PERSPECTIVE

The Genome's Second Code: Non-B DNA Structures as Evolvability Platforms Linking Adaptation, Cancer, and Ageing

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Abstract

Genomes encode not merely the sequences of genes but also the capacity for their own evolution. Beyond the canonical B-form double helix, DNA adopts alternative conformations—G-quadruplexes, R-loops, Z-DNA, and cruciform structures—that cluster preferentially at regulatory loci controlling proliferation, immunity, and development. These non-B DNA structures function as evolvability platforms: regulatory switches enabling expression changes without destroying protein function, and sites where controlled mutagenesis generates adaptive diversity. Here I propose that this structural layer constitutes a second genomic code—one that specifies where and how evolution can act. Unlike the deterministic genetic code mapping codons to amino acids, this structural code operates probabilistically: structure formation depends on ionic conditions, superhelical tension, and protein binding, and this context-dependence is precisely what enables dynamic regulation. I synthesize evidence demonstrating that non-B DNA structures are enriched at promoters and 5'UTRs of oncogenes, serve as targets for structure-specific enzymes in meiosis and adaptive immunity, and become drivers of pathology when maintenance mechanisms falter. Most strikingly, I identify aging as the progressive dysregulation of these same evolvability platforms—a model supported by direct evidence of R-loop accumulation in aged stem cells and by the accelerated aging phenotypes caused by helicase deficiency. This framework resolves apparent paradoxes in the literature and reveals therapeutic opportunities at the intersection of cancer biology and gerontology.

Introduction

The central dogma of molecular biology established that genetic information flows from DNA sequence to RNA to protein (Crick, 1970). Yet genomes harbor information beyond primary sequence—information encoded in three-dimensional structure that specifies not what genes do, but where and how they can change. This distinction carries profound implications: while coding mutations alter protein function and are often deleterious, regulatory mutations modulate expression levels and can enable phenotypic adaptation without functional destruction (Wray, 2007; Carroll, 2008; Wittkopp & Kalay, 2012).

Recognition that DNA adopts conformations beyond the Watson-Crick double helix dates to the 1970s, when Rich and colleagues first crystallized left-handed Z-DNA (Rich & Zhang, 2003). In the decades since, G-quadruplexes (G4s), R-loops, cruciforms, and triplex structures have been characterized in vitro and—with increasing sophistication—visualized in living cells (Hänsel-Hertsch et al., 2018). What were initially dismissed as structural curiosities or pathological aberrations are now understood to be widespread, evolutionarily

conserved, and functionally significant (Bacolla et al., 2006; Wang & Vasquez, 2006). The development of G4 ChIP-seq has enabled genome-wide mapping of these structures in chromatin, revealing their dynamic regulation across cell types and conditions (Hänsel-Hertsch et al., 2018).

Several observations converge to demand explanation. First, non-B DNA-forming sequences do not scatter randomly across the genome; they cluster at regulatory regions of genes controlling cell proliferation, particularly oncogene promoters (Huppert & Balasubramanian, 2006). Second, structure-specific enzymes—AID, RAG, SPO11, and the APOBEC family—have evolved to recognize DNA topology rather than sequence alone, enabling mutagenesis at structurally defined sites (Nishana & Raghavan, 2012; Qiao et al., 2017). Third, the same structures that enable beneficial diversification cause pathological instability when maintenance mechanisms become overwhelmed (García-Muse & Aguilera, 2019). Fourth, R-loops accumulate in aged hematopoietic stem cells coincident with declining RNA export capacity, directly linking structure dysregulation to tissue aging (Chen et al., 2025). Fifth, diseases of accelerated aging—Werner syndrome, Bloom syndrome, Rothmund-Thomson syndrome—result from mutations in helicases that resolve non-B structures (Ababou, 2021; Lu et al., 2020; Oshima et al., 2016).

These observations point toward a unifying principle: non-B DNA structures constitute evolvability platforms that specify where evolutionary change preferentially occurs. In this Perspective, I develop this framework systematically, demonstrating how the alignment of specific structures with specific genomic domains creates a regulatory architecture for directed variation. I extend this framework to cancer, where evolvability mechanisms become co-opted for malignant adaptation, and to aging, where their progressive dysregulation drives tissue dysfunction. Throughout, I identify therapeutic implications emerging from this unified view.

Section 1: The Structural Evolvability Framework

1.1 A Vocabulary of Non-B DNA Structures

Before examining their genomic distribution and function, we must establish the structural vocabulary. G-quadruplexes form when guanine-rich sequences fold into four-stranded structures stabilized by Hoogsteen hydrogen bonding between planar G-tetrads stacked upon one another. Computational analyses initially identified approximately 376,000 potential G4-forming sequences in the human genome based on the canonical $G_{3+}N_{1-7}G_{3+}N_{1-7}G_{3+}N_{1-7}G_{3+}$ motif (Huppert & Balasubramanian, 2005; Todd et al., 2005); experimental G4-seq has since confirmed formation of more than 700,000 distinct G4 structures in human genomic DNA under conditions that stabilize folding, though their formation in vivo is dynamic and context-dependent (Chambers et al., 2015; Hänsel-Hertsch et al., 2018). Notably, recent mapping has revealed that i-motif structures—four-stranded structures formed by cytosine-rich sequences on the complementary strand—also form in living cells and overlap with G4s, suggesting coordinated regulation (Zanin et al., 2023).

R-loops arise during transcription when nascent RNA hybridizes with template DNA, displacing the non-template strand as a single-stranded bubble. Far from being rare accidents, R-loops can extend over kilobases and are enriched at CpG islands, gene terminators, and immunoglobulin switch regions (Crossley et al., 2019; Petermann et al., 2022). Their formation depends on sequence context—G-rich template strands favor RNA:DNA hybrid stability—but also on transcription rate, superhelical tension, and the availability of RNA processing factors. Z-DNA adopts a left-handed helix at alternating purine-pyrimidine

sequences under conditions of negative supercoiling; computational estimates suggest approximately 100,000 Z-DNA-forming sequences occur in the human genome, with enrichment near transcription start sites of actively expressed genes, though direct genome-wide experimental mapping remains technically challenging (Rich & Zhang, 2003). Cruciform structures form at inverted repeat sequences that can extrude into four-way junctions during replication or under torsional stress (Wang & Vasquez, 2006).

Each structure presents distinct biochemical properties that define its regulatory potential—and its vulnerability. G4s expose guanines to oxidative modification and block replicative and transcriptional polymerases, requiring specialized helicases for resolution (Teng et al., 2021). R-loops expose single-stranded DNA to cytidine deaminases while creating barriers to transcription-replication progression (Hamperl et al., 2017). Z-DNA recruits ADAR enzymes for A-to-I RNA editing and activates innate immune sensors such as ZBP1 (Herbert, 2019). Cruciforms present hairpin arms that serve as substrates for structure-specific nucleases (Brázda et al., 2011; Lilley, 2017). These properties determine how each structure interfaces with cellular machinery—and what pathological consequences follow when that interface fails.

A critical feature of this structural vocabulary is its context-dependence (Figure 1). Unlike the genetic code—where AUG deterministically specifies methionine regardless of cellular conditions—non-B DNA structure formation is probabilistic and regulatable. G4 folding depends on potassium concentration, molecular crowding (Miyoshi & Sugimoto, 2008), and the availability of G4-binding proteins (Lane et al., 2008; Biffi et al., 2013; Spiegel et al., 2020); R-loop persistence depends on transcription rate and RNA processing efficiency; Z-DNA formation requires negative superhelical tension generated by transcription. This conditional nature is not a weakness of the structural code but rather the source of its regulatory power: cells can modulate structure formation in response to developmental signals, environmental conditions, and metabolic states.

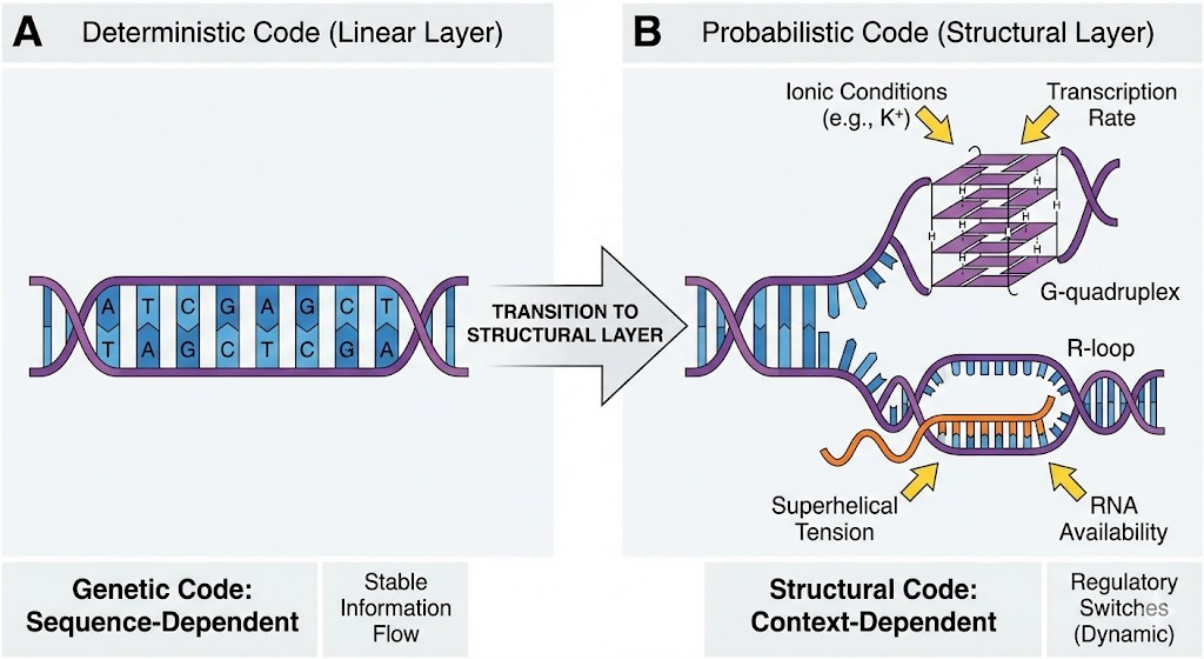

Figure 1: Describes the dual coding systems (deterministic genetic code vs. probabilistic structural code) and the factors modulating structure formation

