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Harnessing the power of genomics for detection, diagnosis, and mechanistic analysis of cellular responses

Raga Krishnakumar
Systems Biology
September 21, 2016

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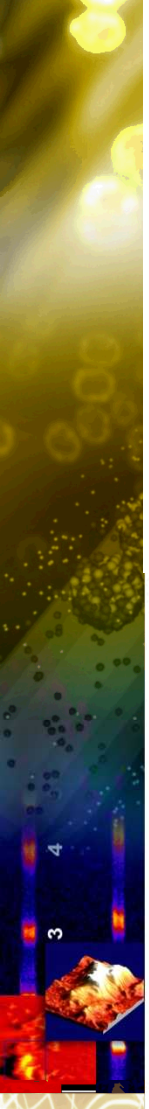

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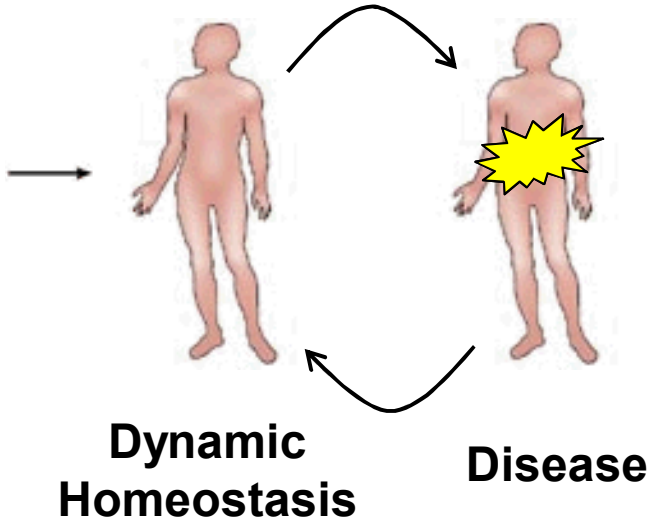
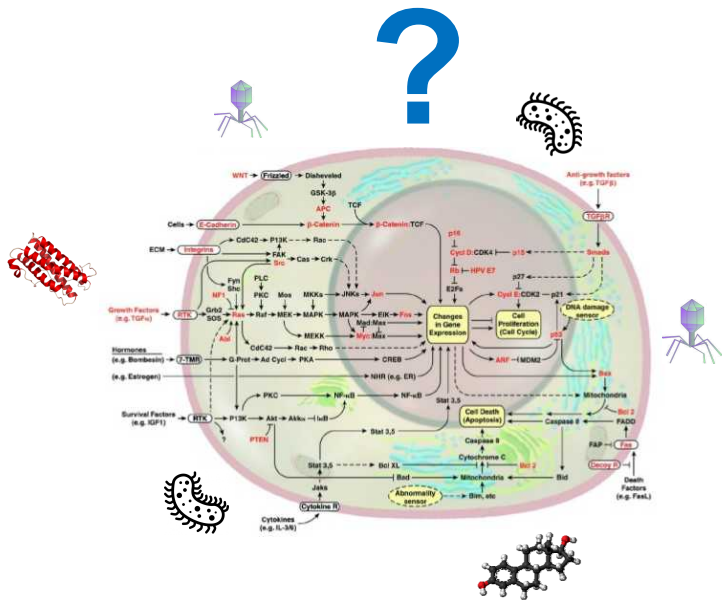
Cells constantly respond to their environment, and make decisions that govern development and disease



Modified from O'Connor and Crystal 2006 (Nature Reviews Genetics)

Cells constantly respond to their environment, and make decisions that govern development and disease

Capture environmental changes and cellular responses



Functional studies of molecular mechanisms

Systems-level integrated data analysis

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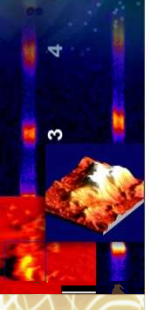
Cells constantly respond to their environment, and make decisions that govern development and disease



Capture environmental changes and cellular responses

GENOMICS IN THE ERA OF NEXT GENERATION SEQUENCING

Functional studies of molecular mechanisms

Systems-level integrated data analysis

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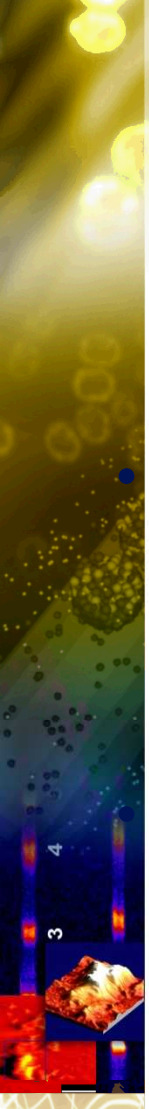
Overview

- Combinatorial genomics reveals novel molecular mechanisms during cell fate transitions in early development

Applying lessons learnt for engineering therapeutic cells for personalized immune therapy



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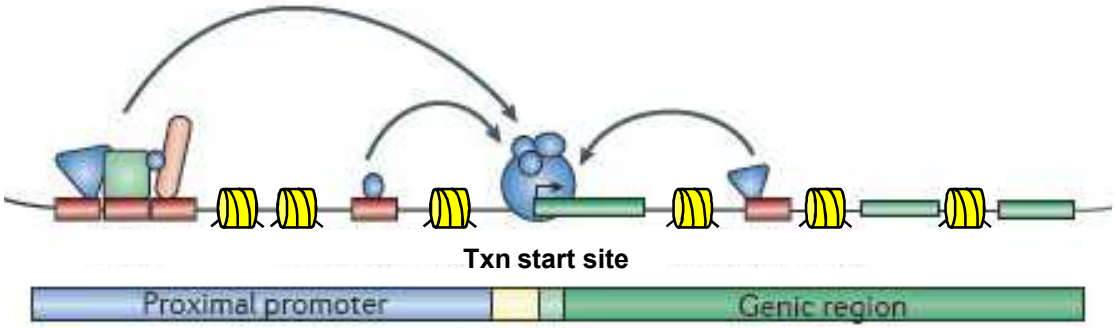
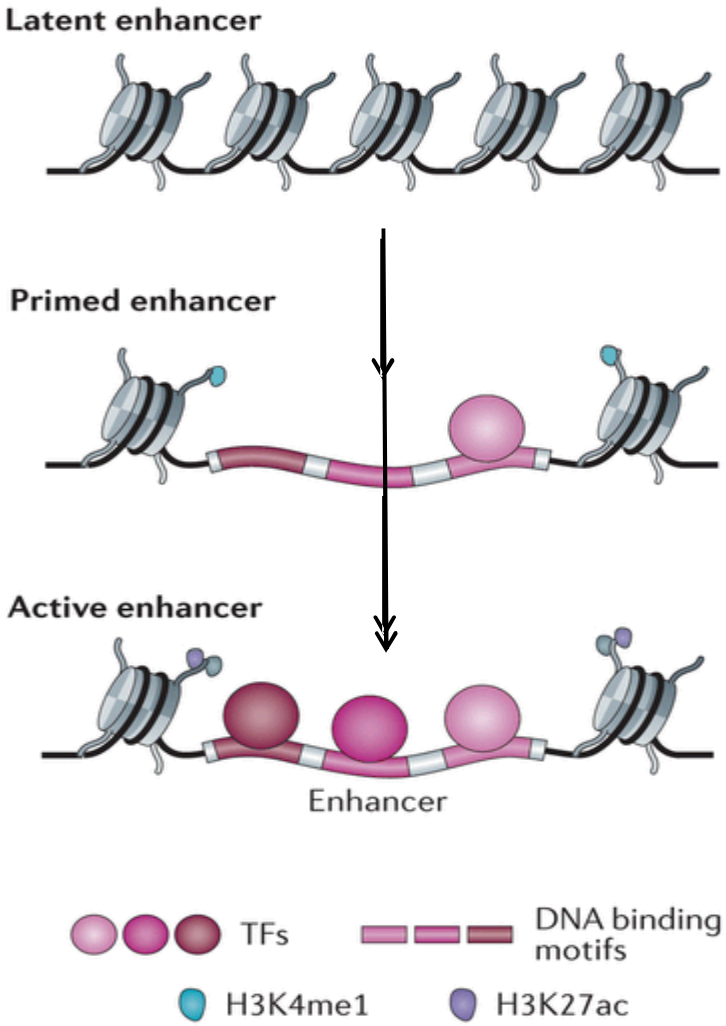
Transcriptional regulation in eukaryotes



Chromatin-bound enzymes (what) + Sequence specific TFs (where and when)

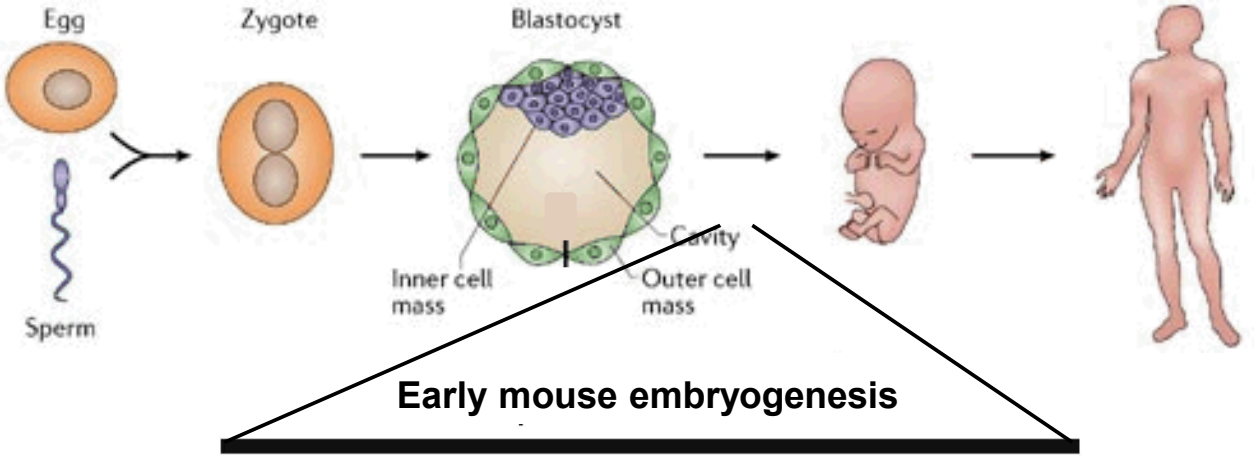
Lenhard et al 2012 (Nature Reviews Genetics)

Enhancers act as molecular switches for their cognate genes



Shlyueva et al 2014 (Nature Reviews Genetics)

Rapid cell fate choices occur in early development

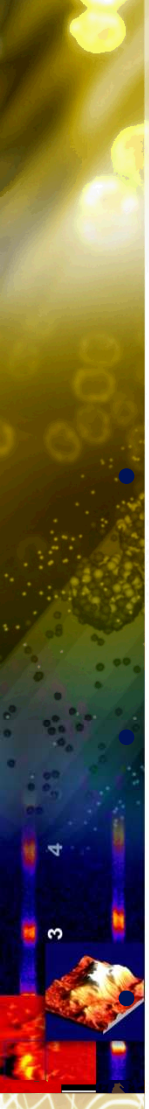


Embryonic stem cells (**ESCs - red**) ---> Epiblast cells (**EpiCs – yellow**)

Parchem et al 2014 (Cell Stem Cell)

Enhancer function in pluripotent cell fate choices

- Is gene expression regulated by enhancer priming in early mouse embryonic development?
- What factors regulate the switch between primed and active enhancers?
- What is the mechanism of this regulation?



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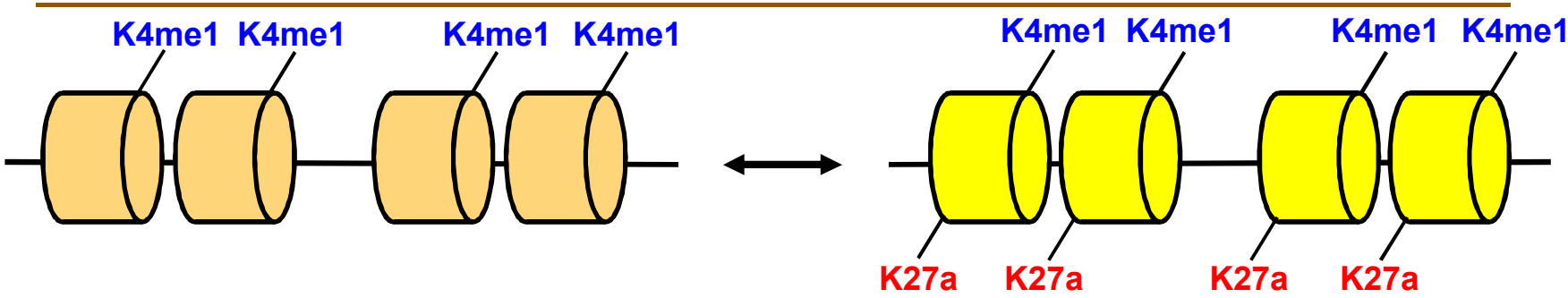
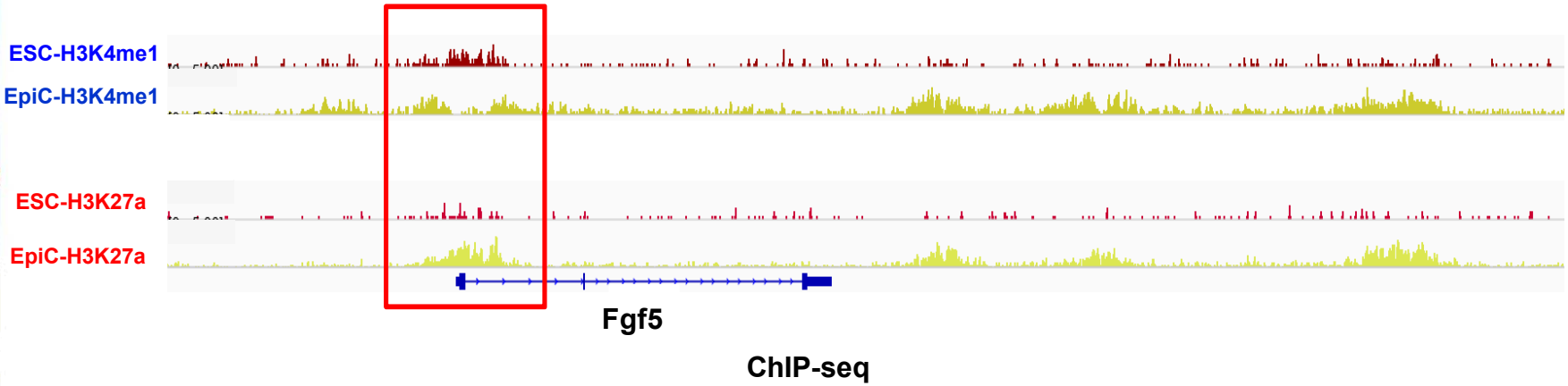
Profiling of enhancer chromatin in pluripotent cells



'primed' – H3K4me1+

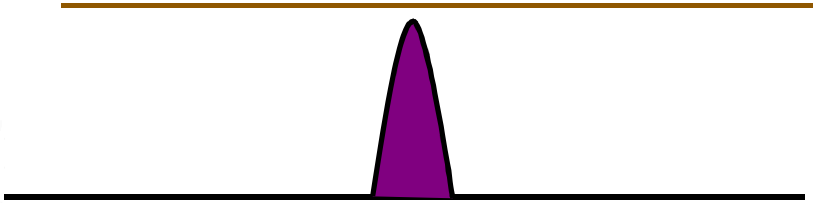
'active' – H3K4me1+ H3K27ac+

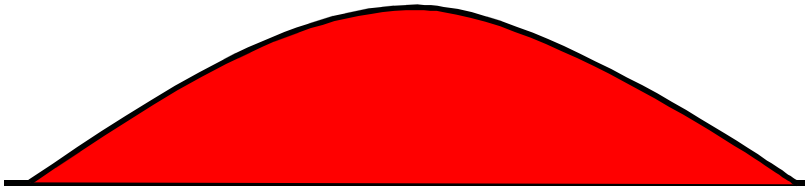


ChIP-seq

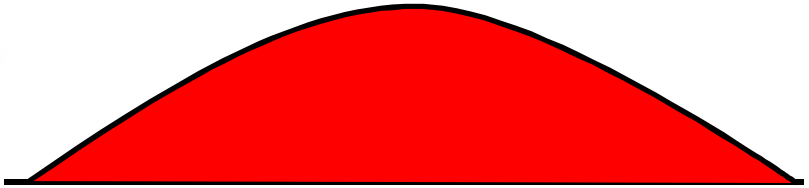
How can we consolidate the information and get something biologically meaningful out of it?

Peaks vs domains – understanding the chromatin landscape

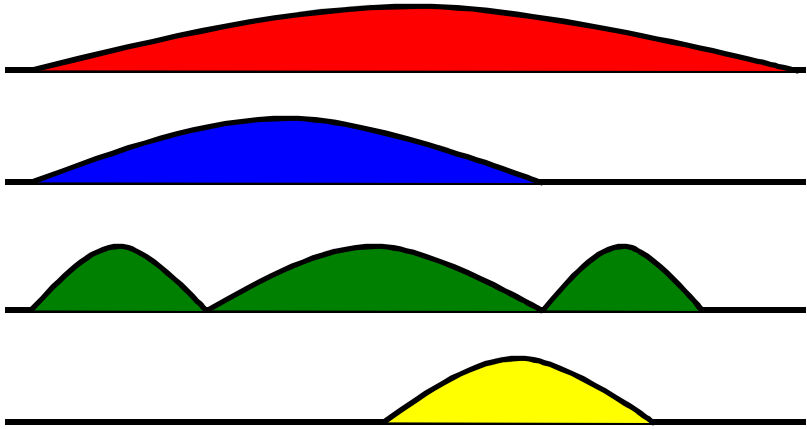


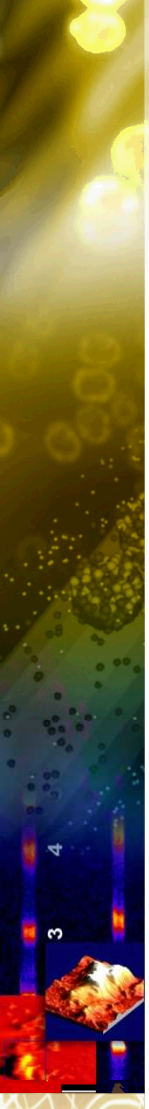


vs



vs





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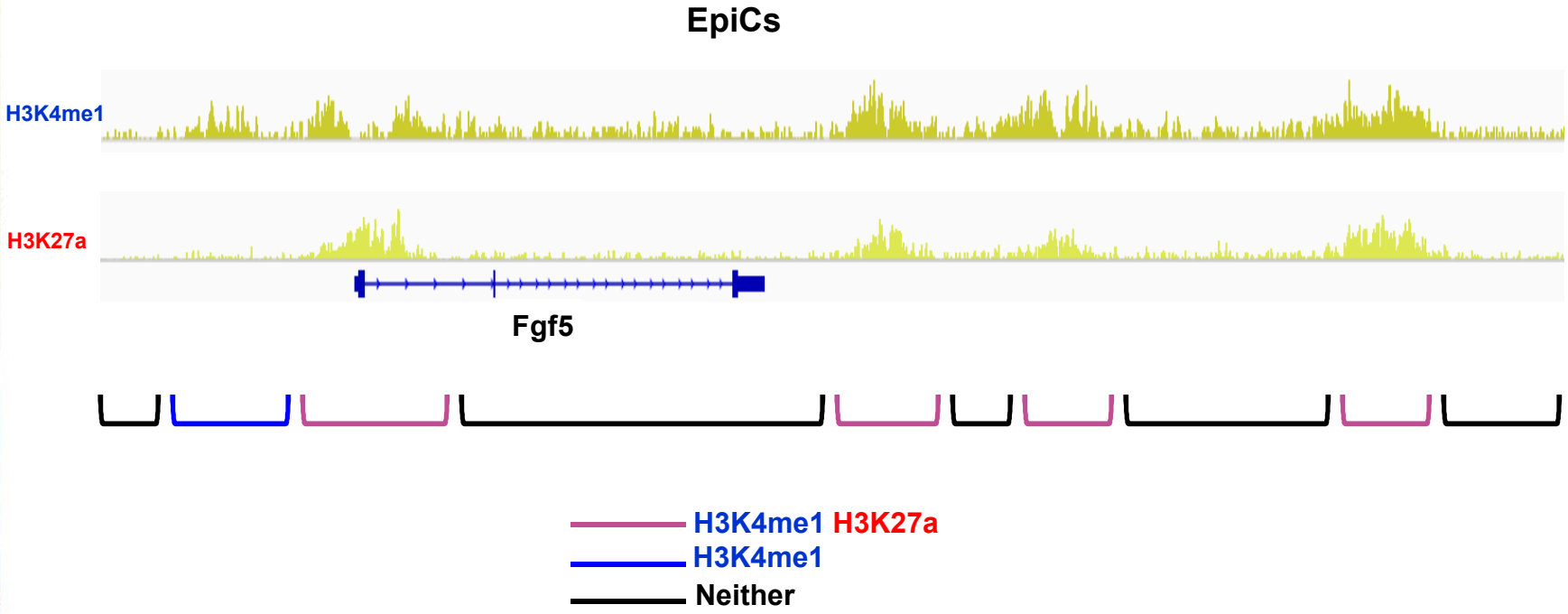
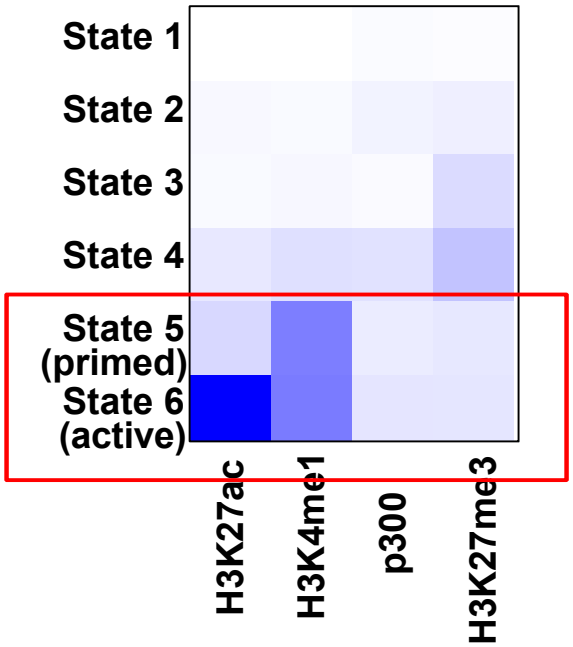
Profiling of enhancer chromatin in pluripotent cells

