National and Kapodistrian
UNIVERSITY OF ATHENS

Laboratory of Histology-Embryology
Molecular Carcinogenesis Group

nature COMMUNICATIONS

A high-resolution 3D epigenomic map reveals insights into the creation of the prostate cancer transcriptome

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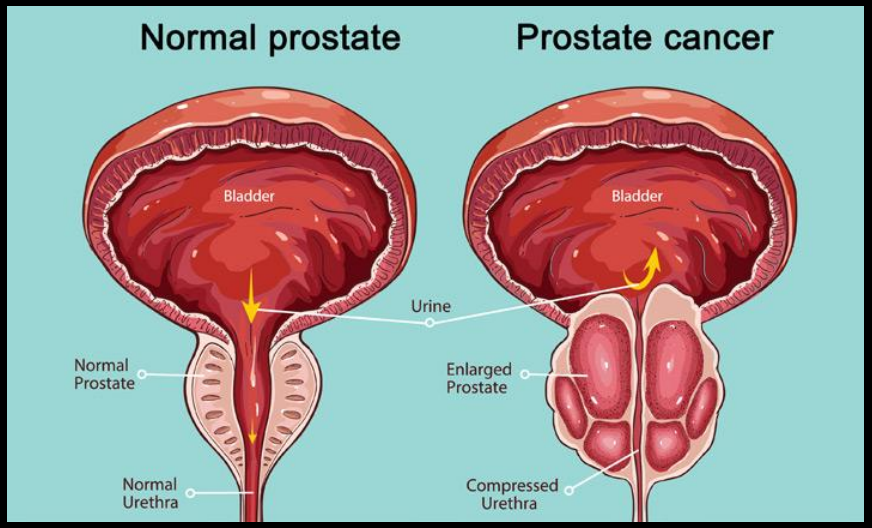
MSc in Molecular Biomedicine

nature

INTRODUCTION

▪Prostate Cancer



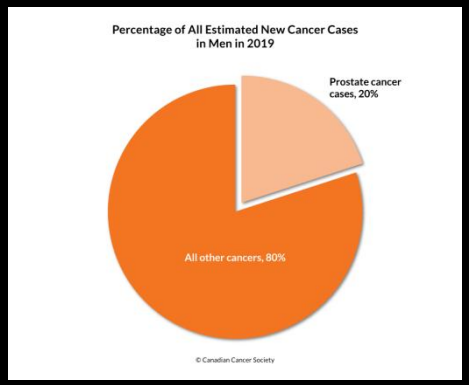
- form of cancer that develops in the prostate gland
- many risks associated with the disease:
  - ✓genetic and environmental factors including
  - ✓genetic susceptibility
  - ✓age
  - ✓race

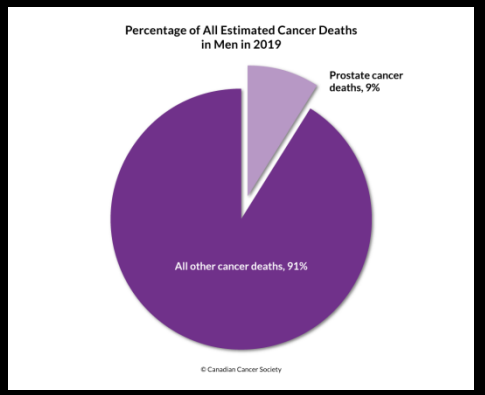
relatively low mutation rate compared with other cancers and few chromosomal loss or gains

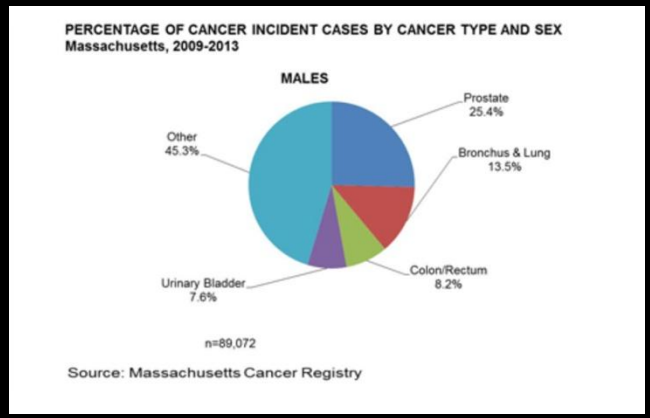
Two main molecular groups:
- ERG rearrangements → TMPRSS2 gene
- E3 ubiquitin ligase adapter SPOP and/or deletion of CDH1

▪Prostate Cancer Statistics

- second most common cancer in men
- second-leading cause of cancer deaths for men