1.2 Domain-Specific Distribution: Structure Meets Function

The fundamental observation motivating this framework is that non-B DNA structures are not randomly distributed (Figure 2). In a landmark computational analysis, Huppert and Balasubramanian (2006) demonstrated that promoter regions show striking enrichment of G4 motifs: more than 40% of human gene promoters contain at least one computationally predicted G4-forming sequence—a proportion far exceeding what chance would predict. Even more remarkable, regions that are both nuclease-hypersensitive and within promoters exhibit a 230-fold enrichment compared to the genome average. Experimental validation using G4-seq and G4 ChIP-seq has confirmed that a substantial fraction of these predicted structures do form in cells, though the precise overlap between computational prediction and in vivo formation varies with cell type and conditions (Chambers et al., 2015; Hänsel-Hertsch et al., 2018). Recent advances in strand-specific G4 sequencing (ssG4-seq) have further refined our understanding, revealing that over 95% of G4s localize to enhancers and promoters across mammalian species, with promoters containing dual-strand G4s exhibiting significantly stronger transcriptional activation than those with single-strand G4s (Li et al., 2025). This is not statistical noise; it reflects functional selection operating over evolutionary time.

The enrichment pattern extends to specific gene classes in ways that illuminate function. Analysis of the Non-B DNA database reveals clustering of G4-forming sequences in promoters of genes controlling cellular proliferation, including MYC, KRAS, KIT, HSP90, and VEGF (Francisco & Paulo, 2017). Machine learning approaches have further demonstrated that G4 proximal enhancer-promoter pairs correlate with gene expression in a tissue-specific manner, with some pairings acting as developmental switches (Konovalov et al., 2025). Rezzoug et al. (2016) identified a family of seventeen G4 motifs sharing greater than 90% sequence identity with the canonical MYC promoter G4, distributed across different chromosomes and associated with genes involved in stem cell maintenance and neural development. This family structure—conserved G4 regulatory elements controlling functionally related genes—suggests that G4-mediated regulation represents an ancient architectural motif for coordinating proliferation programs.

The distribution extends beyond promoters in revealing ways. Cayrou et al. (2015) characterized replication origins genome-wide in mouse embryonic stem cells at unprecedented resolution, distinguishing initiation sites from broader initiation zones. Their analysis confirmed the presence of Origin G-rich Repeated Elements (OGREs) at most origins, coinciding with nucleosome-depleted regions upstream of initiation sites. Origins could be grouped into three classes: Class 1 origins, relatively isolated and low-efficiency, enriched for asymmetric AC repeats; Class 2 origins, particularly rich in enhancer elements; and Class 3 origins, the most efficient, associated with open chromatin and polycomb-enriched regions. Each class displayed distinct G4 patterns, suggesting that the chromatin environment shapes how G4 structures contribute to replication timing.

In the 5'UTR, G4s operate as translational switches. Bugaut and Balasubramanian (2012) established that RNA G4s influence translation of clinically relevant genes, with effects depending critically on position. Kumari et al. (2008) demonstrated this position-dependence directly: a G4-forming sequence in the NRAS 5'UTR repressed translation when situated

within the first 50 nucleotides from the cap, but had no significant effect when located more distally. This positional specificity indicates that G4s function not as simple roadblocks but as context-sensitive regulatory elements whose effects depend on where ribosomes encounter them.

R-loops display their own domain specificity. They are enriched at CpG islands, gene terminators, and—most dramatically—immunoglobulin switch regions, where they can extend beyond one kilobase in length (Niehrs & Luke, 2020; Yu et al., 2003). This distribution reflects both the sequence requirements for R-loop formation (G-rich template strands) and the functional contexts in which R-loops serve regulatory purposes. At switch regions, R-loop formation exposes the displaced G-rich strand for AID-mediated deamination, initiating the double-strand breaks required for class switch recombination. The precision of this targeting—enabling somatic hypermutation within switch regions while protecting flanking sequences—illustrates how structure specifies the boundaries of beneficial mutagenesis.

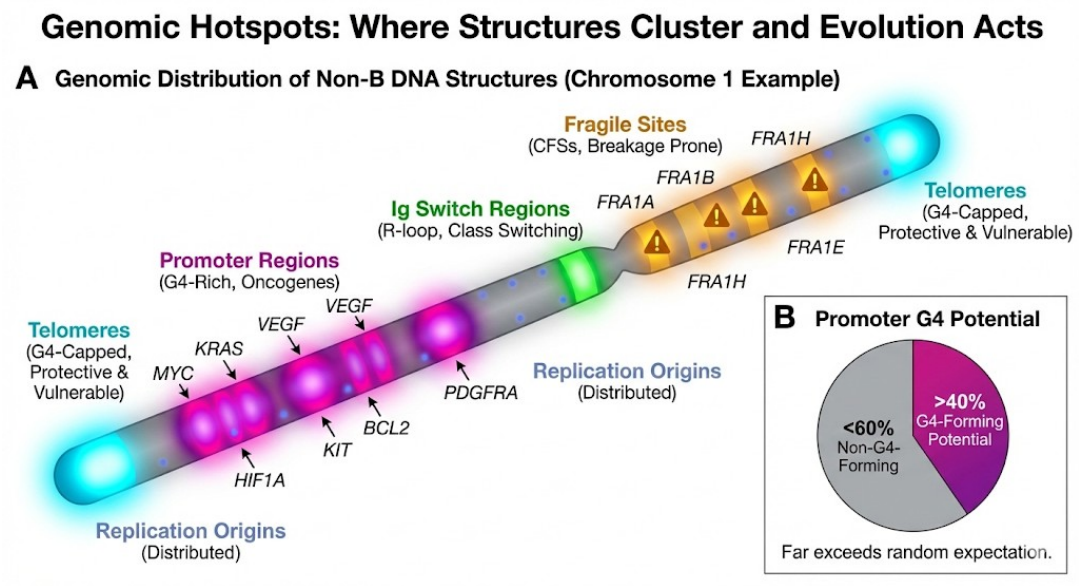

Figure 2: Explains the non-random genomic distribution with specific examples (MYC, KRAS, VEGF promoters; telomeres; switch regions; fragile sites) and the >40% promoter statistic

1.3 The Alignment Principle: Matching Structure to Regulatory Need

The patterns described above suggest a design principle that I term the alignment principle: specific non-B DNA structures are matched to specific regulatory domains based on their biochemical properties and the regulatory requirements of those domains (Table 1). It should be emphasized that this alignment likely arose through natural selection operating on regulatory architectures over evolutionary time (Wray, 2007; Stern & Orgogozo, 2008)—sequences that happened to form beneficial structures at functionally appropriate locations were retained, while those causing deleterious effects were eliminated. The result is a genome that appears "designed" for evolvability but actually reflects the accumulated outcome of selection for regulatory flexibility.

At promoters, G4 structures provide transcriptional switches whose operation depends on structure rather than sequence per se. The MYC promoter illustrates this mechanism with

particular clarity. The nuclease-hypersensitive element III (NHE III) forms a G4 structure that recruits transcription factors and histone modifiers (Esain-Garcia et al., 2024). What makes this system remarkable was revealed by Esain-Garcia et al. (2024), who used CRISPR-mediated ablation of the endogenous MYC G4 to dissect its function. When they replaced the native G4-forming sequence with a different sequence encoding the KRAS G4, transcription was restored—demonstrating that the structure itself, not the underlying sequence, constitutes the functional element. G4 loss triggered de novo nucleosome deposition and reduced RNA polymerase recruitment, establishing that the G4 maintains an open chromatin state permissive for transcription. The promoter reads the structure, not the sequence.

At 5'UTRs, RNA G4s provide translational switches that modulate protein output in response to cellular conditions. Cancer cells, with their elevated demands for oncogenic proteins, become particularly dependent on helicases that resolve 5'UTR G4s. The eIF4A helicase-dependent translatome is highly enriched for mRNAs with G/C-rich 5'UTRs capable of forming G4s, including transcripts encoding G-protein constituents, cyclins, and protein kinases (Modelska et al., 2015). This creates a therapeutic vulnerability: inhibiting eIF4A preferentially blocks translation of G4-containing oncogenic mRNAs while sparing normal transcripts.

