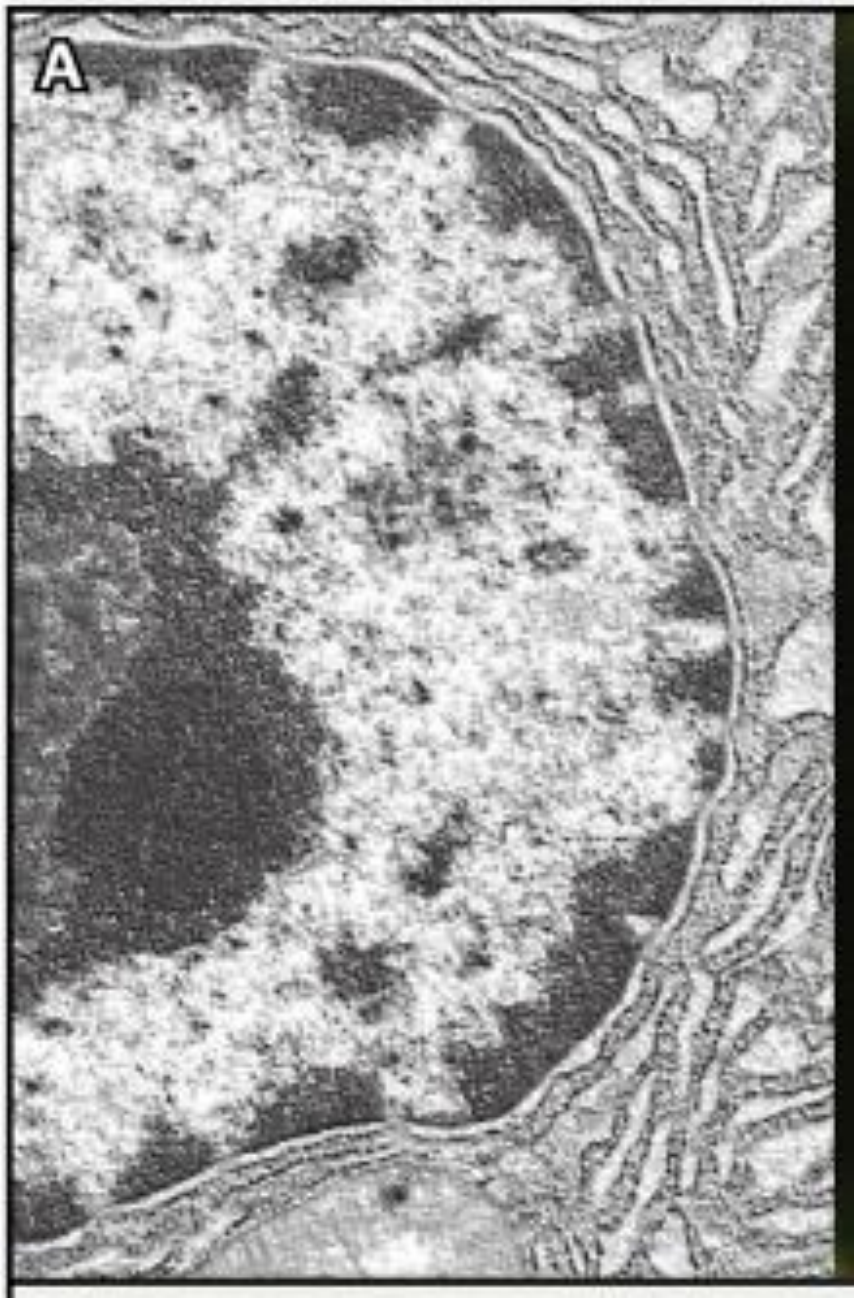
Ch 1 – L 4.2



NL-Associated Heterochromatin
(A) Electron micrograph of part of a mouse cell nucleus. Densely stained chromatin is closely associated with the NL, but is also present around nucleoli and in patches elsewhere in the nucleus

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The role of nuclear lamina in gene regulation

Peripheral localization of heterochromatin depends on proteins in the nuclear lamina, a network of proteins that line the inner side of the NE (Figure 1a and b) [10•]. Genomic sites that contact the nuclear lamina have been mapped by DamID [11] and are called lamina-associated domains (LADs) [12]. LADs range in size from tens of kilobases to several megabases, are relatively gene poor, have low transcriptional activity and are enriched in repressive chromatin marks. LADs make up nearly 40% of human and mouse genomes.

Dam ID

Geyer et al., 2011

What is Dam ? use UniProt

Dam ID

N6-methyladenine (m6A) is the product of the addition of a methyl group (CH_3) at position 6 of the adenine. This modified nucleotide is absent from the vast majority of eukaryotes.

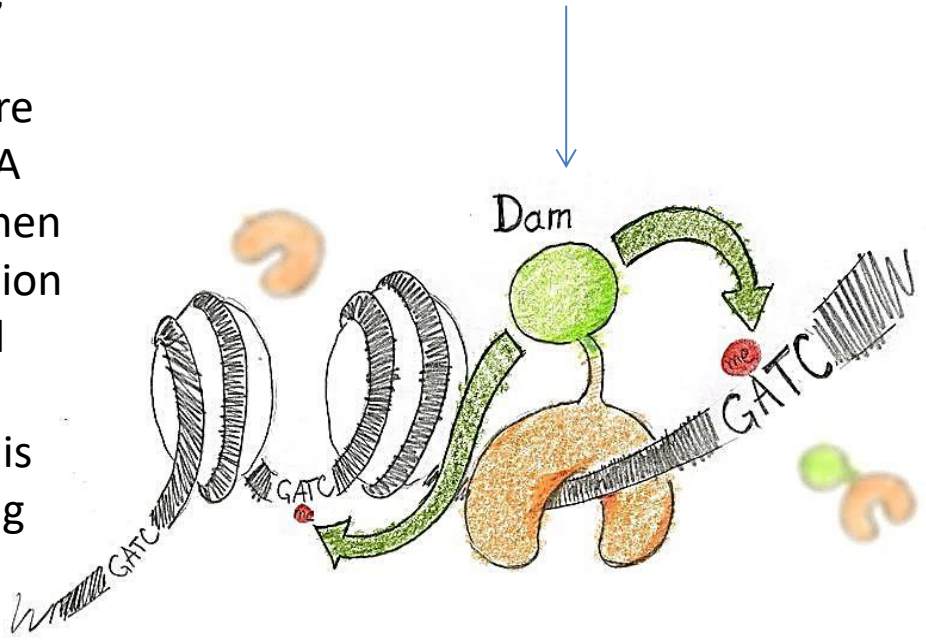
Methyl PCR (mePCR)

In this assay the genome is digested by DpnI, which cuts only methylated GATCs. Double-stranded adapters with a known sequence are then ligated to the ends generated by DpnI. A PCR with primers matching the adaptors is then carried out, leading to the specific amplification of genomic fragments flanked by methylated GATCs. In practice, ligation products are digested by DpnII prior PCR amplification. This enzyme cuts non-methylated GATCs, ensuring that only fragments flanked by *consecutive* methylated GATCs are amplified.

