

Functional Genomics Overview

RORY STARK

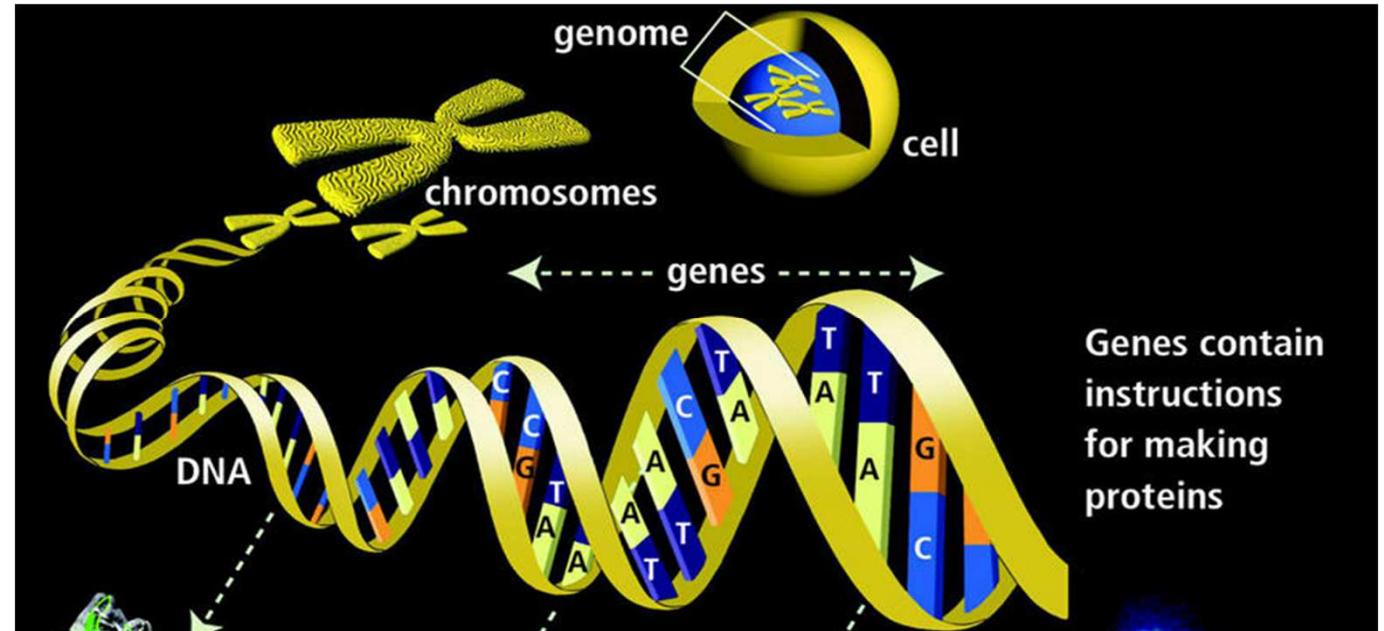
PRINCIPAL BIOINFORMATICS ANALYST
CRUK – CAMBRIDGE INSTITUTE

18 SEPTEMBER 2017

Agenda

- What is Functional Genomics?
- RNA Transcription/Gene Expression
- Measuring Gene Expression
 - Microarrays
 - High-throughput Sequencing
- Transcriptional Regulation
 - Transcription factors
 - Epigenetics
 - Post-transcriptional regulation

The Genome



- Each cell contains a complete copy of the genome, distributed along chromosomes (compressed and entwined DNA)
- 3×10^9 (3Gb) base pairs in human DNA: 6 meters in each cell!
- Encodes blueprint for all cellular structures and activities and which cells go where (somehow...)

Functional Genomics: Sequence vs. Function



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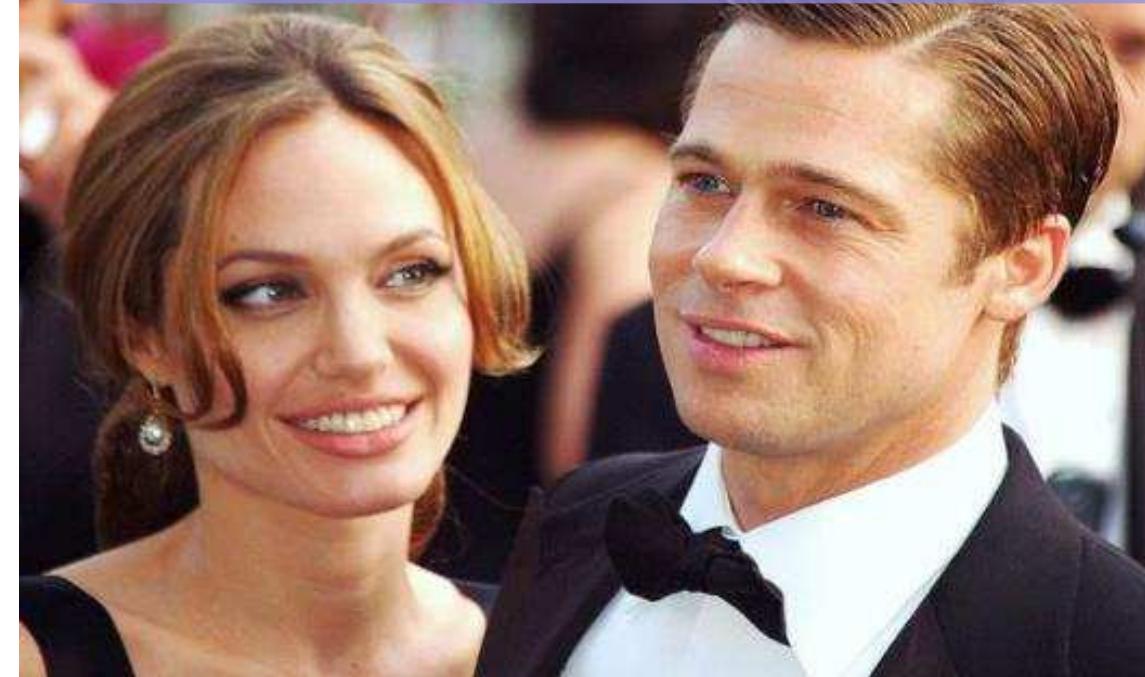
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What accounts for the difference in phenotype?



Different Genomes!



