Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Comment

The chromatin state paradox in alternative lengthening of telomeres

Mengjie Xiong[1], Wenxian Zheng[1], Tongxin Jia[1], Kailong Hou[1], Jiawei Liu[1], Runzhi Liu[1], Jing Liu[1,2], Kun Wang[3,*], Ruoyu Zhou[1,*], Shuting Jia[1,2*]

[1] Laboratory of Molecular Genetics of Aging and Tumor, Medical School, Kunming University of Science and Technology, Kunming, 650500, P.R. China

[2] The Academy for Cell and Life Health, Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming, 650500, P.R. China

[3] Department of Thoracic Surgery, Anning First People's Hospital, The Affiliated Hospital of Kunming University of Science and Technology, Kunming, 650500, P.R. China

Article Info

Article history:

Received: January 25, 2026
Accepted: April 11, 2026
Published: April 30, 2026

Use your device to scan and read the article online

Abstract

Telomeres are protected by the shelterin complex and consist of TTAGGG repeats. Their gradual erosion triggers replicative senescence unless counteracted by telomerase or the telomerase-independent Alternative Lengthening of Telomeres (ALT) pathway, utilized by 10-15% of cancers. Accumulating evidence indicates that the decision to activate ALT is intimately linked to the plasticity of telomeric chromatin. This review integrates recent data showing that both constitutive and facultative heterochromatin marks shape ALT activity. We summarize the dynamic regulation of telomeric chromatin and explore the contrasting evidence for two models: the 'open telomeric chromatin model,' where loss of constitutive heterochromatin (e.g., reduced H3K9me3) promotes ALT by increasing chromatin accessibility for homologous recombination (HR) factors, and the 'closed telomeric chromatin model,' where a specific gain of heterochromatic features (e.g., H3K9me3 or H3K27me3) facilitates ALT by creating a specialized phase-separated environment that promotes telomere clustering and break-induced replication (BIR). Resolving this paradox is crucial for understanding ALT initiation and for developing promising synthetic-lethal strategies against ALT-dependent cancers.

Keywords: Telomere, ALT, Heterochromatin, Histone Modification.

1. Introduction

Human telomeres localize to the termini of linear chromosomes to protect against degradation and end-to-end fusions[1]. In most eukaryotes, telomeric DNA consists of TTAGGG tandem repeats bound by the shelterin protein complex. The "end-replication problem" causes the progressive loss of nucleotides from the 3' ends of linear chromosomes during cell division, leading to gradual shortening of telomeres[2]. Once telomeres reach a critical length, known as the Hayflick limit, they become dysfunctional and trigger activation of the DNA damage response (DDR), ultimately leading to cellular senescence or apoptosis[1,3]. To achieve replicative immortality, tumor cells must therefore overcome this telomere shortening barrier[4, 5]. Most tumors extend telomeres through reactivating telomerase, while approximately 10–15% utilize a telomerase-independent mechanism known as Alternative Lengthening of Telomeres (ALT)[6]. ALT activation is usually associated with specific phenotypes, including colocalization of ALT telomeres with promyelocytic leukemia protein (PML) nuclear bodies (forming structures called ALT-associated PML bodies, APBs), heterogeneous telomere length, telomere sister-chromatid exchange (T-SCE), and extrachromosomal telomeric repeat DNA (ECTR, such as C-rich circle)[7]. However, the mechanisms underlying ALT activation are not fully understood. A prevalent hypothesis posits that ALT initiation requires the accumulation of replication stress at telomeres[8, 9], which sustains endogenous DNA damage, thereby triggering break-induced replication (BIR) at telomeres to maintain telomere length[10, 11].

Increasing evidence demonstrates that alterations in telomeric chromatin—such as histone modifications, dysregulation of histone chaperone complexes, and the presence of variant repeats—can modulate replication stress and consequently influence ALT activation[12]. However, the highly specialized and dynamic nature of telomeric chromatin complicates the precise delineation of its role

* Corresponding author.
E-mail address: shutingjia@kust.edu.cn (S. Jia); ruoyuzhou@kust.edu.cn (R. Zhou); wangkunkm@126.com (K. Wang).
Doi: http://dx.doi.org/10.14715/cmb/2026.72.4.7

in ALT cells. This has led to two seemingly contradictory models: one where a loss of heterochromatin ('open' state) promotes ALT, and another where a specific gain of heterochromatin ('closed' state) is facilitative. In this review, we first summarize the dynamic regulation of telomeric chromatin. We then critically evaluate the evidence for these contrasting models by examining key chromatin remodelers, histone modifiers, and readers involved in ALT initiation and maintenance. Finally, we discuss the therapeutic implications of targeting telomeric chromatin plasticity in ALT-positive cancers. This review focuses on recent studies (primarily from the past decade) elucidating the role of chromatin structure in the ALT pathway in mammalian systems. Literature was identified through searches of PubMed and Google Scholar using keywords including 'Alternative Lengthening of Telomeres,' 'telomeric heterochromatin,' 'H3K9me3,' 'ATRX,' 'chromatin remodeling,' and 'phase separation.' Priority was given to primary research articles establishing mechanistic links between chromatin modifiers, remodelers, and ALT phenotypes.

2. Dynamic regulation of telomeric chromatin state

The nucleosome is the basic unit of chromatin, consisting of DNA wrapped around octamers of the core histones H2A, H2B, H3, and H4. In mammalian cells, chromatin is structurally categorized into two types: euchromatin and heterochromatin. Heterochromatin is a key architectural feature of eukaryotic chromosomes and endows particular genomic regions with specific functional properties. Heterochromatin is distinguished from euchromatin by its highly condensed state through cell cycle. It plays vital roles in chromosome segregation, genome stability maintenance, gene expression silencing and elements transport[13]. Chromatin state is defined epigenetically by specific histone modifications, for instance, heterochromatin, which is predominantly found at pericentromeres, telomeres, and other silent regions, is frequently marked by trimethylation of histone H3 lysine 9 (H3K9me3)[14].

Heterochromatin could be further subdivided into constitutive and facultative heterochromatin based on its dynamic nature and specific marks. Facultative heterochromatin regions are characterized by high density of H3K27me3, H3K9me2, and H4K20me and relatively dynamic in response to developmental or environmental signals, thus enabling rapid reprogramming of gene expression[15]. In contrast, constitutive heterochromatin regions are rich in H3K9me3 and H4K20me3 and preferentially target repetitive DNA regions, such as centromeres and telomeres, and maintains a highly condensed state[16].

The chromatin state of human telomeres is complex and somewhat paradoxical. While they exhibit strong enrichment of facultative heterochromatin marks like H4K20me, H3K27me3, and H3K9me2[17], recent studies suggest that human telomeres may also display euchromatin characteristics such as H4K20me1 and H3K27ac. A chromatin immunoprecipitation (IP) combined with high-throughput sequencing (ChIP-seq) study in human $CD4^+$ T cells, for instance, revealed that H3K9me3 enrichment primarily occurs in subtelomere and centromere, rather than in telomere regions[18]. In addition, the histone methylation pattern at telomeres may also correlate with telomere length variations in different cell types. In general, cells with shorter telomeres exhibited low histone density, and the modification level of H3K9me3 and H4K20me3 reduced in the telomeres of *Terc*$^{-/-}$mice[19]. Additionally, the loss of telomere repeats leads to a reduced heterochromatin features and nucleosome density[20, 21].

The heterochromatic state of telomeres and subtelomeres is essential for their function, including telomeric integrity and transcriptional regulation[22]. TERRA is a long noncoding RNA transcribed from telomeres[23]. The transcription of TERRA is up-regulated by the open telomeres and subtelomere chromatin state[24]. DNA methyltransferase 3B (DNMT3B) genetic mutations were found in lymphoblastoid and fibroblast cells from patients of ICF syndrome (Immunodeficiency, Centromeric instability, Facial anomalies syndrome), these mutations lead to subtelomeric DNA hypomethylation, ICF cells then consequently exhibit advanced telomere replication timing and elevated levels of transcripts of TERRA[25]. TERRA, together with two other processes--the binding of the shelterin complex to telomeric DNA and histone tail modifications--collaborates to dynamically regulate telomeric heterochromatin state. Disruptions in these processes can lead to reduced telomere DNA synthesis efficiency, activation of ALT pathway and compromised telomere protection[17].

Given the established correlation between telomeric heterochromatin state and tumor, it has been gradually revealed that chromatin plasticity plays an important role in tumor progression, particularly in tumors rely on ALT pathway. However, it remains controversial whether a loss or a gain of heterochromatin promotes ALT initiation. The specific molecular mechanisms of heterochromatin regulation were applied at ALT telomeres, and whether these mechanisms are the causes of ALT initiation, or they are just the consequence of the replication stress inherent to ALT-positive cells? These questions still require further elucidation.

