

*Eminent Toxicologist
Lecture Series*

Society of Toxicology

Eminent Toxicologist Lecture Series

Reprogramming of the Genome by Toxic Injury

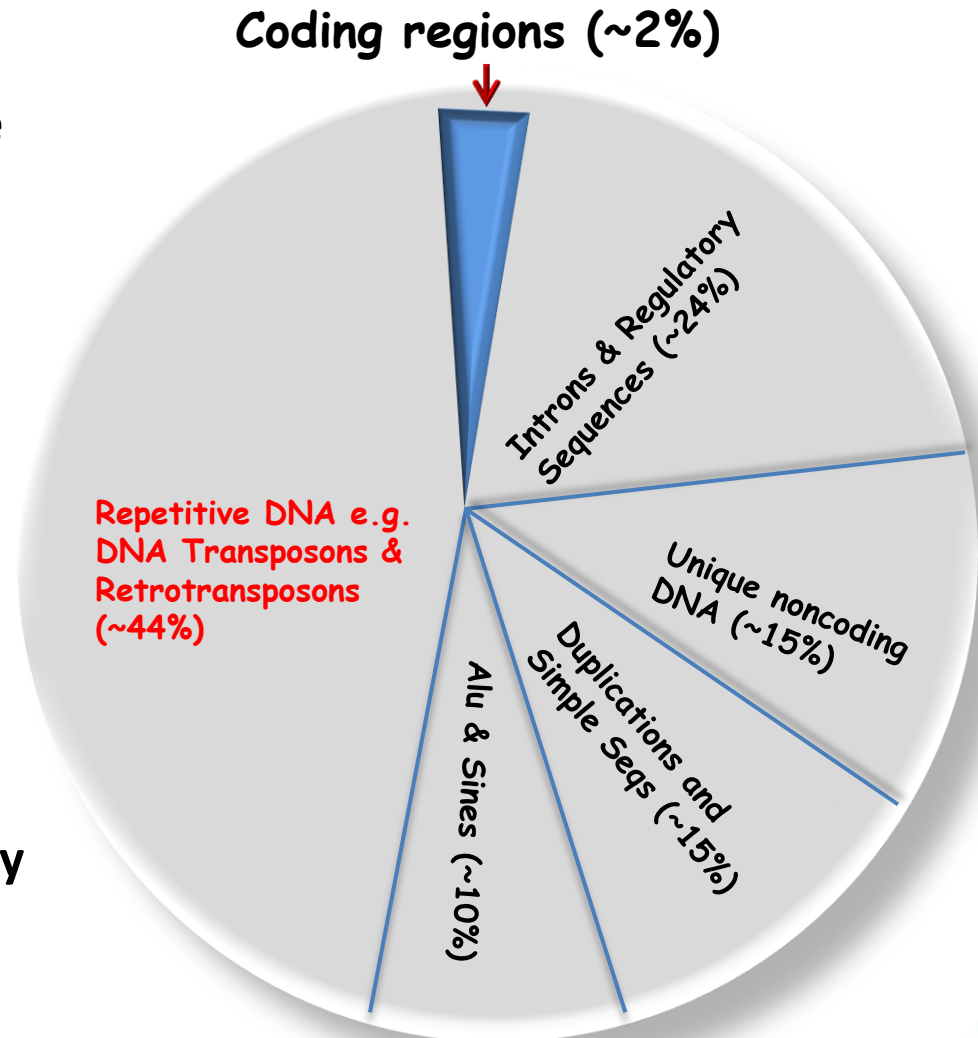
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Society of Toxicology

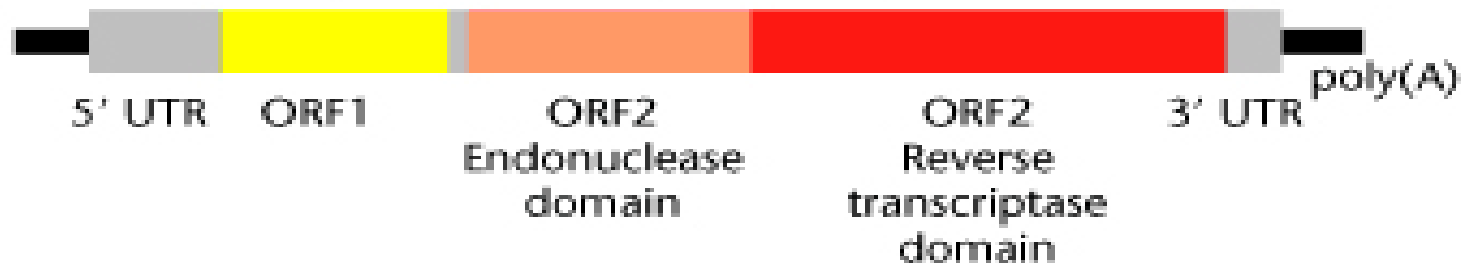
The Human Genome

- The human genome is stored on 23 chromosome pairs and encompasses over 3 billion DNA base pairs.
- Protein coding sequences comprise less than 2% of the genome.
- Aside from genes and regulatory sequences, the genome contains vast regions of DNA with poorly characterized functions.



