


Review

CENP-B: Cornerstone of Kinetochores in Centromeres of Human Chromosomes

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Abstract

Each chromosome contains a centromere, the site at which the kinetochore assembles to ensure accurate chromosome segregation during cell division. Centromeric chromatin, which anchors the kinetochore, includes three core proteins: Centromere Protein A (CENP-A), CENP-B, and CENP-C. Among these, CENP-B is unique for its sequence-specific DNA binding to a 17–base pair element known as the CENP-B box within the alpha-satellite DNA. CENP-B contains an N-terminal DNA-binding domain and a C-terminal dimerization domain that together enable juxtaposition of distant CENP-B boxes and promote higher-order centromeric structure. CENP-B also interacts directly with CENP-A and CENP-C, thereby facilitating kinetochore assembly. The CENP-B box includes two CpG dinucleotides that, when methylated, reduce CENP-B binding and limit recruitment of CENP-A and CENP-C. The recently completed human genome assembly (T2T-CHM13) revealed centromeric regions with low CpG methylation, termed centromere dip regions, that coincide with active, unmethylated CENP-B boxes. The uniform density of these unmethylated sites across chromosomes contributes to balanced kinetochore–spindle attachment. The CENP-B gene shows no pathogenic alterations in the American Association for Cancer Research (AACR) GENIE cancer cohort (211,526 patients), underscoring its conserved role in chromosome stability.

Keywords: human genome; chromosome; centromere; kinetochore

1. Introduction

Centromeres are chromosomal regions that define where kinetochores assemble to mediate chromosome movement during cell division. They are characterized by arrays of 171-bp alpha-satellite DNA repeats and a specialized chromatin structure enriched in the histone H3 variant Centromere Protein A (CENP-A) [1–7]. The centromeric chromatin forms the foundation of the kinetochore and includes three essential proteins—CENP-A, CENP-B, and CENP-C—that together recruit additional centromere-associated complexes (CENP-HIKM, CENP-LN, CENP-OPQUR, and CENP-TWSX) to form the constitutive centromere-associated network (CCAN) [8–11]. The CCAN then anchors the outer kinetochore KMN network (KNL1C, MIS12C, and NDC80C), which connects to spindle microtubules to drive chromosome segregation.

Despite decades of study, the repetitive nature of alpha-satellite DNA hindered precise mapping of centromeres until recently. This limitation obscured understanding of how CENP-A, CENP-B, and CENP-C coordinate to ensure faithful segregation. With advances such as the T2T-CHM13 genome assembly, these questions can now be addressed in detail. This review summarizes current insights into the roles of CENP-A, CENP-B, and CENP-C in centromere organization and kinetochore function, with particular emphasis on CENP-B.

2. CENP-B Gene and Protein

The CENP-B gene, located on chromosome 20p13, encodes a 65.2-kDa protein of 599 amino acids [12] (Table 1). CENP-B is distinctive among centromeric proteins as it binds DNA directly in a sequence-specific manner. Its N-terminal helix-loop-helix motif (amino acids 1–125) mediates DNA binding, while its C-terminal dimerization domain (amino acids 540–599) enables protein dimerization [13,14] (Fig. 1). The protein recognizes a 17-bp sequence, the CENP-B box (YTTTCGTTGGAARCGGGA) (Y = Cytosine / Thymine; R = Guanine / Adenine) within centromeric alpha-satellite DNA [15,16]. The presence of the underlined nine-nucleotide core recognition motif is critical for binding. Through dimerization, CENP-B can juxtapose two distant CENP-B boxes, facilitating the formation of higher-order chromatin structures and compact centromeric organization via DNA looping and protein-protein interactions [13,17] (Fig. 2, Ref. [18]). This intrinsic ability to bridge distant DNA sites positions CENP-B as a key architectural factor that prepares centromeric chromatin for subsequent epigenetic and structural regulation.

2.1 Affinity Binding of CENP-B Protein With CENP-B DNA Box

Kinetic analyses determined a dissociation constant (Kd) of 6.3×10^{-8} M for the CENP-B/CENP-B box interaction, placing it at the lower end of known protein-DNA binding affinities (10^{-8} – 10^{-11} M) [19–23] Table 2. This



Table 1. Overview of CENP-A, CENP-B and CENP-C genes and proteins.

Gene	Chromosome locus	Size (Amino Acids)	Molecular mass (kDalton)
CENP-A	2p23.3	140	16.0
CENP-B	20p13	599	65.2
CENP-C	4q13.2	943	106.8

CENP, Centromere Protein.

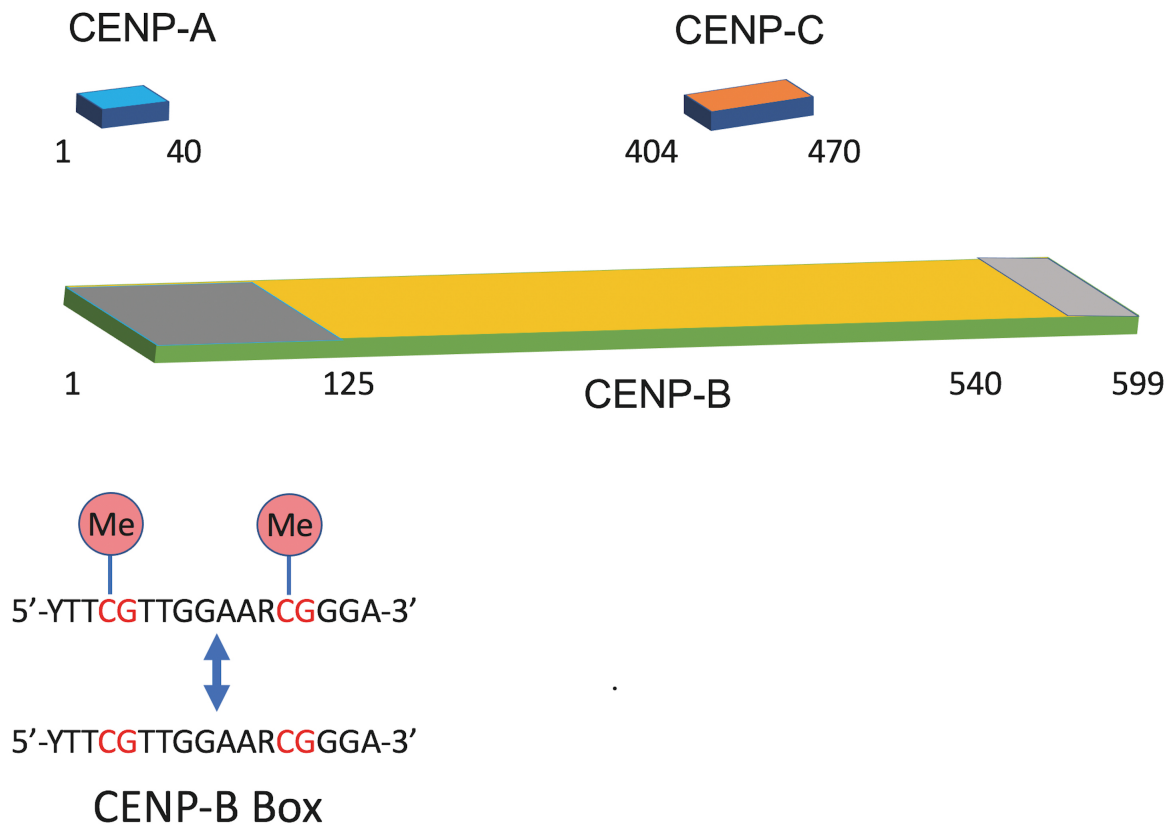


Fig. 1. Domain organization of CENP-A, CENP-B, and CENP-C and sequence features of the CENP-B box. Schematic representations illustrate the relative positions of functional domains within centromere proteins. CENP-A contains an N-terminal domain (residues 1–40). CENP-C is shown with its annotated domain spanning residues 404–470. CENP-B includes an N-terminal DNA-binding domain (dark grey, residues 1–125) and a C-terminal dimerization domain (light grey, residues 540–599). The consensus CENP-B box sequence (5'-YTT**CG**TTGGAAR**CG**GGA-3') is shown below, highlighting the two Cytosine Guanine (CG) dinucleotides (red) that can undergo cytosine methylation (Me), which can influence CENP-B binding.

moderate yet specific affinity is consistent with CENP-B's role in dynamically shaping centromeric chromatin while remaining sensitive to regulatory cues such as DNA methylation.

2.2 Crystal Structure of CENP-B

CENP-B forms homodimers that bind two CENP-B boxes, producing a tetrameric complex [17]. The crystal structure of the N-terminal DNA-binding region (residues 1–129) in complex with the CENP-B box DNA was resolved at 2.5 Å [24]. The domain comprises two helix-turn-helix motifs bound to adjacent major DNA grooves. The DNA adopts a 'kink-straight-kink' conformation, with a total bend of 59°, largely due to phosphate bridging by

an arginine-rich helix. This unique geometry likely contributes to centromere-specific chromatin architecture.

