

# **The Complex Genome: From Structure to Function**

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## **Overview**

In eukaryotes, the genome is extensively compacted inside the nucleus, hierarchically organized at a multiscale of structural units. One of the most critical roles of these delicately regulated structures is the control of transcriptional dynamics. Transcriptional regulations have been recognized as the primary regulatory mechanisms coordinating cellular activities, responses, cell type and fate determinations. No doubt, regulations on transcription are hugely achieved by changes in regulatory factors and structural configuration. Yet, with more than two hundred years of study on the genome, we are just starting to realize the complexity lies within ...

The course is designed for students to learn:

- 1) Basic knowledge of the genomic structure and its role in transcriptional regulation.
- 2) Cutting-edge techniques and bioinformatics used in the field.
- 3) Practical experience in bioinformatic analysis.
- 4) Frontier research principles and methodology.
- 5) Mysteries and dilemmas remained unsolved.

## **Schedule**

Each class comprises 1 hour of lecture and 1 ~ 1.5 hour of discussions/presentations/hands-on sessions, subject to change based on the progress of study.

Literature discussion is scheduled for week 1, 2, 4, 5, 7, 8. A research article and a related synopsis will be posted one week prior to the discussion. Students should read the article and complete the synopsis before the discussion. For each discussion, 1 ~ 2 students will be selected as discussion leader to guide the discussion.

Week 2, 3 will have hands-on sessions, which requires Win/Mac setup, specific details to be determined.

Week 6 will have a group presentation on Microscopy, which is the essential technology for week 6 lecture content. Topics and requirements will be given on week 4.

Week 9 and 10 is scheduled for research proposal project. Recommended topics will be provided on week 6. However, students can pick other topics related to the course and discuss with the instructor no later than week 9.

# Syllabus

## Week 1 – 1

### DNA, Chromatin, and Transcription

Introduction to the structure basis of DNA, nucleosomes, chromatins, and nucleus; Histone modifications and DNA methylations; Elements of genes and the transcription program; *Cis*- and *trans*-regulation.

- [Discussion paper 1: Discovery of P-TEF, an important TF](#)  
Marshall, N. F. & Price, D. H. Control of Formation of Two Distinct Classes of RNA Polymerase II Elongation Complexes. *Mol. Cell. Biol.* **12**, 2078-2090 (1992).
- Additional readings: *Perspectives on transcription regulation*  
Lis, J. T. A 50 year history of technologies that drove discovery in eukaryotic transcription regulation. *Nat. Struct. Mol. Biol.* **26**, 777-782 (2019).

## Week 1 – 2

### Methods to Discovery

Quantification of gene expression (from RNA to proteins); Functional analysis of genes: fluorescent reporter assay, *in situ* hybridization, genetic perturbations (RNAi, CRISPR/Cas system, and protein degrons); Introduction to omics. [Paper 1 discussion](#)

- [Discussion paper 2: Protein degrons](#)  
Nishimura, K., Fukagawa, T., Takisawa, H., Kakimoto, T. & Kanemaki, M. An auxin-based degron system for the rapid depletion of proteins in nonplant cells. *Nat. Methods* **6**, 917-922 (2009).
- Following paper: *Advanced protein degrons*  
Yesbolatova, A. et al. The auxin-inducible degron 2 technology provides sharp degradation control in yeast, mammalian cells, and mice. *Nat. Commun.* **11**, 5701 (2020).

## Week 2

### Sequence, Omics, and Bioinformatics I

Three generations of sequencing; Sequence alignment; Genomics and genome element (GWAS); Transcriptomics (RNA-seq & nascent RNA-seq). [Paper 2 discussion](#); [Hands-on session: Sequence alignment & database searching](#).

- [Discussion paper 3: Hi-C for long-range DNA interaction sequencing](#)  
Lieberman-Aiden, E. et al. Comprehensive Mapping of Long-Range Interactions Reveals Folding Principles of the Human Genome. *Science* **326**, 289-293 (2009).

- Additional readings: *Review of high throughput sequencing*  
Reuter, Jason A., Spacek, D. V. & Snyder, Michael P. High-Throughput Sequencing Technologies. *Mol. Cell* **58**, 586-597 (2015).

### **Week 3**

#### **Sequence, Omics, and Bioinformatics II**

**Functional annotation (GO & KEGG); Epigenomics (ATAC-seq, ChIP-seq, BS-seq); Hi-C; Screening and lineage tracing.** *Hands-on session: ChIP-seq analysis using Galaxy.*

- *Discussion paper 4: Loop extrusion model*  
Fudenberg, G. *et al.* Formation of Chromosomal Domains by Loop Extrusion. *Cell Rep.* **15**, 2038-2049 (2016).
- Additional readings: *Reviews of epigenetic technologies*  
Rivera, Chloe M. & Ren, B. Mapping Human Epigenomes. *Cell* **155**, 39-55 (2013).  
Mehrmohamadi, M., Sepehri, M. H., Nazer, N. & Norouzi, M. R. A Comparative Overview of Epigenomic Profiling Methods. *Front. Cell Dev. Biol.* **9** (2021).