3. Regulation of telomeric chromatin state and ALT activation

3.1 The role of chromatin remodeling complexes in ALT

It has been reported recently that chromatin remodeling complexes play crucial roles in the initiation and maintenance of ALT by dynamically modulating heterochromatin accessibility through precise regulation of nucleosome positioning and histone variant deposition at telomeres. In eukaryotes, chromatin remodelers can be classified into four families based on protein similarity and domain architecture: (i) SWI/SNF, (ii) ISWI (imitation switch), (iii) INO80 (inositol requiring mutant 80), and (iv) CHD (chromodomain helicase DNA-binding)[26].

The regulation of telomere heterochromatin on ALT was originally derived from the study of alpha-thalassemia mental retardation X-linked (ATRX) and its binding chaperone, the death-domain associated protein (DAXX). ATRX is a chromatin remodeling factor and transcriptional regulator that belongs to the SWItch/Sucrose NonFermentable (SWI/SNF) protein family[27]. The ATRX-DAXX complex deposits histone variant H3.3 at chromatin repetitive sequences, such as telomeres, ribosomal DNA (rDNA), and centromeres[28, 29], to maintain a repressive heterochromatic state and prevent genomic instability[30]. Notably, ATRX mutations are present in over 90% ALT tumors, underscoring a strong association between ATRX/DAXX dysfunction and ALT activation[6, 31, 32]. Consistently, restoring ATRX expression in ATRX-defi-

cient ALT cells significantly suppresses ALT-related phenotypes[33]. Several mechanisms have been proposed for how ATRX suppresses ALT activity, including suppressing DDR and homologous recombination (HR) at ALT telomeres[34]; resolving G-quadruplexes (G4) and TERRA hybrid structures by its helicase activity; inhibiting MRE11-mediated resection of stalled replication forks; and preserving proper chromatin architecture[33, 35, 36]. Many of the above models are related to ATRX's role in heterochromatin homeostasis. For example, the ATRX/DAXX-mediated deposition of histone H3.3 at telomeres limits the formation of G4 structures, thereby inhibiting replication fork stalling caused by G4 accumulation and its activated HR repair mechanisms in ALT cells[37]. However, in ATRX-DAXX deficient ALT cells, H3.3 was deposited by HIRA (another histone H3.3 chaperone) compensatory to restrict telomeric single-stranded DNA and 'bad' TERRA R-loops accumulation that threaten ALT cancer cell viability[38].

In addition to ATRX, other subunits of the SWI/SNF complex are also implicated in ALT regulation. For instance, the catalytic subunit BRM (SMARCA2) of the SWI/SNF complex is recruited to the promoters of the shelterin components TRF1 and TRF2, where it promotes their transcription by remodeling chromatin into an open conformation. Depletion of BRM in ALT-positive VA13 cells leads to telomere dysfunction and replication defects, indicating that the remodeling complex is essential for maintaining telomere stability and ALT cell survival[39-41]. The role of ATRX and BRM highlight the crucial function of chromatin remodeling protein complexes in regulating ALT.

Like the SWI/SNF complex, the INO80 complex, driven by its core ATPase Ino80, participates in telomere. Deletion of INO80 subunits in yeast induces ALT-like phenotypes, including telomere elongation and formation of extrachromosomal telomeric circles[42]. In mammalian cells, INO80 is critical for the generation of ssDNA to promote HR (homologous recombination) of dysfunctional telomeres. Conditional deletion of *mIno80* in mouse embryonic fibroblasts (MEFs) results in decreased telomere sister chromatid exchanges at dysfunctional telomeres, increased frequency of fragile telomeres, and activation of cellular senescence programs[43]. These studies suggest that the chromatin remodeling complexes play important roles in HR-mediated telomere replication.

Additional support for the role of chromatin remodeling factors in ALT regulation comes from a large-scale IP and mass spectrometry study of DAXX, which identified heterochromatin protein 1 (HP1) and HP1-binding protein 3 (HP1BP3) as its interacting proteins. Notably, HP1BP3 was found to be specifically enriched at telomeres in ALT cells. Functional studies demonstrated that inhibition of HP1BP3 resulted in decreased H3K9me3 levels and heterochromatin integrity, which ultimately led to increased ALT activity. This suggests that HP1BP3 helps maintaining ALT telomere chromatin compaction by facilitating H3K9me3 occupancy. In addition, HP1BP3 can also en-

Table 1. Heterochromatin marks and associated methylation/demethylation enzymes involved in ALT maintenance.

Histone marks/Process	Related Proteins	Molecular Function/Mechanism	Telomeric chromatin state	Effect on ALT	References
H3K9me3	SETDB1	H3K9 methyltransferase	Compaction	Promotes/ Suppresses	[45, 46]
	CHAMP1	Recruits SETDB1 to enhance H3K9me3.	Compaction	Promotes	[47]
	KDM4B	H3K9 demethylase	Relaxation	Promotes	[48]
	TRIM28	Stabilizes SETDB1; Telomeric accumulation of H3K9me3.	Compaction	Suppresses	[46]
	LAP2α	Recruits HDAC1 to enhance H3K9me3 deposition.	Relaxation	Suppresses	[49]
	ORCA	Induces chromatin condensation and enhances H3K9me3 deposition.	Compaction	Suppresses	[50]
	HP1BP3	Telomeric accumulation of H3K9me3; Stabilizes heterochromatin via HP1 & DAXX binding.	Compaction	Suppresses	[44]
H3.3 deposition	HIRA	H3.3 chaperone; maintains telomeric accessibility.	Relaxation	Promotes	[38]
	ATRX/ DAXX	H3.3 deposition at telomeric heterochromatin.	Compaction	Suppresses	[51]
H4 hypoacetylation	NuRD-ZNF827	Histone deacetylase complex; promotes HR	Compaction	Promotes	[52]
H3K27me3	PRC2	Catalytic component (EZH2) responsible for H3K27 methylation.	Compaction	Promotes	[53]
H3K36me2	KDM2A	H3K36 demethylase	NM	Suppresses	[54]
H3K23ac	TRIM24	Reader of H3K23ac	NM	Promotes	[55]

hance chromatin compaction by clustering multiple H1 proteins for nucleosome condensation[44]. Collectively, these findings suggest that the telomere heterochromatin state is specifically regulated in ALT cells which may be important for the activation of ALT mechanism.

3.2 Telomeric constitutive heterochromatin state plays an important role in the activation and maintenance of ALT mechanism

The contrasting models of H3K9me3 regulation at telomeres in normal and ALT-positive cells are summarized in Figure 1. Panel (a) illustrates the heterochromatic state of telomeres in normal cells, where ATRX recognizes H3K9me3-enriched chromatin to suppress homologous recombination and prevent ALT activation. Panel (b) depicts the 'open telomeric chromatin model', in which disruption of heterochromatin maintenance leads to reduced H3K9me3 levels, increased chromatin accessibility, and enhanced recruitment of homologous recombination factors, thereby promoting ALT. In contrast, panel(c) shows the 'closed telomeric chromatin model', where a specific gain or reorganization of heterochromatic features, particularly H3K9me3, facilitates telomere clustering, phase separation, and break-induced replication to support ALT activity.

Histone methylation, catalyzed by histone methyltransferases (HMTs), is a key determinant of telomeric chromatin state. For instance, the H3K9me3 mark deposited by the H3K9-specific methyltransferases SUV39H1/2 at telomeres recruits HP1, which drives chromatin condensation via liquid-liquid phase separation (LLPS), thereby ensuring telomere integrity[56, 57]. Depletion of SUV39H1/2 reduces H3K9me3 level and HP1 recruitment, leading to telomere decompaction and aberrant homologous recombination, ultimately compromising telomere stability (Figure 1, a)[57, 58].

In addition to regulating chromatin structure, histone methylation also plays important roles in regulating telomeric DDR signaling. For instance, the lysine demethylases (KDMs) of H3K36, including KDM2A and KDM4A, function in promoting DNA damage repair pathways. When DSBs are formed, ATM phosphorylates KDM2A and blocks its demethylase activity, which finally increases H3K36me2 level around DSBs sites and facilitates the recruitment of the MRN (MRE11-RAD50-NBS1) complex to promote HR[59]. Besides, DSBs trigger the ubiquitin-dependent degradation of KDM4A. Since KDM4A and 53BP1 sharing same binding sites near DSBs, the reduction of KDM4A facilitates the recruitment of 53BP1 to DSBs, thus facilitates c-NHEJ repair system[60]. Emerging evidence indicates that numerous KDMs and HMTs are involved in the regulation of ALT mechanisms primarily by either modulating constitutive heterochromatin marks (e.g., H3K9me3, H4K20me3) at telomeres or by participating in the telomeric DDR regulation[61, 62].