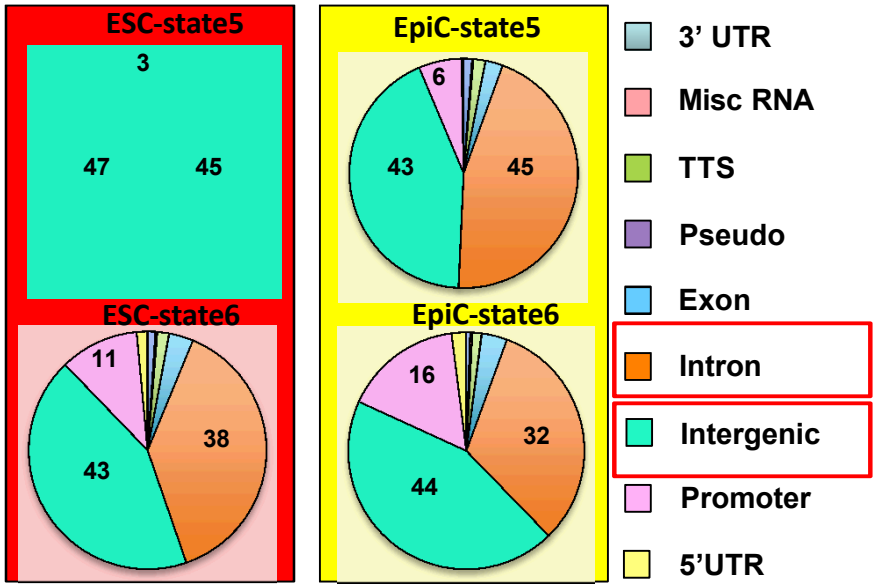


- Unknown combinations of marks
  - Unbiased discovery
- Use HMM to identify states (H3K4me1, H3K27ac, H3K27me3, p300)

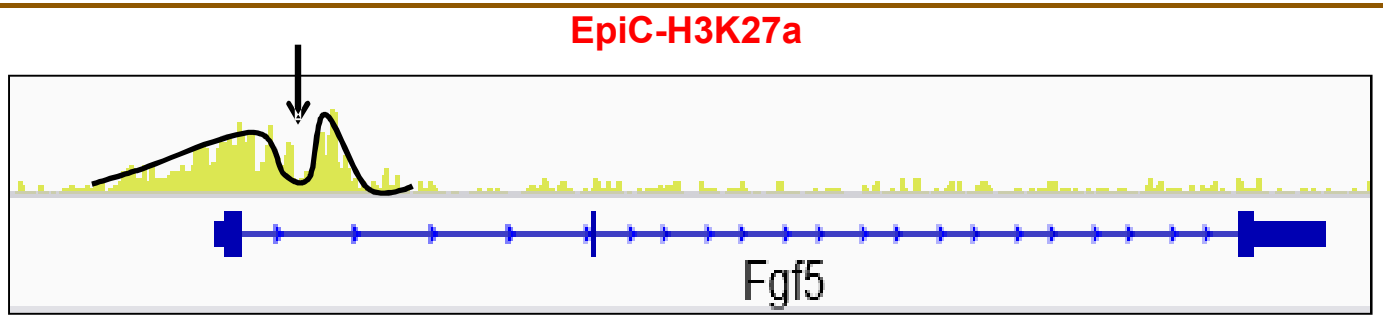
Ernst and Kellis 2012 (Nature Methods)

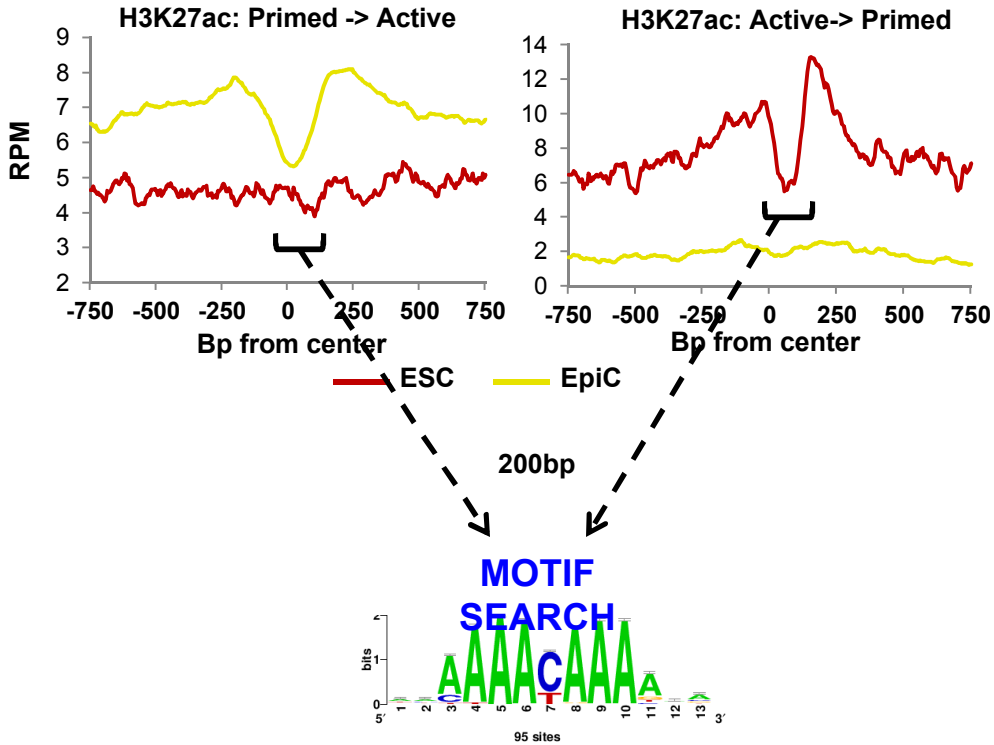
ChromHMM identified dynamic primed and active enhancer chromatin





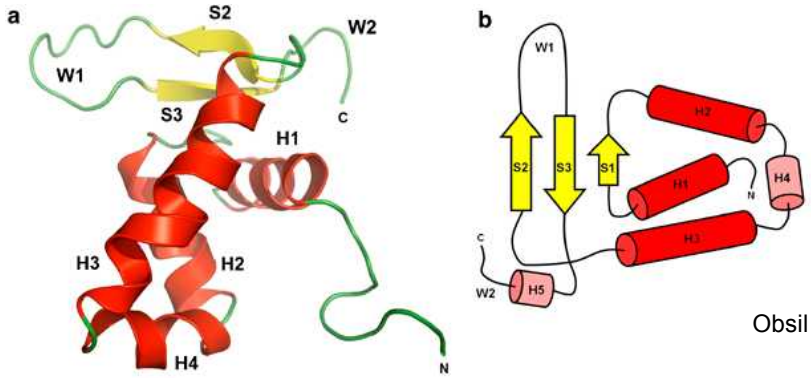
Enhancers transitioning between primed and active are enriched for the Foxd3 motif





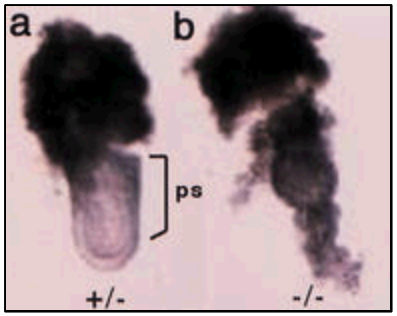
Foxd3 is essential in early embryonic development

Forkhead family of transcription factors – involved in many early embryonic fate decisions



Obsil and Obsilova 2008 (Oncogene)

Foxd3 KO embryos fail to form a primitive streak and die by e7.5

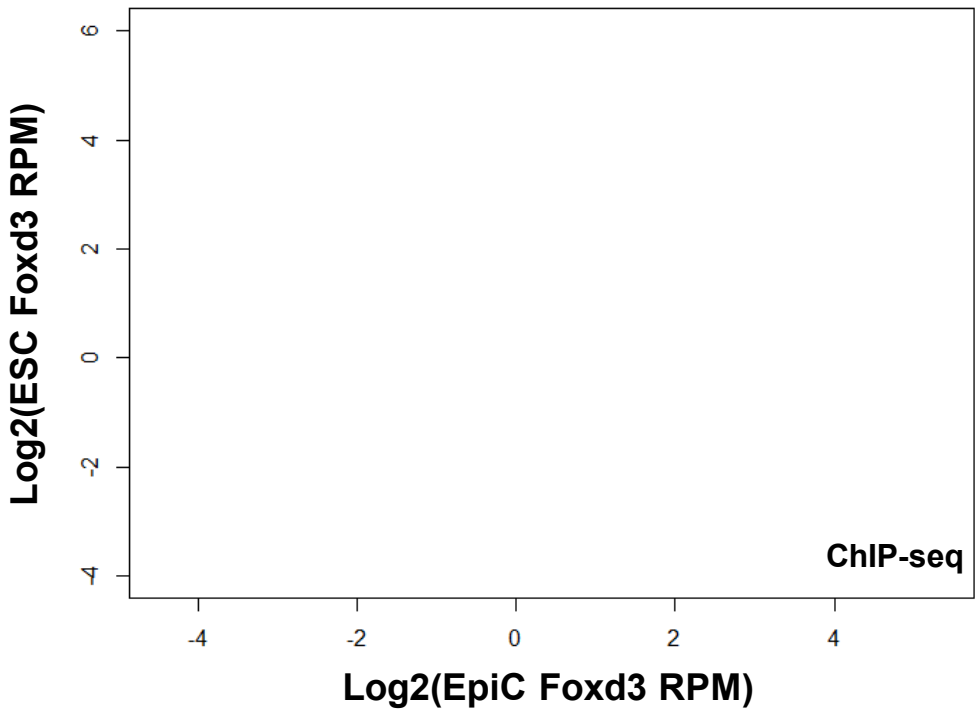


Hanna et al 2002 (Genes and Dev)

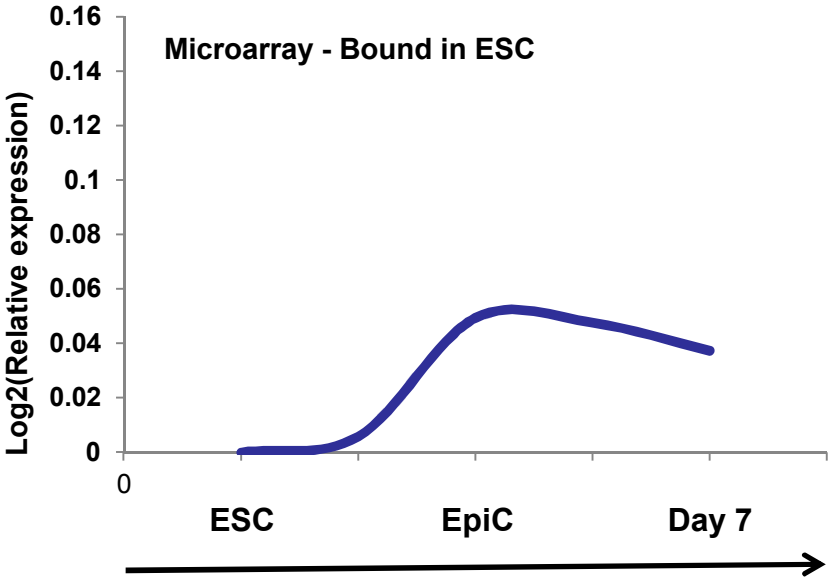
Foxd3 re-localizes during the transition from naïve to primed pluripotency

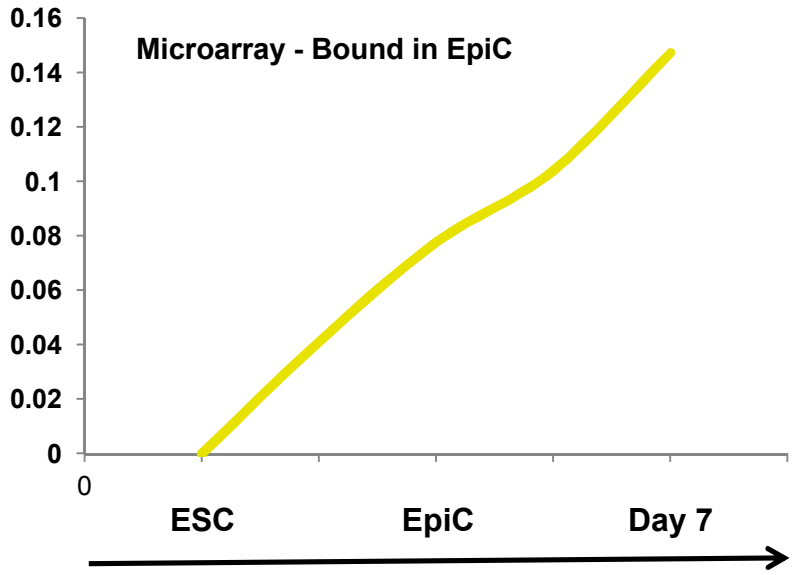
Foxd3 ChIP-seq peak finding using MACSv1.4

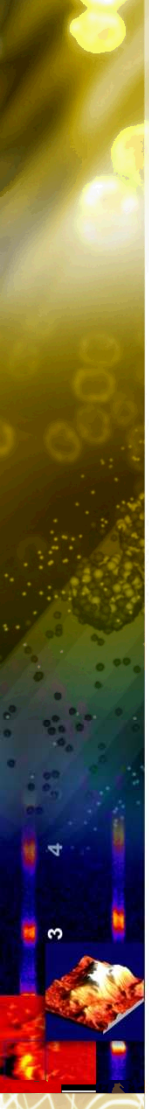
In ESCs (y-axis) and EpiCs (x-axis)



Foxd3 primes genes for later expression







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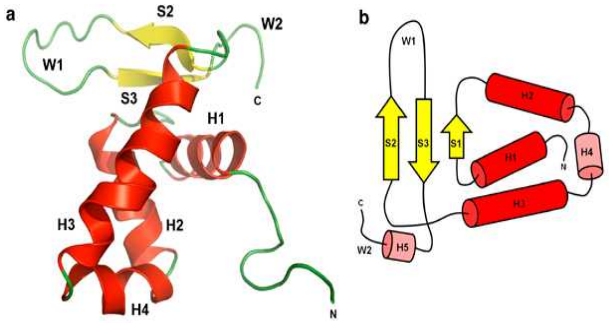
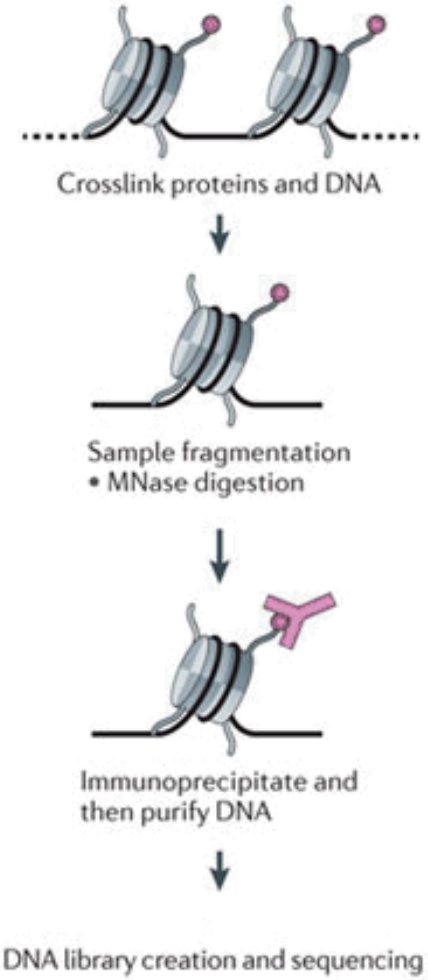
Does Foxd3 initiate enhancers or engage pre-existing ones?

Winged helix domain can access nucleosome-occluded chromatin

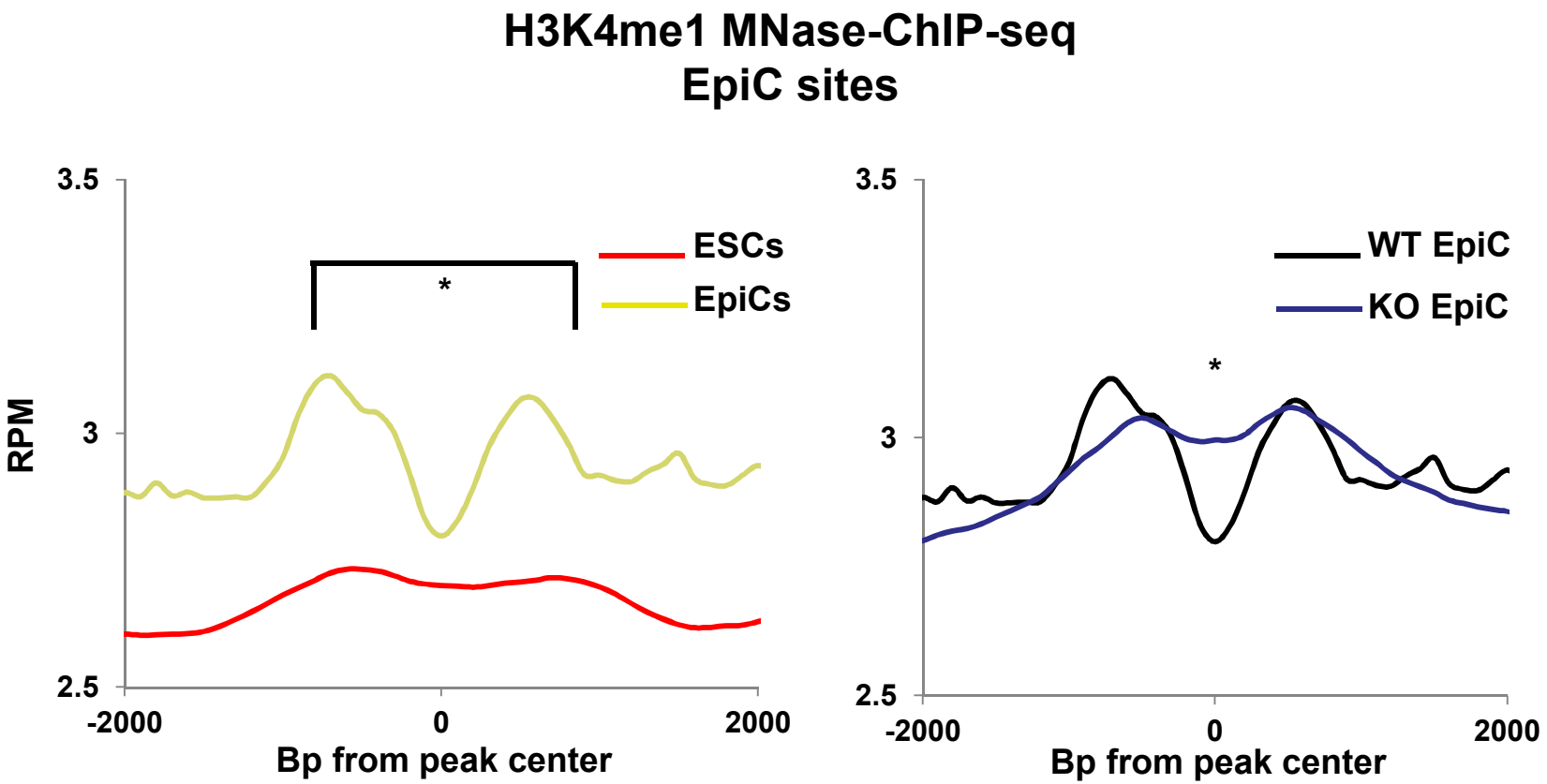


MNase-ChIP-seq

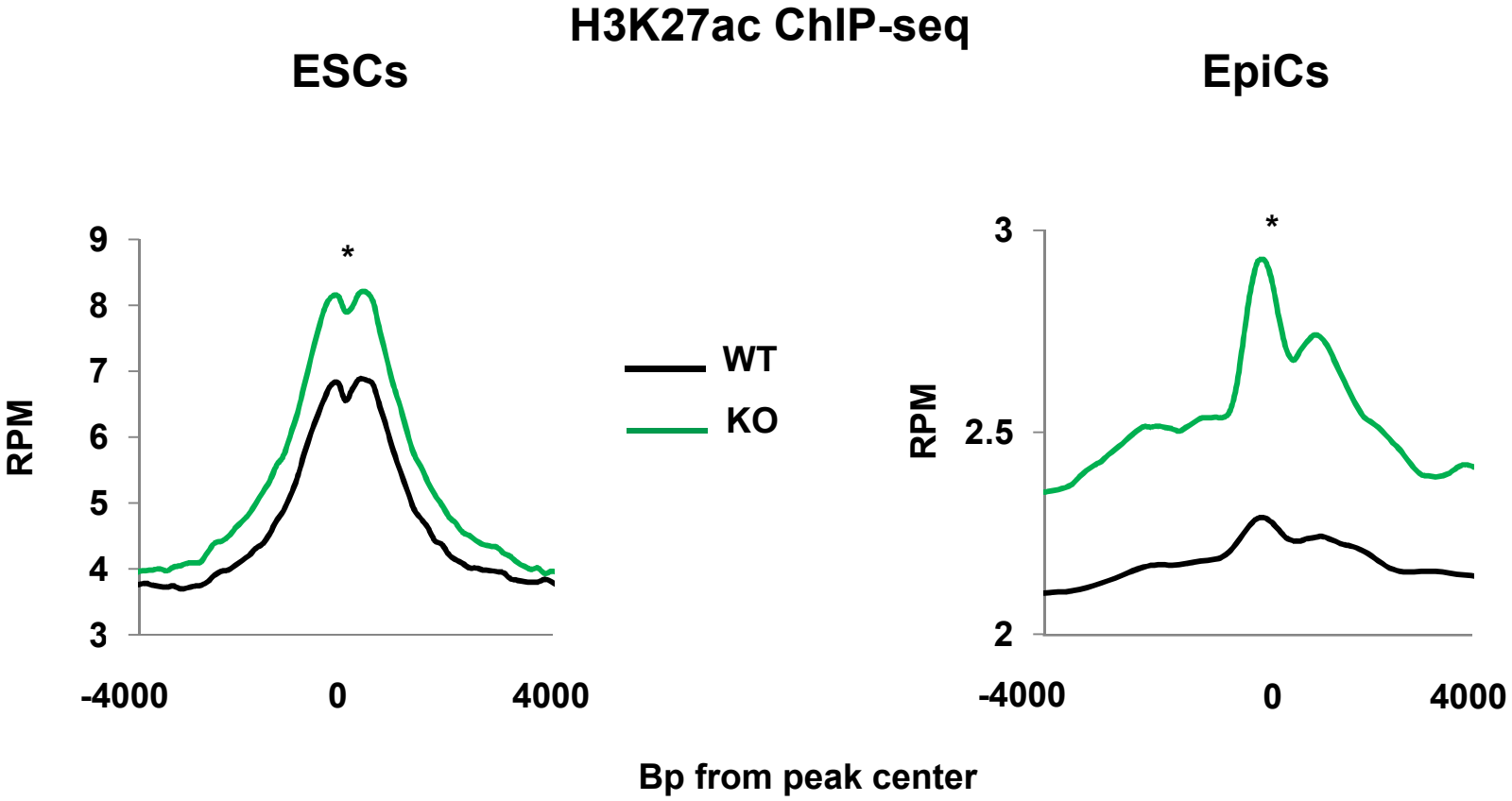


Furey 2012 (Nature Reviews)