At replication origins, G4 structures contribute to the specification of initiation timing and efficiency through mechanisms that remain incompletely understood but clearly involve chromatin accessibility. The three origin classes identified by Cayrou et al. (2015)—each with distinct G4 signatures—suggest that G4s participate in establishing the chromatin landscape that determines when and how efficiently origins fire.

At immunoglobulin switch regions, R-loops combine with G4 formation to create the substrate for controlled mutagenesis. Transcription through the switch region generates an R-loop that persists because of the G-rich nature of the template strand; the displaced non-template strand, also G-rich, forms G4 structures that further stabilize the exposed configuration (Refaat et al., 2023; Yu & Lieber, 2019). HNRNPU binds these RNA-DNA G4 structures, regulating the accumulation of R-loops and single-stranded DNA that serve as AID substrates. The result is a precisely targeted mutational mechanism: AID accesses its substrate within switch regions while the rest of the genome remains protected.

This alignment of structure with function is not merely correlative—disrupting the alignment causes disease. When the wrong structure forms at the wrong location, or when structures persist beyond their regulatory purpose, pathology results. The R-loops that enable class switch recombination cause genome instability when they accumulate at non-immunoglobulin loci (García-Muse & Aguilera, 2019). This dual nature—regulatory when controlled, pathological when dysregulated—is central to understanding both cancer and aging.

Table 1. Alignment of Non-B DNA Structures with Genomic Domains

Structure	Primary Genomic Location	Regulatory Function	Key Biochemical Properties	Cancer Exploitation	Aging Vulnerability
G-quadruplex	Promoters (>40% of genes, computational	Transcriptional switching, translation control,	Blocks polymerases, recruits structure-	TERT promoter mutations destabilize repressive G4s;	Helicase insufficiency → persistent G4s →

Structure	Primary Genomic Location	Regulatory Function	Key Biochemical Properties	Cancer Exploitation	Aging Vulnerability
	prediction), 5'UTRs, telomeres, replication origins	telomere capping, origin specification	specific factors, excludes nucleosomes, presents oxidation-vulnerable guanines	oncogene promoter G4 destabilization increases expression	replication stress; 8-oxoG accumulation destabilizes telomeric G4s → accelerated shortening
R-loop	Switch regions, CpG islands, gene terminators, long genes	Class switch recombination, transcription termination, chromatin modification	Exposes ssDNA for deaminases, creates transcription-replication collision sites, activates DNA damage signaling	Off-target AID activity at non-Ig R-loops → lymphomagenesis; transcription-replication conflicts → genome instability	Alyref decline → RNA retention → R-loop accumulation → HSC exhaustion; MCM8 dysfunction → reproductive aging
Cruciform	Inverted repeats, fragile sites, stressed replication forks	Replication stress response, fragile site marking	Exposes ssDNA hairpin arms, creates nucleosome-depleted regions, serves as nuclease substrate	APOBEC targeting of hairpin ssDNA → kataegis at fragile sites (limited direct evidence for cruciform-specific targeting)	Fragile site expression increases with age → chromosomal instability
Z-DNA	Alternating purine-pyrimidine sequences, active gene promoters	ADAR-mediated A-to-I editing, innate immune activation	Left-handed helix recognized by ZBP1, serves as ADAR substrate	Aberrant editing may enable immune evasion (emerging area with limited direct evidence)	Dysregulated editing → potential autoimmunity; ZBP1 activation → inflammation (mechanistic links less established than for G4/R-loop)
Trinucleotide repeats	5'UTRs, promoters, introns of disease genes	Tunable expression via length polymorphism	Hairpin formation enables replication slippage; R-loops form at expanded	Repeat expansion → oncogene dysregulation in specific contexts	Repeat expansion diseases show age-dependent toxicity due to chaperone network

Structure	Primary Genomic Location	Regulatory Function	Key Biochemical Properties	Cancer Exploitation	Aging Vulnerability
			repeats		remodeling

Section 2: Evolvability Platforms in Action

The same structural features that cluster at regulatory regions serve as substrates for enzymes that generate beneficial genetic diversity (Figure 3). This section examines three paradigmatic examples: meiotic recombination, adaptive immunity, and cancer mutagenesis.

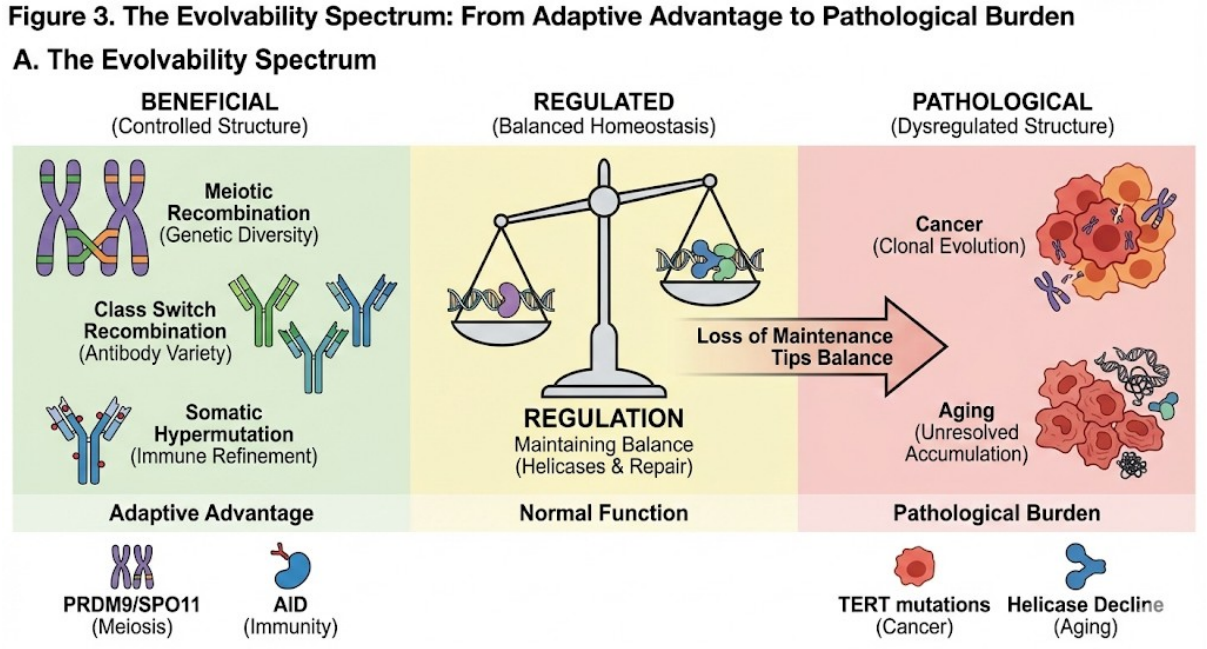

Figure 3: Presents the evolvability spectrum from beneficial (meiosis, immunity) through regulated homeostasis to pathological (cancer, aging), with regulation as the determining factor

2.1 Meiotic Recombination: PRDM9 and the Architecture of Directed Variation

Meiotic recombination offers the paradigm for understanding how DNA structure specifies mutational targeting. The process is not random; recombination concentrates at hotspots whose positions are determined by the zinc-finger protein PRDM9. This protein binds specific DNA sequences through its variable zinc finger array and deposits H3K4me3 and H3K36me3 histone marks that attract SPO11, the topoisomerase that creates the programmed double-strand breaks initiating meiotic recombination (Davies et al., 2016).

But PRDM9 does more than mark sequences—it creates a chromatin architecture conducive to recombination. Lange et al. (2016) revealed this architecture by mapping double-strand breaks at nucleotide resolution: DSBs occur within narrow zones nestled between methylated nucleosomes, in regions of open chromatin accessible to the recombination machinery. The stereotyped hotspot structure—PRDM9 binding sites flanked by positioned nucleosomes with a central nucleosome-depleted zone—creates a physical environment optimized for controlled DNA breakage and repair. Tock and Henderson (2018) showed that in plants and other species lacking PRDM9, DSB hotspots similarly localize to nucleosome-depleted regions associated with regulatory elements and transcription factor binding. The conserved principle is that chromatin structure—not merely sequence—determines where meiotic recombination occurs.

The evolutionary significance of this targeting extends far beyond individual meioses. PRDM9 is the only mammalian speciation gene yet identified; its rapid evolution drives changes in recombination landscape that contribute to reproductive isolation between incipient species (Davies et al., 2016). The genome does not merely experience recombination passively—it directs recombination to specific sites through structural encoding, and changes in that encoding can drive speciation.

When this exquisitely regulated system becomes dysregulated, cancer can result. Houle et al. (2018) made the unexpected discovery that PRDM9 is aberrantly expressed in approximately 20% of tumors. In these cancers, the meiotic recombination machinery activates at somatic sites, generating genome instability. Structural variant breakpoints in PRDM9-expressing tumors cluster at known meiotic hotspots—the same sites that normally experience controlled recombination during gametogenesis. The machinery evolved to generate beneficial germline diversity becomes, when activated in somatic cells, an engine of malignant genome evolution.

