

# 基因组学 2023

## Lecture 11

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生命科学学院  
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2023/11/27

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2 3D Genome

- 3C 系列技术

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- RNA 修饰: m6A RIP/CLIP-seq, GLORI-seq
- K4-K36 domain、K4-K27 bivalent domain、ChromHMM

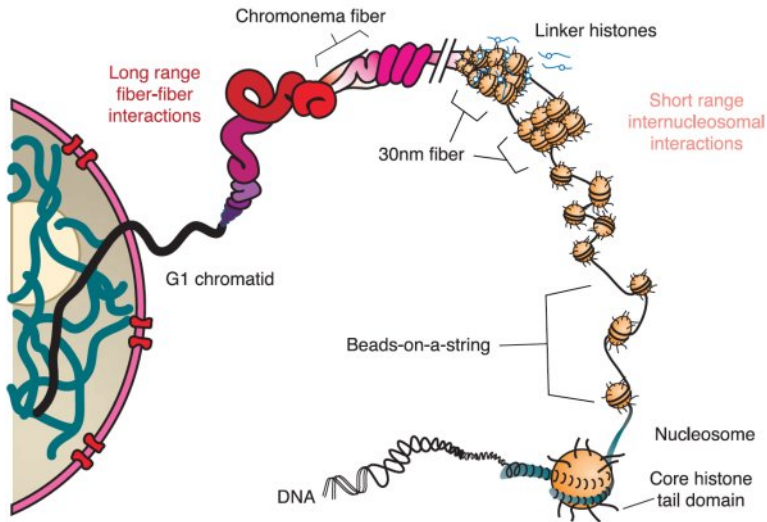
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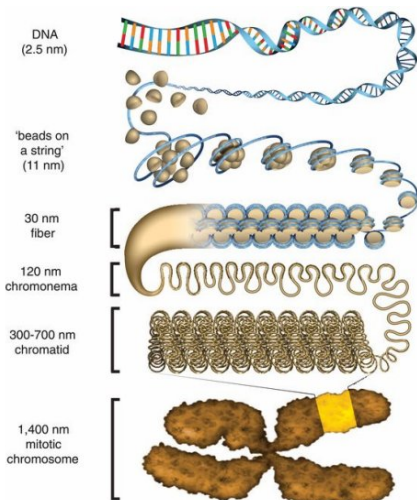
# Beyond genome sequence



Horn, 2002, Science 297: 1824

# 基因组三维空间结构

- 染色体由DNA与组蛋白共同组成
- 染色体从一级结构（绳珠模型）到四级超螺旋折叠结构
- DNA分子一共被压缩了8400倍左右，形成了复杂的三维空间结构
- 正是这些折叠和压缩，使得基因在细胞中的分布复杂而又有序



ChromEMT: Visualizing 3D chromatin structure and compaction in interphase and mitotic cells. *Science* 2017

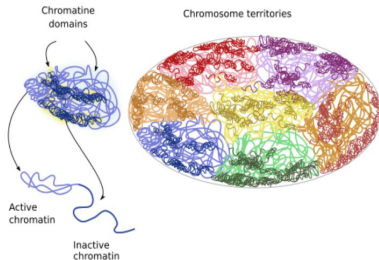
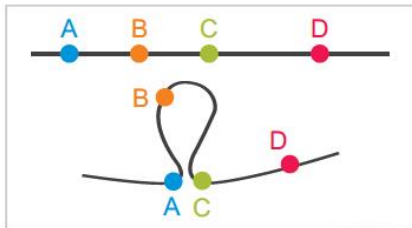
# 三维基因组学 (3D Genomics)

- 基因组三维空间结构与功能的研究简称**三维基因组学**

在考虑基因组序列、基因结构及其调控元件的同时，对基因组序列在细胞核内的三维空间结构，及其对基因转录、复制、修复和调控等生物过程中功能的研究。

- 为什么要研究三维基因组？

科学家们发现，调控元件在空间结构上并不是在染色体上呈线性地一字依次排开，这些离散的调控元件并不能有效地解释很多基因的调控结果和机制。由此，猜测其与基因组的三维空间结构相关。

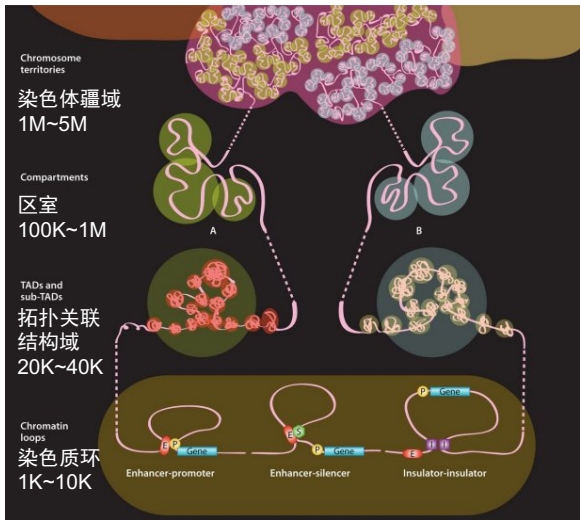


# 染色质三维空间结构

随着测序分辨率的增加，研究者发现基因组的三维空间结构从大到小依次分为：

- 染色体疆域
- 区室
- 拓扑关联结构域
- 染色质环

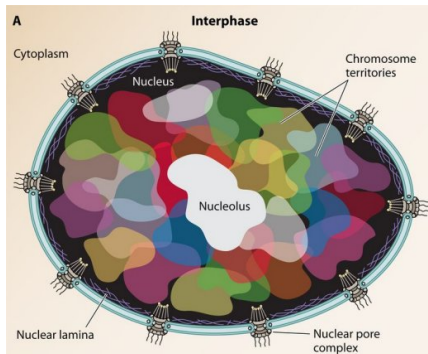
An Overview of Genome Organization and How We Got There: from FISH to Hi-C. *Microbiol Mol Biol Rev* 2015



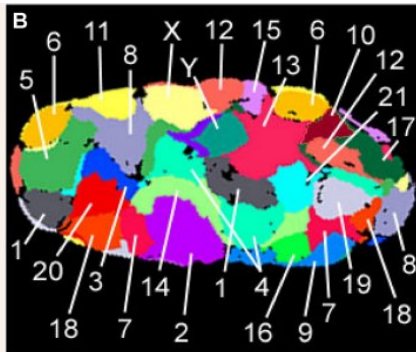


# 染色体疆域

染色体疆域（Chromosome Territories）：指每条染色体都占据着一个独特的区域，同一染色体上的交互频率高于不同染色体之间的交互频率。交互频率随着基因座之间的线性距离增加而呈指数级下降。



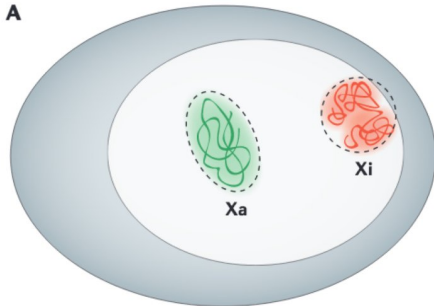
Development 2016, 143 (6) :910



PLoS Biology 2005, 3(5): e157

# Chromosome Territories

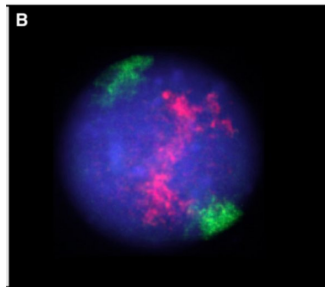
Distinct 3D X-chromosome structures: more condensed Xi