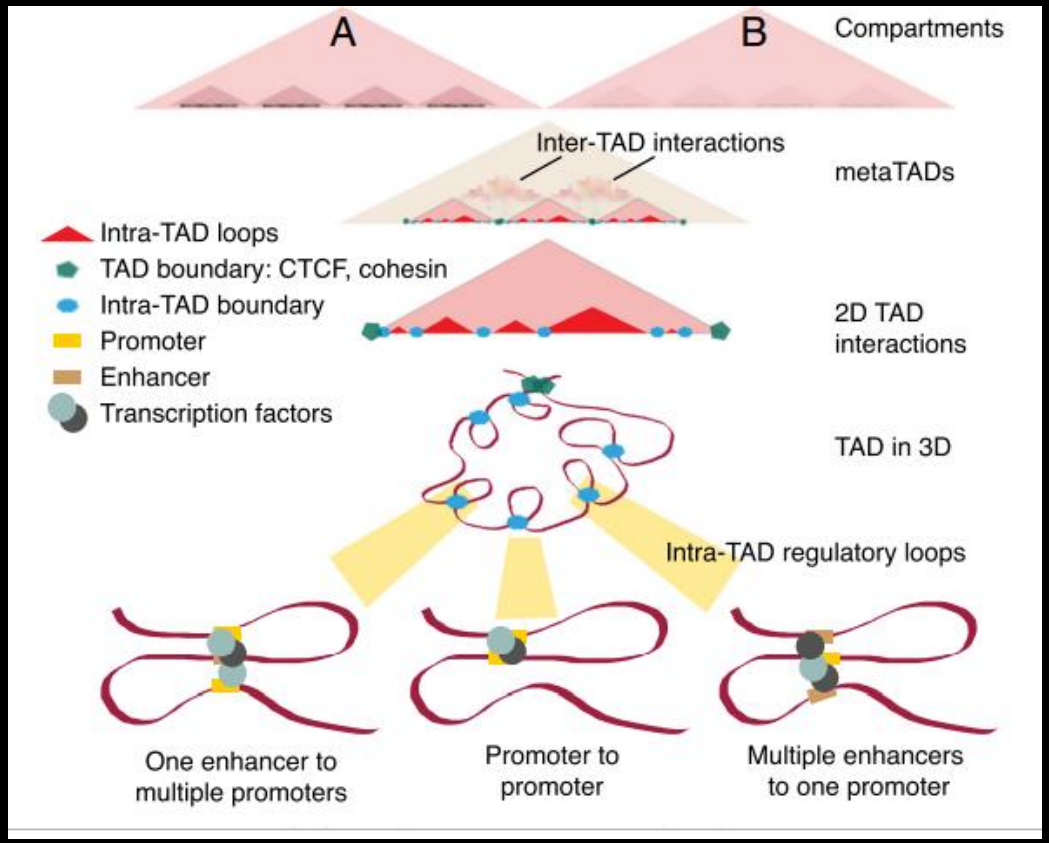






INTRODUCTION

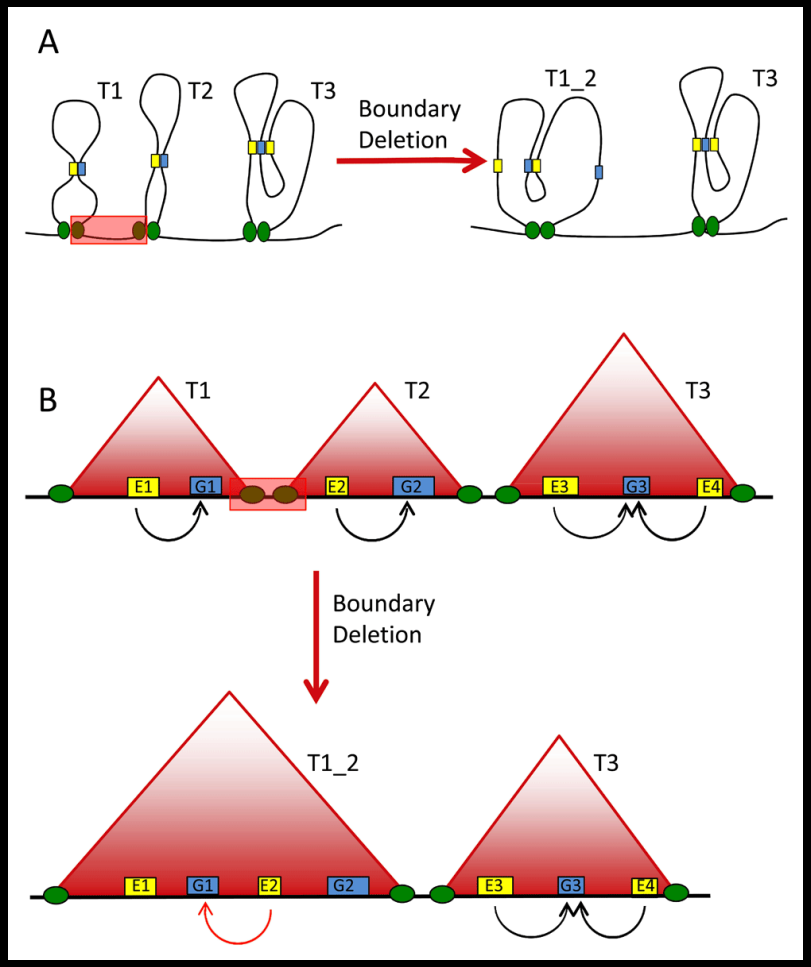
- **three-dimensional (3D) organisation of human genome**

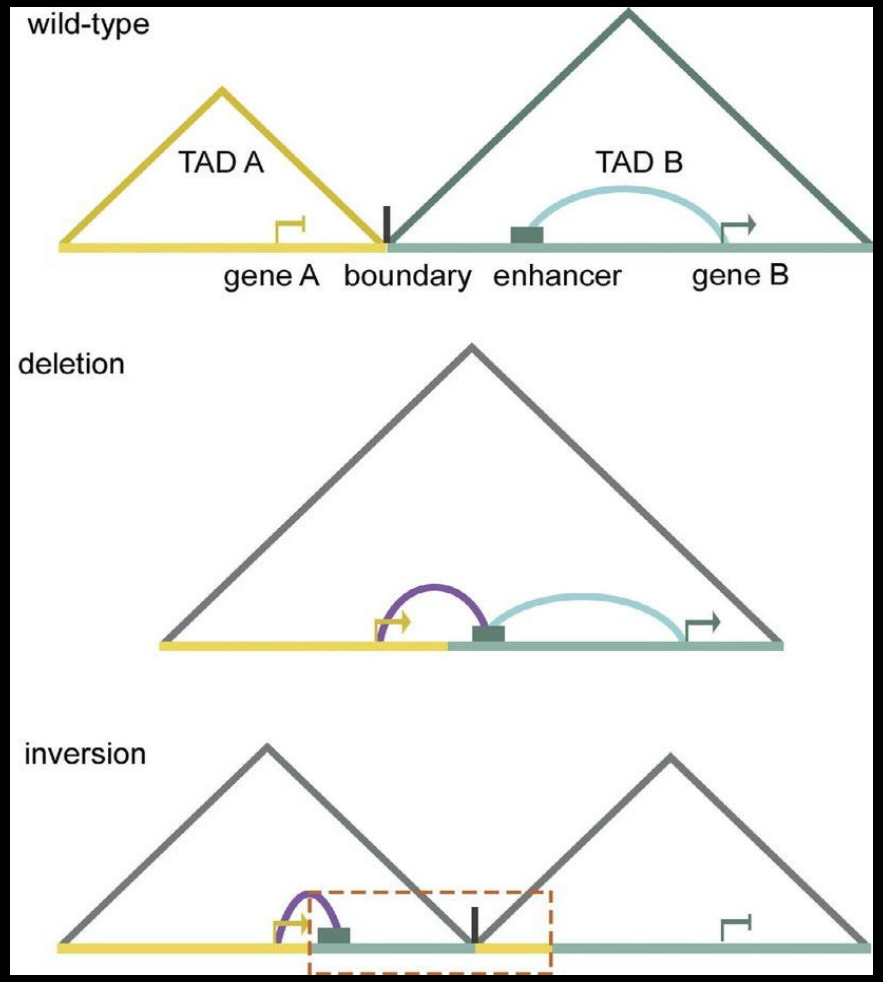


• active and inactive compartments
• topologically associating domains (**TADs**) → regions of high within contact frequency
• protein–protein interaraqctions: cohesin complexes and CTCF → long-range chromatin looping
• The epigenetic state of a TAD can influence gene expression levels of genes within a given TAD