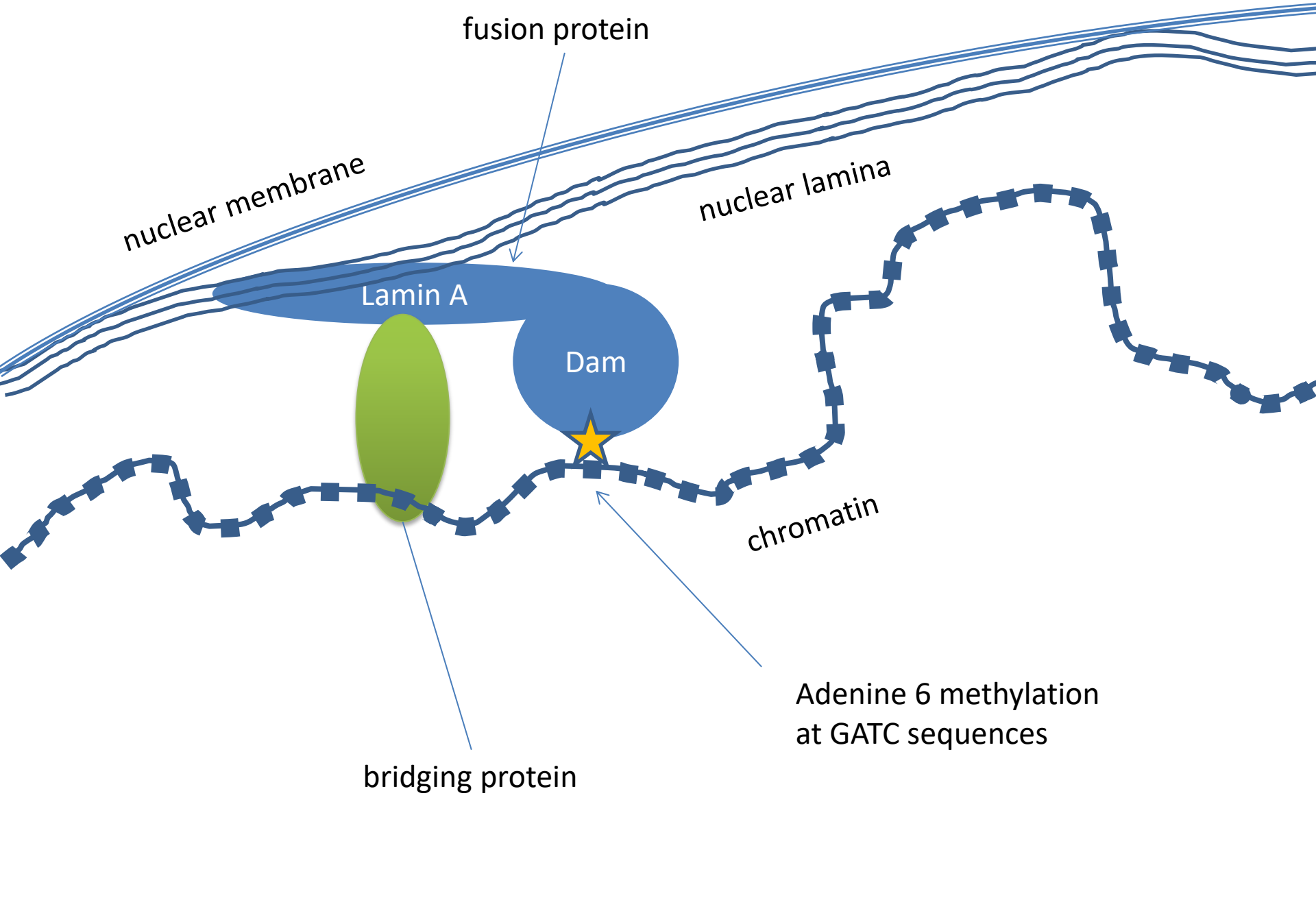
Dam-ID is a proximity assay

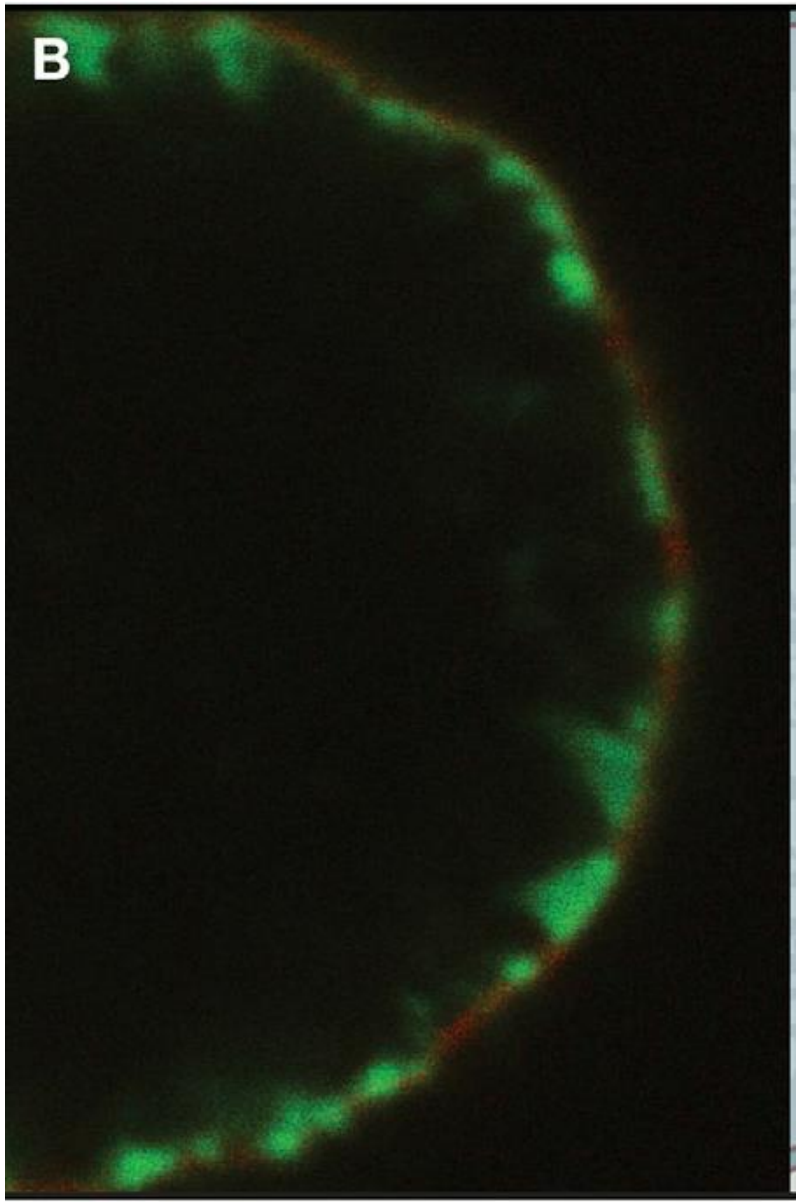
Example:

Fusion protein
TF + Dam



This figure refers to a DNA-binding protein


fusion protein
nuclear membrane
nuclear lamina
Lamin A
Dam
chromatin
bridging protein
Adenine 6 methylation
at GATC sequences



(B) Labeling of DNA-NL contacts in a cultured human cell by co-expression of Dam-Lamin B1 and a GFP-tagged m6A-tracer protein that binds to adenine-methylated DNA (green). Image by Jop Kind. A single confocal section is shown. Lamin B1 is shown in red.

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LADs = Lamina-Associated Domains

Parts of the chromosomes that are attached to the nuclear lamina by either

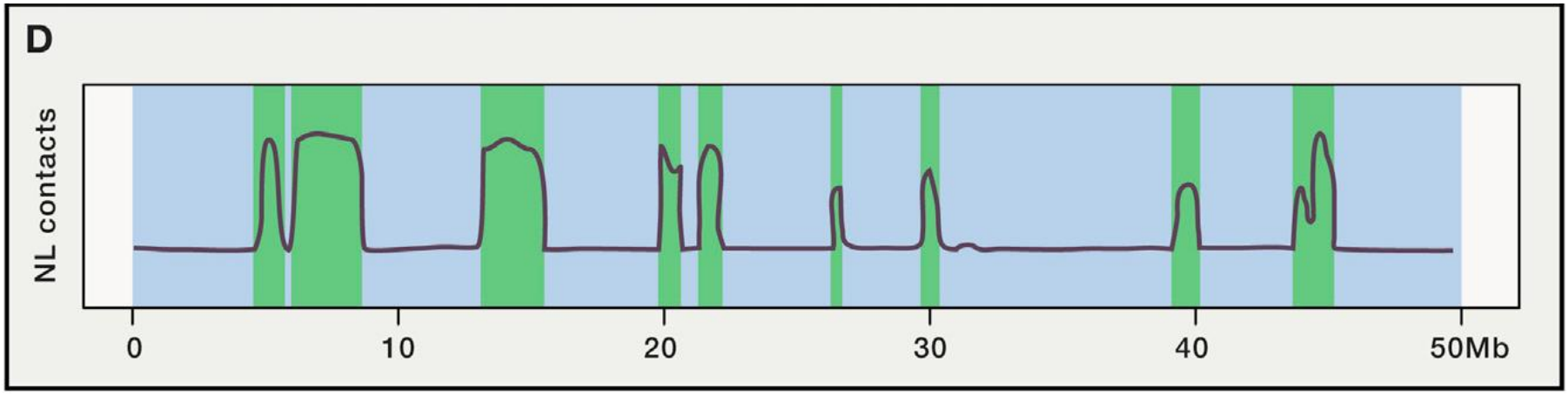
1) direct interaction ?

or

2) Nucleosome–mediated interactions

mediated by Lamina components or other associated proteins

LADs make up nearly **40%** of human and mouse genomes



(D) Schematic representation of a DamID track of interactions with the NL along part of a mammalian chromosome, illustrating the size range, relative sharply defined edges, and broad distribution of LADs. LADs are highlighted in green, inter-LAD regions in blue.

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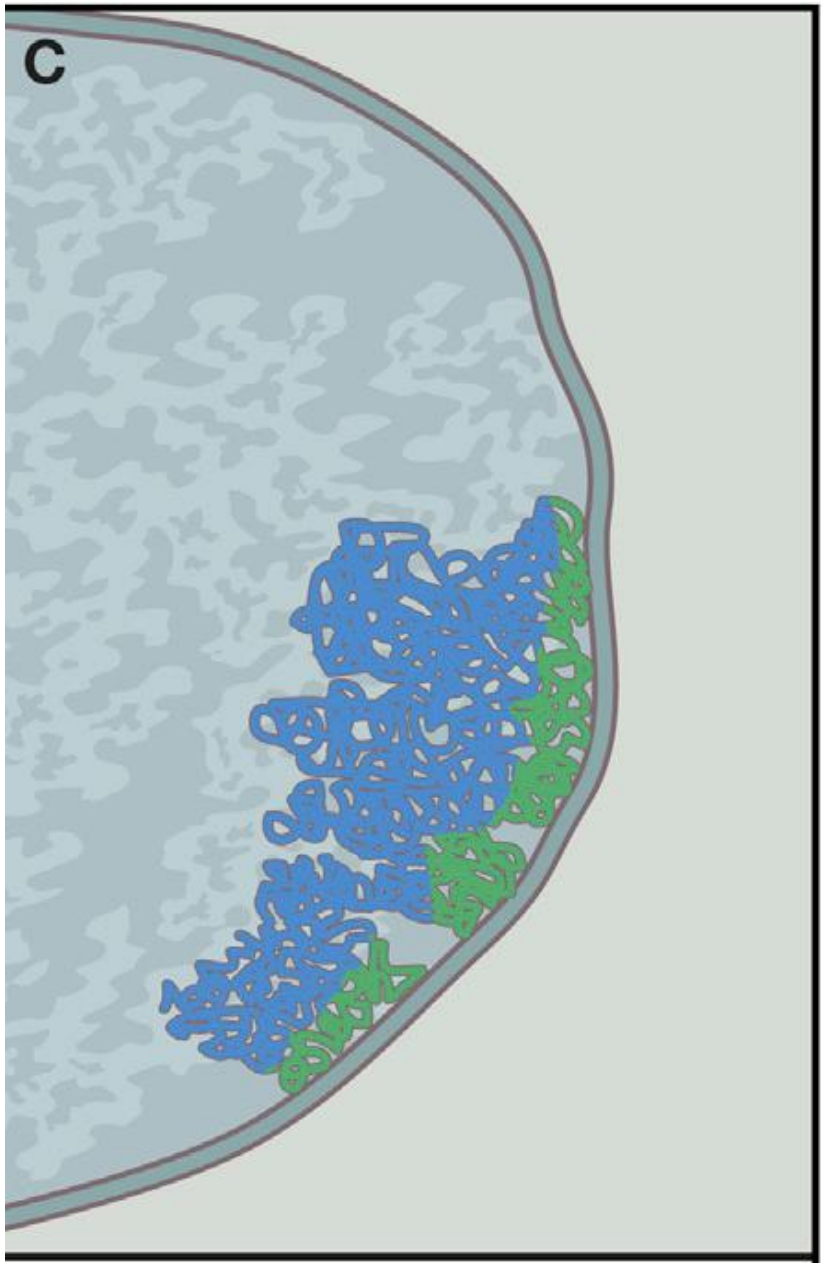

(C) Cartoon model illustrating how a chromosome (blue) is associated with the NL through multiple LADs that jointly form a heterochromatin layer (green). Only one chromosome is depicted. LADs that jointly form a heterochromatin layer (green). Only one chromosome is depicted.