Active and inactive X chromosomes (Xa, Xi)

**The X chromosome in space.** *Nature Reviews Genetics* 2017

Gene-rich chromosomes in the center: allowing more contacts

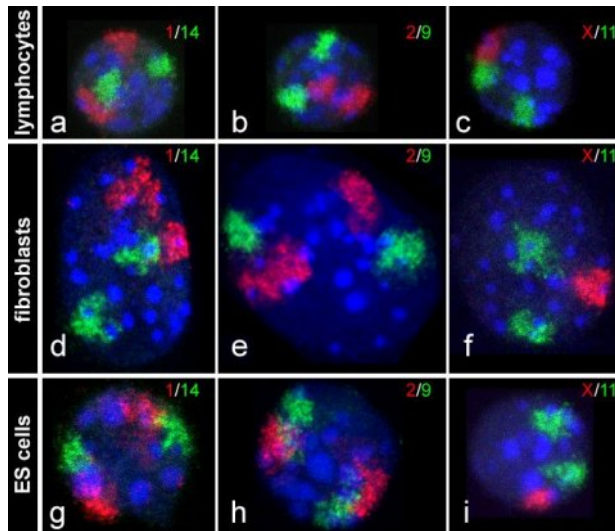


Gene-rich human chromosome 19 (red) and gene-poor chromosome 18 (green)

**Genome Architecture: Domain Organization of Interphase Chromosomes.** *Cell* 2013



# Chromosomes occupy nucleus in their own territories

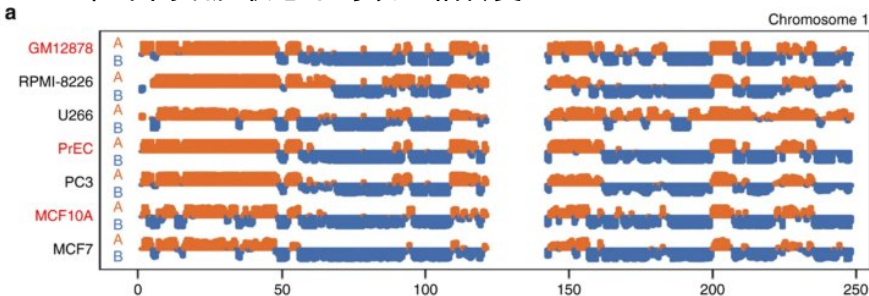


3D-FISH

Mayer et al., BMC Cell Biol (2005)

# 区室

区室（Compartments A/B）：根据互作图谱，能够将基因组近似分为A（常染色质-转录活跃区域），B（异染色质-转录非活性区）。A/B区室在染色体上相间分布，并且在不同细胞状态下可以互相转变。

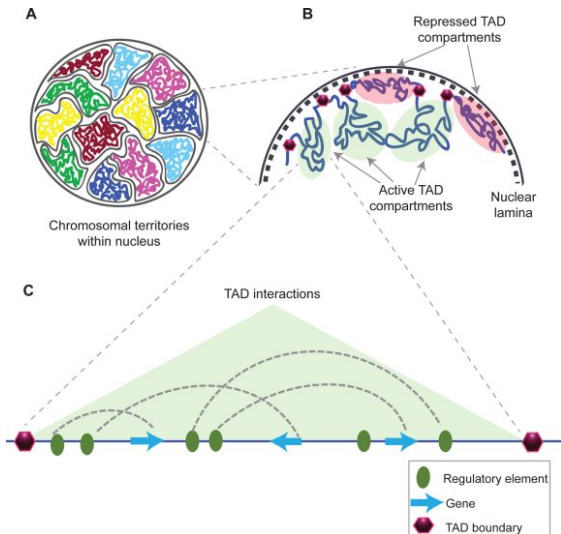


3D genome of multiple myeloma reveals spatial genome disorganization associated with copy number variations. *Nature Communications* 2017



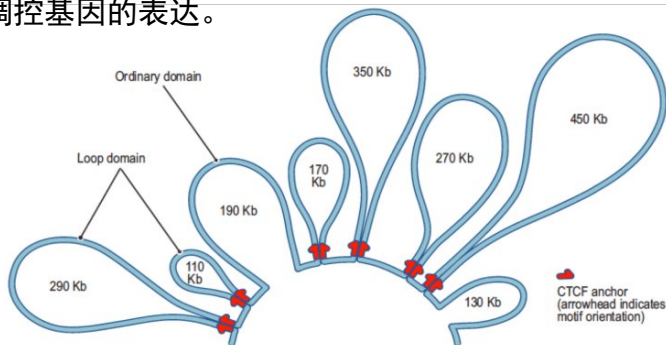
# 拓扑关联结构域

拓扑关联结构域  
(Topologically Associating Domains, TAD)：是区室下的亚结构，长度为300Kb-1Mb，具有TAD内部互作频率高，TAD间互作频率低的特点。其边界富集CTCF、持家基因、tRNAs、SINE 反转录转座子等DNA元件。



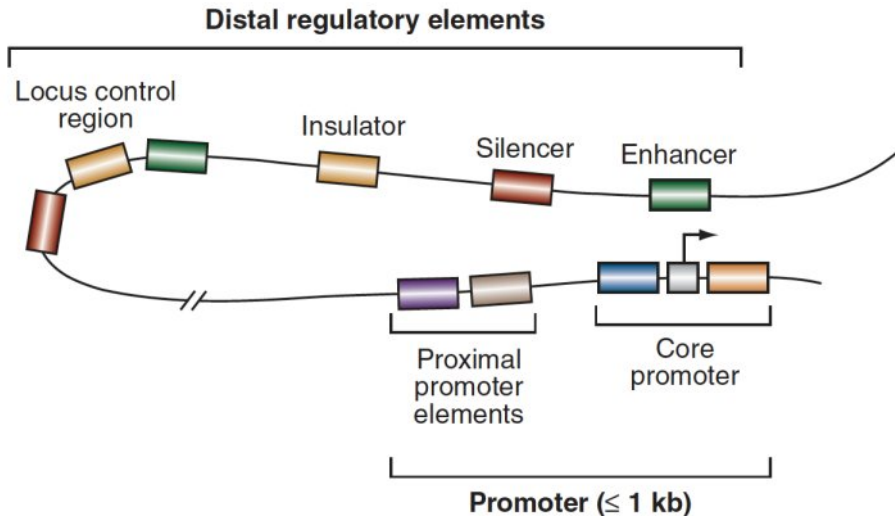
# 染色质环

染色质环（Chromatin loop）：也可称为交互峰（interaction peaks），由染色体上相距较远的两个片段构成，其在线性空间中虽相距较远，但在三维空间结构中却具有显著的近距离交互作用。调控元件（如增强子）便可以通过这种结构远距离调控基因的表达。



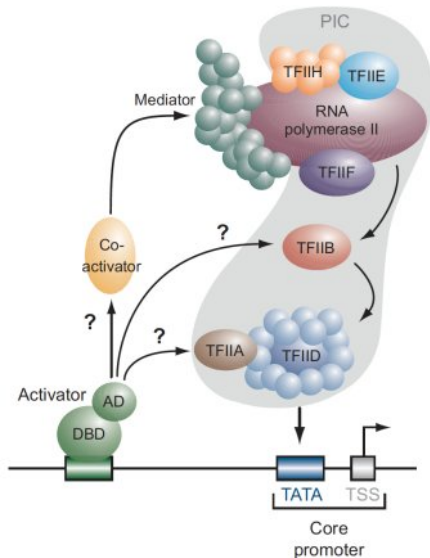
CTCF is a transcription factor encoded by the CTCF gene and involved in regulation of chromatin architecture.