CpG methylation within the CENP-B box can interfere with binding. Modeling of the CENP-B (1–129)–methylated DNA complex showed that methyl groups on cytosines cause steric clashes with CENP-B residues Thr44 and Arg125 [25]. The short alpha-helix (residues 120–129) includes four arginines (Arg125, 127, 128, 129) that penetrate the DNA major groove. Arg125 specifically forms a hydrogen bond with guanine 16, recognizing the second CpG dinucleotide, while the other arginines bind phosphate backbones. CpG methylation disrupts this precise interaction, reducing binding specificity.

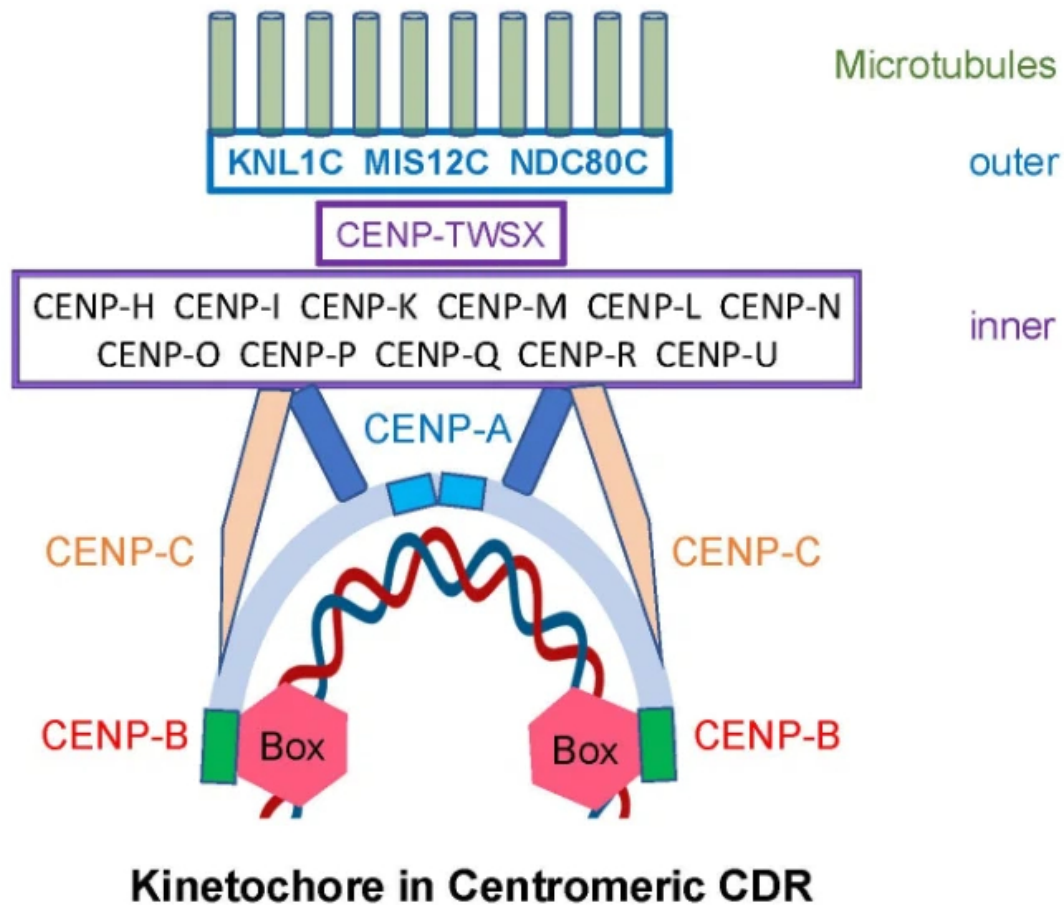


Fig. 2. Schematic model of human kinetochore with cornerstone of CENP-B protein and anchor of unmethylated CENP-B boxes in centromeric dip region (CDR). CENP-B dimer binds to two CENP-B boxes (red hexagons) within α -satellite DNA and interacts with CENP-A and CENP-C to assemble the multi-component inner kinetochore. The inner kinetochore (purple boxes) consisting of CCAN (constitutive centromere-associated network, including CENP-H, -I, -K, -M, -L, -N, -O, -P, -Q, -R and -U) and CENP-TWSX bridges the centromeric chromatin to the outer kinetochore. The outer kinetochore (blue box), consisting of the KMN network (KNL1C, MIS12C and NDC80C) forms the major microtubule-binding interface. Together, these components establish the structural linkage between centromeric DNA and spindle microtubules. Figure reprinted with permission from “Parl (2024) [18]”.

Table 2. Binding affinity of CENP-B with CENP-B box and other protein-DNA modules.

Protein	DNA	Kd (M)	Reference
CENP-B	CENP-B box	6.3×10^{-8}	[19]
MSH2/MSH3	50-mer Oligonucleotide Duplex	7.7×10^{-8}	[20]
c-Myb	Myb Binding Site	3.8×10^{-9}	[21]
ER alpha	Estrogen Response Element (ERE)	2.4×10^{-10}	[22]
p53	p53 Consensus Binding Site	3.1×10^{-11}	[23]

The dimerization domain (residues 540–599), solved at 1.65 Å, consists of two antiparallel alpha-helices forming a symmetric four-helix bundle with extensive hydrophobic contacts [13]. The monomeric domain is unstable in solution, as its hydrophobic surface (~34% of total area) would be solvent-exposed. The N-terminal loops of the dimer project outward, ideally positioned to engage two separate CENP-B boxes through the DNA-binding domains.

This structural configuration supports its role in linking distant centromeric DNA sites and promoting chromatin compaction. Together, these structural insights provide a mechanistic basis for how CENP-B integrates DNA sequence recognition with higher-order centromeric architecture.

2.3 Epigenetic Modification of CENP-B Box

A short inverted repeat (TTGGAA) within the CENP-B box contains CpG dinucleotides at both ends, which can be methylated (Fig. 1). This methylation significantly reduces CENP-B's binding affinity to its box—almost to the level of nonspecific DNA interaction—since CENP-B preferentially binds to unmethylated DNA [25]. Thus, CpG methylation establishes an epigenetic switch that modulates whether CENP-B can contribute to chromatin organization, linking sequence-specific binding to broader regulatory programs.

2.4 Epigenetic Maps of Human Centromeres and Centromere Dip Region (CDR)

Human centromeres consist of 1500–30,000 copies of imperfectly repeated alpha-satellite DNA sequences [26]. Until 2022, their repetitive nature hindered complete sequencing. The Telomere-to-Telomere (T2T) Consortium resolved this by mapping all human centromeres, including their epigenetic features, in the T2T-CHM13 reference genome [27,28].

Using these data, the locations of CENP-B boxes were identified as kinetochore anchor points [18]. Notably, each centromere contains a single hypomethylated region called the centromeric dip region (CDR), which colocalizes with CENP-A and CENP-B enrichment [27,28]. Most CENP-B boxes are methylated except those within the CDR, where CENP-B dimers bind to adjacent unmethylated boxes. These unmethylated regions likely play a crucial role in kinetochore assembly. This epigenomic organization provides a functional transition from CENP-B's molecular properties to its chromosome-level role in defining the kinetochore-competent domain.

2.5 Epigenetic Mechanisms in Human Centromeres

Two key epigenetic processes define human centromeres: histone modification and DNA methylation [1, 18,25,29]. The former involves replacement of canonical histone H3 with CENP-A, which establishes the foundation for centromere-specific chromatin, while the latter regulates CENP-B binding through methylation status of the CENP-B box. Hypomethylation within the CDR enhances CENP-B binding and supports kinetochore formation, illustrating how DNA sequence and epigenetic state cooperate to define centromere identity (Fig. 3A–D). Together, these mechanisms create a chromatin environment that enables subsequent recruitment of key kinetochore proteins.

2.6 Interaction of CENP-B With CENP-A and CENP-C

CENP-A, CENP-B, and CENP-C play central roles in chromosome segregation. Binding of CENP-B dimers to adjacent unmethylated CENP-B boxes in centromeric DNA initiates kinetochore assembly. These boxes act as anchors, positioning CENP-B as a cornerstone of the kinetochore by directly linking CENP-A and CENP-C [26] (Figs. 1,2).

This anchoring function provides a mechanistic bridge between the epigenetic landscape described above and the structural organization of the kinetochore. The interaction between CENP-B and CENP-A occurs through their N-terminal tails, while CENP-B binds to CENP-C via regions homologous to the human CENP-C central domain and CENP-C motif [26,30]. These coordinated interactions ensure that epigenetically defined centromeric chromatin is translated into a stable and functional kinetochore complex.

2.7 Kinetochore-Spindle Interface and Mechanical Uniformity

The kinetochore is a large protein complex connecting centromeric DNA to spindle microtubules during mitosis and meiosis, ensuring proper genome segregation [29]. Its primary function is to form stable, load-bearing attachments between sister chromatids and spindle fibers, transmitting the pulling forces generated by depolymerizing microtubules to separate chromatids toward opposite centrosomes during anaphase [29,31,32].

