3rd Lecture: Epigenetic Gene Regulation

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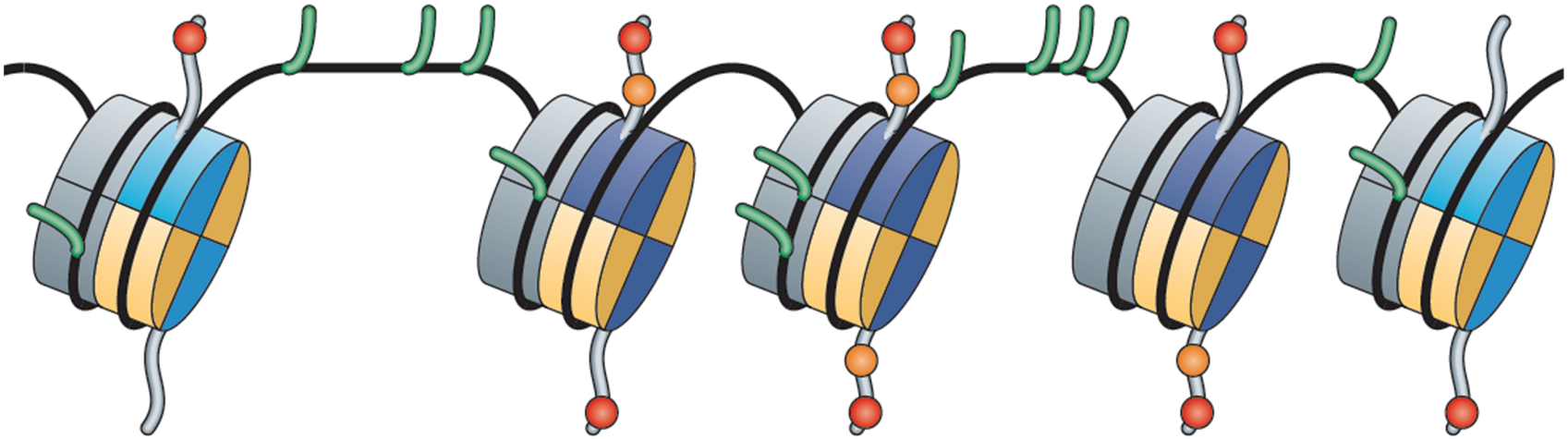
Lecture overview

1. Introduction to Epigenetic Gene Regulation



What is epigenetics, really?

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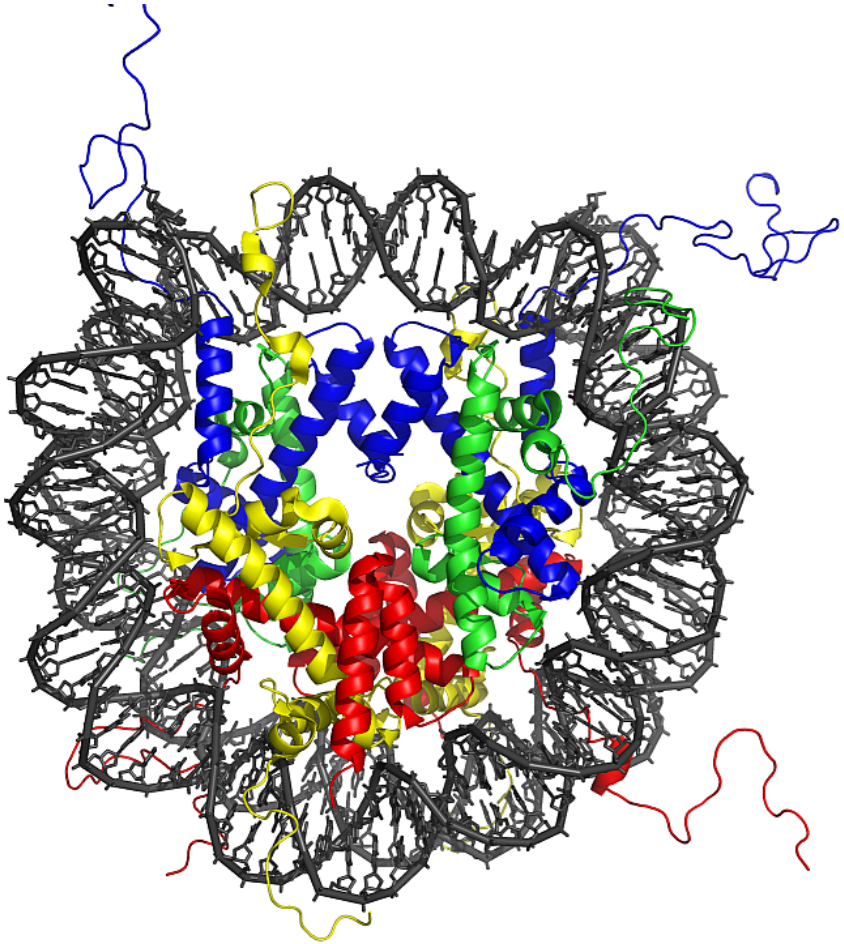
Original (and still used) definition:

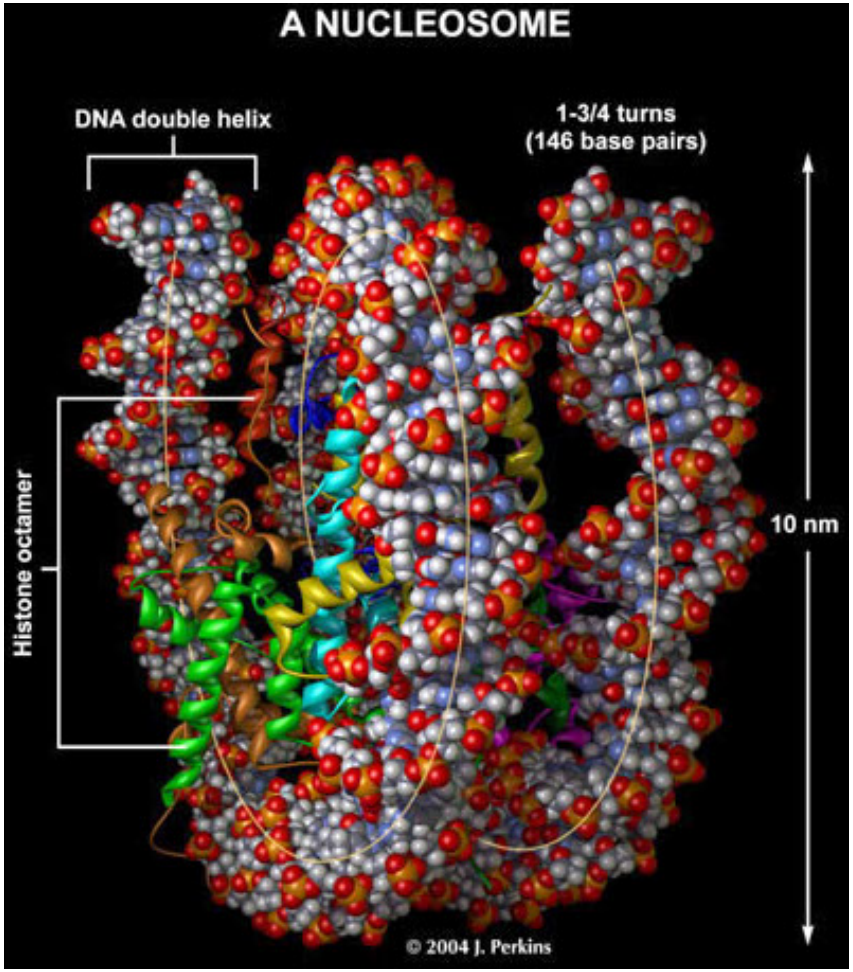
"The study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in the DNA sequence." (Riggs et al. 1996)

Now:

Anything chromatin-related

Nucleosome-DNA structure

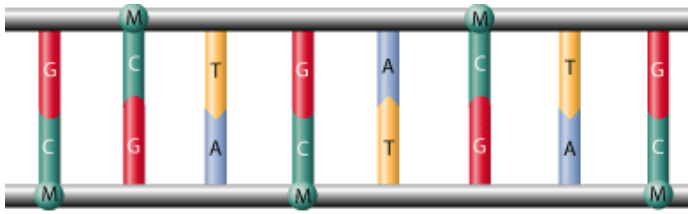


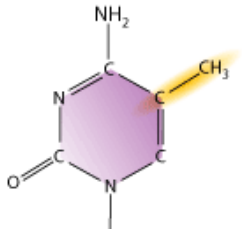


Epigenetic keyplayers

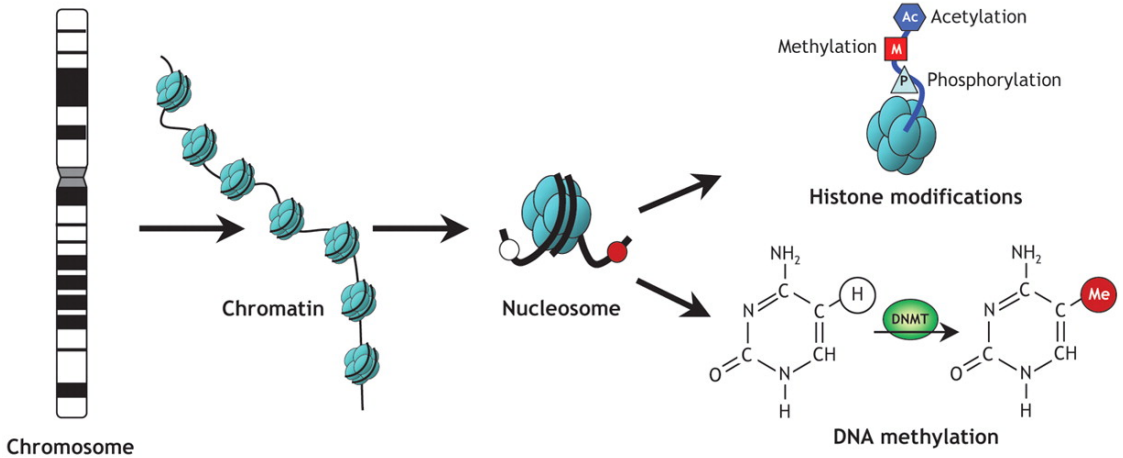
- Histone modifications: methylation, acetylation, phosphorylation, ubiquitylation, SUMOylation, propionylation, butyrylation, formylation, citrullination, proline isomerization, ADP ribosylation, tyrosine hydroxylation, lysine crotonylation, ...
- Histone variants: e.g., for H3: CenH3/CENP-A, H3.1, H3.2, and H3.3, H3.T
- Nucleosome occupancy and spacing
- Histone replacement (turnover) and nucleosome eviction
- Chromatin packaging
- DNA methylation (yielding Methylcytosine and subsequently Hydroxymethylcytosine)