INTRODUCTION

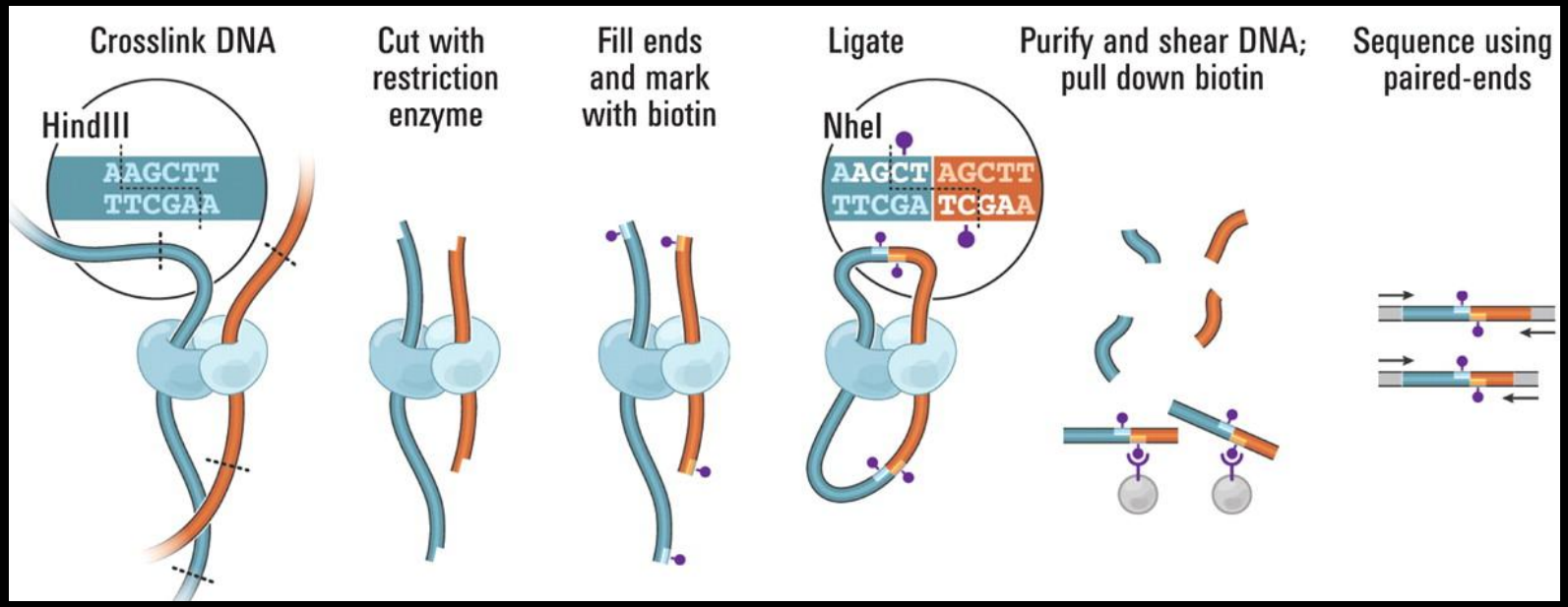
- **The epigenetic state of a TAD can influence gene expression levels of genes within a given TAD**





INTRODUCTION

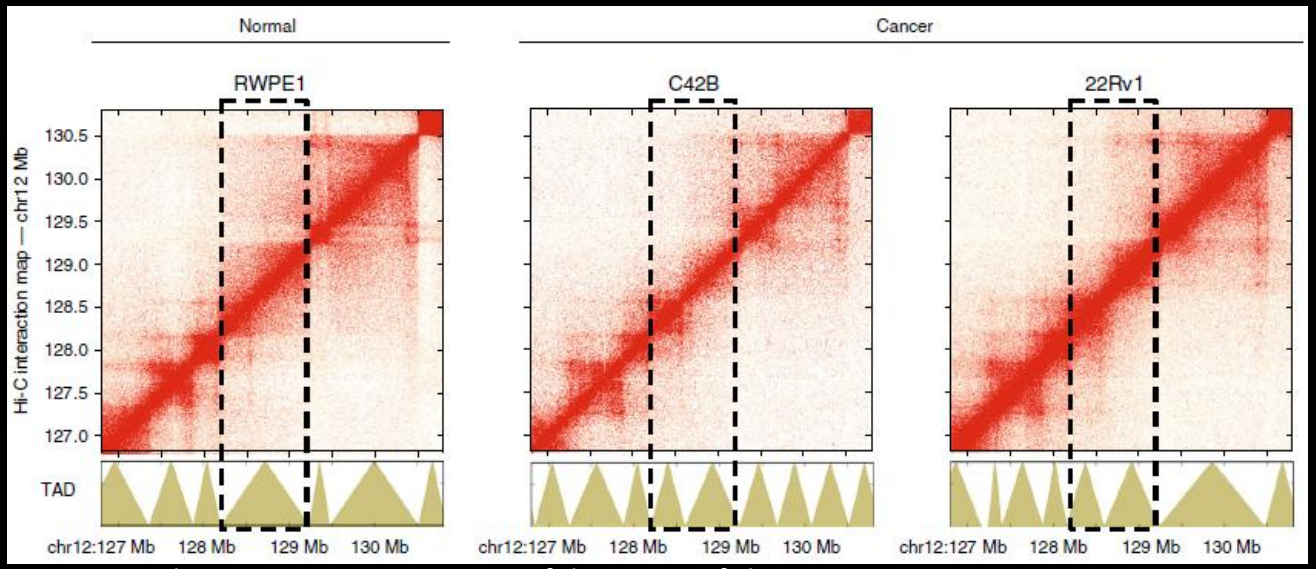
- **Hi-C, a genome-wide chromatin conformation capture assay**



• mapping the very large structural TADs
• high-resolution intra-TAD chromatin interactions between regulatory elements
• the epigenetic state → ChIP-seq of specifically modified histones, which mark regulatory elements
• TFs involved in chromatin interactions → motif analyses i.e. DNase-seq, ATAC-seq, or NOMe-seq (Nucleosome Occupancy and Methylome sequencing) → NDRs → Chip-seq

