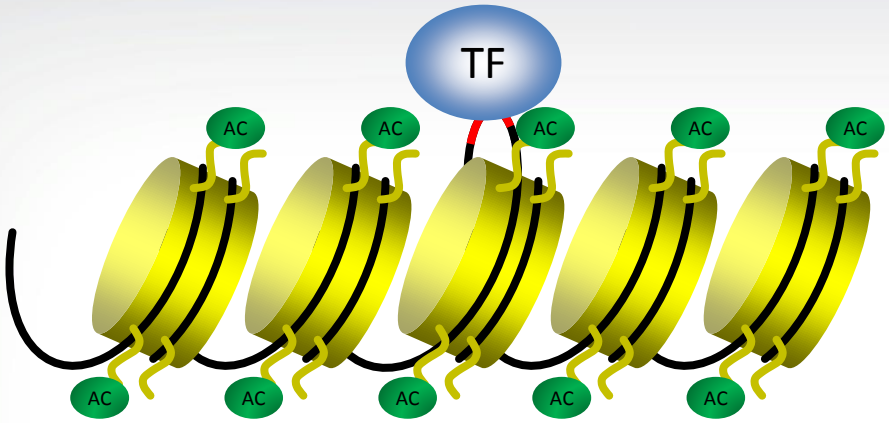
Epigenetic Marks

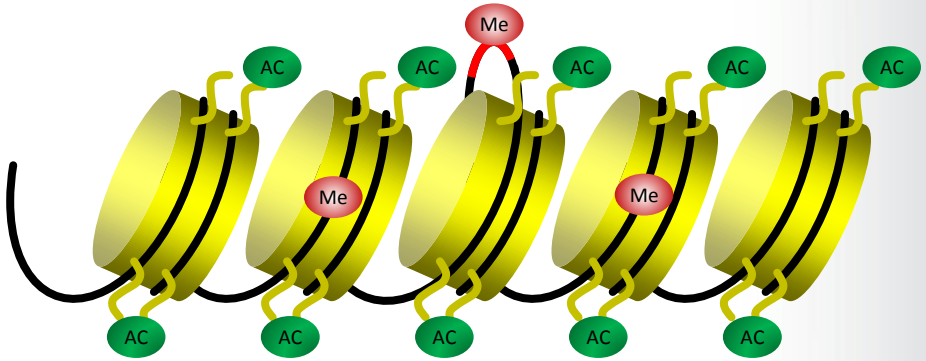
- Three categories of epigenetic marks
  - DNA Methylation
  - Histone Modification
  - Noncoding RNA
- Considerations for risk assessment
  - Demonstrated causative relationship or biologically plausible association with adverse outcome
  - Demonstrated dose-response relationship
  - Mechanistic understanding desirable (AOP)
  - Reproducible, cost-effective, facile measurement
- Based on above considerations, DNA methylation and noncoding RNA mechanisms most amenable to risk assessment *today*

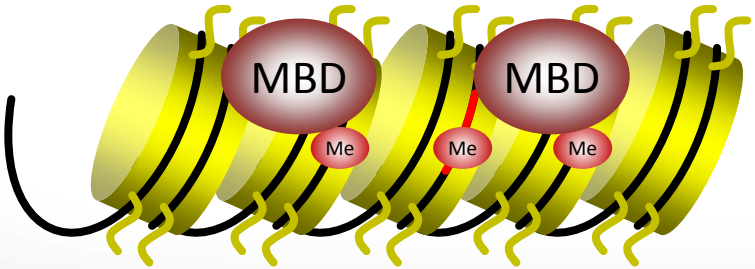
Epigenetic Marks

- DNA Methylation
  - Occurs at 5’ position of C residues in CpG dinucleotides
  - 60 to 70% of CpG sites methylated in mammals
  - Generally associated with gene silencing
  - Catalyzed by DNA methyltransferase (DNMT) using SAM as cofactor
  - Five mammalian DNMT (1, 2, 3A, 3B, 3L)
    - DNMT1 catalyzes maintenance methylation
    - DNMT3A & 3B catalyze *de novo* methylation
  - Direct effect by excluding transcription factor binding
  - Indirect effect by recruiting methyl-CpG binding domain proteins, and in turn, ATP-dependent chromatin remodeling proteins and HDACs
  - CpG Islands (CGI)
    - ≥200 bp & ≥ 50% GC content
    - Present in ~40% mammalian promoters
    - Often hypomethylated
  - X chromosome Inactivation and Imprinting