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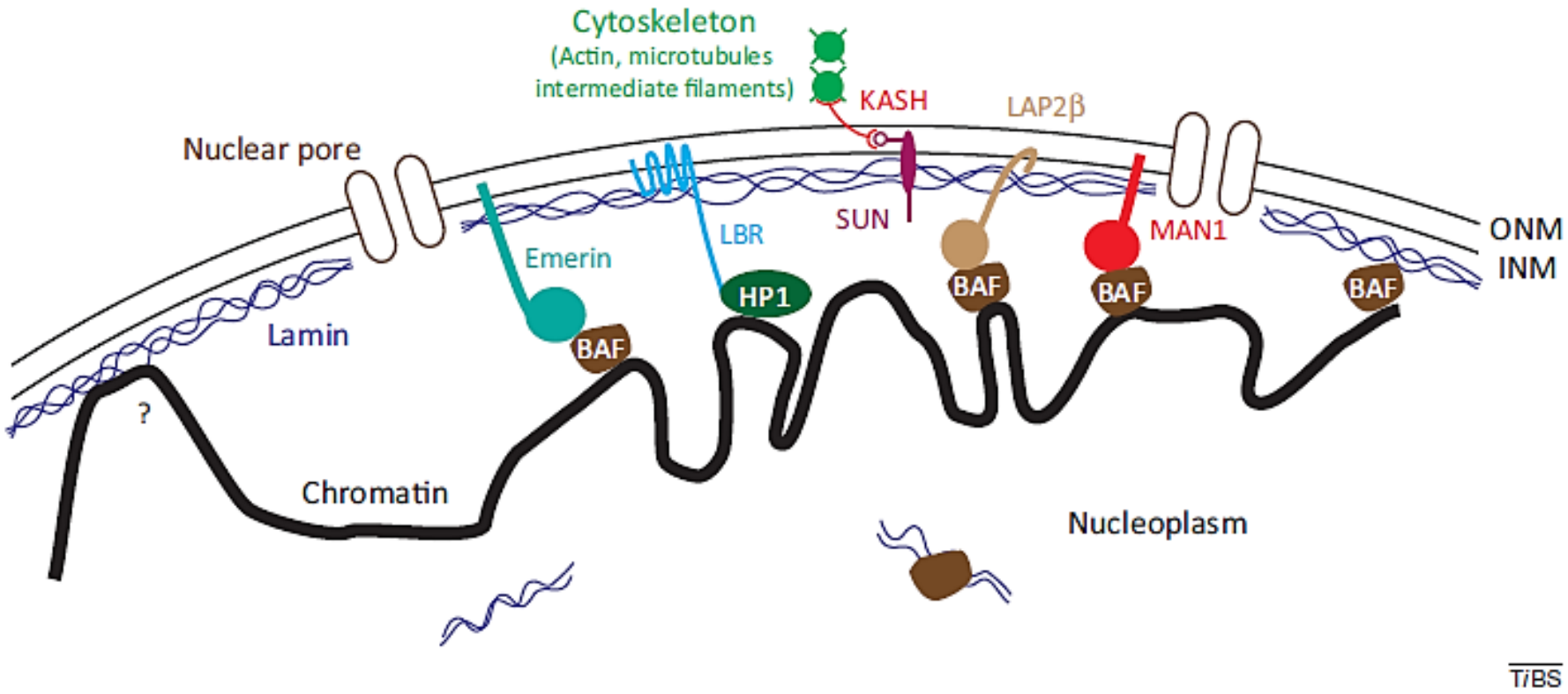
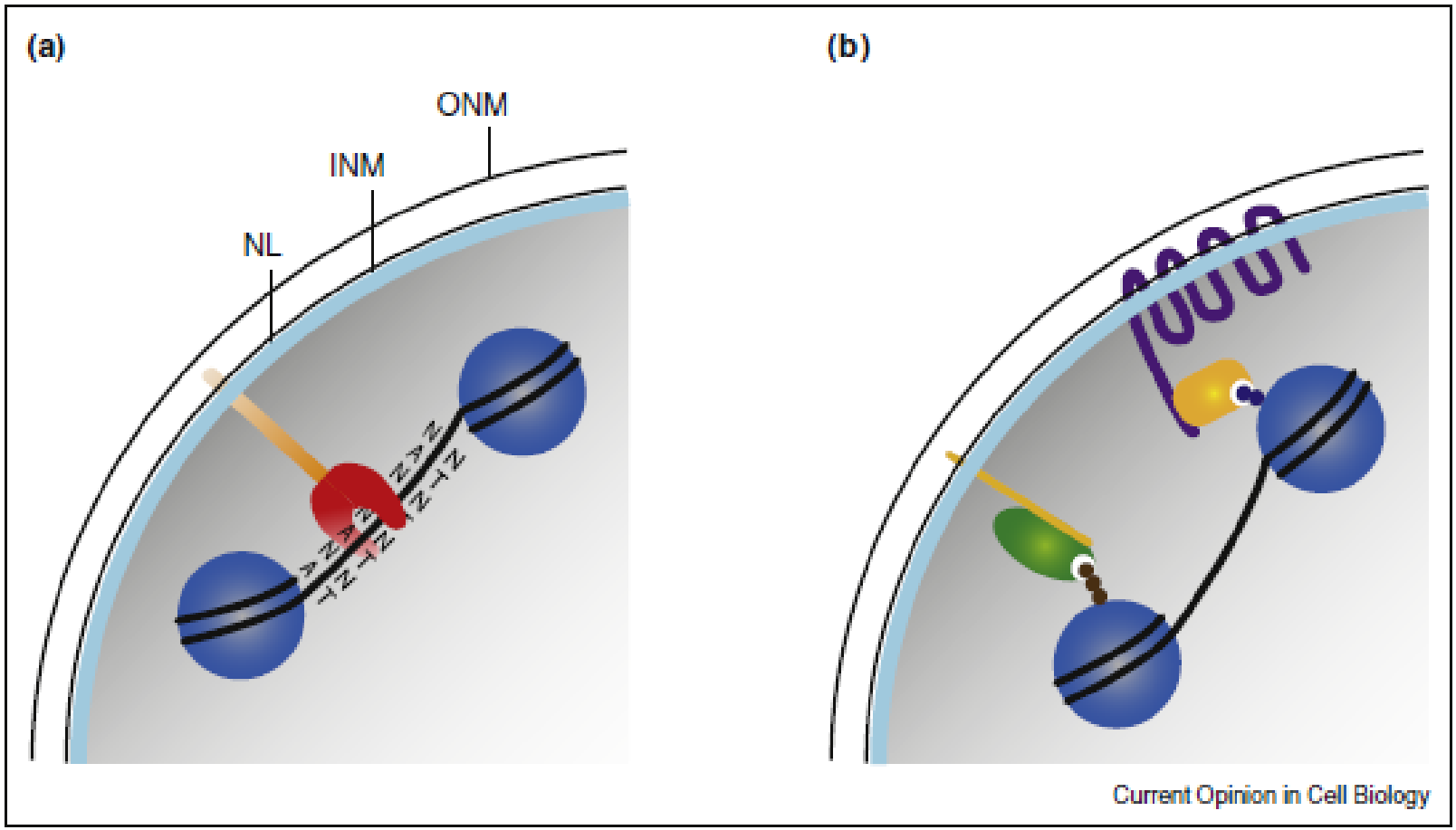


Figure 1. Schematic view of the nuclear envelope (NE). Lamins form a meshwork of intermediate filament proteins underlying the inner nuclear membrane (INM), where they interact with several distinct classes of transmembrane proteins. One class contains the NE-associated proteins Emerin, LAP2b (lamina-associated polypeptide 2b) and MAN1 (target of MAN autoimmune antibody 1), which interact via a conserved LEM (Lamina-associated-polypeptide, Emerin and MAN1)-related domain with the small protein barrier-to-autointegration factor (BAF), which has affinity for chromatin. A second example is the lamin B receptor (LBR), which contains seven transmembrane domains that span the INM. It interacts directly with the chromatin binding protein HP1 or in mammals with H4K20me through a Tudor domain. Finally, SUN (Sad1–UNC84 homology) domain proteins span the INM, and interact with lamins on the nucleoplasmic side and with KASH (Klarsicht/ANC-1/Syne Homology) domain proteins in the perinuclear space. KASH proteins (called Nesprins in human) span the outer nuclear membrane (ONM) and contact the cytoskeleton. Up to 10% of lamins and BAF are also found in the nucleoplasm in some cells.

Towbin et al., Trends in Biochem Sci., 38:356-363, 2013



Cartoon model depicting two possible non-exclusive mechanisms responsible for the tethering of LADs to the NL.

(a) DNA-binding factors anchored to the NL may recognize specific sequence motifs enriched in LADs;

(b) Certain proteins interacting with the NL may bind specific histone modifications, such as H3K9 methylation. INM = inner nuclear membrane; ONM = outer nuclear membrane.

Amendola & van Steensel, Curr. Op. Cell Biol., 28:61–68, 2014

How many proteins ?

Moodle Activity 4

Table 1. Features of Mammalian LADs

	LAD	Inter-LAD
Gene density	low[a]	high
Gene expression	low	high
Hi-C compartment	B	A
Replication timing	late	early
Retroelements	LINE[a]	SINE
Sequence A/T content	high[a]	low
Histone marks	H3K9me2, H3K9me3, (H3K27me3)	aH3K4me1, H3K4me3, H3K27ac, other active marks
Pericentric heterochromatin	frequent	rare
Nucleolus association	frequent	infrequent

[a]More pronounced in cLADs compared to fLADs (Meuleman et al., 2013).

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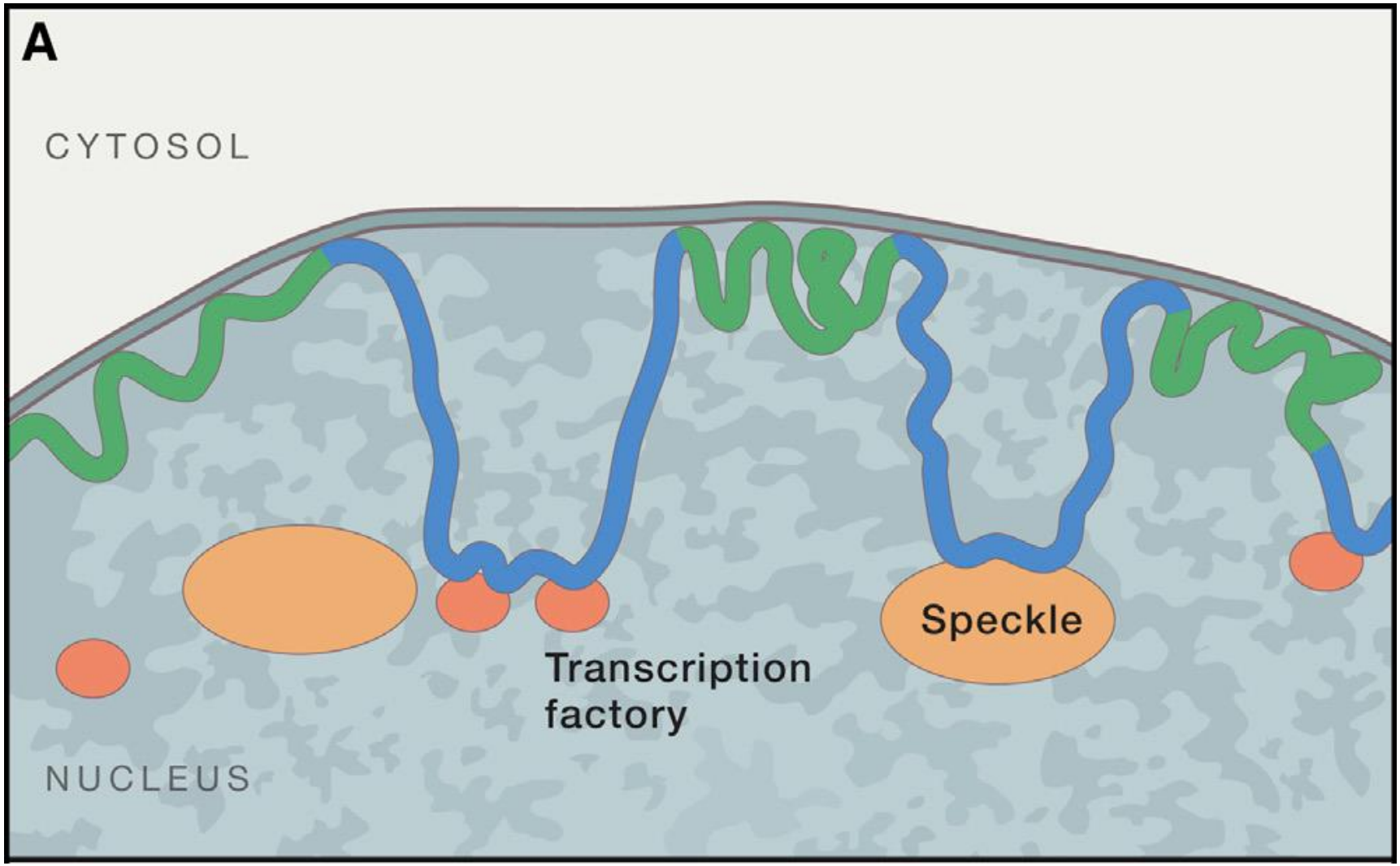
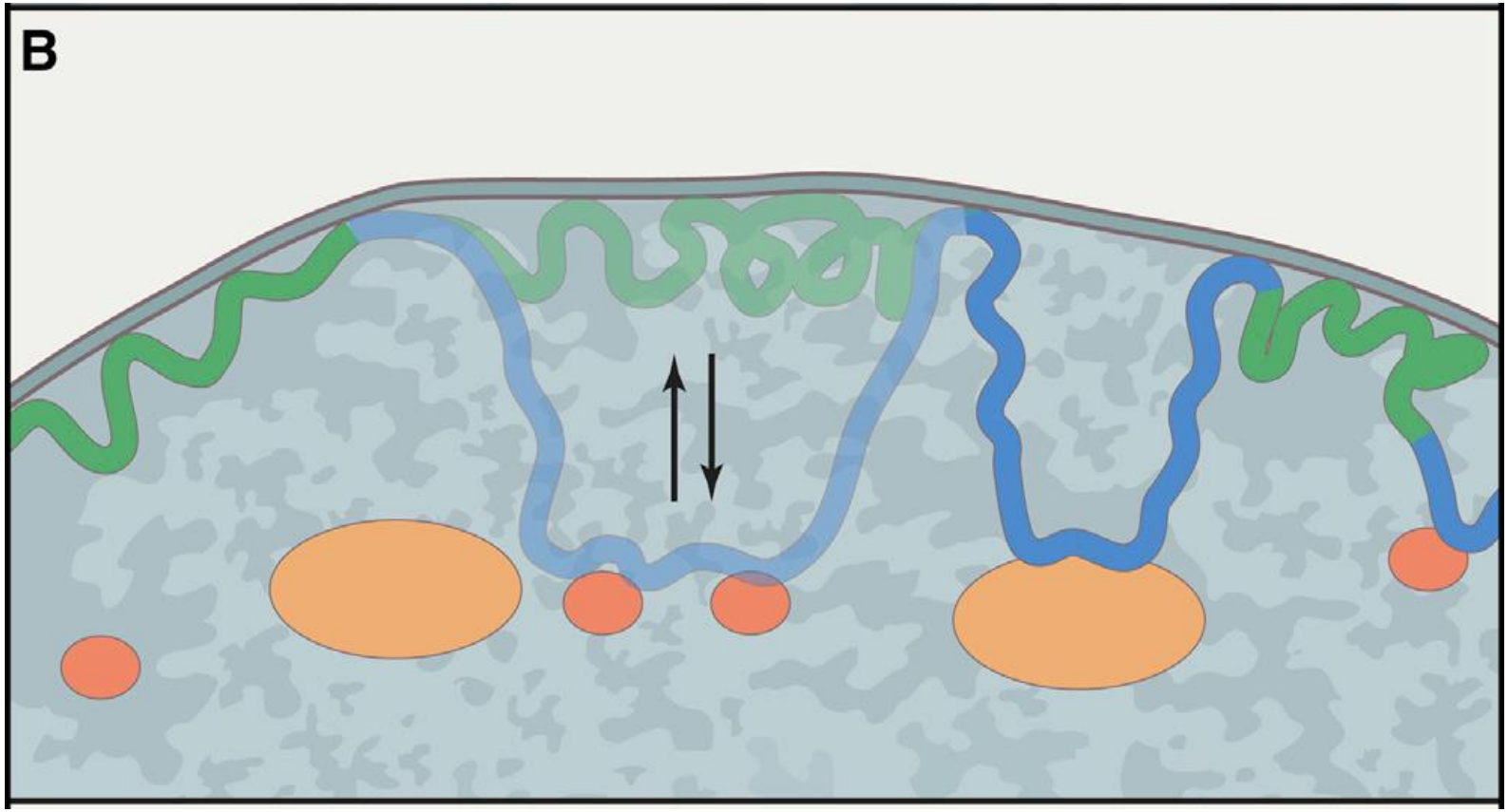