1. Cancer-specific TADs that lead to transcriptome changes

- **high-resolution chromatin contact interaction profiles**

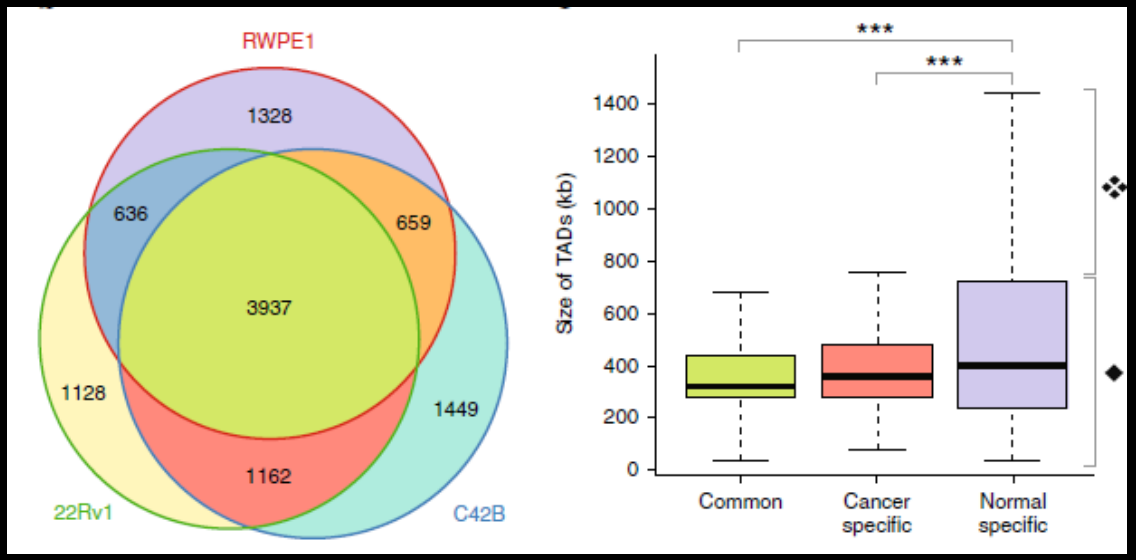


In situ Hi-C chromatin interaction maps of the region of chromosome 12q24

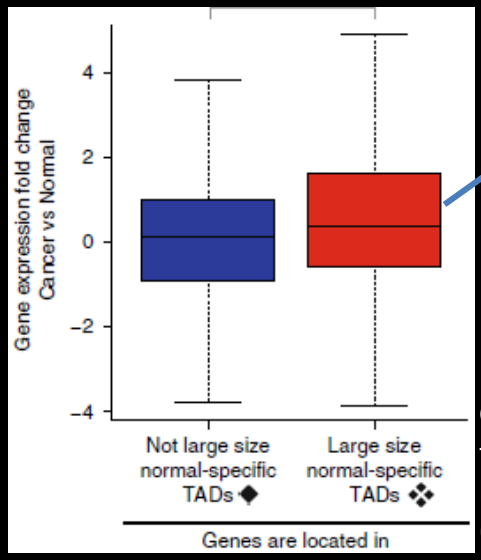
- **Number of TADs**

RWPE1 → 6565
C42B → 7205
22Rv1 → 6861

80% reproducable between replicates



Venn diagram showing the overlap of TADs and TAD sizes

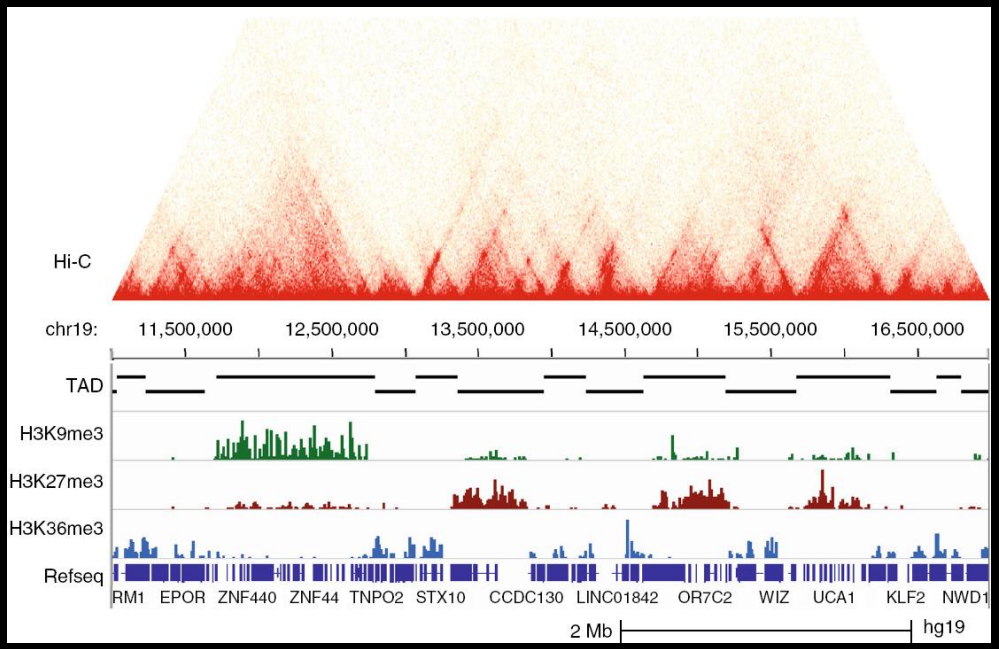


520 large size TADs → 850 smaller in cancer

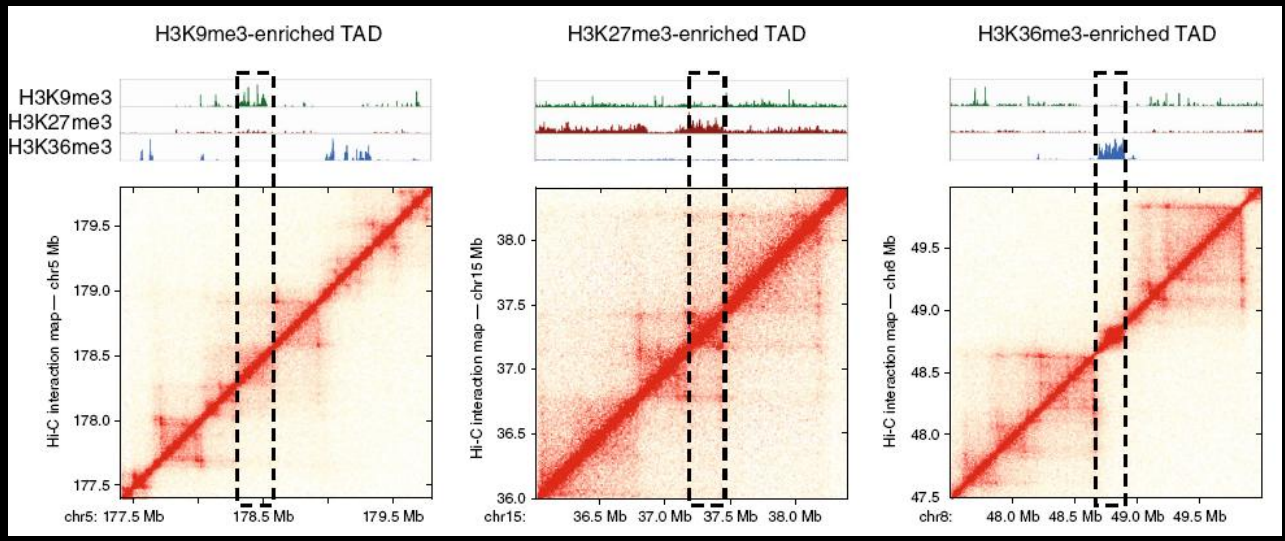
Gene expression fold change between cancer (C42B) vs normal (RWPE1) cells

2. Common TADs that can change chromatin states

- **Overall nature of the chromatin state of all TADs**



In situ Hi-C chromatin interaction map with histone-mark ChIP-seq tracks of a region of chromosome 19p13 in C42B