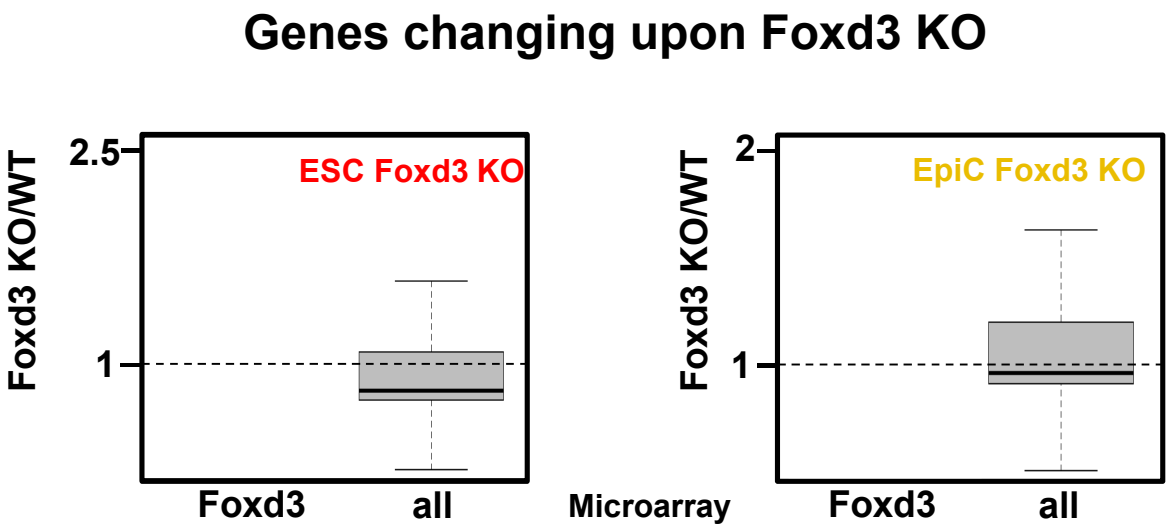
Foxd3 establishes nucleosome-free enhancers



Foxd3 suppresses enhancer acetylation



Foxd3 knockout increases expression of neighboring genes



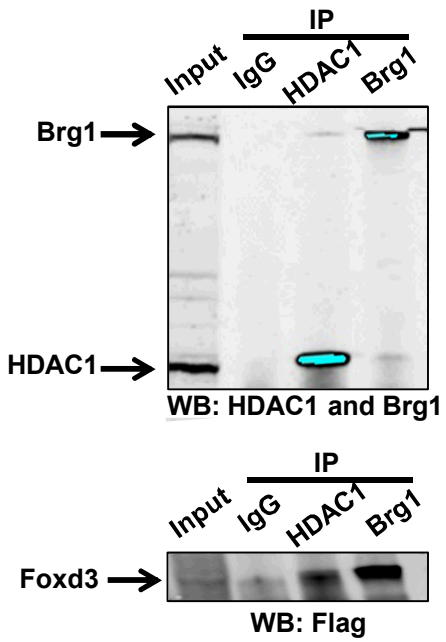
What is the mechanism of Foxd3 action at enhancers?

Foxd3 is in a complex with Brg1 and HDAC1

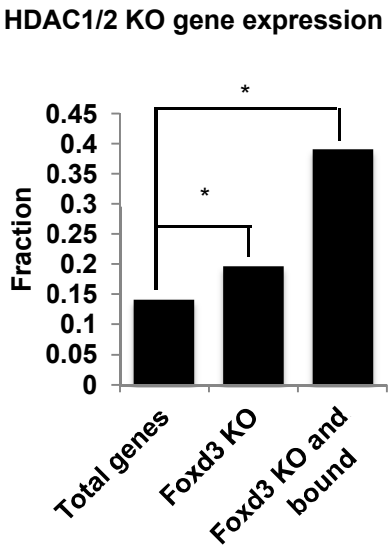
H3K27 deacetylation – HDAC1/2 (NURD complex)

Chromatin remodeling – Brg1 (SWI/SNF complex)

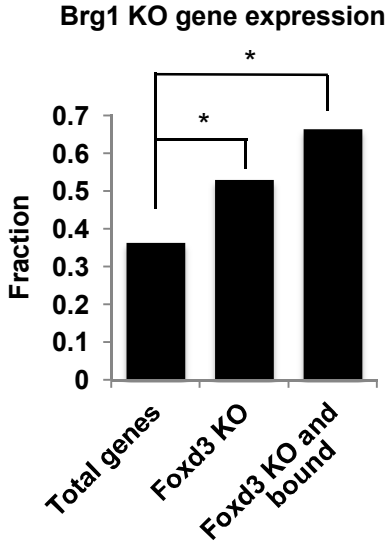
Immunoprecipitation



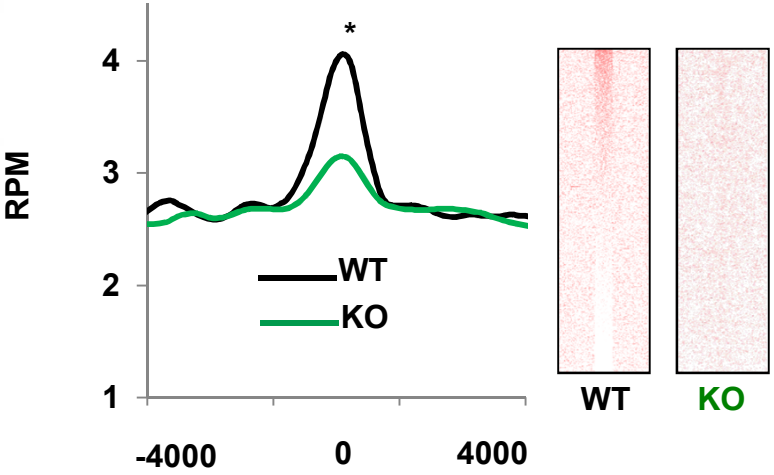
Foxd3 recruits BRG1 and HDACs to simultaneously establish and repress enhancers

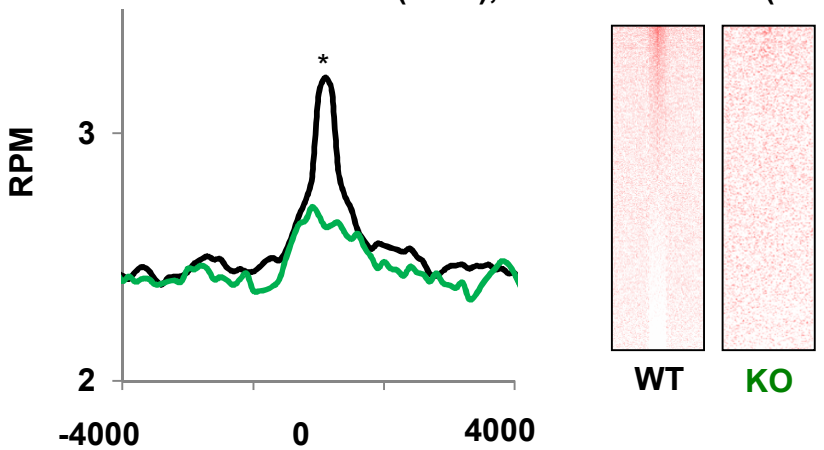


* Chi-squared test
p-value < 2.2e-16

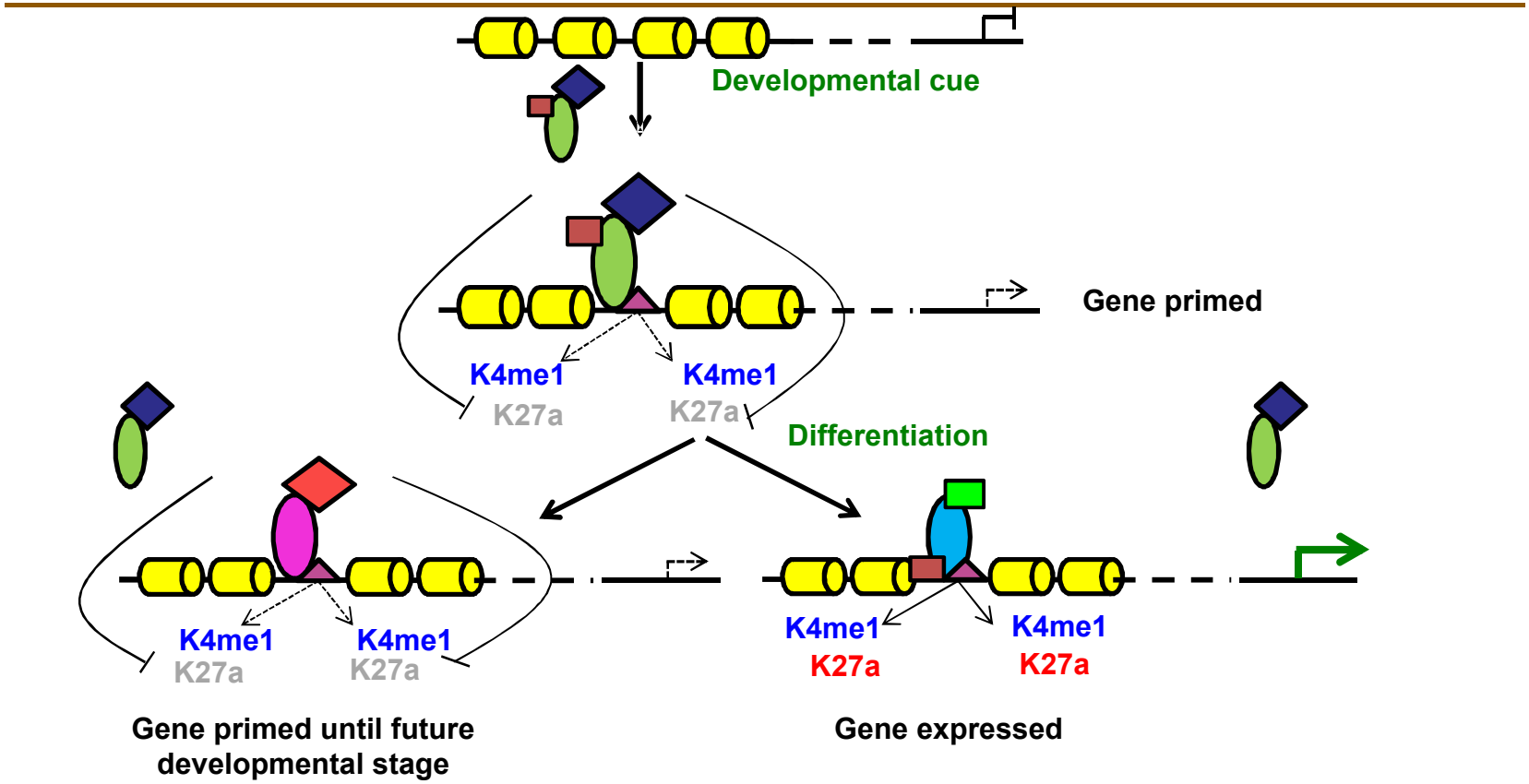


Ho et al (2013), Jamaladdin et al (2014)





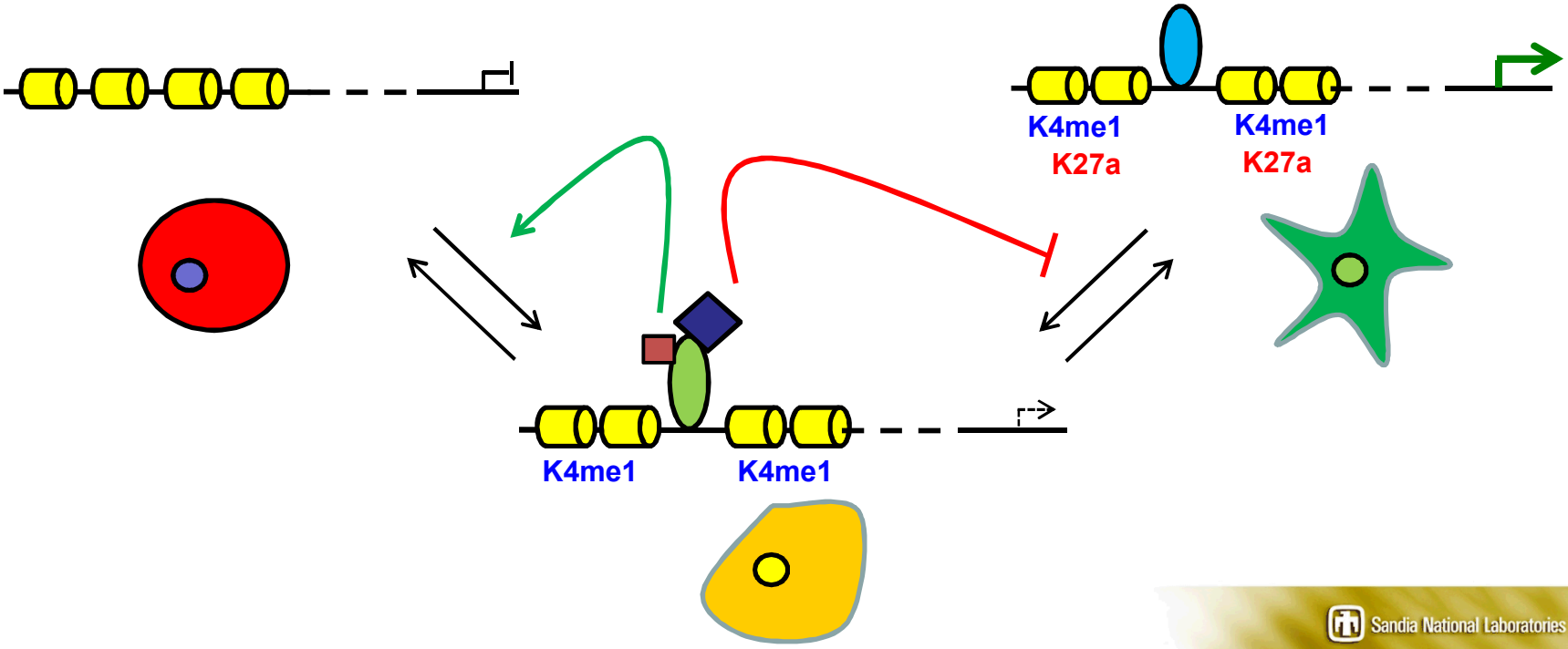
Foxd3 establishes and primes developmental enhancers



Krishnakumar et al 2016 (Cell Stem Cell)

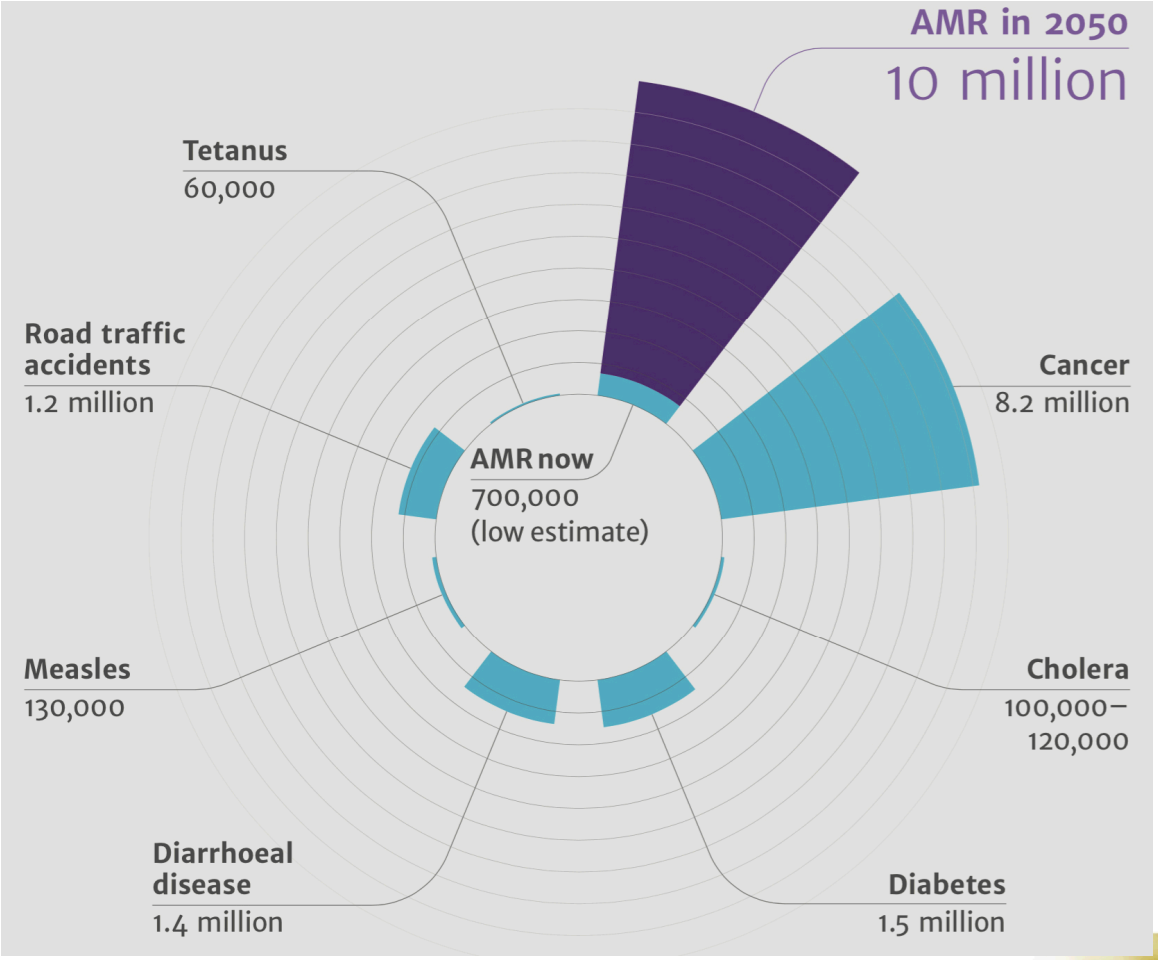
Broad implications

- Metastable, intermediate states during cellular responses and transitions
- Identification of the regulators requires integrative genomic analysis of chromatin and gene expression



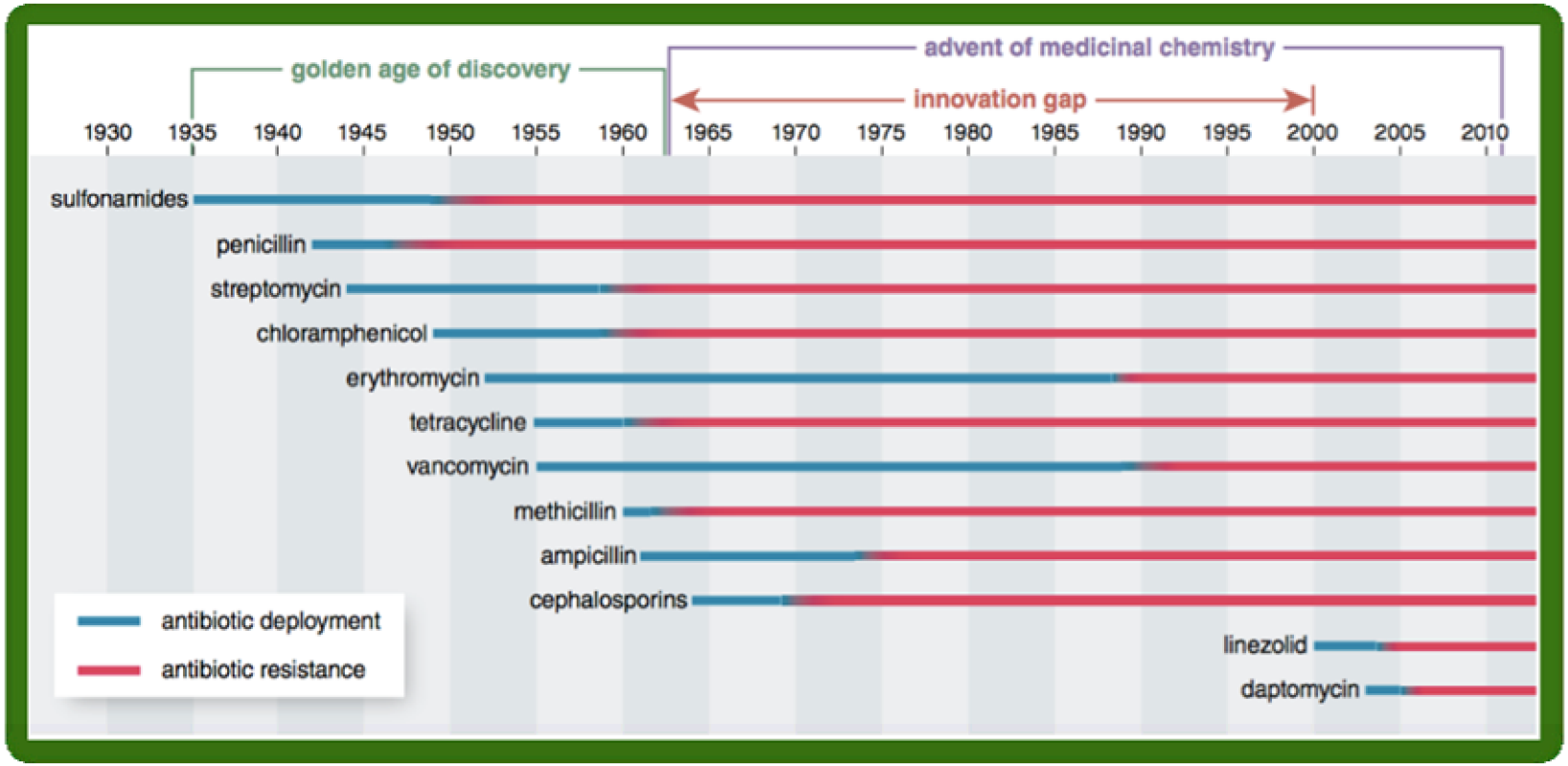
Antimicrobial resistance – a global health crisis