Figure 2. Dynamic Compartmentalization of Chromosomal Domains
(A) Besides anchoring of LADs (green) to the NL, other regions (blue) may be tethered to nuclear structures that are permissive for transcription (orange), such as transcription factories (tf) or splicing factor speckles (speckles). *VanSteelsen 2017*

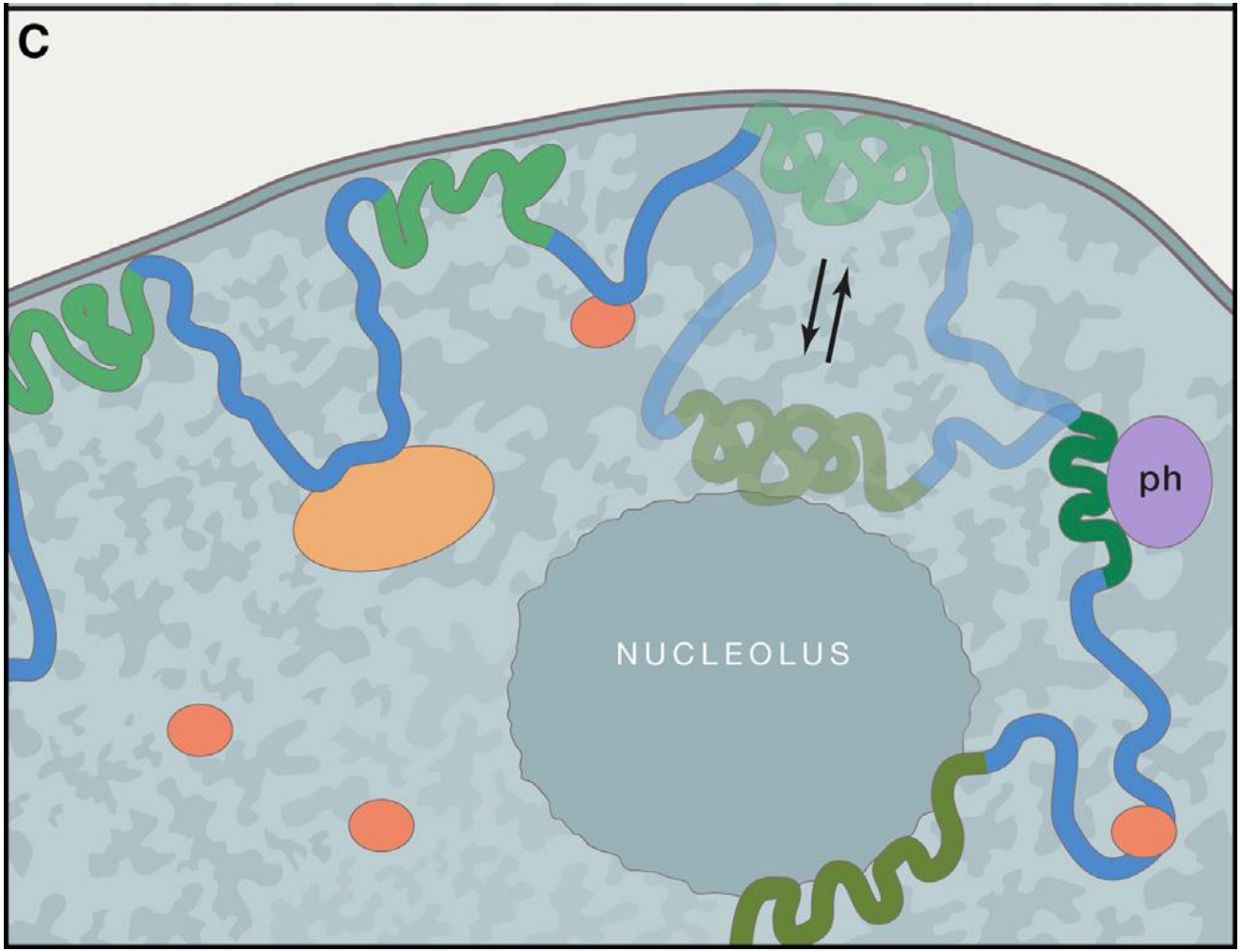


(B) Some LADs (semi-transparent green) contact the NL erratically (i.e., in a subset of cells) and may become transcriptionally active when associated with a permissive compartment (semi-transparent blue).

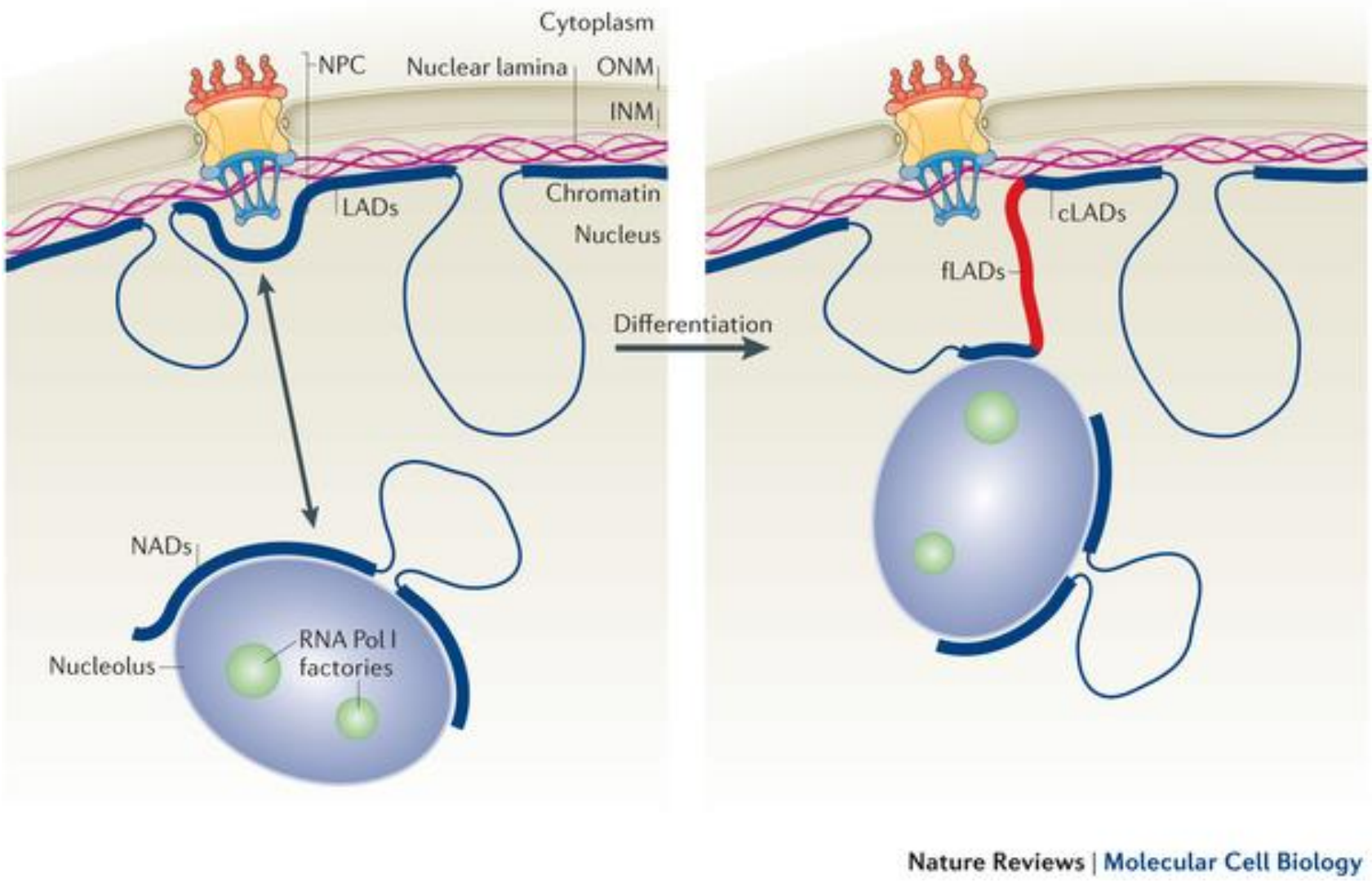
Another location of inactive (heterochromatic) loci is at the nucleolar periphery

These domains are called **NADs** (Nucleolus-associated domains)

Dynamic studies have shown that after mitosis LADs re-distribute in part. Some LADs re-distribute to nucleolar periphery (i.e. they become NADs).



(C) Some LADs are apparently stochastically distributed between the NL, nucleoli, and pericentromeric heterochromatin (ph), which are all repressive environments.