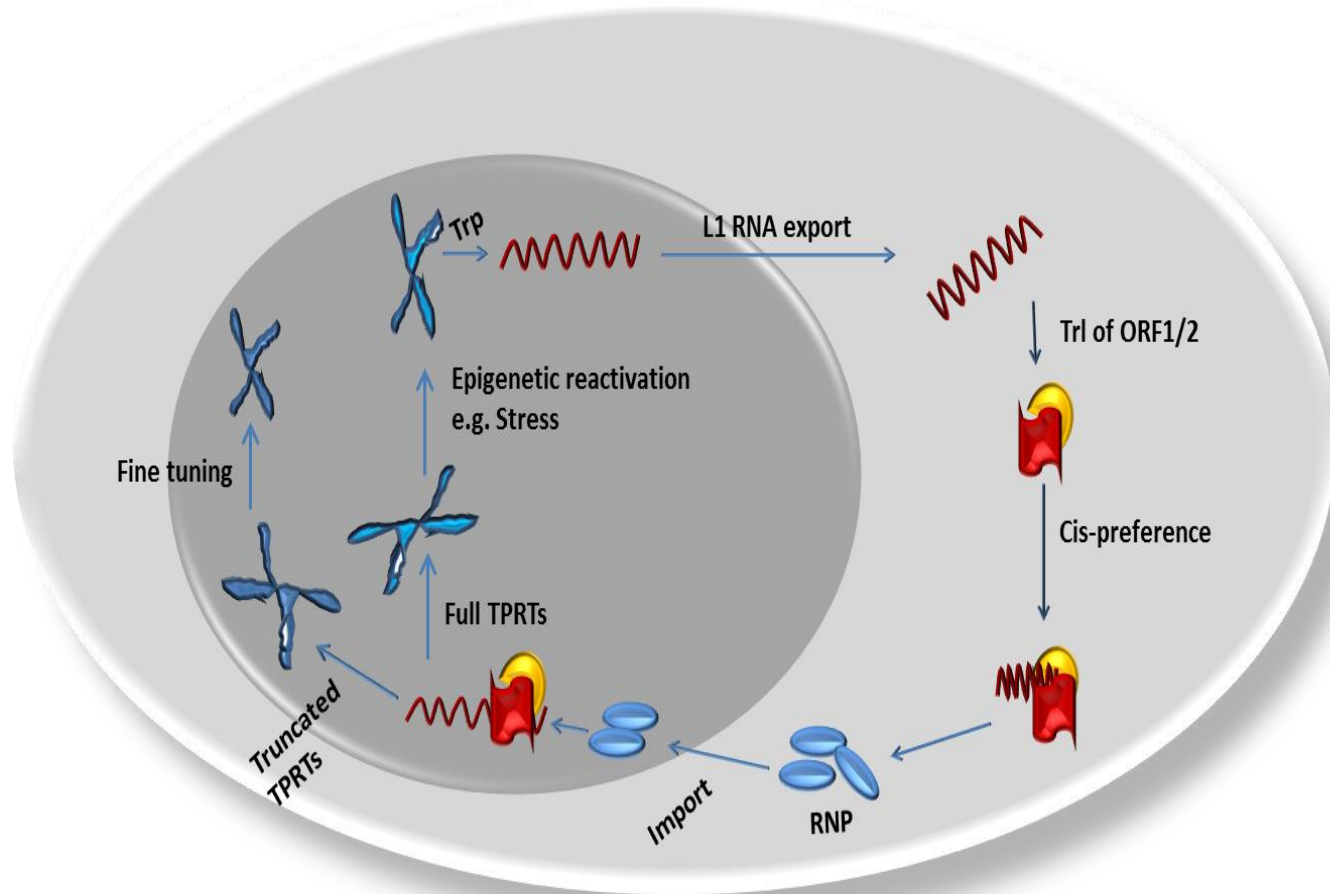
LINE-1 Retroelements

LINE (long interspersed repeat element)



- ✓ **5'UTR:** Contains a promoter region with regulatory sequences that bind YY1, RunX3, and E2F/Rb
- ✓ **ORF1:** RNA-binding protein that shuttles L1 RNA
- ✓ **ORF2:** Endonuclease and reverse transcriptase activities
- ✓ **~600,000 copies per genome (17%)**
- ✓ **~100 full length active in the human genome**
- ✓ **Silenced by DNA methylation and reactivated by reversal of epigenetic marks.**