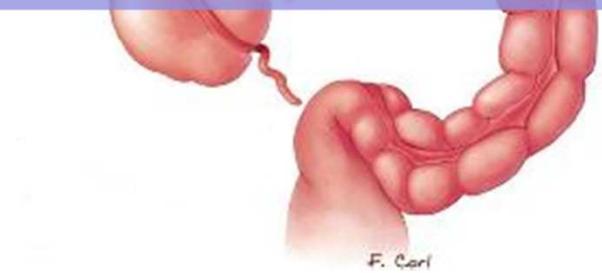
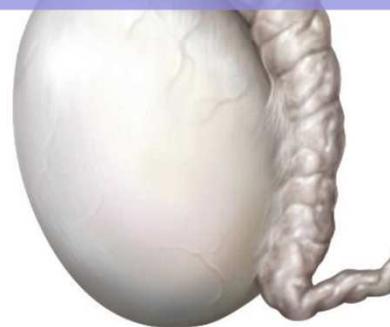
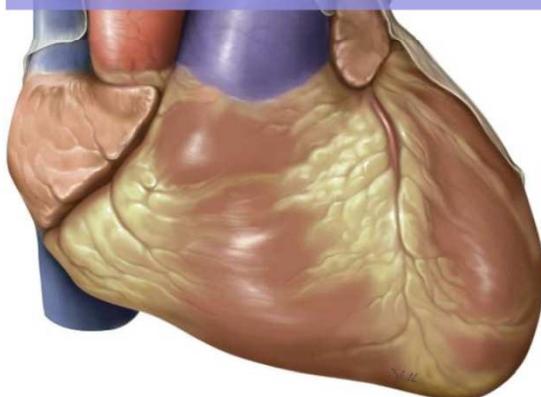
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What accounts for the difference in phenotype?



Different Functions!



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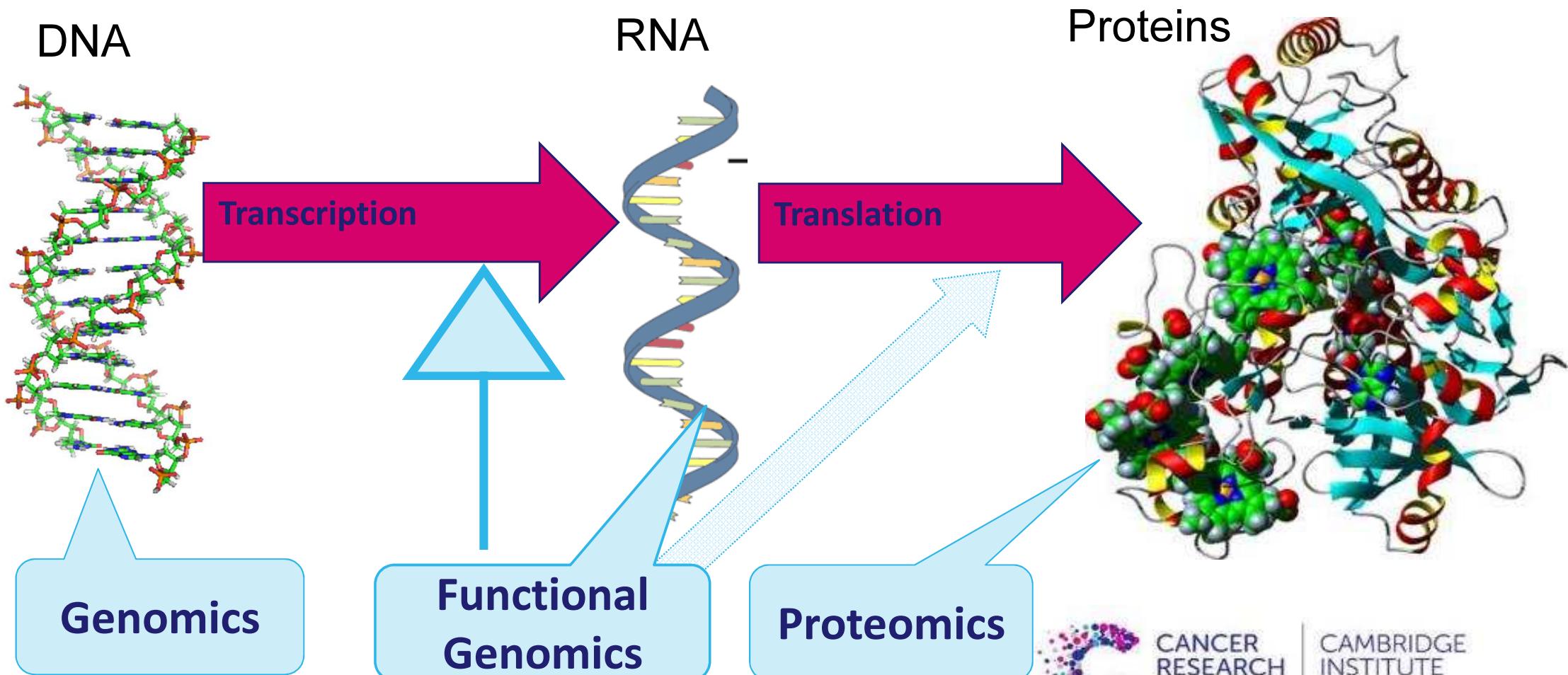
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The Central Dogma of Molecular Biology



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So, what is functional genomics?

- Where *sequence-based genomics* looks at the structure and components of genomes, and analyses the similarities and differences between genomes...
- *Functional genomics* looks at **how genomes result in cellular phenotypes**, and analyses differences in how the same genome functions differently in different cells, and how changes in genomes alter function



Gene Expression (RNA Transcripts)



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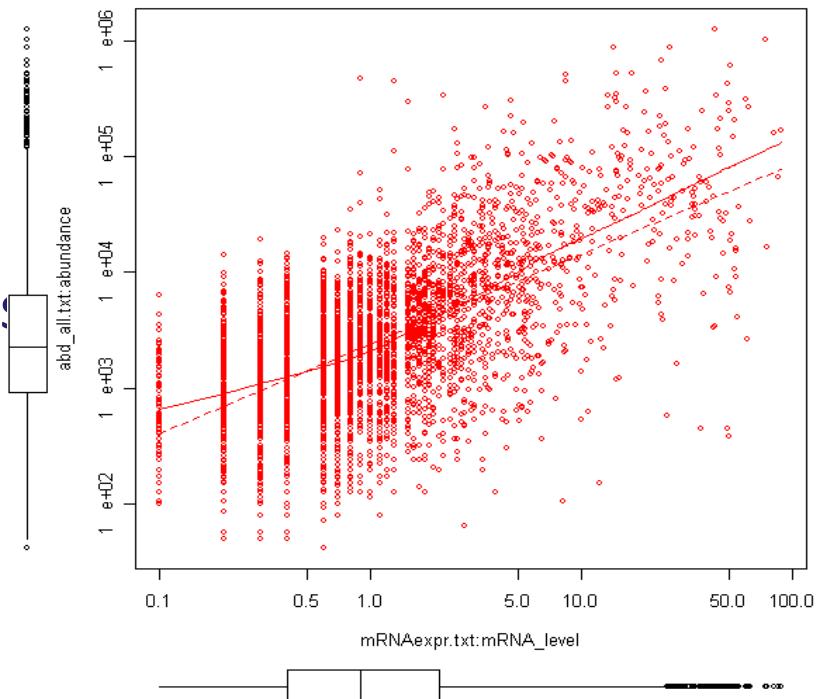
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Gene expression experiments

- Measure the **expression** levels of many genes in parallel
- Ideally, we'd measure all protein levels
- However, proteomics is difficult!
- Instead, use mRNA ("transcript") levels as a **proxy** for protein levels
- (How good a proxy is RNA?)
- Several good ways to measure RNA
- Analyses:
 - Expression levels
 - **Differences in expression levels (DE)**
 - Patterns of expression
 - Splicing and isoforms

mRNA vs Protein levels in Yeast
 $R=0.44$



Ghaemmaghami et al Nature 2003

What kinds of samples are we interested in?