3.2.1 Open telomeric chromatin model: reduced telomeric H3K9me3 level activates ALT

H3K9me3 is the predominant constitutive heterochromatin mark in mammalian cells. Recent studies have shown that reduced H3K9me3 epigenetic marks is closely related to the activation of ALT, since this histone modification keep chromatin in an open state and allows the access of HR factors[46, 57, 63]. A study investigating chromatin state and ALT activity revealed that telomeric H3K9me3 is reduced at ALT telomeres, suggesting a more open telomeric chromatin may favor ALT mechanism[63]. Several HMTs and HDMs(Histone Demethylases) are involved in the regulation of telomeric H3K9me3, and are associated with ALT activation (Table 1). Depletion of the H3K9-specific methyltransferases SUV39H1/2 causes a loss of pericentromeric H3K9me3, thereby triggering typical ALT phenotypes[57], indicates SUV39H-mediated high telomeric H3K9me3 level protecting telomeres and inhibiting ALT activation (Figure 1, a). Subsequent research showed that ALT telomeric H3K9me3 heterochromatin marks are mainly regulated by SETDB1, a highly specific euchromatin methyltransferase targeting H3K9 to H3K9me2/3[64]. In synergy with the co-repressor TRIM28/KAP1 (a core component of the human silencing hub (HUSH) complex), SETDB1-mediated H3K9me3 triggers chromatin compaction, converting euchromatic regions into transcriptionally silenced heterochromatin-like states[65, 66]. A study has found that TRIM28 preferentially at ALT telomeres and interacts with telosome complex, which stabilizes SETDB1 to maintain the modification level of telomere H3K9me3 and inhibits ALT activity in tumor cells [46]. Consistently, another research in mouse embryonic stem cells has revealed that Dcaf11 targets TRIM28 for ubiquitination-mediated degradation, leading to the removal of heterochromatic H3K9me3 at telomere/subtelomere regions and activating the Zscan4-mediated ALT mechanism[67].

In addition, several H3K9me3-binding proteins are also involved in regulating H3K9me3 modifications on telomere chromatin thus related to the ALT mechanism. For instance, H3K9me3 can directly interact with ATRX[68], which may contribute to ALT mechanism via ATRX inhibition (Figure 1, a). The H3K9me3 occupancy on telomere is bound by HP1BP3, which stabilizes the compacted structure of telomere chromatin and inhibits ALT phenotypes and ALT tumor cell growth [44]. The nuclear envelope protein lamin-associated polypeptide 2α (LAP2α) recruits histone deacetylase HDAC1 to telomeres, leading to histone deacetylation that driving heterochromatic compaction by elevating H3K9me3 deposition and stabilizing HP1 anchoring. Thus, LAP2α deficiency causes pathological chromatin decondensation and increases telomeric DNA accessibility and promotes ALT activation[49]. Notably, methotrexate (MTX), a drug widely used to treat osteosarcoma, traps STAT5 in its unphosphorylated, active form. This enables STAT5 to bind HP1α, leading to heterochromatin stabilization and promotion of H3K9me3[69]. In patient-derived xenograft (PDX) models of LAP2α-deficient ALT-positive osteosarcoma, MTX treatment reverses chromatin decompaction, suppresses ALT-driven recombination, and significantly inhibits tumor proliferation[49], validating heterochromatin restoration as a synthetic lethal strategy against ALT tumors. Origin Recognition Complex-Associated (ORCA/LRWD1) is recruited to telomeres of ALT cells through a specific interaction with the MMS21-SUMOylated shelterin complex. This recruitment enables ORCA to orchestrate a repressive chromatin architecture by facilitating the deposition of the H3K9me3 heterochromatin mark. The establishment of this compact chromatin state directly impedes the access and loading of key homologous recombination mediators, including RPA and RAD51, thereby effectively suppressing the initiation of telomere recombination. Consequent-

ly, the loss of ORCA triggers heterochromatin decondensation, which in turn drives the hyper-activation of the ALT pathway, as evidenced by increased APBs formation and elevated telomere sister chromatid exchanges[70] (Figure 1, b). These findings indicate that the reduction in telomeric heterochromatin could promote ALT, possibly due to increased telomeric accessibility benefits replication and HR. It is also worth noting that, decreased H3K9me3 may accumulate more telomeric DNA damage, which is proved by either deletion of TRIM28 or HP1BP3 caused a decrease of telomeric H3K9me3 level, accompanied by the elevated telomeric damage foci[44, 46], suggesting the replication stress induced by de-heterochromatinization may also be a potential trigger for ALT (Table 1).

3.2.2 Closed telomeric chromatin model: elevated telomeric H3K9me3 level contributes to the occurrence of ALT

Although numerous studies suggest that the loss of the heterochromatin mark H3K9me3 can activate ALT, other studies have reported completely opposite findings (Table 1). For example, in ALT cells, ATRX deficiency prevents the deposition of the histone variant H3.3 at telomeres[71]. This leads to unopposed, persistent demethylase activity of histone demethylase KDM4B, resulting excessive depletion of the critical heterochromatic marks of H3K9me3/2 and HP1α binding. The erosion of these constitutive heterochromatin marks not only disrupts telomere maintenance but also impedes the formation of APBs in ALT cells[48] , This observation suggests that ALT activation requires not merely a reduction of heterochromatin, but the maintenance of a minimal or properly organized H3K9me3 pool. In this context, excessive KDM4B activity leads to over-erasure of H3K9me3, disrupting HP1-dependent telomeric compartmentalization and impairing APB formation, thereby suppressing ALT (Figure 1, c). However, in the LAP2α-deficient U2OS, the elevated H3K9me3 and HP1 at telomeres caused by KDM4B knockdown instead yields decreased C-circle[49]. The contradictory result has also been observed in SETDB1 study. As we mentioned above, SETDB1 suppresses ALT by maintaining the H3K9me3 heterochromatin mark on telomeric DNA[46]. However, it has also been demonstrated that SETDB1 catalyzes H3K9me3 modification specifically at telomeres to recruit HR-related factors such as BRCA1 and SMC5/6 complex, ultimately activating ALT in the absence of ATRX[45]. In addition, further studies have found that the CHAMP1-POGZ complex directly recruits SETDB1 through zinc finger scaffold interactions, and promotes HR at ALT telomeres[47] (Figure 1, c). These conflicting results indicate that the H3K9me3 state may not directly engage in the activation or inhibition of ALT, further investigation of DNA methylation molecular mechanism in ALT tumors need to be explored.

Taken together, the seemingly contradictory roles of H3K9me3 in ALT regulation suggest that its function cannot be explained by a simple gain-or-loss model. One possible explanation is cell-type specificity: distinct ALT-positive cell lines or tumor types may differ in their chromatin remodeling landscape, DNA damage response capacity, or dependency on specific recombination pathways, leading to context-dependent outcomes of H3K9me3 modulation. In addition, the genomic context of H3K9me3 deposition may be critical. H3K9me3 enrichment at subtelomeric