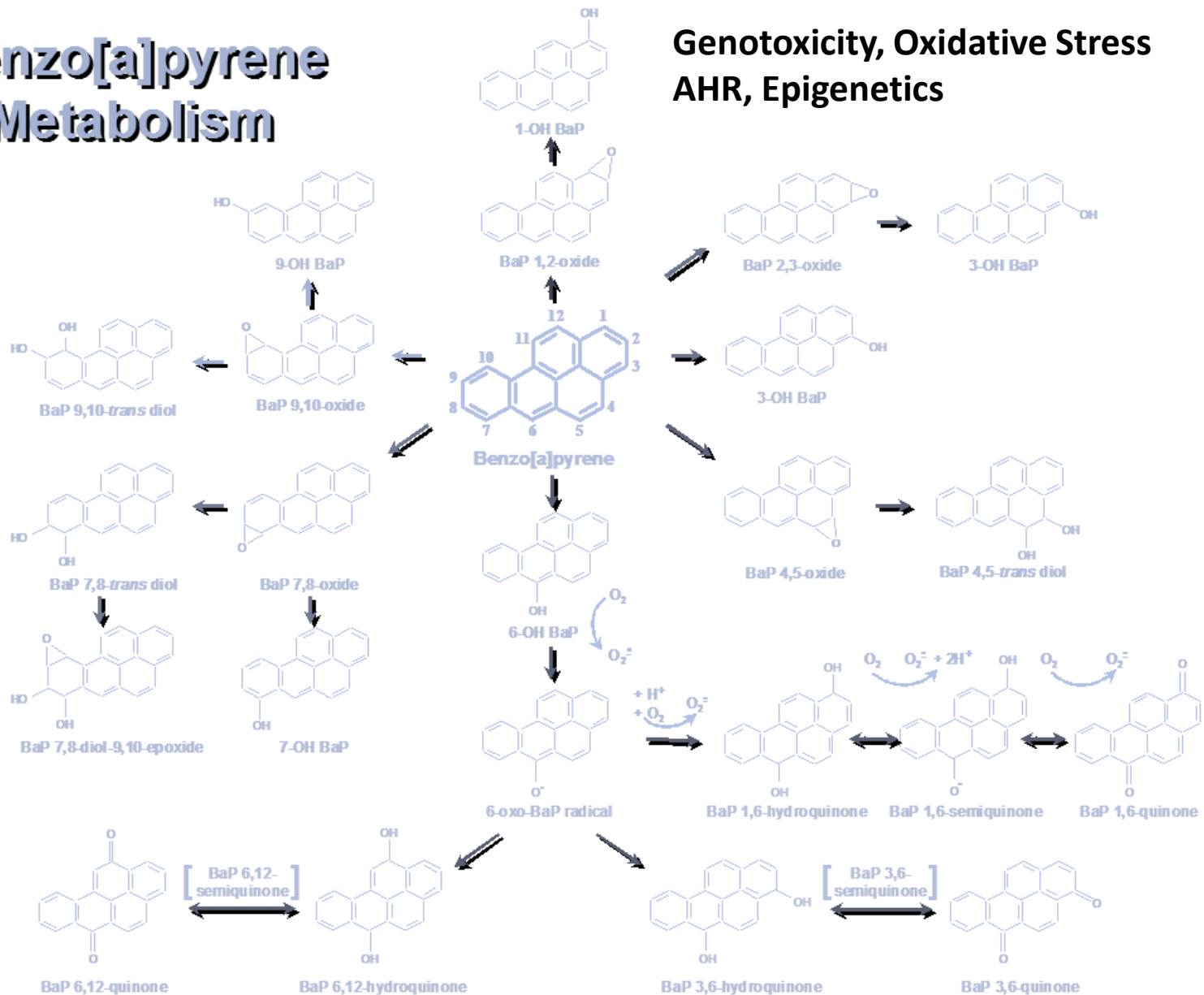
Life Cycle of LINE-1



Toxic injury by BaP reactivates L1 retrotransposition via epigenetic mechanisms

Benzo[a]pyrene Metabolism

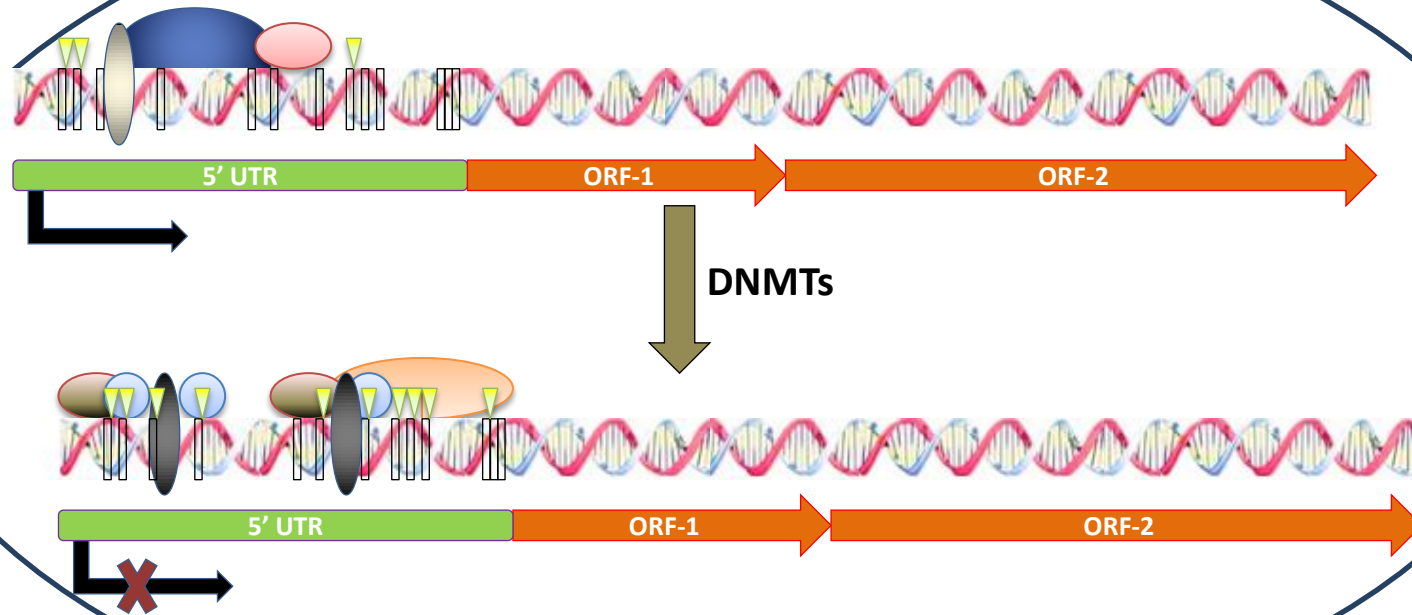
Genotoxicity, Oxidative Stress AHR, Epigenetics











What is Epigenetics?

- ✓ Heritable changes in gene expression without changes in DNA sequence.
- ✓ Assembly of protein complexes on chromatin to activate or silence genes.
- ✓ If not properly controlled can mediate adverse health effects.
- ✓ Environmental toxicants can alter epigenome to cause disease.
- ✓ **DNA Methylation and Histone Modifications are two well characterized epigenetic pathways.**

L1 Silencing During Embryonic Development




 Sequence-specific TF
 RNA Pol complex
 Activator
 Unmethylated CpG

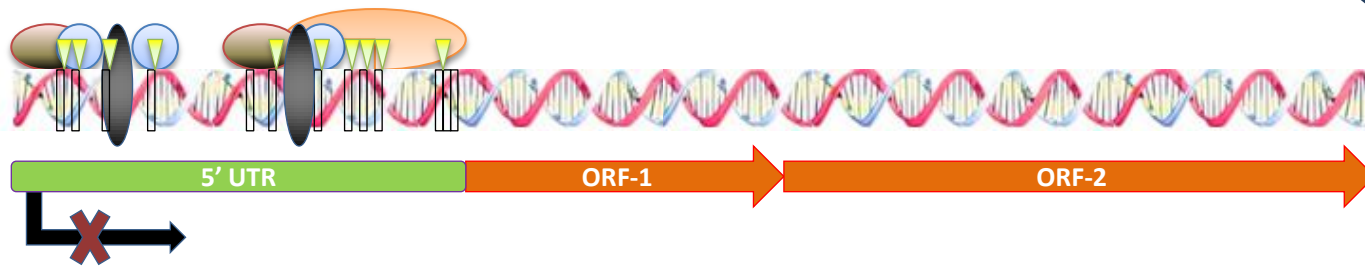
 Sequence-specific repressor
 Methyl-binding protein
 DNA methyl-transferase
 Methylated CpG