2.2 Adaptive Immunity: G4 Recognition by AID

The adaptive immune system deploys structure-specific enzymes to generate antibody diversity through controlled mutagenesis. Activation-induced deaminase (AID) mediates both class switch recombination—changing antibody isotype from IgM to IgG, IgA, or IgE—and somatic hypermutation—the iterative mutation and selection that increases antibody affinity. For decades, the mechanism by which AID targets switch regions while sparing most of the genome remained mysterious. The answer involves structure.

Qiao et al. (2017) demonstrated that G4-containing substrates mimicking immunoglobulin switch regions are superior AID substrates compared to unstructured single-stranded DNA. Their structural analysis revealed why: AID possesses a bifurcated substrate-binding surface that simultaneously captures two single-stranded overhangs. This geometry corresponds precisely to what G4 structures present—loops of single-stranded DNA connecting the G-tetrads. The enzyme has evolved to recognize a structural signature, not a sequence.

The functional importance of this G4 recognition was established definitively by Yewdell et al. (2020), who identified a Hyper-IgM syndrome mutation that specifically disrupts AID's ability to bind G4s while leaving its catalytic activity intact. Mice bearing this mutation showed a striking phenotype: they completely lacked class switch recombination and somatic hypermutation. The mutation didn't destroy AID's enzymatic activity—it destroyed its ability to find its natural substrates. G4 recognition is not an incidental property of AID; it is essential for adaptive immunity.

R-loops complement G4s in creating the mutagenic substrate at switch regions. Transcription through the highly repetitive, G-rich switch sequences generates persistent R-loops—the RNA:DNA hybrid is stabilized by the G-richness of both the RNA and the template strand (Yu et al., 2003). Meanwhile, the displaced non-template strand, being C-poor, is susceptible to forming G4 structures. HNRNPU binds these switch region G4s and modulates R-loop accumulation, fine-tuning the availability of AID substrate (Refaat et al., 2023).

The interconversion between G4 and R-loop structures is actively regulated. Ribeiro de Almeida et al. (2018) discovered that the DEAD-box helicase DDX1 binds G4 structures in intronic switch transcripts and converts them into S-region R-loops, thereby targeting AID to switch regions. Chemical stabilization of G4-RNA diminished R-loop levels over switch regions, confirming that G4 structures serve as intermediates in the AID-targeting pathway. This reveals G4s not as passive damage but as programmable targeting devices that direct enzymatic modification to specific loci. The coordination of multiple non-B structures creates a sophisticated targeting mechanism that enables the precise, localized mutagenesis required for class switching.

Tang and MacCarthy (2021) extended this principle to variable region genes, showing that many IGHV3 and IGHV4 family genes—targets of somatic hypermutation—contain sequences predicted to form G4 structures. G4 potential in variable regions correlates with higher mutability of WRC hotspots on the same strand, suggesting that G4 formation contributes to the targeting of somatic hypermutation beyond the switch regions.

2.3 V(D)J Recombination and RAG

Similar principles operate in V(D)J recombination, the process that assembles antigen receptor genes from dispersed gene segments. The RAG1/RAG2 recombinase cleaves at recombination signal sequences (RSS) flanking V, D, and J segments, enabling their joining into functional receptor genes. Nishana and Raghavan (2012) made a surprising discovery: non-B DNA structures can substitute for the RSS heptamer when present alongside a nonamer sequence. Moreover, the chromosomal fragile region involved in BCL2 translocations—a driver of follicular lymphoma—is susceptible to RAG cleavage through a nonamer-dependent mechanism.

This finding explained a long-standing puzzle: how do RAGs, designed for controlled diversification at immunoglobulin and T cell receptor loci, generate the chromosomal translocations that cause lymphoid malignancies? The answer is that RAGs recognize structure as well as sequence. Nilavar et al. (2020) identified the specific domain responsible—the ZnC2 module in RAG1's central domain is required for binding to non-B DNA structures. When non-B DNA forms at chromosomal locations outside the intended receptor loci, RAGs can cleave there, generating the translocations that initiate lymphomagenesis.

2.4 Cancer Mutagenesis: APOBEC and Replication Stress

While AID expression is restricted to B cells and RAGs to lymphoid development, the APOBEC family of cytidine deaminases provides a structure-dependent mutagenesis mechanism operative across many cancer types. These enzymes evolved for antiviral defense—deaminating cytosines in retroviral replication intermediates—but in cancer cells they become agents of somatic mutation. Their substrate is single-stranded DNA, a structure that becomes abundant at stressed replication forks and within non-B DNA conformations.

The mechanistic link between structure and mutation deserves explicit statement: non-B DNA structures create the single-stranded DNA substrate that deaminases require. G4

formation on one strand necessarily leaves the complementary strand single-stranded. R-loops expose the displaced non-template strand. Cruciform arms present single-stranded hairpin loops. Replication fork stalling at these structures extends the window during which single-stranded regions remain accessible. Structure does not merely correlate with mutation—it generates the biochemical substrate on which mutagenic enzymes act.

The relationship between APOBEC mutagenesis and replication stress has been illuminated by several key studies. Hoopes et al. (2016) demonstrated that APOBEC3A and APOBEC3B preferentially deaminate the lagging strand template during DNA replication—the strand that transiently exists as single-stranded DNA awaiting Okazaki fragment synthesis. Seplyarskiy et al. (2016) quantified this strand bias: more than 33% of dispersed APOBEC-induced mutations in human cancers occur on the lagging strand, reflecting the extended single-stranded DNA exposure inherent to discontinuous synthesis. When replication forks stall, this single-stranded region expands, providing extended substrate for APOBEC action. The processivity of APOBEC enzymes—their tendency to deaminate multiple cytosines before dissociating—means that stalled forks experience concentrated mutagenesis. Buisson et al. (2017) demonstrated that APOBEC3A binding to single-stranded DNA at replication forks triggers replication stress and double-strand breaks, creating a feed-forward loop: APOBEC activity generates damage that further stalls replication, exposing more substrate for APOBEC action.

Mertz et al. (2017) showed that transcription-associated mutagenesis by APOBEC is potentiated at collision sites between transcription and replication machineries, where R-loops and exposed single-stranded DNA accumulate. The genomic regions most susceptible to these collisions are those with high transcription rates and late replication timing—regions enriched for structure-forming sequences. Petljak et al. (2022) characterized APOBEC3A and APOBEC3B activity in cancer cells, finding that APOBEC3A generates the majority of mutations and operates during S-phase at replication forks, while APOBEC3B contributes fewer mutations but can act outside of replication. Both enzymes target single-stranded DNA, but their distinct expression patterns and kinetics create different mutational signatures.

This structure-dependence explains the distinctive mutation patterns—termed kataegis, from the Greek for thunderstorm—observed in cancer genomes. Lada et al. (2012) provided direct evidence linking APOBEC activity to kataegis by expressing a hypermutagenic sea lamprey cytidine deaminase in yeast: the resulting mutation distribution showed localized clusters strikingly similar to those found in human tumors. When a replication fork stalls at a structure-forming sequence, processive APOBEC action generates hypermutation in the exposed single-stranded region (Rebhandl et al., 2015). The non-B DNA structure determines not just whether APOBEC acts but precisely where the resulting mutations cluster.

The prevalence of APOBEC mutagenesis across cancer types underscores its importance. Roberts et al. (2013) identified APOBEC signatures in breast, head and neck, lung, bladder, and cervical cancers, among others. Alexandrov et al. (2020), in the most comprehensive analysis to date, found APOBEC signatures among the most common mutational processes across the cancer spectrum. In some tumors, APOBEC-mediated mutations constitute the dominant source of genetic diversity—the fuel for clonal evolution and therapeutic resistance.

2.5 A Common Principle Emerges

These examples—meiotic recombination, class switch recombination, V(D)J recombination, and APOBEC mutagenesis—reveal a common principle: DNA structure functions as an information substrate specifying where mutagenesis occurs. This creates a dual information layer in the genome. Primary sequence encodes proteins; structural potential encodes

evolvability—the map of sites where genetic change can occur without destroying essential functions (Kirschner & Gerhart, 1998; Wagner, 2008).

This framework resolves what otherwise appear as paradoxes. Why do non-B DNA structures persist evolutionarily despite their association with genome instability (Maizels & Gray, 2013; Zhao et al., 2010)? Because they provide regulated evolvability essential for adaptation—in meiosis, in immunity, in the fine-tuning of gene expression. Why do cancer mutations cluster at regulatory regions? Because these regions contain the evolvability platforms that enable expression changes without functional destruction. Why do the same structural mechanisms underlie beneficial diversity and pathological instability? Because the distinction lies not in the structures themselves but in their regulation. Controlled formation enables adaptation; uncontrolled accumulation drives disease.

Section 3: When Evolvability Becomes Pathology

3.1 Cancer: Evolvability Co-opted

Cancer evolution exploits the same evolvability platforms that enable normal adaptation—representing the pathological end of the evolvability spectrum (Figure 3). Nowhere is this more evident than at the TERT promoter, where mutations C228T and C250T constitute the most common non-coding driver mutations across human cancers (Tornesello et al., 2023). These mutations occur within G4-forming regions and exert their oncogenic effects through structure modulation.