- Different **tissues**, same organism
 - human brain/human liver
- Same tissue, different **organism**
 - human liver/mouse liver
 - wt/ko
- Same tissue, same organism, different **condition**
 - benign/tumour
 - treated/untreated
- Time course (effect of treatment over time)
- *In vivo* vs *In vitro*
- ...

Measuring Gene Expression



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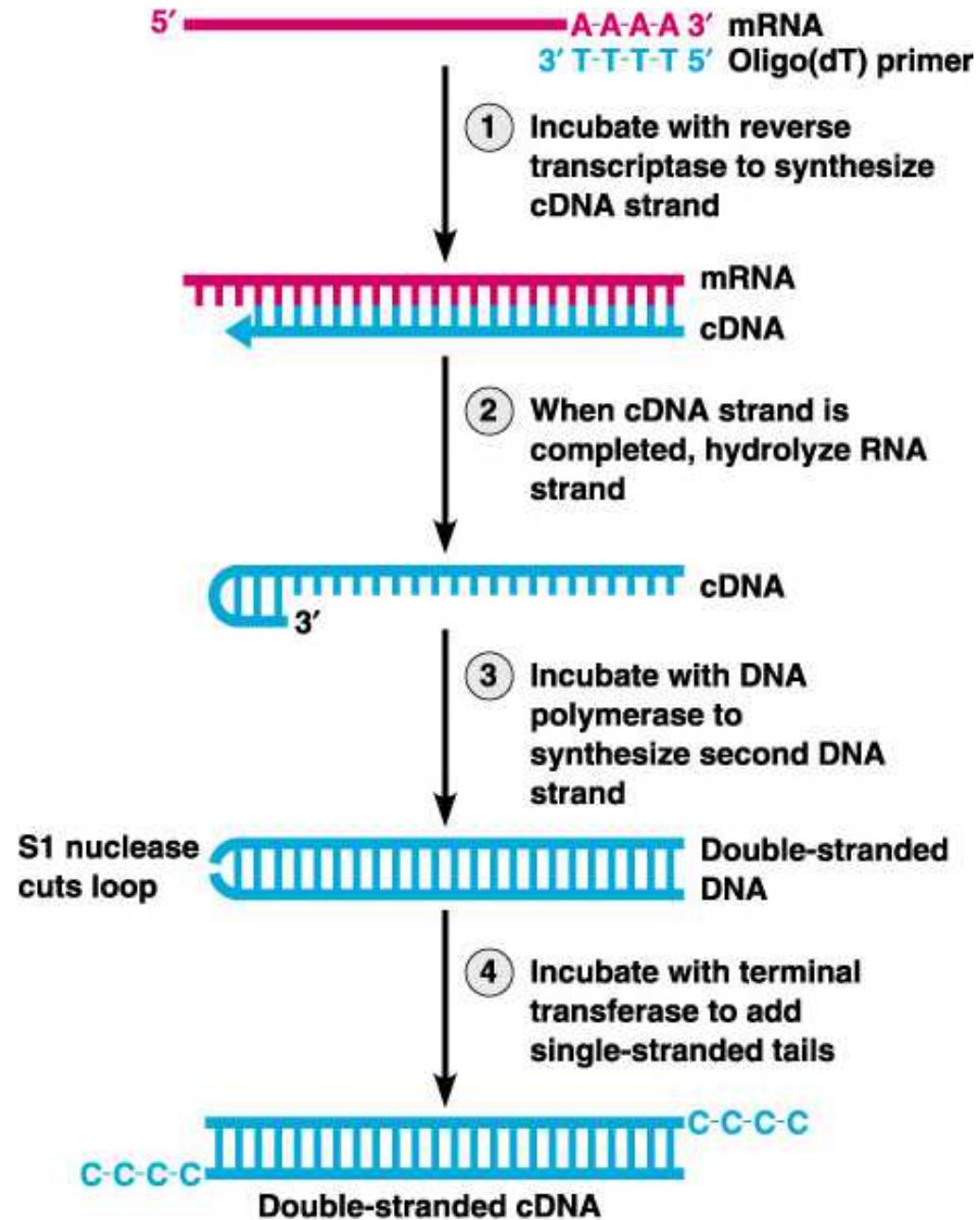


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Reverse transcription (mRNA -> cDNA)

- Most RNA-seq involves large **populations** of cells (10^{6-7})
- Most RNA-seq involves sequencing **cDNA** synthesized using reverse transcription
- Most RNA-seq involves significant **amplification** of cDNA molecules via PCR