DNMT



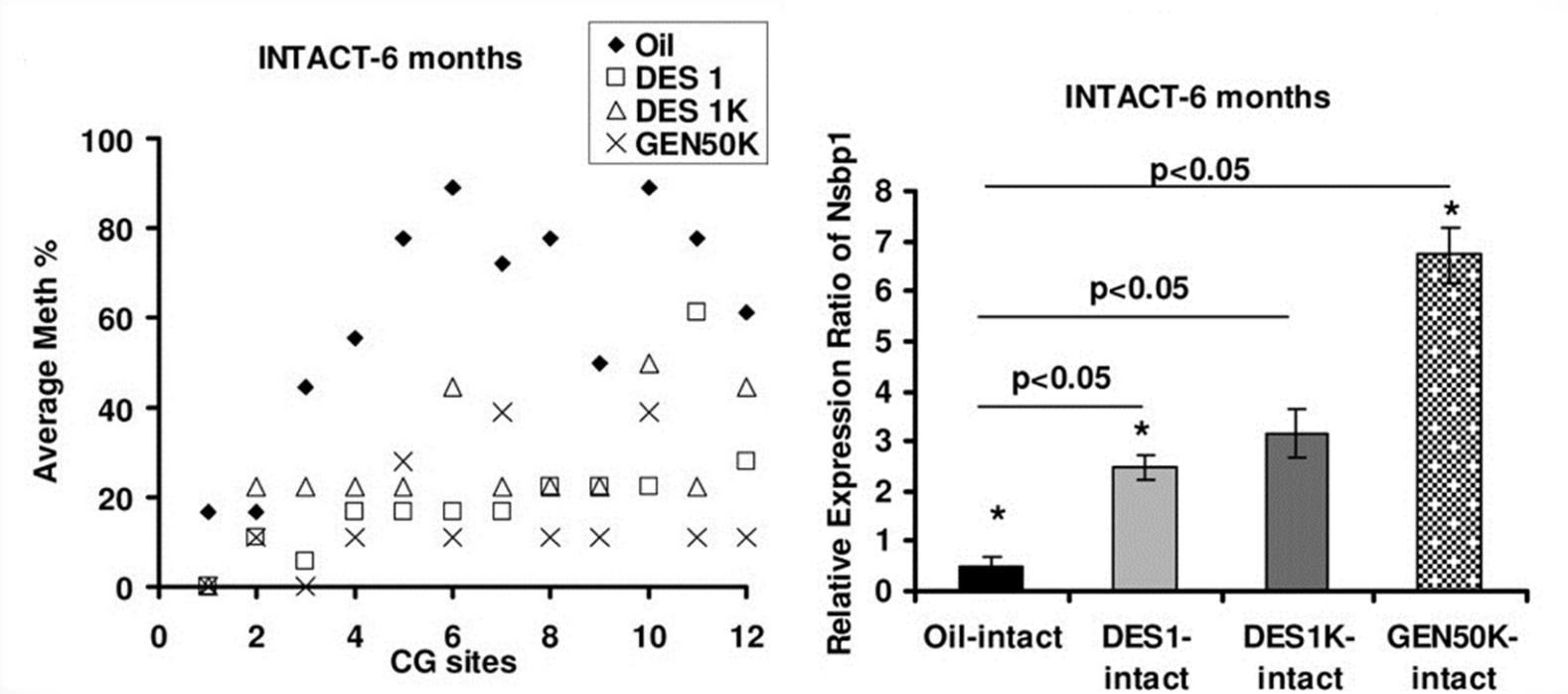


- Methyl binding domain proteins
- ATP-Dependent Chromatin Remodeling
- Histone deacetylase

