

*Eminent Toxicologist
Lecture Series*

Society of Toxicology

Eminent Toxicologist Lecture Series

**Environmental Epigenomics: The
Developmental Origins of Health
and Disease**

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Society of Toxicology

Eminent Toxicologist Lecture Series

Learning Objectives

1. Exposure to chemicals early in life while our tissues are undergoing development can alter normal physiological responses in adulthood
2. This “Developmental Programming” can determine risk of diseases such as heart disease, obesity, and cancer in adulthood
3. This suggests that adult risk of diseases, such as cancer, may be determined by environmental exposures that occurred in early life, possibly decades, before disease presentation

Human Health Impact: Identification of the “imprint” left by developmental programming may be useful for identification of exposed individuals, as a biomarker for disease susceptibility in adult life, and because the epigenome is “plastic” may be reversible with lifestyle or pharmacological interventions

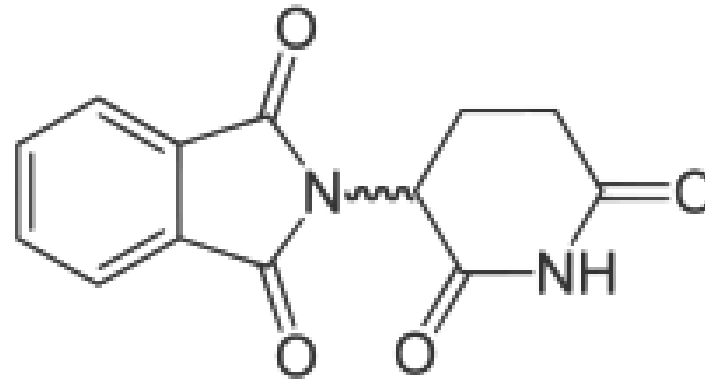
Our Early Life Environment Impacts Us as Adults

What we learned
in the 60's

Congenital Abnormalities

- Limb malformations
- Spina bifida
- Neurological deficits

- Thalidomide
- Folate deficiency
- Alcohol



Thalidomide

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What we learned
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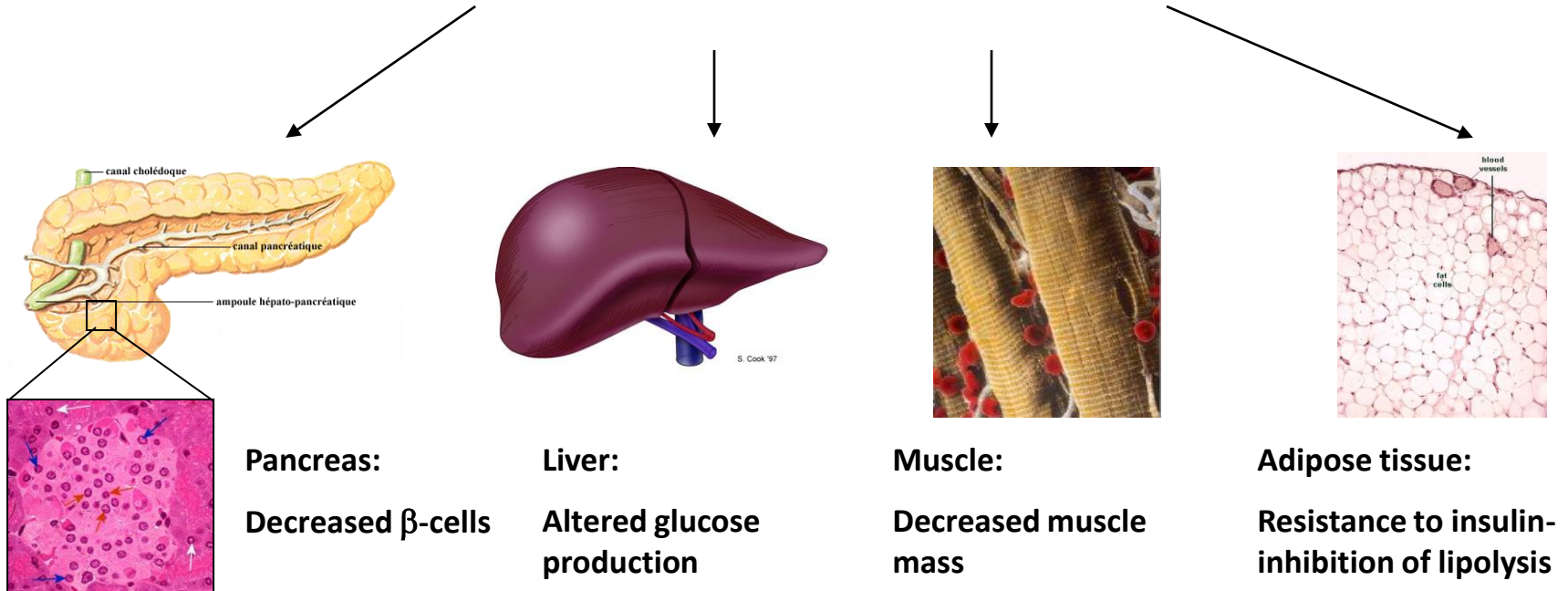
Physiological
“Set-points”

- Type II Diabetes
- Hypertension
- Obesity

- Fetal Environment in the
Womb

Reprogramming of Physiological “Set-points”: Glucose Metabolism

Nutrient Deficiency *in utero*

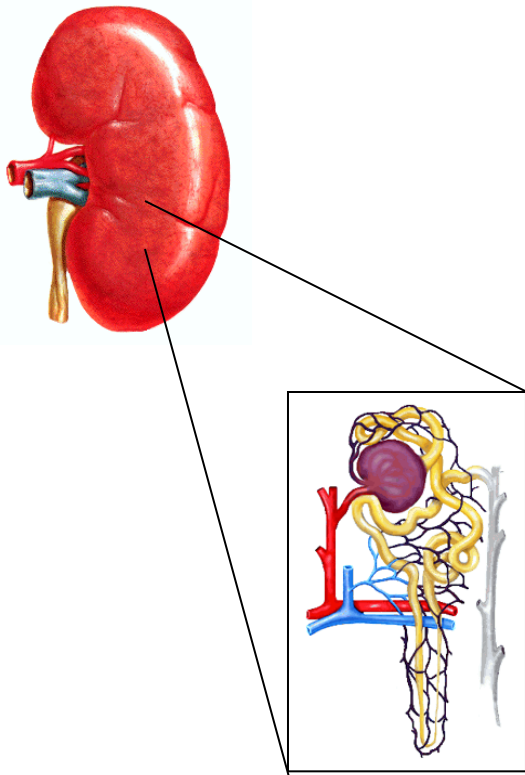


Type II Diabetes

- Diminished pancreas function
- Insulin resistance

- Glucose intolerance

Relationship to Intrauterine Growth Retardation (IUGR) and Hypertension



- IUGR (or low protein diet in experimental animals) results in decreased nephron number
- This results in sodium retention and a compensatory increase in glomerular filtration rate (GFR) in the remaining nephrons
- Increased GFR leads to glomerulosclerosis, setting up a cycle of increasing GFR and glomerulosclerosis
- Ultimately more nephron loss occurs, leading to perturbed RAS and increased arterial blood pressure

Fetal Origin of Adult Disease: The Barker Hypothesis

- 1989 David Barker found an inverse relationship between birthweight and death from heart disease in England and Wales
- Since birthweight is a surrogate for intra-uterine nutrition, individuals who were small at birth experienced poor maternal environment *in utero*
- Studies confirmed by “Dutch Hunger Winter” when food supplies to occupied Netherlands were cut off by Nazis. Individuals born during this time had high incidence as adults of insulin-resistance
- “Developmental Origins of Health and Disease (DOHaD) now confirmed for
 - **Coronary heart disease**
 - **Hypertension**
 - **Type II diabetes**

Developmental Origins of Health and Disease: Ethical, Legal, Social ELSI Implications

- Men with low taxable income are known to have higher rates of coronary heart disease
- However, effect of low income is confined to men with low ponderal index (thin at birth and low fetal growth)
- Cause? Individuals small at birth have persistent alterations in stress responses (increased cortisol levels etc.)
- Men not thin at birth may be more resistant to the effects/stress of low income

Epigenomic Plasticity During Development Allows “Pre-Adaptation” Programming to the Adult Environment

**...but creates a vulnerability if this programming is
disrupted that can have life-long consequences**

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Physiological
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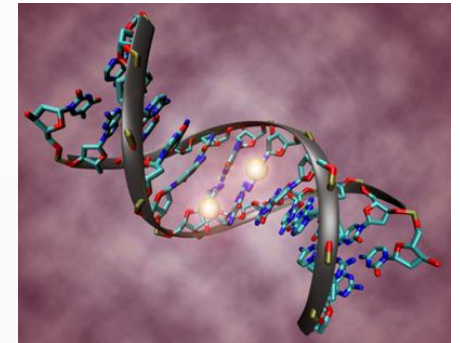
- Type II Diabetes
- Hypertension
- Obesity

- Fetal Environment in the
Womb



What we know in
2015

Molecular
(re)Programming



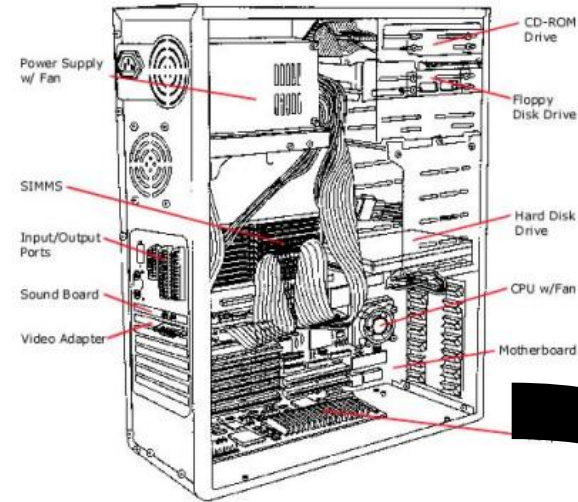
- Early Life Exposures

Programming the Epigenome



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DNA = Hardware inherited at birth



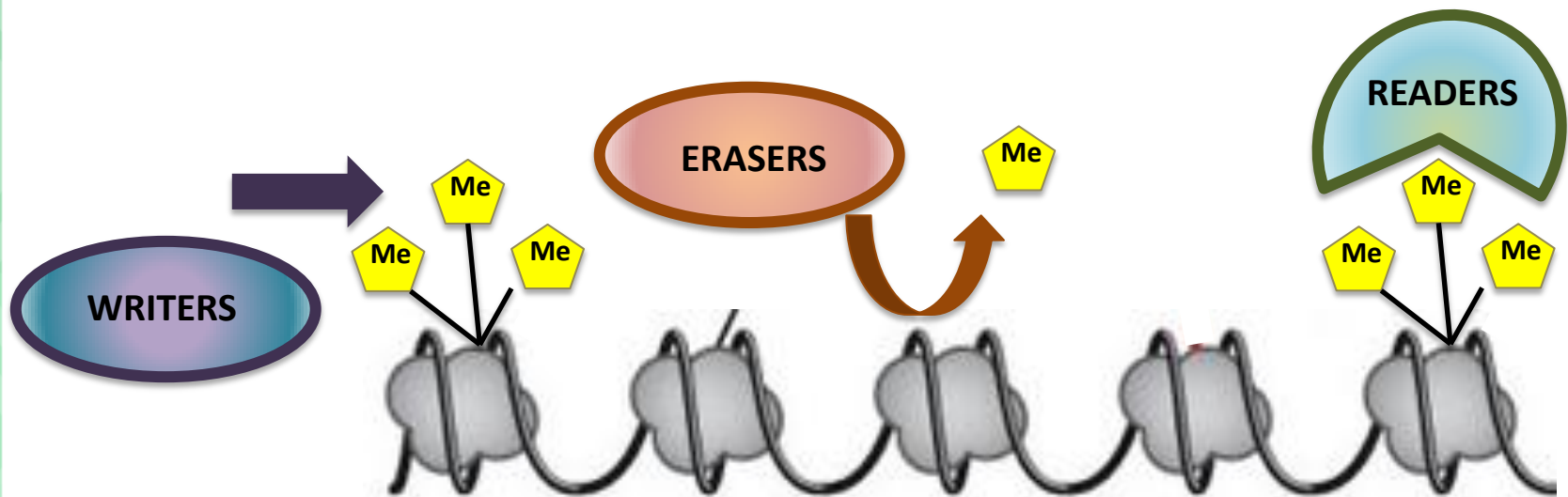
Epigenome = Software installed during development that determines how cells and tissues function throughout the life course



Programming the Epigenome

- During development, the genome of cells that make up tissues and organs becomes “programmed” to specify their function in the adult
- Much like when installing new software on a computer, the health of the developing organism depends on a proper “install” of the epigenome
- Disrupting the process during the “install” phase will dramatically alter how the “software” or “programming” functions in the future

Epigenetic Programming by “Readers, Writers, and Erasers” of the Epigenome



Writers:

Methyltransferases
Acetylases
Kinases

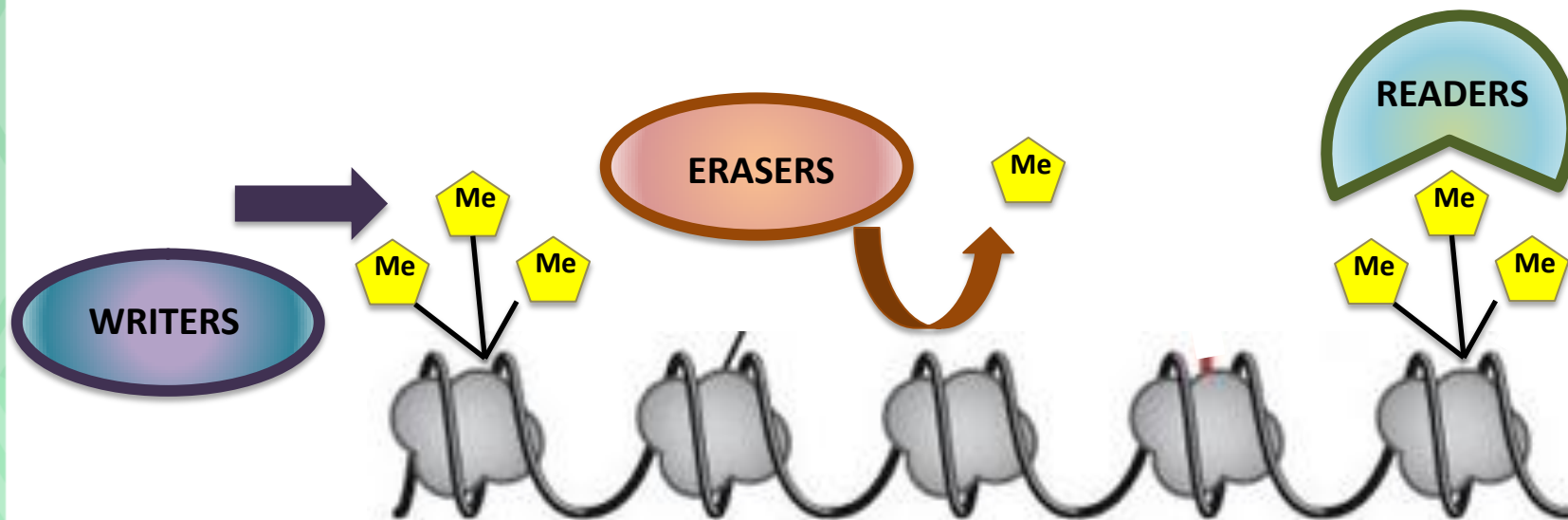
Erasers:

Demethylases
Deacetylases
Phosphatases

Readers:

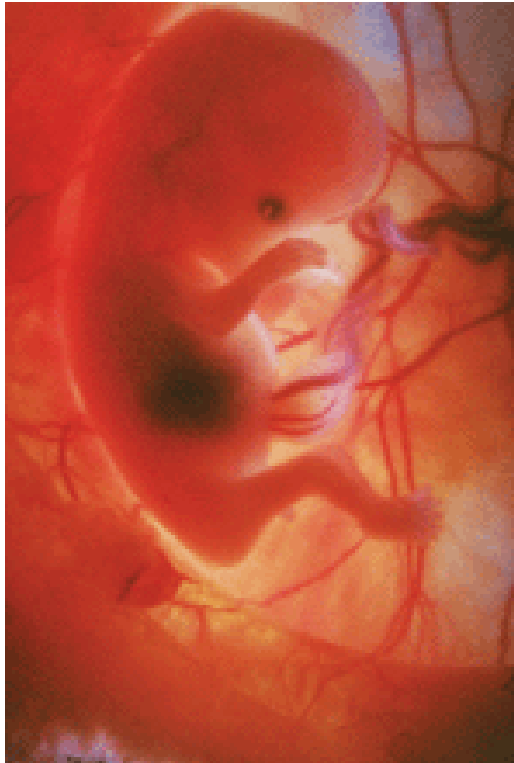
Bromodomain
Chromodomain
DNMTs

Development: Establishment and Maintenance of Transcriptional Memory



- Epigenetic “writers”, the histone methyltransferase EZH2 in the Polycomb Repressive complex (PRC2) and MLL in the COMPASS complex, add distinct “methyl marks” to histone tails, H3K27me3 and H3K4me3 respectively, to **repress or activate gene expression**.
- Plasticity is obtained erasers, such as the histone acetyltransferases (HATs), such as the histone demethylases (HDMs) and deacetylases (HDACs), that **remove epigenetic marks**
- **These marks, established during development, are inherited across subsequent cell divisions to maintain “transcriptional memory and differentiation**
- “Readers” such as transcription factors and DNA methyltransferases (DNMTs) then act on these epigenetic marks to regulate gene transcription in differentiated cells and tissues

Developmental (re)Programming

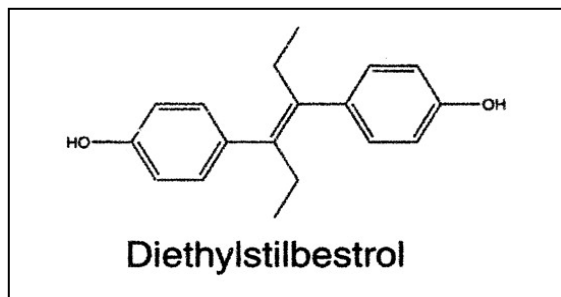


Exposure of developing tissues or organs to an adverse stimulus or insult during critical periods of development that can permanently reprogram normal physiological responses in such a way as to give rise to disease later in life

Endocrine Disrupting Compounds (EDCs), Developmental Reprogramming and Cancer Risk

- Many compounds in our environment, both naturally occurring and man-made, mimic the female hormone estrogen.

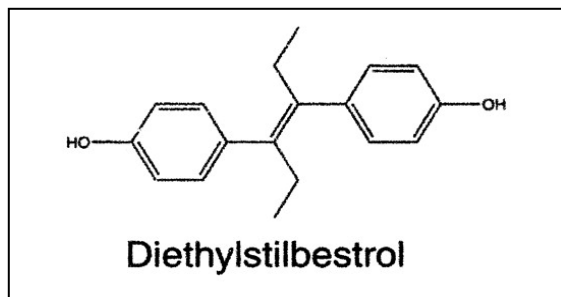
Endocrine Disrupting Compounds (EDCs), Developmental Reprogramming and Cancer Risk



- DES is a pharmaceutical estrogen that binds to the ER and transactivates estrogen-responsive genes.
- Administered to pregnant women for miscarriage prevention in 1940-70's.
- Causes reproductive tract abnormalities and otherwise rare cervicovaginal clear-cell cancer in female offspring exposed in utero

- As a potent estrogen that is not bound by steroid hormone binding proteins such as AFP that protect the developing uterus from maternal hormones, DES is a useful “tool” compound
- However, women are no longer exposed to this drug, posing the question: “Can developmental reprogramming be caused by relevant environmental estrogens?”

Endocrine Disrupting Compounds (EDCs), Developmental Reprogramming and Cancer Risk



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BPA

- Bisphenol A is used in the production of epoxy resins and polycarbonate plastics.
- A recent CDC survey detected BPA in 95% of Americans sampled
- Significant levels also found in cord blood of newborns



Genestein

- Phytoestrogen that act as estrogen receptor agonist
- Isoflavone found in soybeans and legumes
- Dietary exposure from soy products including soy-based infant formulas

Endocrine Disrupting Compounds (EDCs), Developmental Reprogramming and Cancer Risk

- Many compounds in our environment, both naturally occurring and man-made, mimic the female hormone estrogen.
- Inappropriate exposure to these “xenoestrogens” is an environmental exposure that can participate in gene-environment interactions and increase cancer risk

Gene-Environment Interactions: Developmental (re)Programming Cancer Risk

Traditionally gene-environment interactions are believed to contribute to cancer by causing the accumulation of mutations that ultimately leads to cancer

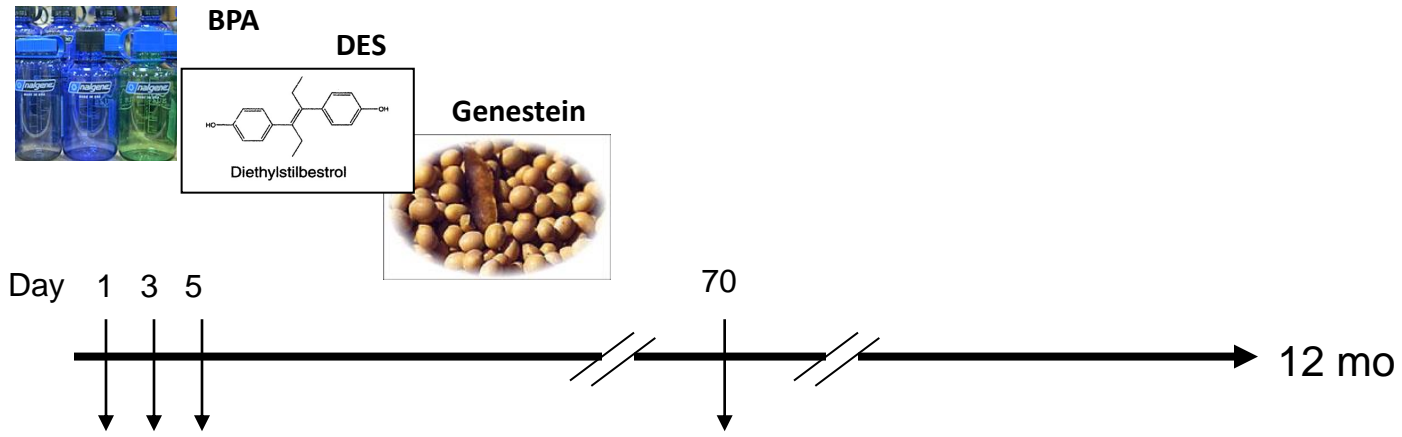
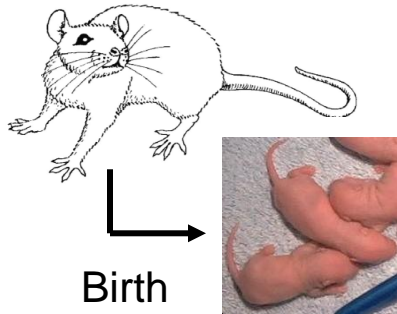
Examples include:

- Slight variations (polymorphisms) in genes for metabolizing enzymes that enhance or diminish the potency of carcinogens in cigarettes
- Defects in DNA repair genes that compromise the cell's ability to repair DNA damage induced by environmental agents such as UV

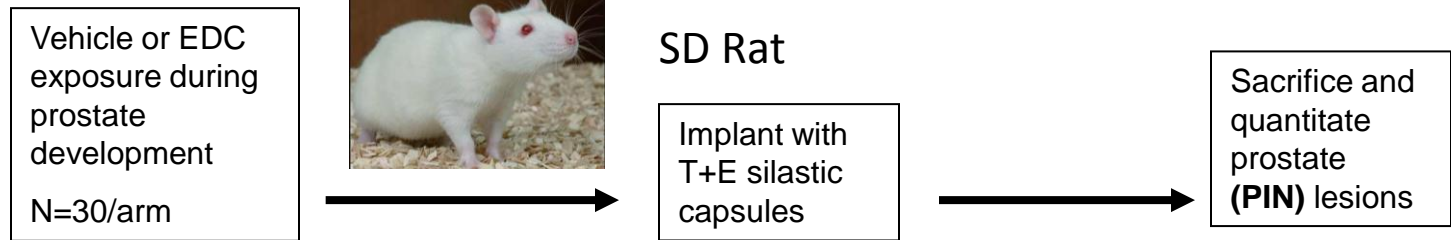
However, we proposed that exposure to chemicals during critical times of development might “reprogram” cells and tissues in a way that could influence cancer risk for the rest of an individual's life

Developmental Reprogramming by Environmental Estrogens

Uterus



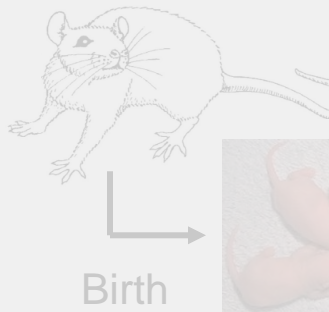
Prostate



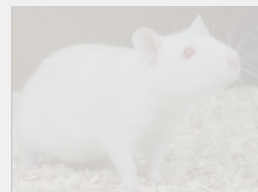
Developmental Reprogramming by Environmental Estrogens

What these models have in common:

- Exposure to an environmental estrogen
- During development of the target tissue when epigenetic “programming” is normally be installed
- Endpoint that is affected is a hormone-dependent tumor
- Effect is seen many months/years after the “developmental (re)programming has occurred