From Pombo & Dillon, 2015

Other structural components of cell nuclei

Effects of nuclear pore proteins on gene positioning

Multiple constituents of the NE contribute to genome organization. In addition to lamins, components of the nuclear pore complex (NPC) show chromatin association

Geyer et al., 2011

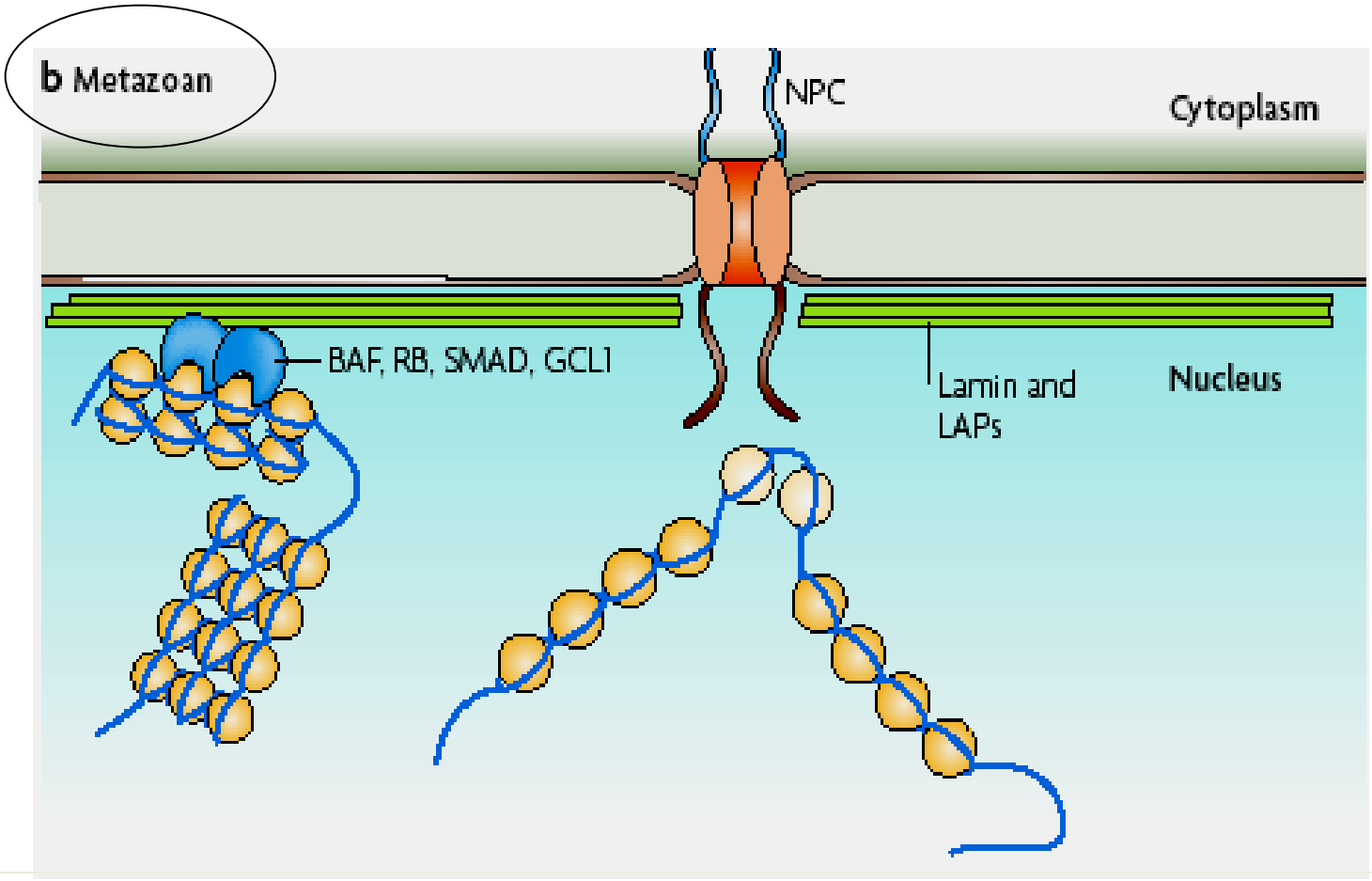


Figure 2 | **The nuclear periphery in metazoans and yeast.** In eukaryotic cells, the nuclear compartment is separated from the cytoplasm by the inner and outer nuclear membranes. This membrane bilayer is perforated by nuclear pores, which are constituted by a large multiprotein complex (the nuclear pore complex (NPC)) that is composed of about 30 proteins. This nuclear membrane, together with the pores, is commonly referred as the 'nuclear envelope' (NE).

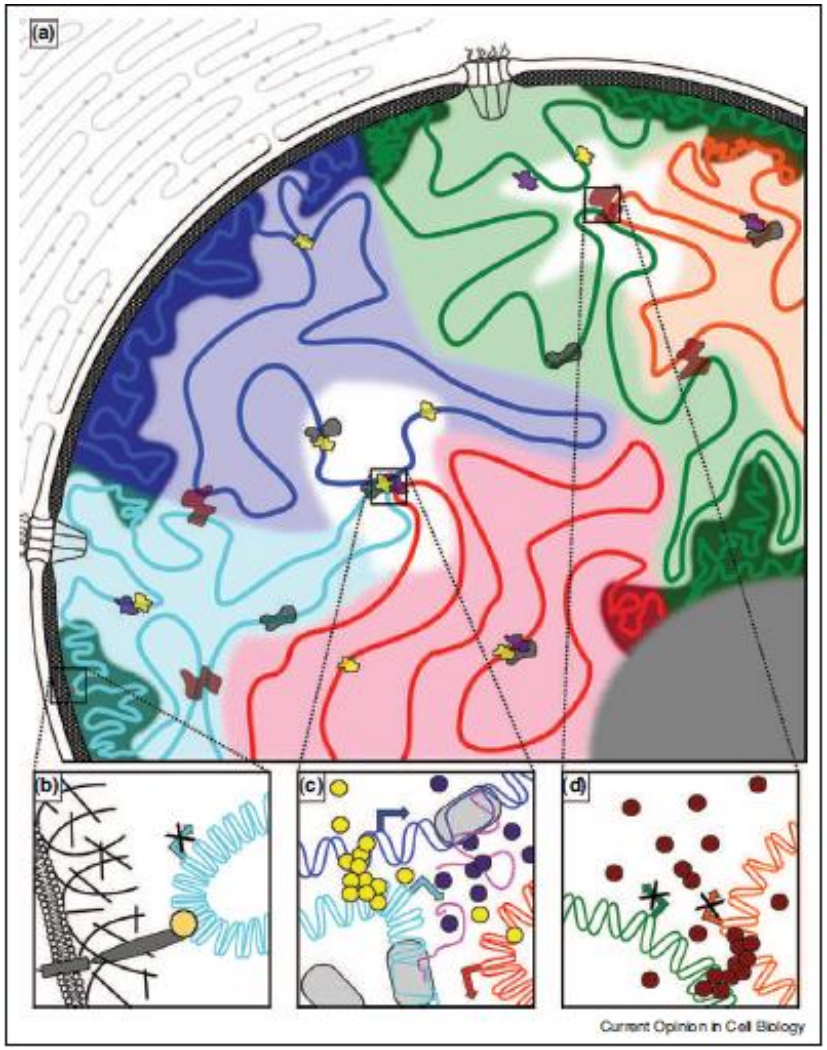
b | In metazoan nuclei, the nuclear envelope is underlaid by a continuous meshwork of lamins and lamin-associated proteins (LAPs), which preferentially associate with inactive chromatin regions. Increasing evidence implicates interactions of chromatin with various nuclear-envelope components in gene repression as well as gene activation. BAF, barrier to autointegration factor; GCL1, germ-cell-less homologue; RB, retinoblastoma 1.

Contribution of transcriptional machinery to nuclear organization

The interchromosomal space located between CTs contains a variety of nuclear substructures, referred to by many names including foci, speckles, bodies and spots (Figure 1a). The number and composition of these bodies depends on cell type [25]. Nuclear bodies are enriched in specific factors, such as those involved in transcription and RNA processing. While the function of these bodies has been challenging to discern, recent studies demonstrate an important role in nuclear organization.