AMR deaths relative to other major causes of death



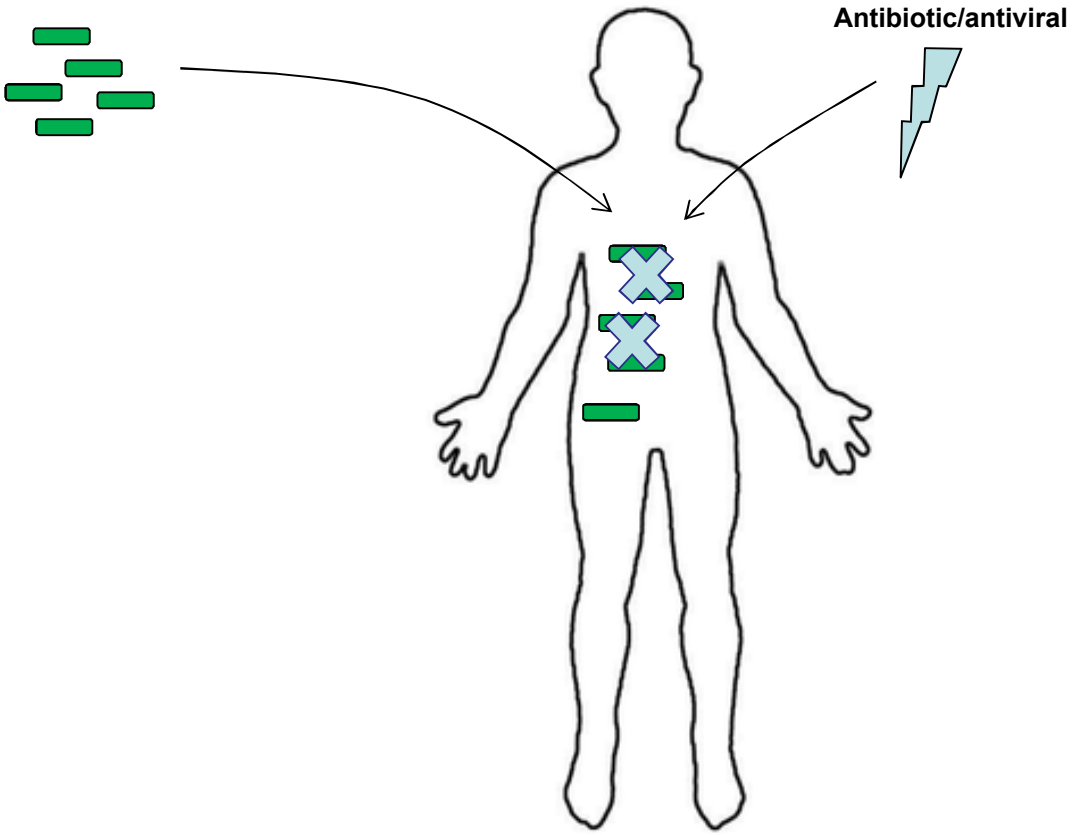
Review on Antimicrobial Resistance (www.amr-review.org)

Antimicrobial resistance – a global health crisis



https://antimicrobialresistance101.wordpress.com

Solutions to antibiotic resistance



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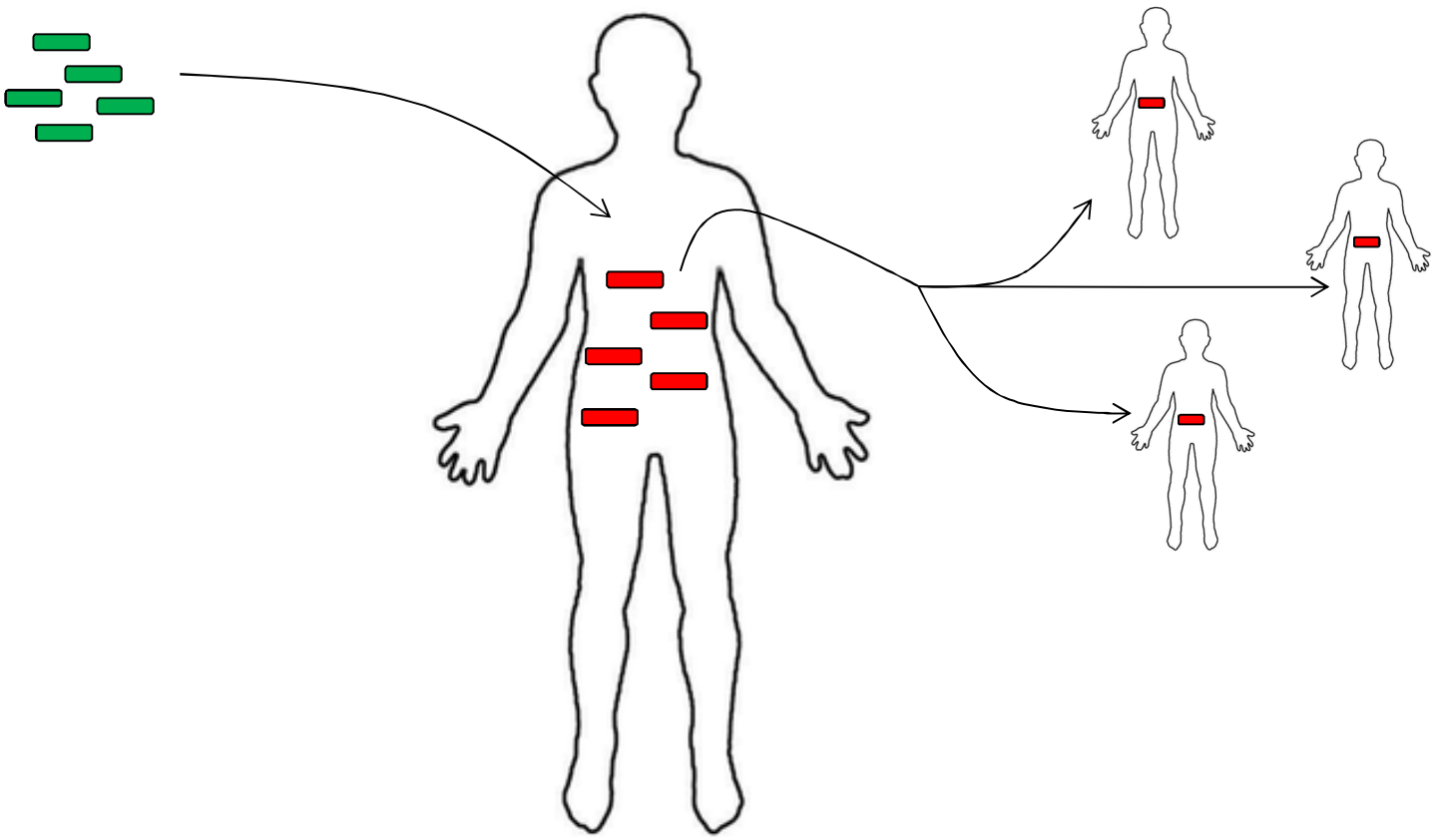
Solutions to antibiotic resistance



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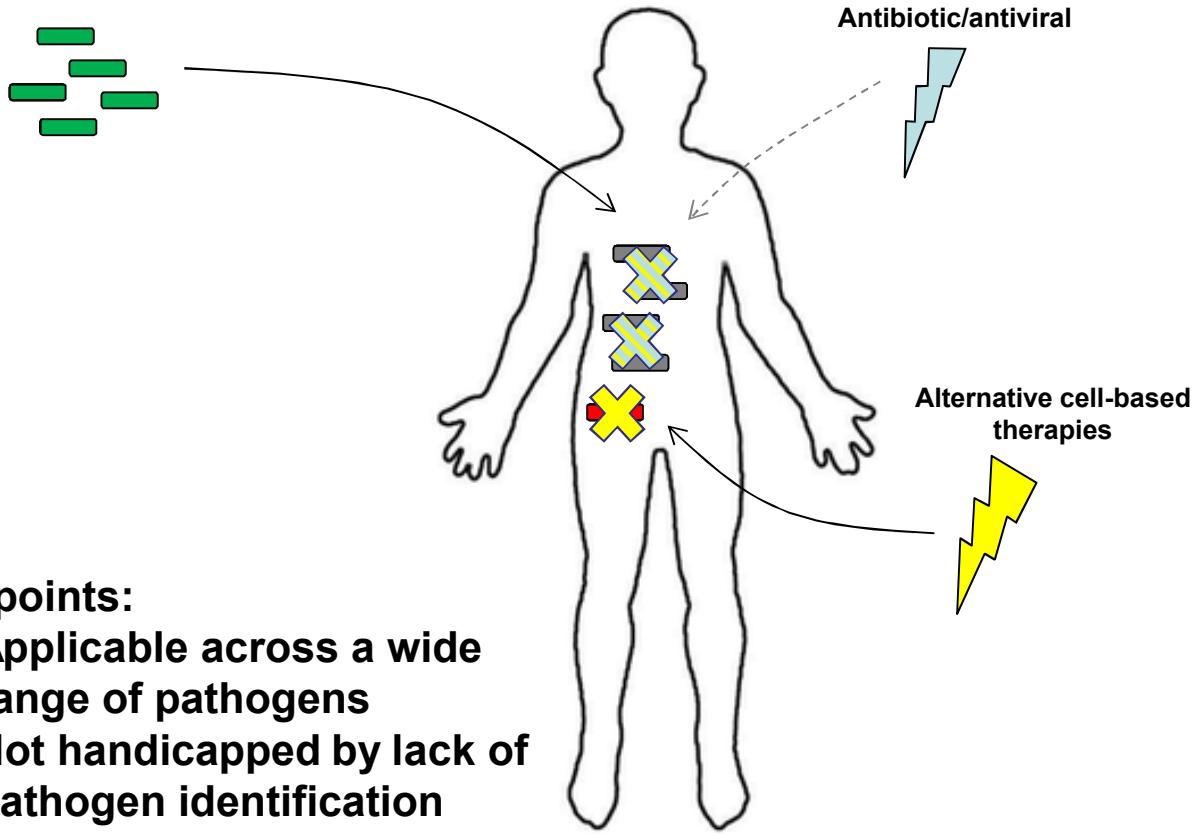
Solutions to antibiotic resistance



Key points:

- **Applicable across a wide range of pathogens**
- **Not handicapped by lack of pathogen identification**

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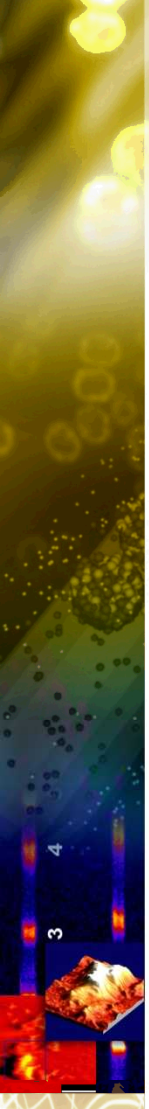
New cell-based approaches to antibiotic resistance

- Infections with no cure/AMR infections spread unchecked, and eventually can lead to death (parallels in presentation and prognosis with cancer, except with the added “bonus” of being contagious!)
- Cell-based therapies (i.e. immunotherapy) have been very successful in treating a variety of diseases including, especially cancer and autoimmune
- Challenges to tackle – make cell-based infection therapies:
  - Cheap
  - Fast
  - Effective
  - Safe



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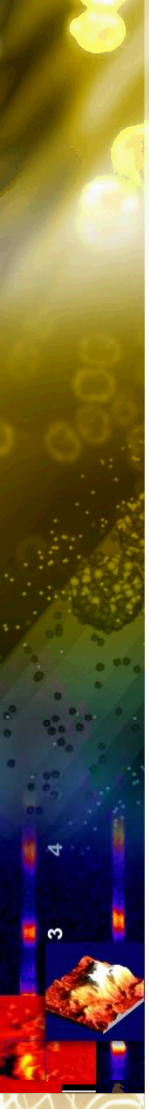
New cell-based approaches to antibiotic resistance

- We hypothesize that by using ***gene editing technology*** (i.e. CRISPR/Cas9), we can rapidly convert large amounts of easily accessible cells to therapeutic cells.

- We plan to use mesenchymal stromal cells (MSCs) as our therapeutic cell type of interest

- MSCs are found in ***bone marrow***, ***adipose tissues***, and ***umbilical cord tissue***, among other sources



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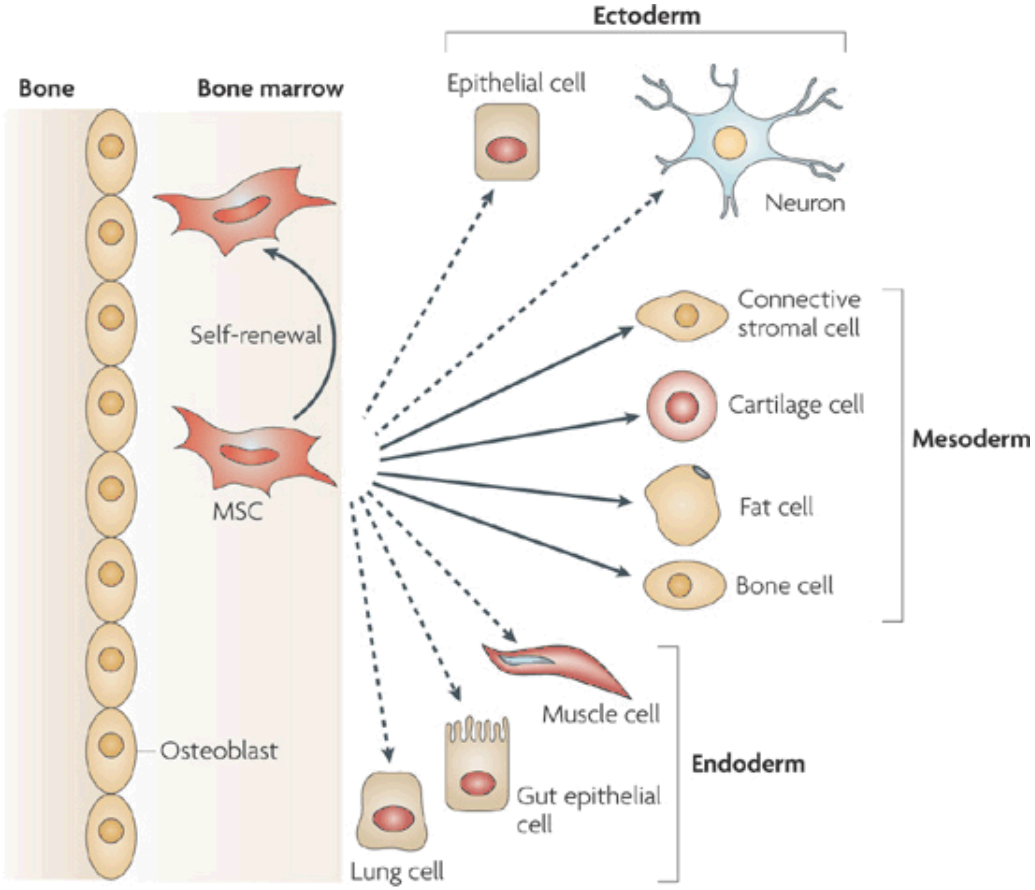
MSCs can divide and regenerate tissue

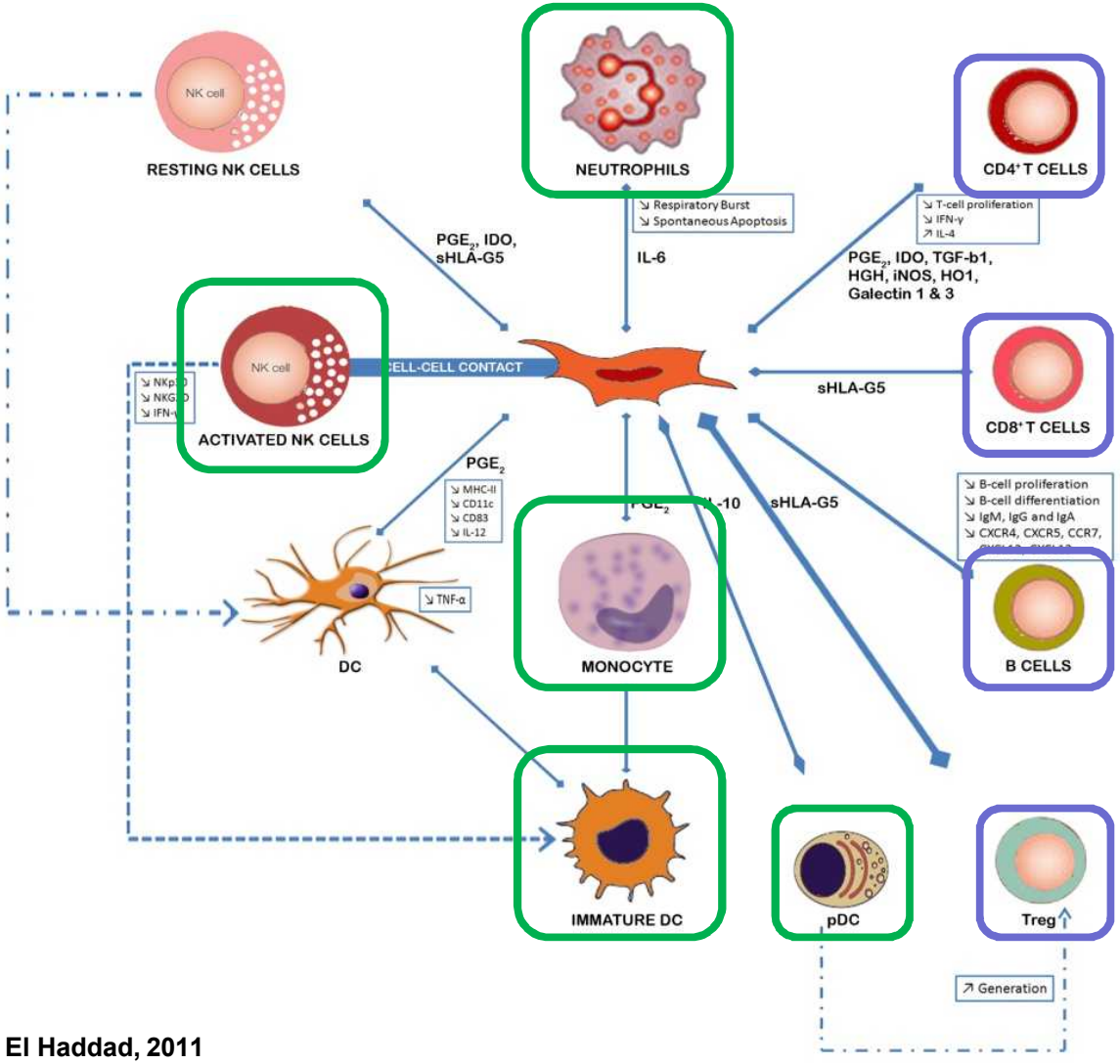
- MSCs can be passaged in culture, and differentiated down many lineages (multipotent)



Nature Reviews | Immunology

Uccelli et al, 2008

MSCs are immunomodulatory



Innate immue system

Adaptive immue system

In addition, MSCs are hypoimmunogenic, making them a good candidate for both autologous and allogeneic therapy

El Haddad, 2011

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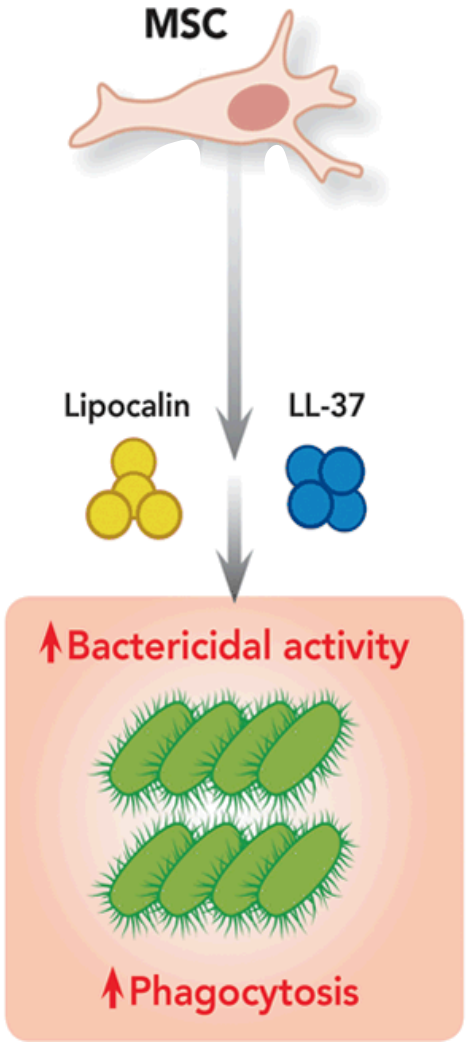
MSCs are antimicrobial

- MSCs secrete peptides that directly target pathogens
- Lipocalin inhibits bacterial growth by scavenging enterobactin and 'starving' them of iron
- LL-37 intercalates in and causes changes to the cell membrane of bacteria, eventually causing lysis



Monsel et al, 2014

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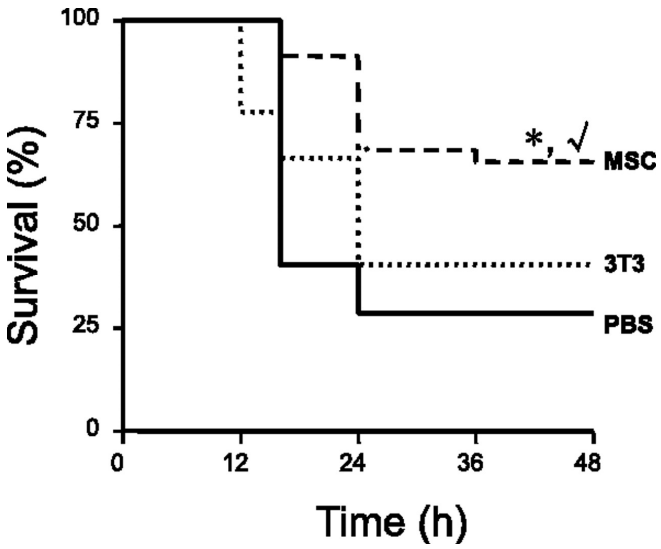
MSCs and infection in the literature

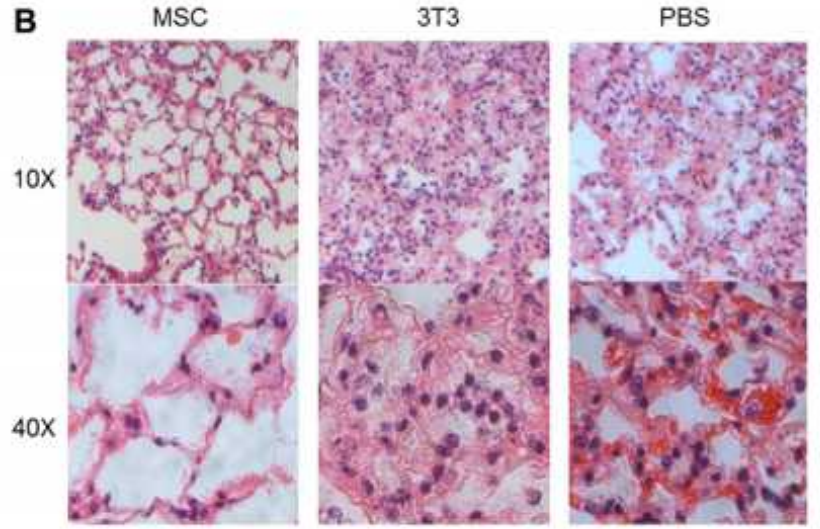
- There are numerous examples of MSCs being used to clear infection and improve survival, including in ***sepsis***, ***lung infection*** and ***wound infection***.