The normal TERT promoter contains a G4 structure that recruits repressor complexes, maintaining telomerase silencing in somatic cells. Saha et al. (2017) demonstrated that this G4 is essential for occupancy of NME2 and the REST repressor complex on the TERT promoter. The recurrent cancer mutations destabilize this repressive structure while simultaneously creating novel ETS transcription factor binding sites—the C→T transition converts the sequence CCGGAA to the ETS consensus motif TTGGAA. The result is a regulatory switch flip: repression converts to activation, telomerase reawakens, and cells acquire unlimited replicative capacity.

Why do these two positions—and only these two—serve as universal oncogenic hotspots? The answer lies at the intersection of three constraints (Heidenreich & Kumar, 2017). First, ssDNA exposure: G4 folding must expose these specific cytosines to mutagenic enzymes. Second, mutator enzyme specificity: both positions occur in TpC dinucleotide context, the preferred substrate for APOBEC cytidine deaminases (McDaniel et al., 2020). Third, functional consequence: the C→T transition at these positions—and essentially no others in the promoter—creates the GGAA motif recognized by ETS transcription factors, specifically recruiting the GABP complex to activate telomerase expression (Mancini et al., 2018). The TERT promoter hotspots exist precisely where all three constraints align: positions where structure exposes substrate, mutators preferentially act, and single-base changes flip regulatory state. This represents structural logic encoded in DNA sequence—programmed positions where mutations can create oncogenic function.

This mechanism—structure-mediated regulatory switching—operates throughout cancer genomes. G4 ligands that stabilize the MYC promoter G4 suppress MYC transcription, demonstrating that structure controls this oncogene's expression (Esain-Garcia et al., 2024). Conversely, mutations destabilizing promoter G4s unleash oncogene expression. The PCAWG Consortium (2020), analyzing whole-genome sequences across cancer types, found that cancer genomes contain on average 4-5 driver mutations when combining coding and

non-coding elements. Approximately 5% of cases showed no identified coding drivers—suggesting that non-coding, potentially structure-mediated mechanisms drive these cancers (Rheinbay et al., 2020).

The 5'UTR provides another theater for structure-mediated oncogenesis. Zeraati et al. (2017) systematically identified cancer mutations within or near 5'UTR G4s, finding 217 mutations with 33 predicted to destabilize and 21 to stabilize these structures. Functional validation confirmed the predictions: destabilizing mutations increased translation of encoded proteins, while stabilizing mutations decreased translation. For BCL2—the anti-apoptotic protein whose overexpression promotes cancer cell survival—G4-destabilizing mutations increased protein output, conferring survival advantage.

Microsatellite instability (MSI) cancers illustrate evolvability platform exploitation in a different register. van Wietmarschen et al. (2020) discovered that MSI cancers harbor massive expansions of TA-dinucleotide repeats that form non-B DNA structures capable of stalling replication forks. These cancers become dependent on WRN helicase for survival: WRN unwinds the expanded repeats, preventing fork collapse and the chromosome shattering that would otherwise kill the cell. This dependence creates a therapeutic vulnerability—WRN inhibition is synthetic lethal specifically in MSI cancers, precisely because of their acquired dependence on non-B DNA processing.

3.2 Translocation Breakpoints: Predetermined by G4 Landscape

The chromosomal translocations that define many cancers are not random—their breakpoints are predetermined by the genomic distribution of non-B DNA structures. This principle has been established through detailed analysis of translocation breakpoints across lymphoid malignancies.

In Burkitt lymphoma, the t(8;14) translocation juxtaposes MYC with the immunoglobulin heavy chain locus. Kumari et al. (2023) demonstrated that the MYC breakpoint region forms R-loops in a transcription-dependent manner, with the non-template strand folding into stable parallel G-quadruplexes. R-loop and G4 formation proved mutually exclusive conformations of the same sequence, with AID capable of binding to single-stranded regions in either structure. The mechanism proceeds through a defined pathway: MYC transcription generates R-loops; the displaced strand adopts G4 conformation; AID targets the exposed single-stranded DNA; cytosine deamination creates uracil; base excision repair generates abasic sites; and unprocessed abasic sites convert to double-strand breaks that can join with DSBs at immunoglobulin loci.

The pattern extends to other lymphoid translocations. In T-cell acute lymphoblastic leukemia, the t(10;14) translocation activates HOX11. Nambiar et al. (2013) identified two G4-forming motifs at the HOX11 breakpoint cluster, demonstrating that these structures block both transcription and replication. In follicular lymphoma, the t(14;18) translocation deregulates BCL2. Javadekar et al. (2017) characterized the BCL2 major breakpoint region as forming multiple non-B DNA structures including G4s, triplexes, and cruciforms—a convergence of structure-forming potential that explains the region's fragility (Table 2).

The implications extend beyond individual translocations to a general principle: the mutation and translocation spectrum of any cancer is shaped by which G4 structures are accessible—determined by chromatin state and lineage (Hänsel-Hertsch et al., 2016)—which mutator enzymes are expressed, and which genomic positions allow functional mutations. Beyond translocations, copy number variation breakpoints are similarly enriched in G4-forming sequences and tandem repeats (Bose et al., 2014). In B-cell malignancies, AID mediates

translocations through both RAG-dependent mechanisms in precursor cells and direct activity in mature B cells (Nadeu et al., 2020). This is not random mutagenesis—it is programmed mutagenesis at genomic positions where structure exposes substrate and single changes create oncogenic function.

Table 2. Chromosomal Translocations Mediated by Non-B DNA Structures

Translocation	Cancer	Breakpoint Structure	Reference
t(8;14) MYC/IgH	Burkitt lymphoma	R-loop + G4	Kumari et al., 2023
t(10;14) HOX11/TCR	T-ALL	G4 motifs	Nambiar et al., 2013
t(14;18) BCL2/IgH	Follicular lymphoma	Cruciform + G4 + triplex	Javadekar et al., 2017
IgH switch regions	Various B-cell	G4-RNA → R-loop	Ribeiro de Almeida et al., 2018

3.3 Neurodegeneration: When Evolvability Platforms Overwhelm Cellular Defenses

If cancer represents evolvability co-opted, neurodegeneration reveals what happens when evolvability mechanisms exceed cellular capacity for control. Trinucleotide repeat expansion diseases—Huntington's disease, fragile X syndrome, myotonic dystrophy, the spinocerebellar ataxias—arise from unstable repetitive sequences that expand beyond functional thresholds (Koshy & Zoghbi, 1997).

The repeat sequences form non-B DNA structures that drive both the expansion process and the resulting toxicity. CAG repeats form hairpin structures that cause replication slippage during DNA synthesis; R-loops at expanded repeats trigger additional instability through mechanisms involving transcription-coupled repair (Wojciechowska & Krzyzosiak, 2011). The same structural features that enable tunable gene expression through length polymorphism—a form of evolvability—become pathological when expansion exceeds the cell's capacity to manage the resulting structures.

Toxicity in these disorders shows striking age-dependence, pointing to the intersection of evolvability and aging. Lee and Kim (2006) documented this pattern in Huntington's disease: CAG-induced neurodegeneration manifests with characteristic age-dependent progression, the age of onset correlating inversely with repeat length. Thiruvalluvan et al. (2020) uncovered a mechanism linking this age-dependence to changes in protein homeostasis during development. They found that polyglutamine aggregation occurs in differentiated neurons but not in neural progenitors. The difference lies in chaperone networks: during neuronal differentiation, the chaperone landscape undergoes drastic rewiring, including loss of DNAJB6, an anti-amyloidogenic chaperone that suppresses polyglutamine aggregation. Neural progenitors possess intrinsic protection that neurons lack.

New repeat expansion diseases continue to emerge, expanding the landscape of structure-mediated neurodegeneration. Boivin et al. (2021) identified GGC repeat expansion in NOTCH2NLC as causing neuronal intranuclear inclusion disease. The repeats lie within an upstream open reading frame and are translated into polyglycine-containing proteins that accumulate in intranuclear inclusions, causing locomotor deterioration, neuronal loss, and premature death. This defines a new disease class—"polyG diseases"—distinct from polyglutamine disorders but sharing the common thread of structural instability in repetitive sequences.

Perhaps most intriguing is the emerging connection between repeat expansion mechanisms and common neurodegenerative diseases. Suh et al. (2019) demonstrated that ATXN1—the spinocerebellar ataxia type 1 gene containing CAG repeats—modulates Alzheimer's disease risk. Ataxin-1 normally promotes CIC-mediated repression of BACE1, the β-secretase that initiates amyloidogenic processing of APP. Loss of ataxin-1 function elevates BACE1 and increases amyloid-β pathology; polyglutamine-expanded ataxin-1 also elevates BACE1 through a post-transcriptional mechanism. The repeat expansion system thus interfaces directly with the amyloid pathway driving Alzheimer's disease.

Section 4: The Aging Connection

4.1 Direct Evidence: R-Loop Accumulation in Aged Stem Cells

Before considering indirect evidence from progeroid syndromes, we begin with direct observation of structure dysregulation during normal aging. Chen et al. (2025) documented age-related R-loop accumulation in hematopoietic stem cells (HSCs), the cells responsible for lifelong blood production. Using DRIP-seq and immunofluorescence, they found that R-loops accumulate in aged HSCs coincident with DNA damage markers γH2AX and RPA. Critically, they traced the mechanism to declining expression of Alyref, a component of the TREX complex required for nuclear RNA export. As Alyref levels fall with age, RNA accumulates in the nucleus, hybridizing with DNA to form R-loops. These persistent R-loops create replication barriers and activate DNA damage responses.