Hi-C maps for a H3K9me3-, H3K27me- and H3K36me3-enriched TAD

2. Common TADs that can change chromatin states

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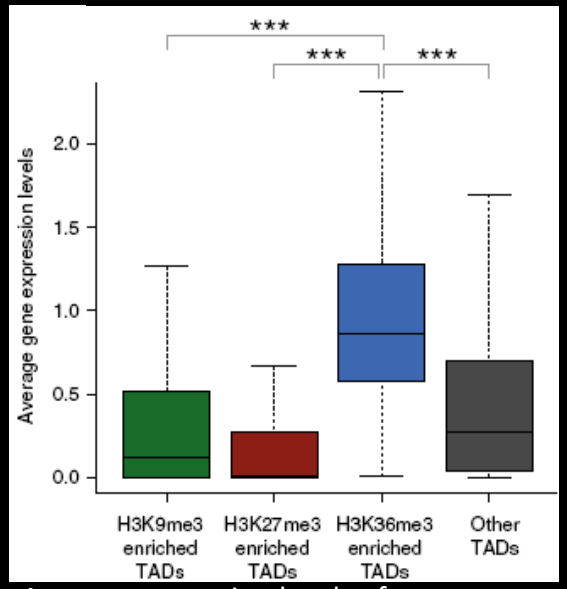
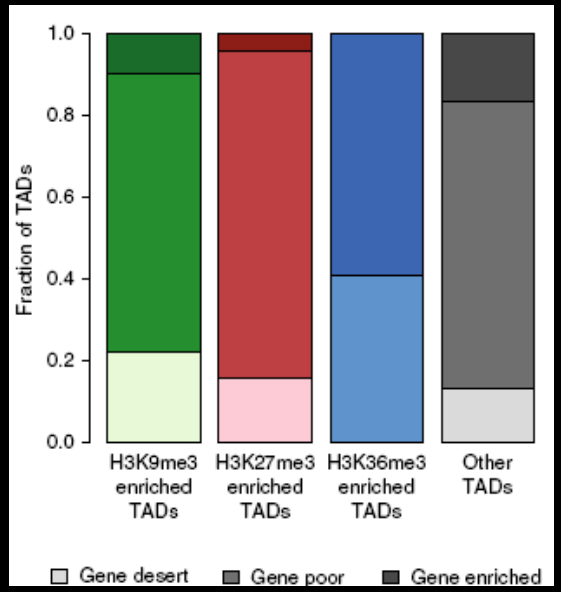
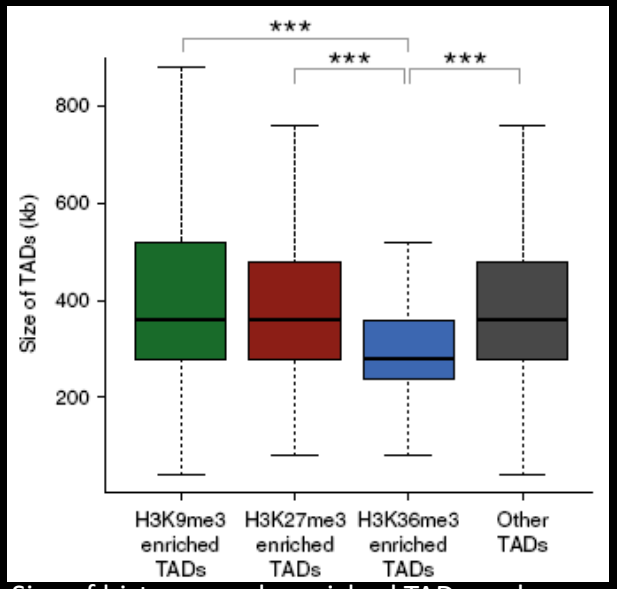


Average expression levels of genes



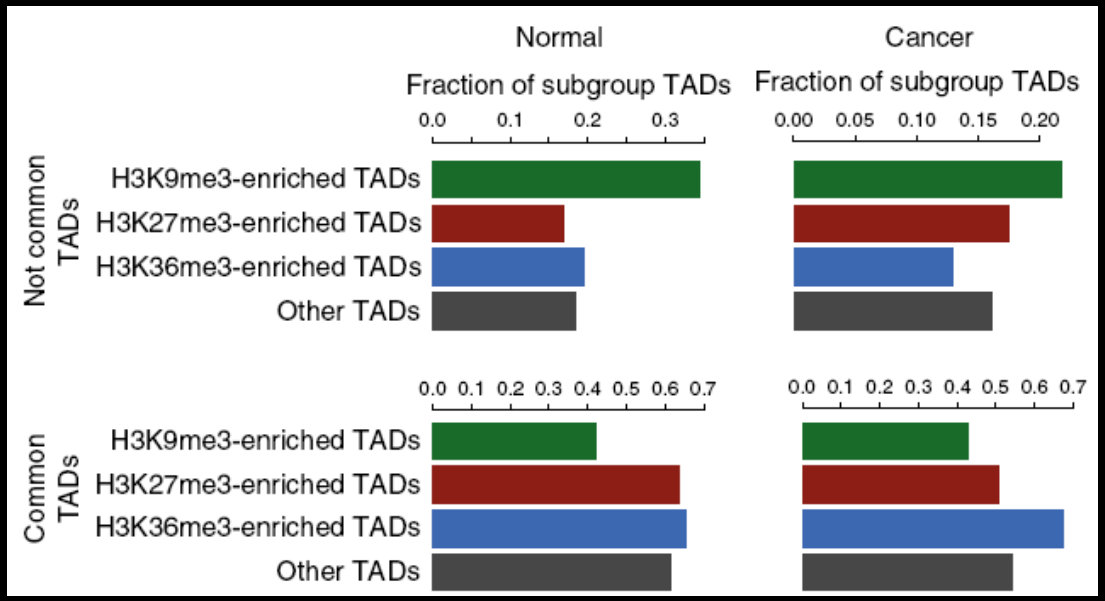
Fraction of gene desert (light), gene poor (mid), and gene enriched (dark) TADs



Size of histone mark-enriched TADs and other TADs

2. Common TADs that can change chromatin states

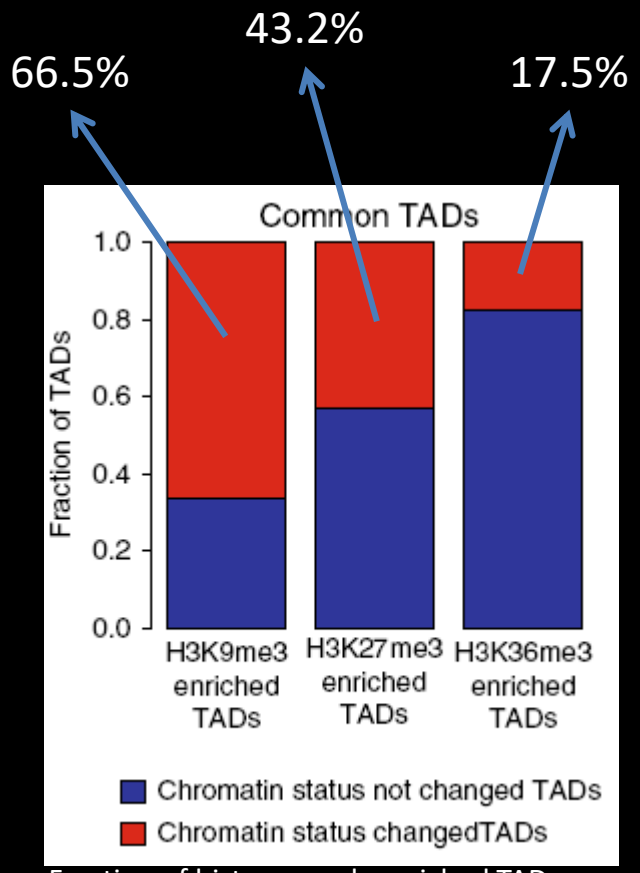
- **The epigenetic influence on genes within the TADs - Epigenetic states and expression levels**