Classical micromanipulation experiments using calibrated glass needles measured the maximum force exerted by the spindle on a single chromosome at 7×10^{-5} dynes [33]. The stability of kinetochore–microtubule connections depends on tension: correct attachments generate defined tension, while incorrect ones lack it. The spindle assembly checkpoint monitors kinetochore stretching, ensuring only tension-bearing connections persist [34–36].

Across all human chromosomes, centromeres span about 407 Mb, with kinetochores comprising 5.0 Mb (1.2%) [27,28,37]. Although there is no correlation between centromere and kinetochore size ($p = 0.77$), the number and density of unmethylated CENP-B boxes—anchoring kinetochores to centromeric DNA—are crucial for uniform spindle mechanics. While box numbers vary four-fold among chromosomes, their density varies less than two-fold, averaging 2.61 ± 0.33 per kilobase [18], maintaining consistent spindle pull across centromeres.

Because CENP-B engages directly with centromeric DNA and contributes to kinetochore positioning, this uniformity in CENP-B cassette density produces a regularized and predictable array of anchor points for kinetochore formation. In turn, it supports the generation of consistent spindle traction forces across chromosomes of very different sizes and repeat architectures. By minimizing heterogeneity in the number of microtubule attachment sites per unit DNA, centromeres avoid imbalances in spindle pulling strength that could otherwise distort kinetochore geometry, increase merotelic attachments, or elevate segregation error rates.

Thus, the uniform density of unmethylated CENP-B boxes acts as a force-balancing mechanism: it standardizes kinetochore-DNA coupling strength across the karyotype, ensuring that each chromosome experiences comparable spindle forces despite inherent structural differences.

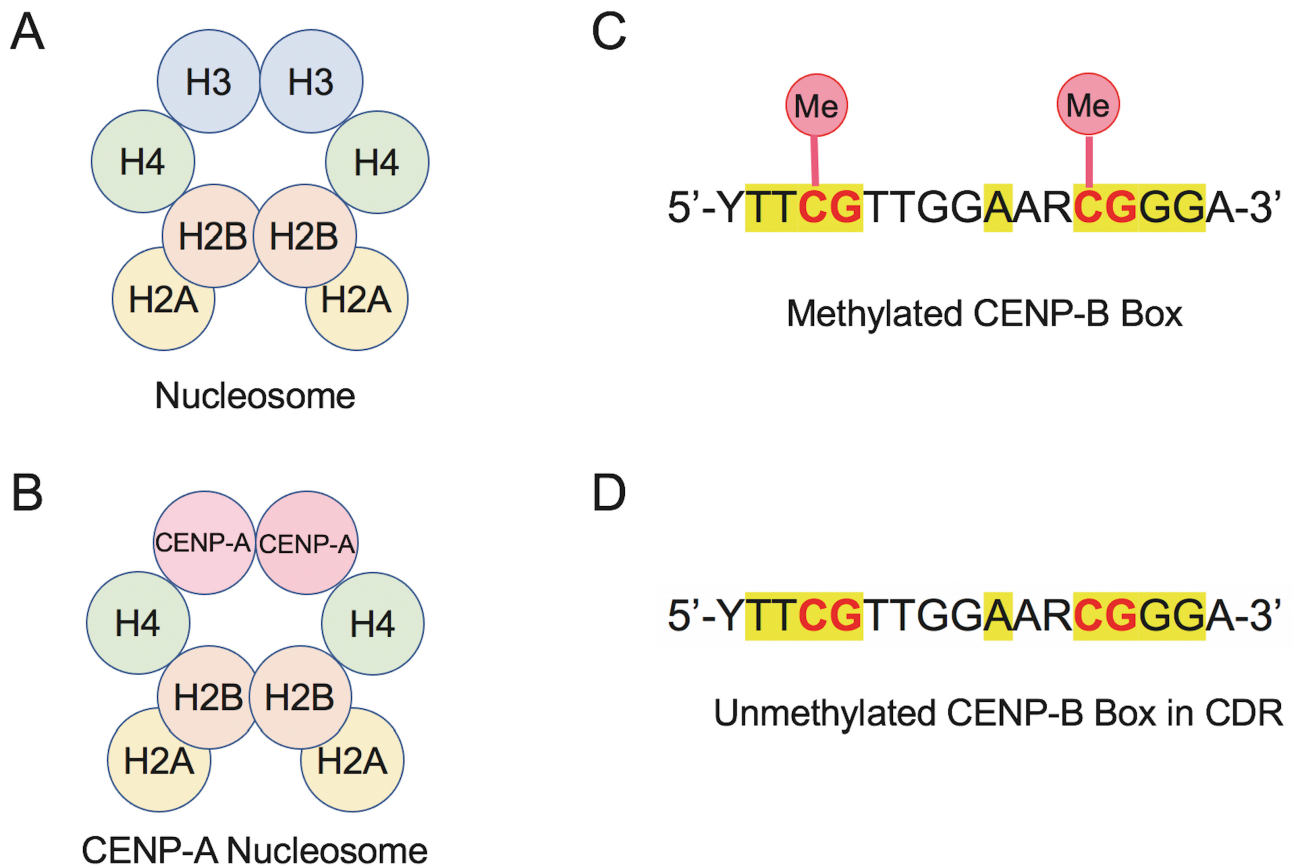


Fig. 3. Comparison of canonical and CENP-A nucleosomes and methylation states of the CENP-B box to illustrate epigenetic mechanisms in human centromeres. (A) Schematic of the canonical nucleosome containing histones H3, H4, H2A and H2B. (B) CENP-A nucleosome in which canonical H3 is replaced by the centromere-specific histone variant CENP-A. (C) Sequence of the methylated CENP-B box, highlighting CpG dinucleotides (yellow) with cytosine methylation (Me), which reduces CENP-B binding. (D) Unmethylated CENP-B box variant found in centromeric dip region (CDR), retaining CpG motifs but lacking cytosine methylation, which increases the binding affinity for CENP-B protein.

This design principle likely represents an evolutionary solution to mitigate mechanical asymmetry and maintain robust genome transmission during mitosis.

Circos plots illustrate kinetochore positions within centromeres of human chromosomes (Fig. 4, Ref. [18]). Although overall patterns are inconsistent, acrocentric chromosomes (13–15, 21, 22) show kinetochores positioned asymmetrically within centromeres, displaced away from telomeres.

2.8 CENP-B Box and P α (pJ α) DNA Motif

In addition to the CENP-B box, human alpha satellite DNA contains a second conserved sequence, the pJ α (pJal α) motif, which specifically binds the pJ α protein [38]. This 17-bp motif (TTCCTTTTYCACCRTAG; Y = C/T, R = G/A) differs from the CENP-B box (YTTCGTTGGAARCGGGA) and lacks a CpG dinucleotide, making epigenetic modification unlikely.

The pJ α motif occurs alongside the CENP-B box and contributes to the evolutionary diversity of alpha satellite

DNA, especially in primates [39]. In lower primates, most alpha satellite monomers contain the pJ α -binding motif (Type A). In contrast, great apes, including humans, possess both Type A and Type B monomers, suggesting the evolutionary emergence of the CENP-B box [38,40]. Interestingly, no pJ α gene is listed in the human GeneCards database [41], raising the question of whether such a gene exists in lower primates.

2.9 CENP-B and Cancer

Cancer cells often display an abnormal number of chromosomes—aneuploidy—a condition linked to centromere instability and chromosome segregation errors [40]. Given that CENP-B is crucial for accurate chromosome segregation, researchers have investigated whether alterations in the CENP-B gene contribute to aneuploidy and cancer development [42,43].

To explore this, I examined the CENP-B gene in the American Association for Cancer Research (AACR) Genie Cohort, the largest publicly available cancer database, ac-