Example of DNA Methylation

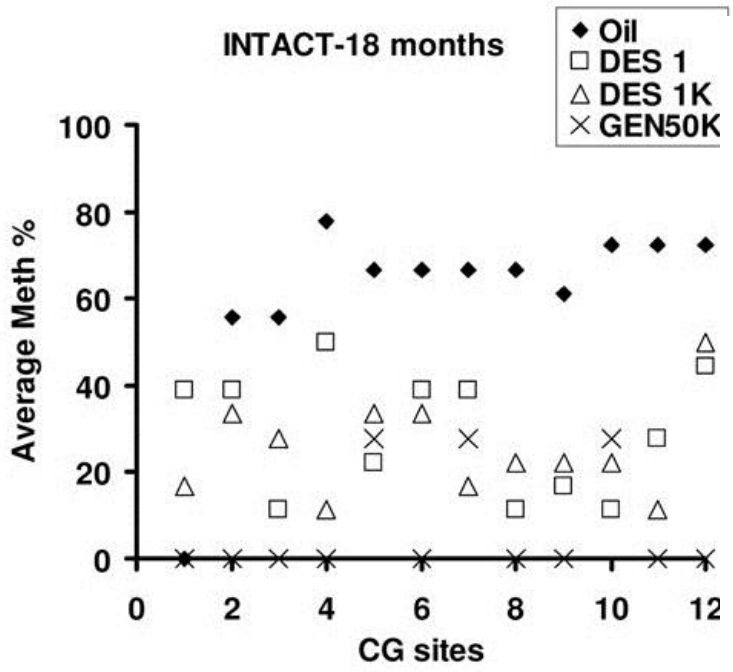
- Study reported by Tang *et al*.
  - Tang WY, Newbold R, Mardilovich K, Jefferson W, Cheng RYS, Medvedovik M, and Ho SM. Persistent hyomethyltion in the promoter of nucleosomal binding protein 1 (*Nsbp*1) correlates with overexpression of *Nsbp*1 in mouse uteri neontally exposed to diethylstilbesterol (DES) or genistein (GEN). *Endocrinology* 149:5922-5931, 2008
- Neonatal exposures
- Methylation-sensitive restriction endonuclease fingerprinting performed to identify differentially methylated DNA sequences
- Of fourteen (14) candidate genes identified, *Nsbp*1 selected for further study because of important role in chromosome remodeling
  - Downregulation of *Nsbp*1 results in suppression of tumor growth and metastatic phenotype
- CpG island (CGI) within *Nsbp*1 promoter minimally methylated in immature, control mice, but increasing methylation and gene silencing with age
- Effect of DES or GEN treatment?