# A typical gene regulatory region



Maston, 2006, Annu. Rev. Genomics Hum. Genet. 7:29

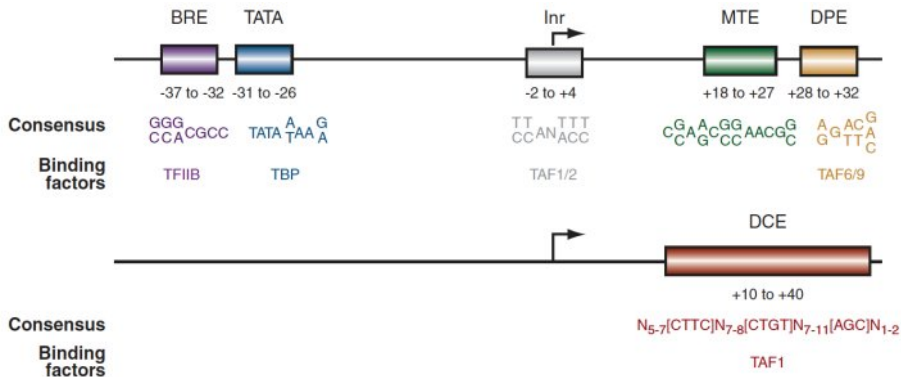
# The eukaryotic transcriptional machinery



Maston, 2006, Annu. Rev. Genomics Hum. Genet. 7:29

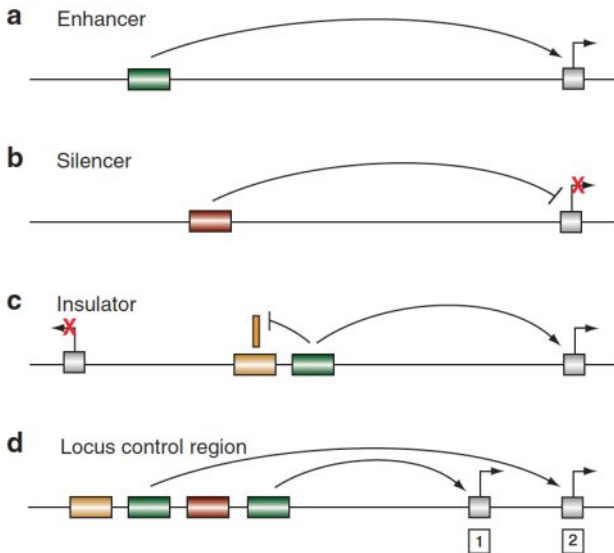


# Core promoter elements



Maston, 2006, Annu. Rev. Genomics Hum. Genet. 7:29

# Distal transcriptional regulatory elements



Maston, 2006, Annu. Rev. Genomics Hum. Genet. 7:29

- 基因组 3D 结构的检测?
- 基因组 3D 结构的形成和维持机制?
- 基因组 3D 结构如何调控基因表达?
- 基因组 3D 结构的动态变化和作用? (发育, 疾病, 病毒感染等?)

# 三维基因组基本实验技术

- 荧光显微实验方法：荧光原位杂交技术Fluorescence in situ hybridization (FISH), 2D-FISH、3D-FISH
- 染色体构象捕获：通过消化和重连空间上接近的染色体片段来确定不同位点之间的空间交互
  - Chromosome Conformation Capture (3C)
  - Chromosome Conformation Capture on Chip (4C)
  - Chromosome Conformation Capture Carbon Copy (5C)
  - High-throughput Chromosome Conformation Capture (Hi-C)
  - Chromatin Interaction Analysis by Paired-End Tag sequencing (ChIA-PET): 加入了免疫共沉淀技术，鉴定特定蛋白介导的染色质交互和空间结构



Job Dekker  
UMASS

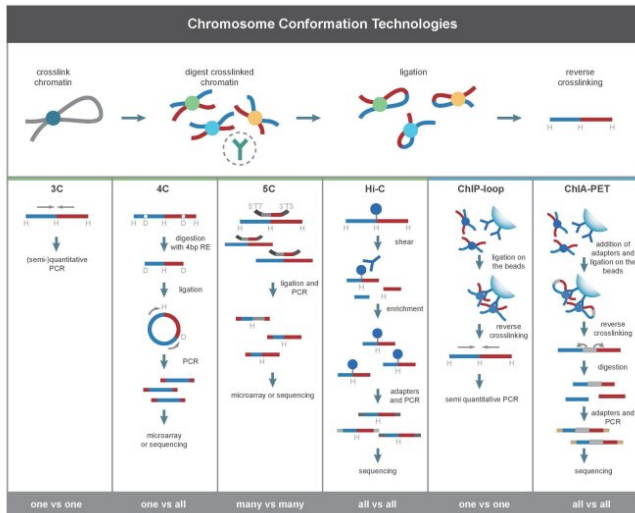
Dekker J, Rippe K, Dekker M, Kleckner N.  
**Capturing Chromosome Conformation.** *Science* 2002

# 染色体构象捕获

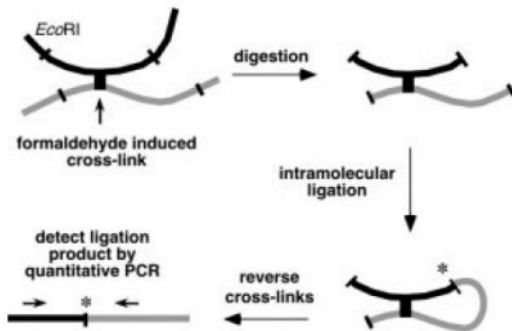
	互动方式	覆盖范围	检测方法	技术限制	研究应用
3C	单点对单点	通常小于1Mb	位点特异性PCR	通量低	确定已知启动子和增强子之间的相互作用
4C	单点对多点	全基因组范围	高通量测序	仅限于一个视角	与已知LCR (locus control regions)互作的全部基因和基因组元件
5C	多点对多点	通常小于1Mb	高通量测序	覆盖有限	确定染色体特定区域内的完整高级结构
Hi-C	全部互动	全基因组范围	高通量测序	—	所有染色体内和染色体外的相互左右
ChIP	特定蛋白介导的单点对单点	通常小于1Mb	位点特异性PCR	依赖于染色质交联的蛋白因子，忽略了其他的互动	依靠已知启动子和增强子之间的相互左右，确定特殊转录因子的功能
ChiA-PET	特定蛋白介导的全部互动	全基因组范围	高通量测序		构建已知转录因子介导的染色质互作网络

# 3C技术

染色体构象捕获技术是通过一种定量手段(PCR产物的有和无、产量的高和低)对DNA之间是否存在相互作用这一定性问题进行研究。主要经过甲醛交联、限制性酶切、稀释和连接、解交联、DNA纯化与PCR鉴定。通过一对分别与选定的2段DNA配对的引物进行PCR扩增,通过PCR产物的有无、产量的高低等,就可以对是否存在相互作用进行判断。