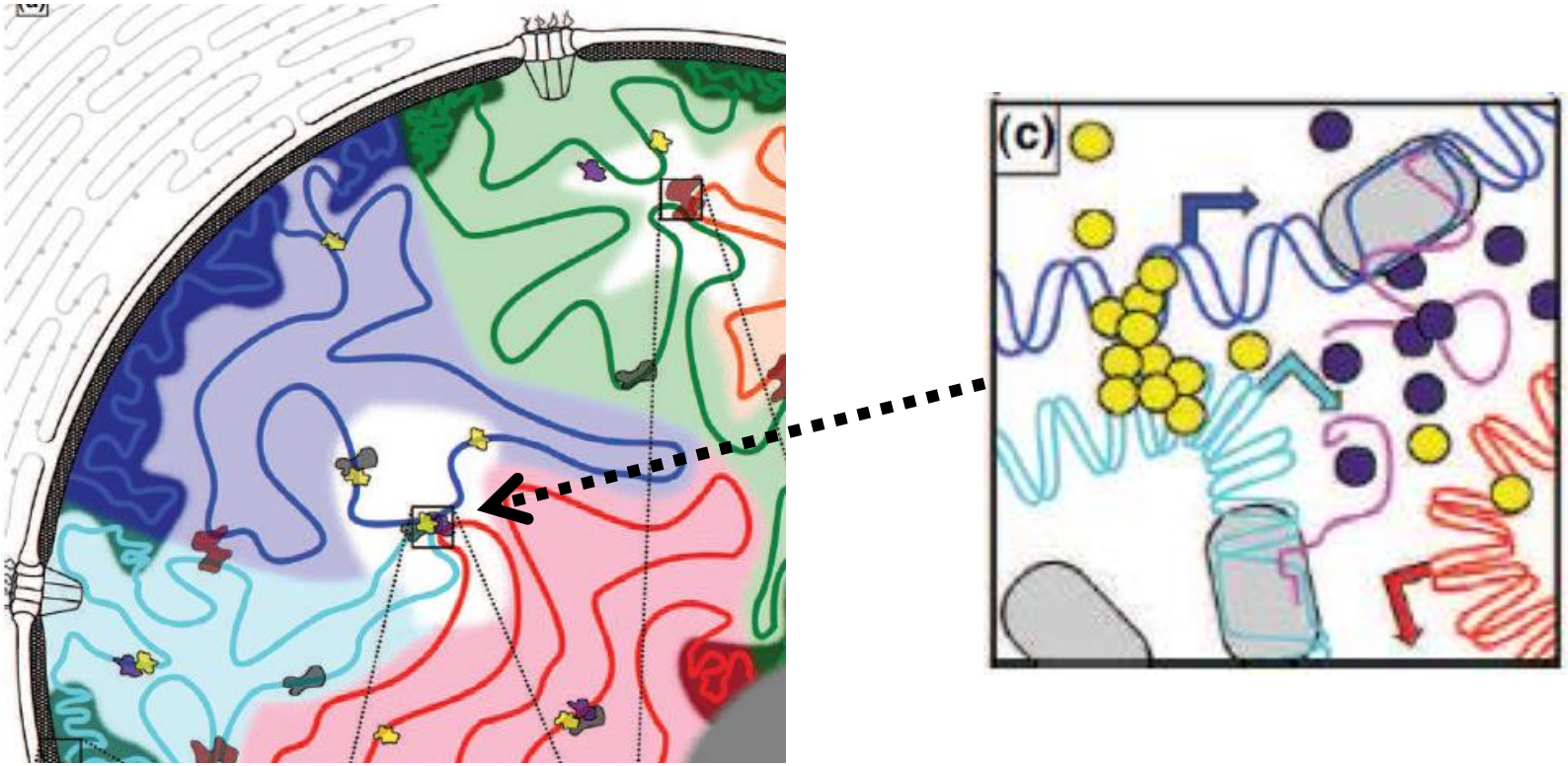
Foci – speckles – bodies - spots

Geyer et al., 2011



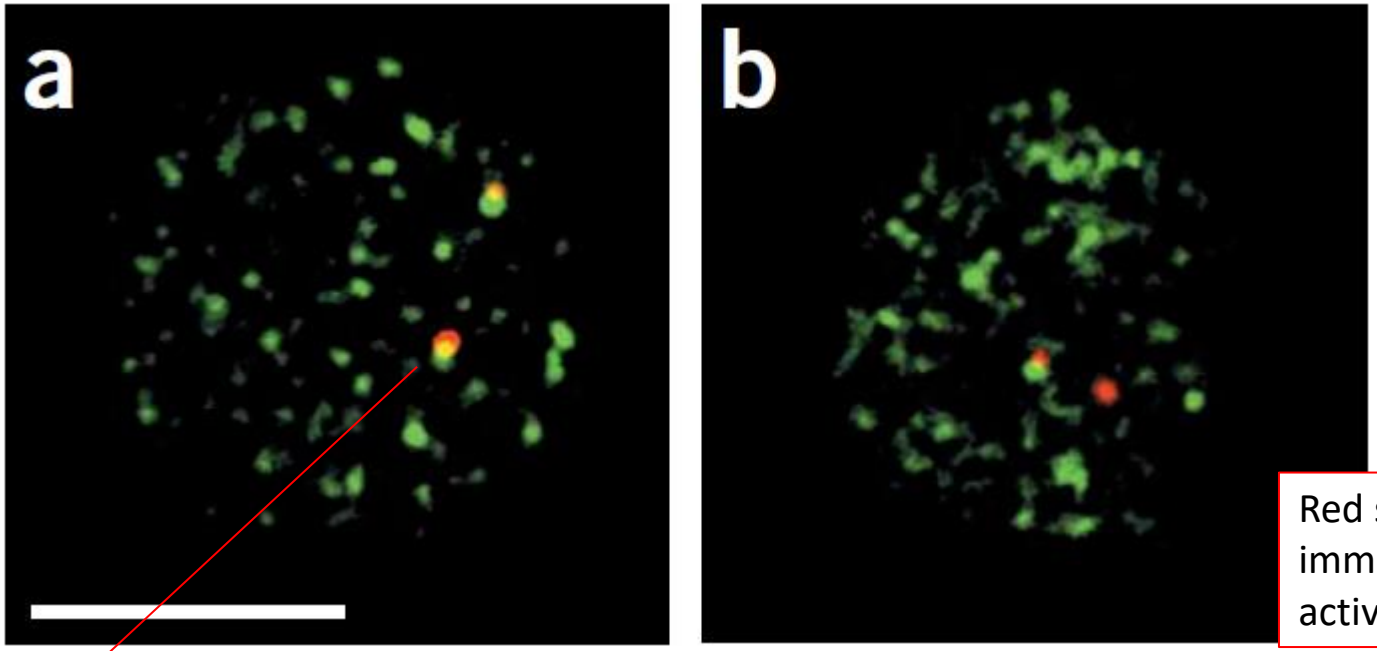
Regulatory machinery, RNA Polymerases, splicing factors, repressive complexes and others, form «bodies» in the interchromosomal spaces

Geyer et al., 2011



Actively transcribing RNA Polymerase II exhibits a non-uniform distribution within interchromosomal spaces (Figure 1a). These 'transcription factories' have varying composition, with some enriched for RNA splicing factors, such as SC35 [26] (Figure 1c).

Geyer et al., 2011



Red spot: RNA immuno-FISH

RNA immuno-FISH

green spots: RNA Pol II immunostaining

Red spots: DNA immuno-FISH of an active gene (see legend)

DNA immuno-FISH

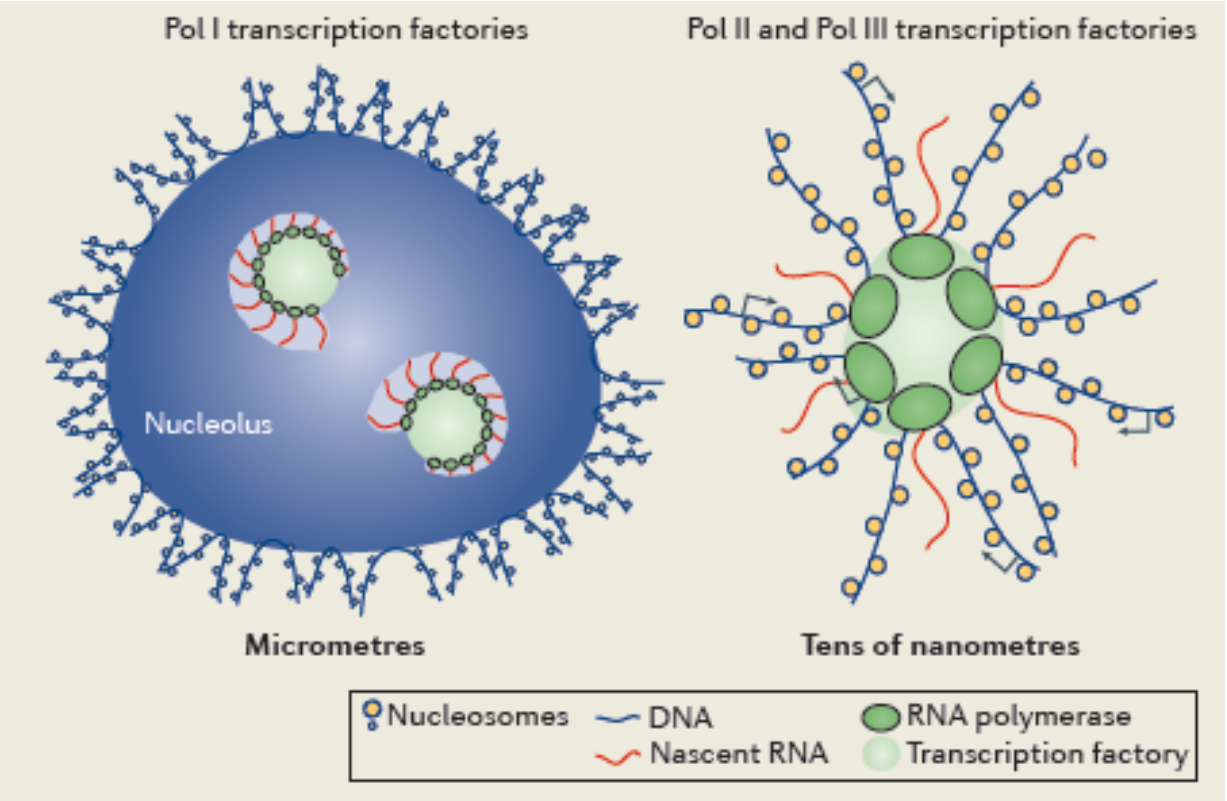
Figure 4 Actively transcribed genes associate with RNAP II foci. (a) RNA immuno-FISH of Hbb-b1 transcription (red) with RNAP II staining (green) in anemic spleen erythroid cells. Scale bar, 5 mm. (b) DNA immuno-FISH of Eraf (red) with RNAP II staining (green).

from Osborne et al., Nature Genetics 36(10):1065-71 · November 2004

Foci – speckles – bodies - spots

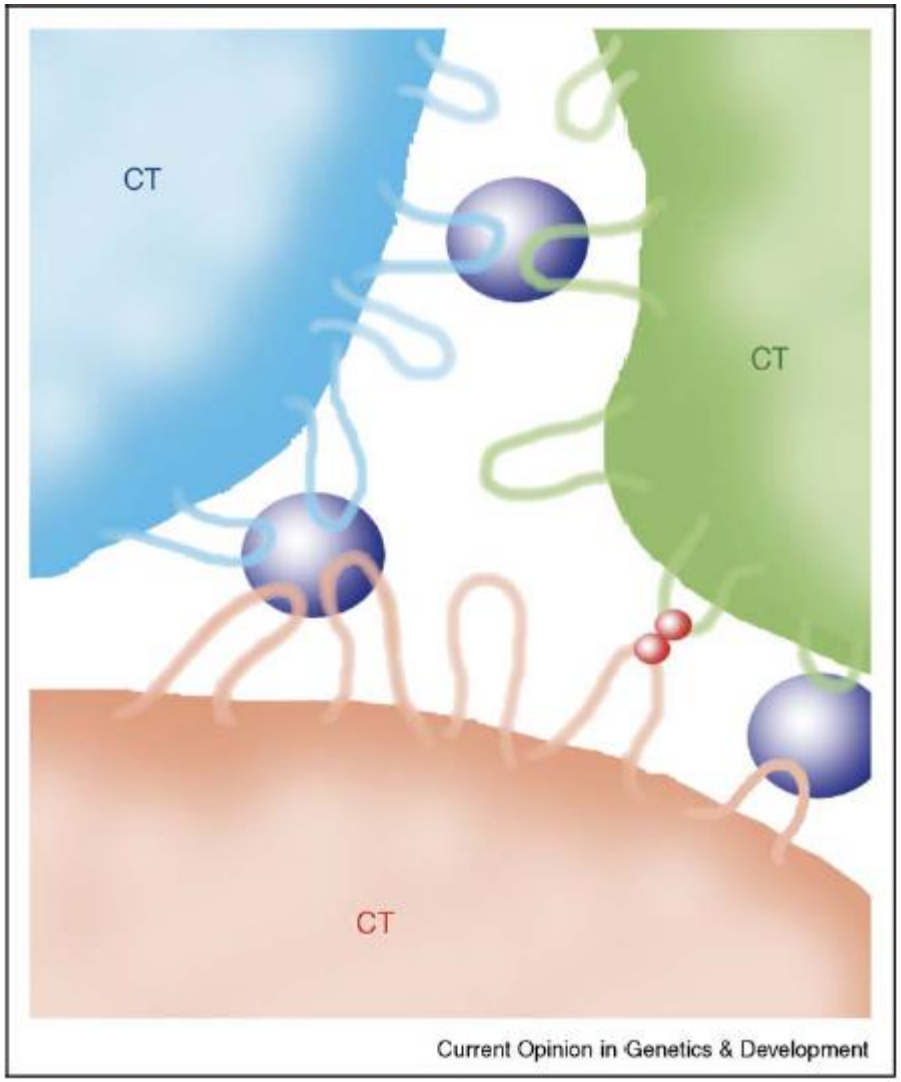
These supramolecular organizations (organelles) contain RNA Polymerases (many copies) and associated factors, including proteins involved in RNA processing (splicing factors, poly-Adenylation enzymes, capping enzymes etc.)

Transcriptional Factories



Pol I factories are found within **nucleoli** (see the figure, left), which in HeLa cells contain on average of 500 active enzymes and about four 45S rRNA genes, each of which is transcribed simultaneously by approximately 125 enzymes.
Individual nucleoli bring together 45S rRNA genes from clusters present in different chromosomes, constituting one of the first known examples of gene expression coordinated within a single structure

Pombo & Dillon 2015

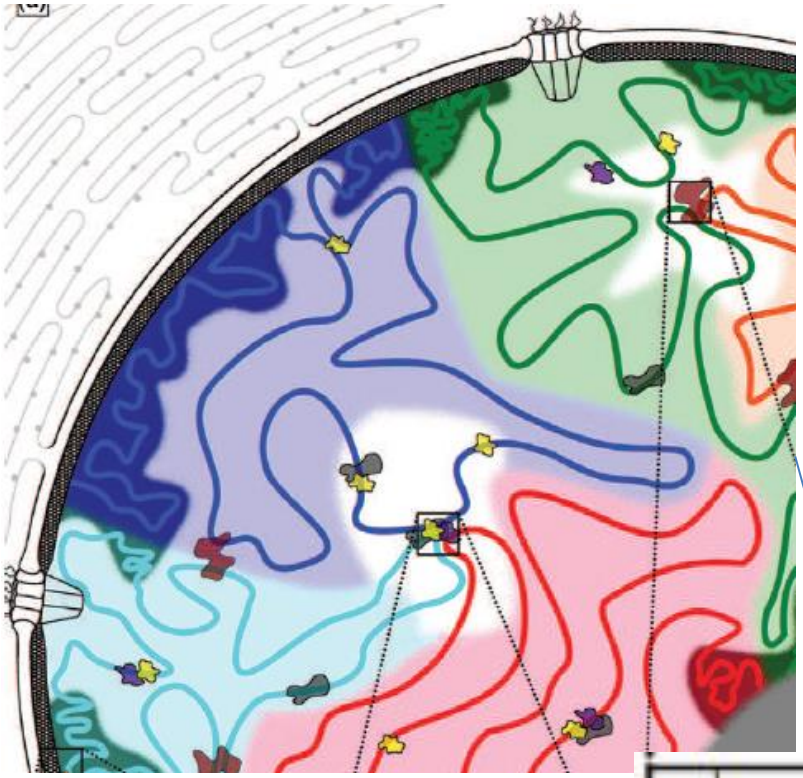


Nuclear organization. Three different chromosome territories (CTs) are shown. Potentially active genes in *cis* and *trans* dynamically engage transcription factories (blue spheres). Most 'active' genes spend the majority of their time outside transcription factories and are transcriptionally inactive. Other types of regulatory interactions (red spheres) might possibly sequester genes or inhibit mobility and factory contact.