Survival in a *Pseudomonas aeruginosa* model of peritoneal sepsis



Krasnodembskaya et al, 2012

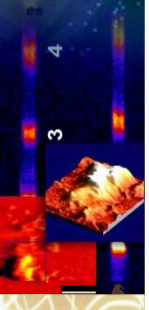
Histology (H&E) in a *Escherichia coli* model of lung infection



Gupta et al, 2017

Challenges ahead

- High enough quantities of high-quality, homogeneous MSCs for therapy
- Being able to fine-tune MSC function in a context-specific manner



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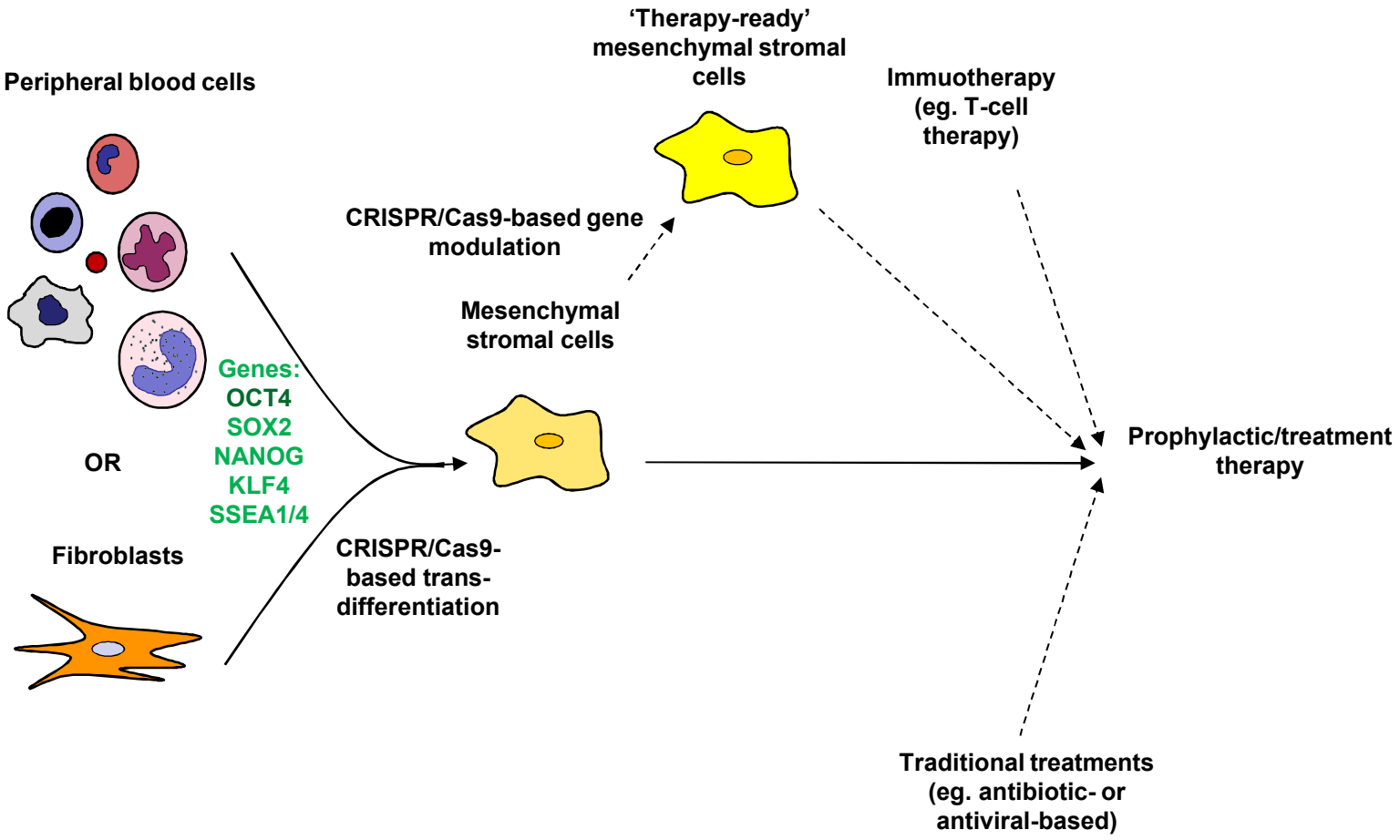
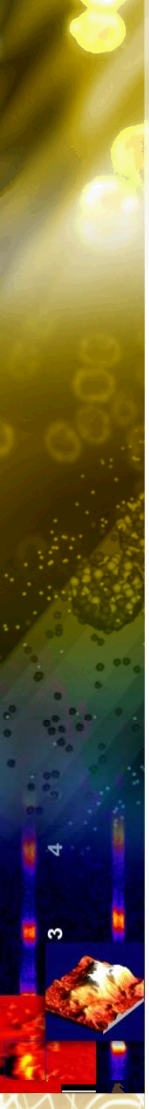
Engineering ‘therapy-ready’ MSCs



Engineering 'therapy-ready' MSCs

- Optimize the ***production of therapy-ready mesenchymal stromal cells*** (MSCs)
- Use ***gene editing technology*** for easy and reversible changes in gene expression
- Use both large-scale unbiased and logic-based experimental and bioinformatic approaches to optimize the cells
- Use in vitro and in vivo models of bacterial infection to test the resulting cells



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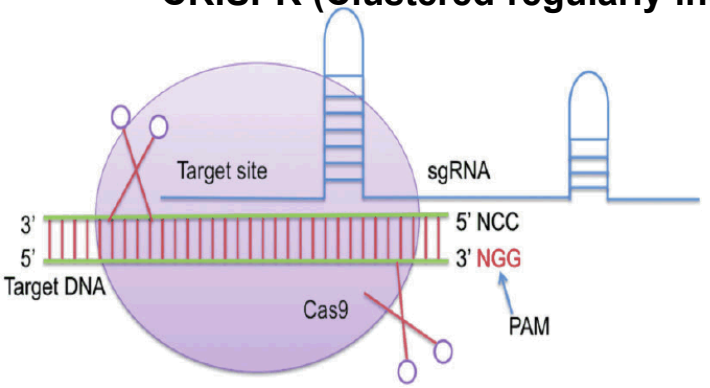
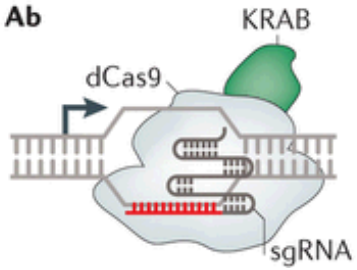
Harnassing CRISPR/Cas9 gene editing technology

CRISPR (Clustered regularly interspaced short palindromic repeats)

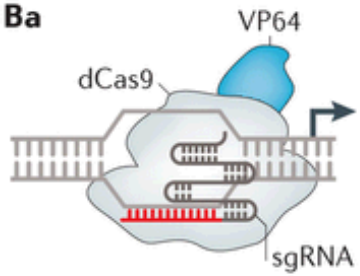


- Inactive Cas9 can be used as a targeting method for other proteins ('Break the scissors' – Jonathan Weissman)

CRISPRi - inhibition



CRISPRa - activation



Kaur et al, 2015

Shalem et al, 2015

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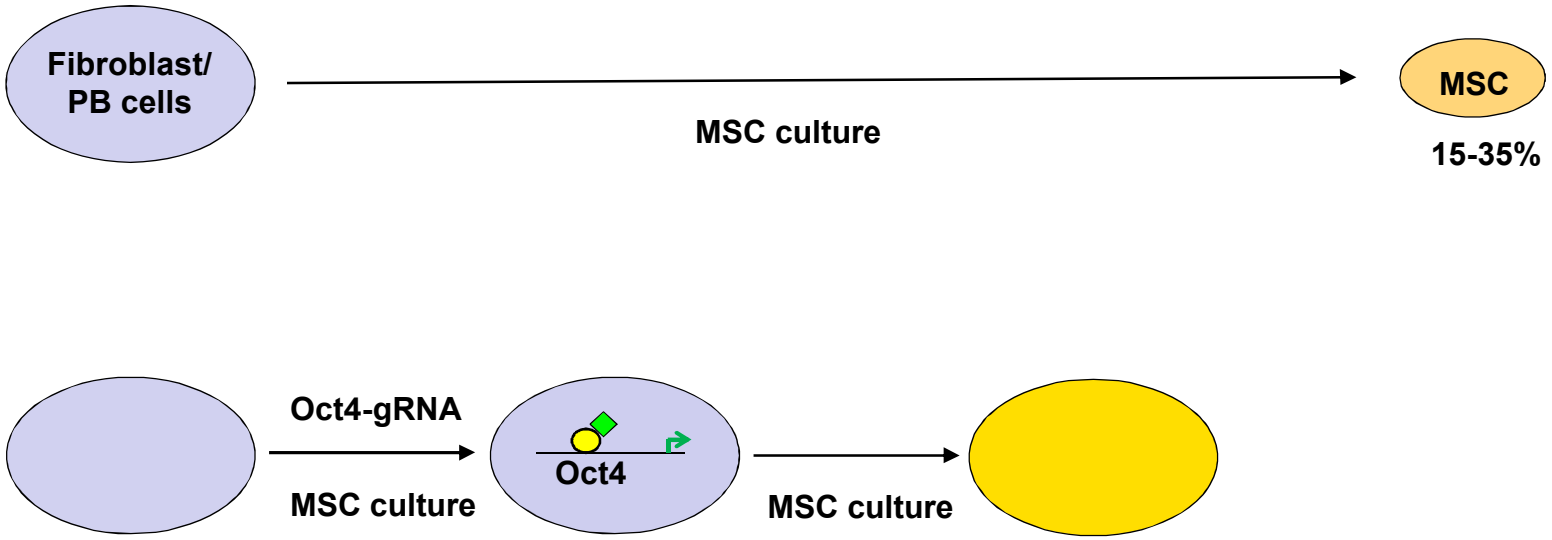
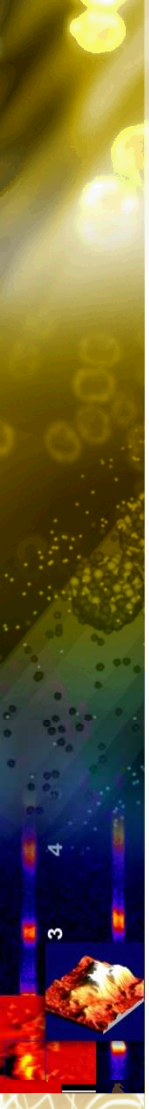
Altering endogenous gene expression profiles to modulate cell fate

- Optimize the transition from fibroblasts to MSCs

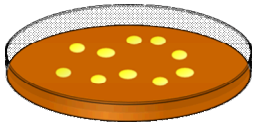


Meng et al, 2013
Lai et al, 2017
Pan et al, 2017

Verifying MSC potency



MSCs



Colony-forming ability of bacteria (direct contact with cells or conditioned media)

Burkholderia thailandensis lung infection model

Ability of MSCs to affect infection rates/survival of other cells, such as macrophages, upon exposure to bacteria

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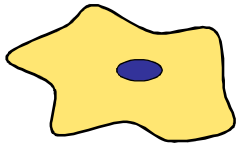
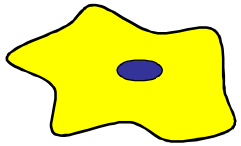
Fine-tuning MSCs to enhance their potency

Mesenchymal stem cells

‘Therapy-ready’ mesenchymal stem cells



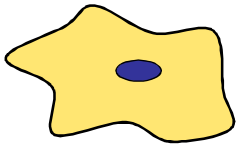


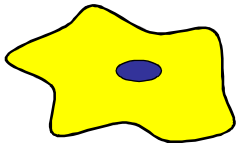
Δ Culture conditions

Mesenchymal stem cells

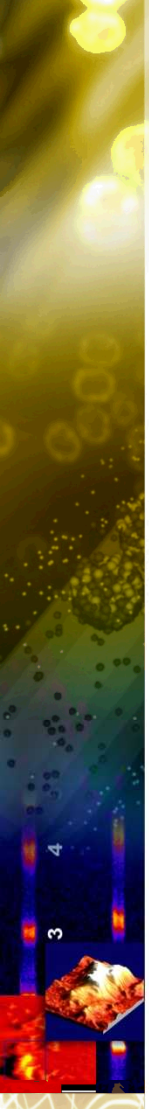
Examples:
LL-37
MHC1/2
HLA-G
PGE2
IDO

‘Therapy-ready’ mesenchymal stem cells





CRISPR/Cas9-based gene modulation



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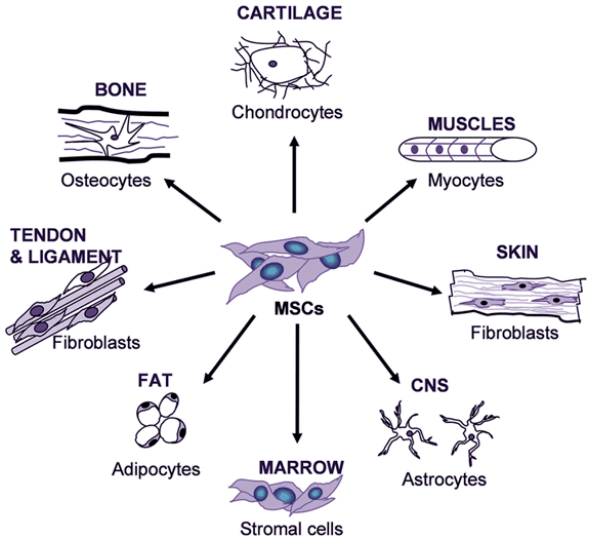
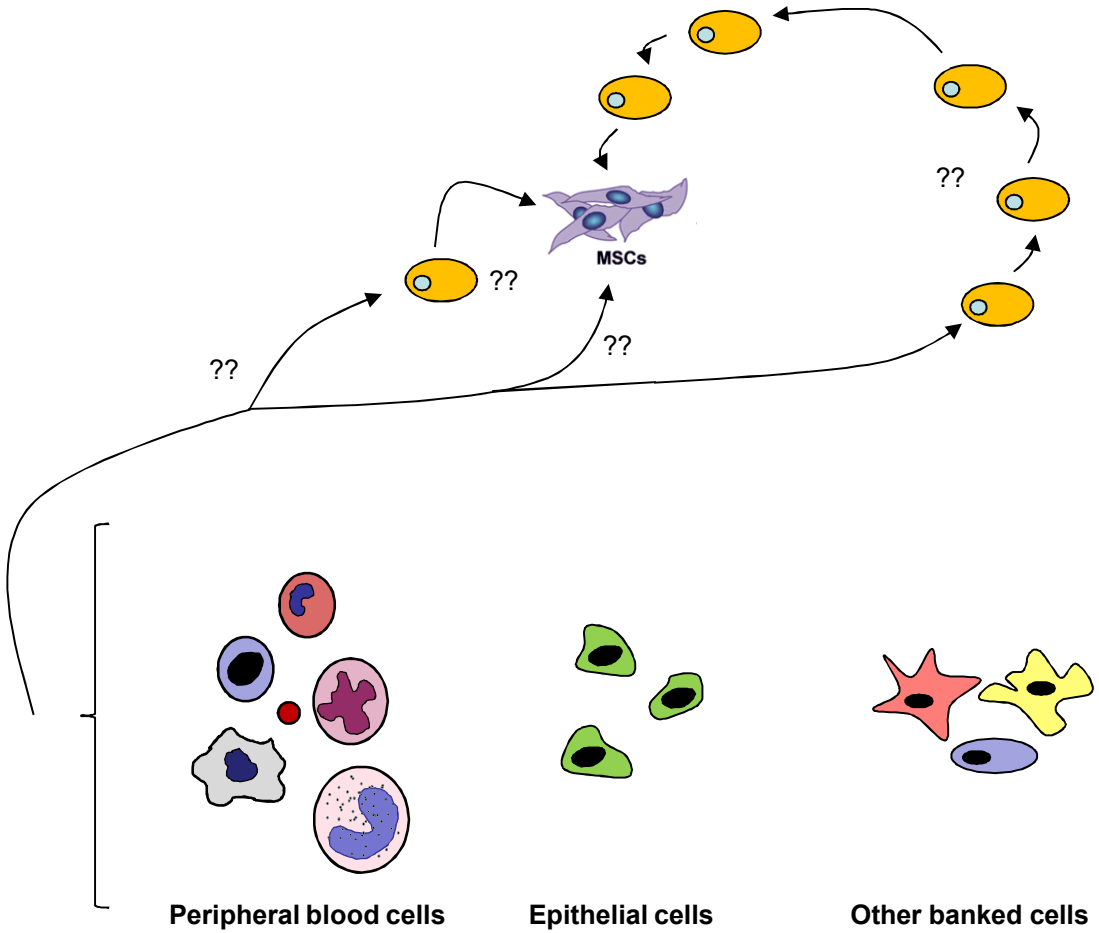
Expanding the types of cells that can be converted to MSCs



stemology.co.uk

Regenerative Medicine, 2006

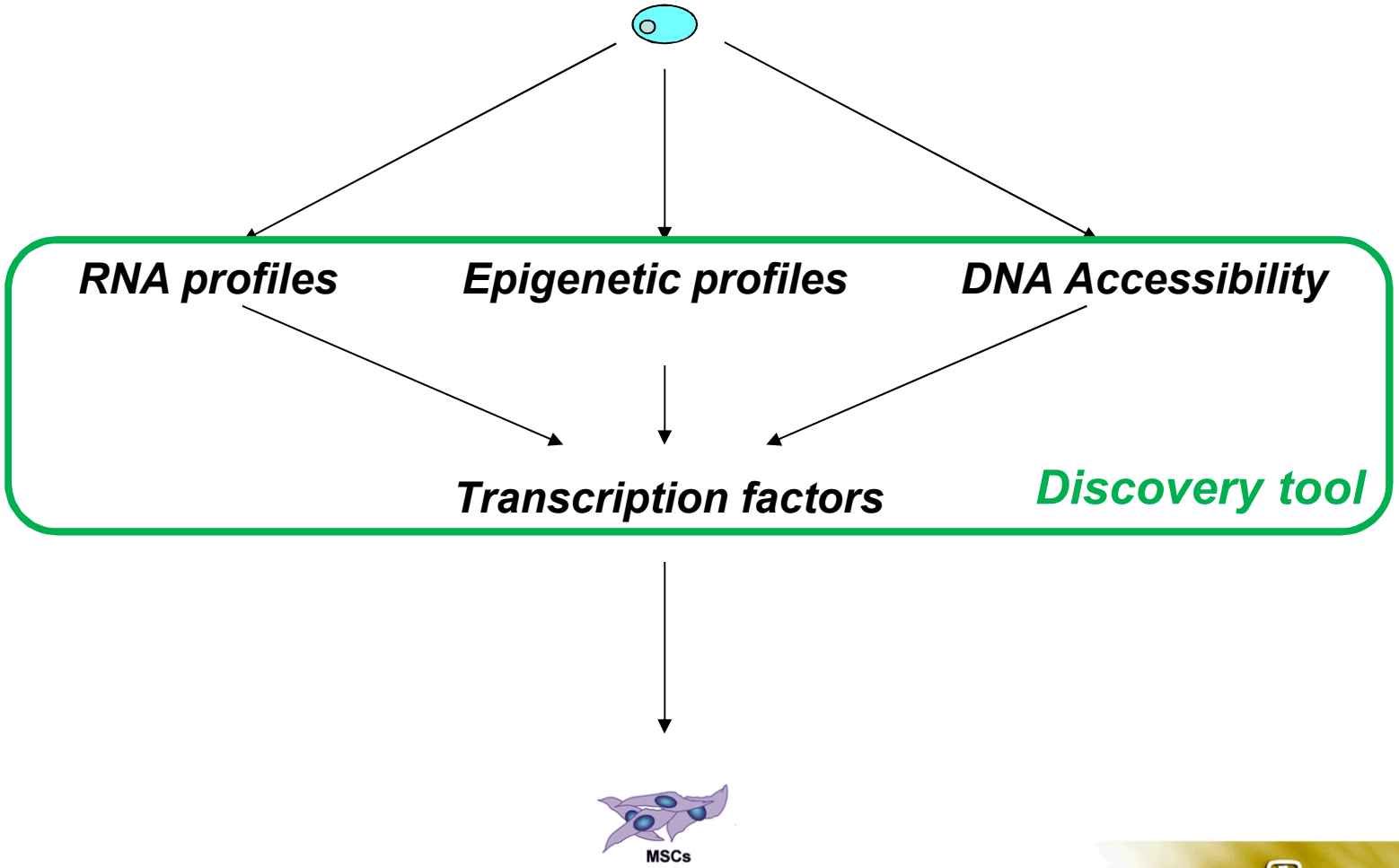
Expanding the types of cells that can be converted to MSCs



stemology.co.uk

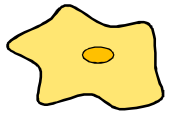
Regenerative Medicine, 2006

Bioinformatic analysis to identify key factors for converting cells



Clinical uses

MSCs

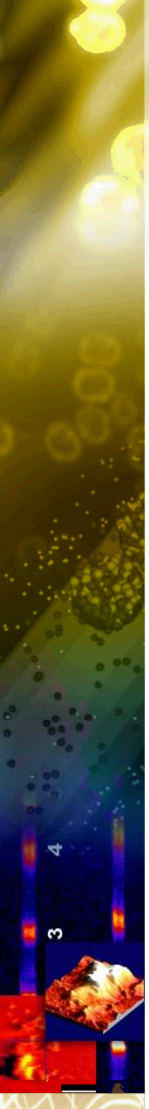


Acute therapy in infection – stand-alone or together with antibiotics or other immune cells)

In vitro potentiation of immune cells (T-cells) or antigen presentation (B-cells)

Prophylaxis – antigen presentation or immune system potentiation

Collecting, synthesizing and deciphering genomic information for engineering cell fates



Capture environmental changes and cellular responses

GENOMICS IN THE ERA OF NEXT GENERATION SEQUENCING

Functional studies of molecular mechanisms

Systems-level integrated data analysis

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Acknowledgements

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University of California
San Francisco

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Barbara Panning (UCSF)
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8623, 8625 and 8621