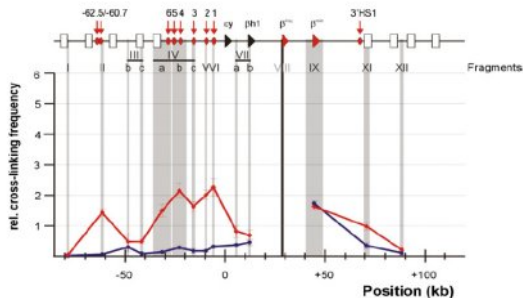
# Chromosome Conformation Capture (3C)



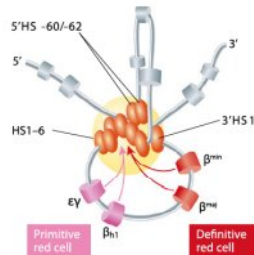
Dekker J et al., 2002, Science, 295:1306

# 3C example data and active chromatin hub

A



B

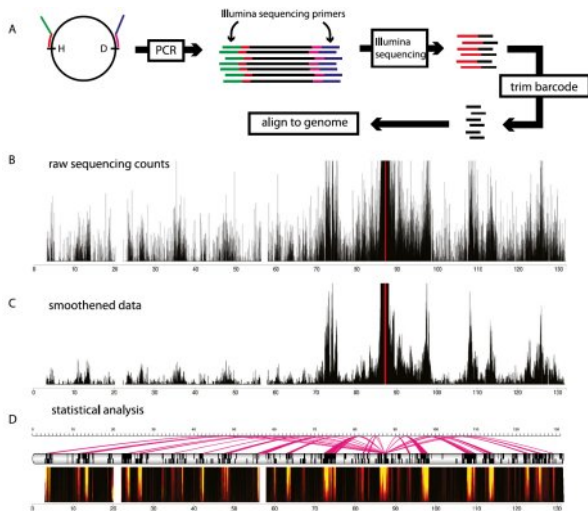


A. Anchor:  $\beta - globin^{maj}$ , 黑色竖线; 调控元件: 红色箭头; Liver ( $\beta^{maj}$  激活): 红色线表示的互作频率; Brain ( $\beta^{maj}$  沉默): 蓝色线。

B. ACR: Active Chromatin Hub model.



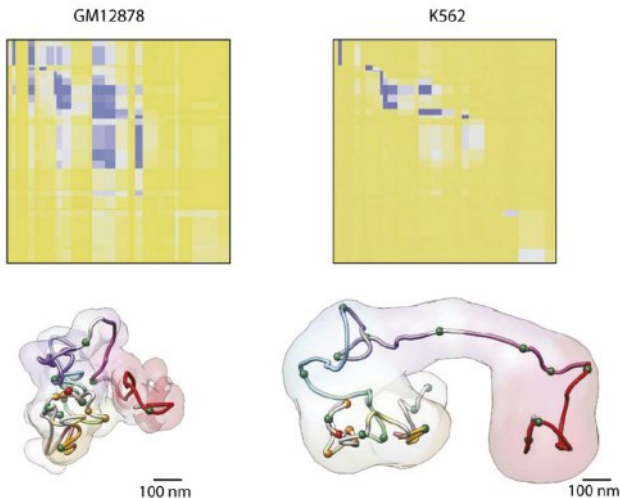
# 4C-seq example data and analysis



Ligation junctions of a given genomic site (viewpoint or bait) are PCR-amplified using viewpoint-specific primers.

Wit, 2012, Genes & Dev. 26:11

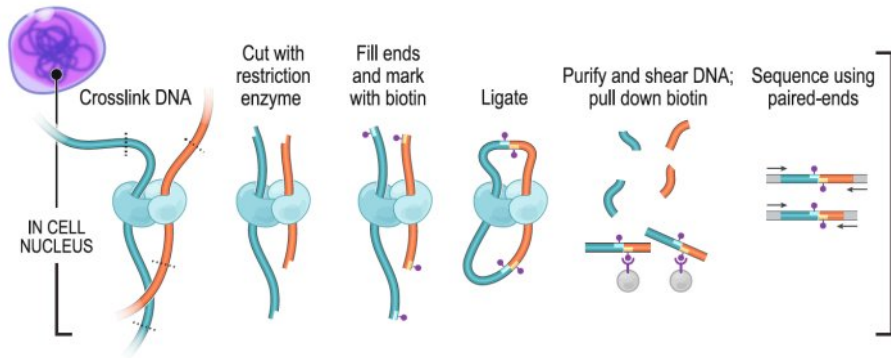
# 5C and interaction frequencies



5C results across 500 kb around the inactive and active  $\alpha$ -globin locus (in GM12878 and K562 cells, respectively)

Wit, 2012, Genes & Dev. 26:11

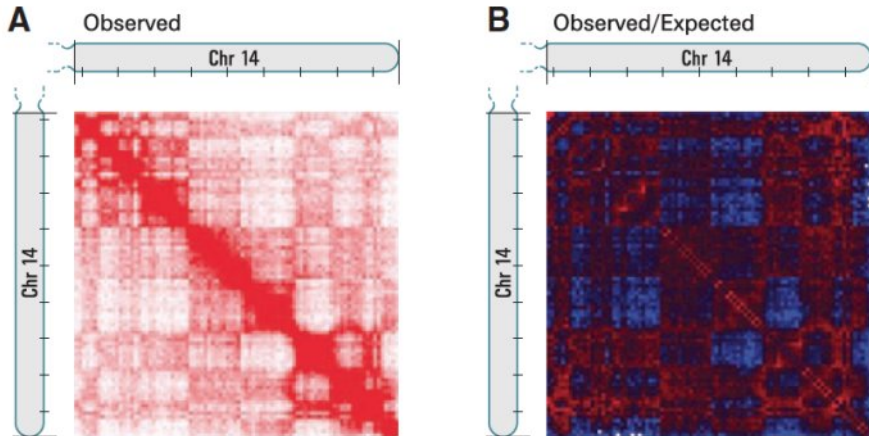
# Hi-C to probe the 3D architecture of whole genome



甲醛交联，固定由蛋白质介导、空间临近的染色质片段；内切酶切断染色质；末端加入 biotin；连接空间临近 DNA；纯化和打断 DNA、富集有 biotin 的片段；高通量测序和分析。

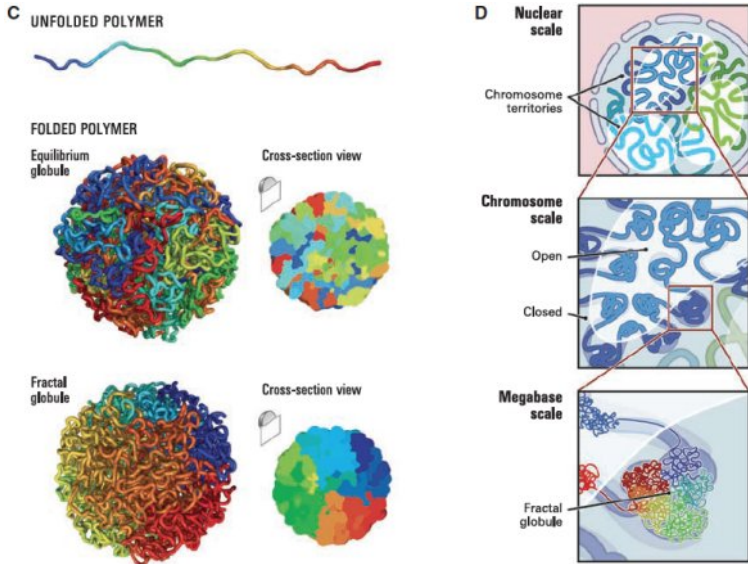
Lieberman-Aiden, 2009, Science 326:289; Rao, 2015, Cell 159:1665