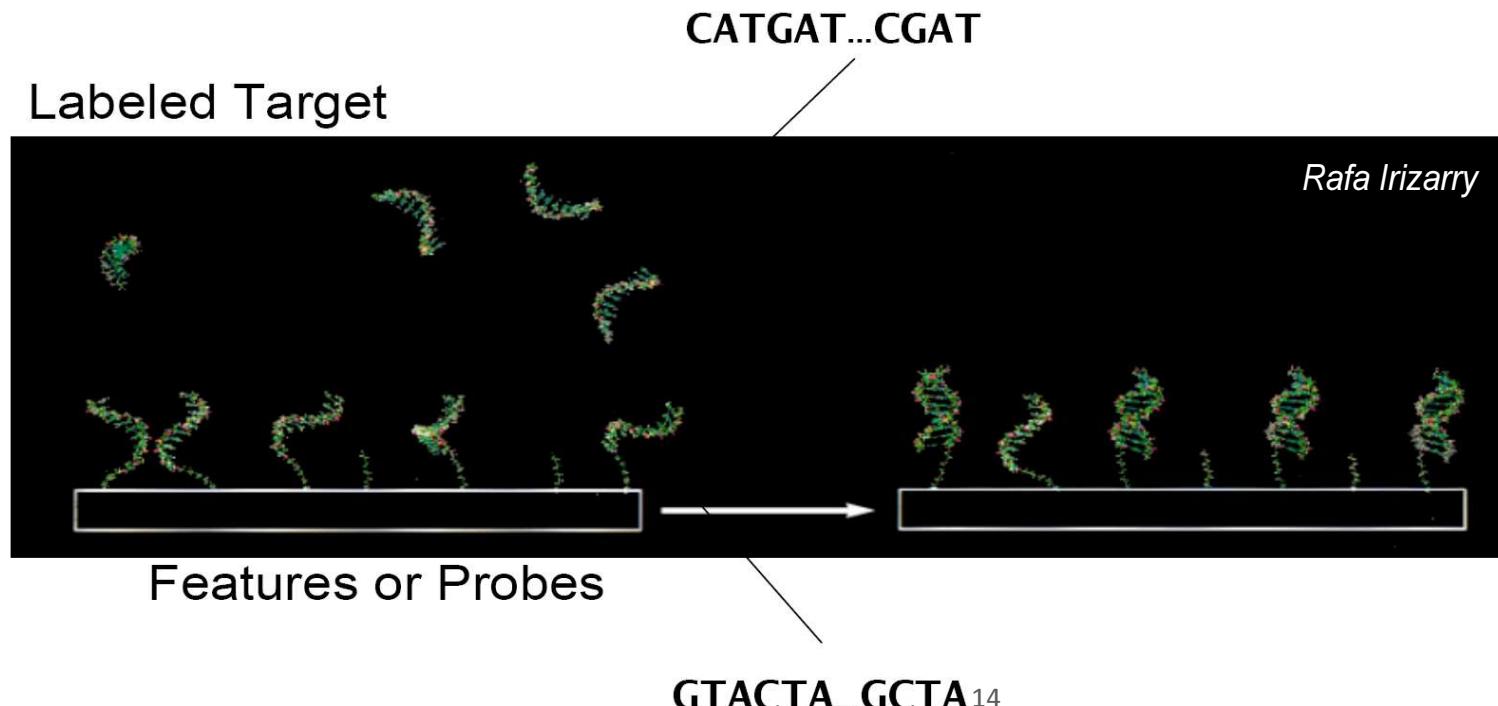
Measuring cDNA: Microarrays

Use hybridization to measure abundance of mRNA transcripts

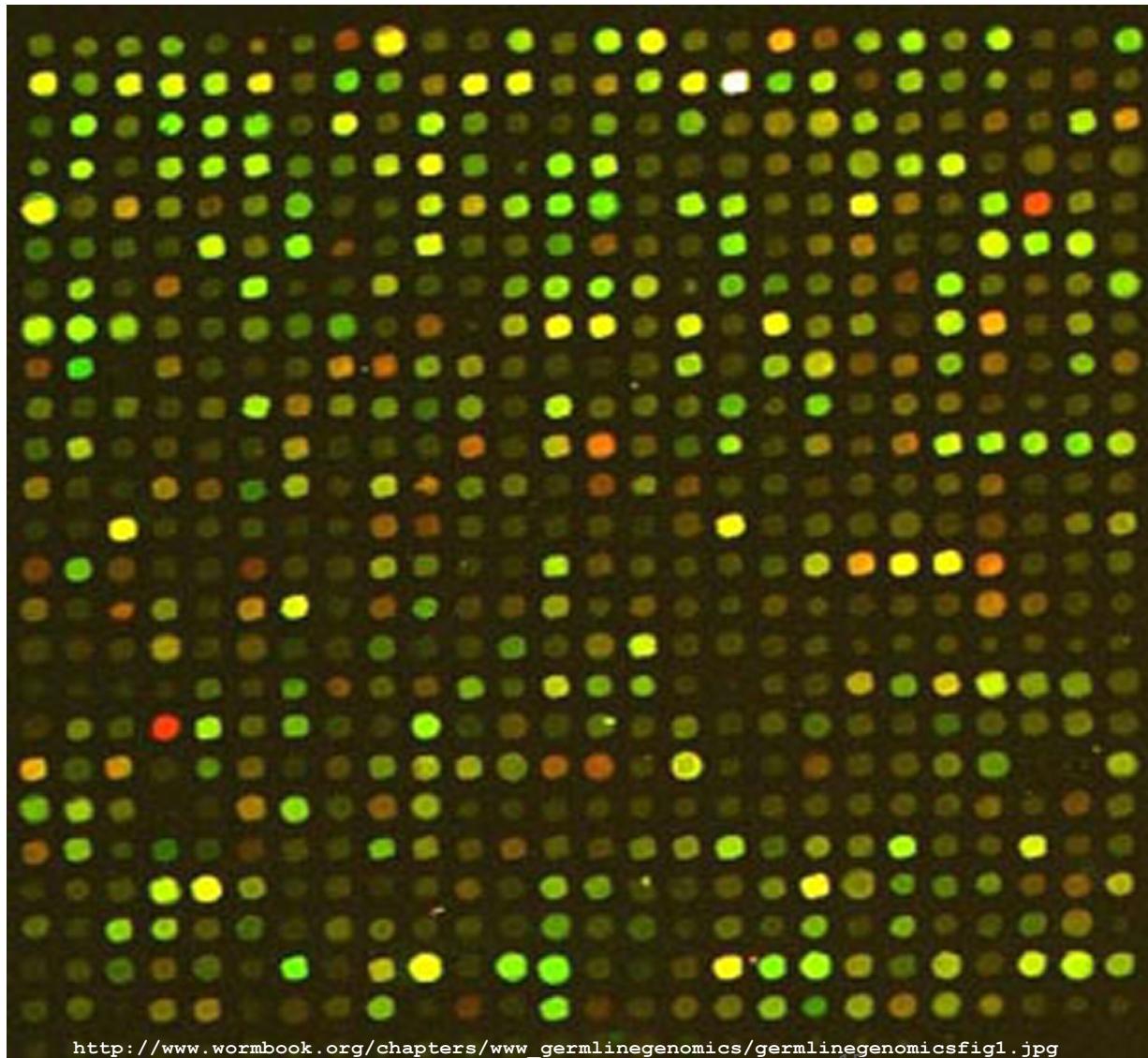
Fix “probes” to a solid support

Hybridize labeled samples of mRNA to probes

Use labels to measure hybridization intensity



Microarrays: Scanning



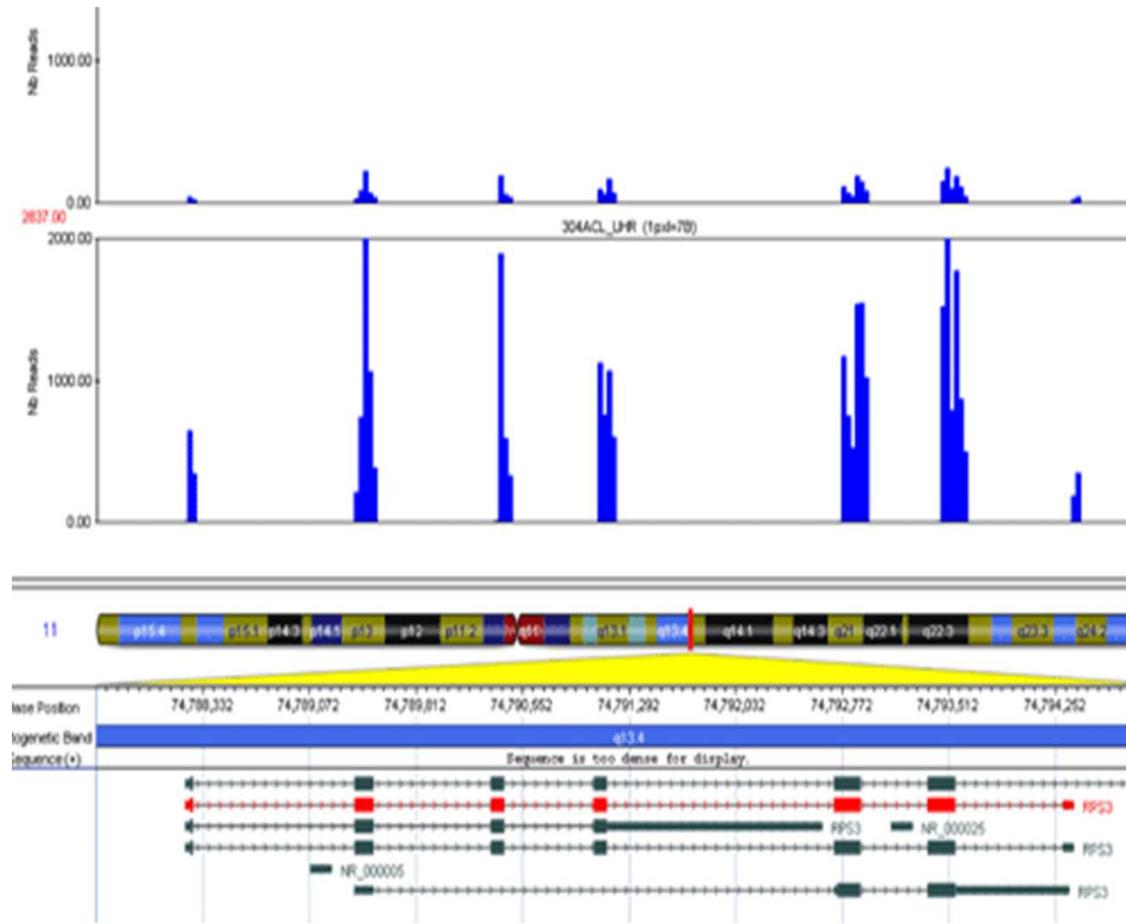
http://www.wormbook.org/chapters/www_germlinegenomics/germlinegenomicsfig1.jpg