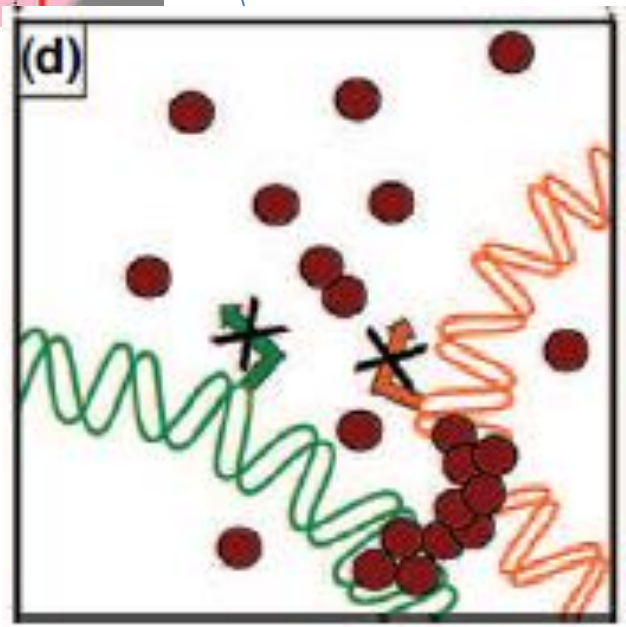
Transcriptional repressors contribute to nuclear organization. This has been well documented for Polycomb group (PcG) proteins. PcGs play a role in the developmental repression of *Hox* gene clusters, which display an evolutionarily conserved chromosome arrangement and span megabases of DNA. Extensive studies of *Hox* genes in Drosophila demonstrated that transcriptional repression depends upon PcG proteins that associate with Polycomb Response Elements* (PREs) distributed throughout the gene clusters [30]. Repression correlates with coalescence of PREs into nuclear foci, termed Polycomb bodies that are present in the nuclei of most, but not all, tissues (Figure 1d) [31].

* In Mammals no PRE have been identified so far

Geyer et al., 2011



Polycomb bodies
Transcriptional Repressors



Borders of LAD, NAD domains

Borders of «loops» protruding from CTs towards transcriptional factories or other functional complexes

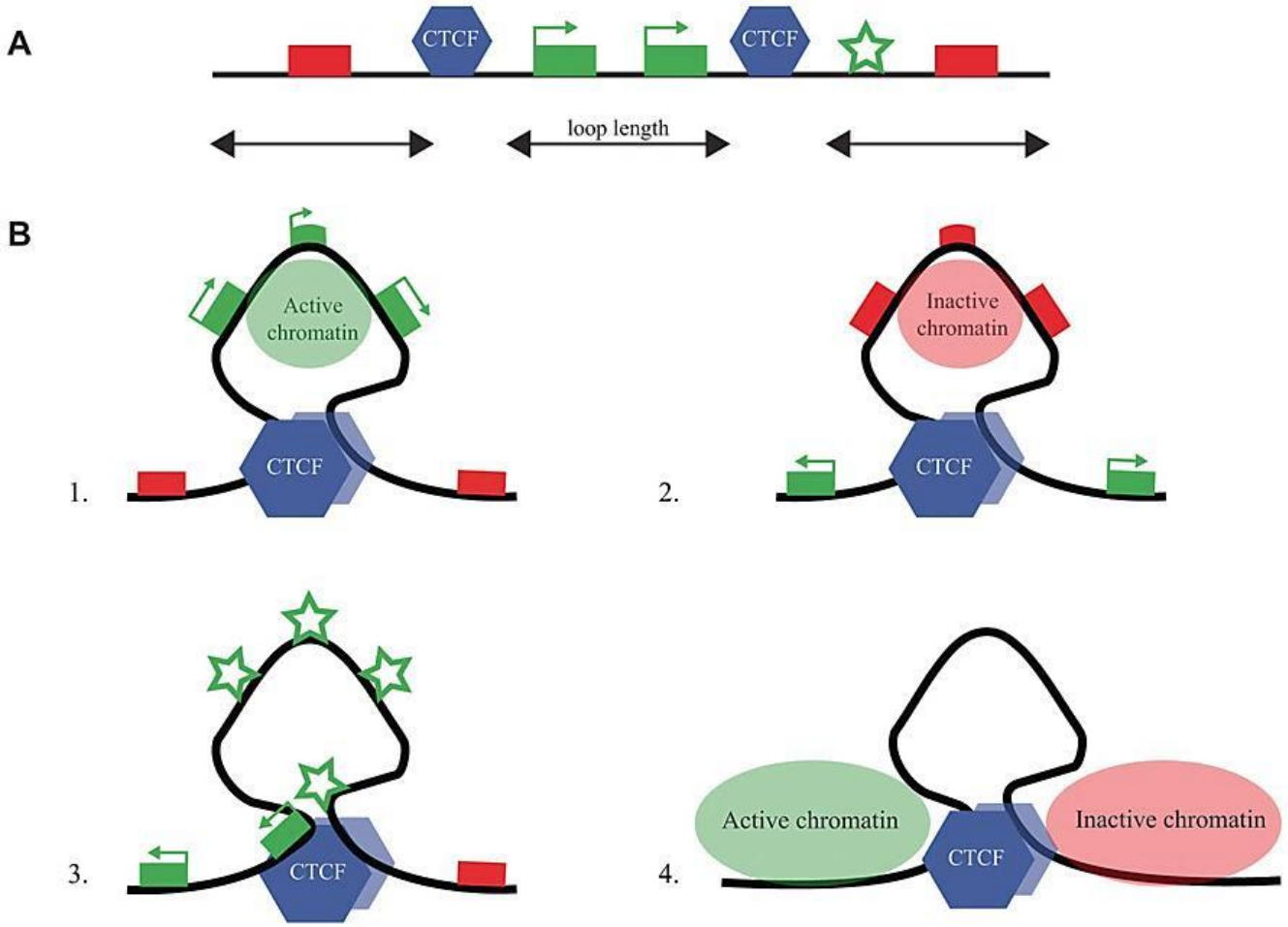
Border regions, also called «insulators» are usually very rich in genes, often highly transcribed (e.g. tRNA genes) and contain binding sites for insulator proteins, such as CTCF

CCCTC-binding factor

Insulator proteins also contribute to nuclear organization by establishing long-range and inter-chromosomal associations [35]. The insulator binding protein CCCTC binding factor (CTCF) is a multiple zinc finger protein with a highly conserved DNA binding domain.

CTCF participates in inter-chromosomal and intra-chromosomal interactions that regulate transcription. Interactions between CTCF and partner proteins such as cohesin and lamins might be crucial for positioning genes into subnuclear compartments.

Geyer et al., 2011



REVIEW ARTICLE

Front. Genet., 17 October 2012 | doi: 10.3389/fgene.2012.00217

Chromatin loops, gene positioning, and gene expression

Sjoerd Holwerda and Wouter de Laat*

Technical

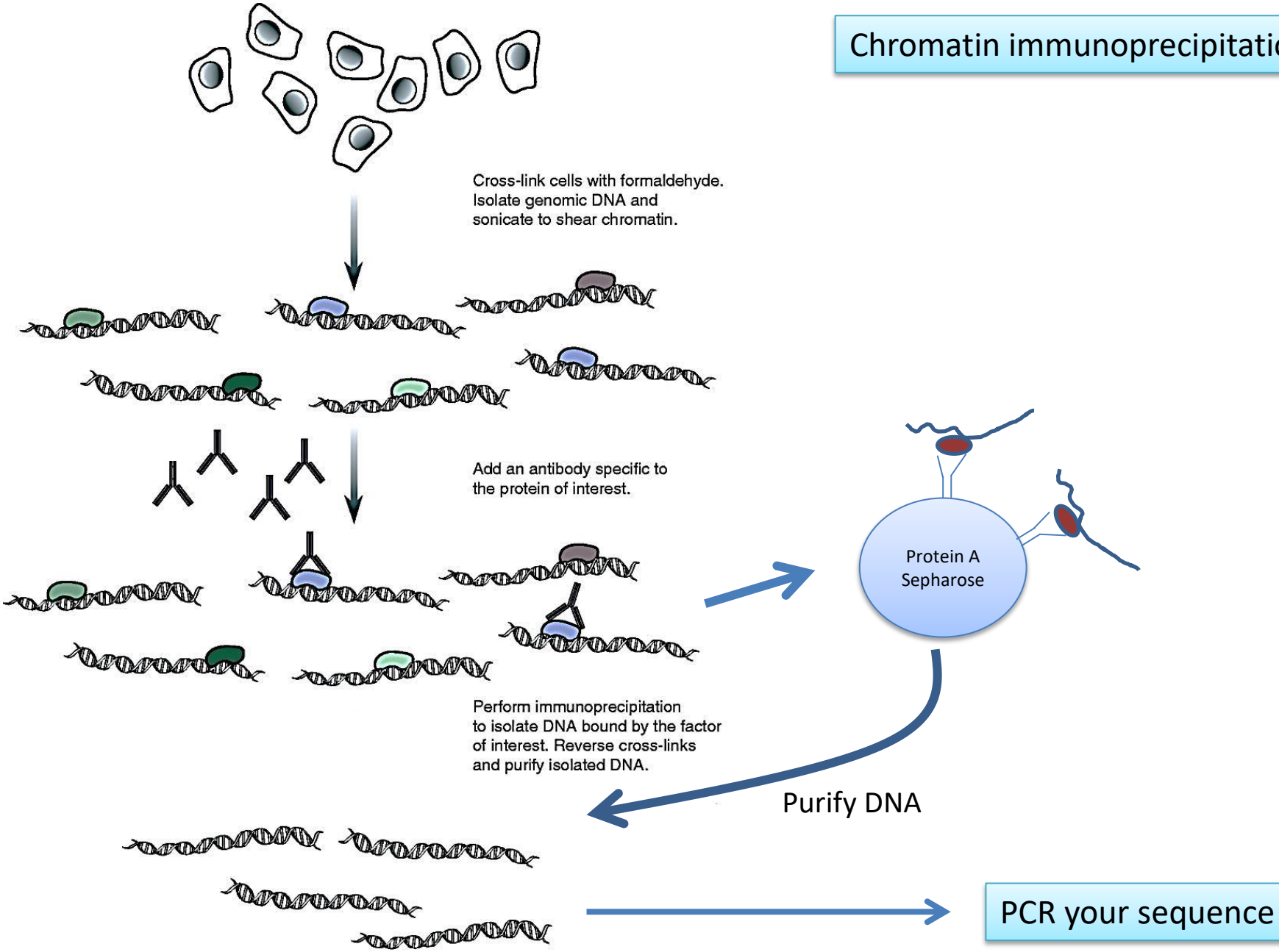
- Dam-ID
- Chromatin Immunoprecipitation
- 3C – Chromosome Conformation Capture