Fraction of histone mark-enriched TAD subgroups

Hypothesis

Large, heterochromatic TADs that form in normal prostate cells → split into smaller, active TADs during neoplastic transformation?



Fraction of histone mark-enriched TAD subgroups

2. Common TADs that can change chromatin states

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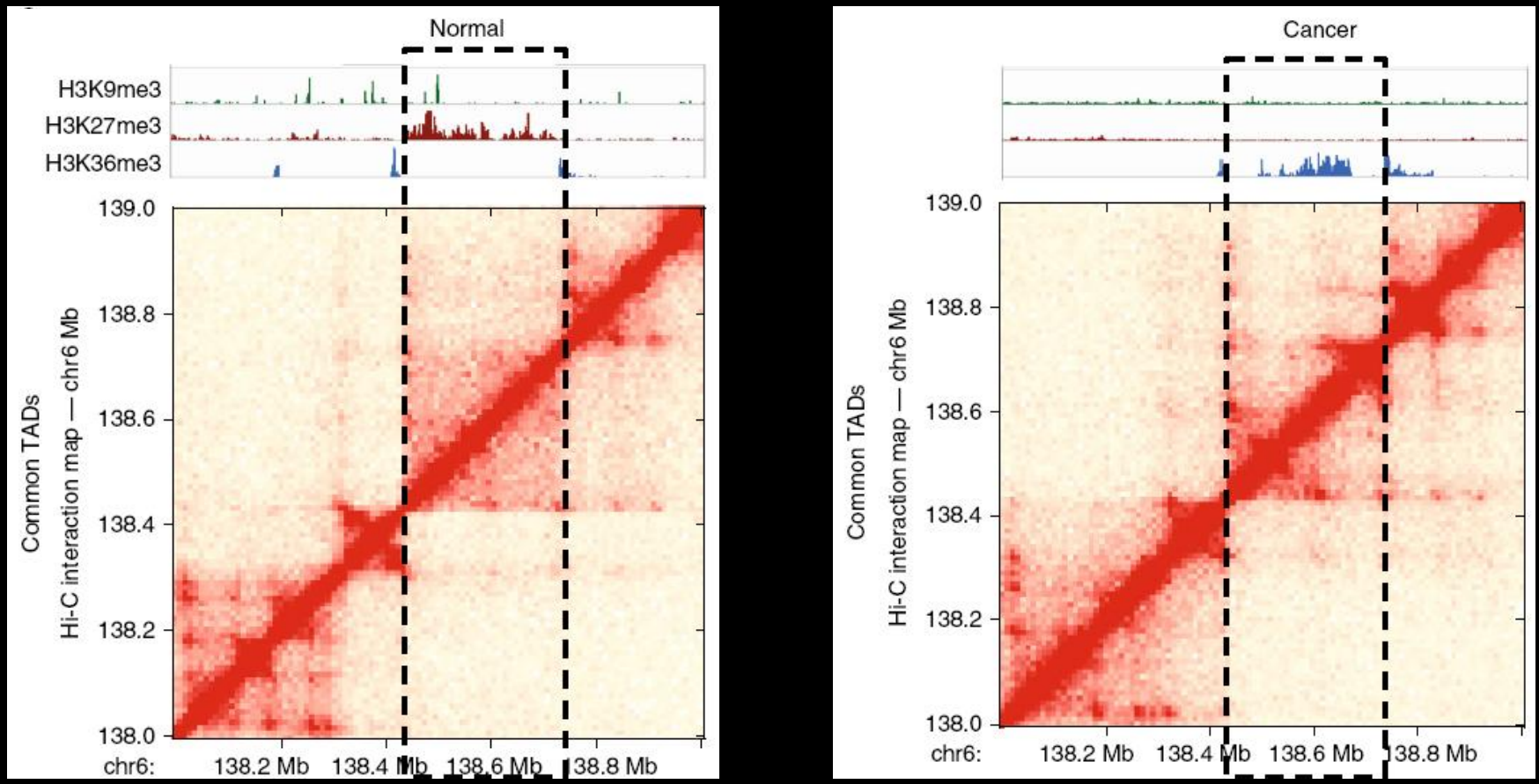


Example of a common TAD located at chromosome 6q23 that changed epigenetic status between normal and prostate cancer cells

~2000 genes in the sets of changed epigenetic state TADs:

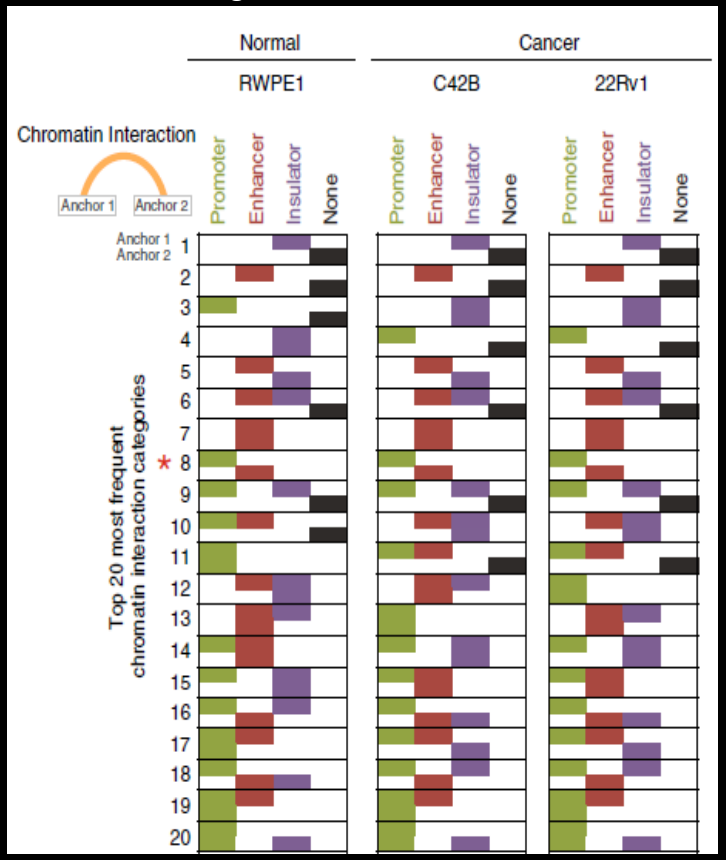
✓>40% of genes changed expression level significantly