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Typically less than 1 inch width, spot diameter ! 0.1 mm

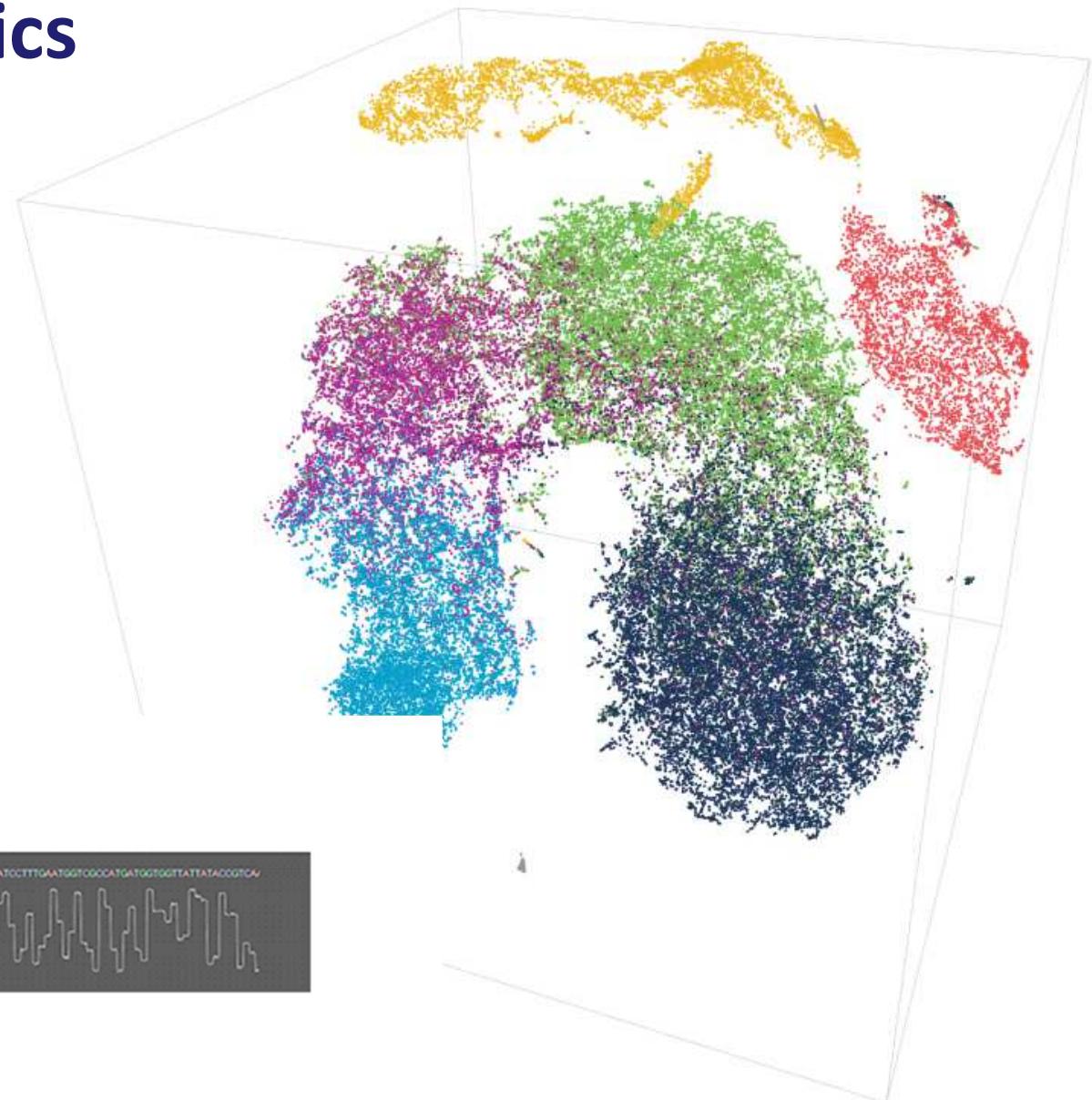
Measuring cDNA: RNA-seq

- High-throughput sequencing allows us to sequence a representative **sample** of the cDNA population “directly”
- Each sequence “read” is **aligned** back to a reference genome/transcriptome to see where it was transcribed from
- We can **count** how many transcripts came from each gene