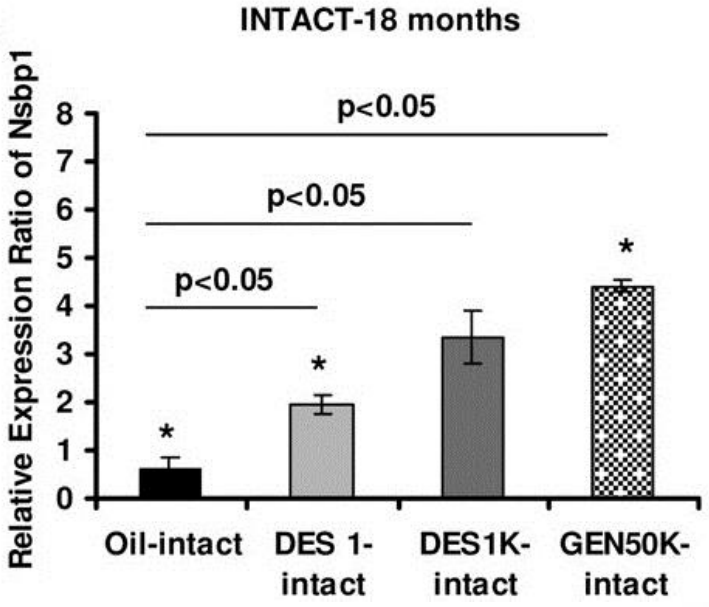
Example of DNA Methylation



From Tang *et al. Endocrinology* 149:5922-31, 2008

Example of DNA Methylation





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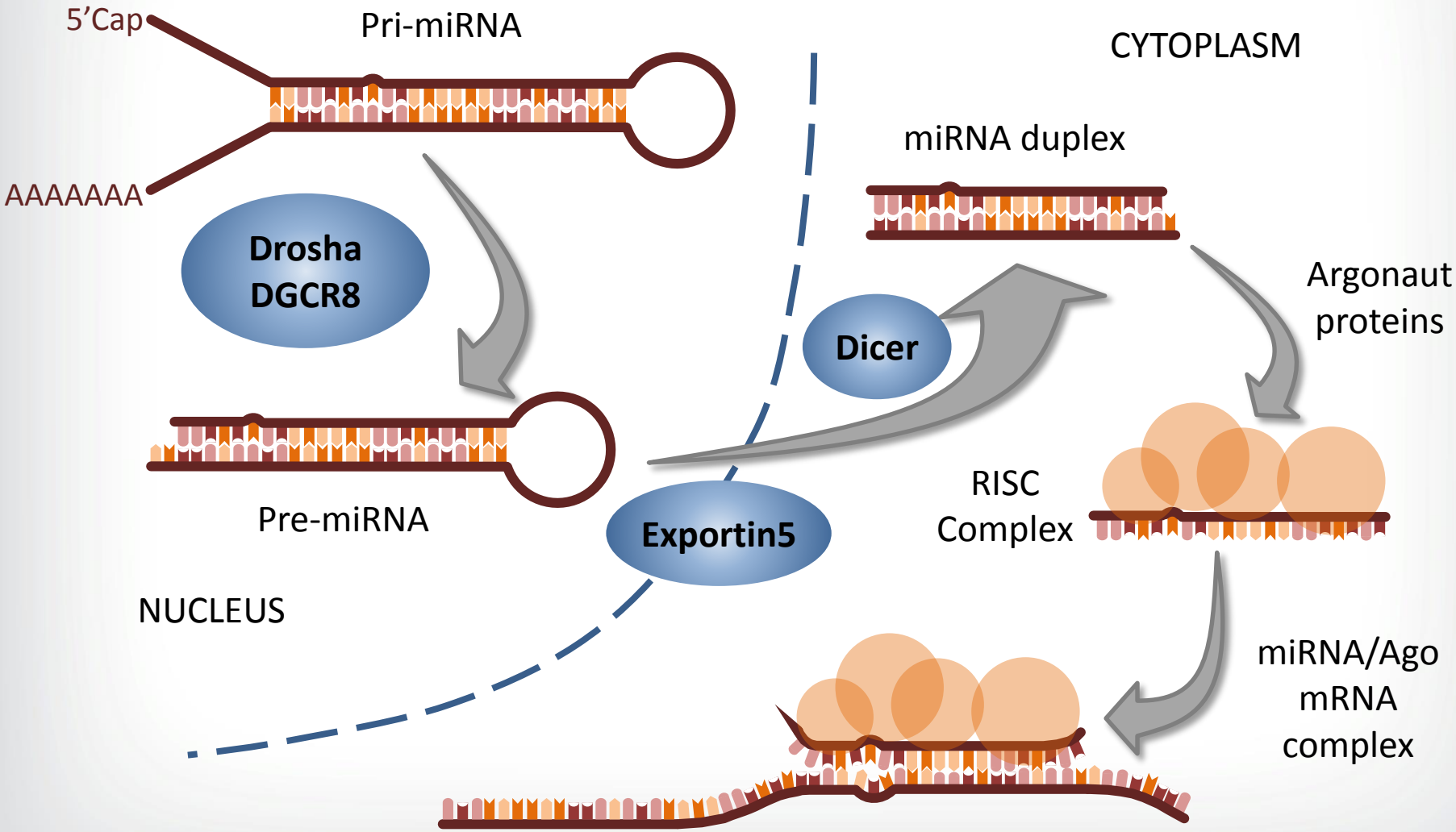
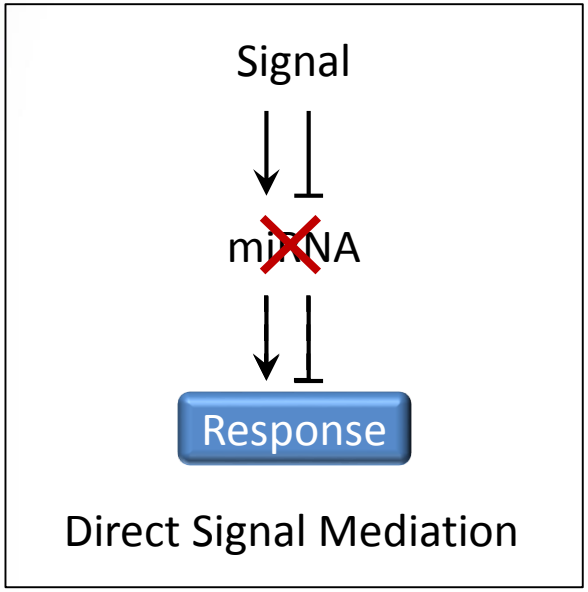
Epigenetic Marks