✓~50% increase in the number of differentially expressed gene

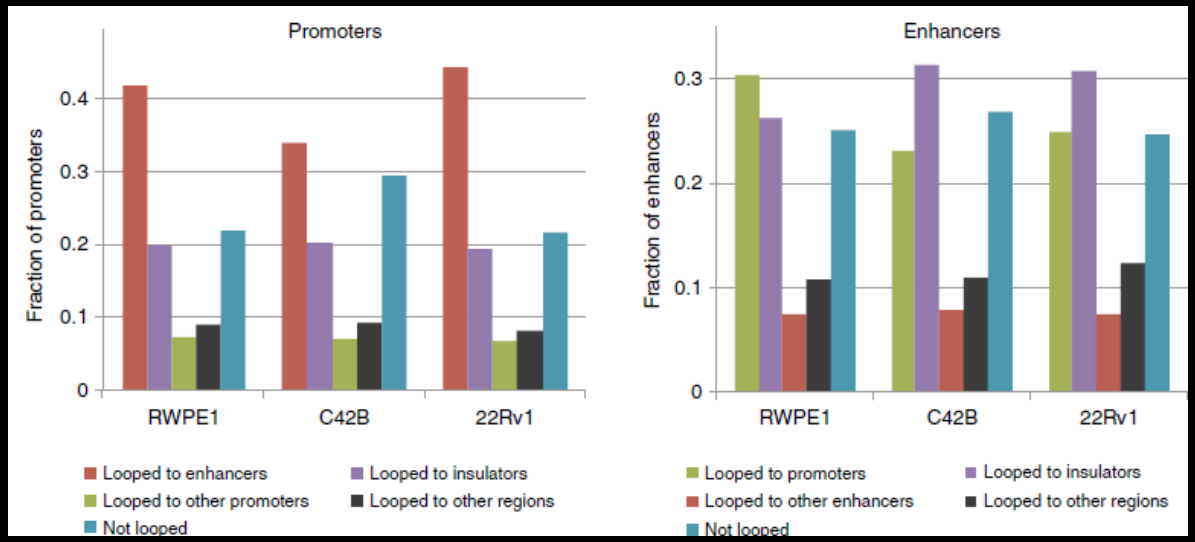
3. Enhancer–promoter loops in prostate cancer cells

- high-resolution chromatin contact loops

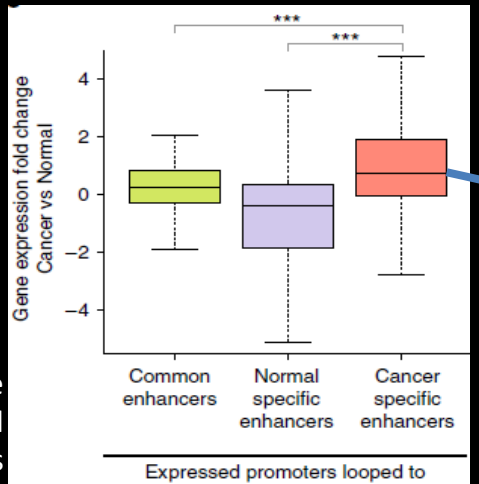
regulatory loops → chromatin anchors overlapped with
- ✓active promoters
- ✓active enhancers
- ✓ CTCF-binding sites



Top 20 most frequent chromatin interaction categories



Fraction of active promoters or enhancers that loop to enhancers, insulators, promoters, other genomic regions or not looped.

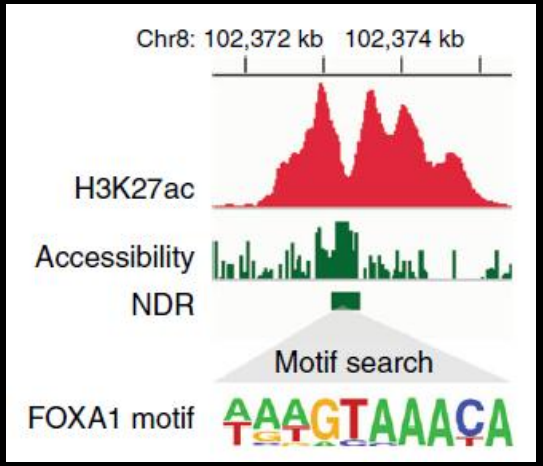


Gene expression fold change between cancer and normal (RWPE1) cells

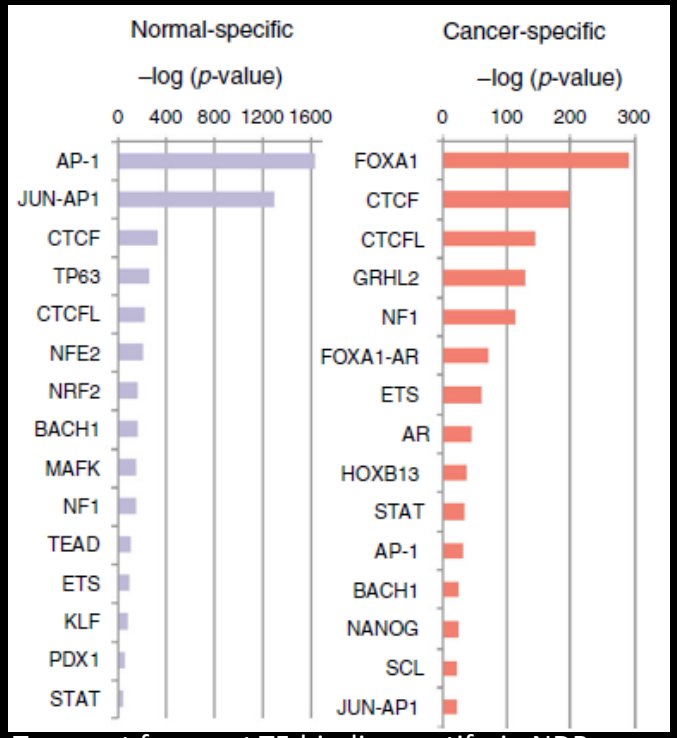
Cancer specific loops contribute to an increase in gene expression

4. TFs at cell-type-specific enhancers that loop to promoters

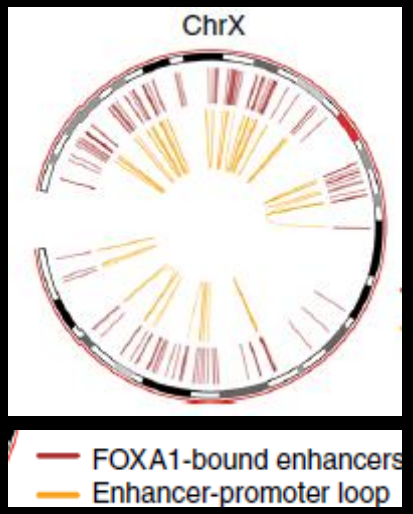
- TF-binding motifs within the active enhancers that loop to promoters