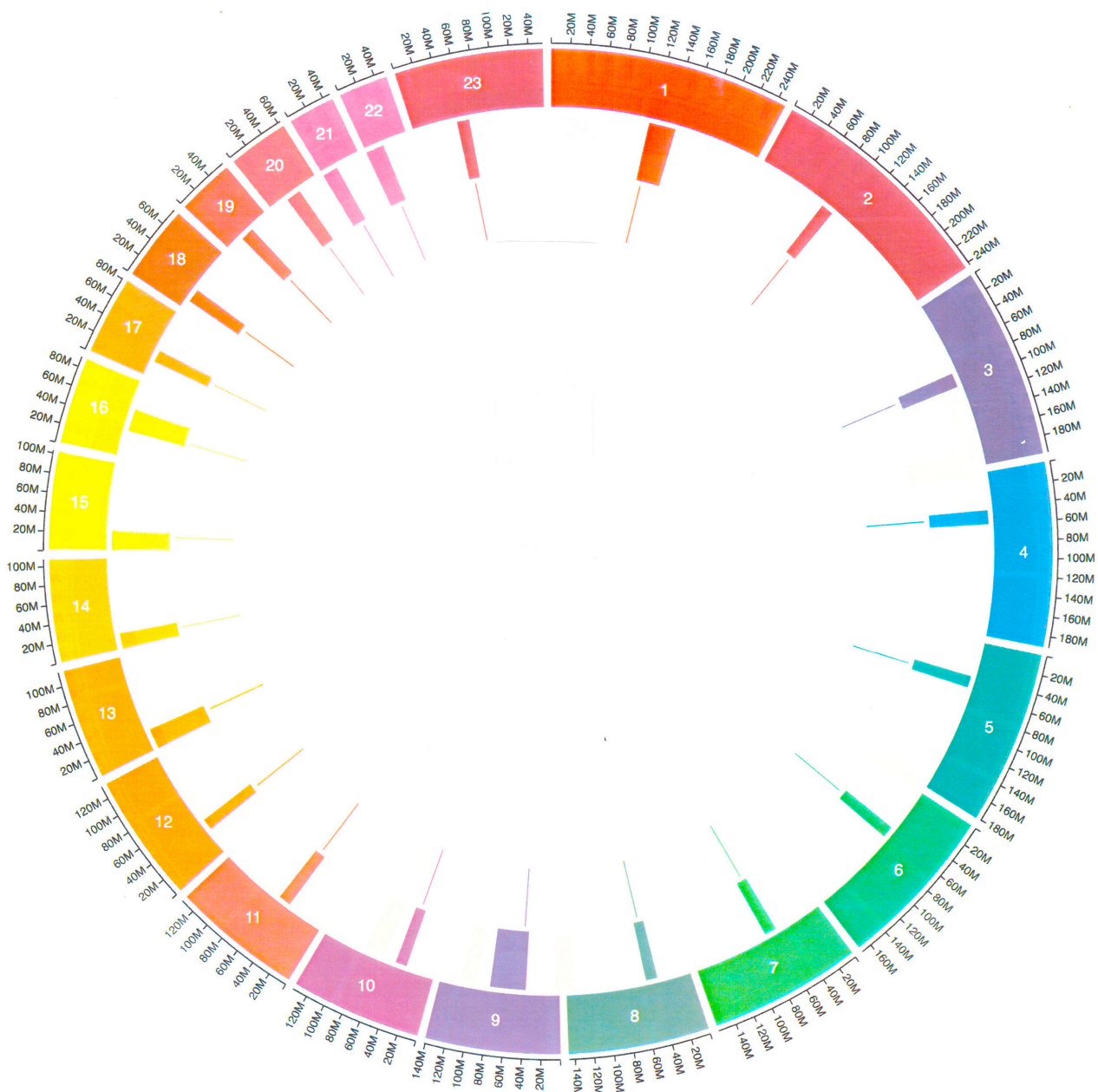


Fig. 4. Circos plots illustrating centromeric and kinetochore-associated features across human chromosomes. Circular genomic maps display chromosomes 1–5 (top), 6–12 (middle), and 13–23 (bottom), with each chromosome represented as a color-coded arc in the outer track. Centromeres (middle track) and kinetochores (inner track) are indicated by inward-facing blocks, while outward tick marks denote positional labels along each chromosome. The plots highlight the organization and relative positioning of centromeres and kinetochores across chromosome groups, enabling visual comparison of variation in centromeric structure throughout the human genome. Figure reprinted with permission from [18].

cessed through cBioPortal [44–46]. Version 18.0 includes 211,526 patients and 250,018 tumor samples analyzed for genetic alterations such as point mutations, copy number variations, and structural variants [47]. Remarkably, the dataset revealed a complete absence of genetic alterations in the CENP-B gene across all samples (Table 3).

The complete absence of CENP-B mutations, copy-number changes, or structural variants in the AACR GE-

NIE cohort stands out as exceptionally rare for a protein involved in chromosome segregation, especially given the widespread aneuploidy characteristic of cancer. This finding strongly suggests that CENP-B is highly intolerant to genetic disruption.

Several biological principles may explain this intolerance.

Table 3. CENP-A, CENP-B and CENP-C gene alterations in cancer.

Gene	GENIE AACR Cohort	
	211,526 patients	250,018 samples
CENP-A	230 (<1%)	239 (<1%)
CENP-B	0 (0%)	0 (0%)
CENP-C	1 (<1%)	1 (<1%)

AACR, American Association for Cancer Research.

2.9.1 Essential Architectural Role in Centromere Stability

CENP-B binds directly to the CENP-B box within centromeric alpha-satellite DNA, contributing to nucleating centromere structure, reinforcing kinetochore positioning, and stabilizing centromere–microtubule interfaces. Because centromere integrity must be preserved on every chromosome in every cell division, even partial loss of CENP-B function may be incompatible with long-term cell survival. Many cancers tolerate extensive chromosomal chaos—but only to a threshold. Loss of a core centromeric architectural protein may push cells beyond that tolerance, resulting in catastrophic mitotic failure rather than tumor evolution.

2.9.2 CENP-B Mutations may be Lethal Early in Tumorigenesis

Most cancer-driving mutations are positively selected, while neutral or deleterious ones can accumulate. The complete absence of CENP-B mutations suggests: CENP-B loss may cause early cell death, preventing a clone from expanding enough to appear in tumor datasets. Mutant cells may undergo mitotic catastrophe, apoptosis, or be competitively outcompeted by cells with intact centromeres. Thus, CENP-B may be under purifying selection even within cancer, a context where genomic instability is often tolerated.

2.9.3 High Functional Constraint Due to CENP-B's Multidomain Protein Architecture

CENP-B contains a DNA-binding helix–turn–helix domain, a dimerization domain, and a centromere-targeting domain. Mutations in any of these regions could disrupt DNA recognition, protein–protein interactions, or kinetochore linkage. Such multidomain proteins often exhibit ultra-low mutational tolerance because small amino acid changes can propagate structural breakdown across domains. This helps explain why CENP-A and CENP-C show rare mutations (<1%), whereas CENP-B shows none. Even slight perturbations may be nonviable.

2.9.4 Dependence of Kinetochore Mechanics on CENP-B Density

CENP-B boxes—when unmethylated—provide consistent anchoring points that normalize spindle traction across chromosomes. A mutation that disrupts either binding affinity or dimer stability could alter spacing or stability

of kinetochore attachment sites, produce asymmetric spindle forces, and increase merotelic attachments or lagging chromosomes. Cancer cells already operate near the edge of mitotic tolerance; losing CENP-B may push them into unsustainable levels of instability.

2.9.5 Redundancy Limitations: CENP-B May Occupy a Non-replaceable Functional Niche

Some centromere proteins (e.g., CENP-A) have partial redundancy through chaperones and deposition machinery. In contrast CENP-B is the only protein that binds a defined DNA motif within alpha-satellite and it plays unique roles in organizing higher-order centromeric arrays. This lack of functional redundancy means that no other protein can compensate for partial or complete CENP-B dysfunction, making mutations more deleterious.

3. CENP-A

The CENP-A gene, located at 2p23.3, encodes a 16-kDa protein of 140 amino acids (Table 1) [48,49]. Biochemical studies have shown that CENP-A is a variant of histone H3, one of the four core histones (H2A, H2B, H3, H4) present in nucleosomes. The C-terminal two-thirds of CENP-A comprise the histone-fold domain, which shares over 60% sequence identity with canonical histone H3, while the N-terminal one-third is unique [50,51] (Fig. 5). This histone-fold domain is essential for targeting CENP-A to centromeric regions [51,52]. CENP-A is the only centromere-specific histone variant, marking centromeric chromatin in most eukaryotes [53]. Thus, centromeres are specialized chromatin domains defined by the enrichment of CENP-A, which replaces histone H3 in centromeric nucleosomes [1,29]. CENP-A nucleosomes are interspersed with canonical H3 nucleosomes containing histone modifications (e.g., H3K4me2), together forming the centromeric structure [5,54]. This mosaic organization provides the molecular backdrop for the specialized structural and regulatory roles that CENP-A nucleosomes play in shaping functional centromeres.

