HESI Workshop on State of the Science: Evaluating Epigenetic Changes

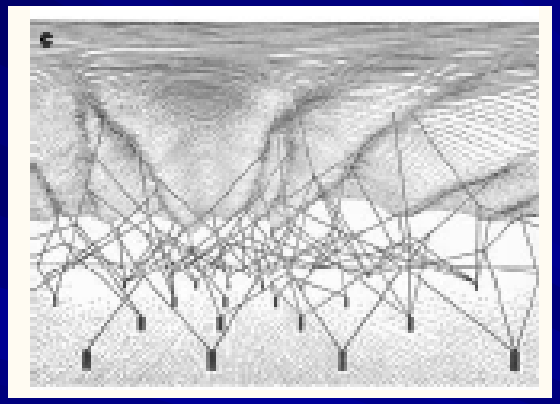
Overview

- How the workshop came into effect
  - Epigenetics: Background Science
  - Epigenetics as an Emerging Issue
- Workshop goals/agenda
- Discussion forum- what was learned from the workshop

What is Epigenetics?

Conrad Waddington

Waddington's Original Concept: The epigenetic landscape Is the underlying effect of variations in cellular metabolism on the respective pathways embryonic cells take and their Ultimate fate determination.



Concept today: Epigenetics: "Above the DNA". Mechanisms that modulate or alter gene expression. Such mechanisms Include DNA methylation pattern, histone pattern/modification and Non coding RNA mechanisms.

Illustrations from Waddington's book *Organizers and Genes*, 1940

Epigenetics: Expanding our Understanding of Cellular Biology

- Epigenetics expands the central dogma of cellular function
  - Transcriptional regulation is influenced by additional factors beyond DNA code or cell signaling
    - Chromatin regulations via histone code patterns/modifications
    - Alteration of DNA methylation resulting in differences in repression or activation of transcription
    - Non coding RNA influences
- As epigenetic mechanisms have become better understood, it has become apparent that such mechanisms could contribute to disease and abnormal development and that agents in the environment may influence such mechanisms

Prenatal Environmental Insults on Adult Disease

- *Dutch Famine Cohort Study (Rosebloom et al 2000)*
- Background: 1944-1945: Holland under German Occupation- population subsided on air-dropped rations
- Average daily dietary intake: 800-1200 calories
- Dutch took careful records of births during this period. High incidence of low birth weight babies during famine period.
- Long term studies have been active to track the implications of prenatal dietary restriction in these babies.

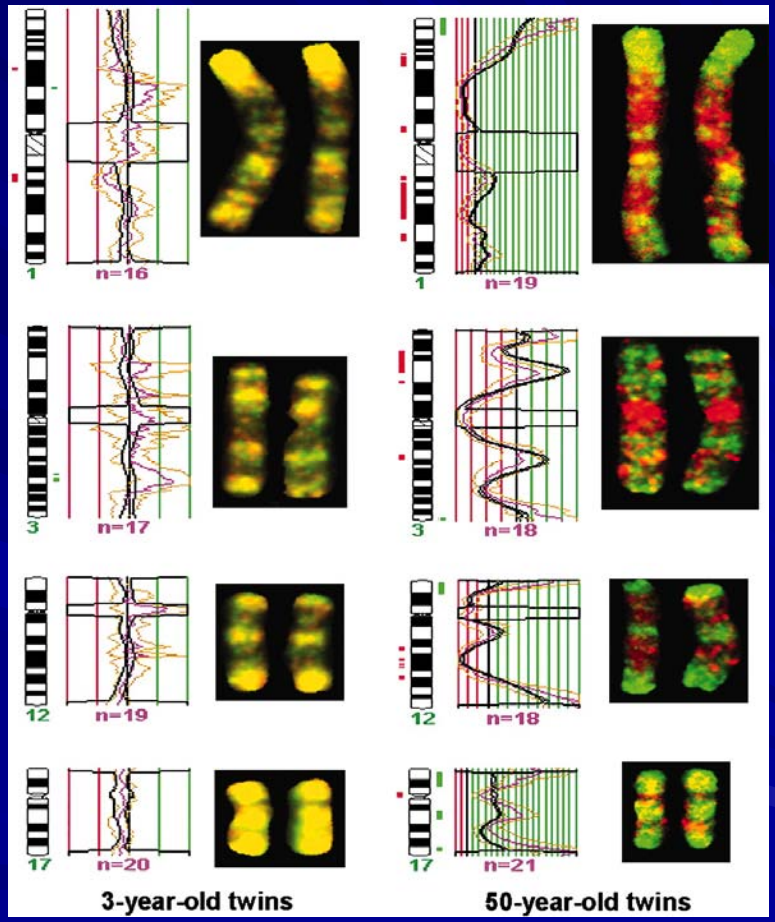
Impact of Prenatal Dietary Restriction

- Dietary restriction during pregnancy:
  - Increased rate of diabetes especially in females
- Dietary restriction during mid gestation:
  - Increased risk of obstructive pulmonary disease and microalbuminera
- Dietary restriction during early gestation:
  - 3-fold increased risk of coronary heart disease