5'-CpG-3'
 3'-GpC-5'
Active

DNMTs

 CH₃
 5'-CpG-3'
 3'-GpC-5'
Inactive

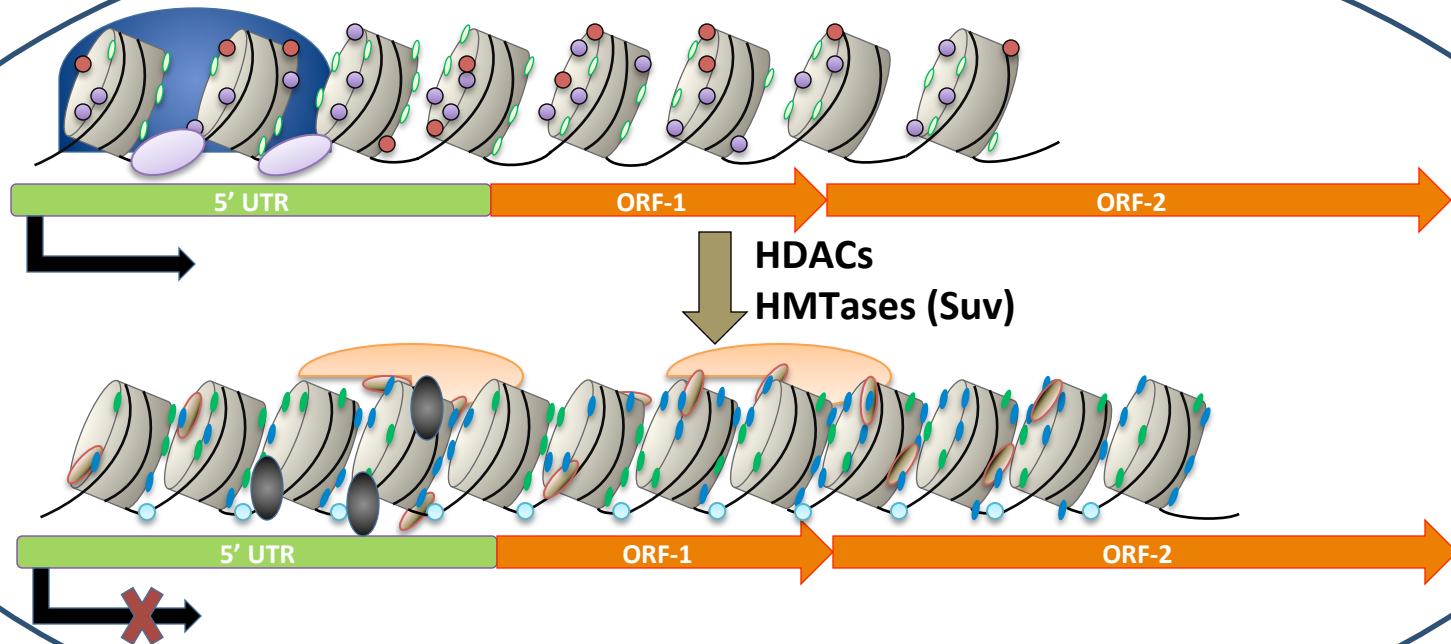
L1 Silencing by E2F/Rb



- ✓ Rb is recruited to both human and mouse L1 5' UTR in vivo.
- ✓ E2F1 and E2F4 bind to both human and mouse L1 5' UTR in vivo
- ✓ E2F binding to L1 is Rb-independent.
- ✓ Rb prevents cells from replicating damaged DNA by inhibiting progression from G1 to S.
- ✓ Rb recruits HDACs that inhibit E2F and facilitate condensation of nucleosomes into heterochromatin.