The crystal structure of human CENP-A bound to its alpha-satellite DNA (147 bp) has been resolved [55]. The DNA is wrapped around a histone octamer composed of H2A, H2B, H4, and CENP-A, in a left-handed orientation. Unlike canonical H3 nucleosomes, only the central 121 base pairs of DNA are visible, while the terminal 13 base pairs at each end are detached from the histone surface. Two regions of CENP-A differ structurally from H3 [56]: the N-terminal α -helix is shorter, influencing DNA-end orientation [57], and loop 1 (the RG-loop), which includes residues Arg80 and Gly81, is solvent-exposed [58,59]. Mutations in this loop reduce CENP-A retention at centromeres, indicating its role in stabilizing centromeric chromatin. Super-resolution microscopy revealed that CENP-A nucleosomes form globular, rosette-like clusters of ~250–300 nm diameter [60]. These structural features collectively explain how

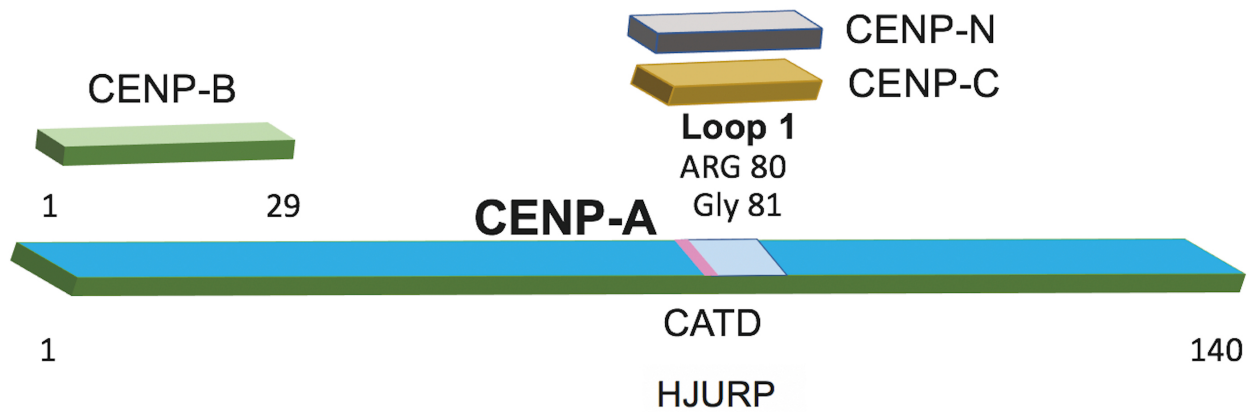


Fig. 5. Domain architecture of CENP-A and its interactions with centromeric proteins. A schematic representation of CENP-A (residues 1–140) indicating regions required for binding to centromere-associated factors. The N-terminal segment (residues 1–29) corresponds to the CENP-B-interacting region. Loop 1 (pale blue), containing Arg80 and Gly81, mediates contacts with CENP-C and CENP-N. The CENP-A targeting domain (CATD; pink) marks the region recognized by the CENP-A chaperone HJURP and is essential for centromere-specific deposition of CENP-A.

CENP-A imparts unique geometric and mechanical properties to centromeric chromatin, distinguishing it from canonical nucleosomes.

CENP-A interacts with CENP-B and CENP-C. The N-terminal tail (residues 1–29) mediates binding to CENP-B [26], while the RG-loop interacts with CENP-C [58]. CENP-A also recruits CENP-N and the Holliday junction recognition protein (HJURP) during centromeric chromatin assembly. Loop 1 of CENP-A binds both CENP-C and CENP-N in a cell cycle-dependent manner [59]. This loop alternates between concealed (compacted chromatin) and exposed (open chromatin) states, enabling CENP-N recruitment during the G1/S transition before cell division. These dynamic interactions position CENP-A as the central coordinator of chromatin architecture and protein recruitment at the centromere.

HJURP acts as a key assembly factor for CENP-A deposition at centromeres [61,62]. The CENP-A-targeting domain (CATD), encompassing loop 1 and the adjacent two-helix region, is critical for HJURP binding. An amino-terminal fragment of HJURP is sufficient to assemble CENP-A nucleosomes *in vitro*, confirming its role as a chromatin assembly factor [63]. HJURP directly binds Mis18 β , a component of the conserved Mis18 complex [64]. Two repeats in HJURP interact simultaneously with the 4:2:2 Mis18: Mis18: M18BP1 complex [65]. Mis18 β depletion impairs both CENP-A and HJURP recruitment, underscoring its importance in CENP-A loading [48,64]. HJURP localization at the centromere is cell cycle-regulated, coinciding with the period of new CENP-A deposition during late telophase and early G1 phase [61,66], distinct from replication-coupled histone loading. Thus, CENP-A propagation depends on a highly choreographed assembly pathway, ensuring faithful maintenance of centromere identity across cell divisions.

Cyclin-dependent kinase 1 (CDK1), the master regulator of mitosis, phosphorylates both CENP-A and HJURP to control centromere assembly timing [67]. CENP-A is phosphorylated at Ser68 during early mitosis by CDK1 [68], disrupting its interaction with HJURP and preventing premature loading. Concurrently, CDK1-mediated phosphorylation of HJURP weakens its binding to Mis18 β , ensuring CENP-A assembly occurs post-mitosis [64]. This dual regulatory mechanism underscores how cell cycle signaling integrates with centromere-specific pathways to preserve genomic stability.

To assess whether CENP-A plays a role in cancer, I examined the gene in the AACR Genie Cohort, Version 18.0 [47]. CENP-A alterations were identified in 230 of 211,526 patients (<1%) and 239 of 250,018 samples (<1%) (Table 3). These included 175 mutations (missense, truncating, in-frame, and splice), 66 copy number aberrations (amplifications and deletions), and 2 structural variants. Some tumors exhibited multiple alterations, but overall, CENP-A aberrations remain rare across cancers [42]. These findings suggest that although CENP-A is essential for chromosome segregation, its direct genetic alteration is an uncommon contributor to tumorigenesis.

4. CENP-C

The CENP-C gene is located at 4q13.2 [69]. It encodes a 106.8-kDa protein composed of 943 amino acids (Table 1). This protein is larger than other kinetochore components such as CENP-A and CENP-B. Its functional size doubles through dimerization, allowing it to act as a structural scaffold that connects multiple kinetochore elements, including CENP-A, CENP-B, and CCAN components in the inner kinetochore, as well as MIS12C and NDC80 in the outer kinetochore (Fig. 2). Through this extensive connec-

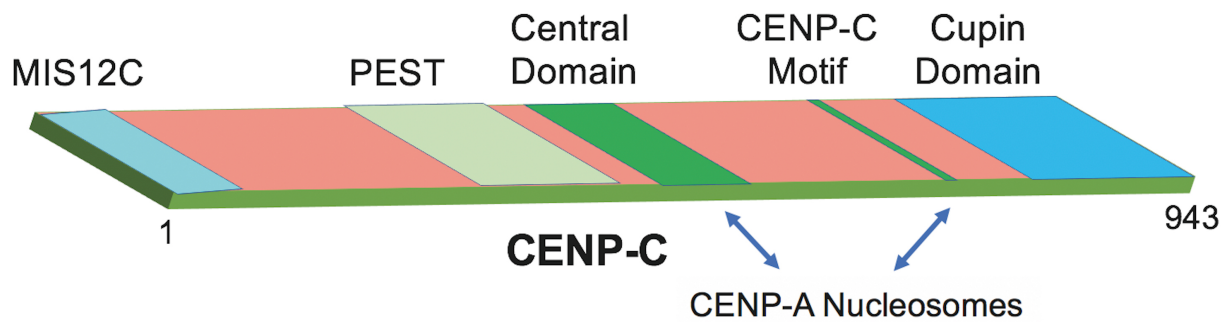


Fig. 6. Domain architecture of CENP-C and regions required for CENP-A nucleosome engagement. Schematic representation of CENP-C (residues 1–943) highlighting major structural and functional domains. The N-terminal MIS12C-interacting region (pale blue) is followed by a PEST sequence (pale green) and a central domain (green). The conserved CENP-C motif (green) lies C-terminal to the central domain and contributes, together with the adjacent cupin domain (blue), to binding CENP-A nucleosomes, as indicated by arrows. The cupin domain additionally mediates CENP-C dimerization, supporting kinetochore assembly.

tivity, CENP-C forms the principal architectural bridge between centromeric chromatin and the microtubule-binding machinery.

CENP-C dimerization is mediated by the cupin domain at its C-terminal end (Fig. 6). This domain has a characteristic β -barrel (jelly-roll) structure containing two conserved histidine-rich motifs [70]. The β -sheet architecture promotes homodimer formation, and interactions between β -strand 4 may enable further self-oligomerization [71]. Such oligomerization provides the mechanical stability required of a protein that anchors the kinetochore's core components.

Human CENP-C contains two CENP-A binding regions: the central domain (amino acids 426–537) and the CENP-C motif (amino acids 735–755), both interacting with the C-terminus of CENP-A [58,72]. This interaction is strengthened by CDK1-mediated phosphorylation at T734, a key mitotic regulator [73]. CENP-C also interacts with CENP-B via conserved Mif2-related regions homologous to those in budding yeast [26,30]. These multivalent binding interfaces enable CENP-C to function as an integrator of centromeric signals and structural elements.

CENP-C organizes CENP-A nucleosomes and recruits components of the constitutive centromere-associated network (CCAN) [72]. Its PEST-rich region (proline, glutamate, serine, threonine) in the N-terminal half binds directly to the CENP-HIKM complex [74]. A conserved N-terminal motif also associates with MIS12C, linking the inner and outer kinetochore [75–77]. Cryo-EM studies have confirmed CENP-C's binding to CENP-N within the CENP-LN complex [9,78], and together with CENP-T, it bridges the inner kinetochore to NDC80 [29]. Finally, CENP-C binds α -satellite RNA, which, although sequence-nonspecific, enhances its DNA affinity [79–82]. This extensive interaction network highlights CENP-C as the primary organizer of kinetochore assembly, ensuring continuity from centromeric chromatin to microtubule attachment sites.