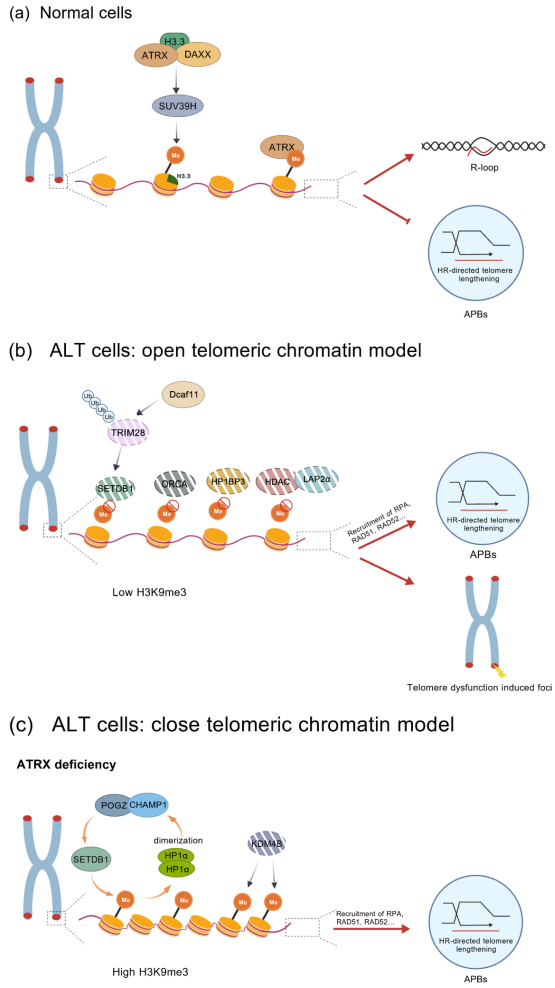


Fig. 1. The figure summarizes the molecular mechanisms by which the H3K9me3 modification level in normal cells and ALT cells models. (a) In normal cells, telomeres maintain a certain level of H3K9me3. The chromatin remodeler ATRX directly binds to telomeric H3K9me3, which inhibits HR and prevents ALT. (b) In ALT cells: open telomeric chromatin model. In this model, dysregulation of multiple pathways converges to cause telomeric heterochromatin decompaction and reduced H3K9me3 levels, thereby activating ALT. Dcaf11-TRIM28 axis: Dcaf11 targets TRIM28 for ubiquitin-mediated degradation. The loss of TRIM28 leads to destabilization of the methyltransferase SETDB1, resulting in decreased H3K9me3 deposition at telomeres; Impaired function of ORCA compromises its role in promoting telomeric heterochromatin condensation, thereby increasing the accessibility for HR factors; Inhibition of HP1BP3 function reduces H3K9me3 levels, disrupts heterochromatin compaction, and promotes ALT. LAP2α-HDAC1 axis impairment: Deficiency of LAP2α abrogates the recruitment of HDAC1 to telomeres, leading to pathological chromatin decondensation, increased telomeric DNA accessibility, and consequently activate ALT.(c) In ALT cells: closed telomeric chromatin model. In this model, a specific mechanism of heterochromatin establishment drives ALT. CHAMP1-POGZ-SETDB1 positive feedback loop: The CHAMP1-POGZ complex directly recruits the methyltransferase SETDB1 and deposits H3K9me3 to ALT telomeres. H3K9me3 is recognized by HP1α, whose dimerization and LLPS properties facilitate the formation of heterochromatin clusters and promote HR. This process further enriches the CHAMP1-POGZ complex, establishing a positive feedback loop that amplifies H3K9me3 signaling and promotes HR. KDM4B is precisely regulated, and its balanced activity helps maintain the pro-recombinogenic heterochromatin state. The slash indicates downregulation or depletion of indicated proteins.

regions could exert different effects compared with its presence on the telomeric repeat array itself, potentially influencing TERRA transcription, replication timing, or telomere–nuclear compartment interactions in distinct ways.

Another important consideration is that ALT regulation may depend not on the global abundance of H3K9me3, but rather on its spatial organization and local distribution. A threshold level, focal enrichment, or specific heterochromatin microdomains of H3K9me3 may be required to promote liquid–liquid phase separation, telomere clustering, and break-induced replication, whereas excessive loss or uniform spreading of this mark could instead lead to uncontrolled DNA damage and ALT suppression. Thus, the balance, localization, and dynamics of H3K9me3—rather than its bulk level alone—are likely to determine whether heterochromatin acts as a barrier or a facilitator of ALT.

Taken together, the seemingly contradictory observations regarding H3K9me3 in ALT regulation do not necessarily reflect cell-type specificity alone, but rather suggest a more integrated, context-dependent model. In this unifying view, a reduction in telomeric H3K9me3 (open chromatin model) may be required to increase chromatin accessibility at dysfunctional telomeres, thereby facilitating the initial recruitment of homologous recombination factors and the detection of replication stress. Conversely, a specific, spatially organized gain or maintenance of H3K9me3 at subtelomeric or focal telomeric regions (closed chromatin model) promotes liquid–liquid phase separation, stabilizes APB formation, and supports processive break-induced replication. Thus, ALT activity does not depend on a simple global increase or decrease of H3K9me3, but rather on a finely tuned equilibrium between locally open and condensed heterochromatin states. Disruption of this balance—whether by excessive loss or aberrant spreading of H3K9me3—compromises ALT, suggesting that the precise spatial and dynamic organization of this mark, rather than its bulk abundance, is the critical determinant of ALT permissiveness.

3.2.3. Other histone modifications that govern telomeric chromatin state

Beyond telomeric H3K9me3, several additional histone marks are also essential for activating or maintaining the ALT pathway. For example, the NuRD complex, is recruited to telomeres by zinc finger protein ZNF827 via its N-terminal RRK motif, induces histone deacetylation and nucleosome repositioning through its ATPase subunit CHD4. This activity establishes a hypoacetylated chromatin state (H4K5ac and H4K8ac increase) that reducing shelterin binding, promoting telomere-telomere interactions, and recruiting HR proteins to initiate recombination process[52]. In a related but distinct pathway, CBP/p300-mediated histone H3K23ac acts as an initiating signal that recruiting the reader protein TRIM24, subsequent SUMOylation of TRIM24 stabilizing RAD51 and BLM, directly facilitating the telomere recombination process[55, 72]. Conversely, the demethylase KDM2A contributes to resolving the recombination state by erasing H3K36me2 marks, creating a permissive environment for the isopeptidase SENP6 to remove excessive SUMO modifications at PML, thereby preventing aberrant telomere clustering and ensuring normal cell division. Thus, the loss of KDM2A considerably suppresses the survival of ALT cells without affecting the viability of non-ALT cells[54, 73, 74] (Table 1).

3.3 The regulation of facultative heterochromatin on ALT telomeres

Very little evidence supports that facultative heterochromatin marks participate in regulating ALT activation, although both facultative heterochromatin and euchromatin marks exist on human telomeres. It has been demonstrated that the H3K27me3/H3 ratio increased 2-fold in BRCA2-deficient ALT-like MEFs compared with non-ALT MEFs, whereas the H3K9me3/H3 ratio did not change significantly[53]. Evidence indicates that H3K27me3 deposition in ALT cells is primarily mediated by the Polycomb Repressive Complex 2 (PRC2) complex. Specifically, PRC2 complex is recruited to telomeres through LLPS, which triggered by the stabilization of G4 upon BRCA2 loss. Once recruited, the methylase subunit (EZH2) catalyzes the methylation of the H3K27, establishing a facultative heterochromatin state at telomeres. This PRC2-mediated heterochromatinization creates a permissive chromatin environment that facilitating the engagement of BIR pathway, thereby directly activating the ALT mechanism[53] (Table 1). Unlike the constitutive heterochromatin, facultative heterochromatin presents temporal and context-dependent feature during development progression, the plasticity makes it a compelling regulator for ALT, a process requiring a delicate balance between telomere stability and recombination. How heterochromatin state is regulated in ALT system and whether crosstalk exists between facultative and constitutive heterochromatin remain outstanding questions in ALT research.

3.4 DNA methylation at subtelomeres: an additional layer of telomeric chromatin regulation

Beyond histone modifications, DNA methylation at subtelomeric regions plays a critical yet underappreciated role in modulating telomeric chromatin state and ALT activity. In normal cells, subtelomeric CpG islands are heavily methylated by DNA methyltransferases such as DNMT3B, which represses TERRA transcription and maintains telomeric heterochromatin integrity. In ICF syndrome, DNMT3B mutations cause subtelomeric hypomethylation, leading to TERRA overexpression, altered replication timing, and telomere dysfunction. Emerging evidence suggests that ALT-positive tumors frequently exhibit aberrant subtelomeric DNA methylation patterns, including both hypomethylation and hypermethylation at distinct loci, which may influence TERRA expression, R-loop formation, and homologous recombination at telomeres. Moreover, TET proteins (Ten-Eleven Translocation dioxygenases) that mediate active DNA demethylation have been implicated in telomere elongation dynamics, although their specific role in ALT remains poorly defined. Thus, future studies integrating DNA methylation profiling with chromatin analysis in ALT cells are necessary to determine whether altered DNA methylation is a cause or consequence of ALT activation, and whether targeting DNA methylation machinery could offer a synthetic-lethal opportunity in ALT cancers[22, 25, 36].