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Harrison Edwards
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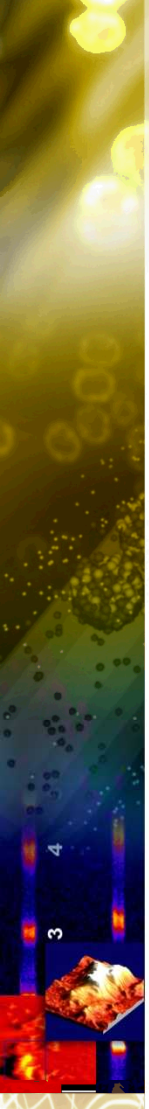
Ken Patel
Joe Schoeniger
Anupama Sinha
Kelly Williams

Funding – Sandia LDRD



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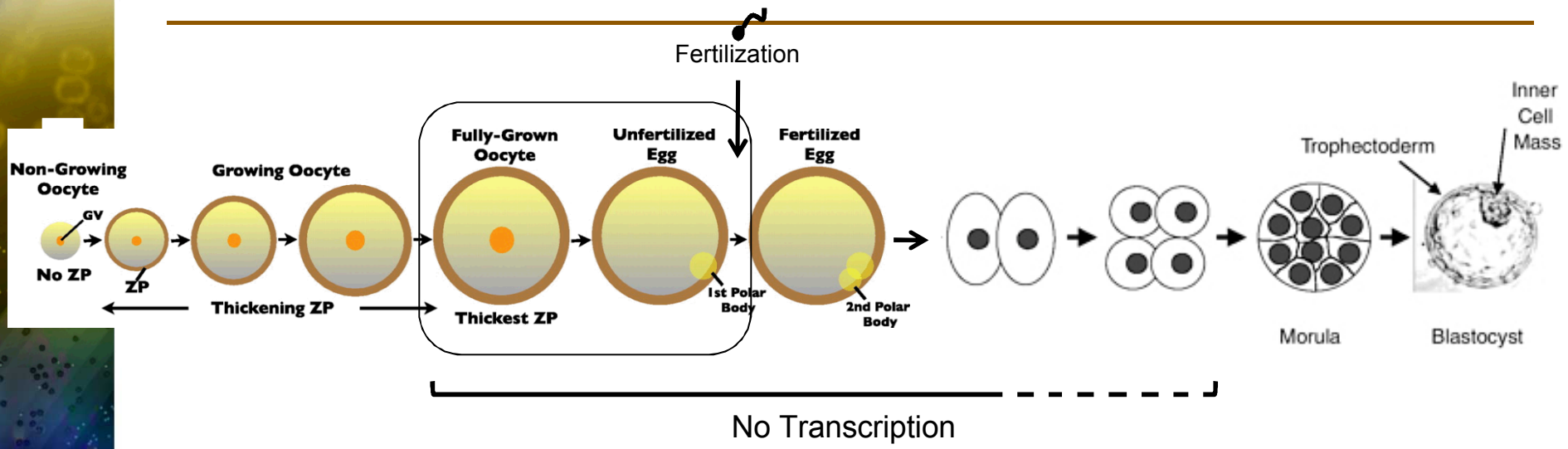
Oogenesis happens in the absence of transcription



No Transcription

Regulation of mRNA stability and translation

RNA seq of GV (fully-grown) and MII (unfertilized) oocytes

Modified from Wassarman (2008) and National Academic Press (2005

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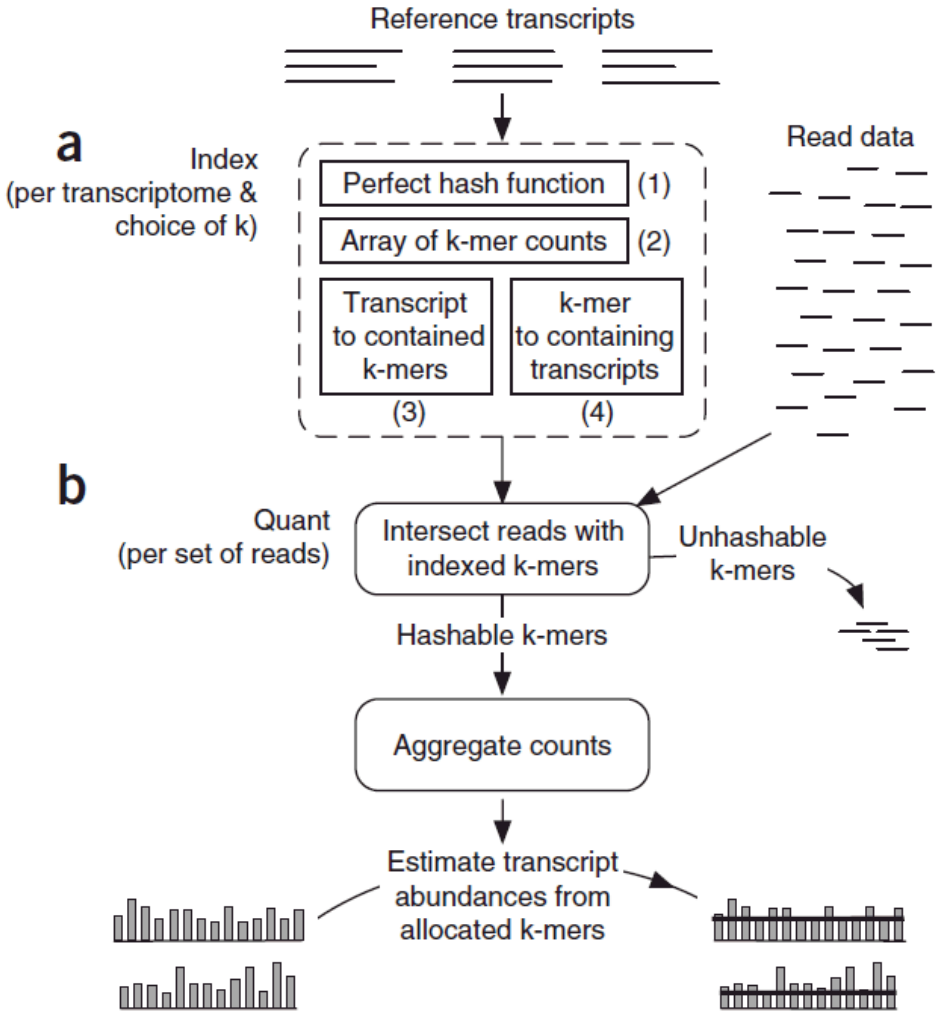
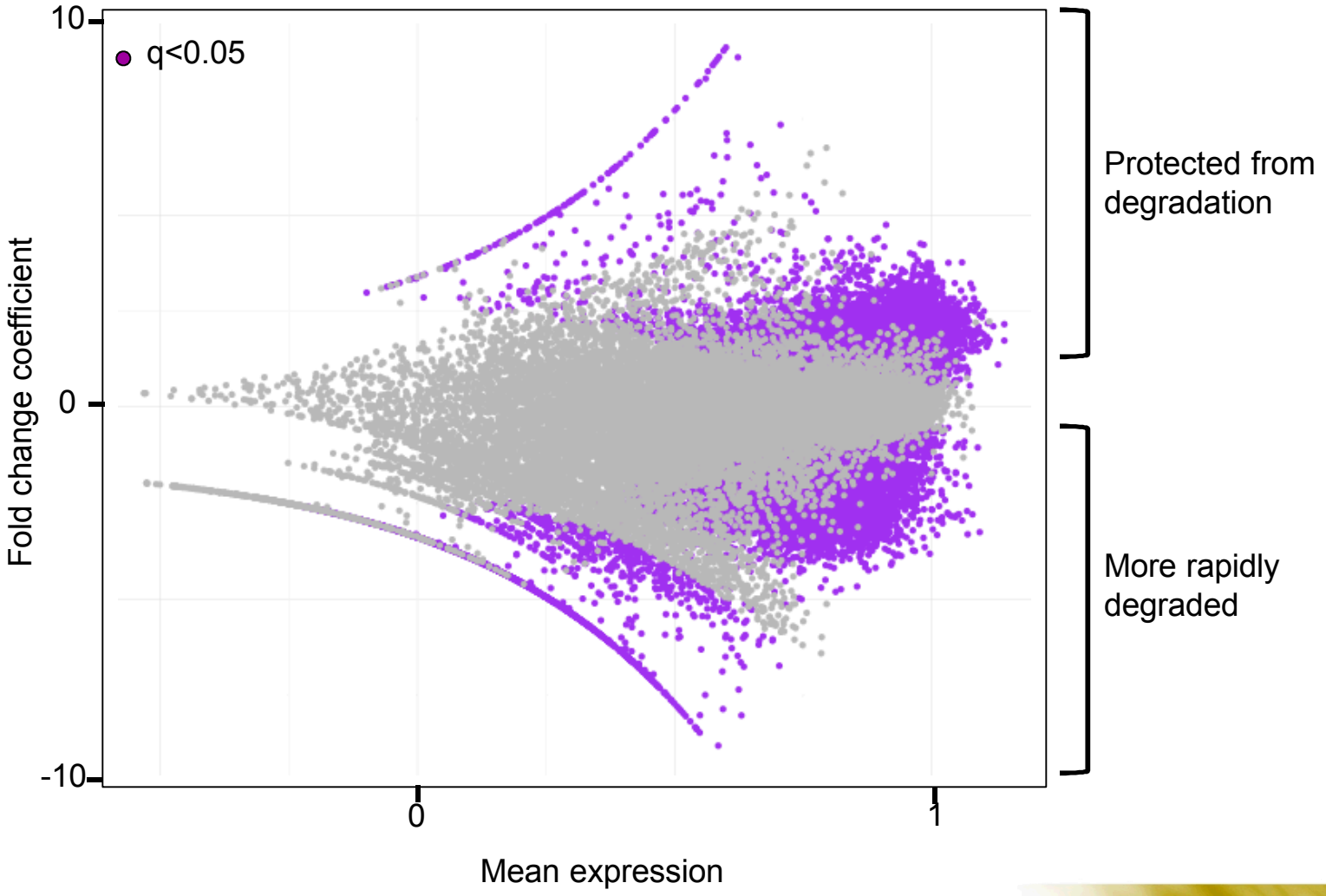
Transcriptome mappers aka "alignment-free mappers"

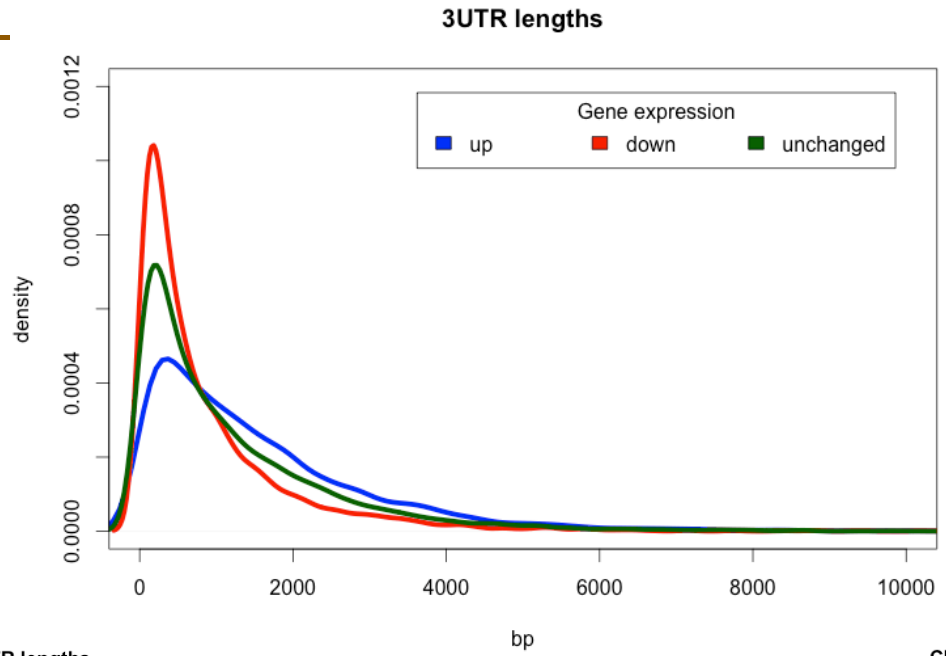


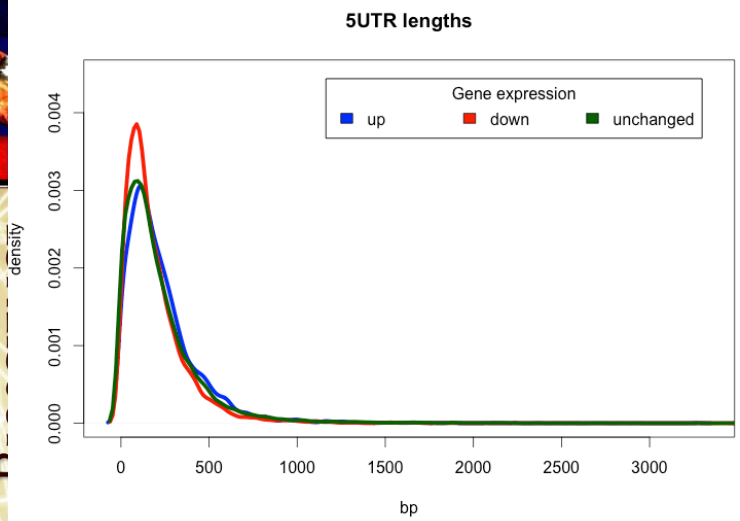
Patro et al 2014

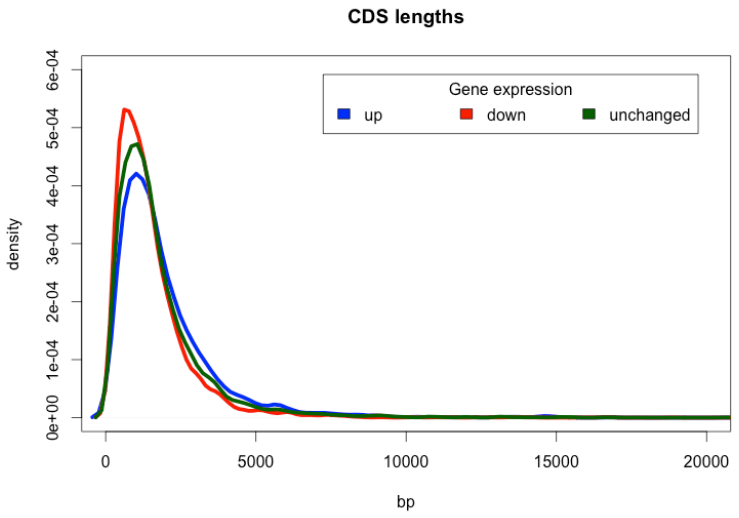
Dramatic changes in RNA stability during oocyte maturation



Changing genes from GV to M2 have different 3'UTR lengths

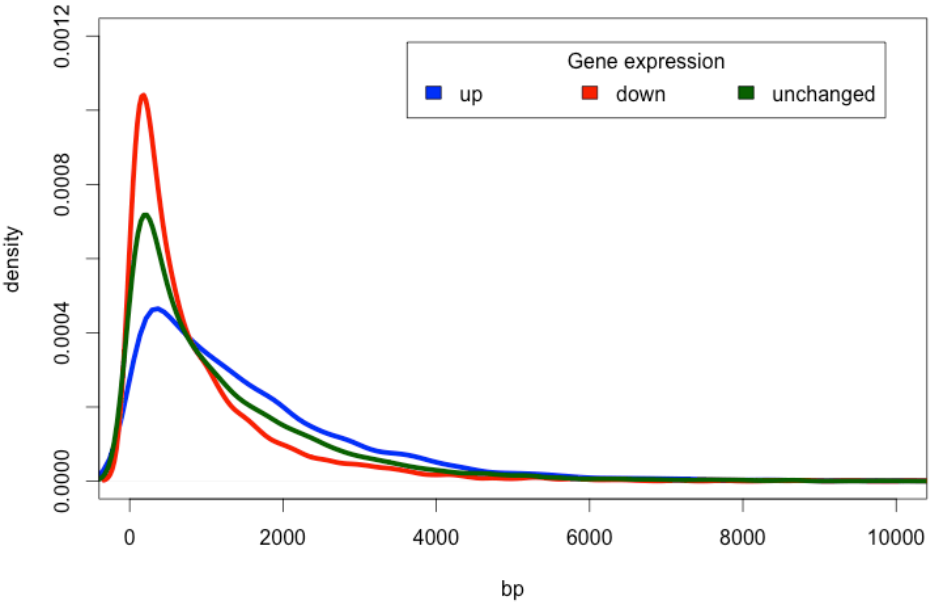




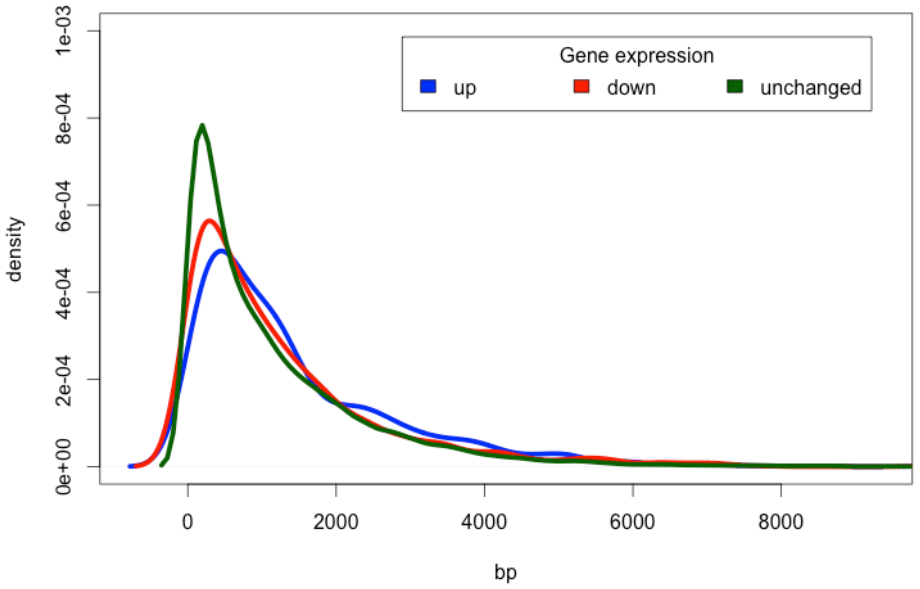


Changing genes from GV to M2 have different 3'UTR lengths

GV to M2



ESC to EpiC



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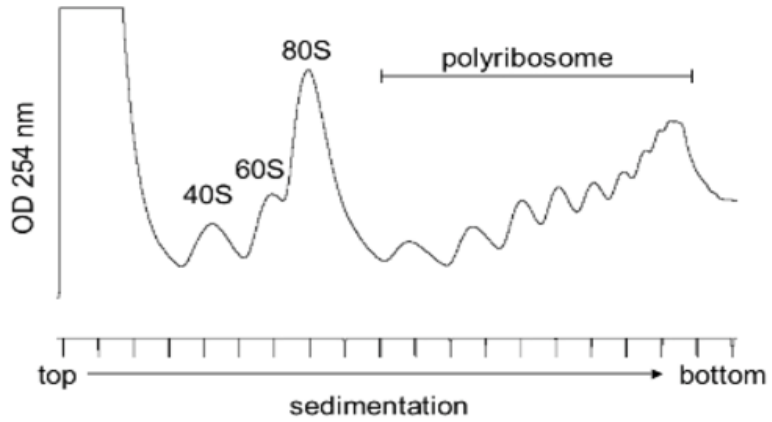
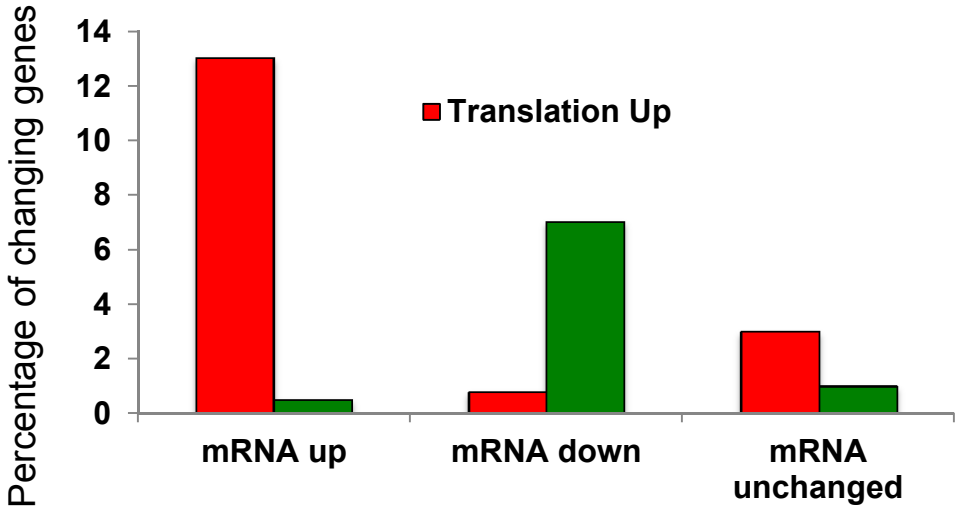
Relationship of stability with translational efficiency

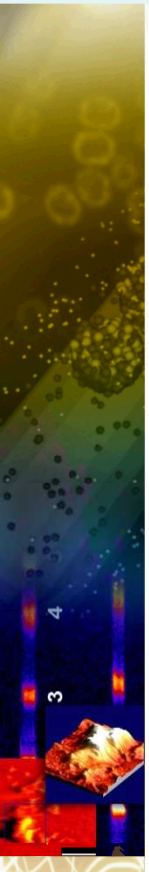


From Bor et al 2010 (Nature Protocol Exchange)



Polysome data from Chen et al 2010 (Genes and

Ongoing analyses/Future directions



- Are there any proteins/structural features in longer UTRs that protect them from degradation?
- Can we stabilize transcripts by manipulating the degradation machinery?
- How do these changes relate to oocyte function?