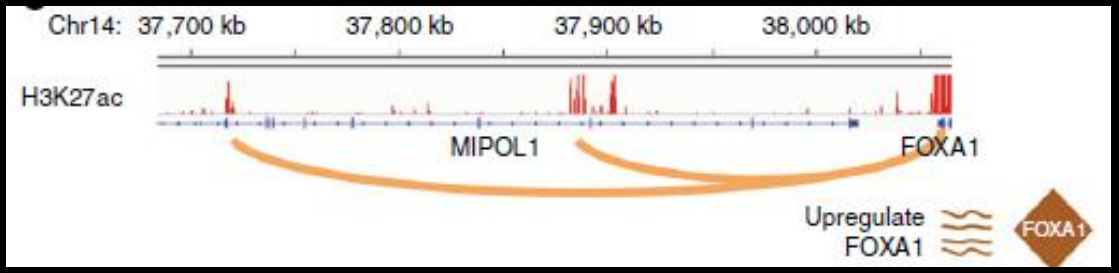
Example of a cancer-specific enhancer



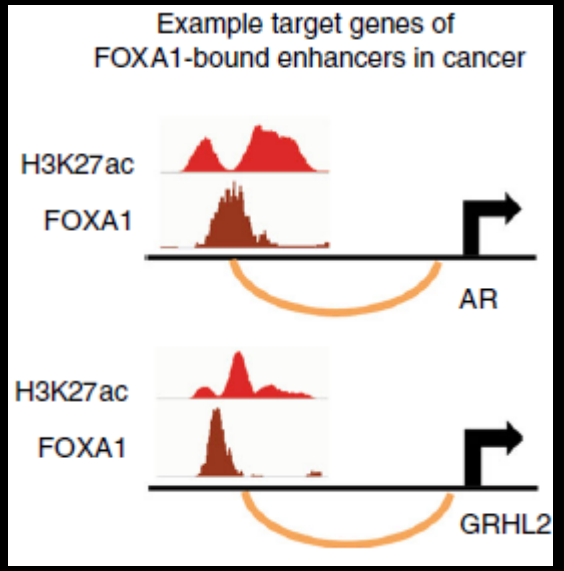
Top most frequent TF-binding motifs in NDRs



Circos plots

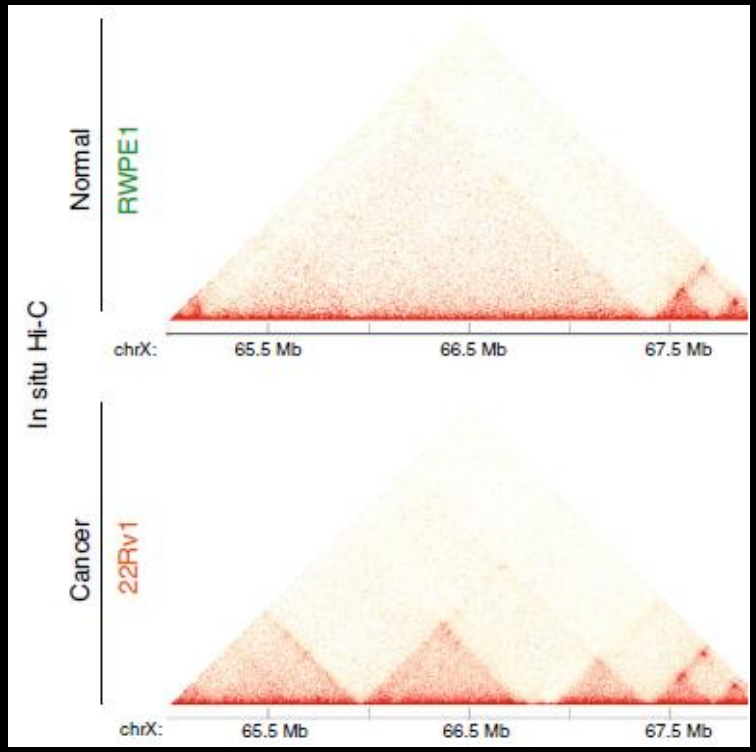


Genome browser snapshot for the FOXA1 promoter in prostate cancer cells (C42B)

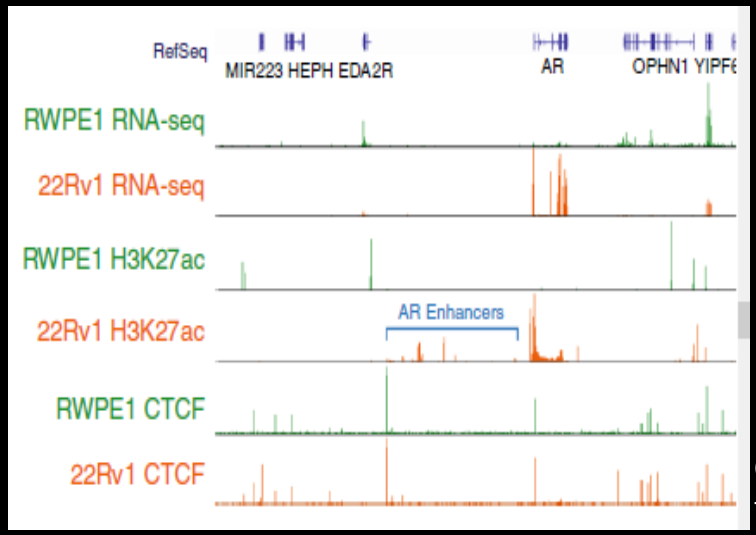


5. The chromatin structure surrounding the AR locus

- Most frequent prostate cancer specific enhancer–promoter loops → gene that is statistically significantly higher expressed in prostate cancer cells



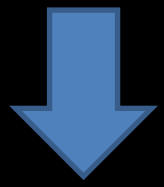
In situ Hi-C chromatin interaction maps for the region surrounding the AR locus



RNA-seq, H3K27ac, and CTCF ChIP-seq tracks for the AR locus.

Discussion

- Three-approach strategy to investigate the interplay between gene regulation and 3D chromatin structure:
  1. TADs that have altered boundaries in normal vs prostate cancer cells plus set of genes whose expression is increased in the cancer-specific TADs
  2. >20% of the common TADs → different epigenetic status and genes located in these TADs showed changes in expression correlating with an epigenetic status change from an inactive to an active TAD
  3. Through chromatin interaction loops → genes regulated by prostate cancer-specific enhancer-promoter loops



✓boundary-changed and epigenetic state-changed TADs
✓enhancer–promoter loops found specifically in prostate cancer cells
✓list of genes regulated by these mechanisms

Thank you

for your attention