The potential role of CENP-C in cancer has been explored primarily in cell models [42]. In the AACR Genie Cohort (v18.0), CENP-C alterations are rare—identified in only one tumor sample (<1%), which contained a structural variant (Table 3). Thus, similar to CENP-A, CENP-C appears to contribute to genome instability chiefly through functional dysregulation rather than frequent genetic alteration.

5. Conclusions

5.1 Summary of Existing Knowledge

Centromeres are a focal point of chromosome biology due to their indispensable role in ensuring accurate chromosome segregation and maintaining genomic integrity. Centromere identity is not defined by primary DNA sequence but instead is governed by epigenetic mechanisms. These include the incorporation of the canonical histone variant CENP-A within centromeric chromatin and the presence of a defined centromeric domain (CDR) that facilitates strong CENP-B binding and the assembly of a functional kinetochore [1,18,25,29]. Collectively, these features reflect a multilayered regulatory system that integrates chromatin composition, DNA methylation, and sequence-specific protein interactions to ensure stable centromere function.

5.2 Proposed Hypotheses

Despite CENP-B's well-documented contributions to centromere structure and chromosome stability, its role in pathological chromosome mis-segregation—including cancer-associated aneuploidy—remains unresolved. The analysis of the AACR GENIE cohort presented here, encompassing more than 211,000 patients and 250,000 tumor samples [47], reveals that CENP-B is remarkably conserved in cancers, with no detectable genetic alterations during tumorigenesis. This striking absence of mutation suggests that CENP-B is not typically compromised at the

level of coding sequence variation. Rather, if CENP-B contributes to oncogenic chromosomal instability, the disruption is likely to arise from epigenetic mis-regulation or altered chromatin dynamics instead of direct mutation [18]. A second hypothesis is that the hypomethylated CDR, required for proper kinetochore assembly, becomes progressively destabilized during early tumorigenesis, promoting chromosome segregation errors. Finally, centromere-associated proteins may form compensatory networks, such that only multi-component perturbations—not single-gene mutations—result in catastrophic mitotic failure.

5.3 Future Research Directions

These hypotheses emphasize that future research should expand beyond sequence-level analysis toward dissecting the regulatory mechanisms that maintain CENP-A deposition, CDR methylation, and CENP-B binding *in vivo*. Addressing these questions will not only promote a deeper understanding of the epigenetic foundations of chromosomal stability but may also identify novel diagnostic and therapeutic targets. Key directions include: High-resolution epigenomic profiling (e.g., DNA methylation, histone acetylation) of centromeres in normal, premalignant, and malignant tissues will be essential for identifying early chromatin alterations associated with aneuploidy. Functional assays that measure CENP-B binding dynamics under controlled methylation states will help determine how CDR epigenetics influence kinetochore stability. Integrating multi-omic data to define how structural and regulatory centromere features are rewired under pathological conditions. Evaluating whether restoring normal centromere chromatin states can reduce aneuploidy or chromosomal instability in model systems. Finally, exploring whether chromatin-modifying enzymes that act on centromeric regions represent therapeutic targets could open new strategies to mitigate chromosome mis-segregation in cancer. Overall, examining the epigenetic and interaction networks that preserve centromere identity offers a powerful path toward understanding—and potentially mitigating—chromosomal instability in human cancers.

Author Contributions

FP: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Software, Resources, Supervision, Validation, Visualization; FP: Writing – original draft; Writing – review & editing. FP: Read and approved the final manuscript. FP: Has participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

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Conflict of Interest

The author declares no conflict of interest.

References

- [1] Fukagawa T, Earnshaw WC. The centromere: chromatin foundation for the kinetochore machinery. *Developmental Cell*. 2014; 30: 496–508. <https://doi.org/10.1016/j.devcel.2014.08.016>.
- [2] Miga KH. Centromere studies in the era of ‘telomere-to-telomere’ genomics. *Experimental Cell Research*. 2020; 394: 112127. <https://doi.org/10.1016/j.yexcr.2020.112127>.
- [3] Willard HF. Chromosome-specific organization of human alpha satellite DNA. *American Journal of Human Genetics*. 1985; 37: 524–532.
- [4] Earnshaw WC, Rothfield N. Identification of a family of human centromere proteins using autoimmune sera from patients with scleroderma. *Chromosoma*. 1985; 91: 313–321. <https://doi.org/10.1007/BF00328227>.
- [5] Sullivan BA, Karpen GH. Centromeric chromatin exhibits a histone modification pattern that is distinct from both euchromatin and heterochromatin. *Nature Structural & Molecular Biology*. 2004; 11: 1076–1083. <https://doi.org/10.1038/nsmb845>.
- [6] Sullivan LL, Chew K, Sullivan BA. α satellite DNA variation and function of the human centromere. *Nucleus (Austin, Tex.)*. 2017; 8: 331–339. <https://doi.org/10.1080/19491034.2017.1308989>.
- [7] Sullivan LL, Sullivan BA. Genomic and functional variation of human centromeres. *Experimental Cell Research*. 2020; 389: 111896. <https://doi.org/10.1016/j.yexcr.2020.111896>.
- [8] Ando S, Yang H, Nozaki N, Okazaki T, Yoda K. CENP-A, -B, and -C chromatin complex that contains the I-type alpha-satellite array constitutes the prekinetochore in HeLa cells. *Molecular and Cellular Biology*. 2002; 22: 2229–2241. <https://doi.org/10.1128/MCB.22.7.2229-2241.2002>.
- [9] Pesenti ME, Raisch T, Conti D, Walstein K, Hoffmann I, Vogt D, *et al.* Structure of the human inner kinetochore CCAN complex and its significance for human centromere organization. *Molecular Cell*. 2022; 82: 2113–2131.e8. <https://doi.org/10.1016/j.molcel.2022.04.027>.
- [10] Yatskevich S, Muir KW, Bellini D, Zhang Z, Yang J, Tischer T, *et al.* Structure of the human inner kinetochore bound to a centromeric CENP-A nucleosome. *Science (New York, N.Y.)*. 2022; 376: 844–852. <https://doi.org/10.1126/science.abn3810>.
- [11] Yatskevich S, Yang J, Bellini D, Zhang Z, Barford D. Structure of the human outer kinetochore KMN network complex. *Nature Structural & Molecular Biology*. 2024; 31: 874–883. <https://doi.org/10.1038/s41594-024-01249-y>.
- [12] Sugimoto K, Yata H, Himeno M. Mapping of the human CENP-B gene to chromosome 20 and the CENP-C gene to chromosome 12 by a rapid cycle DNA amplification procedure. *Genomics*. 1993; 17: 240–242. <https://doi.org/10.1006/geno.1993.1312>.
- [13] Tawaramoto MS, Park SY, Tanaka Y, Nureki O, Kurumizaka H, Yokoyama S. Crystal structure of the human centromere protein B (CENP-B) dimerization domain at 1.65-Å resolution. *The Journal of Biological Chemistry*. 2003; 278: 51454–51461. <https://doi.org/10.1074/jbc.M310388200>.
- [14] Yoda K, Kitagawa K, Masumoto H, Muro Y, Okazaki T. A