Epigenetic Implications on Phenotype

Why do many identical twins gradually look distinct with age?

- **Spanish study**: Fraga M F et al. PNAS 2005;102:10604-10609; Manual Estellar et al 2005
  - Evaluated genome profiles of 40 pairs of monozygotic twins and found variations in methylation (including CpG islands in promoter regions) and histone acetylation in 35% of the twins
- Twins with the greatest difference in profiles were the oldest



Fraga M F et al. PNAS 2005;102:10604-10609;

Epigenetic Implications on Phenotype

Why do many identical twins gradually look distinct with age?

Other Findings:

- There was a correlation in greatest difference in medical history with ones which spent greatest time apart
- These studies were some of the first to link on a molecular level, epigenetic changes in the genome with phenotype
- Implies that environmental influences can impact gene expression via epigenetic mechanisms

Environmental Agents and Epigenetic Insult

Agents in Plastic:
A Possible Implication in Obesity?

Bisphenol A agouti mouse study (Waterland and Jirtle, 2003; Dolinoy, et al 2007)

- Agouti mouse: spontaneous mutant full phenotype presents yellow coat color and obese phenotype.
- Variations in penetrance of phenotype dependent on degree of methylation of agouti locus (loss of methylation=stronger phenotype)
- Pregnant agouti mice fed bisphenol A produce progeny with greater penetrance of agouti phenotype because of demethylation of the locus
- Pregnant agouti mice fed diet supplemented with methyl donating substances (folic acid, vitamin B12, choline, etc) produce progency with more wild type appearance
  - Increases methylation of the IAP promotor of the A^{vy} locus

Epigenetics as an Emerging Issue

- **“Evaluating Epigenetic Changes”**
  - Selected as a top emerging issue by both the public HESI membership and by the Emerging Issues Subcommittee
- What centered epigenetics as an emerging issue:
  - *Underlying science*- adverse effects of some environmental agents may have an epigenetic basis
  - *An immediate issue*- scientists who may need to consider epigenetic insults in context of safety assessment may not be sufficiently versed in the basic concepts and applications of the epigenetic field

Workshop Goals

- Day 1: Provided participants with a general overview of the current state of the field (Day 1)
  - Epigenetics 101
  - Measuring epigenetic changes
- Day 2: Enhanced an understanding of what one needs to know prior to thinking about incorporating an “epigenetic evaluation” into safety assessments
  - What constitutes epigenetic changes
  - Research in epigenetics
- Day 3: Focused discussions on specific questions related to epigenetics and safety assessment

Day 3 Discussion Forum

- A discussion forum addressed the question of what we need to know prior to thinking about incorporating an epigenetic evaluation into safety assessment.
- Workshop participants were divided into 2 breakout groups and discussed the following questions:
  - a. What model systems might be employed to evaluate the ability of a chemical to produce an epigenetic change (affecting the F1 and/or F3 generation)?
  - b. What endpoints/targets might be evaluated?
  - c. What techniques might be employed?
  - d. From the regulatory perspective: When is it appropriate to incorporate “new” science, in this case epigenetics, into the regulatory process?

Output of Workshop

- Considered an initial step to examining this issue as it relates to human health from the perspective of adequate safety evaluation
- A manuscript was accepted for the forum section of Toxicological Sciences that discussed the outcome of the round table discussions.
- Focus of the manuscript
  - Stating areas of consensus was included
  - Surfaced potential areas of difference of opinion as it relates to addressing the questions and to that end, what needs to be further evaluated/refined in terms of integrating epigenetic evaluations into safety assessment
- Next/Future Potential Steps
  - Output of the workshop should provide guidance on how to continue to address this issue from the HESI perspective
  - Potential directions:
    - Workshops on advancements in the field and how they relate to integration into safety assessment
    - HESI-based working groups-Limited focus exploratory work