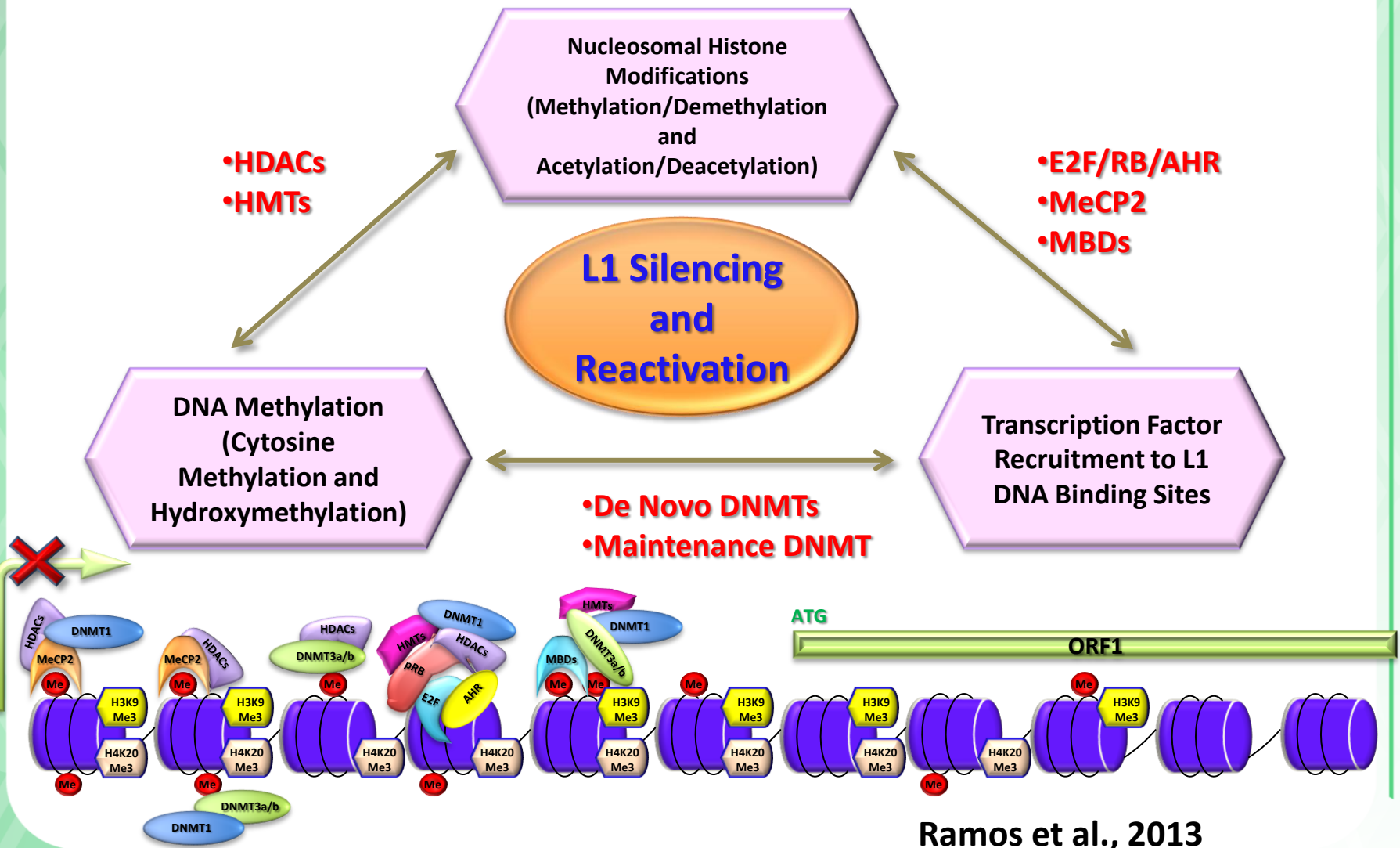
Mutation Research, 2009

L1 Retroelement Silencing: RB Knockdown



- ✓ Rb loss leads to overexpression of endogenous L1 in primary cells.
- ✓ Pharmacological inhibition of HDACs increases L1 expression in human and wild type MEFs, but not Rb-null cells.