- human centromere protein, CENP-B, has a DNA binding domain containing four potential alpha helices at the NH2 terminus, which is separable from dimerizing activity. *The Journal of Cell Biology*. 1992; 119: 1413–1427. <https://doi.org/10.1083/jcb.119.6.1413>.
- [15] Iwahara J, Kigawa T, Kitagawa K, Masumoto H, Okazaki T, Yokoyama S. A helix-turn-helix structure unit in human centromere protein B (CENP-B). *The EMBO Journal*. 1998; 17: 827–837. <https://doi.org/10.1093/emboj/17.3.827>.
- [16] Masumoto H, Masukata H, Muro Y, Nozaki N, Okazaki T. A human centromere antigen (CENP-B) interacts with a short specific sequence in alphoid DNA, a human centromeric satellite. *The Journal of Cell Biology*. 1989; 109: 1963–1973. <https://doi.org/10.1083/jcb.109.5.1963>.
- [17] Chardon F, Japaridze A, Witt H, Velikovskiy L, Chakraborty C, Wilhelm T, *et al.* CENP-B-mediated DNA loops regulate activity and stability of human centromeres. *Molecular Cell*. 2022; 82: 1751–1767.e8. <https://doi.org/10.1016/j.molcel.2022.02.032>.
- [18] Parl FF. Analysis of CENP-B Boxes as Anchor of Kinetochores in Centromeres of Human Chromosomes. *Bioinformatics and Biology Insights*. 2024; 18: 11779322241248913. <https://doi.org/10.1177/11779322241248913>.
- [19] Muro Y, Masumoto H, Yoda K, Nozaki N, Ohashi M, Okazaki T. Centromere protein B assembles human centromeric alpha-satellite DNA at the 17-bp sequence, CENP-B box. *The Journal of Cell Biology*. 1992; 116: 585–596. <https://doi.org/10.1083/jcb.116.3.585>.
- [20] Tian L, Gu L, Li GM. Distinct nucleotide binding/hydrolysis properties and molar ratio of MutSalpha and MutSbeta determine their differential mismatch binding activities. *The Journal of Biological Chemistry*. 2009; 284: 11557–11562. <https://doi.org/10.1074/jbc.M900908200>.
- [21] Tanikawa J, Yasukawa T, Enari M, Ogata K, Nishimura Y, Ishii S, *et al.* Recognition of specific DNA sequences by the c-myc protooncogene product: role of three repeat units in the DNA-binding domain. *Proceedings of the National Academy of Sciences of the United States of America*. 1993; 90: 9320–9324. <https://doi.org/10.1073/pnas.90.20.9320>.
- [22] Klinge CM, Peale FV, Jr, Hilf R, Bambara RA, Zain S. Cooperative estrogen receptor interaction with consensus or variant estrogen responsive elements in vitro. *Cancer Research*. 1992; 52: 1073–1081.
- [23] Szak ST, Pietenpol JA. High affinity insertion/deletion lesion binding by p53. Evidence for a role of the p53 central domain. *The Journal of Biological Chemistry*. 1999; 274: 3904–3909. <https://doi.org/10.1074/jbc.274.6.3904>.
- [24] Tanaka Y, Nureki O, Kurumizaka H, Fukai S, Kawaguchi S, Ikuta M, *et al.* Crystal structure of the CENP-B protein-DNA complex: the DNA-binding domains of CENP-B induce kinks in the CENP-B box DNA. *The EMBO Journal*. 2001; 20: 6612–6618. <https://doi.org/10.1093/emboj/20.23.6612>.
- [25] Tanaka Y, Kurumizaka H, Yokoyama S. CpG methylation of the CENP-B box reduces human CENP-B binding. *The FEBS Journal*. 2005; 272: 282–289. <https://doi.org/10.1111/j.1432-1033.2004.04406.x>.
- [26] Fachinetti D, Han JS, McMahon MA, Ly P, Abdullah A, Wong AJ, *et al.* DNA Sequence-Specific Binding of CENP-B Enhances the Fidelity of Human Centromere Function. *Developmental Cell*. 2015; 33: 314–327. <https://doi.org/10.1016/j.devcel.2015.03.020>.
- [27] Altemose N, Logsdon GA, Bzikadze AV, Sidhwani P, Langley SA, Caldas GV, *et al.* Complete genomic and epigenetic maps of human centromeres. *Science (New York, N.Y.)*. 2022; 376: eabl4178. <https://doi.org/10.1126/science.abl4178>.
- [28] Gershman A, Sauria MEG, Guitart X, Vollger MR, Hook PW, Hoyt SJ, *et al.* Epigenetic patterns in a complete human genome. *Science (New York, N.Y.)*. 2022; 376: eabj5089. <https://doi.org/10.1126/science.abj5089>.
- [29] Musacchio A, Desai A. A Molecular View of Kinetochores: Assembly and Function. *Biology*. 2017; 6: 5. <https://doi.org/10.3390/biology6010005>.
- [30] Suzuki N, Nakano M, Nozaki N, Egashira SI, Okazaki T, Masumoto H. CENP-B interacts with CENP-C domains containing Mif2 regions responsible for centromere localization. *The Journal of Biological Chemistry*. 2004; 279: 5934–5946. <https://doi.org/10.1074/jbc.M306477200>.
- [31] Ault JG, Nicklas RB. Tension, microtubule rearrangements, and the proper distribution of chromosomes in mitosis. *Chromosoma*. 1989; 98: 33–39. <https://doi.org/10.1007/BF00293332>.
- [32] Nicklas RB, Koch CA. Chromosome micromanipulation. 3. Spindle fiber tension and the reorientation of mal-oriented chromosomes. *The Journal of Cell Biology*. 1969; 43: 40–50. <https://doi.org/10.1083/jcb.43.1.40>.
- [33] Nicklas RB. Measurements of the force produced by the mitotic spindle in anaphase. *The Journal of Cell Biology*. 1983; 97: 542–548. <https://doi.org/10.1083/jcb.97.2.542>.
- [34] Maresca TJ, Salmon ED. Welcome to a new kind of tension: translating kinetochore mechanics into a wait-anaphase signal. *Journal of Cell Science*. 2010; 123: 825–835. <https://doi.org/10.1242/jcs.064790>.
- [35] Uchida KSK, Takagaki K, Kumada K, Hirayama Y, Noda T, Hirota T. Kinetochore stretching inactivates the spindle assembly checkpoint. *The Journal of Cell Biology*. 2009; 184: 383–390. <https://doi.org/10.1083/jcb.200811028>.
- [36] Lampson MA, Grishchuk EL. Mechanisms to Avoid and Correct Erroneous Kinetochore-Microtubule Attachments. *Biology*. 2017; 6: 1. <https://doi.org/10.3390/biology6010001>.
- [37] Nurk S, Koren S, Rhie A, Rautiainen M, Bzikadze AV, Mikheenko A, *et al.* The complete sequence of a human genome. *Science*. 2022; 376: 44–53. <https://orcid.org/0000-0003-2983-8934>.
- [38] Romanova LY, Deriagin GV, Mashkova TD, Tumeneva IG, Mushegian AR, Kisselev LL, *et al.* Evidence for selection in evolution of alpha satellite DNA: the central role of CENP-B/pJ alpha binding region. *Journal of Molecular Biology*. 1996; 261: 334–340. <https://doi.org/10.1006/jmbi.1996.0466>.
- [39] Rosandić M, Paar V, Basar I, Glunčić M, Pavin N, Pilas I. CENP-B box and pJalpha sequence distribution in human alpha satellite higher-order repeats (HOR). *Chromosome Research: an International Journal on the Molecular, Supramolecular and Evolutionary Aspects of Chromosome Biology*. 2006; 14: 735–753. <https://doi.org/10.1007/s10577-006-1078-x>.
- [40] Talbert PB, Henikoff S. Centromeres drive and take a break. *Chromosome Research: an International Journal on the Molecular, Supramolecular and Evolutionary Aspects of Chromosome Biology*. 2025; 33: 17. <https://doi.org/10.1007/s10577-025-09777-z>.
- [41] Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, *et al.* The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. *Current Protocols in Bioinformatics*. 2016; 54: 1.30.1–1.30.33. <https://doi.org/10.1002/cpbi.5>.
- [42] Dumont M, Gamba R, Gestraud P, Klaasen S, Worrall JT, De Vries SG, *et al.* Human chromosome-specific aneuploidy is influenced by DNA-dependent centromeric features. *The EMBO Journal*. 2020; 39: e102924. <https://doi.org/10.15252/embj.2019102924>.
- [43] Gamba R, Fachinetti D. From evolution to function: Two sides of the same CENP-B coin? *Experimental Cell Research*. 2020; 390: 111959. <https://doi.org/10.1016/j.yexcr.2020.111959>.
- [44] Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA,