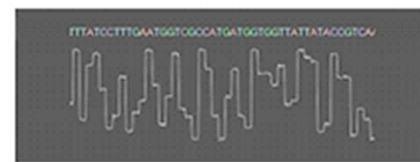
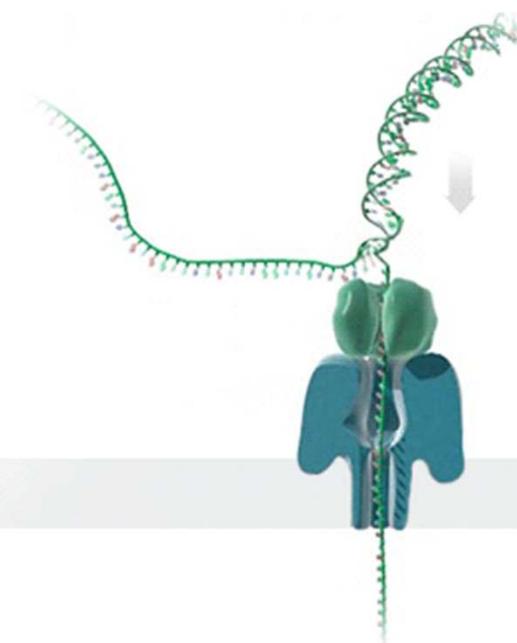
Trends in Transcriptomics

- Single-cell sequencing



- Nanopore Sequencing

- Full-length transcript sequencing
- Direct RNA sequencing



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Beyond Gene Expression: Transcriptional Regulation



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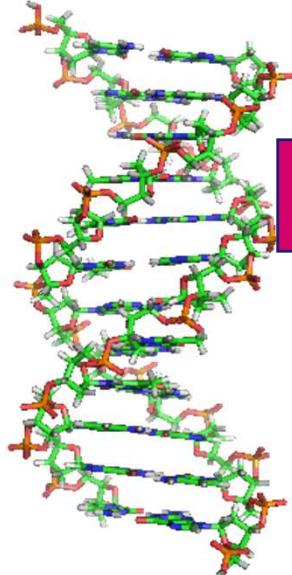


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Transcriptional Regulation

DNA



Transcription

Gene Expression

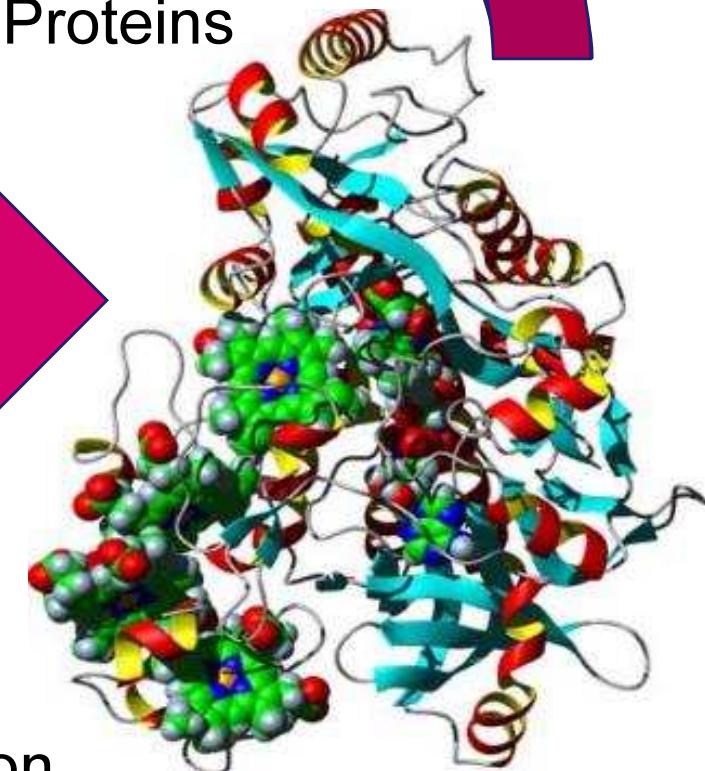
RNA



Translation

Post-transcriptional regulation

Proteins



Genes
Genomes
ACGT

Transcripts
Transcriptomes
ACGU

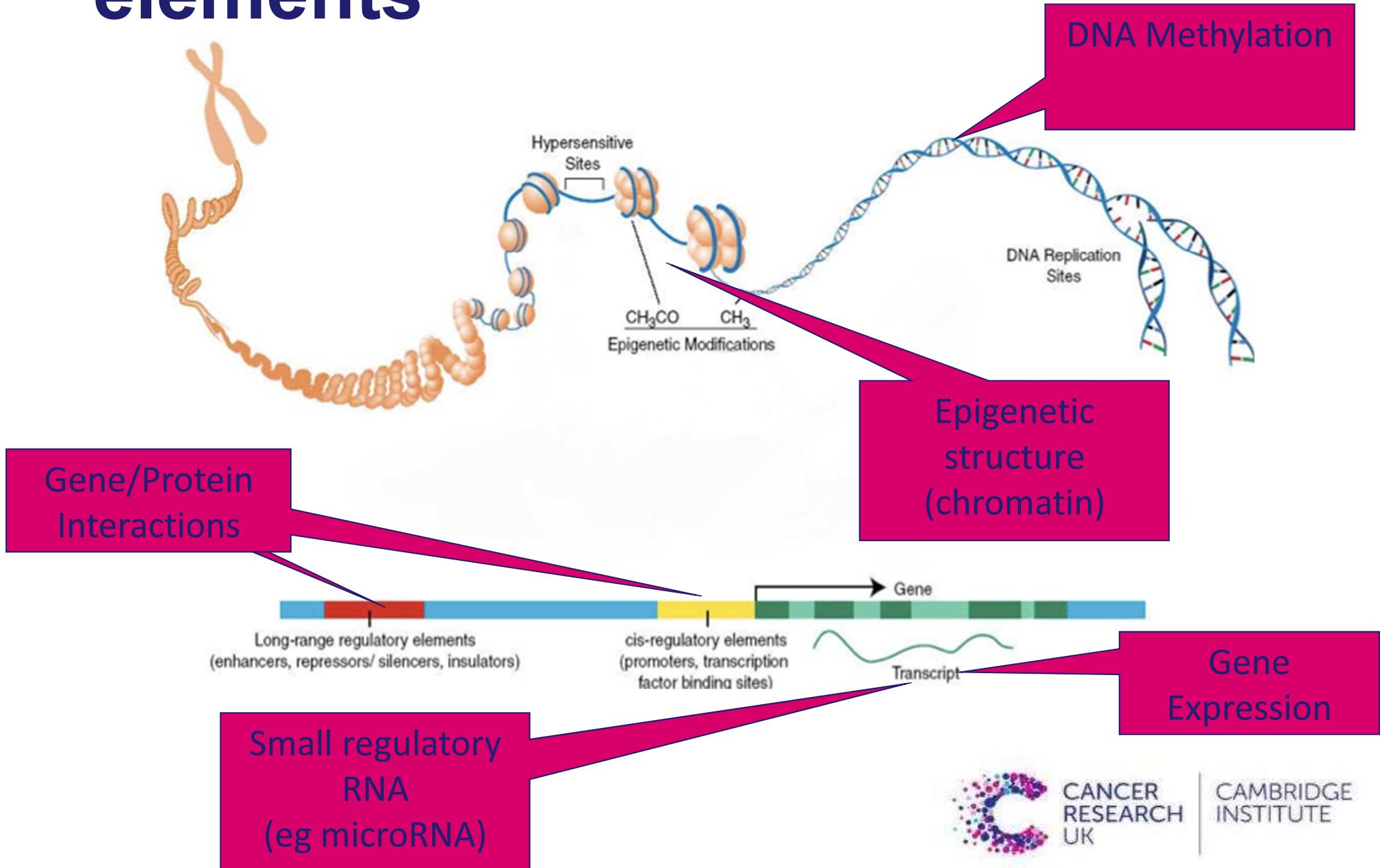
Function
Proteome
Amino Acids
HKDESTNQCUGPAVILMFYW