Integration of the Evidence



Impacts of L1 in the Human Genome

Evolution

Insertion, Duplication, Inversion Mutations

Inhibition of Gene Transcription

Developmental Programming

Alternative Splicing Sites

Exon Skipping

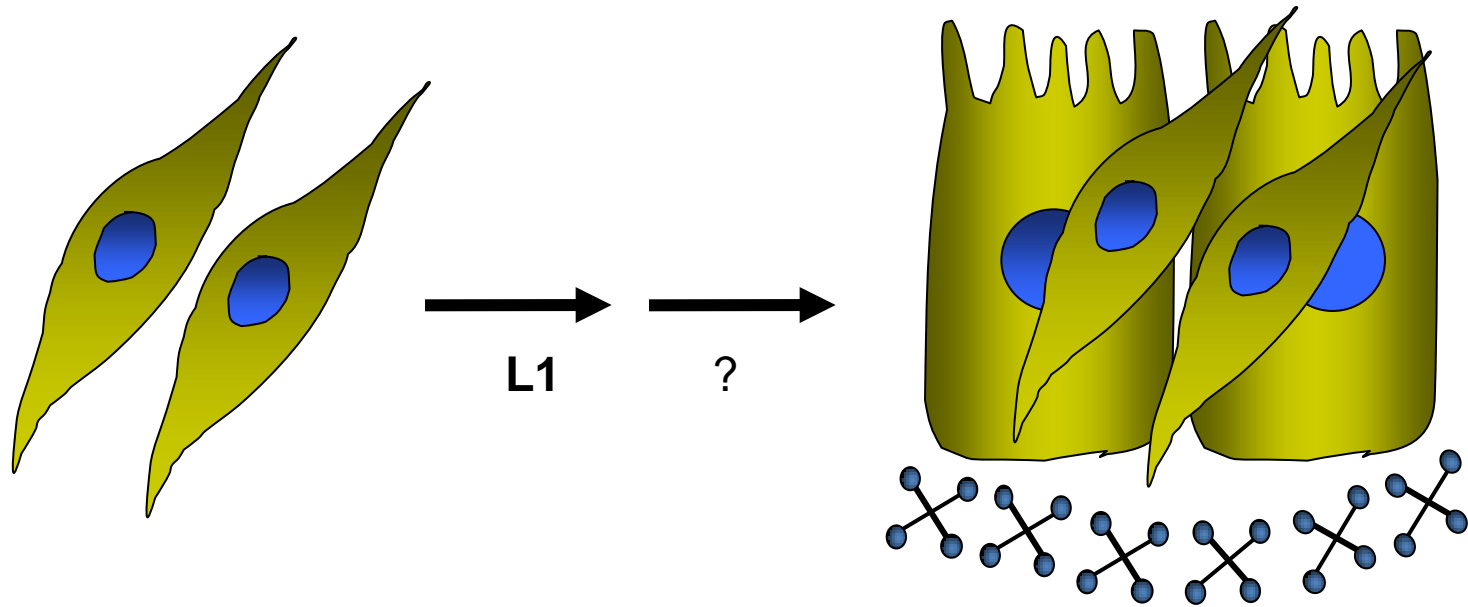
Alternative Promoters

DNA Strand Breaks

DNA Repair

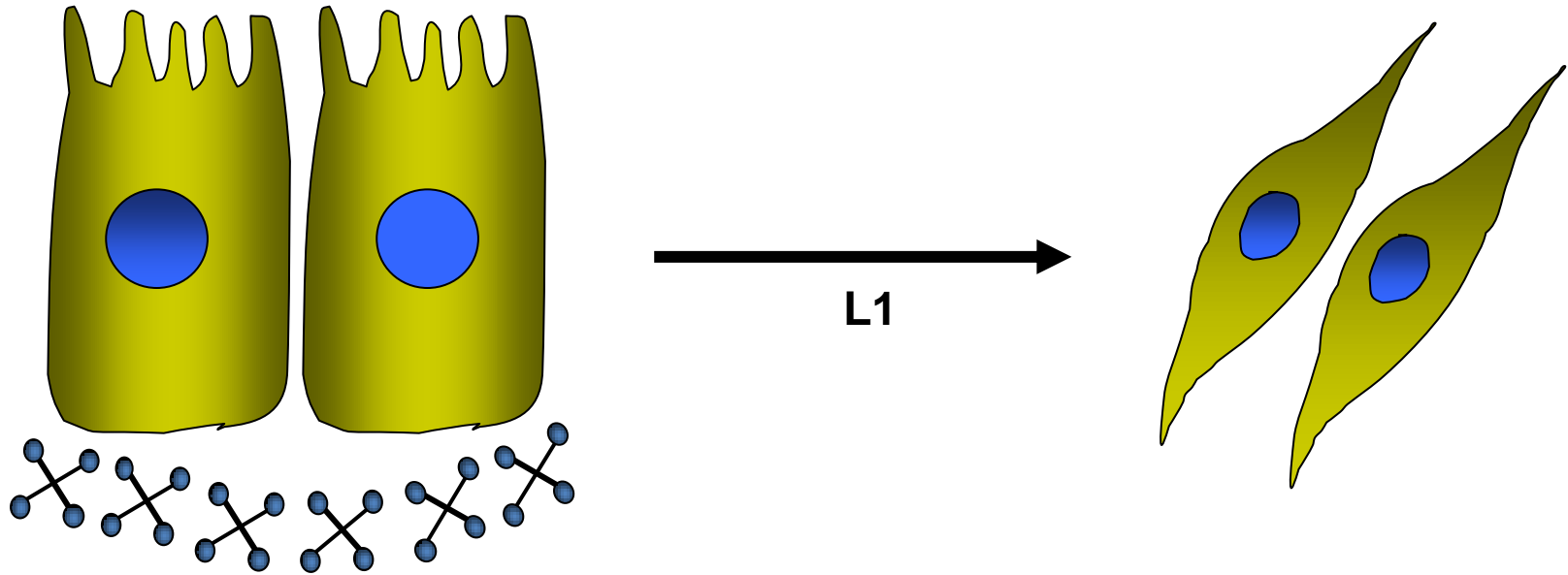