# Hi-C to probe the 3D architecture of whole genome



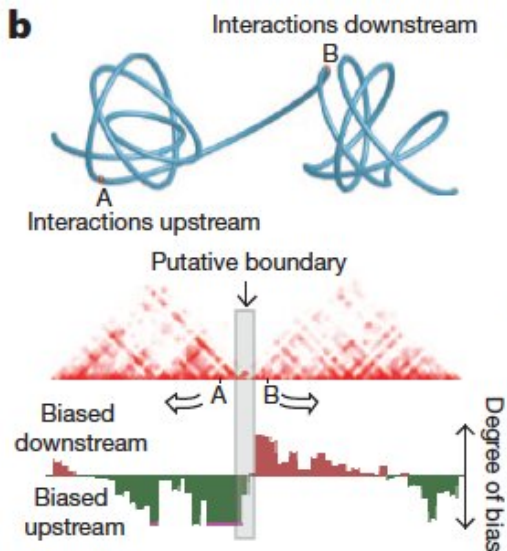
Lieberman-Aiden, 2009, Science 326:289

# Hi-C to probe the 3D architecture of whole genome



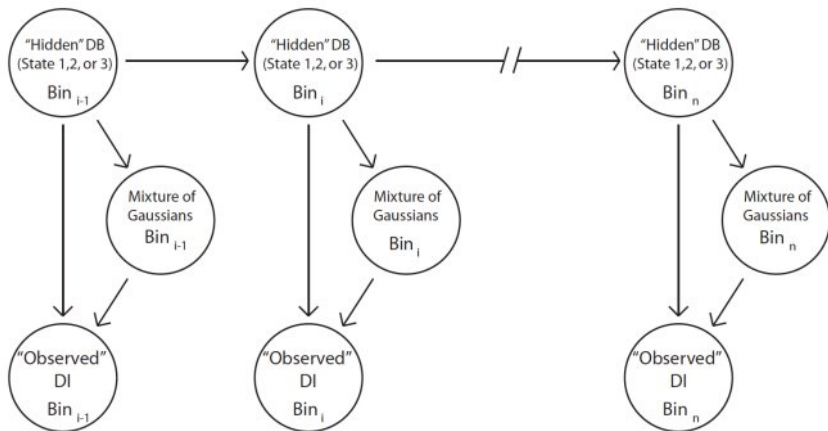
Lieberman-Aiden, 2009, Science 326:289

# Topological domains and directional bias



Dixon, 2012, Nature, 485:376

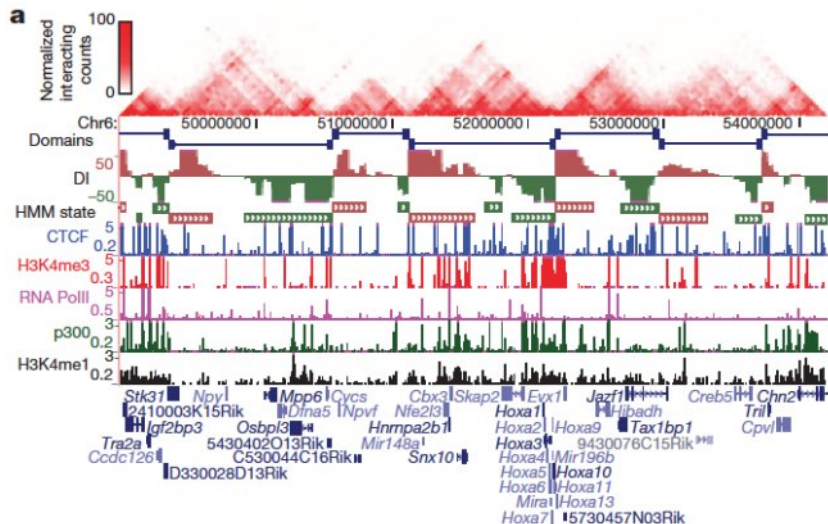
# HMM bias state for directional index



"Upstream Bias" - State 1  
"Downstream Bias" - State 2  
No Bias - State 3

Dixon, 2012, Nature, 485:376

# HiC and Topological domains in mouse ESC

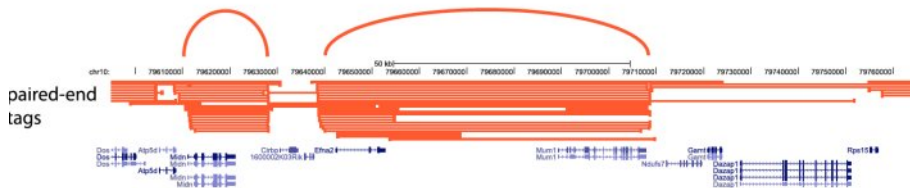


Dixon, 2012, Nature, 485:376

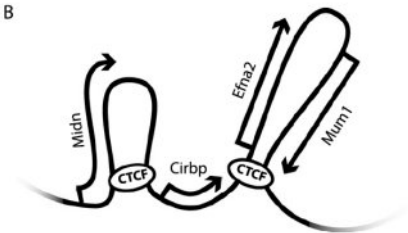


# ChIA-PET and chromosome interactome for CTCF

A



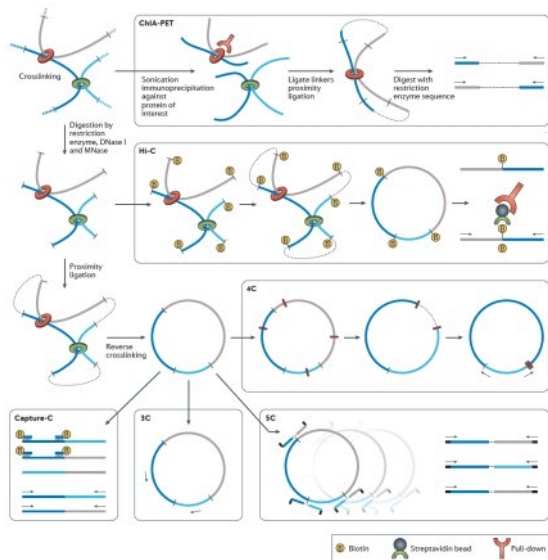
B



ChIA-PET offers insight into the chromosome interactome for the CTCF protein

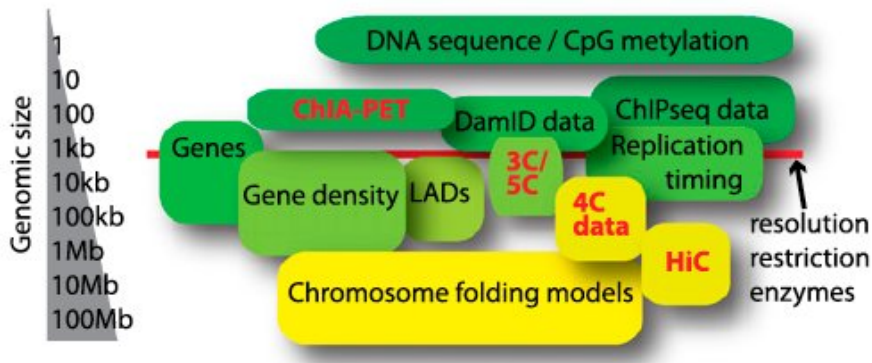