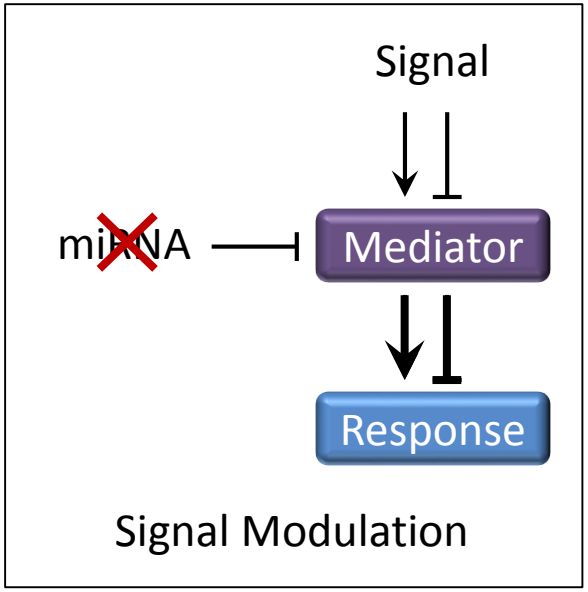
- Non-coding RNA (ncRNA)
  - piwi-interacting RNA (piRNA): 26-31 nt molecules that interact with piwi proteins to silence retrotransposons in germ line cells
  - long ncRNA: > 200 nt, involved in regulating gene transcription, splicing, and translation
  - microRNA (miRNA): 21 to 22 nt
    - Mammalian genomes encode ~800 conserved miRNA
    - Expression highly cell-type specific
    - Function
      - Promote mRNA degradation
      - Destabilize mRNA through shortening of polyA tail
      - Inhibit translation
    - Algorithms predict hundreds of targets for each miRNA
      - Current models suggest fine-tuning gene expression, typically no more than a 2-fold change
      - Coordinated regulation of a suite of transcripts results in a more robust phenotype