4. Discussion

Chromatin structure is an important factor that regulates protein-DNA and protein-protein interactions. Te-

lomeric DNA is not synchronized with the DNA helical repeat and lacks nucleosome positioning signals[75], suggesting that telomeric chromatin condensation and histone modification-associated biological effects are achieved through a pathway distinct from the classical mechanism of bulk chromatin. Due to frequent loss of ATRX/DAXX histone chaperone complex, alterations in recruitment of proteins such as nuclear receptors and chromatin remodelers, and changes in histone modifications of telomeric chromatin, the ALT telomeres are structurally distinct from that of non-ALT tumor cells or normal cells, suggesting that the unique chromatin state of ALT telomeres may be associated with its activation and maintenance[7, 52, 76]. In fact, ALT activity is not dependent on a universally 'open' or 'closed' chromatin state, but rather is closely tied to context-specific chromatin states. For instance, in both 'open' and 'close' telomeric chromatin models, ALT activity is facilitated by certain chromatin state-either the promotion of HR template dissociation through open chromatin or recruitment of telomere-associated proteins and formation of LLPS through compacted heterochromatic state[45, 46, 53]. This highlights that ALT activity depends on dynamic chromatin changes rather than a single 'open' or 'closed' state.

It is also worth noticing that whether these chromatin alterations are a cause or a consequence of ALT. While the suppression of ALT upon restoring ATRX or heterochromatin factors like LAP2α suggests a causative role[49]. RAD51AP1 was reported to interact with chromatin remodeling and modification factors PDS5a, BRG1, ZYMND8 and lead to suppression of TERRA by chromatin modulation and ubiquitination of H2A at telomere DSBs, consequently unchallenged telomeres in ALT cancer cells[77]. The loss of ATRX disrupts the maintenance of heterochromatin at telomeres, leading directly to reduced levels of the H3K9me3 modification. This epigenetic change causes the aberrant expression and accumulation of TERRA and simultaneously destabilizes the chromatin structure at telomeres. Together, these effects hinder the progression of replication forks, creating persistent replication stress. It is within this environment of endogenous replication stress that the homologous recombination-based ALT pathway becomes selectively activated and relied upon to maintain telomere length[7]. It is also possible that the persistent replication stress inherent to ALT cells drives progressive chromatin remodeling. It was found that TERRA R-loops can recruit PRC2, leading to H3K27me3 at telomeres as well as LLPS in ALT-like cells[53]. Replication stress induces dynamic remodeling of chromatin at DNA damage sites, including increased histone acetylation and reduced repressive methylation modifications, which temporarily'opens up' the local chromatin structure. This change facilitates the recruitment of repair factors such as those involved in HR, promoting accurate DDR. It is noteworthy that this replication stress-triggered, unstable chromatin state-particularly its characteristics of promoting recombination and transcription-may create a potential molecular environment for telomere lengthening via abnormal HR.

Future research should aim not only to elucidate the temporal sequence of these events but also to identify the precise readers of ALT-specific chromatin marks. Given that reports on histone reader proteins regulating ALT are currently limited, it is promising that these readers play a crucial role. The catalytic component of the NuRD complex is CHD3 or its isoform CHD4 protein, both of which possess PHD domains and exhibit histone modification reader capabilities[78]. They are recruited to ALT telomeres to maintain an HR-permissive environment[52].

Many studies speculated that APB-associated telomere clustering is dynamic in the nucleus and promotes telomere-telomere recombination[79, 80]. Studies of the dynamic behavior of telomeric DNA repeats showed that, unlike telomerase-positive cells, a majority of telomeres have constrained diffusive movement and tend to cluster in ALT activated cells[79, 81]. It has also been reported that the RAP1-SUN1-mediated telomere-nuclear envelope attachment to prevent hyper-activated telomere-telomere recombination in ALT cells, which suggests the movement of telomeres may be tightly regulated and important for telomeric DNA synthesis[82, 83]. Telomere movement is likely facilitated by SIRT6-SNF2H mediated telomeric chromatin decondensation[84]. It is possible that DNA remodeling activity in ALT cells acts together with several associated DNA repair factors to change the structure of the telomeric DNA, thereby regulating telomere movement, and thus facilitating or maintaining ALT activity.

Both constitutive and facultative heterochromatin marks exist at human telomeres. The role of constitutive marks, particularly H3K9me3, in regulating ALT has been extensively documented, whether it promotes or inhibits ALT activity (Figure 1). However, the role of the facultative heterochromatin marks in ALT has been less studied[53]. It has been proposed that the properties of human telomeric chromatin align more closely with those of facultative heterochromatin[53]. Could the facultative heterochromatin marks serve as a regulator that modulates chromatin compaction or recruitment of histone modification reader under different stress conditions? It is possible that facultative heterochromatin adds another layer of regulation. ALT-like telomeres display a hybrid signature containing both increased H4K20me3 and decreased H4K5ac and H4K8ac[52]. Furthermore, the H3K27me3 allows ALT cells to reversibly toggle between recombination-permissive and refractory states, a plasticity that canonical H3K9me3-based heterochromatin cannot provide. The nuclear lamina plays an important role in maintaining the structural integrity of telomeric chromatin and regulating the choice between constitutive and facultative heterochromatin pathways. The dynamic interaction between nuclear lamina components and telomeres could influence the overall chromatin state at telomeres, contributing to the regulation of telomere maintenance mechanisms such as ALT. Expression of progerin, a truncated unprocessed lamin A protein observed in Hutchinson-Gilford progeria syndrome, reduces both the association of telomeres with LAP2α and the global levels of H3K27me3[85]. This suggests that nuclear lamina integrity could influence the chromatin state at telomeres and affect ALT activity. Whether progerin could also affect LAP2α regulated telomeric H3K9me3 elevation and ALT inhibition? And whether this process is related to its elevated H3K27me3 levels? Further studies will be needed.

Although many studies suggest that ALT is a targetable pathway, there are currently no ALT cancer-specific targeted therapeutics. In a most recent study, Benura Azeroglu, Eros Lazzerini Denchi and colleagues developed a high throughput imaging-based screen assay named TAILS (te-

lomeric ALT in situ localization screen) to identify genes that either promote or inhibit ALT activity. Accordingly, 84 ALT-suppressors and 7 ALT-activators were identified. Besides Fanconi anemia complementation group proteins, genes encoding chromatin remodeling factors or histone chaperones are highly enriched, such as the nucleosome-remodeling factor CHD4 and the chromatin reader SGF29[86], suggesting chromatin state is a promising therapeutic target for ALT-positive cancers. However, very few researches have investigated the inhibition of ALT tumor proliferation by targeting heterochromatin remodeling complexes or histone modification enzymes. Currently, only the following reports are available: the co-treatment with the G4 stabilizer PDS and the EZH2 inhibitor EPZ-6438 selectively inhibited BRCA2-depleted ALT-like fibroblasts[53]. In addition, as we mentioned above, MTX holds potential for treating ALT-positive osteosarcoma by reversing chromatin decompaction, thereby suppressing ALT-associated recombination[49]. Moreover, since the telomeric chromatin compaction has been demonstrated to be essential for LLPS. Disruption of LLPS may provide a further opportunity by which to disrupt the APB formation. For instance, the organic compound 1,6-hexanediol (1,6-HD) has been proved to disrupt LLPS and change chromatin compaction[87]. However, its potential as an ALT inhibitor is still highly speculative, and it is important to note that 1,6-HD may exhibit non-specific effects and toxicity in vivo, which complicates its therapeutic application. Nonetheless, understanding how to therapeutically manipulate the delicate balance of chromatin in ALT cells, such as destabilizing the pro-recombinogenic heterochromatin, holds significant promise for synthetic lethal strategies targeting ALT-positive cancers. However, this approach also presents considerable challenges, particularly in terms of specificity and minimizing off-target effects in a clinical setting.

5. Conclusion

The regulation of Alternative Lengthening of Telomeres by telomeric chromatin presents a compelling paradox, with robust evidence supporting both its activation by open, accessible chromatin and by specialized, condensed heterochromatic states. This apparent contradiction underscores a more fundamental principle: ALT is not governed by a uniform, static chromatin environment. Instead, its initiation and maintenance likely depend on achieving a precise, context-dependent equilibrium of chromatin states, each fulfilling distinct mechanistic roles. The 'open chromatin' model may facilitate the initial recognition of telomeric DNA damage and the recruitment of HR machinery, while the 'closed chromatin' model, particularly through liquid-liquid phase separation, appears crucial for creating discrete nuclear compartments like APBs that promote efficient telomere clustering and BIR.

Key chromatin remodelers and modifiers, such as ATRX/DAXX and SETDB1, emerge not as simple on/off switches but as bidirectional regulators. Their function, whether suppressing or promoting ALT, is determined by a complex interplay of interacting partners, downstream effectors, and the specific cellular and telomeric context. The emerging role of facultative heterochromatin marks, like H3K27me3, introduces an additional layer of plasticity, potentially allowing ALT cells to reversibly toggle between recombinogenic and silent states in response to replication stress.