Vehicle or EDC exposure during prostate development
N=30/arm



SD Rat

Implant with T+E silastic capsules

Sacrifice and quantitate prostate (PIN) lesions

Prostate

Uterus

Vehicle or EDC

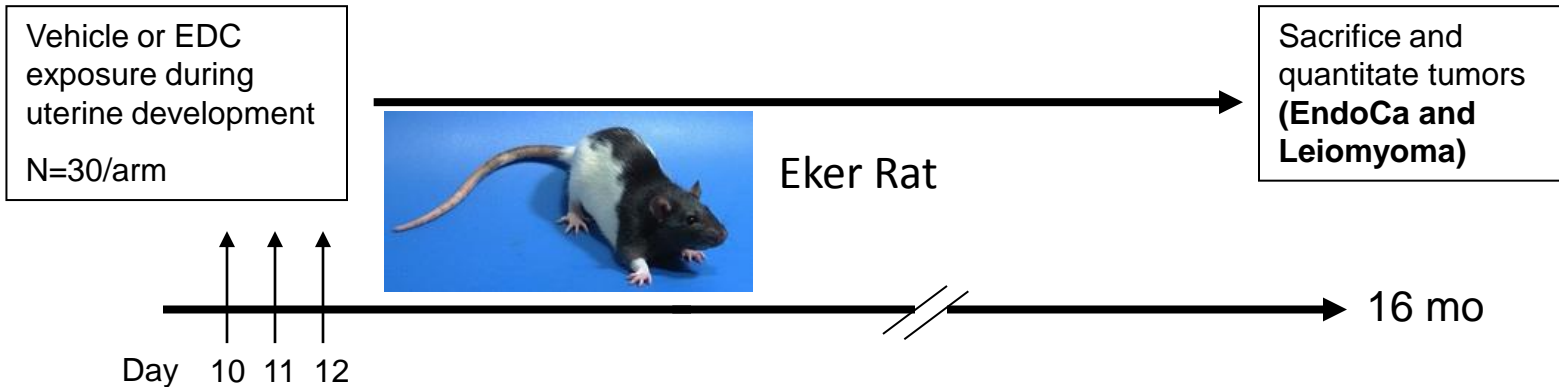
Sacrifice and quantitate tumors (Ca and myoma)

16 mo

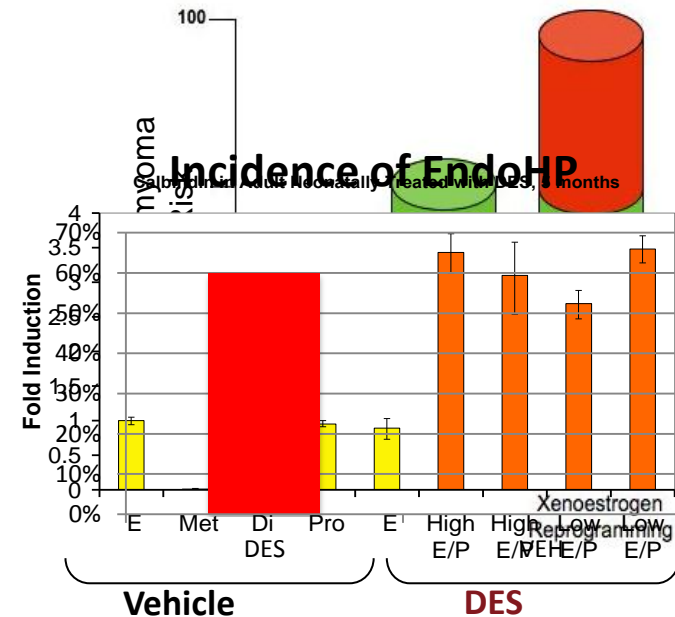
12 mo

Developmental Reprogramming by Environmental Estrogens

Uterus



- Leiomyoma incidence increases from 65% to 100% at 16mo
- EndoHP incidence increases from 0% to 60% at 5 mo
- Over 50% of estrogen responsive genes in the (re)programmed uterus become hyper-responsive to hormone
- Summary: the (re)programmed “hyperestrogenized” phenotype promotes an increase in hormone-dependent uterine tumors

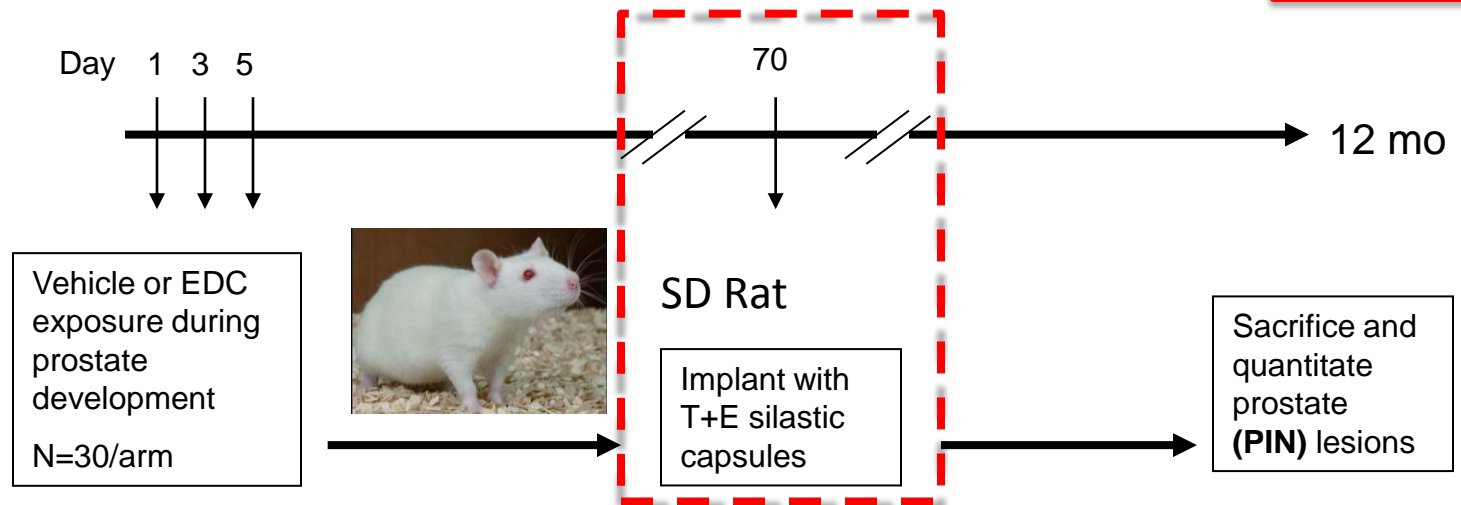


Developmental Reprogramming by Environmental Estrogens

- Incidence of PIN lesions is significantly increased
- Androgen-responsive genes in the KEGG prostate cancer pathway exhibit exaggerated response to testosterone
- Similar to what was seen in the uterus, androgen-responsive genes became hyper-responsive to hormone, promoting development of hormone-dependent lesions in the prostate

| Group | LP dysplasia score (n) | Increase in LP dysplasia score# | Incidence of LP dysplasia score## |
|------------------|---------------------------|---------------------------------|-----------------------------------|
| Oral Veh | 0.97 ± 0.43 (n=24) | - | 15/24 (63%) ^a |
| Oral BPA 2µg/kg | 1.05 ± 0.56 (n=23) | 8% | 15/23 (65%) ^a |
| Oral BPA 10µg/kg | 1.13 ± 0.52 (n=20) | 17% | 15/20 (75%) ^a |
| Oral BPA 50µg/kg | 1.20 ± 0.49 (n=22) | 23% | 20/22 (91%) ^{a,b} |
| SubQ BPA 10µg/kg | 1.23 ± 0.41* (n=27) | 26% | 23/26 (88%) ^c |

Prostate

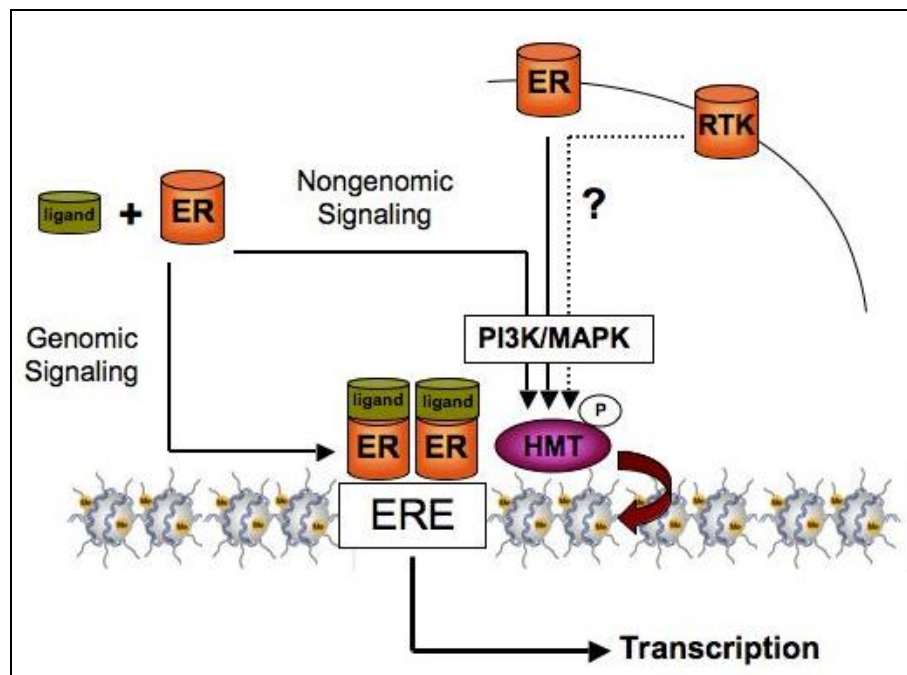


What we learned from these studies:

- Target tissues were (re)programmed to become hyper-responsive to hormones- estrogen-responsive genes in the uterus exhibited an exaggerated response to normal ovarian hormones, and androgen-responsive genes in the prostate became hyper-responsive to testosterone
- (re)Programmed phenotype preceded tumor formation, occurring in the target tissues prior to the onset of tumors

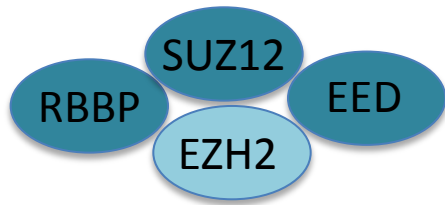
How had the environmental estrogens (re)programmed the tissue to change the way genes responded to hormones?

Model for Modulation of Histone Methyltransferases by Non-genomic Signaling

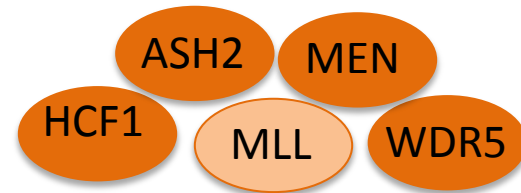


- We focused on environmental xenoestrogens that bind the estrogen receptor and act as endocrine disruptors such as the plasticizer BPA, the pharmacologic estrogen DES and the phytoestrogen genestein
- Like the female hormone estrogen, xenoestrogens activate estrogen receptor to induce genomic (gene transcription/repression) and non-genomic (cell signaling) activity
- **We hypothesized that kinases such as MAPKs, PI3K/AKT, PKA etc. could in turn, phosphorylate epigenetic “readers, writers and erasers” to modify their activity, thus disrupting epigenetic programming during development**