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Transcriptional regulatory elements



Regulatory elements of interest include...

TRANSCRIPTION FACTORS

- ChIP

HISTONE MARKS

- ChIP

DNA METHYLATION

- RRBS
- MeDIP

OPEN CHROMATIN

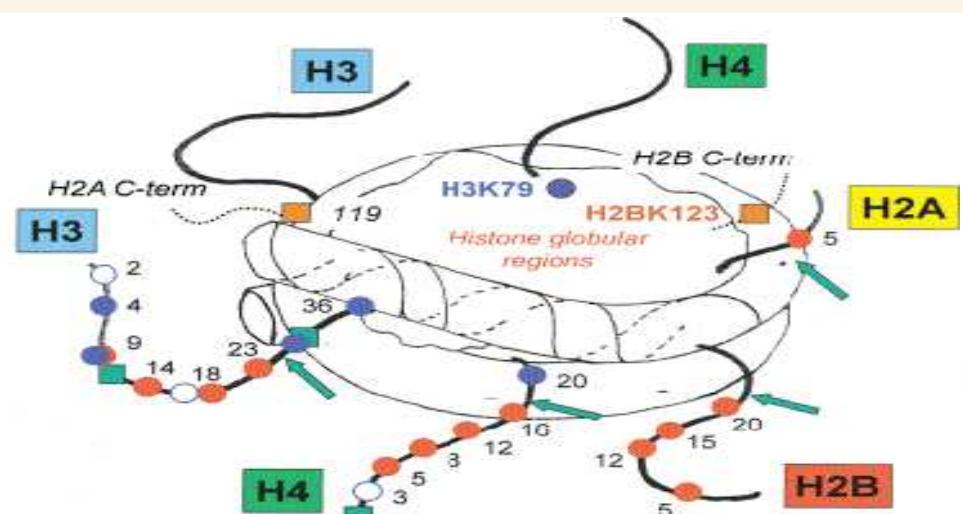
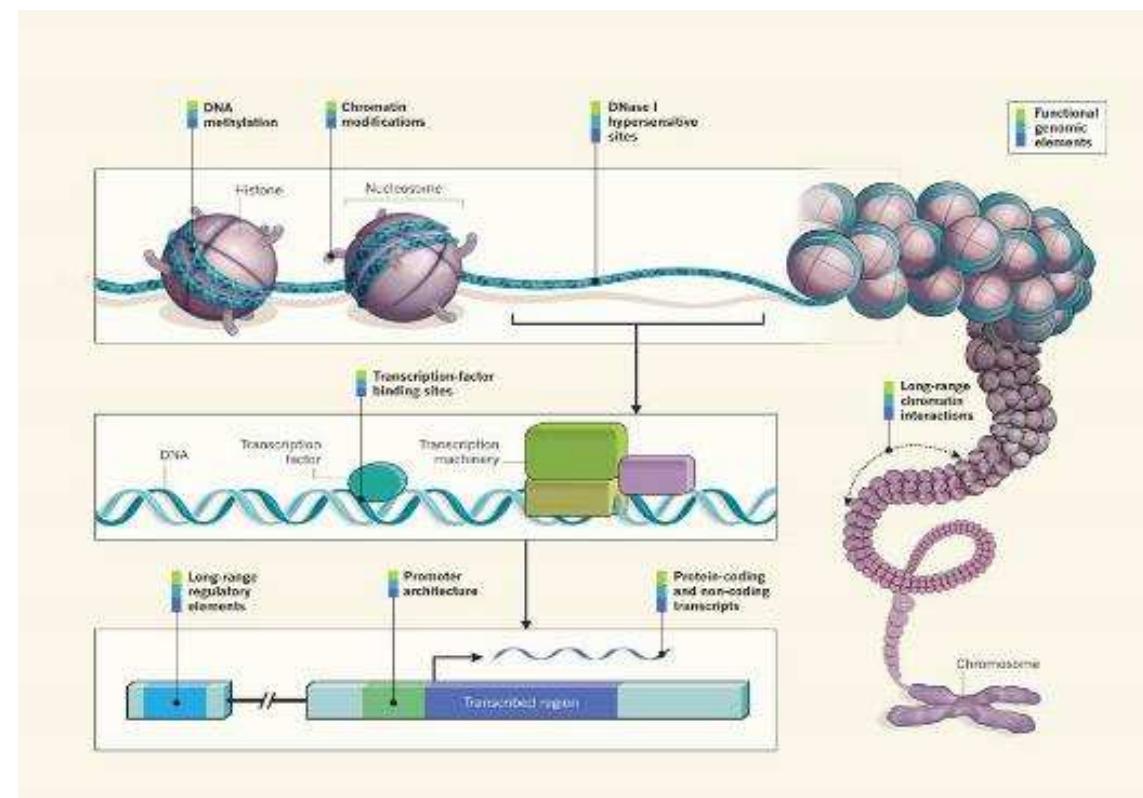
- DNase Hypersensitivity
- ATAC