As research into the ALT mechanism continues to advance, many critical questions remain to be addressed. The following will discuss several important directions that could drive future research.

5.1 Temporal dynamics and causality

The precise temporal dynamics of ALT activity remain to be fully characterized. While recent studies suggest that ALT is triggered under specific chromatin conditions, the causal relationship between chromatin state and ALT activation needs further clarification. Specifically, the timeline of chromatin remodeling events in ALT-positive cells and how these processes influence telomere maintenance require deeper exploration. Longitudinal studies tracking chromatin alterations over time, paired with functional assays, could provide a clearer understanding of cause-and-effect relationships in ALT regulation.

5.2 The role of chromatin readers

Chromatin readers, such as DNA binding proteins and histone modifiers, play a crucial role in the regulation of telomeric chromatin states. However, their specific contributions to ALT are not yet fully understood. Future research should focus on identifying chromatin readers that are recruited to ALT telomeres and their impact on ALT-related processes like homologous recombination. Targeted studies using ChIP and proximity ligation assays could reveal the intricate interactions between chromatin readers and telomere-associated proteins.

5.3 Nuclear architecture and telomere movement

The spatial organization of telomeres within the nucleus appears to be an important factor in ALT activation. Recent evidence suggests that telomere repositioning to specific nuclear compartments correlates with ALT activation. Investigating the role of nuclear architecture in ALT may provide insights into how telomere dynamics influence ALT activity. Live-cell imaging and 3D genome architecture studies could offer new perspectives on the mechanisms underlying telomere movement and its relevance to ALT.

5.4 Therapeutic exploitation

Although the ALT pathway presents a promising synthetic-lethal target, no ALT-specific therapy has yet entered routine clinical practice. However, recent mechanistic insights into telomeric chromatin regulation have opened two distinct therapeutic avenues. First, ALT cells exhibit chronic replication stress and an elevated demand for deoxyribonucleoside triphosphates (dNTPs) to sustain break-induced replication at telomeres. Pharmacological inhibition of dNTP synthesis, for example, with triapine (a ribonucleotide reductase inhibitor), has been shown to selectively impair telomere synthesis and reduce ALT activity in preclinical models. While triapine has been evaluated in clinical trials for other cancers, its efficacy in ALT-positive tumors remains to be specifically tested. Second, liquid-liquid phase separation (LLPS) is essential for the formation of APBs and the clustering of telomeres that facilitates homologous recombination in ALT cells. Disrupting LLPS using compounds that interfere with multivalent protein interactions (e.g., small molecules targeting HP1 dimerization or SETDB1-associated scaffolds)

represents a conceptually novel strategy. Importantly, non-specific aliphatic alcohols such as 1,6-hexanediol are unsuitable for therapeutic use due to their cellular toxicity and off-target effects; instead, next-generation LLPS modulators with improved specificity are required. Beyond these approaches, recent high-throughput screens (e.g., TAILS) have identified additional chromatin-related ALT suppressors and activators, including CHD4 and SGF29, which may serve as druggable nodes. Collectively, targeting the precise balance of telomeric heterochromatin, either by depleting dNTP pools or by disrupting phase-separated recombination compartments, offers a rational, though challenging, path toward synthetic-lethal therapies for ALT-positive cancers [86].

Abbreviations

Alternative Lengthening of Telomeres (ALT), ALT-associated PML body (APB), Alpha-thalassemia/mental retardation X-linked (ATRX), Break-Induced Replication (BIR), Breast Cancer 1 (BRCA1), Breast Cancer 2 (BRCA2), Brahma-related gene 1 (BRG1), Brahma (SMARCA2, BRM), Extrachromosomal telomeric C-rich circle (C-circle), Chromatin Immunoprecipitation followed by sequencing (ChIP-seq), Death-domain associated protein (DAXX), DNA Damage Response (DDR), DNA Methyltransferase 3B (DNMT3B), Double-Strand Break (DSB), Extrachromosomal Telomeric Repeat DNA (ECTR), Enhancer of Zeste Homolog 2 (EZH2), G-quadruplex (G4), Trimethylation of histone H3 at lysine 9 (H3K9me3), Trimethylation of histone H3 at lysine 27 (H3K27me3), Dimethylation of histone H3 at lysine 36 (H3K36me2), Trimethylation of histone H4 at lysine 20 (H4K20me3), Histone Deacetylase 1 (HDAC1), Histone Regulator A (HIRA), Heterochromatin Protein 1 (HP1), HP1-Binding Protein 3 (HP1BP3), Homologous Recombination (HR), Human Silencing Hub (HUSH), Immunodeficiency, Centromeric instability, Facial anomalies syndrome (ICF syndrome), Inositol requiring mutant 80 (INO80), Lysine Demethylase (KDM), Lysine Demethylase 4B (KDM4B), Lamin-associated polypeptide 2α (LAP2α), Liquid-Liquid Phase Separation (LLPS), Mouse Embryonic Fibroblast (MEF), MRE11–RAD50–NBS1 complex (MRN complex), Methotrexate (MTX), Nucleosome Remodeling and Deacetylase (NuRD), Origin Recognition Complex-Associated/ Leucine-rich repeat and WD repeat-containing protein 1 (ORCA/LRWD1), Patient-Derived Xenograft (PDX), Plant Homeodomain (PHD domain), Promyelocytic Leukemia protein (PML), Polycomb Repressive Complex 2 (PRC2), RAD51 recombinase (RAD51), Repressor/activator protein 1 (RAP1), Replication Protein A (RPA), Ribosomal DNA (rDNA), SET Domain Bifurcated Histone Lysine Methyltransferase 1 (SETDB1), Sirtuin 6 (SIRT6), Sucrose Non-Fermentable 2 Homolog (SNF2H), Suppressor of variegation 3-9 homolog 1/2 (SUV39H1/2), SWItch/Sucrose Non-Fermentable (SWI/SNF), Telomeric ALT In situ Localization Screen (TAILS), Telomeric Repeat-containing RNA (TERRA), Telomeric Repeat-binding Factor 1/2 (TRF1/2), Tripartite Motif-containing 28/ KRAB-associated protein 1 (TRIM28/KAP1), Telomere Sister-Chromatid Exchange (T-SCE), Zinc Finger Protein 827 (ZNF827).

Conflict of Interests

The authors declare no competing interests.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

No human or animals were used in the present research.

Informed Consent

The authors declare that no patients were used in this study.

Funding

This work is supported by grants from the Yunnan Fundamental Research Project (202401AT070312) to Ruoyu Zhou; Yunnan "Xing Dian Ying Cai" project (YNWR-QNBJ-2019-240) and joint funding of the Science Foundation of Kunming University of Science and Technology and The First People's Hospital of Yunnan Province (KUST-KH2022007Y) to Shuting Jia.

Generative AI Statement

The author declares that generative AI (artificial intelligence) tools, including Grammarly & QuillBot, were used to enhance the language & clarity of this work. the author takes full responsibility for the accuracy & integrity of the content.

References

1. Shay JW, Wright WE (2019) Telomeres and telomerase: three decades of progress. Nat Rev Genet 20: 299–309 doi: 10.1038/s41576-019-0099-1
2. Brenner KA, Nandakumar J (2022) Consequences of telomere replication failure: the other end-replication problem. Trends Biochem Sci 47: 506–517 doi: 10.1016/j.tibs.2022.03.013
3. Engin AB, Engin A (2021) The Connection Between Cell Fate and Telomere. Adv Exp Med Biol 1275: 71–100 doi: 10.1007/978-3-030-49844-3_3
4. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144: 646–674 doi: 10.1016/j.cell.2011.02.013
5. Bodnar AG, Ouellette M (1998) Extension of life-span by introduction of telomerase into normal human cells. Science 279: 349–352 doi: 10.1126/science.279.5349.349
6. Heaphy CM, de Wilde RF (2011) Altered telomeres in tumors with ATRX and DAXX mutations. Science 333: 425 doi: 10.1126/science.1207313
7. Amorim JP, Santos G (2016) The Role of ATRX in the Alternative Lengthening of Telomeres (ALT) Phenotype. Genes (Basel) 7 doi: 10.3390/genes7090066
8. Hoang SM, O'Sullivan RJ (2020) Alternative Lengthening of Telomeres: Building Bridges To Connect Chromosome Ends. Trends Cancer 6: 247–260 doi: 10.1016/j.trecan.2019.12.009
9. Cesare AJ, Kaul Z (2009) Spontaneous occurrence of telomeric DNA damage response in the absence of chromosome fusions. Nat Struct Mol Biol 16: 1244–1251 doi: 10.1038/nsmb.1725
10. Conomos D, Stutz MD (2012) Variant repeats are interspersed throughout the telomeres and recruit nuclear receptors in ALT cells. J Cell Biol 199: 893–906 doi: 10.1083/jcb.201207189
11. Varley H, Pickett HA (2002) Molecular characterization of inter-telomere and intra-telomere mutations in human ALT cells. Nat Genet 30: 301–305 doi: 10.1038/ng834
12. Lu R, Pickett HA (2022) Telomeric replication stress: the beginning and the end for alternative lengthening of telomeres cancers. Open Biol 12: 220011 doi: 10.1098/rsob.220011