The therapeutic implication was immediate and provides causal evidence: boosting Alyref expression in aged HSCs restored RNA transport, reduced R-loop burden, and rejuvenated cellular function. This intervention demonstrates that R-loop accumulation is not merely correlated with aging but contributes causally to stem cell dysfunction—and that the process is at least partially reversible.

Supporting this mechanism, Pan et al. (2025) demonstrated that the nuclear exosome targeting (NEXT) complex plays a pivotal role in protecting HSCs from R-loop-induced damage. Deletion of ZCCHC8, a core NEXT subunit, led to impaired HSC self-renewal and elevated DNA lesions due to accumulated R-loops from unprocessed nascent transcripts. Notably, ZCCHC8 dysregulation occurs frequently in diffuse large B cell lymphoma, linking R-loop dysregulation to both aging and malignancy.

Reproductive aging follows a parallel path. Wen et al. (2024) showed that MCM8, a helicase critical for DNA replication and repair, partners with DDX5 and DHX9 to resolve R-loops in developing germ cells. Mutations in MCM8 causing premature ovarian insufficiency disrupt DDX5 interaction, leading to R-loop accumulation, genome instability, and impaired primordial germ cell development. The depletion of the reproductive reserve—a defining feature of reproductive aging—connects directly to failed R-loop resolution.

4.2 Helicase Deficiency Syndromes: A Convergent Mechanism

The progeroid syndromes caused by helicase mutations provide complementary evidence that non-B DNA structure processing is essential for longevity. These syndromes are complex, involving multiple mechanisms including replication stress, DNA damage response activation, and telomere dysfunction. This framework proposes that defective structure resolution represents a convergent mechanism contributing to—though not solely causing—the accelerated aging phenotype.

Consider Werner syndrome, caused by biallelic mutations in WRN, a member of the RecQ helicase family. Patients present with accelerated features of aging—atherosclerosis, osteoporosis, cataracts, type 2 diabetes, and shortened lifespan—alongside elevated cancer risk (Oshima et al., 2016). WRN resolves G4 structures and processes stalled replication forks; in its absence, replication stress accumulates specifically at G4-forming sequences. The mechanism involves coordinated protein complexes: Wu et al. (2018) demonstrated that HERC2, an E3 ubiquitin ligase, is critical for BLM and WRN helicase function in G4 suppression. HERC2 facilitates interaction between the helicases and RPA, releasing RPA onto single-stranded DNA to enable G4 resolution. HERC2 depletion or catalytic inactivation increases G4 formation and sensitizes cells to G4-stabilizing compounds—notably, HERC2 expression is frequently reduced in many cancers, potentially explaining their vulnerability to G4-targeting therapies. The helicase-aging connection extends throughout the RecQ family: BLM mutations cause Bloom syndrome with cancer predisposition, growth retardation, and immunodeficiency; RECQL4 mutations cause Rothmund-Thomson syndrome with skeletal abnormalities, poikiloderma, and dramatically elevated osteosarcoma risk (Ababou, 2021; Cheok et al., 2005; Lu et al., 2020).

The connection extends beyond the RecQ family. Watson et al. (2013) demonstrated that ATRX deficiency—ATRX being a SWI/SNF helicase required for chromatin remodeling at repetitive sequences—causes replicative damage specifically at telomeres and pericentromeric heterochromatin, regions enriched for G4 structures. They exposed the consequences by treating ATRX-deficient cells with telomestatin, a G4-stabilizing ligand: DNA damage increased dramatically, confirming that ATRX functions in G4 resolution. ATRX-null mice display a constellation of aging-like phenotypes: lordokyphosis, cataracts, cardiac enlargement, and significantly shortened lifespan. The phenotypic similarity to natural aging suggests—though does not prove—a shared underlying mechanism.

4.3 Aging as Progressive Dysregulation of Evolvability Platforms

Integrating the direct evidence from aged stem cells with the indirect evidence from helicase syndromes, I propose that aging represents the progressive failure of mechanisms maintaining non-B DNA structure homeostasis (Figure 4). The same structures enabling evolutionary adaptation in young organisms become sources of damage when maintenance capacity declines. This framework connects evolutionary theory to gerontology through shared molecular mechanisms.

The key insight is that multiple independent lines of evidence converge on this conclusion. R-loops accumulate in aged HSCs due to declining RNA export (Chen et al., 2025). Reproductive aging involves R-loop accumulation from MCM8 dysfunction (Wen et al., 2024). Telomeric R-loops accumulate at critically short telomeres due to defective RNA degradation (Graf et al., 2017). Each helicase deficiency syndrome—WRN, BLM, RECQL4, ATRX—produces aging-like phenotypes despite affecting distinct aspects of structure processing. The convergence suggests that structure maintenance represents a node whose failure contributes to aging across multiple tissues and contexts.

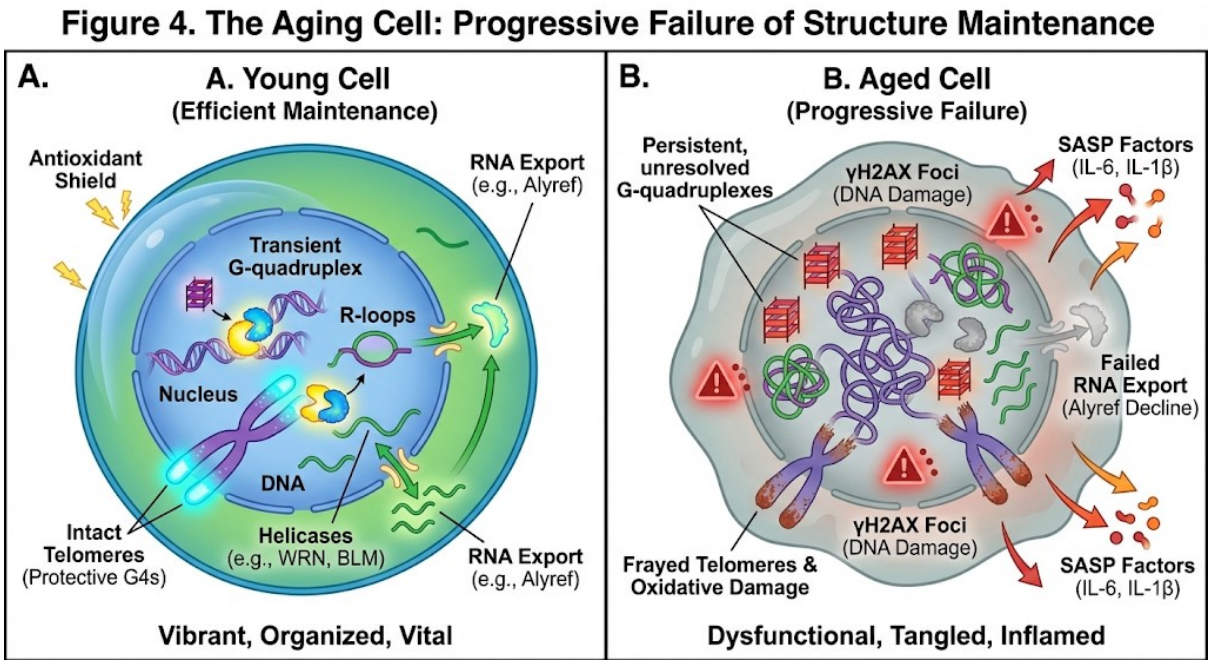

Figure 4: Contrasts young and aged cells with specific molecular mechanisms: helicase activity, Alyref/TREX RNA export, telomeric G4 protection, and the convergence on senescence/SASP

4.4 Oxidative Stress and Telomeric G4s

Telomeres sit at the intersection of structure and aging through their G4-forming potential. Telomeric DNA consists of TTAGGG repeats that readily adopt G4 conformations, with the 3' single-stranded overhang being particularly prone to folding (Riou et al., 2002). These G4 structures contribute to telomere protection but create acute vulnerability to oxidative damage—a vulnerability that increases with age as antioxidant defenses weaken.

Ahmed and Lingner (2018) dissected this vulnerability in mechanistic detail. They demonstrated that PRDX1 (a peroxiredoxin) and MTH1 (a nucleotide pool sanitizer) cooperate to prevent reactive oxygen species from inhibiting telomerase. The problem centers on 8-oxoguanine (8-oxoG), the most common oxidative DNA lesion. When 8-oxoG forms at telomeric 3' ends—the substrate for telomerase extension—it directly inhibits the enzyme. At internal positions within telomeric DNA, 8-oxoG destabilizes G4 folding, compromising telomere architecture. Single-molecule force spectroscopy has quantified these effects: Cheng et al. (2025) showed that a single 8-oxoG lesion at the 5' end of telomeric G4s significantly decreases folding rates and reduces folding free energy by more than half, while 8-oxoG near hairpin termini dramatically enhances end fraying and impedes complete folding. Dual disruption of PRDX1 and MTH1 causes continuous telomere shortening, establishing that antioxidant systems are essential for telomere maintenance.