CHROMATIN STRUCTURE

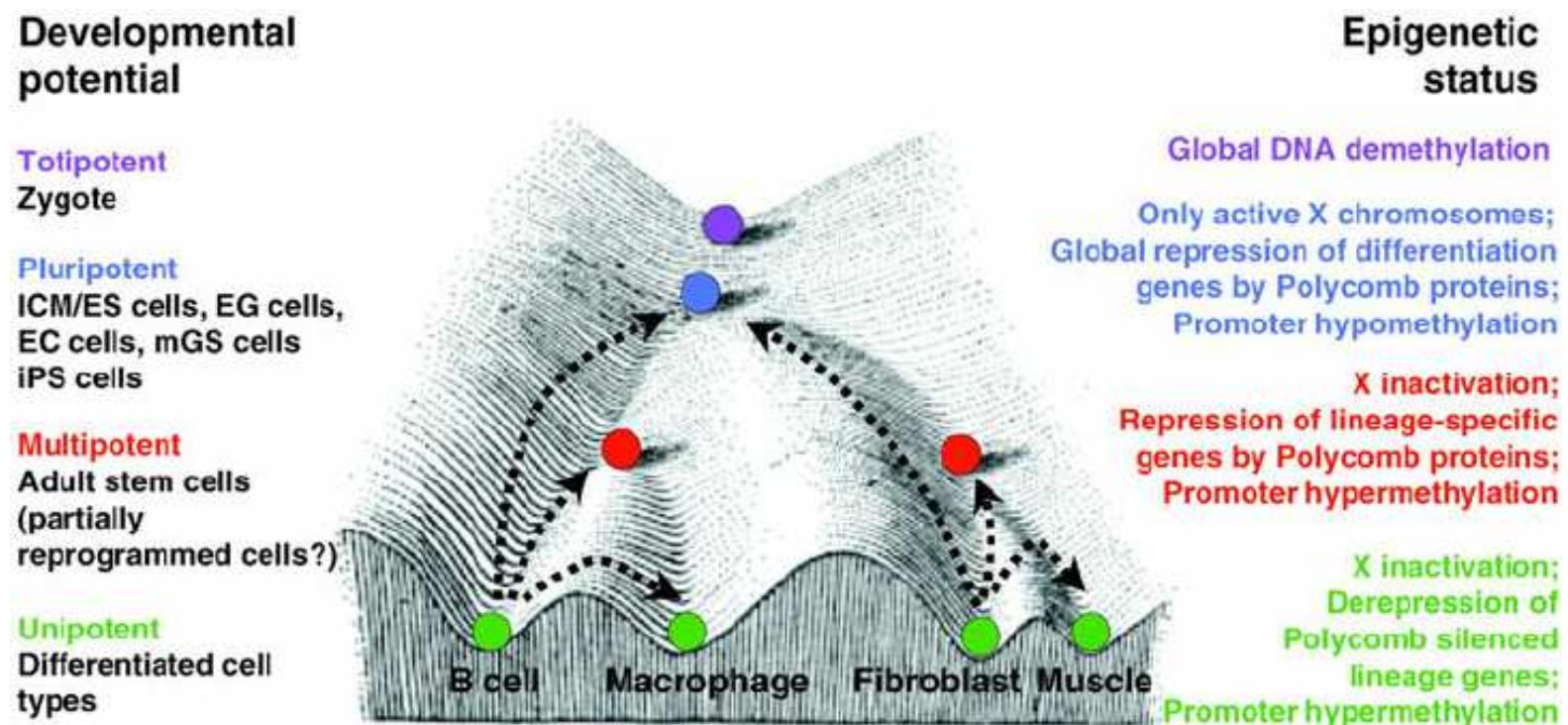
- HiC

RNA POLYMERASE

- Pol II ChIP

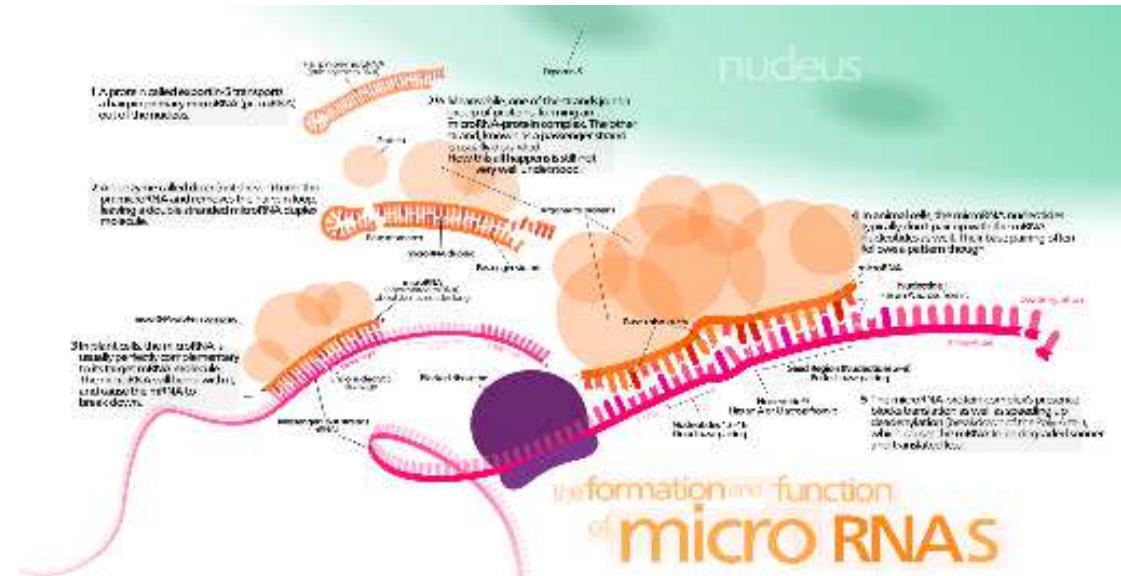


Cell differentiation



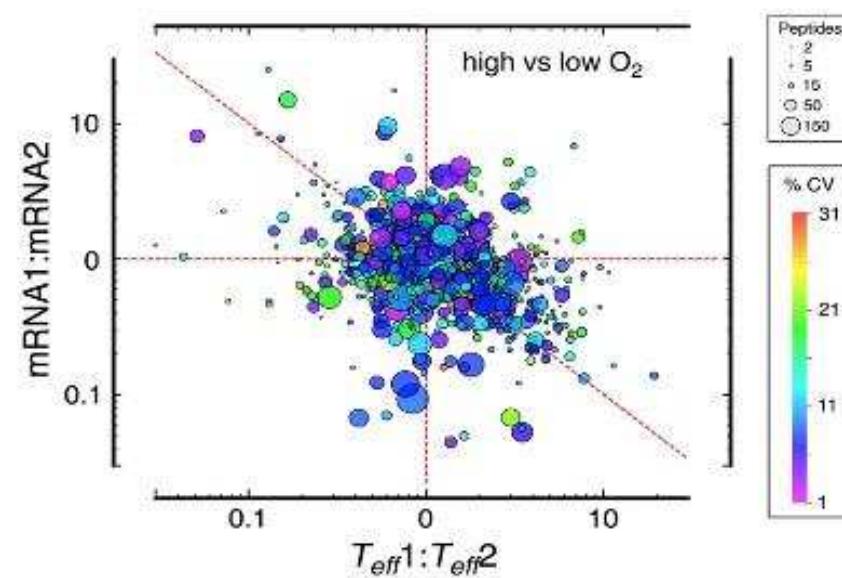
And Beyond...

- Post-transcriptional Regulation



- Translational Efficiency

- DATA INTEGRATION



Acknowledgements

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