Epigenetic keyplayers

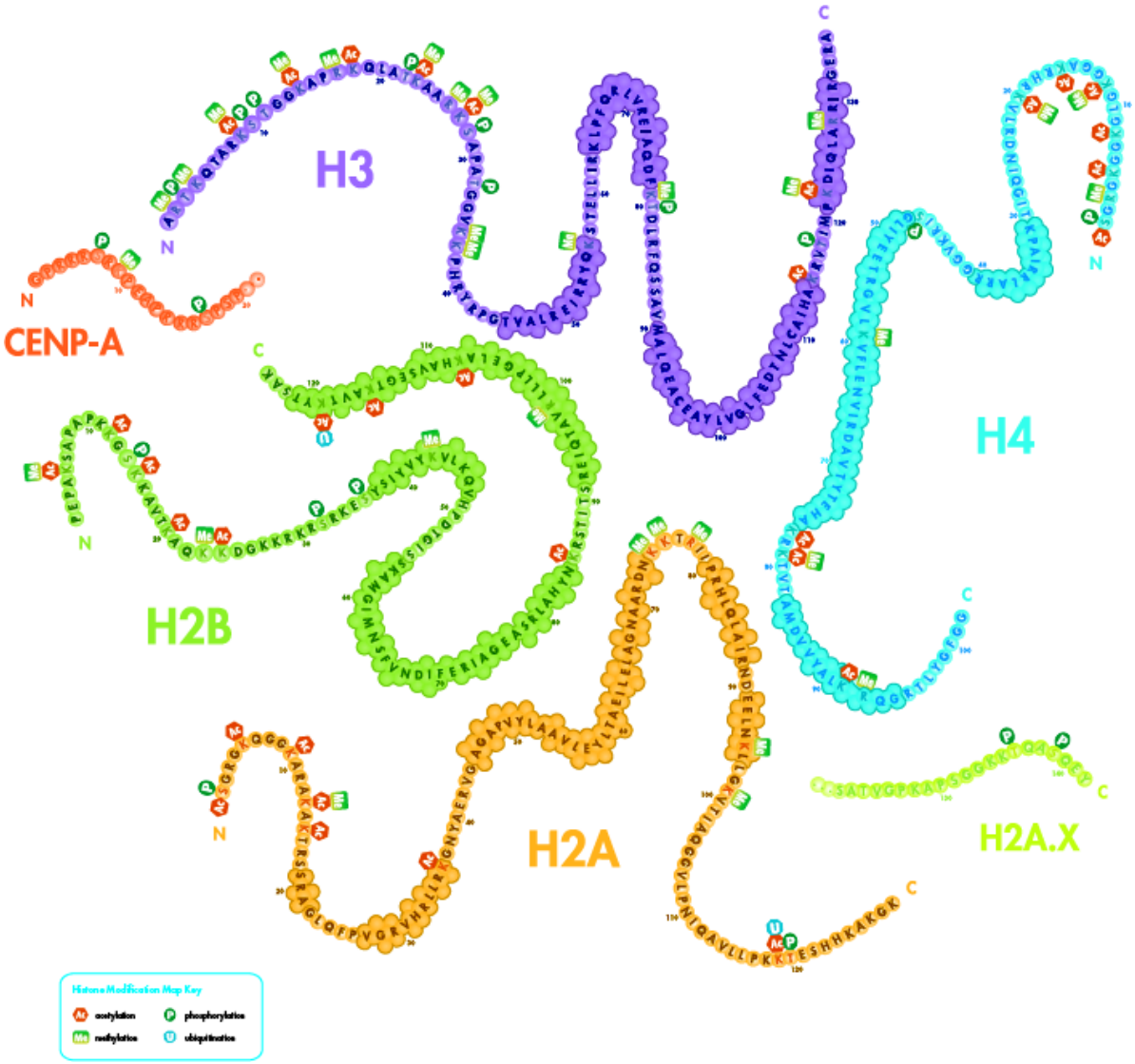




DNA methylation is the addition of a methyl group (M) to the DNA base cytosine (C).



Histones and their post-translational modifications



Histone modifications: Functions and Examples

- Histone methylations and acetylation are the best-studied histone modifications
- Functions
  - involved in a multitude of biological processes, such as transcription, DNA repair, DNA replication, DNA methylation, or possibly even epigenetic inheritance
  - control DNA accessibility by altering either the net charge of the histones or inter-nucleosomal interactions
  - attract or inhibit chromatin binding complexes, causing regulatory changes
- large differences in stability among modifications
- **modifications are reversible**

Histone methylations and acetylations

- Histone methylations
  - effect on transcription dependent on specific residue and methylation degree
  - at lysine (K) or arginine (R) residues
  - K can be mono-, di-, or trimethylated, R only mono- and dimethylated (symmetric and asymmetric)
- Histone acetylations