Development: Establishment and Maintenance of Transcriptional Memory



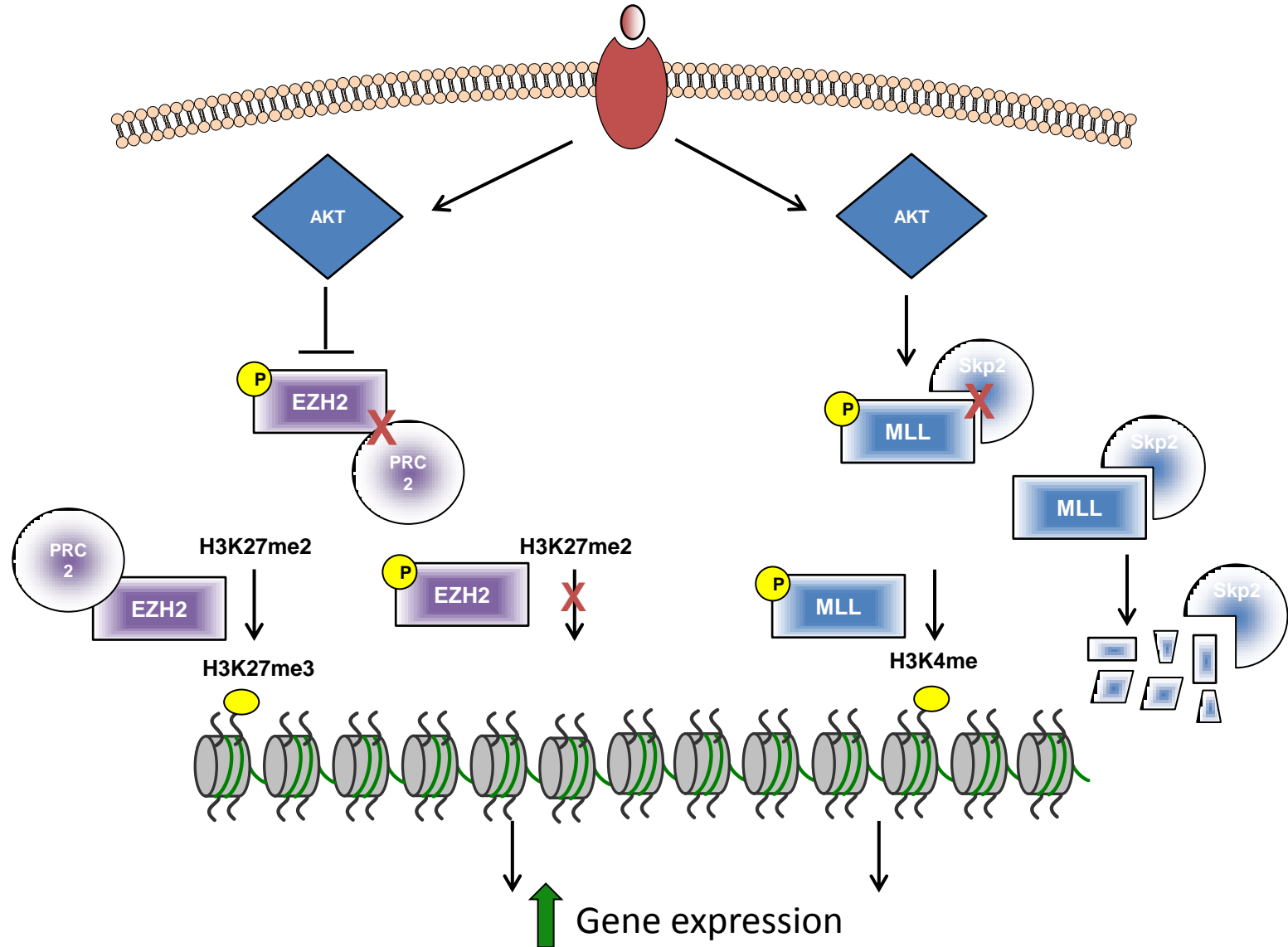
POLYCOMB COMPLEX (PCG2)



COMPASS COMPLEX (MLL)

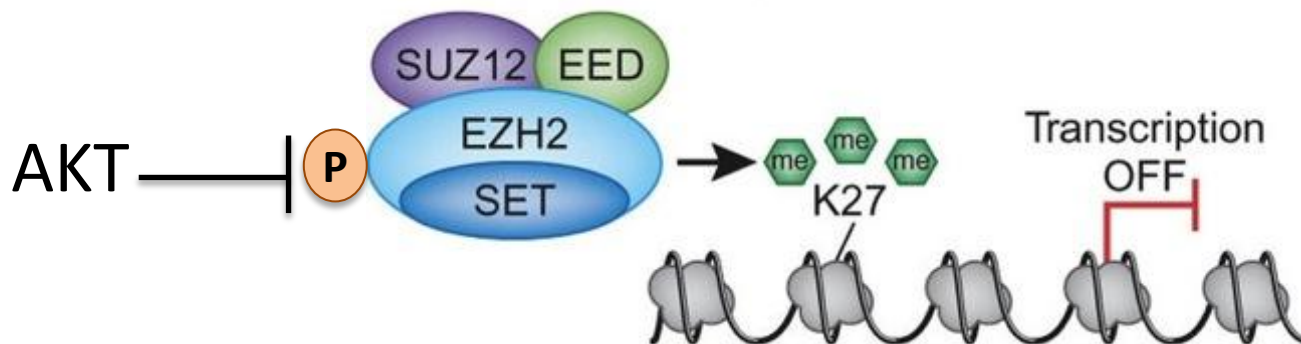
Early studies in *Drosophila* identified Polycomb (PcG) and Trithroax (TrxG) group genes as major histone modifying complexes that impart “transcriptional memory” that *heritably* represses (Polycomb repressive complex = PRC1 and PRC2) or activates (COMPASS complex = MLL) gene expression as cells divide and across the lifespan

Model for Modulation of Histone Methyltransferases by Non-genomic Signaling

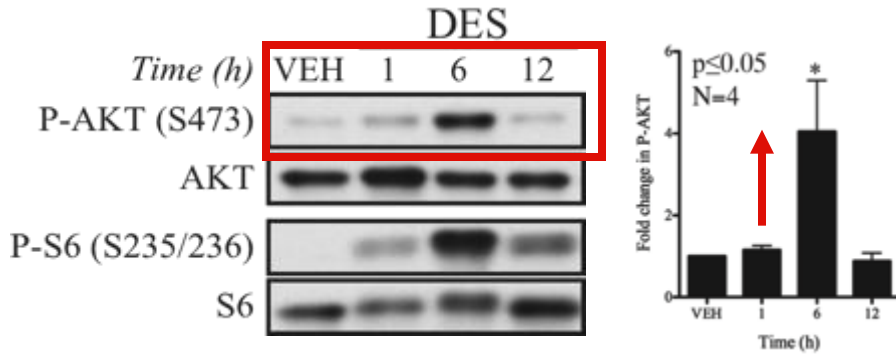


The Epigenetic “Writer” Histone Methyltransferase EZH2

- EZH2 histone methyltransferase is responsible for the histone H3, lysine 27 trimethyl mark (H3K27me3)
- Phosphorylation of EZH2 at serine 21 (S21) inhibits EZH2 methyltransferase activity
- S21 is phosphorylated by AKT when PI3K signaling is activated



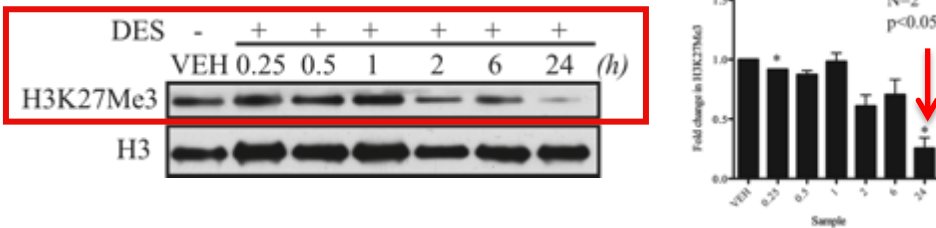
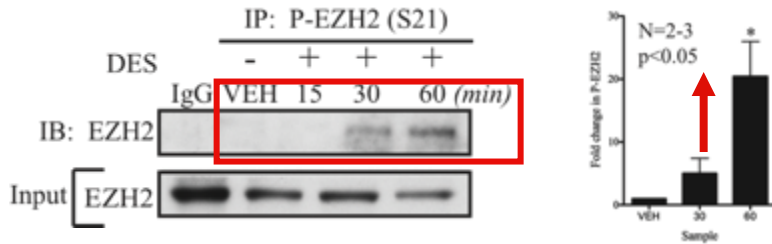
EDCs Modulate Methyltransferase Activity in the Developing Reproductive Tract



- In the developing reproductive tract, DES engages non-genomic signaling and activates AKT as detected by western analysis and IHC

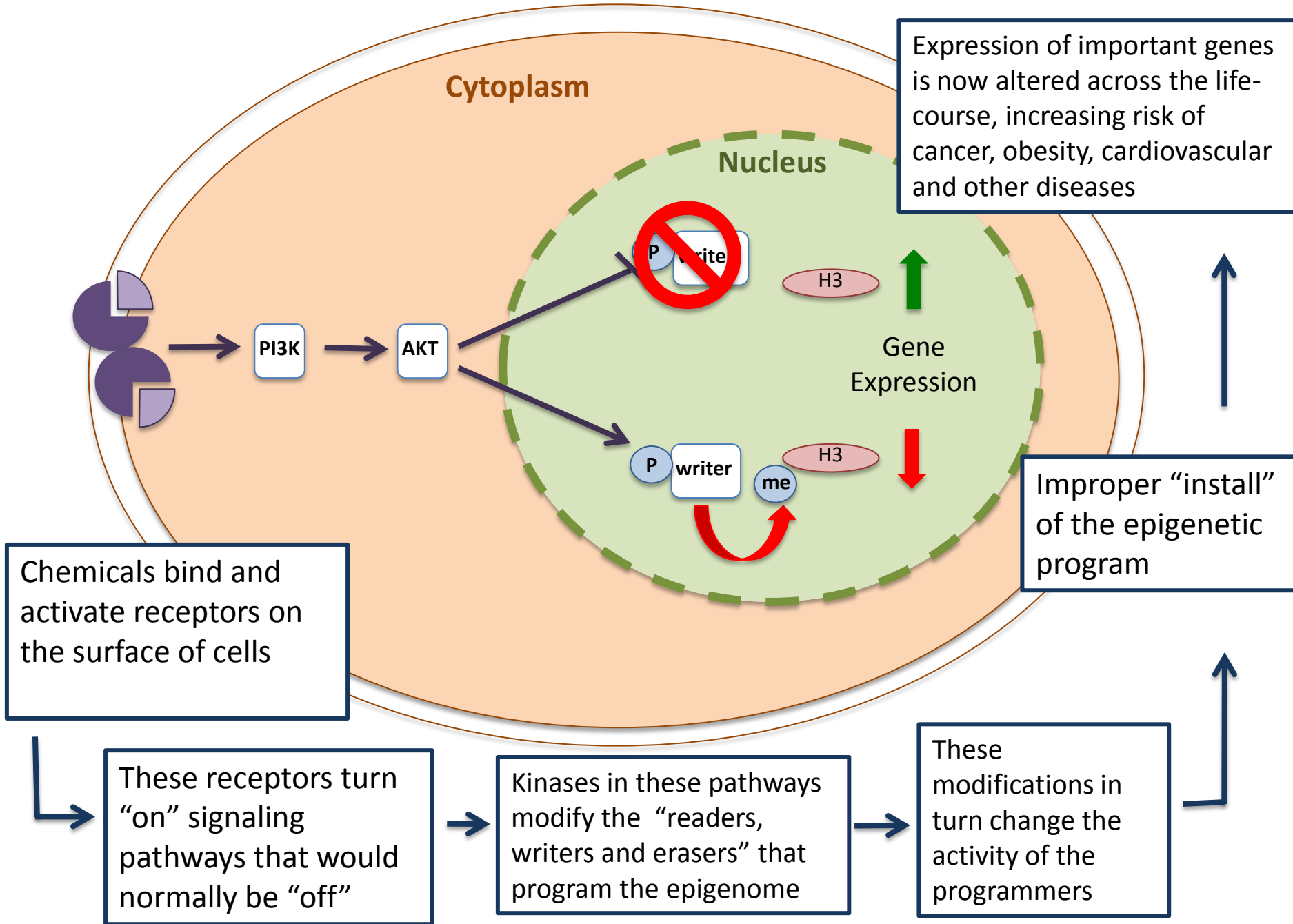
- DES induces rapid phosphorylation of the HMT EZH2 at S21, a target of AKT and a site known to regulate (inhibit) EZH2 activity

- EZH2 phosphorylation correlates with a decrease in the EZH2 methyl mark H3K27Me3 in the developing reproductive tract