13. Allshire RC, Madhani HD (2018) Ten principles of heterochromatin formation and function. Nat Rev Mol Cell Biol 19: 229–244 doi: 10.1038/nrm.2017.119
14. Han C, Srivastava AK (2016) Differential DNA lesion formation and repair in heterochromatin and euchromatin. Carcinogenesis 37: 129–138 doi: 10.1093/carcin/bgv247
15. Morrison O, Thakur J (2021) Molecular Complexes at Euchromatin, Heterochromatin and Centromeric Chromatin. Int J Mol Sci 22 doi: 10.3390/ijms22136922
16. Janssen A, Colmenares SU (2018) Heterochromatin: Guardian of the Genome. Annu Rev Cell Dev Biol 34: 265–288 doi: 10.1146/annurev-cellbio-100617-062653
17. Bettin N, Vaurs M (2025) Epigenetics of Human Telomeres. Cold Spring Harb Perspect Biol doi: 10.1101/cshperspect.a041706
18. Sin JH, Kashyap S (2022) ATF7ip Targets Transposable Elements for H3K9me3 Deposition to Modify CD8(+) T Cell Effector and Memory Responses. J Immunol 208: 1155–1169 doi: 10.4049/jimmunol.2100996
19. Dan J, Chen H, Liu C, Wang L, Xu Y, Wang S, Tao Y, Li Z, Liu D, Chen H, Wang L, Zhao Y, Liu Y, Liu M, Liu Y, Wang L, Chen Z, Liu J, Wang Y, Kang J (2015) Roles for histone acetylation in regulation of telomere elongation and two-cell state in mouse ES cells. J Cell Physiol 230: 2337–2344 doi: 10.1002/jcp.24968
20. Benetti R, García-Cao M (2007) Telomere length regulates the epigenetic status of mammalian telomeres and subtelomeres. Nat Genet 39: 243–250 doi: 10.1038/ng1952
21. Barral A, Déjardin J (2020) Telomeric Chromatin and TERRA. J Mol Biol 432: 4244–4256 doi: 10.1016/j.jmb.2020.03.003
22. Arnoult N, Van Beneden A (2012) Telomere length regulates TERRA levels through increased trimethylation of telomeric H3K9 and HP1α. Nat Struct Mol Biol 19: 948–956 doi: 10.1038/nsmb.2364
23. Rivosecchi J, Cusanelli E (2023) TERRA beyond cancer: the biology of telomeric repeat-containing RNAs in somatic and germ cells. Front Aging 4: 1224225 doi: 10.3389/fragi.2023.1224225
24. Novo CL (2021) A Tale of Two States: Pluripotency Regulation of Telomeres. Front Cell Dev Biol 9: 703466 doi: 10.3389/fcell.2021.703466
25. Yehezkel S, Segev Y (2008) Hypomethylation of subtelomeric regions in ICF syndrome is associated with abnormally short telomeres and enhanced transcription from telomeric regions. Hum Mol Genet 17: 2776–2789 doi: 10.1093/hmg/ddn177
26. Reyes AA, Marcum RD (2021) Structure and Function of Chromatin Remodelers. J Mol Biol 433: 166929 doi: 10.1016/j.jmb.2021.166929
27. Picketts DJ, Higgs DR (1996) ATRX encodes a novel member of the SNF2 family of proteins: mutations point to a common mechanism underlying the ATR-X syndrome. Hum Mol Genet 5: 1899–1907 doi: 10.1093/hmg/5.12.1899
28. Voon HP, Hughes JR (2015) ATRX Plays a Key Role in Maintaining Silencing at Interstitial Heterochromatic Loci and Imprinted Genes. Cell Rep 11: 405–418 doi: 10.1016/j.celrep.2015.03.036
29. Lewis PW, Elsaesser SJ (2010) Daxx is an H3.3-specific histone chaperone and cooperates with ATRX in replication-independent chromatin assembly at telomeres. Proc Natl Acad Sci U S A 107: 14075–14080 doi: 10.1073/pnas.1008850107
30. Voon HP, Wong LH (2016) New players in heterochromatin silencing: histone variant H3.3 and the ATRX/DAXX chaperone. Nucleic Acids Res 44: 1496–1501 doi: 10.1093/nar/gkw012
31. Lovejoy CA, Li W (2012) Loss of ATRX, genome instability, and an altered DNA damage response are hallmarks of the alternative lengthening of telomeres pathway. PLoS Genet 8: e1002772 doi: 10.1371/journal.pgen.1002772
32. Schwartzentruber J, Korshunov A (2012) Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. Nature 482: 226–231 doi: 10.1038/nature10833
33. Clynes D, Jelinska C (2015) Suppression of the alternative lengthening of telomere pathway by the chromatin remodelling factor ATRX. Nat Commun 6: 7538 doi: 10.1038/ncomms8538
34. Qin L, Tang G (2025) ATRX loss inhibits DDR to strengthen radio-sensitization in p53-deficent HCT116 cells. Sci Rep 15: 793 doi: 10.1038/s41598-024-85085-6
35. Leung JW, Ghosal G (2013) Alpha thalassemia/mental retardation syndrome X-linked gene product ATRX is required for proper replication restart and cellular resistance to replication stress. J Biol Chem 288: 6342–6350 doi: 10.1074/jbc.M112.411603
36. Nguyen DT, Voon HPJ (2017) The chromatin remodelling factor ATRX suppresses R-loops in transcribed telomeric repeats. EMBO Rep 18: 914–928 doi: 10.15252/embr.201643078
37. Dyer MA, Qadeer ZA (2017) ATRX and DAXX: Mechanisms and Mutations. Cold Spring Harb Perspect Med 7 doi: 10.1101/cshperspect.a026567
38. Lynskey ML, Brown EE (2024) HIRA protects telomeres against R-loop-induced instability in ALT cancer cells. Cell Rep 43: 114964 doi: 10.1016/j.celrep.2024.114964
39. Wilson BG, Roberts CW (2011) SWI/SNF nucleosome remodellers and cancer. Nat Rev Cancer 11: 481–492 doi: 10.1038/nrc3068
40. Kadoch C, Crabtree GR (2015) Mammalian SWI/SNF chromatin remodeling complexes and cancer: Mechanistic insights gained from human genomics. Sci Adv 1: e1500447 doi: 10.1126/sciadv.1500447
41. Wu S, Ge Y (2020) BRM-SWI/SNF chromatin remodeling complex enables functional telomeres by promoting co-expression of TRF2 and TRF1. PLoS Genet 16: e1008799 doi: 10.1371/journal.pgen.1008799
42. Yu EY, Steinberg-Neifach O (2007) Regulation of telomere structure and functions by subunits of the INO80 chromatin remodeling complex. Mol Cell Biol 27: 5639–5649 doi: 10.1128/mcb.00418-07
43. Min JN, Tian Y (2013) The mINO80 chromatin remodeling complex is required for efficient telomere replication and maintenance of genome stability. Cell Res 23: 1396–1413 doi: 10.1038/cr.2013.113
44. Shi G, Hu Y (2020) A critical role of telomere chromatin compaction in ALT tumor cell growth. Nucleic Acids Res 48: 6019–6031 doi: 10.1093/nar/gkaa224
45. Gauchier M, Kan S (2019) SETDB1-dependent heterochromatin stimulates alternative lengthening of telomeres. Sci Adv 5: eaav3673 doi: 10.1126/sciadv.aav3673
46. Wang C, Songyang Z (2021) TRIM28 inhibits alternative lengthening of telomere phenotypes by protecting SETDB1 from degradation. Cell Biosci 11: 149 doi: 10.1186/s13578-021-00660-y
47. Li F, Zhang T (2025) CHAMP1 complex directs heterochromatin assembly and promotes homology-directed DNA repair. Nat Commun 16: 1714 doi: 10.1038/s41467-025-56834-6
48. Udugama M, Hii L (2021) Mutations inhibiting KDM4B drive ALT activation in ATRX-mutated glioblastomas. Nat Commun 12: 2584 doi: 10.1038/s41467-021-22543-z
49. Wang B, Kou H (2024) LAP2α orchestrates alternative lengthening of telomeres suppression through telomeric heterochromatin regulation with HDAC1: unveiling a potential therapeutic target. Cell Death Dis 15: 761 doi: 10.1038/s41419-024-07116-4
50. Hsu RYC, Lin YC (2020) ORCA/LRWD1 Regulates Homologous Recombination at ALT-Telomeres by Modulating Heterochromatin Organization. iScience 23: 101038 doi: 10.1016/j.isci.2020.101038
51. Clatterbuck Soper SF, Meltzer PS (2023) ATRX/DAXX: Guar-

