

Epigenetic regulation of T cell function

Epigenetics

- Overview
 - **classical definition:** how environment/behaviours imprint on gene expression without altering the DNA sequence, with this gene expression behaviour stably inherited through generations
 - **cell biology definition:** dynamic changes in genome modifications regulate gene expression without altering the DNA sequence
- **Genome packaging**
 - DNA coiled around histones → pack into nucleosomes → compact further into chromosomes
 - tightly packed DNA is largely inaccessible
 - transcriptionally repressive environment
- **Histone modifications and transcriptional regulation**
 - → modification via methylation/acetylation/phosphorylation
 - → target nucleotides on DNA or AAs on histones
 - → can open or close chromatin state:
 - → closed chromatin state = repressed transcription state
 - → open chromatin state = active transcription state
 - e.g. methylation of arginine on histones → open chromatin state → active transcription state
 - **heterochromatin**
 - → **compact** chromatin structure → DNA not accessible to transcriptional machinery → transcriptionally **repressed genes** → associated with H3K9me2, 3 and H327me3 → methylated DNA
 - methylation is typically associated with heterochromatin and transcriptional repression
 - **euchromatin**
 - → **accessible** chromatin structure open for binding to transcriptional machinery → transcriptionally **active genes** → associated with H3K4me2, 3, acetylation, and H2A.Z addition → unmethylated DNA
 - actively transcribed genes tend to be devoid of DNA methylation
- **Transcriptional enhancers**
 - DNA regions typically upstream of target gene
 - role in determining when and where genes are expressed
 - → activator protein binds to enhancer → chromatin structure rearranged to bring protein into close proximity with gene promoter → initiates transcriptional activation of gene
- Mapping the epigenome
 - Chromatin Immunoprecipitation with high throughput sequencing (**ChIP-seq**)
 - identifies modifications
 - **process:** chromatin from cell of interest → fragment genome → fix DNA with protein → binding of antibodies specific for protein → immunoprecipitation of histone complex with DNA → DNA purification → sequence DNA

- Assay for Transposase-Accessible Chromatin using sequencing (**ATAC-seq**)
 - identifies chromatin accessibility (whether it is open/closed)
 - → DNA transposase loaded with marker → inserts marker into genome of interest → insertion of marker only occurs where there is accessible DNA/open chromatin → DNA purification → generate genome-wide maps of chromatin accessibility
 - identify non-coding regulatory elements (transcriptional enhancers)
 - transcriptional enhancers require open chromatin to allow transcription factors to bind

Epigenetic modifications of CD4+ T cell subsets

- Transcription factors
 - determine acquisition of function by naïve T cells via binding to accessible DNA
 - IFN-gamma, IL-12 → **T-bet** activation → **Th1 cells** → IL-2, IFN-gamma
 - IL-4 → **GATA3** activation → **Th2 cells** → IL-4, IL-5
 - TGF-beta, IL-6 → **RoRyt** activation → **Th17 cells** → IL-17, IL-22
- **Th1 locus**
 - IFN-gamma and IL-12 → activation of STAT1, 4, 5 → STAT1 induces T-bet activation → represses GATA3 and drives IFN-gamma gene expression → IFN-gamma production
 - epigenetic regulation of **IFN-gamma locus**:
 - naïve:
 - **repressive** transcriptional epigenetic signature
 - **Th1** → TCR binding + **IL-12/IFN-gamma**:
 - → upregulation of T-bet, STAT4 and STAT5
 - → transition from repressive to **active** state
 - → deposition of active histone PTMs and removal of CpG methylation
 - → chromatin folding brings enhancers in close proximity to drive transcription
 - **Th2** → TCR binding + **IL-4**:
 - → upregulation of GATA3 and STAT6
 - → reinforcement of **repressive** signature
 - → deposition of repressive histone PTMs and extensive CpG methylation
 - i.e. Th2 transcription factors shut down Th1 locus