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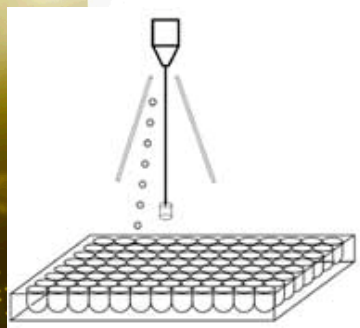
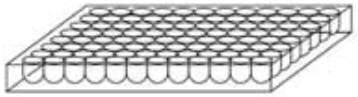
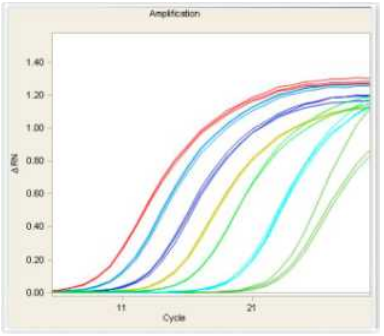
Single-cell miRNA qPCR shows homogeneity in populations

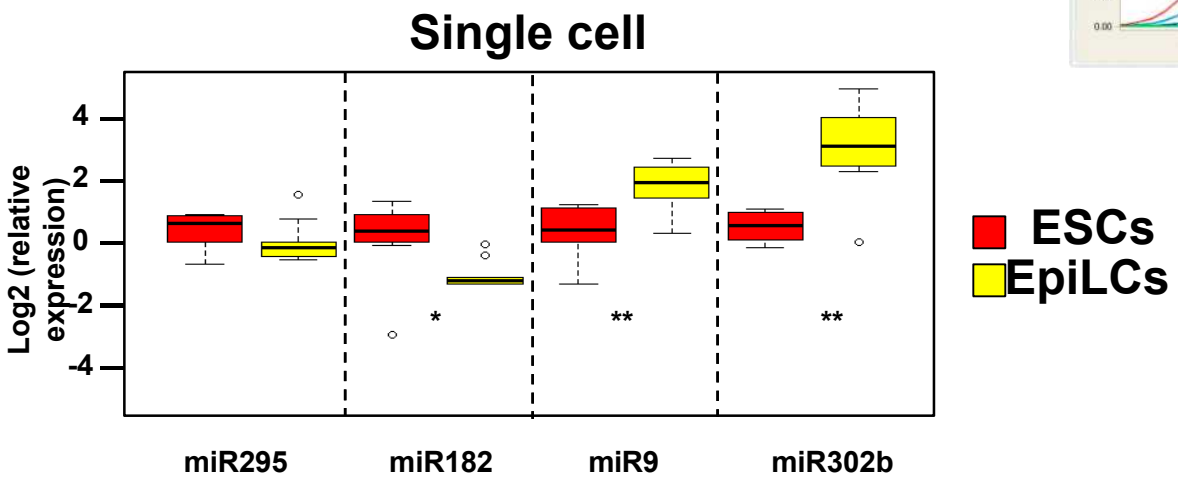


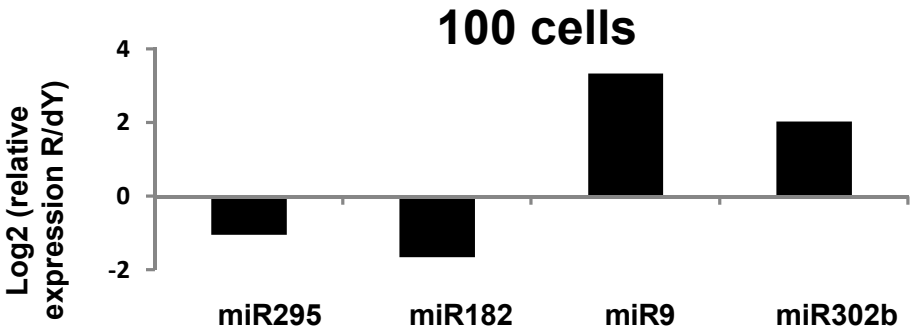
In plate:
Lysis and RT

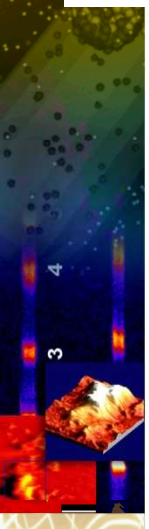


Taqman probe-based qPCR









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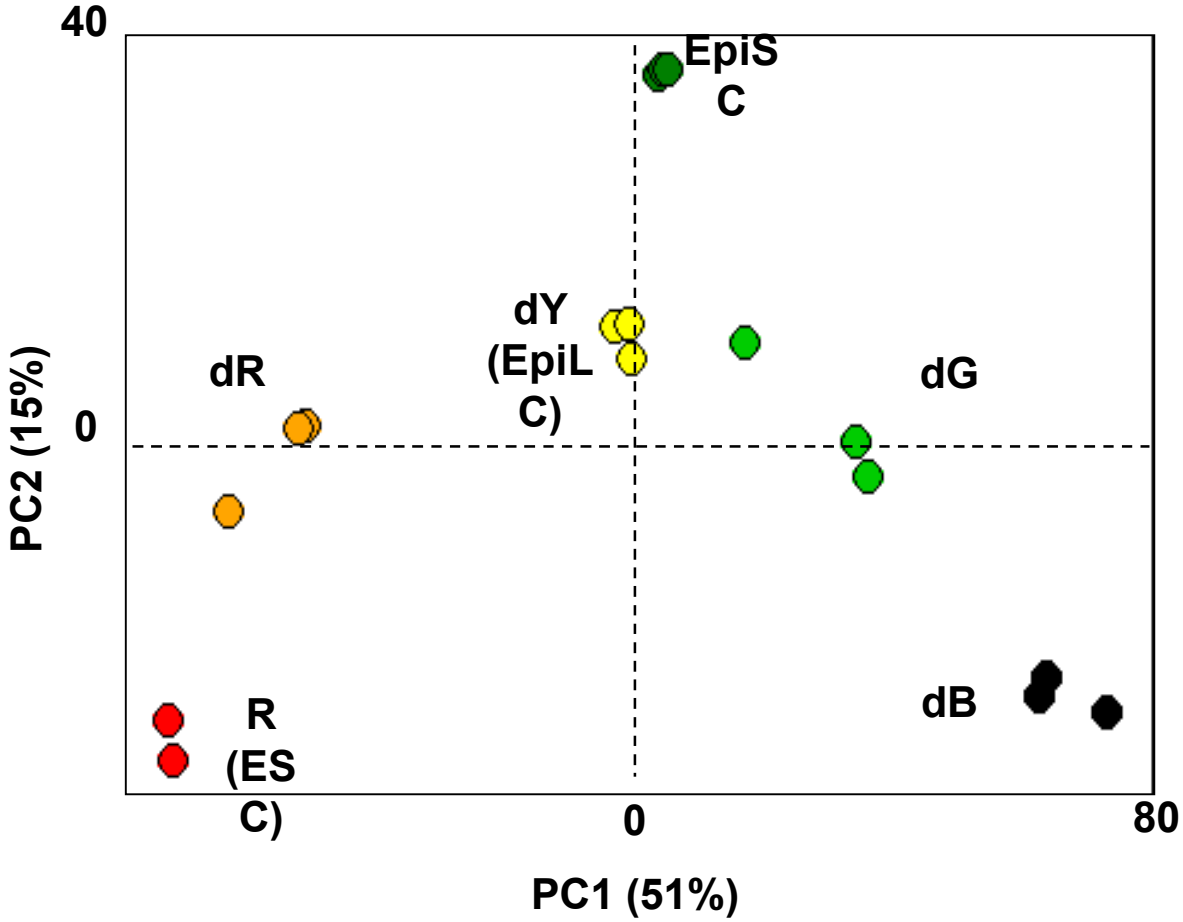
Principal component analysis of populations demonstrates relationships during differentiation



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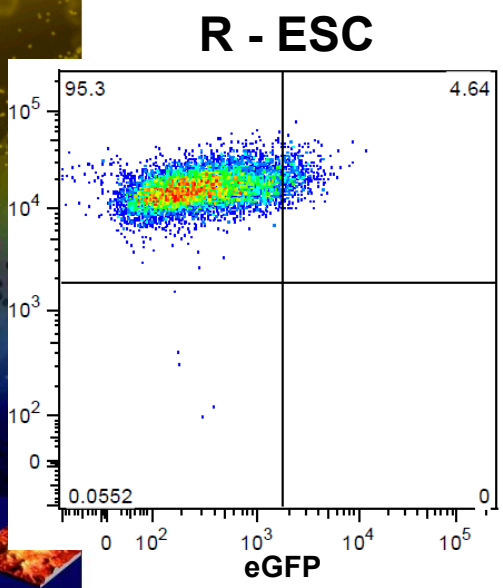
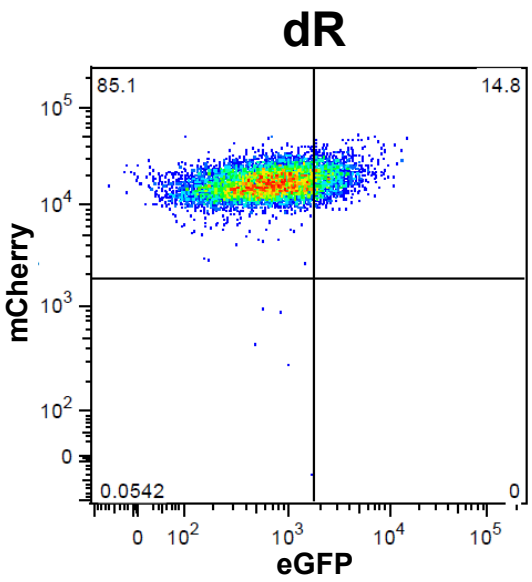
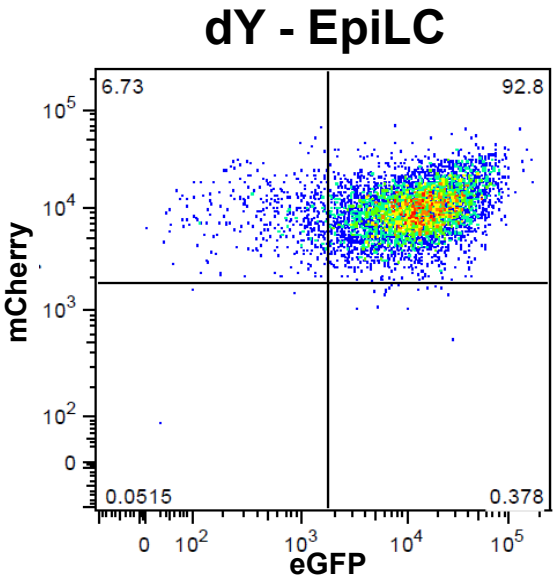
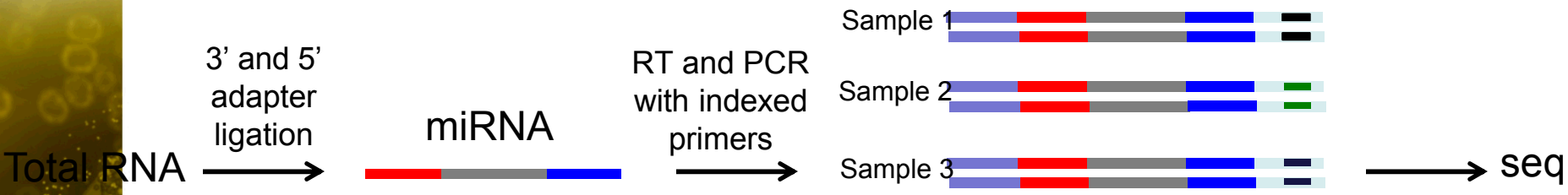
Homogeneous population of ESCs differentiating to EpiLCs

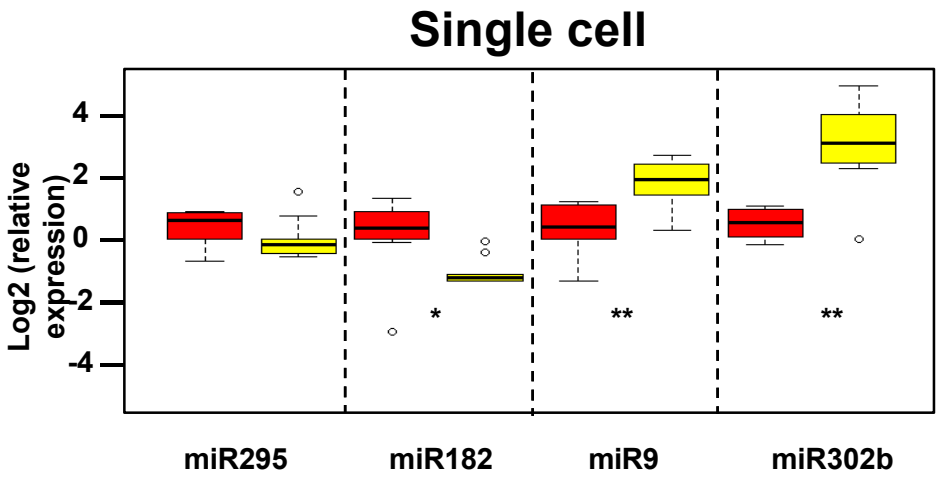


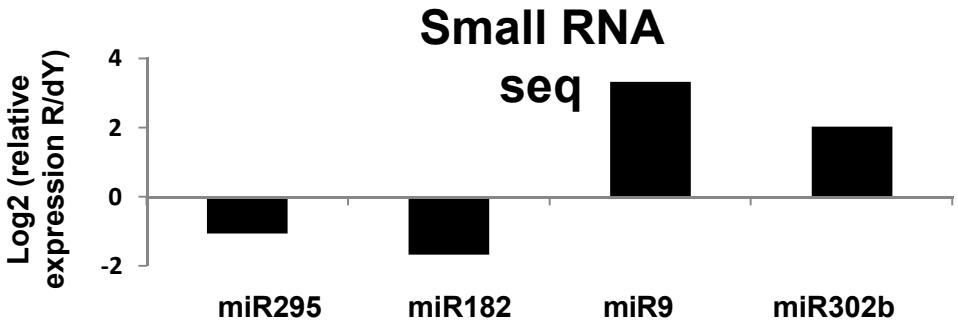




Single-cell miRNA qPCR shows homogeneity in populations







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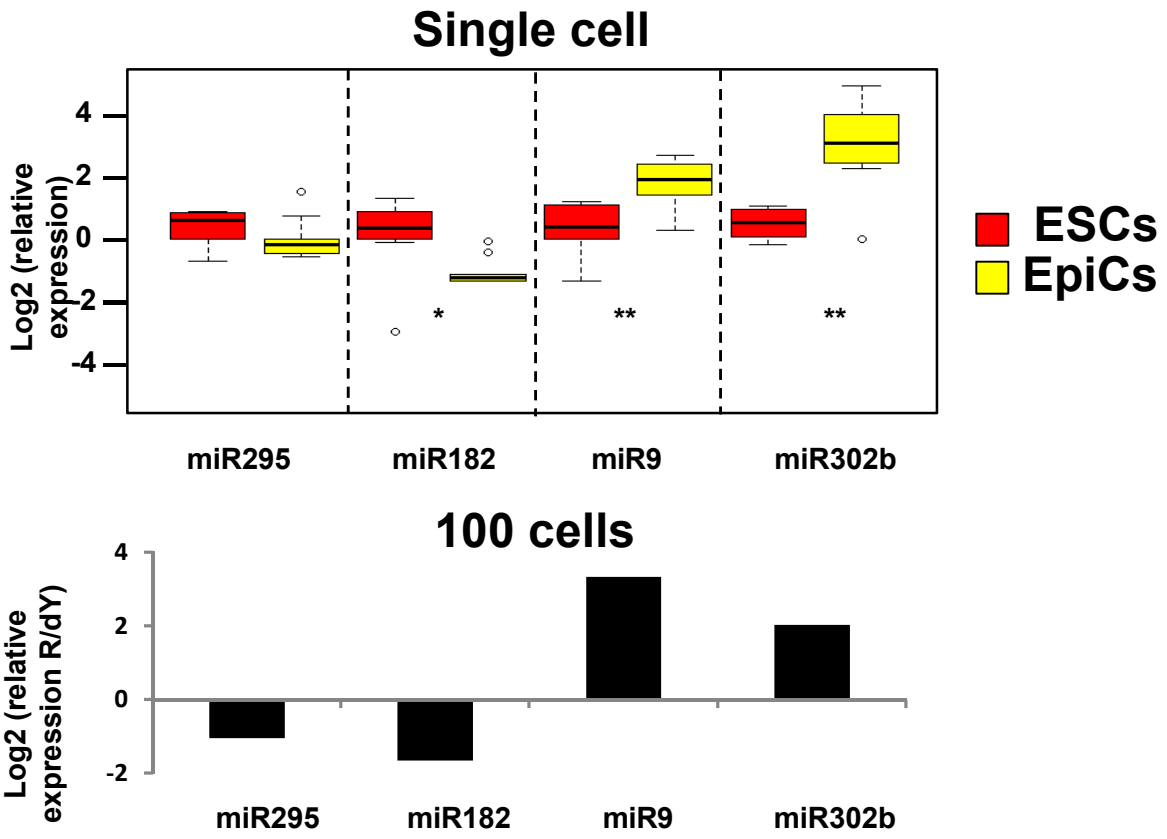
Sandia National Laboratories

Single-cell miRNA qPCR shows homogeneity in populations



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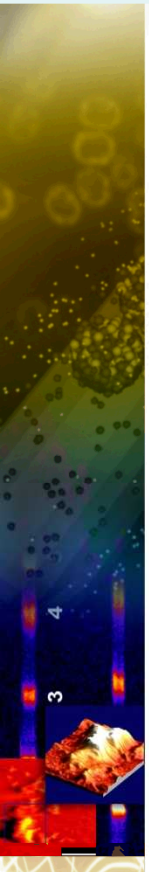
Using ChromHMM to Identify Chromatin Domains in ChIP-seq Data



- Uses hidden markov models to determine combinatorial patterns of chromatin marks
  - H3K4me1
  - H3K27ac
  - H3K27me3
  - p300
- Input: data + number of “states” you want to identify
  - upper limit depending on number of conditions and chromatin marks
- Model re-tested on independent replicates

Ernst and Kellis 2012 (Nature Methods)

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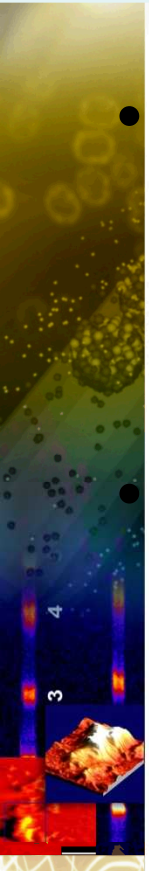
Which genes does Foxd3 regulate?

- Neighboring gene:
  - Didn't focus just on expressed genes, since Foxd3 is known to be a repressor

- In the future need to combine information from a number of data sets to determine cognate genes of regulatory regions:
  - Chromatin landscape
  - Gene expression
  - 3D interaction data
  - Functional experiments



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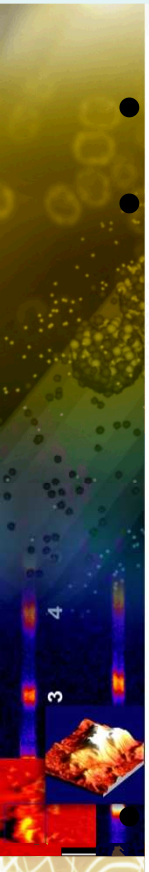
Gene Ontology – linking genomic locations to biological function



- Assigning function to binding – big challenge!
- Gene ontology:
  - Choosing the right background gene set
    - All annotated genes? Expressed genes? If array, genes on array?
  - Choosing the correct statistical test
    - Hypergeometric? Binomial? Chi-squared?
  - Correcting for multiple-hypothesis testing
    - Bonferroni, sidak, FDR?
- Most methods yield similar but not identical results – showing trends vs follow-up on specific conclusions – GoSTAT (Beissbarth and Speed 2004)

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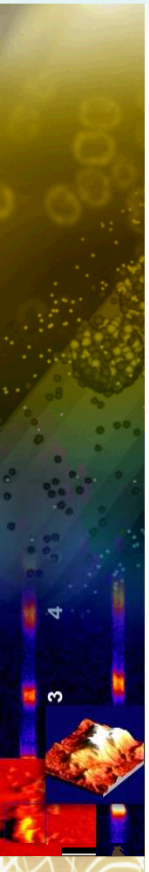
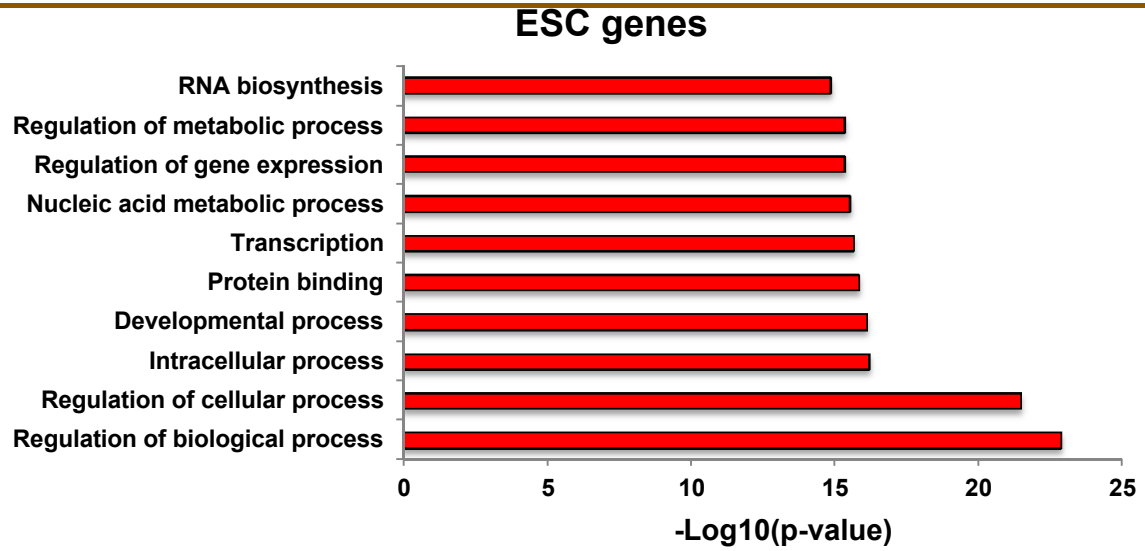
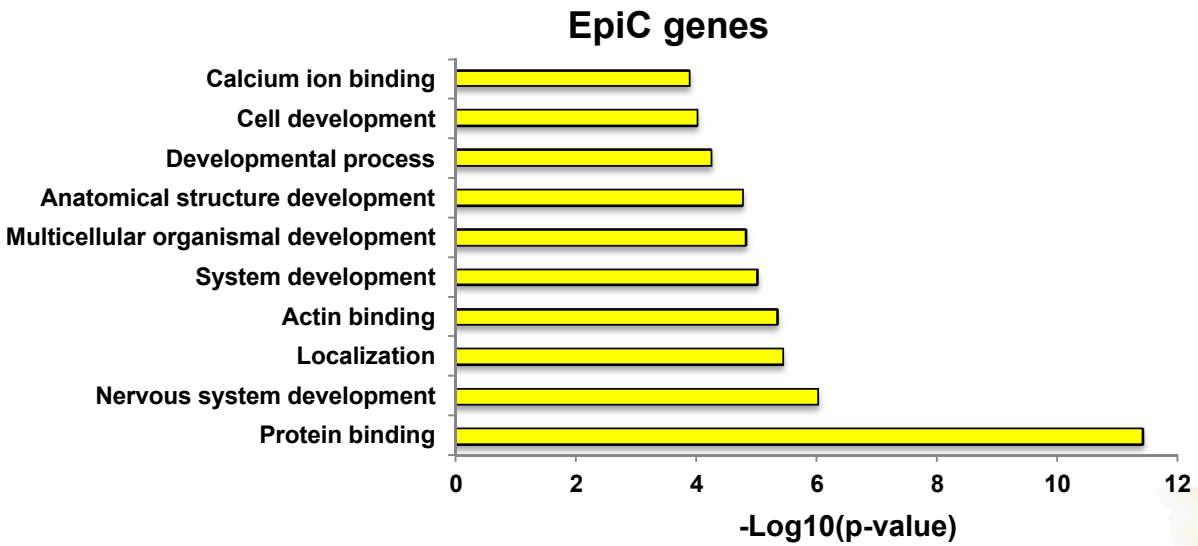
Genes near Foxd3 binding sites are involved in distinct pathways in ESCs and EpiCs