### **Week 4**

#### **Transcriptional Regulation I: Enhancers and Transcription factors**

**Role of enhancers and TFs in transcription initiation, pause-and-release, etc.; Study higher-dimensional architecture of genomes; Model of enhancer-promoter interactions; Enhancer RNA.** *Paper 3 & 4 discussion*

- *Discussion paper 5: Cohesin and chromatin structure*  
Rao, S. S. P. *et al.* Cohesin Loss Eliminates All Loop Domains. *Cell* **171**, 305-320.e324 (2017).
- Parallel study: *CTCF and chromatin structure*  
Nora, E. P. *et al.* Targeted Degradation of CTCF Decouples Local Insulation of Chromosome Domains from Genomic Compartmentalization. *Cell* **169**, 930-944.e922 (2017).
- Additional readings: *Enhancers and promoters*  
Haberle, V. & Stark, A. Eukaryotic core promoters and the functional basis of transcription initiation. *Nat. Rev. Mol. Cell Biol.* **19**, 621-637 (2018).  
Andersson, R. & Sandelin, A. Determinants of enhancer and promoter activities of regulatory elements. *Nat. Rev. Genet.* **21**, 71-87 (2020).  
Li, W., Notani, D. & Rosenfeld, M. G. Enhancers as non-coding RNA transcription units: recent insights and future perspectives. *Nat. Rev. Genet.* **17**, 207-223 (2016).

## Week 5

### Transcriptional Regulation II: Epigenetics

**Epigenetic landscape of the genome; Epigenetic marks of gene activation and silencing; Regulation and dynamics of epigenetics and chromatin remodeling; Epigenetics in development.** [Paper 5 discussion](#)

- [Presentation paper 6: FRAP & RNA FISH for phase separation](#)  
Boija, A. et al. Transcription Factors Activate Genes through the Phase-Separation Capacity of Their Activation Domains. *Cell* **175**, 1842-1855.e1816 (2018).
- Additional readings: *Reviews of epigenetic regulation & profile of histone marks*  
Klemm, S. L., Shipony, Z. & Greenleaf, W. J. Chromatin accessibility and the regulatory epigenome. *Nat. Rev. Genet.* **20**, 207-220 (2019).  
Barski, A. et al. High-Resolution Profiling of Histone Methylations in the Human Genome. *Cell* **129**, 823-837 (2007).

## Week 6

### Transcriptional Regulation III: Condensates and Bursting?

**Lipid-lipid phase separation in cells; Novel (yet skeptical) theory of condensates/ clusters/hubs; Dynamics of condensate; Transcriptional bursting.** [Group presentation on microscopy \(paper 6\)](#)

- [Discussion paper 7: Phase separation at estrogen-induced enhancers](#)  
Nair, S. J. et al. Phase separation of ligand-activated enhancers licenses cooperative chromosomal enhancer assembly. *Nat. Struct. Mol. Biol.* **26**, 193-203 (2019).
- Additional readings: *Perspectives on condensate, loop, and hub model*  
Lim, B. & Levine, M. S. Enhancer-promoter communication: hubs or loops? *Curr. Opin. Genet. Dev.* **67**, 5-9 (2021).  
Furlong, E. E. M. & Levine, M. Developmental enhancers and chromosome topology. *Science* **361**, 1341-1345 (2018).

## Week 7

### A Case of Study – Estrogen Receptor

**Estrogen response pathway; Binding of estrogen receptor; Mechanism of estrogen-induced transcription; MegaTrans enhancers.** [Paper 7 discussion](#)

- [Discussion paper 8: CRISPR perturbations to study enhancer-promoter contact](#)  
Fulco, C. P. et al. Activity-by-contact model of enhancer–promoter regulation from thousands of CRISPR perturbations. *Nat. Genet.* **51**, 1664-1669 (2019).

- Additional readings: *MegaTrans of estrogen-regulated enhancers*  
Liu, Z. *et al.* Enhancer Activation Requires Trans-Recruitment of a Mega Transcription Factor Complex. *Cell* **159**, 358-373 (2014).

## **Week 8**

### **A Case of Study – Enhancer-Promoter Contact and 3D Organization**

**Global analysis of chromatin folding; Advanced technologies (Micro-C & MERFISH); Shaping of enhancer-promoter contact; Discoveries against canonical theory; Problems of heterogeneity.** [Paper 8 discussion](#)

- Additional readings: *Reviews of the 3D genome structure*  
Zheng, H. & Xie, W. The role of 3D genome organization in development and cell differentiation. *Nat. Rev. Mol. Cell Biol.* **20**, 535-550 (2019).  
Hafner, A. & Boettiger, A. The spatial organization of transcriptional control. *Nat. Rev. Genet.* **24**, 53-68 (2023).  
van Steensel, B. & Furlong, E. E. M. The role of transcription in shaping the spatial organization of the genome. *Nat. Rev. Mol. Cell Biol.* **20**, 327-337 (2019).

## **Week 9**

### **The 4D Genome and New Paradigm**

**Summary of the hierarchical 3D structure of chromatin; Reconstruction of the 3D genome; Reorganization of the genome during development; Structure-determined phenotype and diseases.** [Research proposal discussion](#)

- Additional readings: *The 4D Genome*  
Aboelnour, E. & Bonev, B. Decoding the organization, dynamics, and function of the 4D genome. *Dev. Cell* **56**(11), 1562–1573 (2021).  
Dekker, J. *et al.* The 4D nucleome project. *Nature*. **549**, 219–226 (2017).

## **Week 10**

### **Final**

[Research proposal presentation](#)

**Q&A**