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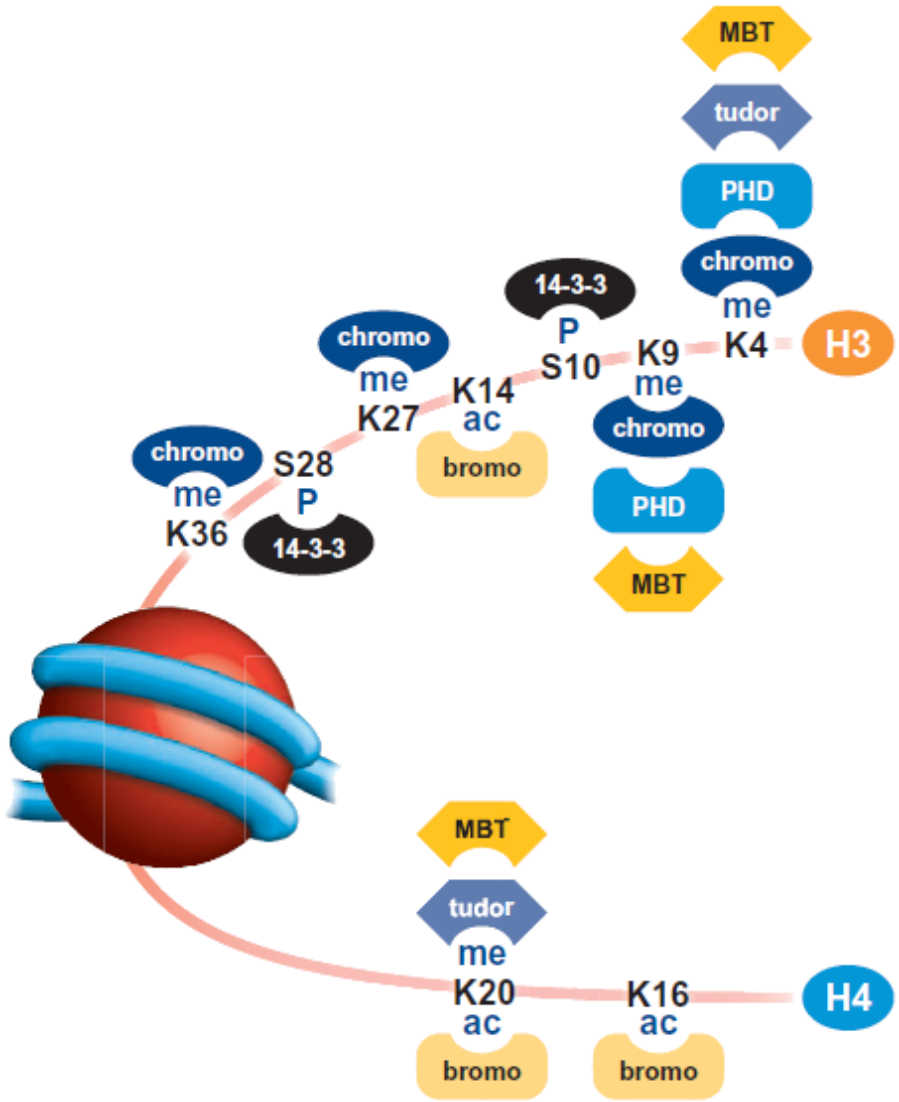
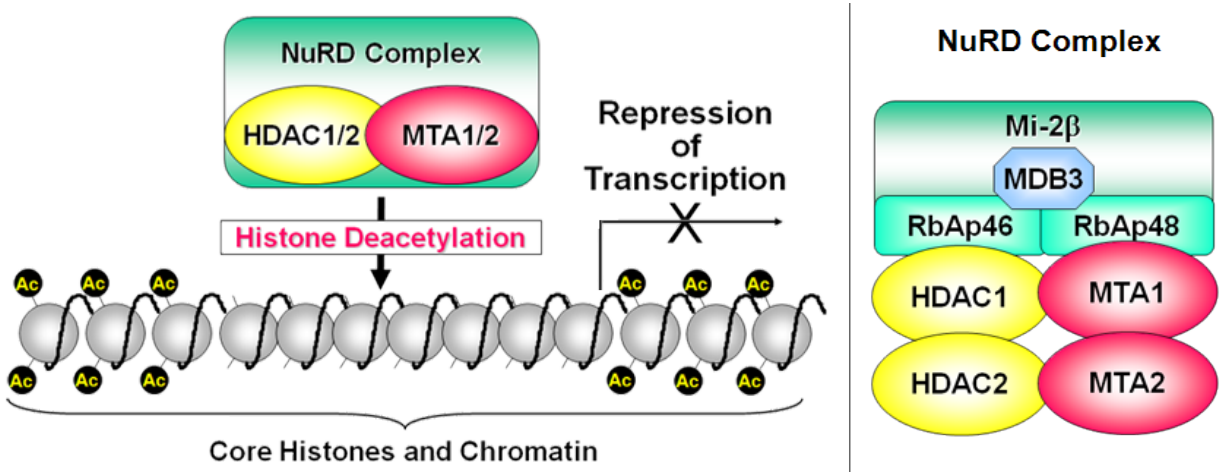
- Histone acetylations
  - generally highly correlated with gene activity
  - only at lysine (K) residues