The consequences for DNA processing enzymes are equally significant. Podbevšek and Plavec (2025) showed that while oxidative lesions generally reduce G4 stability and facilitate enzymatic bypass—particularly when the lesion persists in unfolding intermediates—in some cases 8-oxoG can paradoxically reduce bypass efficiency, trapping processing machinery. Miclot et al. (2021) demonstrated that human telomeric G4s possess remarkable structural stability that allows them to resist oxidative stress producing one or even clustered 8-oxoG

lesions, a capacity that may be essential for their biological role as telomerase inhibitors and that declines with age.

The exceptional oxidative vulnerability of G4s derives from guanine's chemistry. Among the four DNA bases, guanine has the lowest oxidation potential—it is most easily oxidized (Steenken & Jovanovic, 1997). G4 structures, with their stacked G-tetrads concentrating multiple guanines in close proximity, present concentrated targets for oxidative modification. The guanine oxidation (GO) system—comprising OGG1, MUTYH, and MTH1—provides protection, but this protection diminishes with age as repair capacity declines (David et al., 2007; De Rosa et al., 2021; Talhaoui et al., 2016).

Intriguingly, oxidative damage itself can drive G4 formation, creating a feed-forward loop. Roychoudhury et al. (2020) demonstrated that 8-oxoguanine formation in G4-forming sequences triggers the base excision repair pathway, and the subsequent APE1-mediated strand incision facilitates G4 folding. Loss of APE1 abrogated G4 structure formation genome-wide, establishing that oxidative damage and repair actively drive spatiotemporal G4 dynamics. In young cells with robust antioxidant defenses, this pathway enables regulated G4 formation for normal functions. In aging cells with declining protection, accumulating oxidative lesions drive excessive G4 formation that overwhelms resolution capacity—converting a regulatory mechanism into a source of persistent damage.

At telomeres, the intersection of R-loops with aging takes on additional complexity. Graf et al. (2017) discovered that TERRA—telomeric repeat-containing RNA—forms R-loops that accumulate preferentially at critically short telomeres. This accumulation results from defective RNA degradation as telomeres shorten with age. The persistent R-loops activate DNA damage responses and promote recruitment of the recombinase Rad51, potentially initiating the alternative lengthening of telomeres pathway. Telomere length-dependent R-loop regulation thus becomes a determinant of replicative lifespan—and its dysregulation a driver of senescence.

4.5 Convergence on Senescence Signaling

The convergence of R-loop accumulation, telomeric G4 oxidation, and helicase insufficiency on senescence signaling deserves emphasis. Each pathway—through distinct mechanisms—activates the DNA damage response, stabilizing p53 and inducing the cyclin-dependent kinase inhibitors p21 and p16. Persistent DNA damage signaling drives cells into the senescent state characterized by permanent cell cycle arrest, altered metabolism, and secretion of inflammatory factors—the senescence-associated secretory phenotype (SASP) (Birch & Gil, 2020; Coppé et al., 2008; Lopes-Paciencia et al., 2019). The SASP transforms a cell-autonomous damage response into a tissue-level inflammatory program that drives many manifestations of aging.

Margariti et al. (2025) recently articulated how nucleases and helicases converge on this aging axis, demonstrating that defects in these enzymes—through replication stress, DNA damage response activation, and telomere dysfunction—lead to stem cell exhaustion and progressive epigenetic drift that accelerates aging. Salminen (2025) identified HMGB1 as a multifunctional hub connecting DNA damage to inflammaging: released from chromatin during senescence, extracellular HMGB1 activates TLR and RAGE signaling, intensifies SASP, and promotes recruitment of immunosuppressive cells—establishing mechanistic links between cell-autonomous DNA damage and systemic inflammatory aging.

Neurons present special vulnerability to structure-mediated damage. Kajitani et al. (2020) showed that nucleotide excision repair deficiency causes neurological abnormalities in part

through R-loop accumulation. The vulnerability of neuronal tissues reflects their unique transcriptional profile: neurons express exceptionally long genes whose transcription necessarily takes hours to complete, creating extended windows for R-loop formation and for conflicts between transcription and replication or repair machineries. The transcription-blocking lesions that accumulate with age—and that are normally repaired by nucleotide excision repair—compound this vulnerability.

4.6 Somatic Mutation Accumulation at Evolvability Platforms

Vijg and Dong (2020) articulated the somatic mutation theory of aging: the gradual accumulation of DNA mutations throughout life contributes to aging through direct gene inactivation, clonal expansion of mutant cells, and increased transcriptional noise. Zhang et al. (2019) quantified this accumulation with single-cell whole-genome sequencing of B lymphocytes spanning the human lifespan: mutation loads increase from fewer than 500 per cell in newborns to more than 3,000 per cell in centenarians—a six-fold increase reflecting the cumulative toll of replication errors, DNA damage, and imperfect repair.

This suggests that this mutation accumulation concentrates at evolvability platforms (Alexandrov et al., 2013). The same non-B DNA structures that experience elevated mutation rates during beneficial diversification—in meiosis, in immunity—become damage sinks during aging. The mechanistic basis for this concentration is well-established: Kaushal and Freudenreich (2019) showed that secondary structures at common fragile sites (CFSs) not only initiate fragility through replication fork stalling but also inhibit healing, resulting in the characteristic gaps and breaks observed on metaphase chromosomes under replication stress. Twayana et al. (2021) demonstrated that Pol eta, essential for efficient CFS replication, functions to bypass non-B DNA structures; in its absence, pause sites at CFSs associate with non-B DNA-forming motifs, and these same sites embed within regions of increased genetic variation in healthy human populations—suggesting that structure-mediated replication difficulty drives human genetic diversity at these loci.

Several observations support this proposal. First, mutation accumulation in aged tissues occurs predominantly in long-lived stem cells, the cells most dependent on evolvability platform function for their roles in tissue maintenance. Second, the genes most commonly mutated in clonal hematopoiesis—DNMT3A, TET2, ASXL1—encode epigenetic regulators that modify chromatin accessibility at regulatory regions enriched for non-B structures. Third, the chromosomal instability characteristic of aged cells shows preferential localization to fragile sites, many of which contain structure-forming sequences.

4.7 Clonal Hematopoiesis: Evolvability Selection in the Aging Blood System

Clonal hematopoiesis of indeterminate potential (CHIP) provides perhaps the most direct evidence that evolvability mechanisms operate during human aging. CHIP is defined by the clonal expansion of hematopoietic stem cells carrying leukemogenic mutations in individuals without overt hematologic malignancy (Jaiswal & Libby, 2019). Its prevalence increases sharply with age: rare before 40, it affects approximately 10-15% of individuals over 70, with some studies finding prevalence exceeding 20% in those over 80, depending on sequencing sensitivity and variant allele frequency thresholds (Jaiswal et al., 2014; Genovese et al., 2014).

The mutations driving CHIP are remarkably consistent. Roughly 80% occur in just a handful of genes: the DNA methyltransferase DNMT3A, the methylcytosine dioxygenase TET2, and the chromatin regulator ASXL1 (Evans et al., 2019; Marnell et al., 2021). These are not random—they represent strong selection for mutations that provide competitive advantage in

the aging HSC environment. The predominance of epigenetic regulators is telling: these mutations alter chromatin accessibility genome-wide, potentially modifying how cells access evolvability platforms and respond to regulatory signals.

The consequences of CHIP extend far beyond hematologic disease. CHIP confers a two-fold increase in cardiovascular disease risk, independent of traditional risk factors (Jaiswal & Libby, 2019). TET2 mutations specifically lead to increased expression of inflammatory genes—IL-1β, IL-6, inflammasome components—in macrophages, linking clonal evolution to the chronic inflammation that drives atherosclerosis. Yu et al. (2025) characterized the plasma proteomic profile of CHIP across more than 60,000 participants, identifying proteins associated with CHIP that show substantial heterogeneity by driver gene, sex, and race, with enrichment for immune response and inflammation pathways—and critically, identifying shared proteins between CHIP and coronary artery disease that illuminate the mechanistic connection. Koh et al. (2025) emphasized that mutated clones display altered inflammatory profiles with tissue-specific functional consequences, contributing to diseases ranging from atherosclerosis and osteoporosis to heart failure and neurodegenerative conditions. Gumuser et al. (2023) demonstrated that in patients with established atherosclerotic cardiovascular disease, CHIP predicts adverse outcomes: TET2 mutations and spliceosome mutations (SF3B1, SRSF2, U2AF1) carried particularly elevated risk.

Pasupuleti et al. (2023) showed that obesity—an inflammatory state that accelerates aging—exacerbates CHIP. Using mouse models of CHIP driven by heterozygosity for Tet2, Dnmt3a, Asxl1, or Jak2, they demonstrated that obesity-induced inflammation promoted expansion of mutant HSCs. The inflammation and mutant clone expansion created a feed-forward loop, with the mutant cells producing inflammatory cytokines that favored their own competitive advantage. Remarkably, calcium channel blockers (nifedipine, SKF-96365), alone or combined with metformin or IL-1 receptor antagonist (anakinra), suppressed mutant clone growth and partially restored normal hematopoiesis.

This connects evolvability to systemic aging through inflammation. The same somatic selection that operates through evolvability platforms to generate beneficial diversity in immunity becomes, in the aging bone marrow, an engine of clonal expansion that drives cardiovascular disease, myeloid malignancy, and accelerated mortality.