Synopsis of what was Learned from the Discussion Forum

Question 1:
What model systems might be employed to evaluate the ability of a chemical to produce an epigenetic change (affecting the F1 and/or F3 generation)?

http://www.espcr.org/micemut/a001.jpg

Mouse Models

Jirtle and Skinner Nature Reviews; 2007

- Mouse: Most established animal model for evaluating epigenetic change and would be preferred over rat or rabbit (conventional Segment II Repro Tox species)
- Epigenetic background very pliable: changes with age, strain, etc
  - Must use well characterized strains and age matched controls
- Mutant strains (Agouti, Axin, etc): pontentially promising as a phenotypic sensor for epigenetic insult
  - Risk that these models may be over sensitive to test agents due to their affected locus.
  - Unsure whether these mutants have value for human risk assessment:
    - metastable epialleles have not been found in the human genome

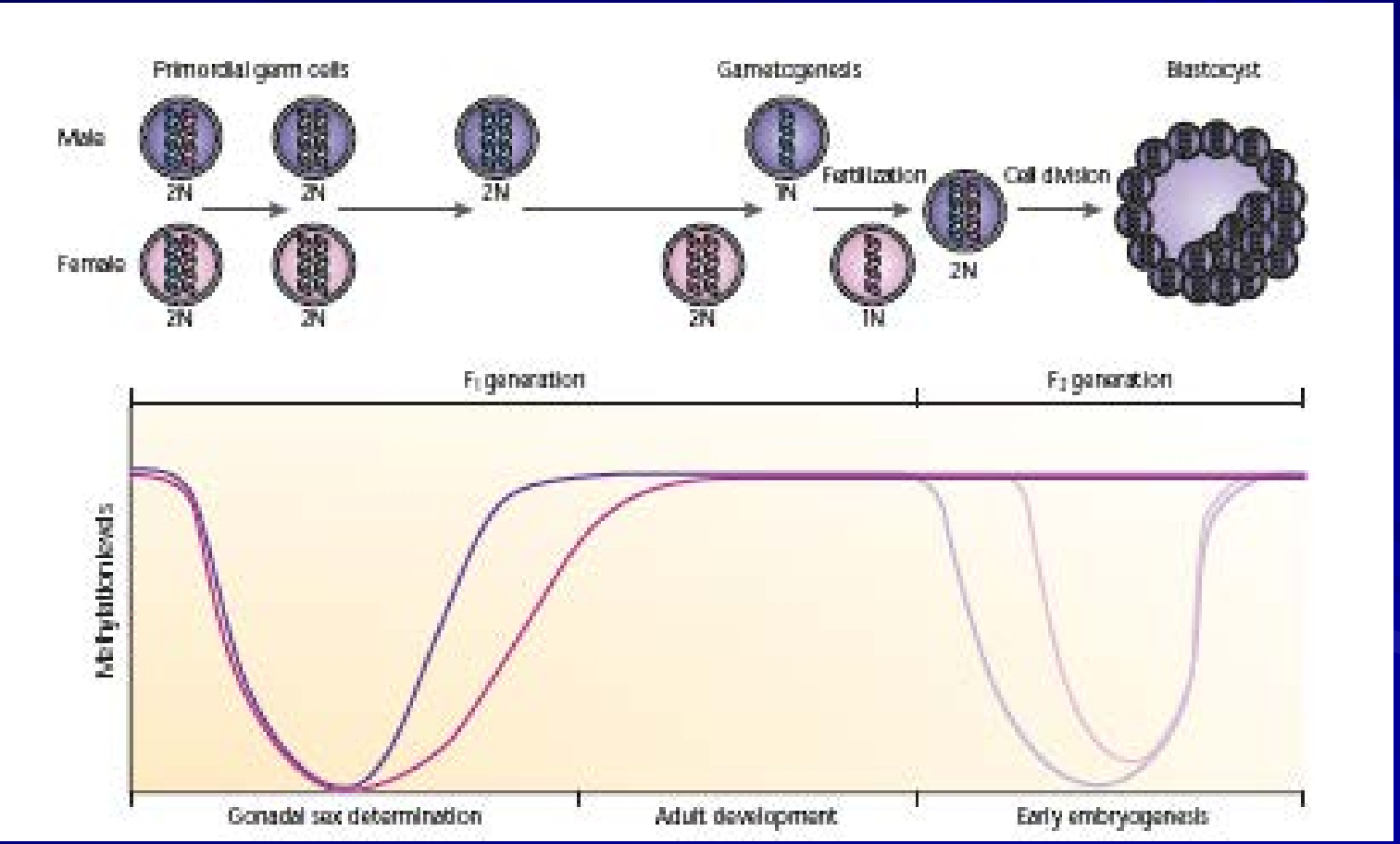
Alternative models

- Various species and mammalian cells may have potential to be used as phenotypic sensors for epigenetic change due to unique properties of the respective model
  - May have promise as hazard identification models but could not be used to evaluate cross-generational effects
  - Potential for cross evaluation of compound across various models to refine mechanism of insults
- Examples:
  - Zebrafish and c elegans: Gene expression is predominantly modulated by non coding RNAS
  - Honey bee external phenotype/behavior influenced primarily by methylation status
  - Drosophila eye color regulated by a combination of epigenetic regulation (histone acetylation, methylation and miRNAs)
  - Mammalian stem cells: possess imprinted genes which are methylated and are typically stable

Question 2: What endpoints/targets might be evaluated?

- **Attributes:**
  - Rapid advancement of tools/reagents for detecting epigenetic changes
- **Challenges:**
  - Delineating adaptive versus adverse epigenetic change (what is an adverse footprint?)
    - Critical targets for profiling: currently unknown
  - Epigenetic change is very dynamic:
    - each cell type has its unique epigenomic profile which can change with age and other factors
    - Critical time points/windows for identifying adverse changes need to be characterized
- **Potential approaches for advancement:**
  - *Hazard identification:* Applying phenotypic sensors (discussed previously) may be advantageous as a means to identify a potential hazard
  - *Target identification:* Establish/execute well designed studies of test substances carefully attuned to dose, exposure times and duration that could potentially elucidate such targets.
  - *References for normal adaptive change:* Establishment of a database that provides a reference for normal profiles of various cell types at various ages