Wit, 2012, Genes & Dev. 26:11

# 3C-based approaches to study chromatin architecture



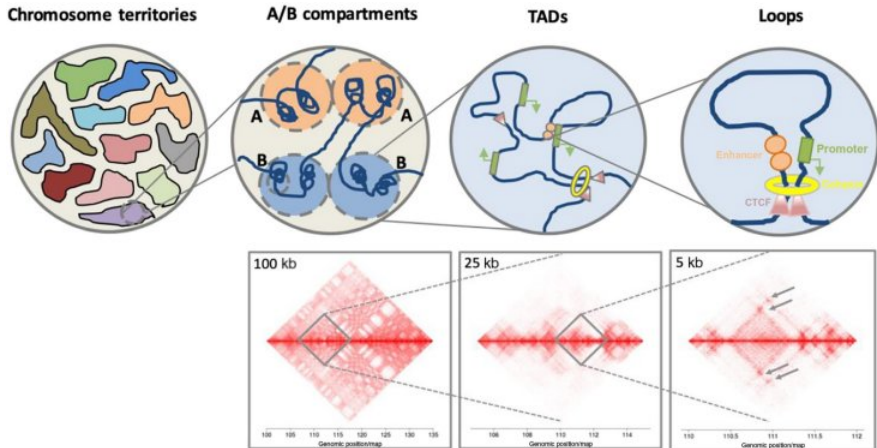
Bonev, 2016, Nat Rev Genetics 17:661

# Length scale of the Seq methods



A length scale shows the resolution of the data that are obtained with various genome-wide methods

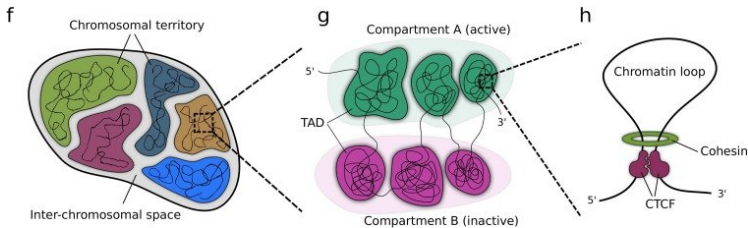
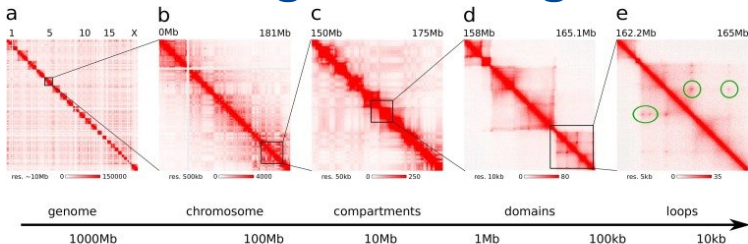
# Chromosome structure at multiple scales



<https://doi.org/10.1016/j.tibs.2019.03.001>

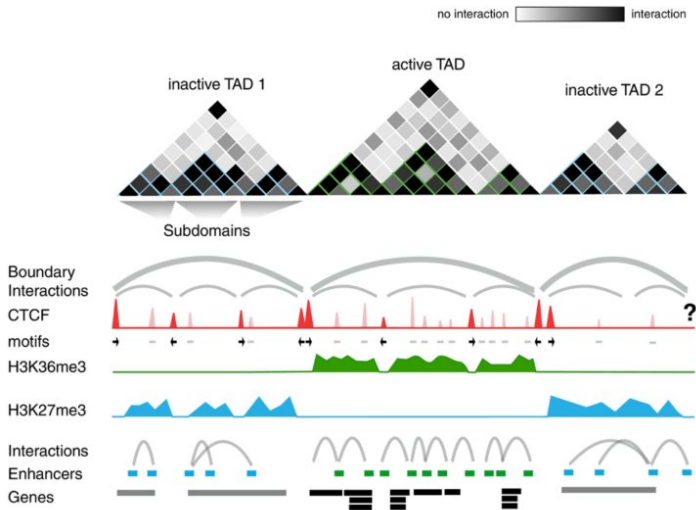
Trends in Biochemical Sciences

# Hierarchical genome organization



Three-dimensional organization and dynamics of the genome. *Cell Biol Toxicol.* 2018

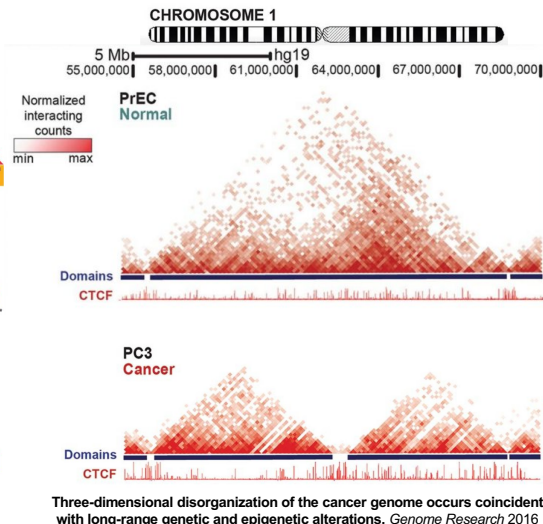
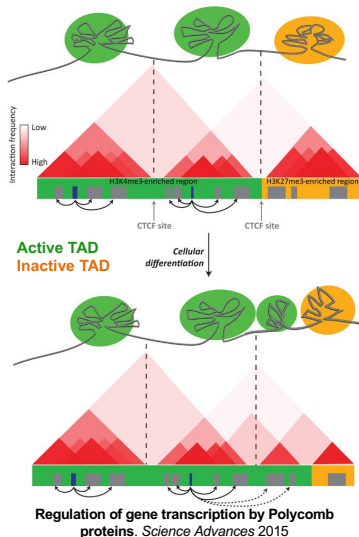
# TADs with histone modifications



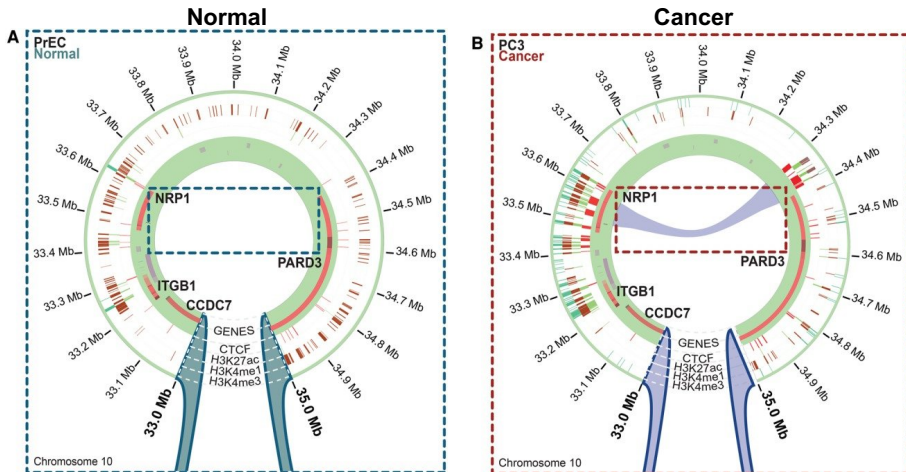
Current Opinion in Genetics & Development