Histone binding domains



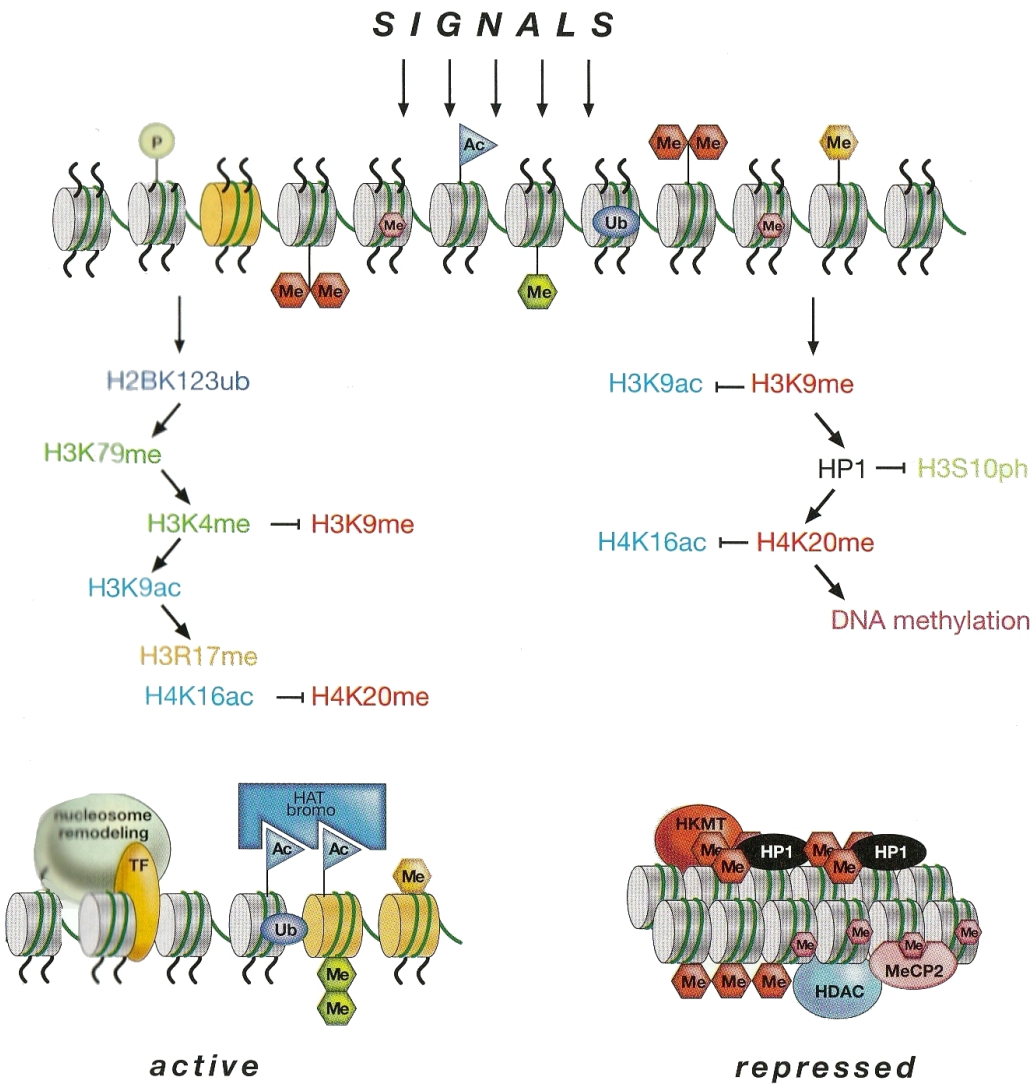
Chromatin remodeling example: The NuRD complex



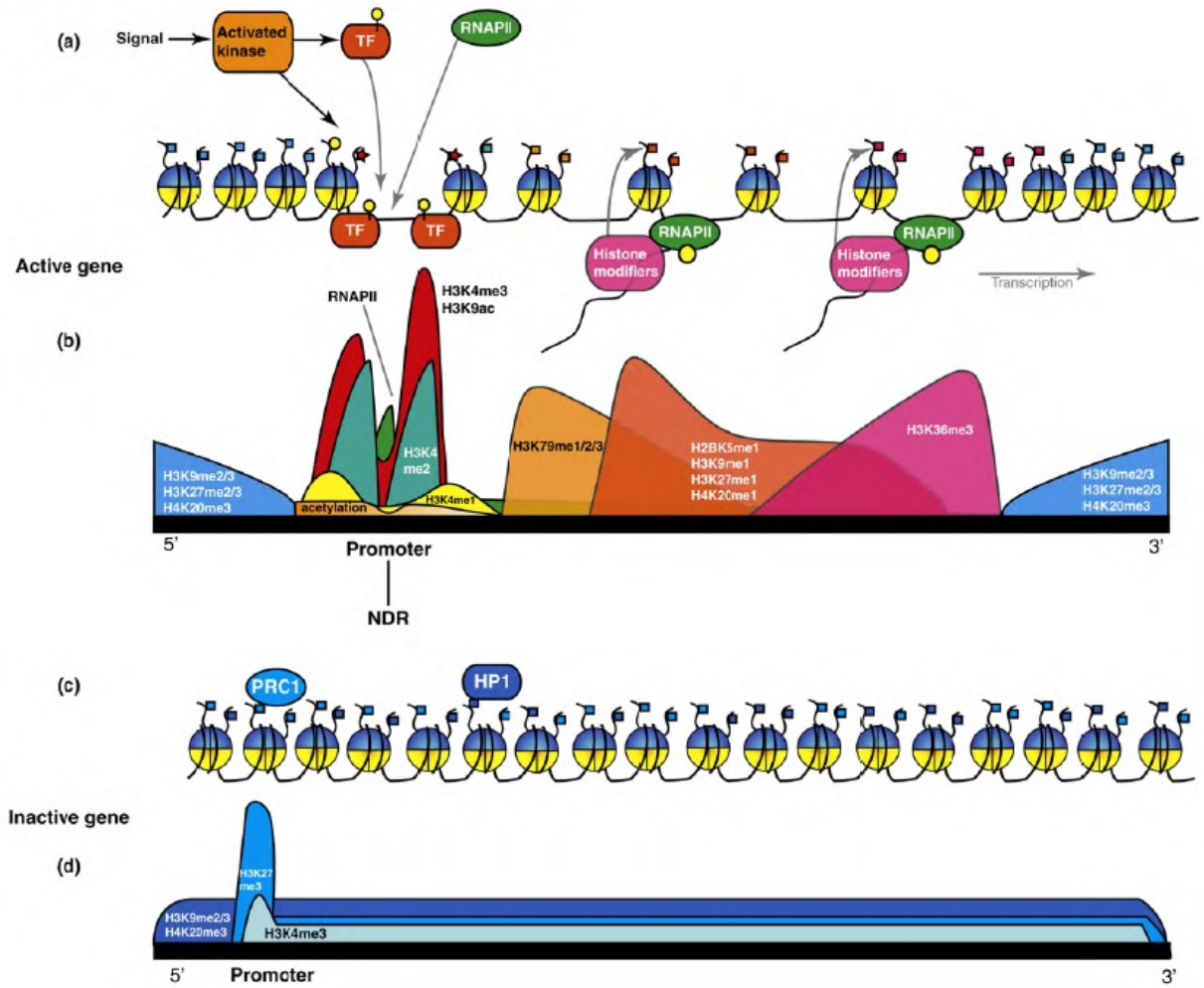
Source: Toh and Nicolson 2009. The role of the MTA family and their encoded proteins in human cancers: molecular functions and clinical implications.