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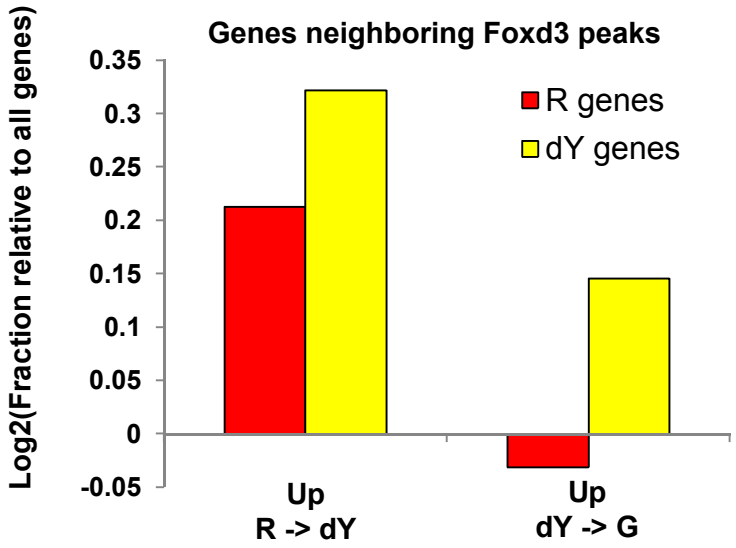
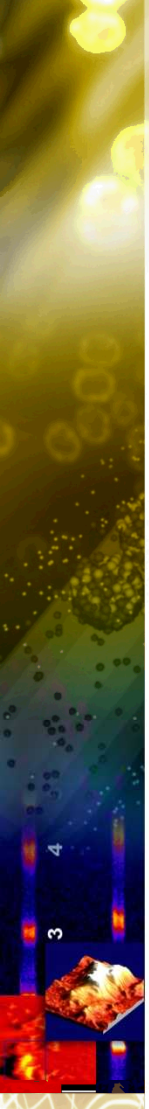

Genes neighboring Foxd3 peaks
Log2(Fraction relative to all genes)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
R genes
dY genes
Up
R -> dY
Up
dY -> G



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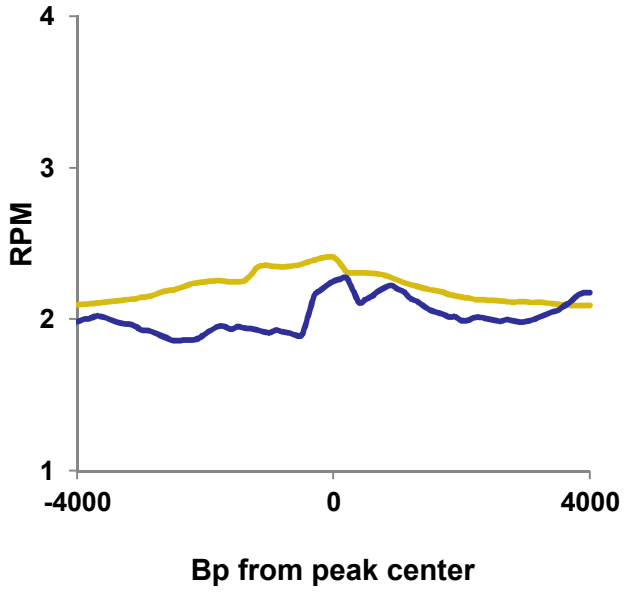
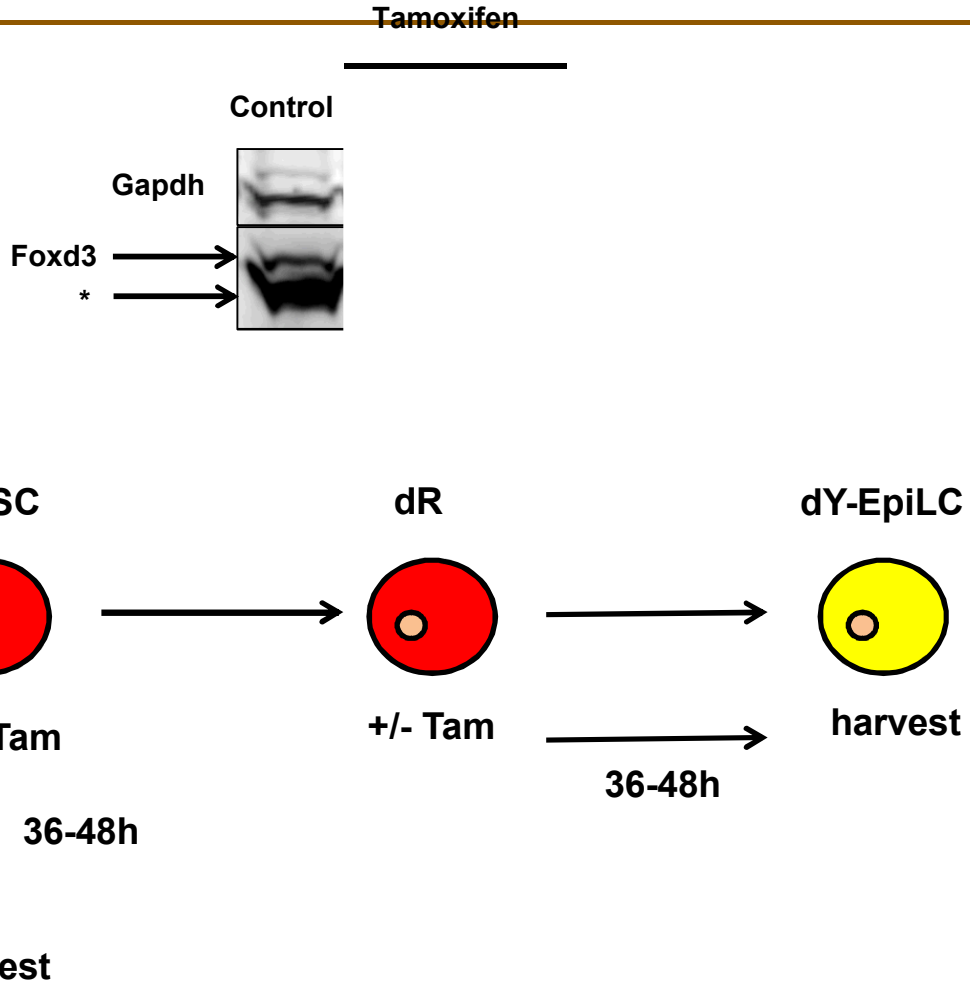
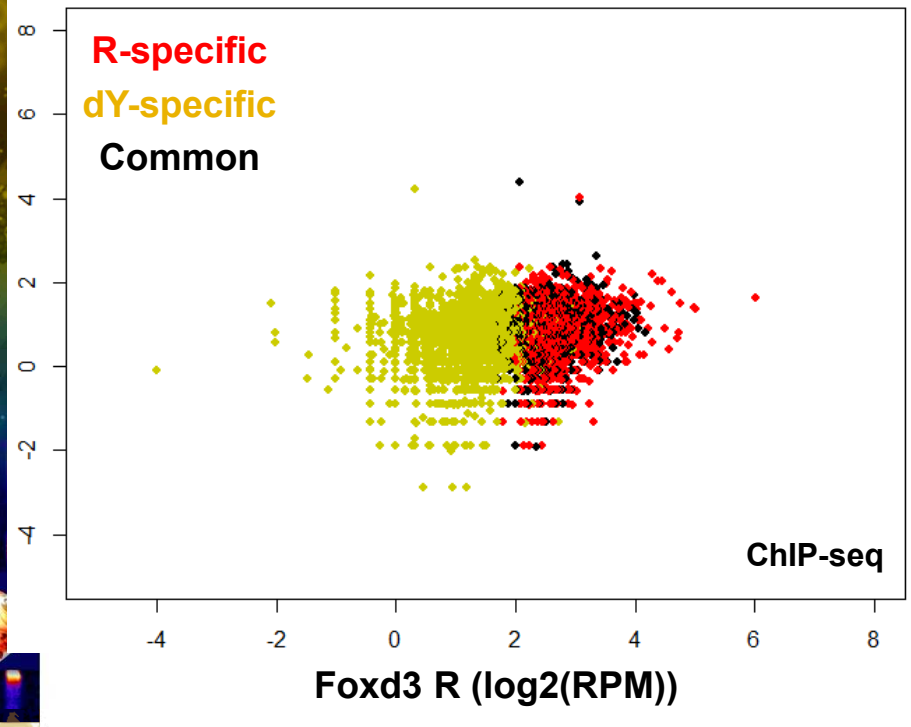
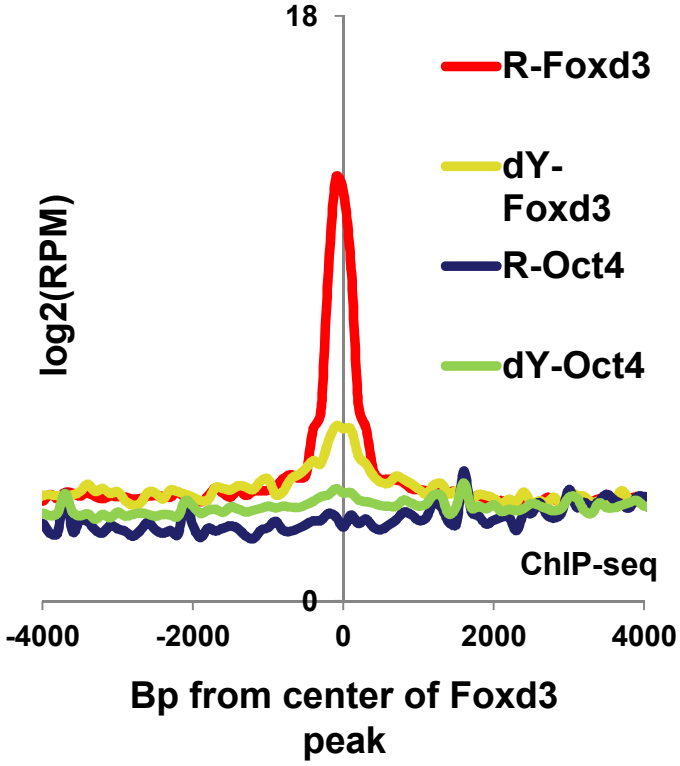


Foxd3 knockout ESCs and EpiLCs



Foxd3 does not co-localize with Oct4





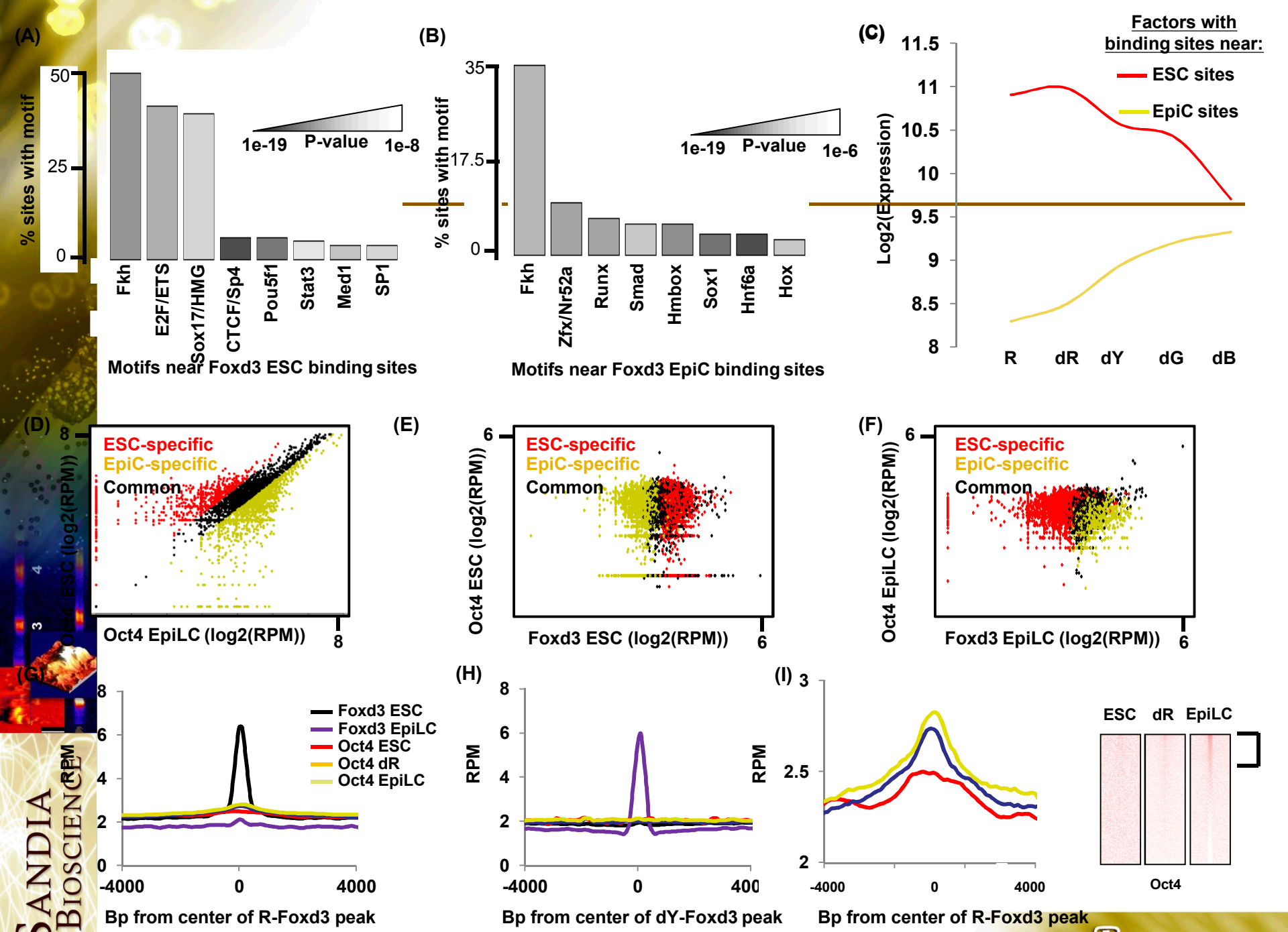


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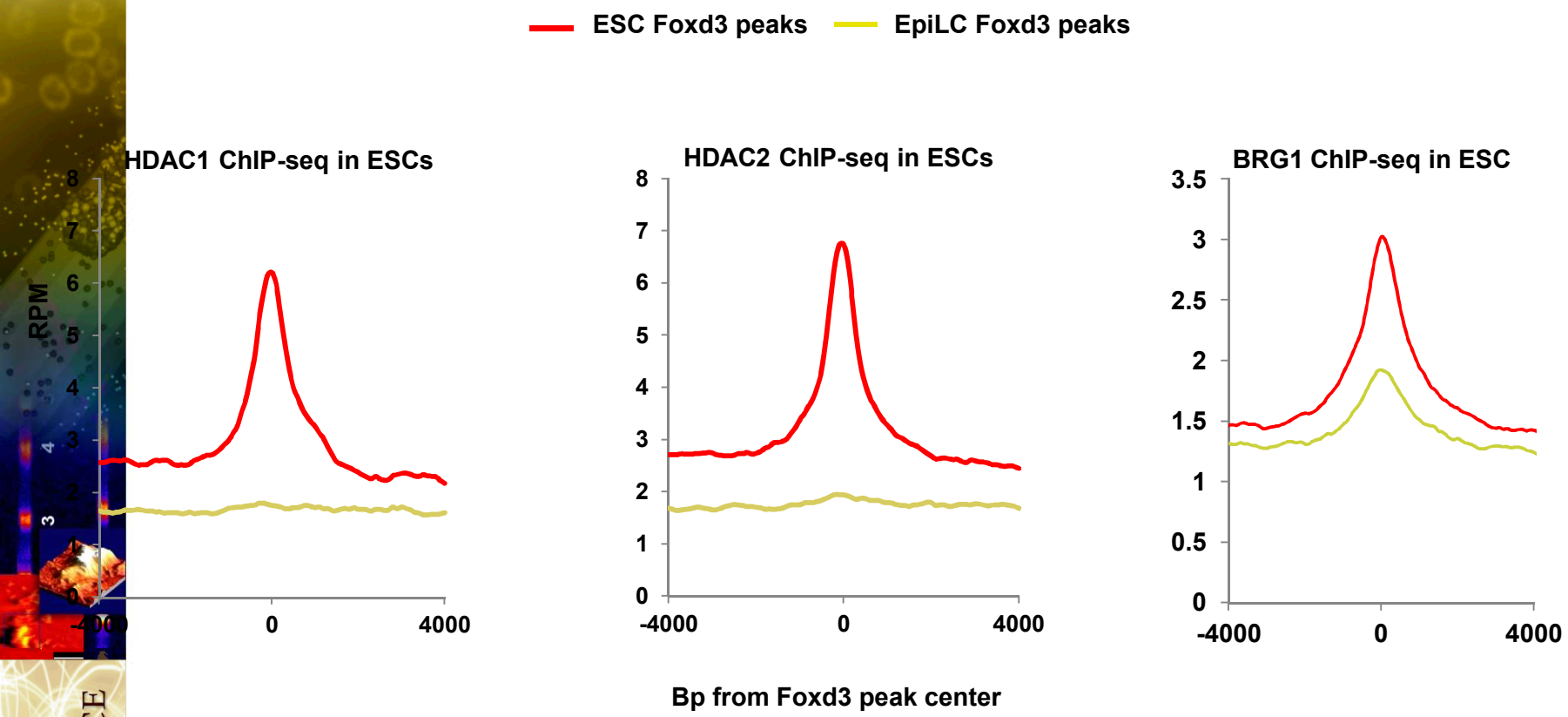
Figure 3

Brg1 and HDAC1/2 globally co-localize with Foxd3 in ESCs



Takebayashi et al 2013 (Epigenetics and Chromatin)

Whyte et al 2012 (Nature)

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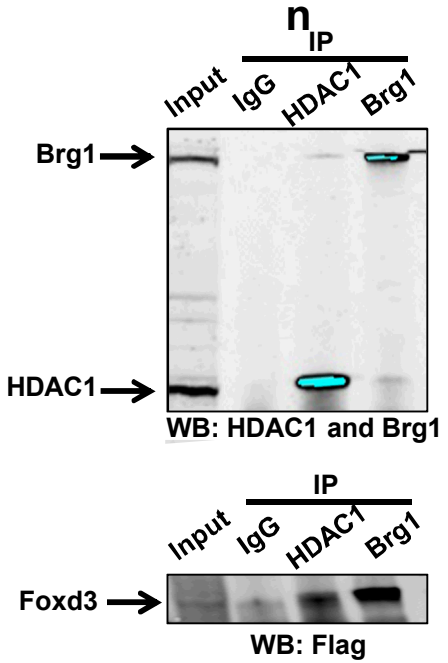
Foxd3 is in a complex with Brg1 and HDAC1

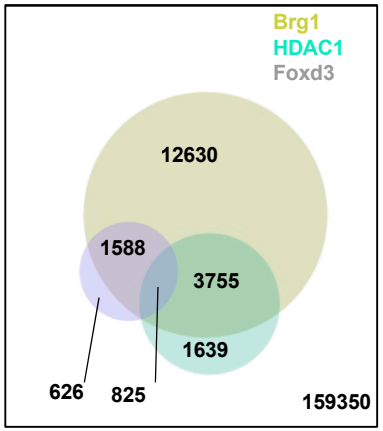
H3K27 deacetylation – HDAC1/2

Chromatin remodeling – Brg1 (SWI/SNF complex)

Immunoprecipitation

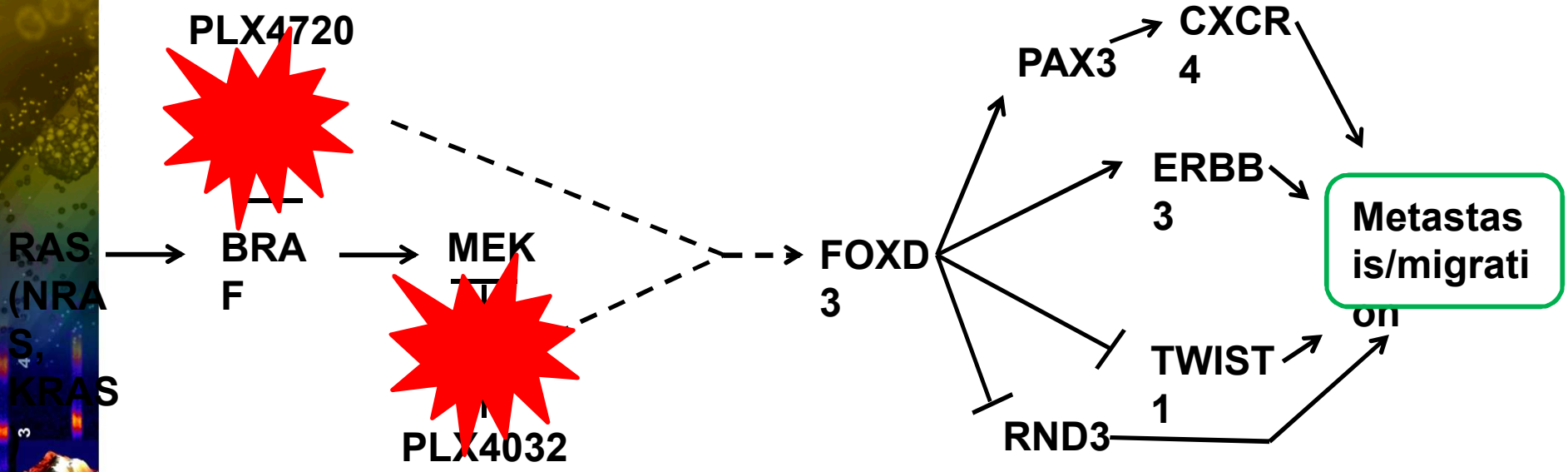


Overlap of ChIP-seq peaks



Chi-squared test p-value < 2.2e-16

Example - Foxd3 expression regulates cell fate in melanoma



Katiyar and Aplin 2011 (Mol Cancer Res)
Kubik et al 2015a, b (JBC)
Weiss et al 2014 (Mol Cancer Res)