<https://doi.org/10.1016/j.gde.2015.11.009>

# TAD with cell differentiation and cancer



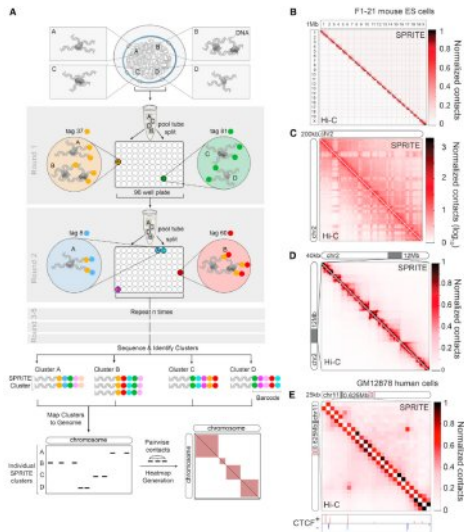
# Differential chromatin interactions



Three-dimensional disorganization of the cancer genome occurs coincident with long-range genetic and epigenetic alterations. *Genome Research* 2016

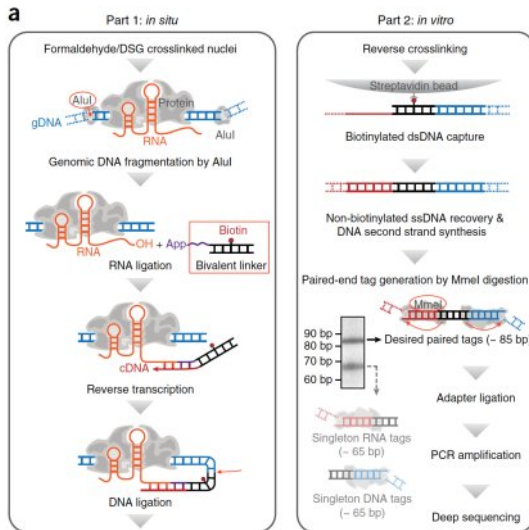


# SPRITE: A Genome-wide Method to Identify Higher-Order DNA Interactions in the Nucleus



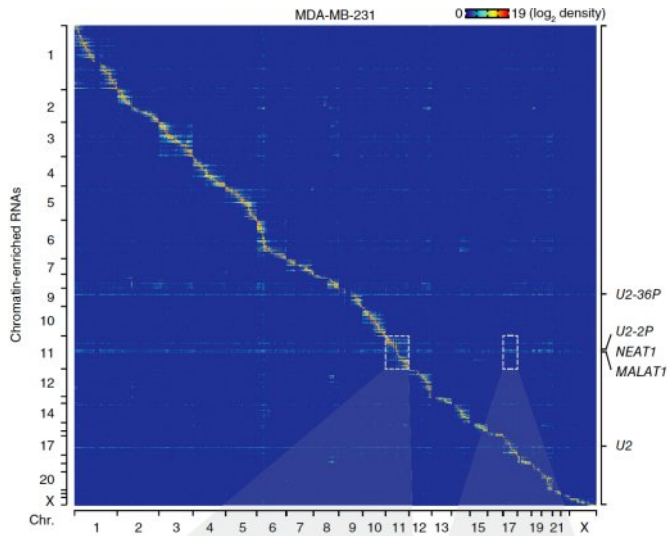
Quinodoz et al, 2018, Cell

# GRID-seq reveals the global RNA–chromatin interactome



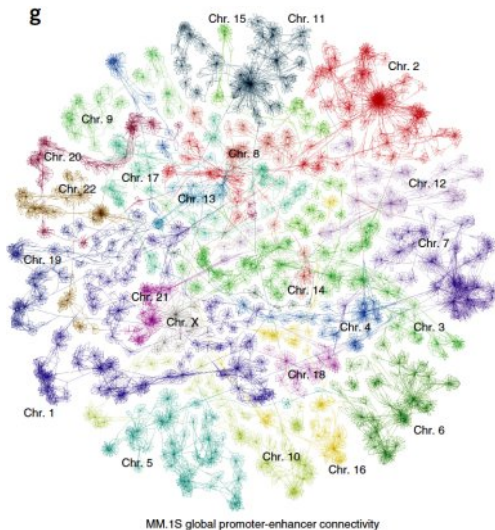
Li, 2017, Nature Biotechnology 35:940

# GRID-seq reveals the global RNA–chromatin interactome



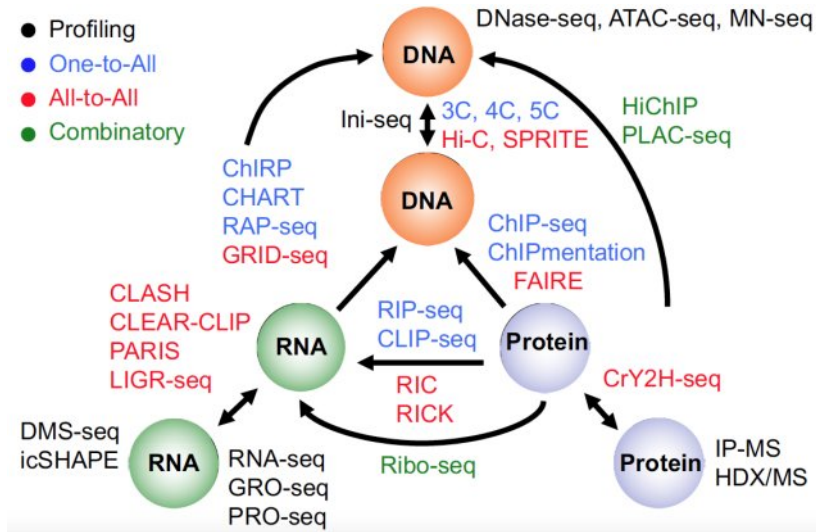
Li, 2017, Nature Biotechnology 35:940

# Self-organized map for whole-genome promoter-enhancer network



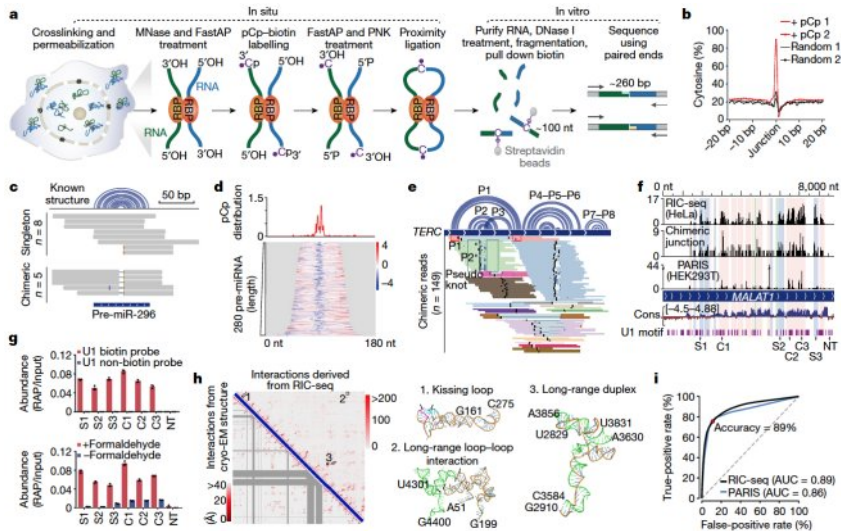
Li, 2017, Nature Biotechnology 35:940

# Technologic Advances Driven Biological Research



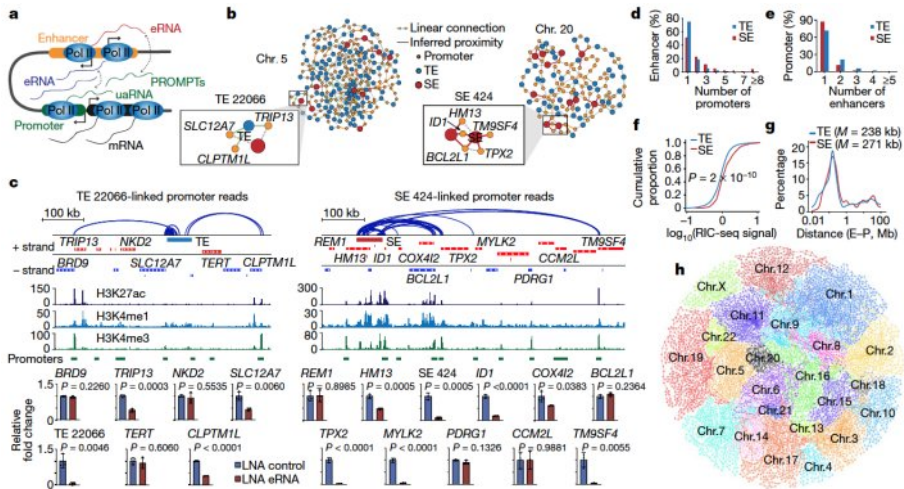
Xiang-Dong Fu, 2018

# RIC-seq captures RNA tertiary interactions



Cai, 2020, Nature 582:432

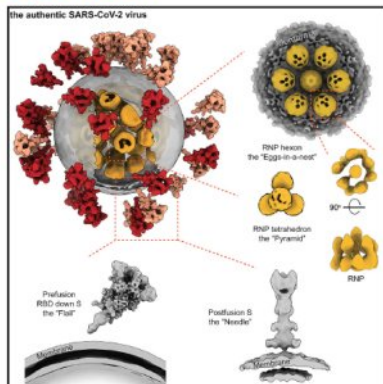
# Enhancer-promoter connectivity map based on pairwise-interacting RNAs



Cai, 2020, Nature 582:432