- NuRD: **Nu**cleosome **R**emodeling and Histone **D**eacetylation complex
- ATP-dependent chromatin remodeling and histone deacetylase (HDAC) activity
- establishes transcriptional repression in vertebrates, invertebrates and fungi

Histone crosstalk and signaling pathways



Summary: Active and silent genes



Source: Barth and Imhof 2010. Fast signals and slow marks:the dynamics of histone modifications.

2. Epigenetics and Bioinformatics

Epigenetics deals with large amounts of data

- No epigenetic analysis without computers possible
- A single experiment may produce Terabytes of data
- Downstream analyses have to be automated and programmed efficiently

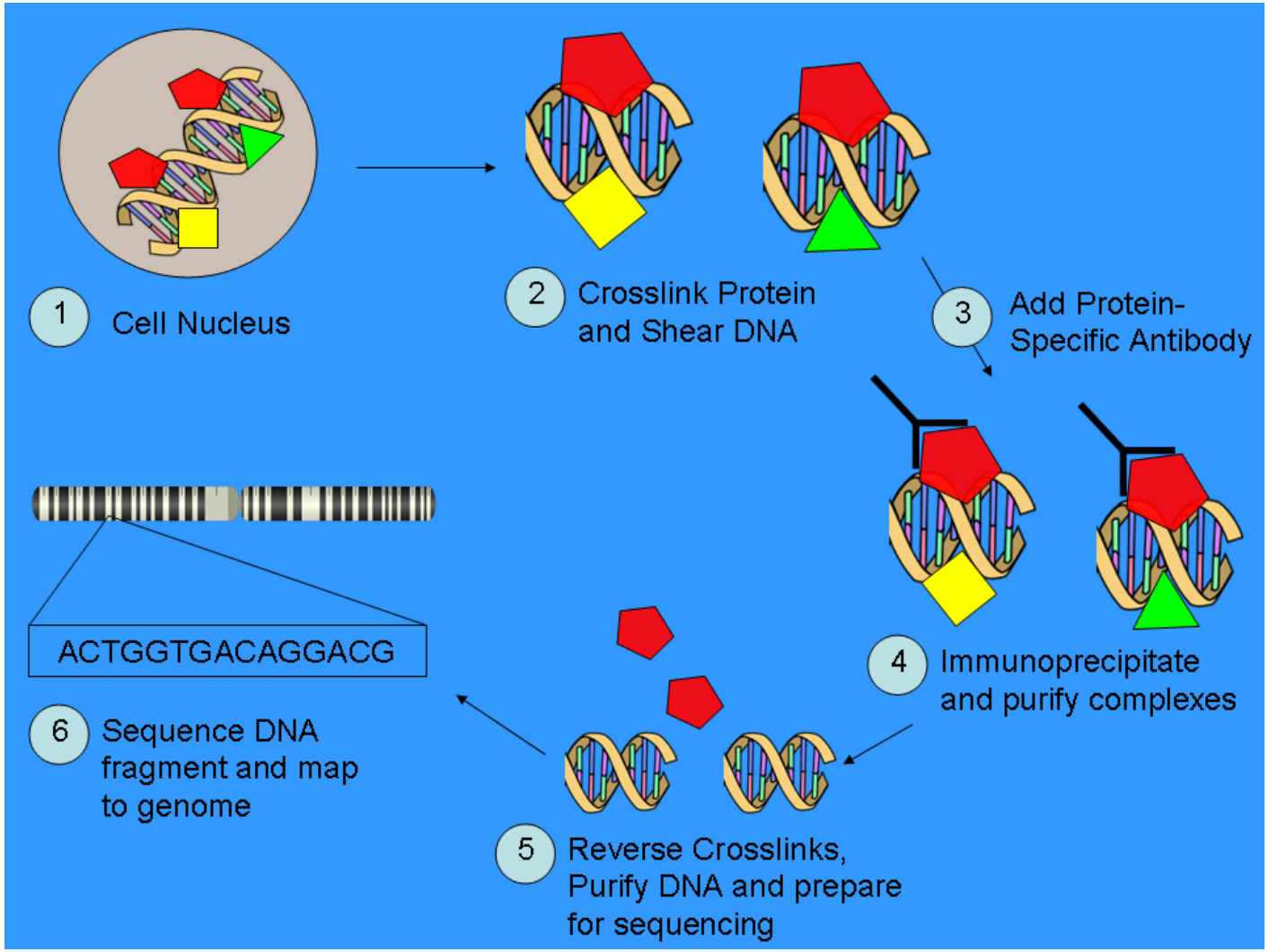
How can histone modification data be obtained?