Genetic Reprogramming

Transition from Normal to Disease Phenotypes : A Precision Medicine Perspective



- ✓ Deficits in cellular differentiation and proliferation
- ✓ Recapitulation of developmental programming

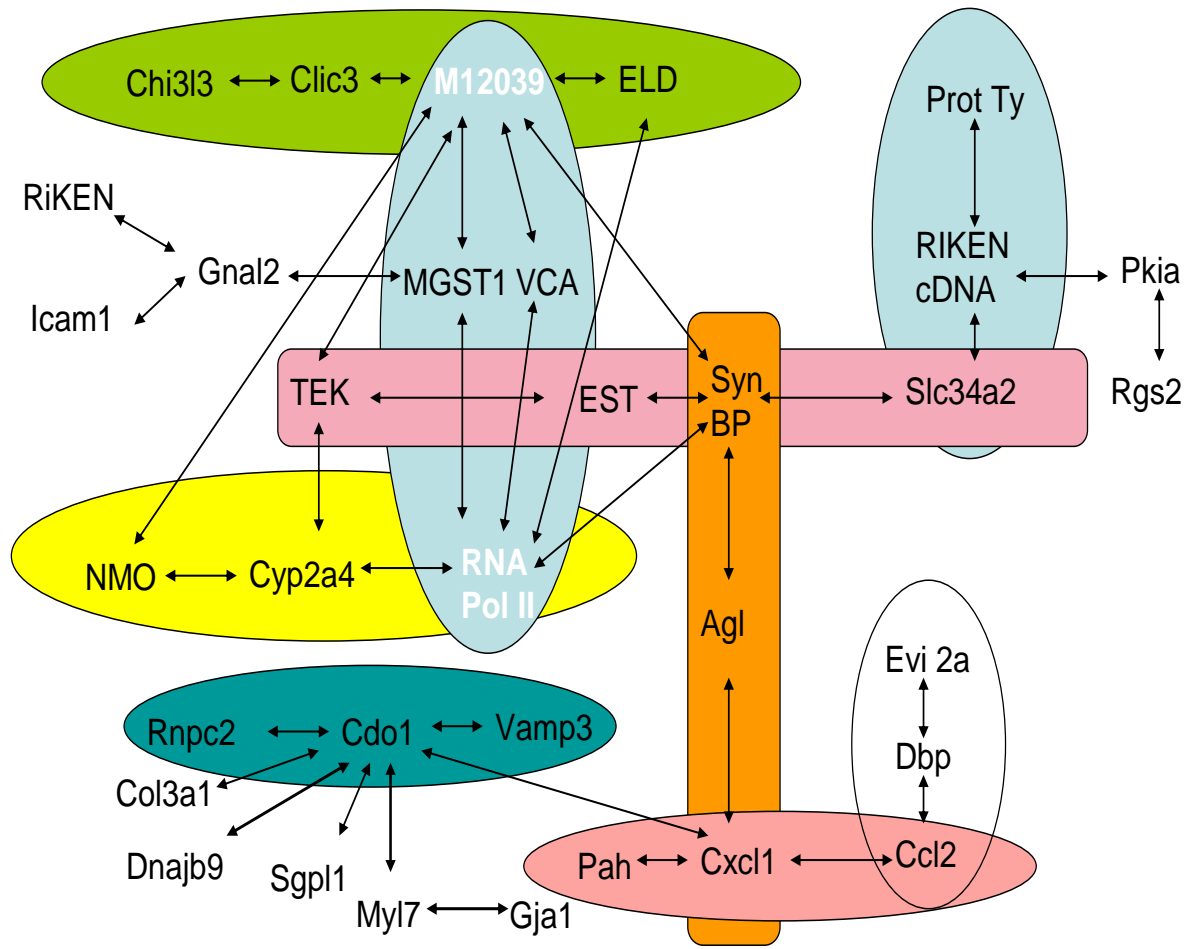
Overexpression of L1 in Epithelial Cells Mimics the Actions of BaP