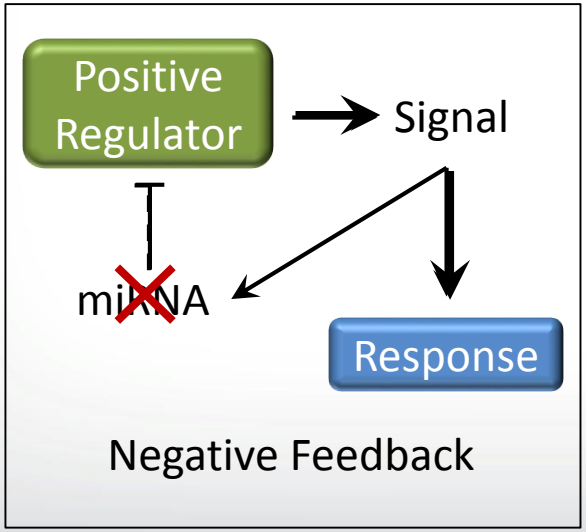
miRNA Synthesis

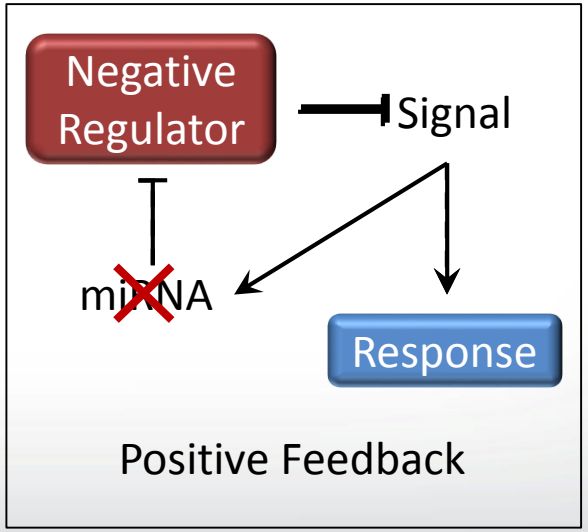


miRNA Function









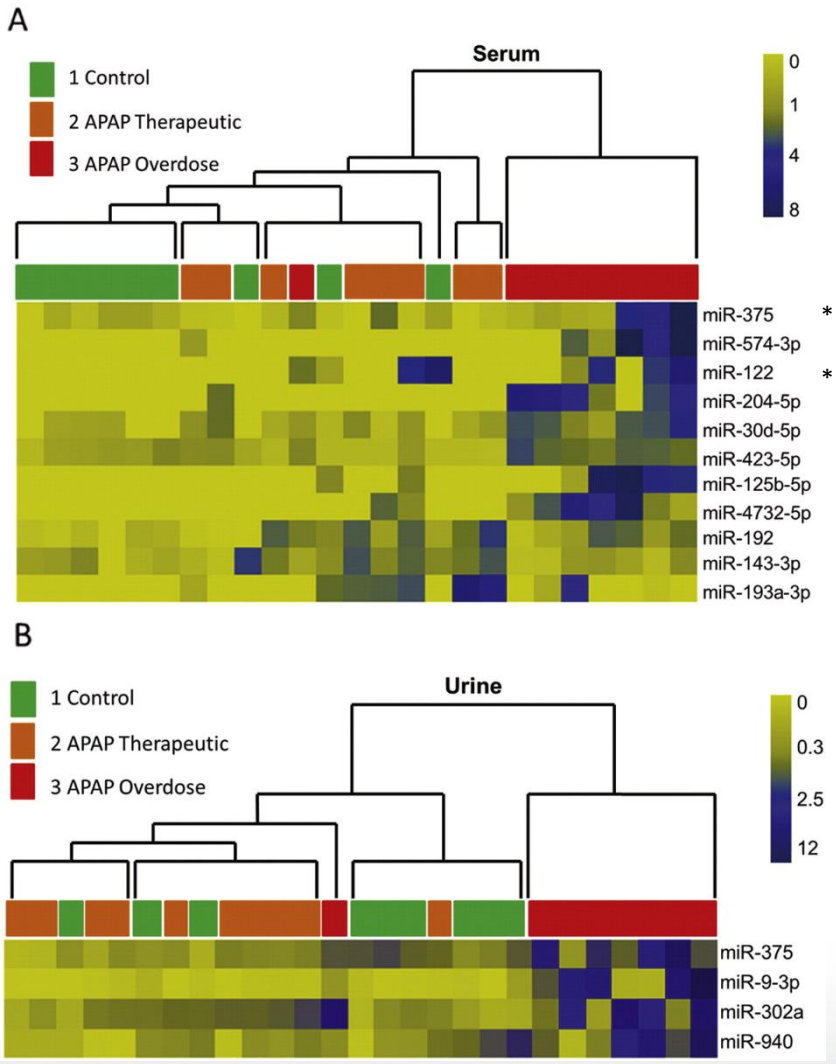
miRNA as Toxicity Biomarkers

- Changes in miRNA expression prior to adverse phenotype and in some cases, appearance in various biological matrices
- Appearance in biological matrices linked to specific cellular responses, including death, proliferation, metabolism, and inflammation
- Advantages of miRNA vs. protein biomarkers
  - Greater stability due to lipoprotein complexes (exosomes)
  - Cell-type specific expression allows linkage of appearance in biological matrix to specific tissues/cells
  - Evolving, high throughput and sensitive detection methods
    - Global sequencing
    - Multiplexed arrays for specific miRNA panels

miRNA Toxicity Biomarker Example

- Study reported by Yang *et al*.
  - Yang X, Salminen WF, Shi Q, Greenhawa J, Gill PS, Bhattacharyy S, Beger RD, Mendrick DL,Mattes WB, and James LP. Potential of extracellular microRNAs as biomarkers of acetaminophen toxicity in children. *Toxicol Appl Pharmacol* 284:180-7, 2015
- Blood and urine samples from 3 cohorts
  - Healthy children with no APAP exposure (N=10)
  - Hospitalized children on standardized APAP therapy (N=10)
  - Children hospitalized due to APAP overdose (N=8) (dose = 59 to 559 mg/kg)
- miRNA quantified using small RNA global sequencing (serum) or PCR-based arrays (urine) with verification by qPCR
- Cluster analysis based on patterns of altered miRNA levels
- Comparison to serum ALT and APAP-protein adducts

miRNA Toxicity Biomarker



* Correlation with APAP-protein adducts, but not serum ALT (r=0.70, p<0.05)

* Correlation with APAP-protein adducts, but not serum ALT (r=0.94, p<0.001)

From Yang *et al. Toxicol Appl Pharmacol* 284:180-7, 2015

Conclusions and Summary

- Of 3 major categories of epigenetic marks, DNA methylation and ncRNA (miRNA) offer the greatest immediate promise for environmental health risk assessment
- Gaps still needing attention
  - Sensitivity and specificity as biomarkers for specific adverse outcomes
    - Incorporation into AOP framework?
  - Better understanding of MOA
  - Better understanding of interindividual variability, what is “normal,” and adaptive vs adverse response
  - Focus on non-cancer end-points
  - Increased focus on environmental chemical exposures at low doses