ChIP-Seq

- Chromatin-Immunoprecipitation followed by (high throughput) sequencing[1]
- many advantages to ChIP-chip (e.g., base pair resolution, no hybdrization issues)
- generally used to identify interactions between proteins and DNA such as transcription factor binding sites
- traditional ChIP protocol can be modified to obtain genome-wide maps of histone modifications or nucleosome positions

[1] Park 2009 - ChIP-seq: advantages and challenges of a maturing technology

Traditional ChIP-Seq workflow



Source: Wikipedia

Main ChIP-Seq workflow for histone modifications

1. native chromatin is used as starting chromatin (no cross-linking needed, histone wrap around DNA naturally)
2. chromatin is sheared by micrococcal nuclease digestion and not by sonication (cuts DNA at the linker), yielding DNA fragments of one or a few intact nucleosomes
3. immunoprecipitating the protein of interest using specific antibodies (coupled to agarose or magnetic beads)
4. purifying and identifying the complex-associated DNA using either **high throughput sequencing (ChIP-Seq)**, microarrays (ChIP-on-chip or ChIP-chip), molecular cloning and sequencing, or PCR

ChIP-Seq background estimation

- no background
- model background using statisical methods (based on the sample mitself)
- negative controls
  - Input-DNA
  - Non-specific antibody (e.g., Immunoglobulin G, IgG)
  - Different tissue (more for transcription factor binding sites)

Steps for the analysis of ChIP-Seq data

1. ChIP-Seq and negative control
2. Bioinformatic analyses:
   - Mapping
   - Peak calling
   - correlation analysis (with respect to other modifications or gene annotation, for example)

Mapping

Important issues

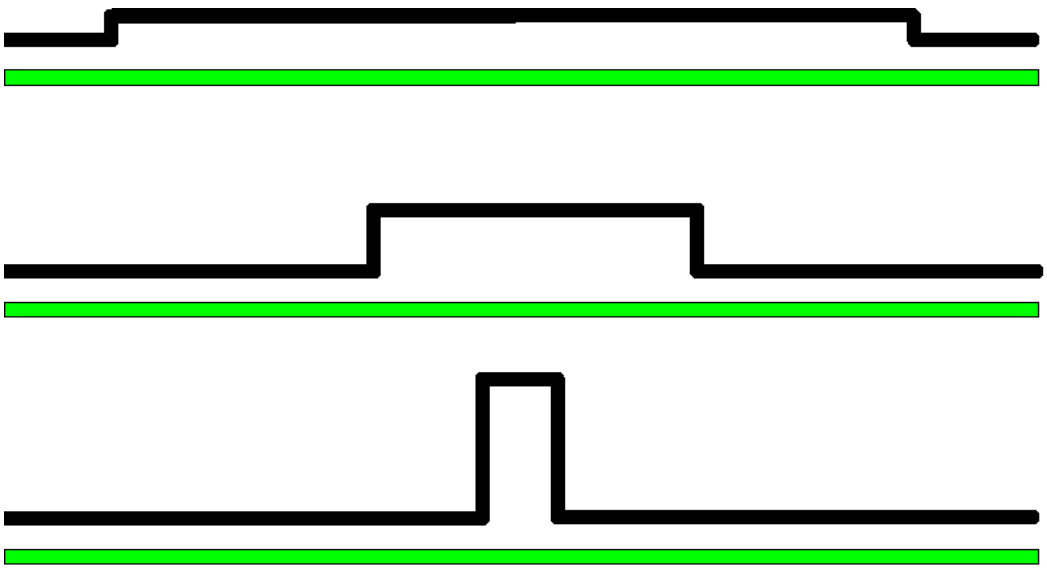
- How to handle non-uniquely mapping reads?
- How to handle reads that have no perfect match?
  - Sequencing error?
  - Quality of the reference genome?
  - Mapping algorithmus exact or a heuristic?
  - SNP?
- Which percentage of the genome is mappable at all with the given read length and the specific parameters?

Peak calling

- extract signal from background noise and identify enriched regions in the genome
- very important, because the majority of the signal may come from noise
- a lot of technical issues influence the results

Peak calling

A lot of bioinformatics programs are available, all with their own flavour and application area.