- et al.* The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discovery*. 2012; 2: 401–404. <https://doi.org/10.1158/2159-8290.CD-12-0095>.
- [45] De Bruijn I, Kundra R, Mastrogiacomo B, Tran TN, Sikina L, Mazor T, *et al.* Analysis and visualization of longitudinal genomic and clinical data from the AACR project GENIE biopharma collaborative in cBioPortal. *Cancer Research*. 2023; 83: 3861–3867. <https://doi.org/10.1158/0008-5472.CAN-23-0816>.
- [46] Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, *et al.* Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Science Signaling*. 2013; 6: p11. <https://doi.org/10.1126/scisignal.2004088>.
- [47] AACR Project GENIE Consortium. AACR Project GENIE: Powering Precision Medicine through an International Consortium. *Cancer Discovery*. 2017; 7: 818–831. <https://doi.org/10.1158/2159-8290.CD-17-0151>.
- [48] Fujita Y, Hayashi T, Kiyomitsu T, Toyoda Y, Kokubu A, Obuse C, *et al.* Priming of centromere for CENP-A recruitment by human hMis18alpha, hMis18beta, and M18BP1. *Developmental Cell*. 2007; 12: 17–30. <https://doi.org/10.1016/j.devcel.2006.11.002>.
- [49] Renaud-Pageot C, Quivy JP, Lochhead M, Almouzni G. CENP-A Regulation and Cancer. *Frontiers in Cell and Developmental Biology*. 2022; 10: 907120. <https://doi.org/10.3389/fcell.2022.907120>.
- [50] Palmer DK, O'Day K, Trong HL, Charbonneau H, Margolis RL. Purification of the centromere-specific protein CENP-A and demonstration that it is a distinctive histone. *Proceedings of the National Academy of Sciences of the United States of America*. 1991; 88: 3734–3738. <https://doi.org/10.1073/pnas.88.9.3734>.
- [51] Sullivan KF, Hechenberger M, Masri K. Human CENP-A contains a histone H3 related histone fold domain that is required for targeting to the centromere. *The Journal of Cell Biology*. 1994; 127: 581–592. <https://doi.org/10.1083/jcb.127.3.581>.
- [52] Yoda K, Ando S, Morishita S, Houmura K, Hashimoto K, Takeyasu K, *et al.* Human centromere protein A (CENP-A) can replace histone H3 in nucleosome reconstitution in vitro. *Proceedings of the National Academy of Sciences of the United States of America*. 2000; 97: 7266–7271. <https://doi.org/10.1073/pnas.130189697>.
- [53] Tromer EC, van Hooff JJE, Kops GJPL, Snel B. Mosaic origin of the eukaryotic kinetochore. *Proceedings of the National Academy of Sciences of the United States of America*. 2019; 116: 12873–12882. <https://doi.org/10.1073/pnas.1821945116>.
- [54] Bergmann JH, Rodriguez MG, Martins NMC, Kimura H, Kelly DA, Masumoto H, *et al.* Epigenetic engineering shows H3K4me2 is required for HJURP targeting and CENP-A assembly on a synthetic human kinetochore. *The EMBO Journal*. 2011; 30: 328–340. <https://doi.org/10.1038/emboj.2010.329>.
- [55] Tachiwana H, Kagawa W, Shiga T, Osakabe A, Miya Y, Saito K, *et al.* Crystal structure of the human centromeric nucleosome containing CENP-A. *Nature*. 2011; 476: 232–235. <https://doi.org/10.1038/nature10258>.
- [56] Tachiwana H, Kagawa W, Kurumizaka H. Comparison between the CENP-A and histone H3 structures in nucleosomes. *Nucleus (Austin, Tex.)*. 2012; 3: 6–11. <https://doi.org/10.4161/nucl.18372>.
- [57] Luger K, Mäder AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature*. 1997; 389: 251–260. <https://doi.org/10.1038/38444>.
- [58] Ariyoshi M, Makino F, Watanabe R, Nakagawa R, Kato T, Namba K, *et al.* Cryo-EM structure of the CENP-A nucleosome in complex with phosphorylated CENP-C. *The EMBO Journal*. 2021; 40: e105671. <https://doi.org/10.15252/emboj.2020105671>.
- [59] Fang J, Liu Y, Wei Y, Deng W, Yu Z, Huang L, *et al.* Structural transitions of centromeric chromatin regulate the cell cycle-dependent recruitment of CENP-N. *Genes & Development*. 2015; 29: 1058–1073. <https://doi.org/10.1101/gad.259432.115>.
- [60] Andronov L, Ouararhni K, Stoll I, Klaholz BP, Hamiche A. CENP-A nucleosome clusters form rosette-like structures around HJURP during G1. *Nature Communications*. 2019; 10: 4436. <https://doi.org/10.1038/s41467-019-12383-3>.
- [61] Dunleavy EM, Roche D, Tagami H, Lacoste N, Ray-Gallet D, Nakamura Y, *et al.* HJURP is a cell-cycle-dependent maintenance and deposition factor of CENP-A at centromeres. *Cell*. 2009; 137: 485–497. <https://doi.org/10.1016/j.cell.2009.02.040>.
- [62] Foltz DR, Jansen LET, Bailey AO, Yates JR, 3rd, Bassett EA, Wood S, *et al.* Centromere-specific assembly of CENP-a nucleosomes is mediated by HJURP. *Cell*. 2009; 137: 472–484. <https://doi.org/10.1016/j.cell.2009.02.039>.
- [63] Barnhart MC, Kuich PHJL, Stellfox ME, Ward JA, Bassett EA, Black BE, *et al.* HJURP is a CENP-A chromatin assembly factor sufficient to form a functional de novo kinetochore. *The Journal of Cell Biology*. 2011; 194: 229–243. <https://doi.org/10.1083/jcb.201012017>.
- [64] Wang J, Liu X, Dou Z, Chen L, Jiang H, Fu C, *et al.* Mitotic regulator Mis18β interacts with and specifies the centromeric assembly of molecular chaperone holliday junction recognition protein (HJURP). *The Journal of Biological Chemistry*. 2014; 289: 8326–8336. <https://doi.org/10.1074/jbc.M113.529958>.
- [65] Pan D, Walstein K, Take A, Bier D, Kaiser N, Musacchio A. Mechanism of centromere recruitment of the CENP-A chaperone HJURP and its implications for centromere licensing. *Nature Communications*. 2019; 10: 4046. <https://doi.org/10.1038/s41467-019-12019-6>.
- [66] Jansen LET, Black BE, Foltz DR, Cleveland DW. Propagation of centromeric chromatin requires exit from mitosis. *The Journal of Cell Biology*. 2007; 176: 795–805. <https://doi.org/10.1083/jcb.200701066>.
- [67] Kapadia N, Nurse P. Spatiotemporal orchestration of mitosis by cyclin-dependent kinase. *Nature*. 2025; 643: 1391–1399. <https://doi.org/10.1038/s41586-025-09172-y>.
- [68] Yu Z, Zhou X, Wang W, Deng W, Fang J, Hu H, *et al.* Dynamic phosphorylation of CENP-A at Ser68 orchestrates its cell-cycle-dependent deposition at centromeres. *Developmental Cell*. 2015; 32: 68–81. <https://doi.org/10.1016/j.devcel.2014.11.030>.
- [69] McKay S, Thomson E, Cooke H. Sequence homologies and linkage group conservation of the human and mouse Cenpc genes. *Genomics*. 1994; 22: 36–40. <https://doi.org/10.1006/geno.1994.1342>.
- [70] Chik JK, Moiseeva V, Goel PK, Meinen BA, Koldewey P, An S, *et al.* Structures of CENP-C cupin domains at regional centromeres reveal unique patterns of dimerization and recruitment functions for the inner pocket. *The Journal of Biological Chemistry*. 2019; 294: 14119–14134. <https://doi.org/10.1074/jbc.RA119.008464>.
- [71] Hara M, Ariyoshi M, Sano T, Nozawa RS, Shinkai S, Onami S, *et al.* Centromere/kinetochore is assembled through CENP-C oligomerization. *Molecular Cell*. 2023; 83: 2188–2205.e13. <https://doi.org/10.1016/j.molcel.2023.05.023>.
- [72] Ali-Ahmad A, Bilokapić S, Schäfer IB, Halić M, Sekulić N. CENP-C unwraps the human CENP-A nucleosome through the H2A C-terminal tail. *EMBO Reports*. 2019; 20: e48913. <https://doi.org/10.15252/embr.201948913>.
- [73] Watanabe R, Hara M, Okumura EI, Hervé S, Fachinetti D, Ariyoshi M, *et al.* CDK1-mediated CENP-C phosphorylation modulates CENP-A binding and mitotic kinetochore localization. *The Journal of Cell Biology*. 2019; 218: 4042–4062. <https://doi.org/10.1083/jcb.201907006>.

- [74] Klare K, Weir JR, Basilico F, Zimniak T, Massimiliano L, Ludwigs N, *et al.* CENP-C is a blueprint for constitutive centromere-associated network assembly within human kinetochores. *The Journal of Cell Biology*. 2015; 210: 11–22. <https://doi.org/10.1083/jcb.201412028>.
- [75] Kong W, Hara M, Tokunaga Y, Okumura K, Hirano Y, Miao J, *et al.* CENP-C-Mis12 complex establishes a regulatory loop through Aurora B for chromosome segregation. *Life Science Alliance*. 2025; 8: e202402927. <https://doi.org/10.26508/lsa.202402927>.
- [76] Przewlaka MR, Venkei Z, Bolanos-Garcia VM, Debski J, Dadlez M, Glover DM. CENP-C is a structural platform for kinetochore assembly. *Current Biology: CB*. 2011; 21: 399–405. <https://doi.org/10.1016/j.cub.2011.02.005>.
- [77] Screpanti E, De Antoni A, Alushin GM, Petrovic A, Melis T, Nogales E, *et al.* Direct binding of Cenp-C to the Mis12 complex joins the inner and outer kinetochore. *Current Biology: CB*. 2011; 21: 391–398. <https://doi.org/10.1016/j.cub.2010.12.039>.
- [78] Pentakota S, Zhou K, Smith C, Maffini S, Petrovic A, Morgan GP, *et al.* Decoding the centromeric nucleosome through CENP-N. *eLife*. 2017; 6: e33442. <https://doi.org/10.7554/eLife.33442>.
- [79] Corless S, Höcker S, Erhardt S. Centromeric RNA and Its Function at and Beyond Centromeric Chromatin. *Journal of Molecular Biology*. 2020; 432: 4257–4269. <https://doi.org/10.1016/j.jmb.2020.03.027>.
- [80] Leclerc S, Kitagawa K. The Role of Human Centromeric RNA in Chromosome Stability. *Frontiers in Molecular Biosciences*. 2021; 8: 642732. <https://doi.org/10.3389/fmolb.2021.642732>.
- [81] McNulty SM, Sullivan LL, Sullivan BA. Human Centromeres Produce Chromosome-Specific and Array-Specific Alpha Satellite Transcripts that Are Complexed with CENP-A and CENP-C. *Developmental Cell*. 2017; 42: 226–240.e6. <https://doi.org/10.1016/j.devcel.2017.07.001>.
- [82] Du Y, Topp CN, Dawe RK. DNA binding of centromere protein C (CENPC) is stabilized by single-stranded RNA. *PLoS Genetics*. 2010; 6: e1000835. <https://doi.org/10.1371/journal.pgen.1000835>.