One (or two) single loci

versus

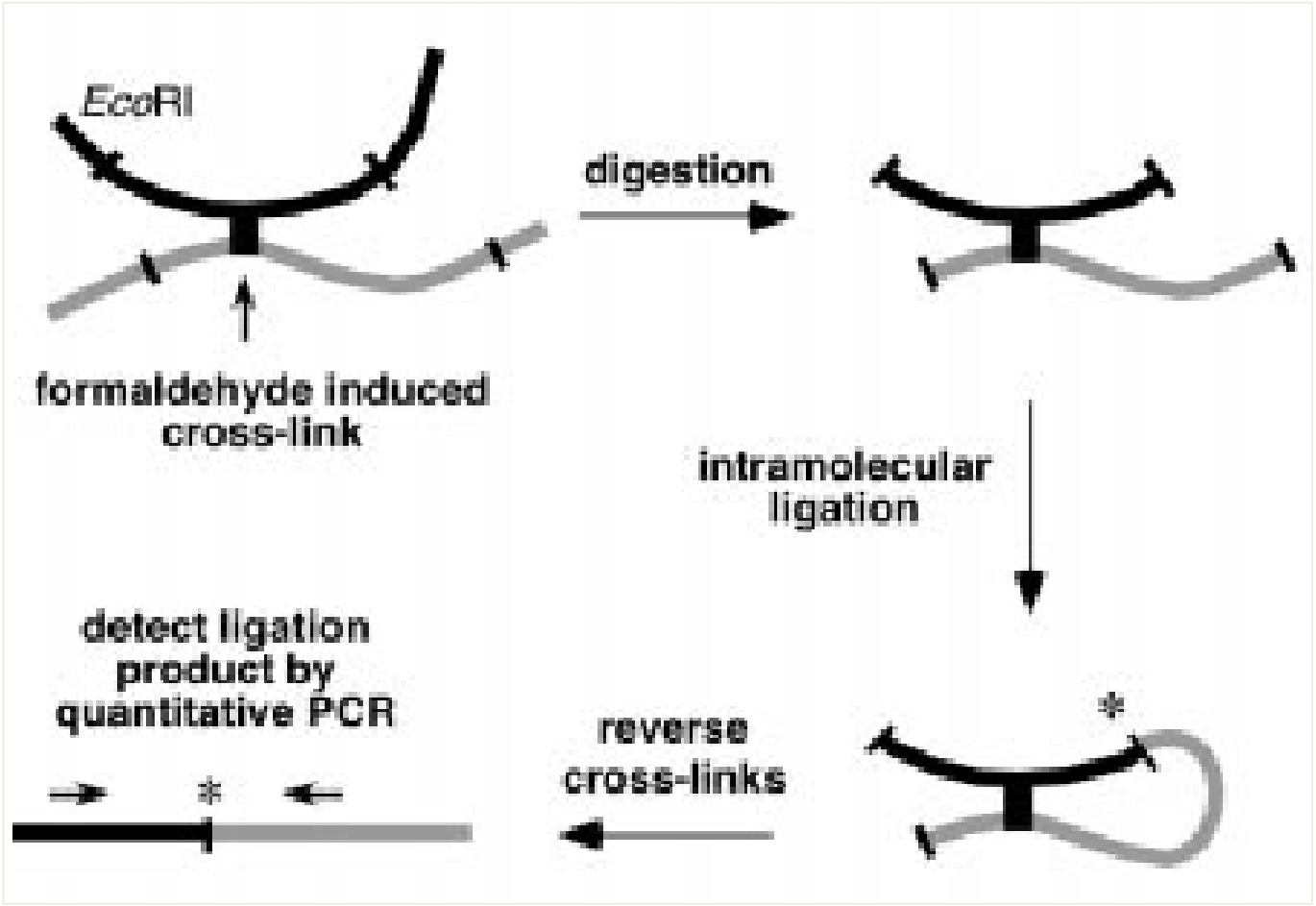
Genome-wide

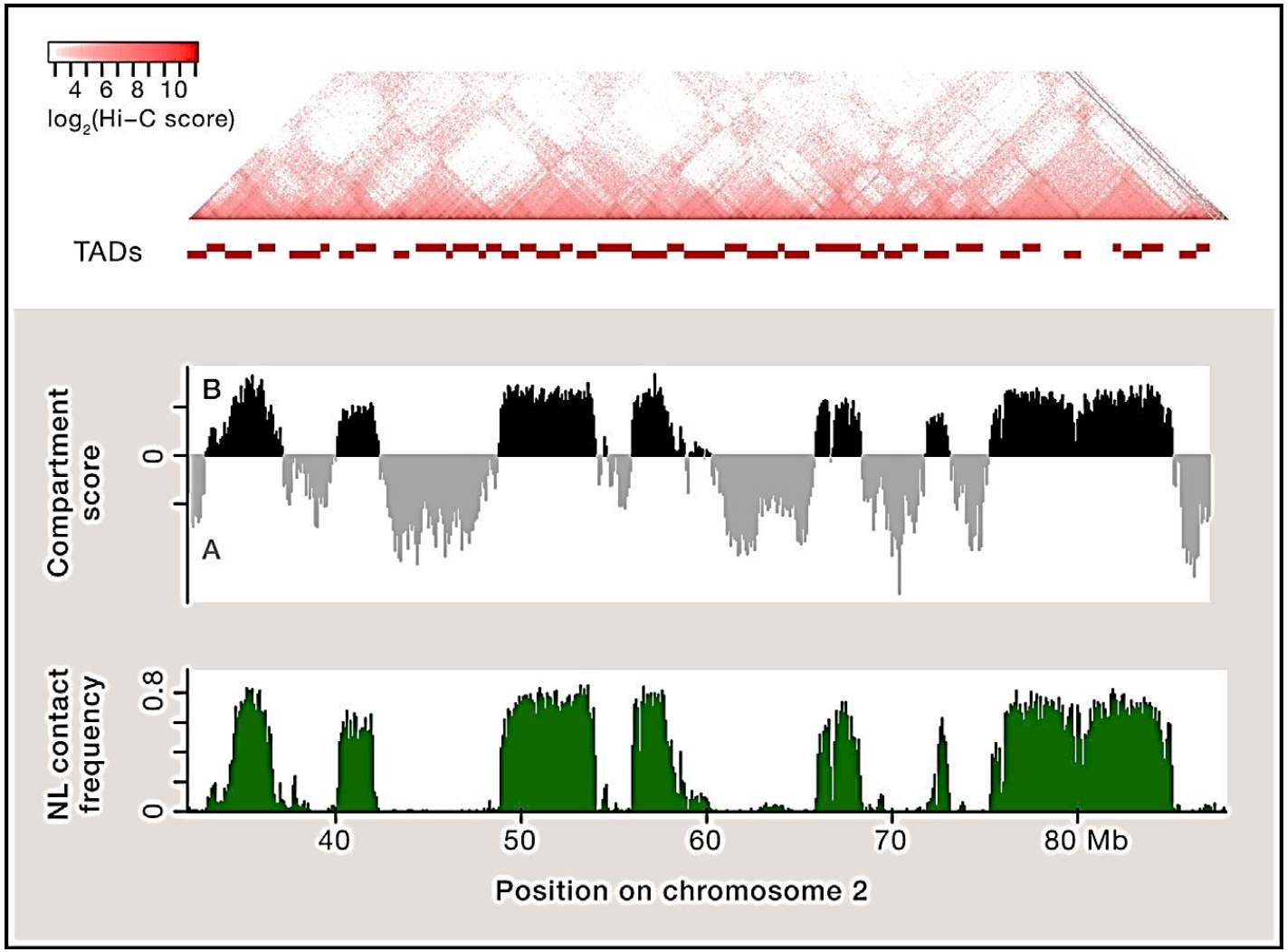
Chromatin immunoprecipitation



PCR your sequence

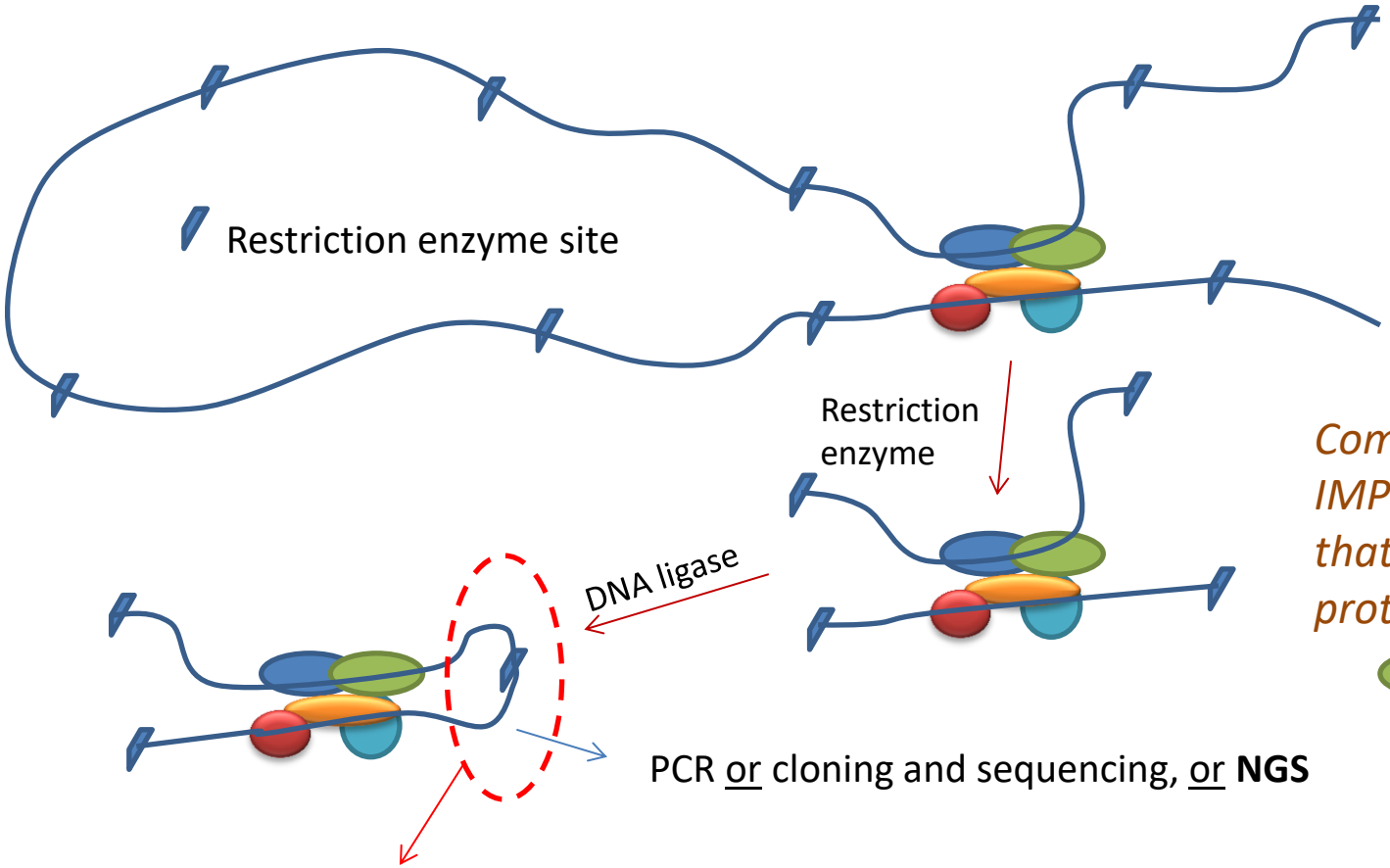
The basic methodology to study Enhancer-Promoter interaction is 3C assay = chromosome conformation capture





LADs Compared to Domains as Identified by Hi-C.
Comparison of a NL contact frequency profile and Hi-C data in human KBM7 cells (see Data Analysis). Note the remarkably strong similarity of NL contact frequencies to the compartment A/B profile and the partial similarity to TAD structure.

Long-range interactions are studied with 3C (Chromatin Conformation Capture) or different genome-wide scale variants (4C, 5C, Hi-C, ChIA-PET).



Complexes may also be IMPT using an antibody that recognizes a specific protein → ChIA-PET

PCR for single interaction.
Generate libraries to NGS for genome-wide studies

Note: from this scheme nucleosomes are omitted