Cell

### Graphical Abstract



## Authors

Hangping Yao, Yutong Song,  
Yong Chen, ..., Yigong Shi,  
Lanjuan Li, Sai Li

### Correspondence

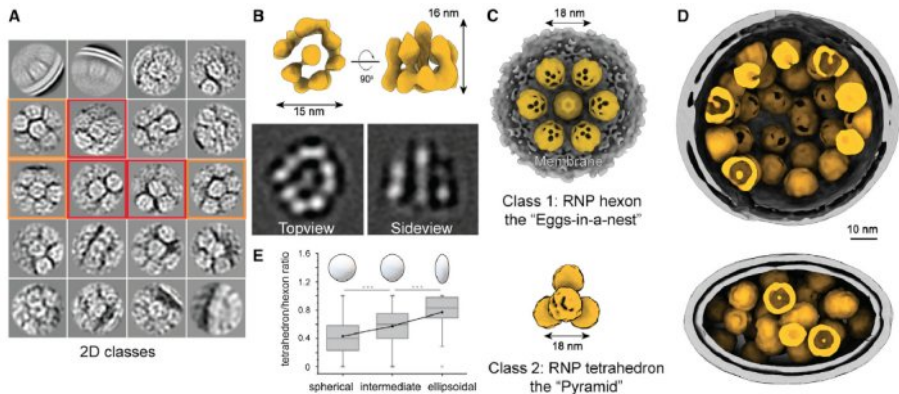
ljli@zju.edu.cn (L.L.),  
sai@tsinghua.edu.cn (S.L.)

## In Brief

Combined imaging analyses of 2,294 intact virions from the authentic SARS-CoV-2 virus resolve the S protein in pre- and postfusion conformations and characterize the molecular architecture of SARS-CoV-2 at high resolution.

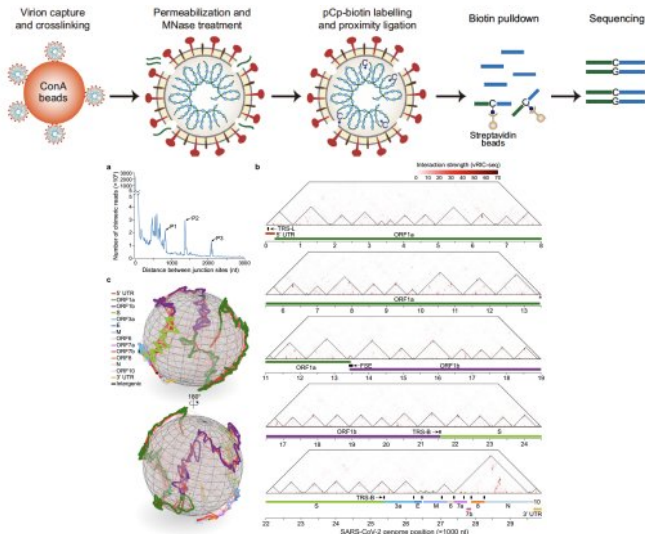


# Native Assembly of the Ribonucleoproteins (RNPs)



Yao, 2020, Cell 183:730

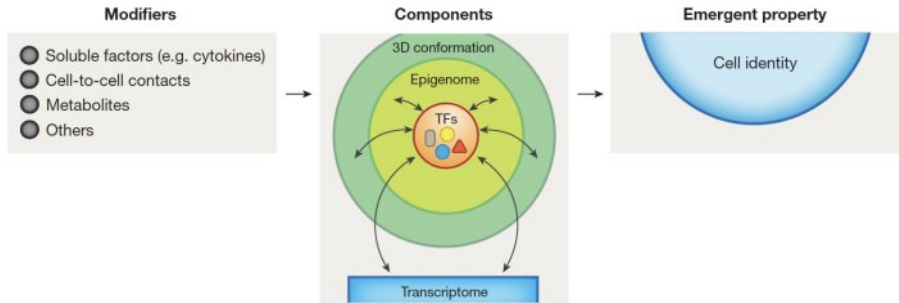
# The architecture of the SARS-CoV-2 RNA genome inside virion by vRIC-seq



Cao et al., 2021, Nat Comm 12:3917

# Ongoing researches ...

- 方法改进：更少的细胞 (tag-HiC)、更高分辨率 (Hi-C 3.0)、...
- 基因组的结构和功能、...
- 4D genome、...



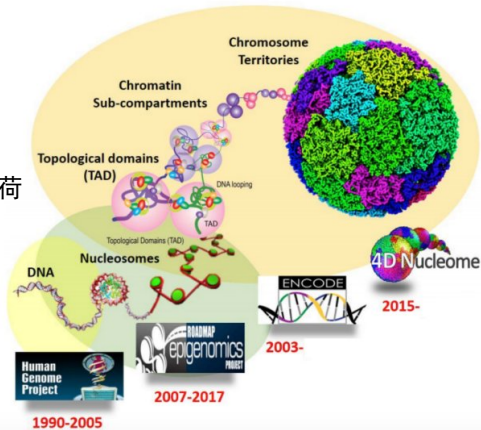
# 4D基因组学

基因表达和调控随着时间的变化而变化，研究时间动态变化下的基因组三维结构和功能，称之为4D基因组学

- 细胞周期过程
- 细胞发育和分化的不同阶段
- 正常细胞向疾病细胞转变过程
- 细胞受到外界刺激（如用药、荷尔蒙、温度变化、病毒入侵）



<https://www.4dnucleome.org>



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基因组学 - Genomics



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