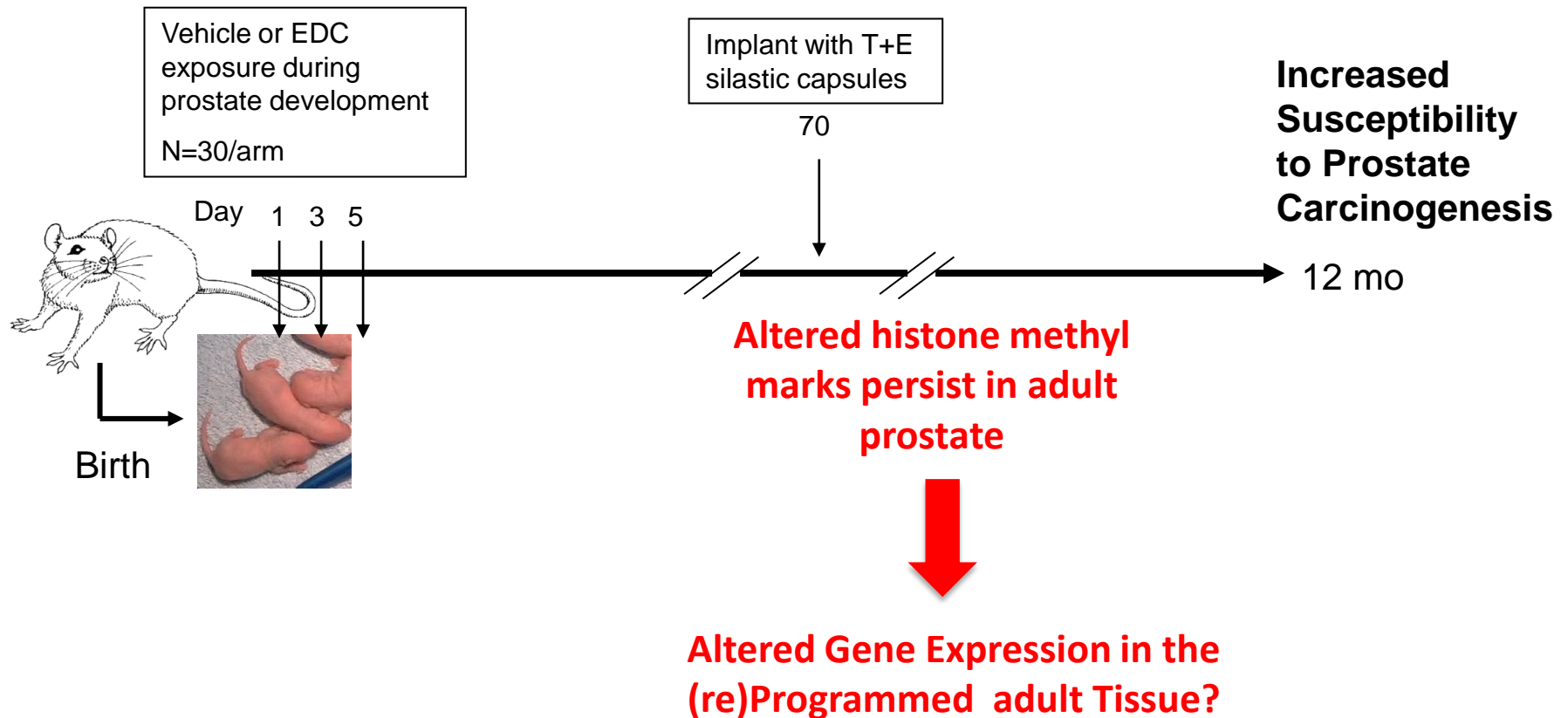


EDCs Modulate Methyltransferase Activity in the Developing Reproductive Tract

First demonstration that an EDC could engage the epigenetic machinery in a developing tissue to disrupt the epigenome



Gene-specific Developmental (re)Programming

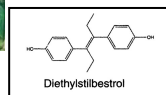


Environmental Estrogen Exposure



BPA

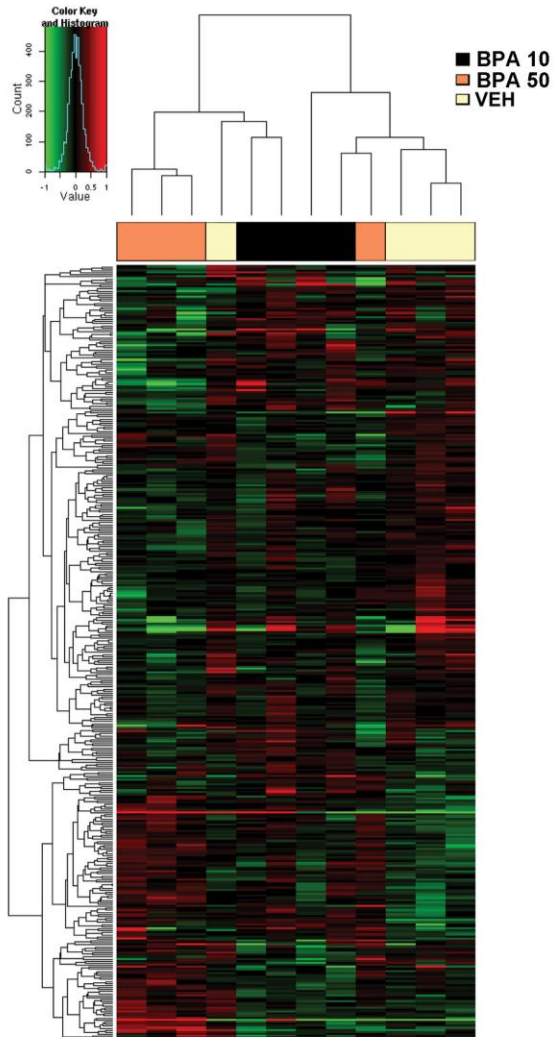
DES



Genestein

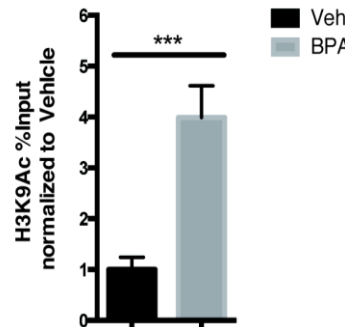
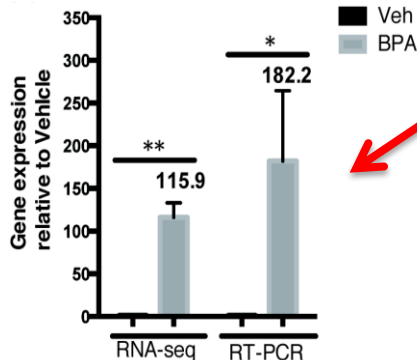
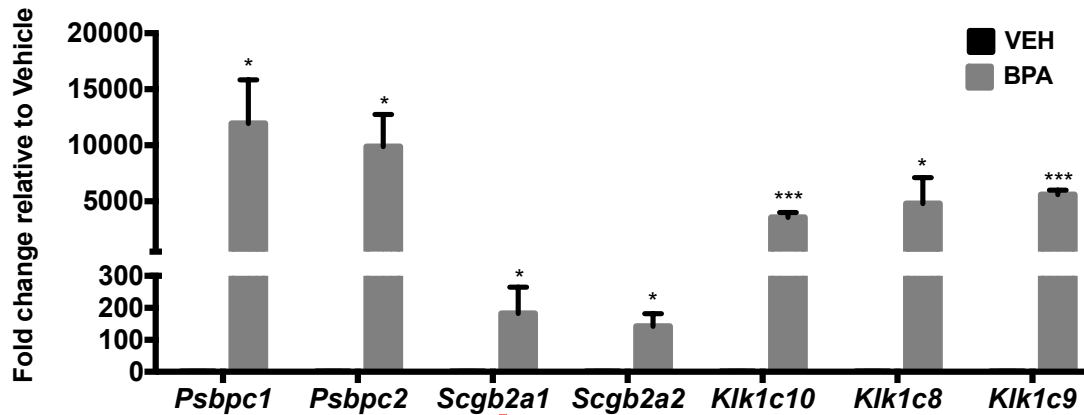
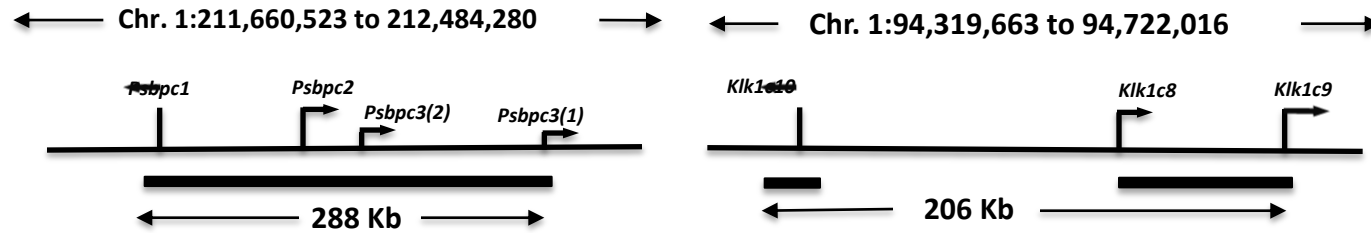


Reprogramming of Gene Expression in the Prostate



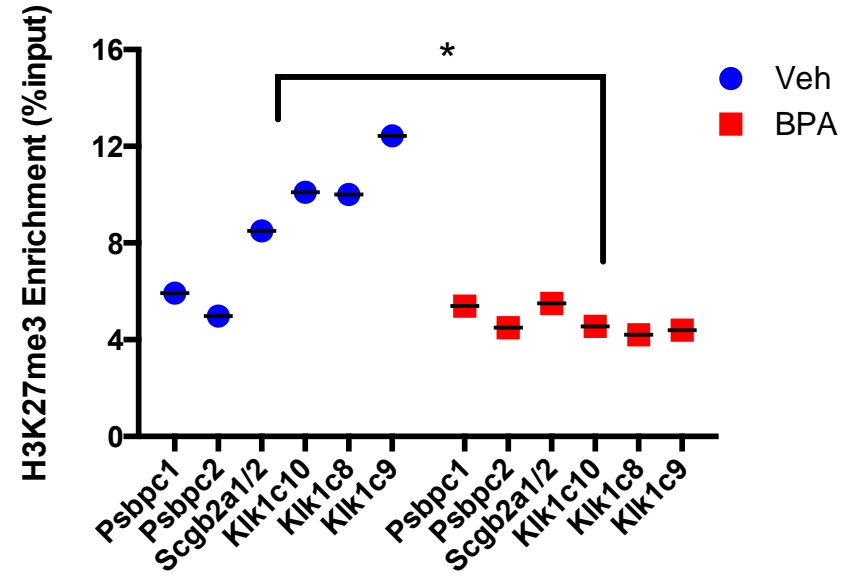
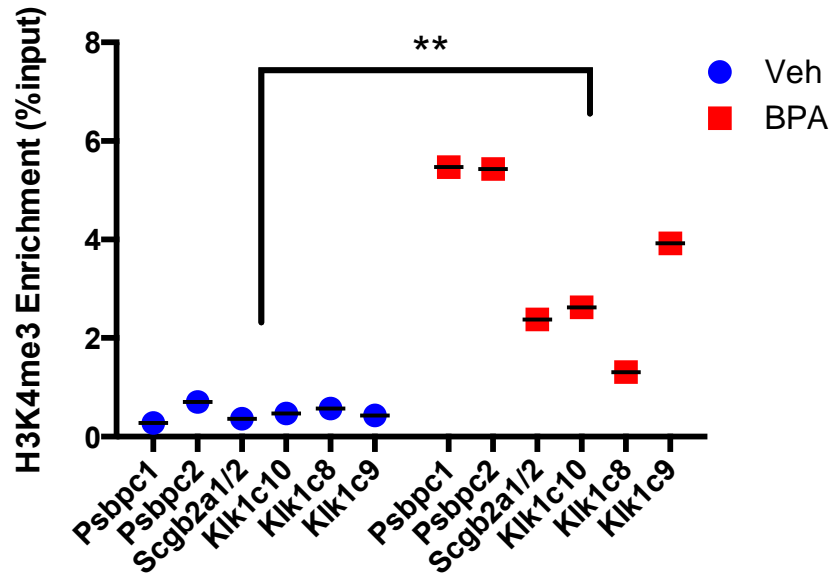
- In the adult prostate, greatest difference in gene expression by RNA-seq was between vehicle and oral BPA (BP50) prostates
- Comparison between these two groups identified two gene clusters on chromosome 1 that were significantly over-expressed in the (re)programmed prostates