ding the Genome against the Hazards of ALT. Genes (Basel) 14 doi: 10.3390/genes14040790
52. Conomos D, Reddel RR (2014) NuRD-ZNF827 recruitment to telomeres creates a molecular scaffold for homologous recombination. Nat Struct Mol Biol 21: 760–770 doi: 10.1038/nsmb.2877
53. Lee JJ, Kim H (2024) Disruption of G-quadruplex dynamicity by BRCA2 abrogation instigates phase separation and break-induced replication at telomeres. Nucleic Acids Res 52: 5756–5773 doi: 10.1093/nar/gkae251
54. Li F, Wang Y (2023) Histone demethylase KDM2A is a selective vulnerability of cancers relying on alternative telomere maintenance. Nat Commun 14: 1756 doi: 10.1038/s41467-023-37480-2
55. Kim D, Bhargava R (2024) TRIM24 directs replicative stress responses to maintain ALT telomeres via chromatin signaling. bioRxiv doi: 10.1101/2024.10.18.618947
56. Lachner M, O'Carroll D (2001) Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature 410: 116–120 doi: 10.1038/35065132
57. García-Cao M, O'Sullivan R (2004) Epigenetic regulation of telomere length in mammalian cells by the Suv39h1 and Suv39h2 histone methyltransferases. Nat Genet 36: 94–99 doi: 10.1038/ng1278
58. He Q, Kim H (2015) The Daxx/Atrx Complex Protects Tandem Repetitive Elements during DNA Hypomethylation by Promoting H3K9 Trimethylation. Cell Stem Cell 17: 273–286 doi: 10.1016/j.stem.2015.07.022
59. Cao LL, Wei F (2016) ATM-mediated KDM2A phosphorylation is required for the DNA damage repair. Oncogene 35: 301–313 doi: 10.1038/onc.2015.81
60. Mallette FA, Mattiroli F (2012) RNF8- and RNF168-dependent degradation of KDM4A/JMJD2A triggers 53BP1 recruitment to DNA damage sites. Embo J 31: 1865–1878 doi: 10.1038/emboj.2012.47
61. Li F, Deng Z (2019) ATRX loss induces telomere dysfunction and necessitates induction of alternative lengthening of telomeres during human cell immortalization. Embo J 38: e96659 doi: 10.15252/embj.201796659
62. Gabellini D, Pedrotti S (2022) The SUV4-20H Histone Methyltransferases in Health and Disease. Int J Mol Sci 23 doi: 10.3390/ijms23094736
63. Episkopou H, Draskovic I (2014) Alternative Lengthening of Telomeres is characterized by reduced compaction of telomeric chromatin. Nucleic Acids Res 42: 4391–4405 doi: 10.1093/nar/gku114
64. Fukuda K, Shinkai Y (2020) SETDB1-Mediated Silencing of Retroelements. Viruses 12 doi: 10.3390/v12060596
65. Czerwińska P, Mazurek S (2017) The complexity of TRIM28 contribution to cancer. J Biomed Sci 24: 63 doi: 10.1186/s12929-017-0374-4
66. Ivanov AV, Peng H (2007) PHD domain-mediated E3 ligase activity directs intramolecular sumoylation of an adjacent bromodomain required for gene silencing. Mol Cell 28: 823–837 doi: 10.1016/j.molcel.2007.11.012
67. Le R, Huang Y (2021) Dcaf11 activates Zscan4-mediated alternative telomere lengthening in early embryos and embryonic stem cells. Cell Stem Cell 28: 732–747.e739 doi: 10.1016/j.stem.2020.11.018
68. Dhayalan A, Tamas R (2011) The ATRX-ADD domain binds to H3 tail peptides and reads the combined methylation state of K4 and K9. Hum Mol Genet 20: 2195–2203 doi: 10.1093/hmg/ddr107
69. Loyola AC, Zhang L (2019) Identification of methotrexate as a heterochromatin-promoting drug. Sci Rep 9: 11673 doi: 10.1038/s41598-019-48137-w
70. Giri S, Prasanth SG (2015) Association of ORCA/LRWD1 with repressive histone methyl transferases mediates heterochromatin organization. Nucleus 6: 435–441 doi: 10.1080/19491034.2015.1102814
71. Drané P, Ouararhni K (2010) The death-associated protein DAXX is a novel histone chaperone involved in the replication-independent deposition of H3.3. Genes Dev 24: 1253–1265 doi: 10.1101/gad.566910
72. Tsai WW, Wang Z (2010) TRIM24 links a non-canonical histone signature to breast cancer. Nature 468: 927–932 doi: 10.1038/nature09542
73. Zhou JC, Blackledge NP (2012) Recognition of CpG island chromatin by KDM2A requires direct and specific interaction with linker DNA. Mol Cell Biol 32: 479–489 doi: 10.1128/mcb.06332-11
74. Iuchi S, Paulo JA (2019) Lysine-specific demethylase 2A enhances binding of various nuclear factors to CpG-rich genomic DNAs by action of its CXXC-PHD domain. Sci Rep 9: 5496 doi: 10.1038/s41598-019-41896-6
75. Fajkus J, Trifonov EN (2001) Columnar packing of telomeric nucleosomes. Biochem Biophys Res Commun 280: 961–963 doi: 10.1006/bbrc.2000.4208
76. Udroiu I, Sgura A (2019) Alternative Lengthening of Telomeres and Chromatin Status. Genes (Basel) 11 doi: 10.3390/genes11010045
77. Kaminski N, Wondisford AR (2022) RAD51AP1 regulates ALT-HDR through chromatin-directed homeostasis of TERRA. Mol Cell 82: 4001–4017.e4007 doi: 10.1016/j.molcel.2022.09.025
78. Längst G, Manelyte L (2015) Chromatin Remodelers: From Function to Dysfunction. Genes (Basel) 6: 299–324 doi: 10.3390/genes6020299
79. Molenaar C, Wiesmeijer K (2003) Visualizing telomere dynamics in living mammalian cells using PNA probes. Embo J 22: 6631–6641 doi: 10.1093/emboj/cdg633
80. Draskovic I, Arnoult N (2009) Probing PML body function in ALT cells reveals spatiotemporal requirements for telomere recombination. Proc Natl Acad Sci U S A 106: 15726–15731 doi: 10.1073/pnas.0907689106
81. Ferreira HC, Towbin BD (2013) The shelterin protein POT-1 anchors Caenorhabditis elegans telomeres through SUN-1 at the nuclear periphery. J Cell Biol 203: 727–735 doi: 10.1083/jcb.201307181
82. Yang CW, Hsieh MH (2021) Nuclear envelope tethering inhibits the formation of ALT-associated PML bodies in ALT cells. Aging (Albany NY) 13: 10490–10516 doi: 10.18632/aging.202810
83. Jegou T, Chung I (2009) Dynamics of telomeres and promyelocytic leukemia nuclear bodies in a telomerase-negative human cell line. Mol Biol Cell 20: 2070–2082 doi: 10.1091/mbc.e08-02-0108
84. Gao Y, Tan J (2018) SIRT6 facilitates directional telomere movement upon oxidative damage. Sci Rep 8: 5407 doi: 10.1038/s41598-018-23602-0
85. Chojnowski A, Ong PF (2015) Progerin reduces LAP2α-telomere association in Hutchinson-Gilford progeria. Elife 4 doi: 10.7554/eLife.07759
86. Azeroglu B, Khurana S (2025) Identification of modulators of the ALT pathway through a native FISH-based optical screen. Cell Rep 44: 115114 doi: 10.1016/j.celrep.2024.115114
87. Ulianov SV, Velichko AK (2021) Suppression of liquid-liquid phase separation by 1,6-hexanediol partially compromises the 3D genome organization in living cells. Nucleic Acids Res 49: 10524–10541 doi: 10.1093/nar/gkab249