- ✓ Associated with EMT in HepG2 Cells and Bronchial Epithelial Cells
- ✓ Deficits are associated with disruption of L1 genetic regulatory network.

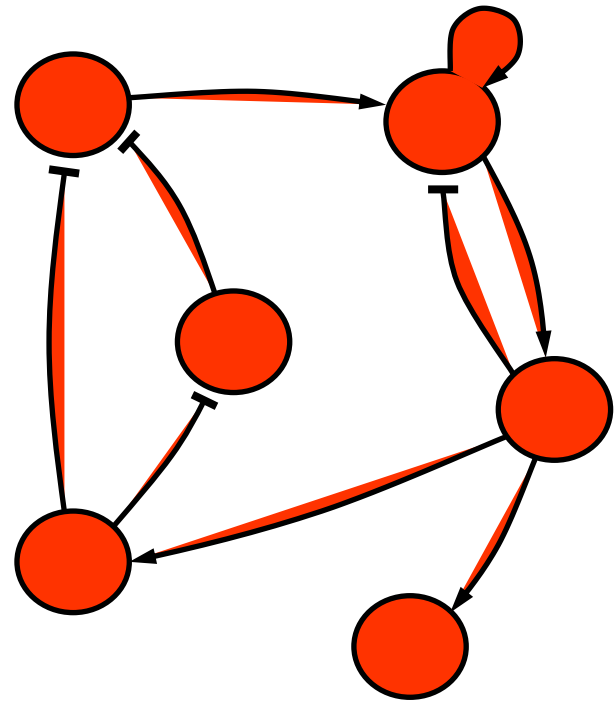
Molecular Oncology, 2014

L1 Regulatory Networks

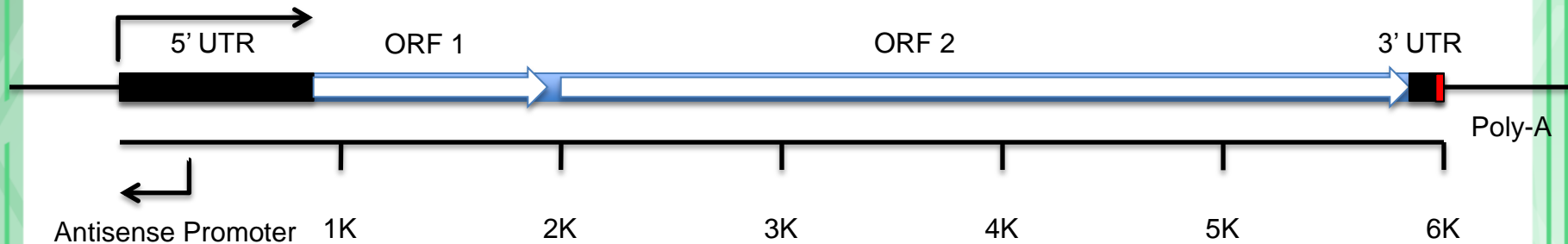


Biological Inference

- ✓ Genetic knockdown of AHR or other targets by si-RNA.
- ✓ Gene expression within the predicted regulatory network measured by RT-PCR
- ✓ PAS proteins identified as members of the L1regulatory network



L1 and Precision Medicine



- ✓ **PM is a medical model that emphasizes use of genetic and molecular data to optimize prevention and treatment.**
- ✓ **Current standards of care are based on epidemiological data and not genetic variability or molecular taxonomy of disease.**
- ✓ **Molecular assays can be used to stratify disease, select proper medication, or tailoring dosages to prevent adverse outcomes and toxicity.**
- ✓ **L1 is highly polymorphic, with varying levels of L1 activity in response to toxic injury correlated to disease onset or severity in human populations.**

Closing Remarks

The Future of Toxicology
Acknowledgements