Reprogramming of Gene Expression in the Prostate



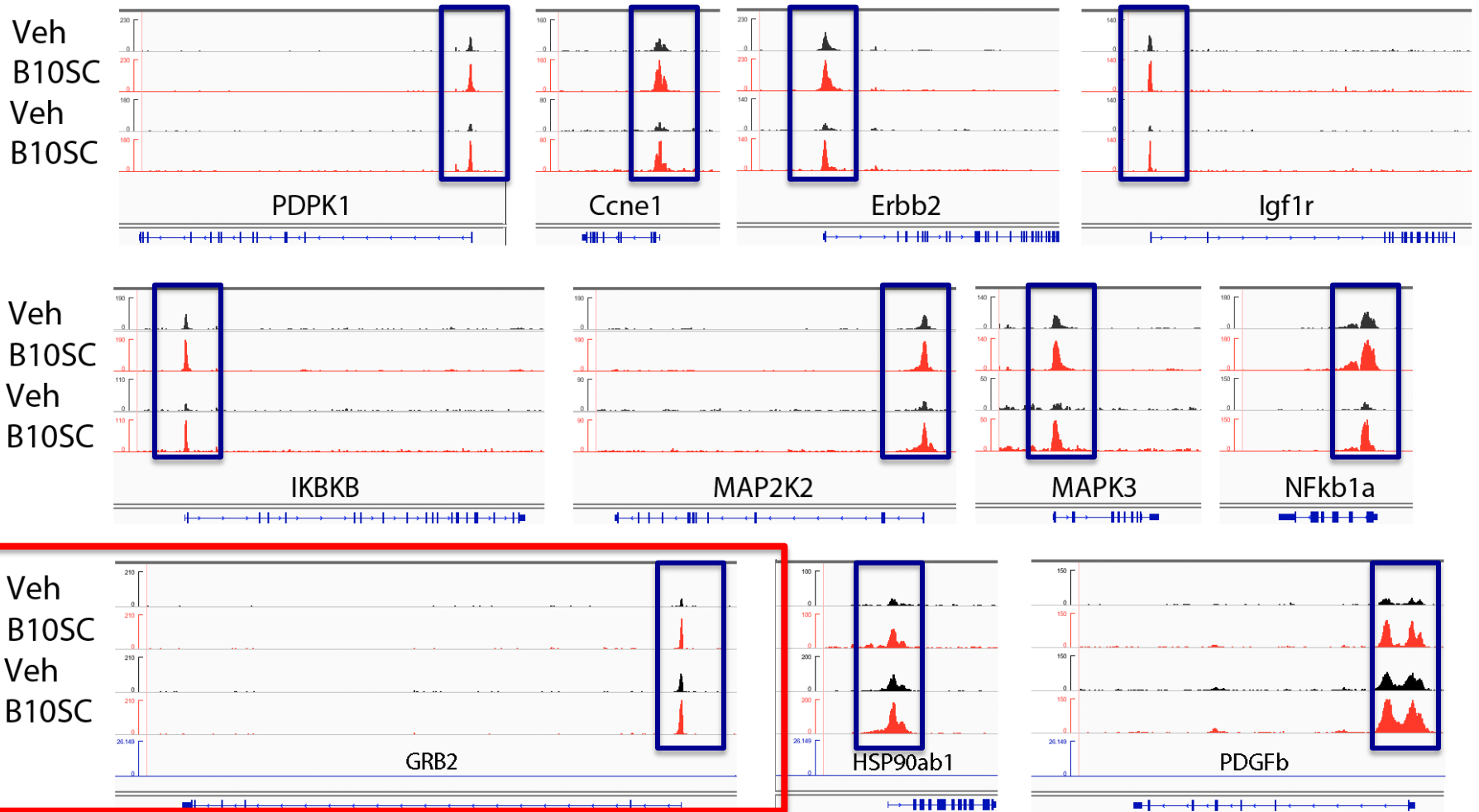
- Two gene clusters containing the secretoglobin and kallikrein gene clusters were overexpressed in reprogrammed prostates
- RNA-seq data were confirmed by RT-PCR and commensurate increase in H3K9Ac

Activating H3K4me3 is Increased and Repressive H3K27me3 is Decreased in Reprogrammed Genes



Increased expression of the secretoglobulin and kallikrien gene clusters was associated with a significant increase in the activating H3K4me3 mark (MLL) and decrease in the repressive H3K27me3 mark (EZH2)

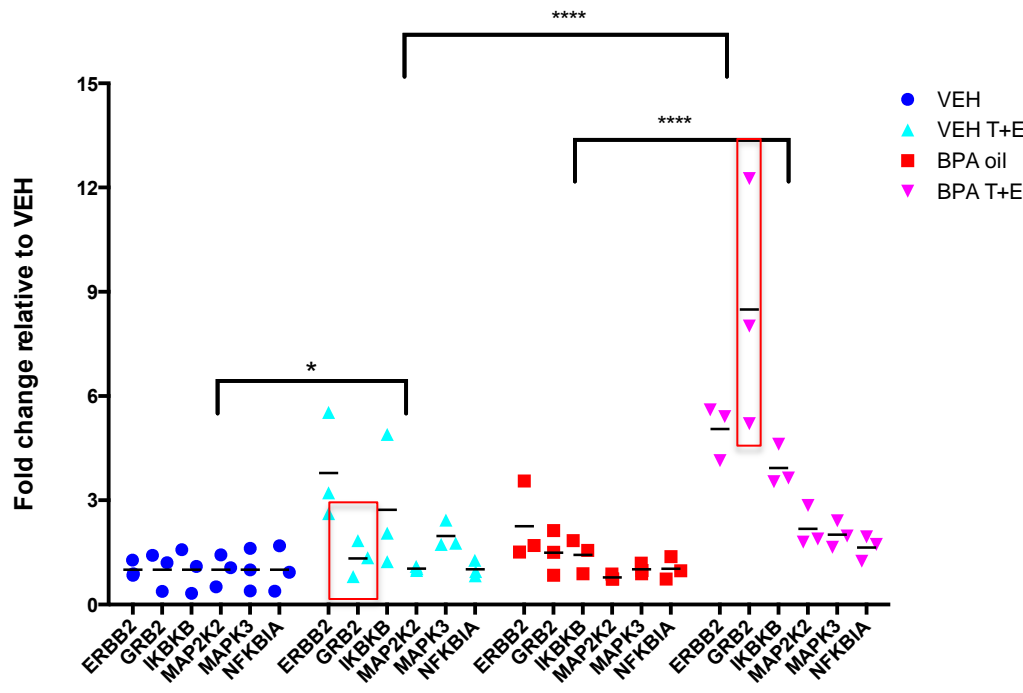
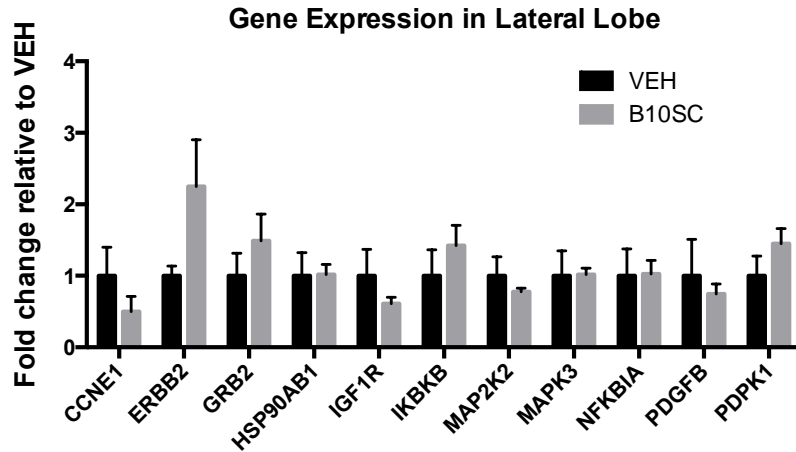
Activating H3K4me3 is Increased and Repressive H3K27me3 is Decreased in KEGG Prostate Cancer Pathway Genes



Black = VEH controls

Red = BPA exposed

Reprogrammed Genes Exhibit Increased H3K4me3 in the Adult d70 Prostate



- Increased H3K4me3 at reprogrammed genes observed by ChIP-seq was confirmed with directed ChIP
- However, these genes did not exhibit any change in basal expression in the adult prostates of BPA-exposed rats
- Treatment of adult rats with testosterone revealed an exaggerated response to hormone i.e. genes had become hypersensitive to androgens

Gene-specific Developmental (re)Programming

Vehicle or EDC exposure during prostate development
N=30/arm

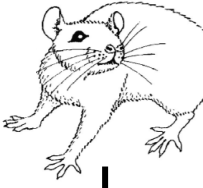
Implant with T+E silastic capsules

d70

Increased Susceptibility to Prostate Carcinogenesis

12 mo

Day 1 3 5



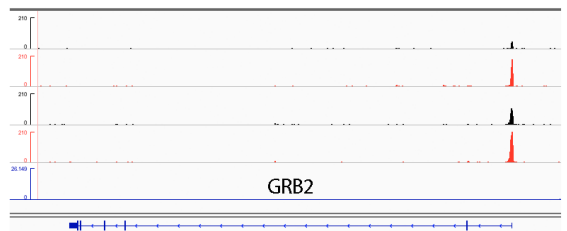
Birth



Neonatal changes in histone methyl marks

Altered histone methyl marks persist in adult prostate

Veh
B10SC
Veh
B10SC



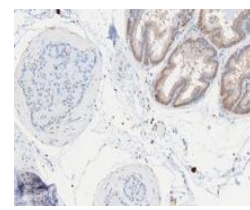
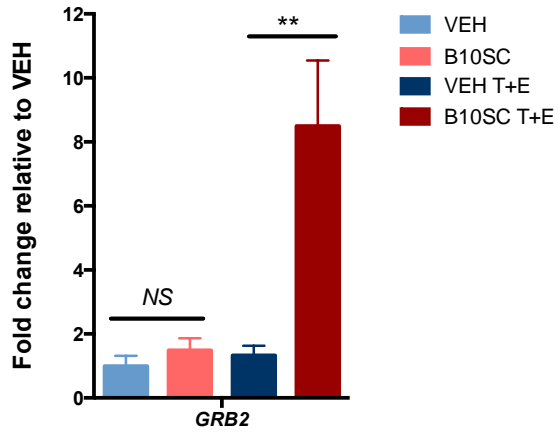
Change in activity of epigenetic programmer(s)

?

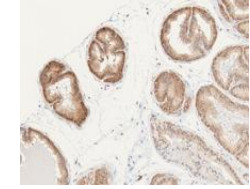


Environmental Estrogen Exposure

Altered Gene Expression in the (re)Programmed Adult Tissue

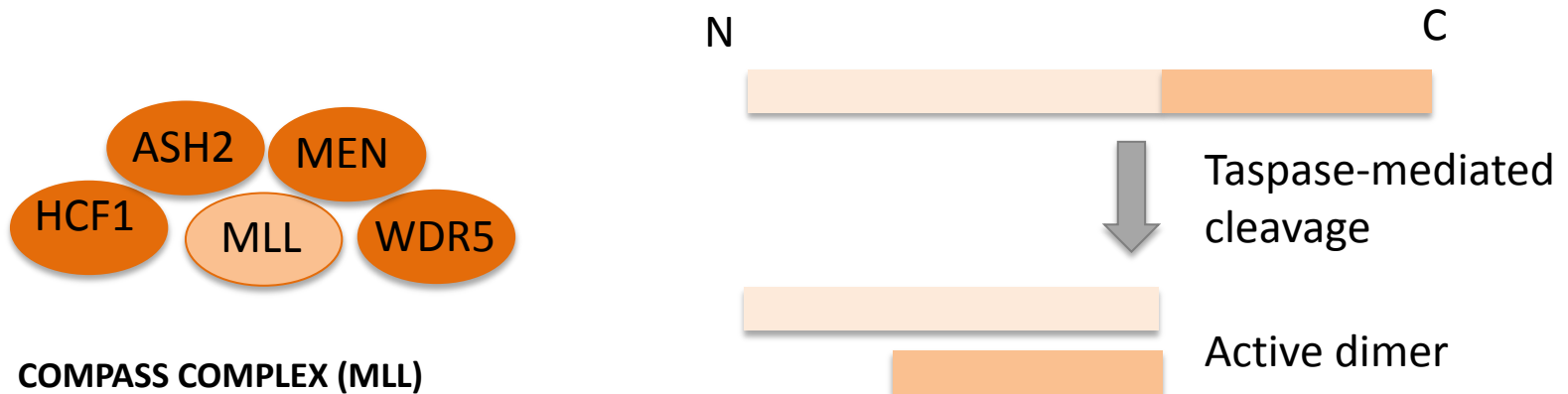


VEH



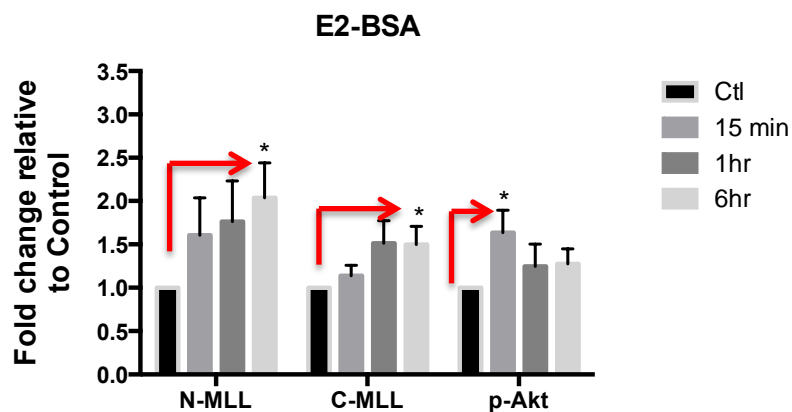
BPA

Development: Establishment and Maintenance of Transcriptional Memory

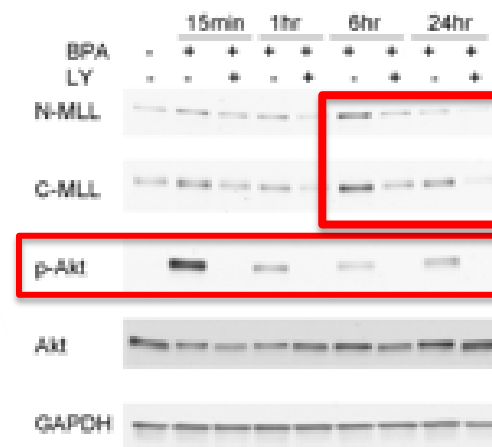
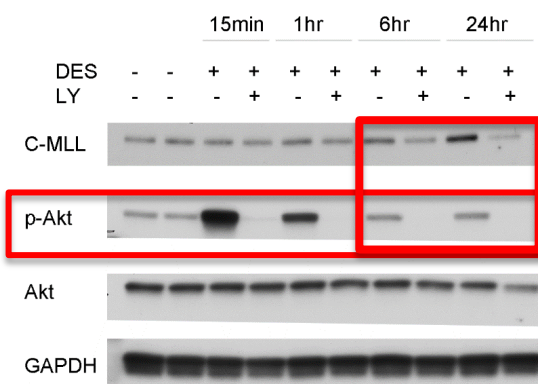
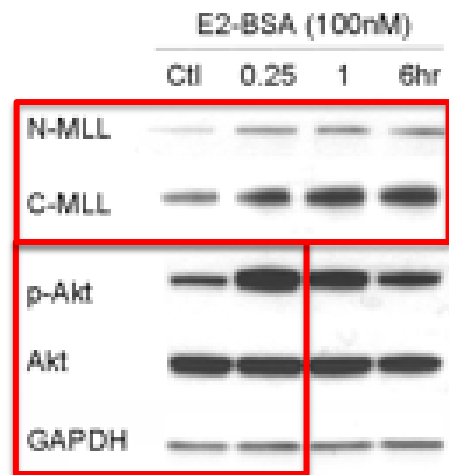