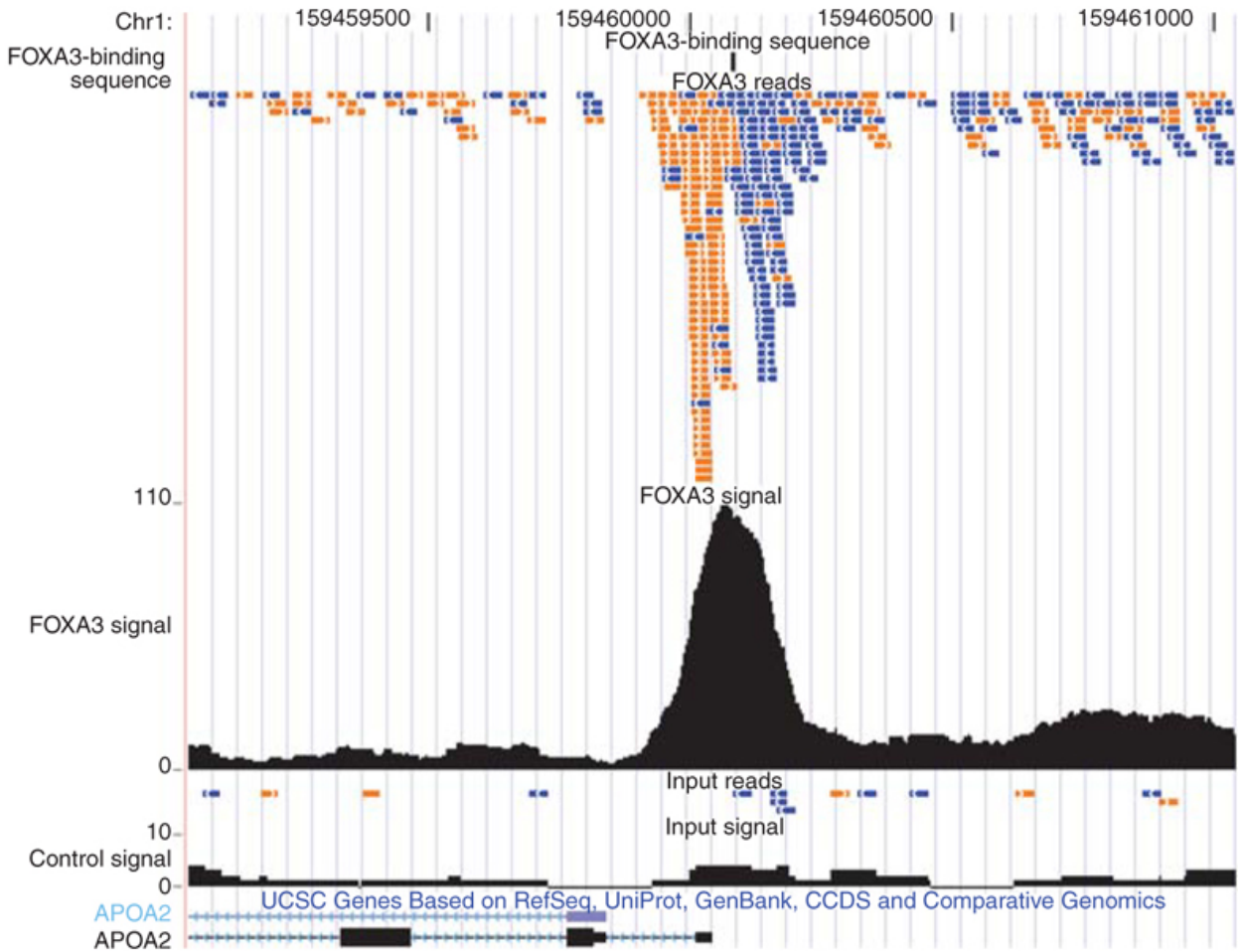


Long-range
(e.g., histone modifications)

Mid-range
(e.g. polymerase binding)

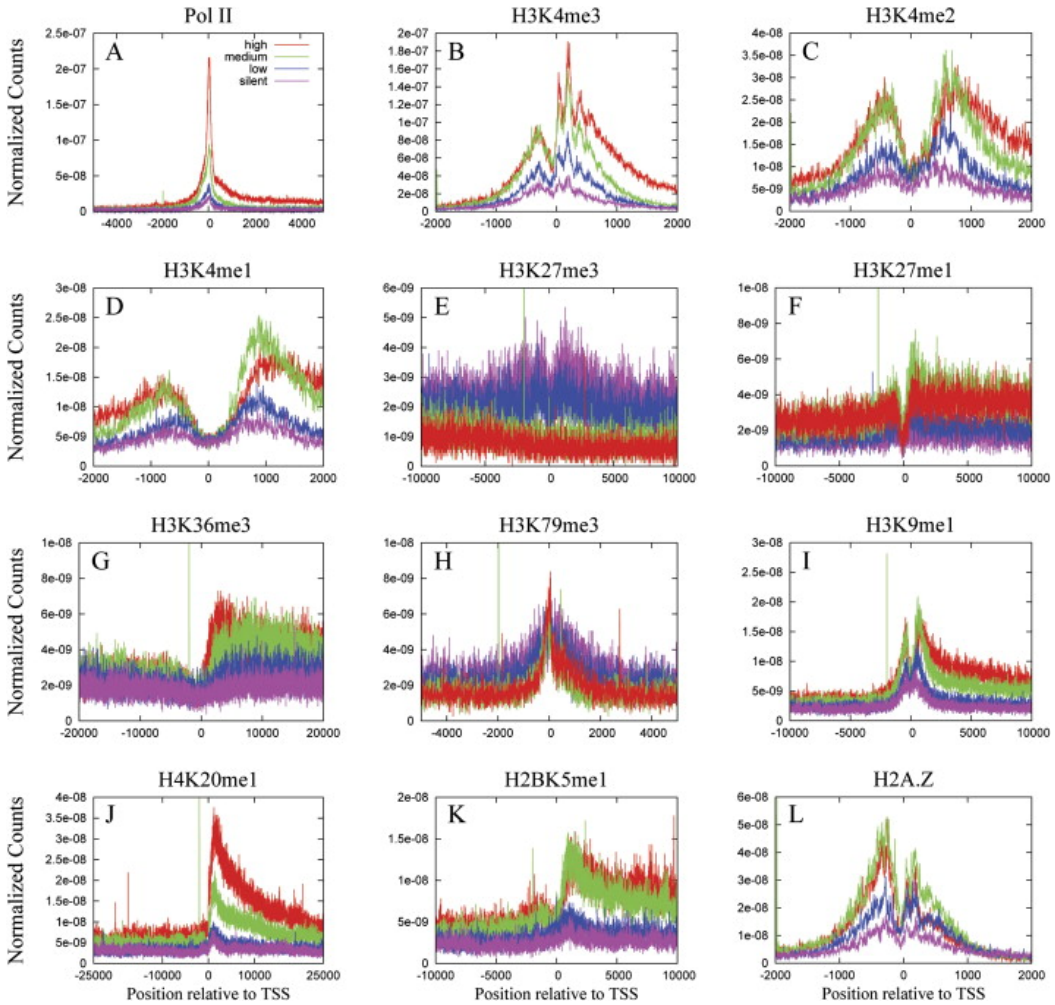
Punctate
(e.g. TF binding)

Peak calling example



Source: Shah 2009. Chromatin immunoprecipitation sequencing (ChIP-Seq) on the SOLiD system.

Bioinformatics analyses of histone modifications



Source: Barski et al. 2007. High-Resolution Profiling of Histone Methylations in the Human Genome.

3. Summary and Take-Home Message(s)

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- Epigenetic mechanisms are inherently involved in gene regulation.
- It has yet to be determined if transcription is cause or consequence of epigenetic mechanisms.
- Bioinformatics methods are crucial for the analysis of epigenetic data, which is peppered with a multitude of issues and limitations

Thank you for your attention!

Are there any questions?

Enjoy the long weekend!