Finding Critical Windows of Sensitivity Associated with Epigenetic Change: *Altered Methylation Status During Development*



Jirtle and Skinner Nature Reviews 2007

Question 3
What techniques might be employed?

- Human and mouse array-based platforms are well established
- Platforms for rat are emerging (Nimblegen arrays)
- Bisulfate-based deep sequencing for methylation change can be performed on any species.
  - Historically labor intensive but technology is improving
- Gap: How is the data interpreted?
- Need more bioinformatics input in this area to aid in identification and interpretation of epigenetic footprints
  - Need to identify an approach to target critical time and cell type(s) for detecting epigenetic insult
  - Need an approach to delineate normal adaptive versus adverse change

Question 4

Regulatory Perspective:

When is it appropriate to incorporate “new” science, in this case epigenetics, into the regulatory process?

Responses to Question 4: Regulatory Perspective

- Current state of the science is emerging and is not ready for incorporation into the regulatory process
- Need to clearly identify the substance as having adverse epigenetic effects
  - Apply robust model systems, study designs and endpoints for identifying and characterizing adverse epigenetic change
  - Presenting epigenetic footprints that are clearly shown as adverse in nature in context of normal reference controls
- Need to be able to thoughtfully apply to human risk assessment and the relevance to public health concerns

Take Home Messages from the Workshop

- The field of epigenetics is evolving at a very rapid pace but there is still a great deal needs to be learned prior applying it thoughtfully to safety assessment.
- Gaps to be refined in the future:
  - Better characterization of predictive model systems
  - Establishing reference ranges for epigenetic signatures that can delineate normal adaptive change from aberrant insults
  - Thoughtful integration of this analysis into in vivo study design strategies
- Workshop provided an impetus for focusing attention on the areas where research and new thinking are needed to better understand the role of epigenetics and its relationship to safety assessment.

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Steering Team Members:

Dr. Chunhua Qin – Merck

Dr. Herman Autrup – University of Aarhus, Denmark

Dr. Adriana Doi- BASF

Dr. Klaus Olejniczak - Federal Institute for Drugs and Medical Devices, Germany

Dr. John Rogers – U.S. Environmental Protection Agency

Dr. Randy Jirtle - Duke University

Dr. *Michael Bolger – U.S. Food and Drug Administration*

Dr. Ammie Bachman - ExxonMobil

Dr. Jerry Heindel- U.S. National Institute of Environmental Health Sciences

Dr. Rick Storer - Merck

Ms. Syril Pettit – HESI

Dr. Jay Goodman- Michigan State University (Program Co-Chair)

Dr. Karen Augustine – Bristol-Myers Squibb (Program Co-Chair)