- The COMPASS complex, which contains the histone methyltransferase MLL, is responsible for “writing” the epigenetic H3K4 histone methyl mark
- MLL must be cleaved into N- and C-terminal fragments that assemble as the active MLL complex

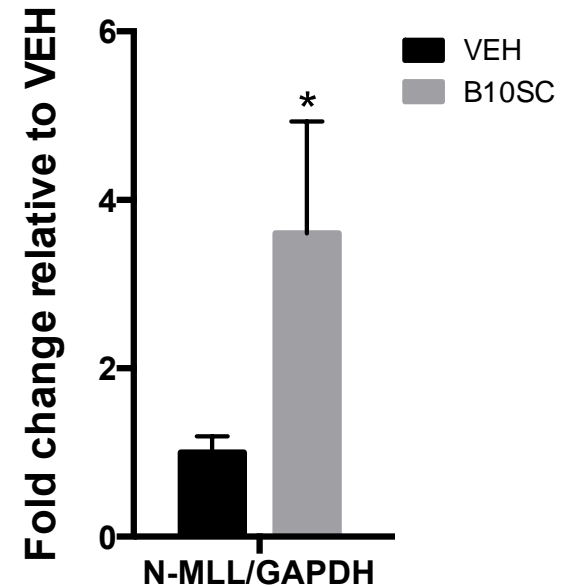
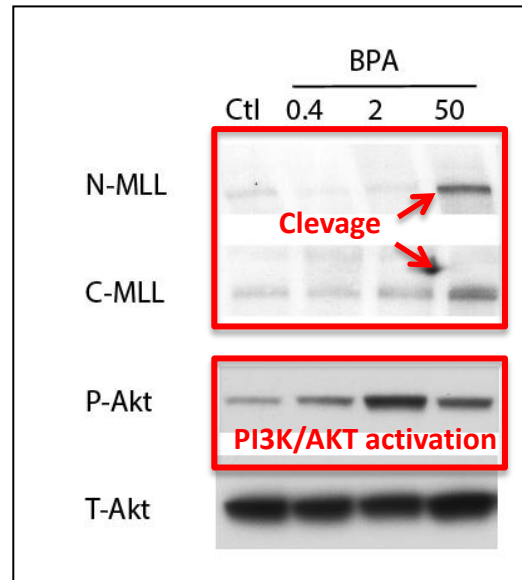
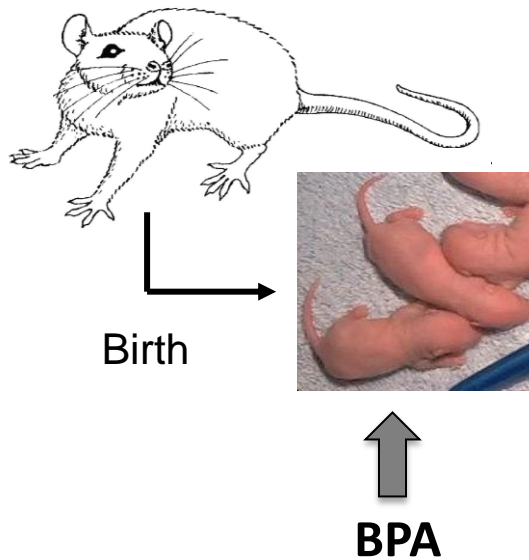
MLL Activation (Cleavage) Induced by Non-Genomic ER Signaling



- AKT and MLL are rapidly activated in the absence of genomic ER activity
- PI3K/AKT signaling is rapidly activated by DES and BPA *in vitro*
- Activated (cleaved) MLL increases between 6 and 24hrs
- MLL activation is inhibited by PI3K/AKT inhibitor LY and wortmannin (data not shown)

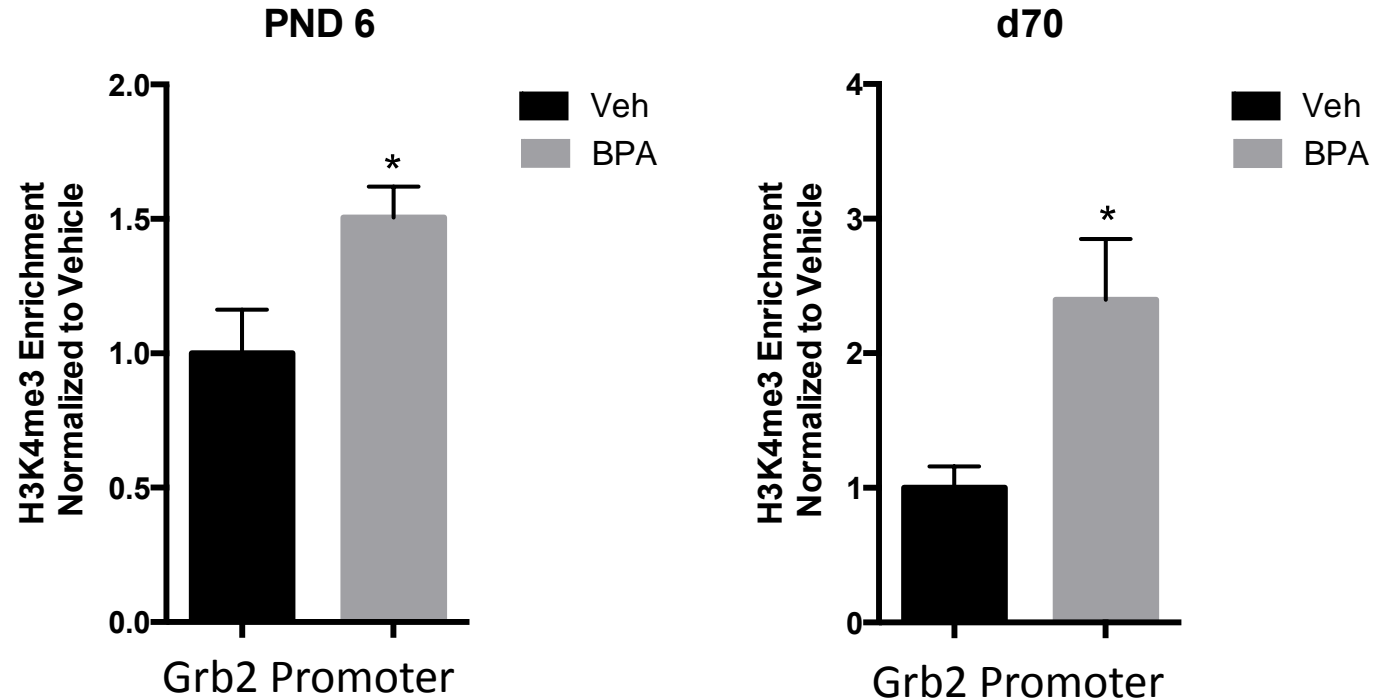


Increased MLL N- and C-Fragment Activation (Cleavage) by Xenoestrogens



- Concordant with induction of non-genomic signaling by BPA *in vivo* in the developing prostate, MLL cleavage to the active form is observed
- Similar data obtained with other environmental estrogens (DES, genistein) that induce PI3K/AKT non-genomic signaling

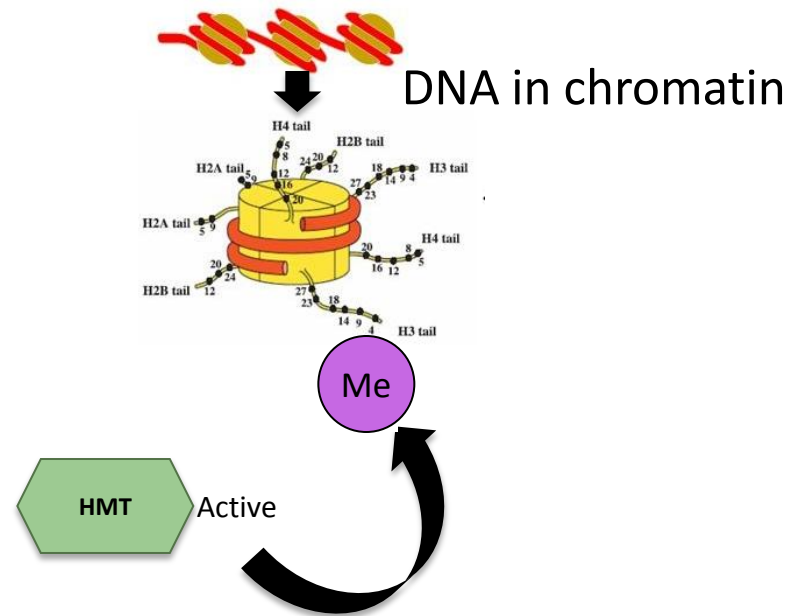
Reprogrammed Genes Exhibit Increased H3K4me3 in Neonatal and Adult Prostate



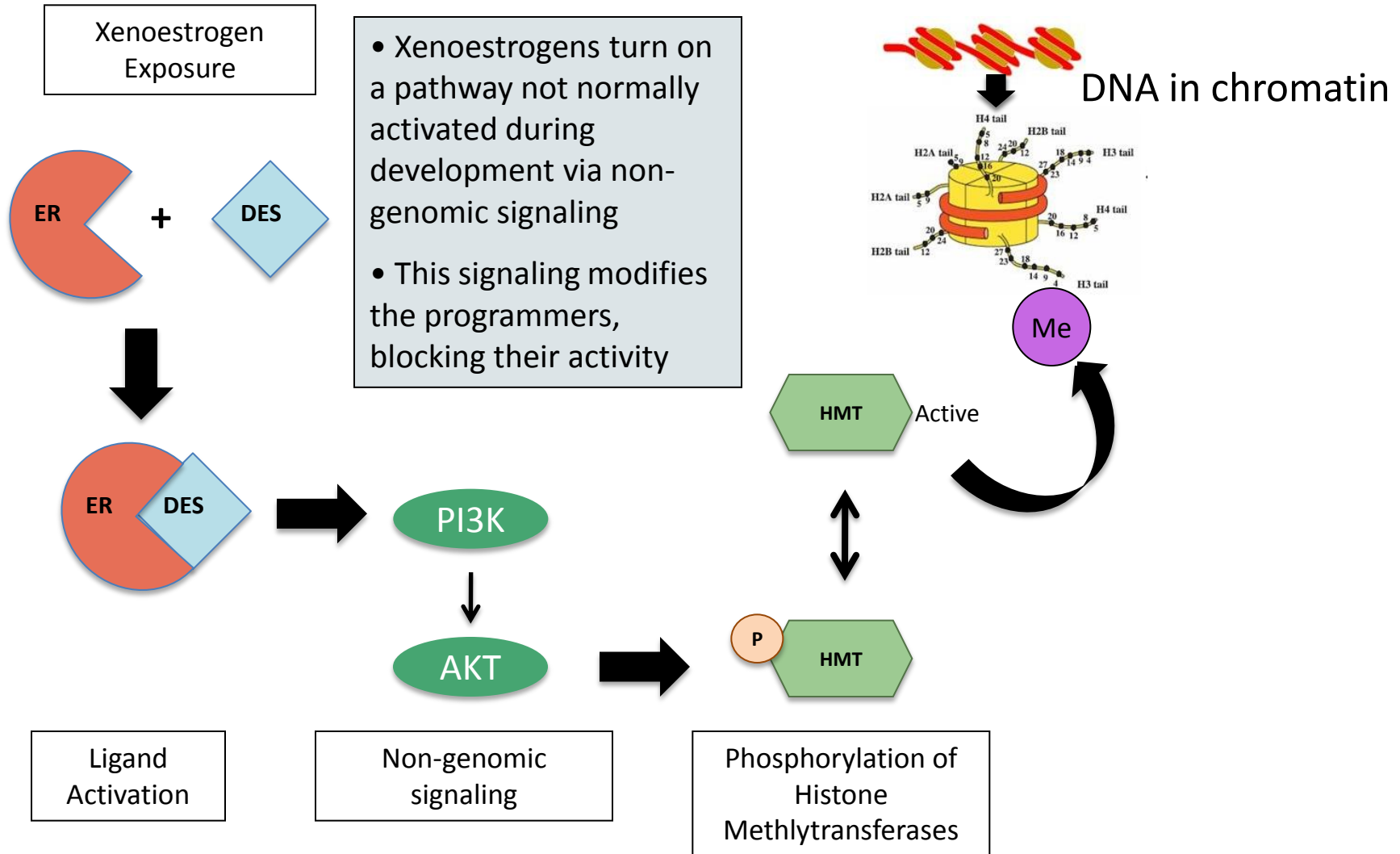
- BPA induced an increase in activating H3K4me3 methylation of genes that were targeted for (re)programming
- Increased H3K4me3 persisted in the adult prostate for genes such as Grb2 that were (re)programmed to become hyper-responsive to androgens

How is Epigenetic Reprogramming Happening?

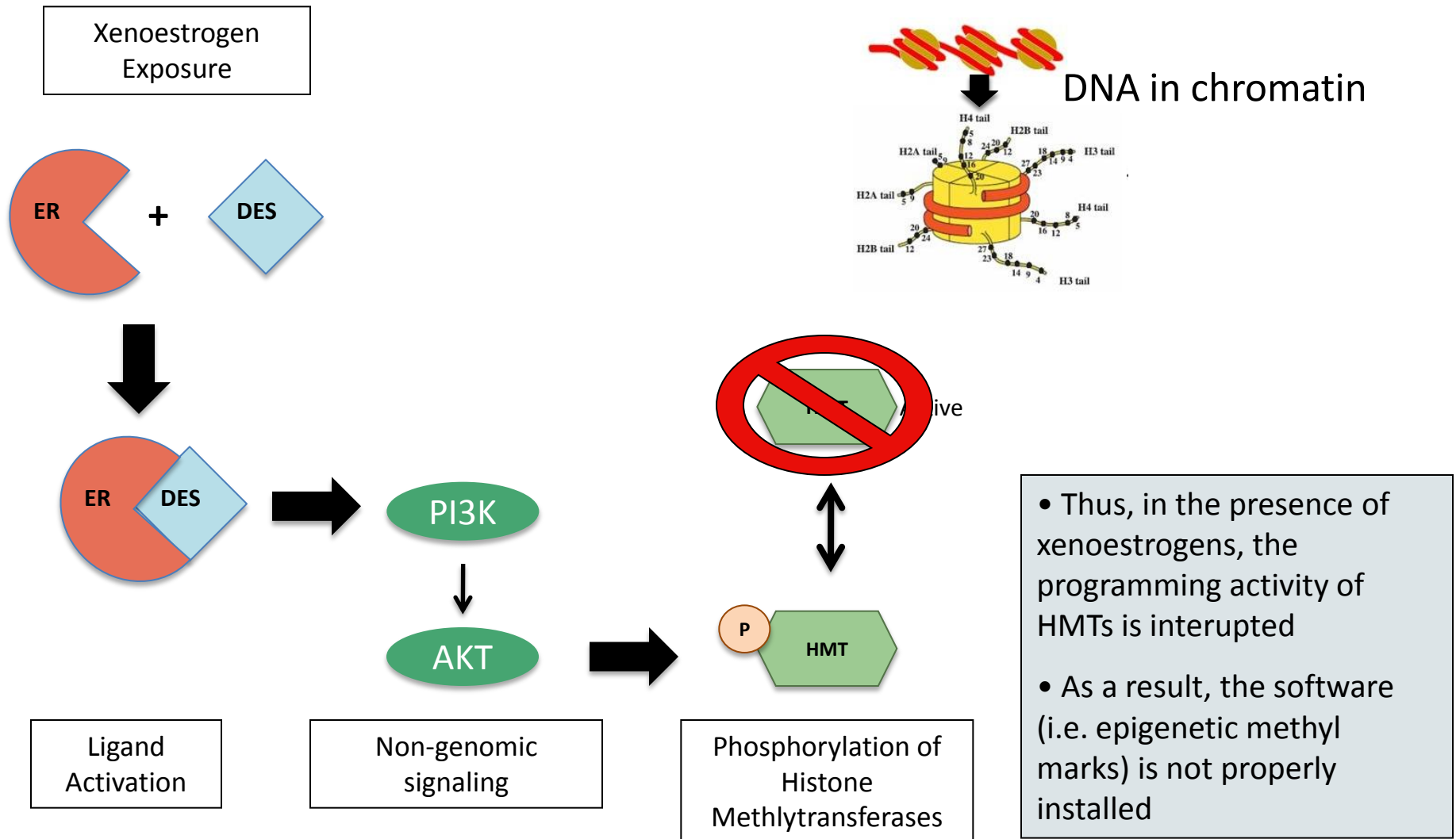
- The epigenome (i.e. software of the genome) is being programmed during development
- Enzymes (programmers) such as histone methyltransferases (HMT) are installing the software
- The histone methyl “marks” installed by these enzymes are permanent, i.e. stable, and inherited after each cell division
- These histone methyl marks function as the epigenetic software that determines how cells respond to estrogen



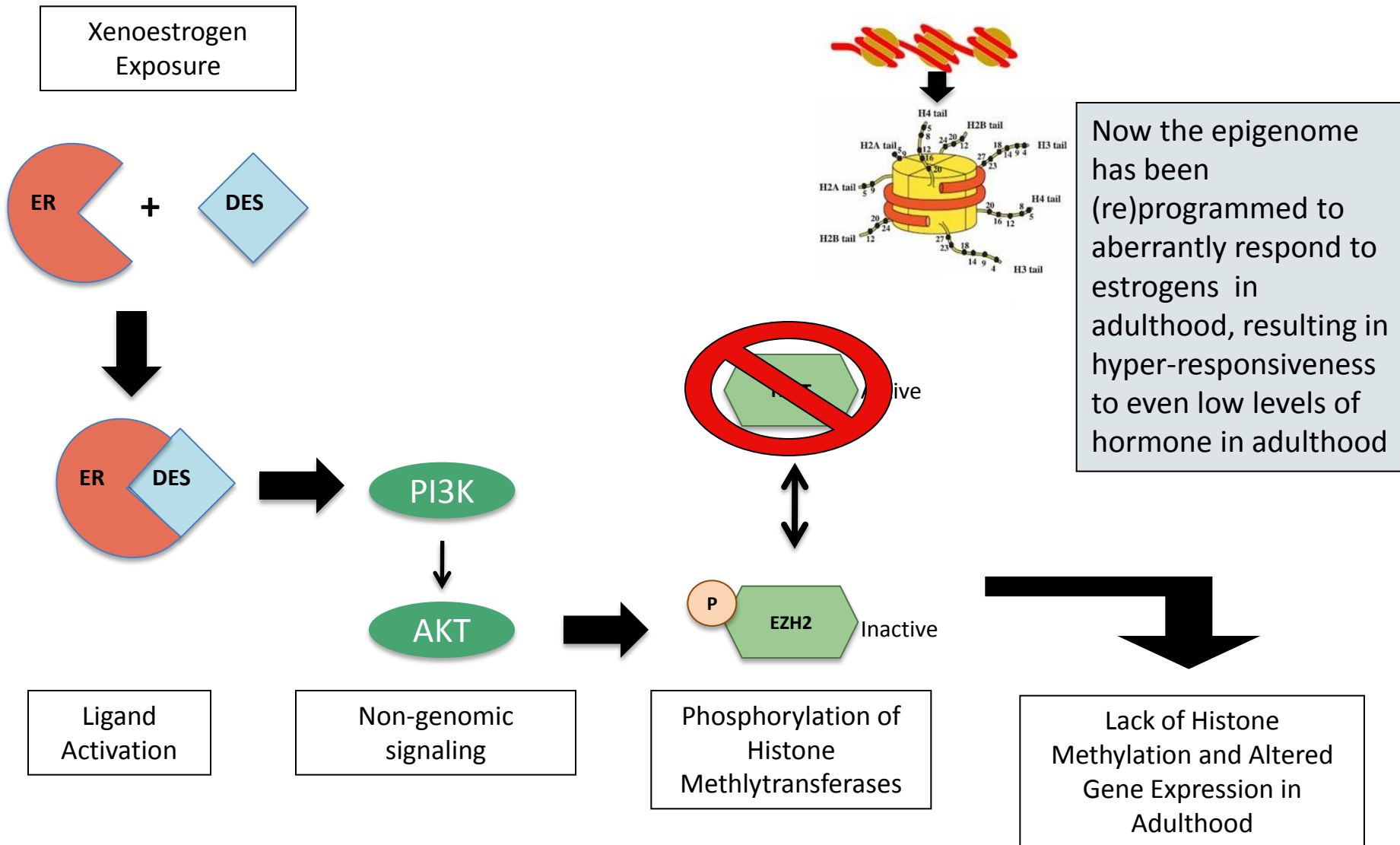
How is Epigenetic Reprogramming Happening?



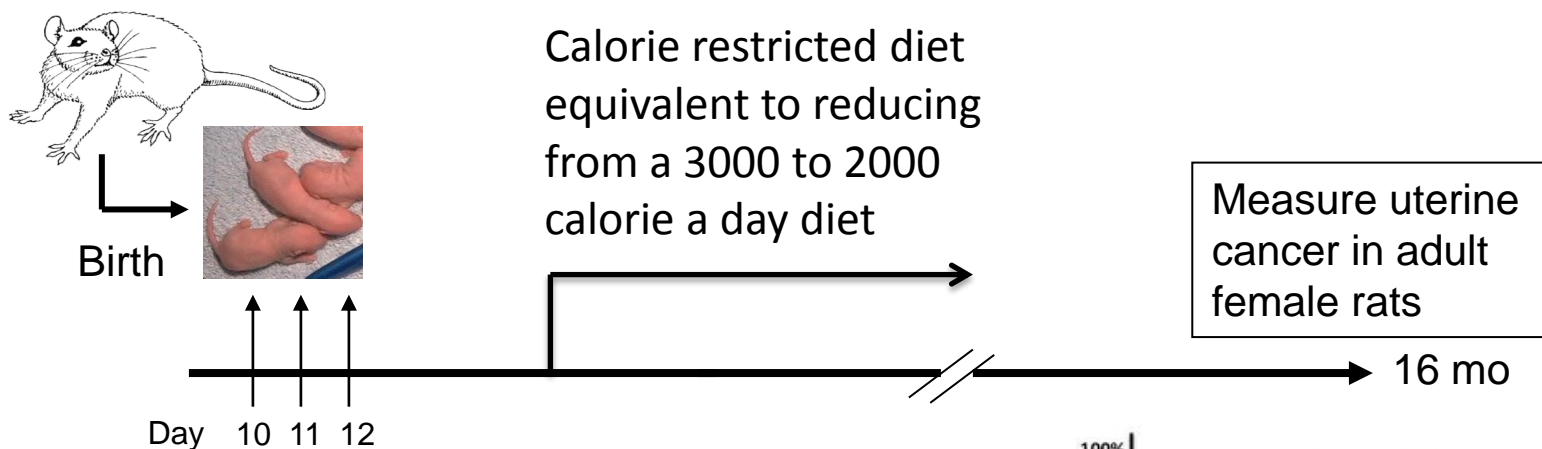
How is Epigenetic Reprogramming Happening?



How is Epigenetic Reprogramming Happening?

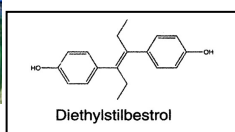


Dietary Interventions Can Reverse the Effects of Developmental Reprogramming



BPA

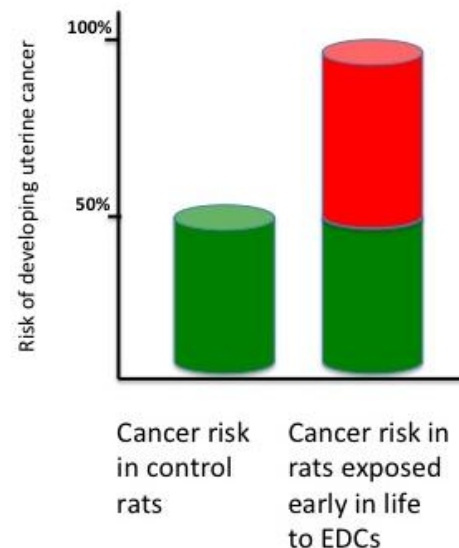
DES



Genestein



Environmental estrogens, termed “endocrine disrupting compounds” or EDCs



What Does it Mean for Human Health?

- Exposure to EDCs (and perhaps other environmental agents) early in life while our tissues are undergoing development can alter normal physiological responses in adulthood
- This “Developmental (re)Programming” can determine risk of diseases such as cancer in adulthood
- This implies that adult cancer risk, for example, may be determined by environmental exposures that occurred in early life, possibly decades, before disease presentation
- Identification of the “imprint” left by developmental programming may be useful for identification of exposed individuals and as a biomarker for cancer susceptibility in adult life
- Interventions later in life (dietary, lifestyle, pharmacologic) may be able to reverse the adverse health effects of developmental (re)programming