Section 5: Synthesis and Therapeutic Implications

5.1 The Unified Model

The evidence converges on a unified model connecting evolution, cancer, and aging through shared evolvability mechanisms (Figure 3). Non-B DNA structures constitute evolvability platforms that specify where genetic change preferentially occurs. In evolutionary contexts, this enables regulatory adaptation without functional destruction—the raw material for phenotypic change without the burden of deleterious protein alterations. In cancer, these platforms become co-opted for malignant evolution through mutations that flip regulatory switches. In aging, progressive failure of structure maintenance converts evolvability platforms from regulatory assets to sources of chronic damage and inflammation.

The key insight is that regulation—not structure per se—determines outcome. Controlled G4 formation enables transcriptional switching; uncontrolled G4 persistence causes replication stress, helicase exhaustion, and DNA damage. Controlled R-loop formation enables class switch recombination and gene regulation; uncontrolled R-loop accumulation drives genome instability, senescence, and tissue dysfunction. Controlled repeat polymorphism enables

expression tuning; uncontrolled repeat expansion causes neurodegeneration. The structures are double-edged swords whose effects depend entirely on whether cellular machinery maintains control.

This framework explains observations that otherwise resist interpretation. Non-B DNA structures persist evolutionarily because their regulatory benefits outweigh their risks—in young organisms with intact maintenance systems. Cancer mutations concentrate at regulatory regions because these harbor the evolvability platforms that enable expression changes without killing cells through protein dysfunction. The same mechanisms underlie beneficial diversity and pathological instability because the distinction is regulatory, not structural. Aging diseases result from helicase mutations because helicases are the engines of structure maintenance, and their failure unleashes the pathological potential inherent in evolvability platforms.

5.2 Therapeutic Opportunities

This framework reveals therapeutic opportunities spanning oncology and gerontology (Table 3; Figure 5).

For cancer, targeting structure-dependent vulnerabilities offers selective mechanisms for killing tumor cells while sparing normal tissue. The WRN dependence of microsatellite-unstable cancers—a dependence arising from acquired non-B DNA structure accumulation—creates a therapeutic window for WRN inhibitors (van Wietmarschen et al., 2020). G4-stabilizing compounds can restore silencing of oncogenes with destabilized promoter G4s, potentially reversing the regulatory switch flip that drives malignant transformation (Kaulage et al., 2018). The dependence of cancer cells on eIF4A helicase for translation of G4-containing oncogenic mRNAs provides another targetable node, with inhibitors such as silvestrol showing promising activity (Modelska et al., 2015).

For aging, the framework suggests strategies to enhance structure maintenance and prevent damage accumulation. Helicase enhancement—activating WRN, ATRX, or related enzymes—could reduce G4-associated replication stress in aged cells (Croteau et al., 2014; Mendoza et al., 2016). R-loop resolution through RNase H activation or TREX complex enhancement could prevent the nuclear RNA accumulation that drives stem cell exhaustion (Cerritelli & Crouch, 2009). The Chen et al. (2025) demonstration that Alyref restoration rejuvenates aged HSCs provides proof-of-concept that structure-targeted interventions can reverse aging phenotypes. Antioxidant strategies specifically protecting telomeric G4s—targeting PRDX1 or MTH1 pathways—could reduce the oxidative damage that accelerates telomere loss.

Figure 5. Therapeutic Convergence: Shared Targets, Opposite Goals

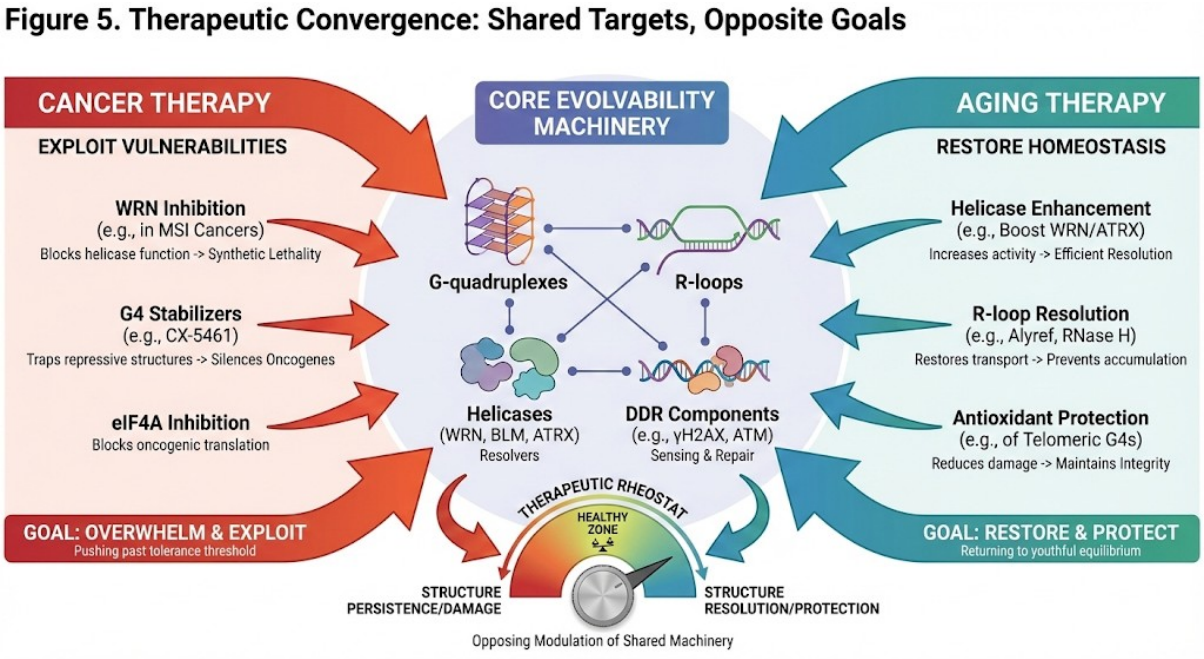

Figure 5: Describes therapeutic strategies targeting the same machinery with opposite goals: cancer therapy to exploit/overwhelm vs. aging therapy to restore/protect

For CHIP-associated disease, interventions targeting the inflammatory consequences of clonal expansion show promise. IL-1β inhibition with canakinumab has already demonstrated cardiovascular benefit in patients with elevated inflammatory markers (Ridker et al., 2017). Pasupuleti et al. (2023) showed that calcium channel blockers can suppress mutant clone growth, potentially preventing both hematologic and cardiovascular complications. The MutSβ-MutLβ-FANCJ axis recently identified by Isik et al. (2024) as mediating replication restart at G4/R-loop sites represents another potential target for modulating structure-associated pathology.

The crucial distinction is that cancer therapy aims to exploit evolvability platforms co-opted by malignant cells, while aging therapy aims to restore regulation of platforms whose dysregulation drives decline. The shared molecular targets—G4 structures, R-loops, the helicases that resolve them—enable therapeutic translation between fields, with lessons learned in cancer potentially applicable to aging and vice versa.

Table 3. Therapeutic Strategies Targeting Evolvability Platforms

Target	Disease Context	Therapeutic Goal	Mechanism	Status/Examples
WRN helicase	MSI cancers	Synthetic lethality	Block processing of expanded TA repeats → replication fork collapse → cell death	Preclinical development of small molecule WRN inhibitors
G4 structures	MYC/KRAS-driven cancers	Oncogene silencing	Stabilize repressive promoter G4s → suppress	Benzimidazole-carbazole ligands; pyridostatin; CX-5461 in

Target	Disease Context	Therapeutic Goal	Mechanism	Status/Examples
			oncogene transcription	clinical trials
eIF4A helicase	Cancers with G4-rich oncogenic mRNAs	Translation inhibition	Block unwinding of 5'UTR G4s → reduce oncogenic protein synthesis	Silvestrol, hippuristanol in preclinical studies
MutSβ-FANCJ axis	Tumors with transcription-replication conflicts	Replication stress exploitation	Block G4/R-loop resolution → synthetic lethality in repair-deficient cells	Mechanistic studies ongoing
RecQ helicases	Aging, progeroid syndromes	Structure resolution enhancement	Increase G4/R-loop processing capacity → reduce replication stress	Helicase activators under development
TREX complex/Alyref	HSC aging	R-loop prevention	Enhance RNA export → reduce nuclear RNA accumulation → prevent R-loop formation	Gene therapy approaches; small molecule screens
RNase H	R-loop-associated pathology	R-loop resolution	Degrade RNA strand of R-loops → restore transcription and replication	Enzyme activation strategies
PRDX1/MTH1	Telomere aging	Antioxidant protection	Prevent 8-oxoG incorporation → protect telomeric G4 structures	Antioxidant targeting approaches
IL-1β pathway	CHIP-associated CVD	Anti-inflammatory	Block inflammatory output of TET2-mutant macrophages	Canakinumab demonstrated CV benefit in CANTOS trial
Calcium channels	CHIP progression	Clone suppression	Reduce mutant HSC/P expansion → restore normal hematopoiesis	Nifedipine, SKF-96365 effective in mouse models
DNAJB6 chaperone	Repeat expansion diseases	Aggregate prevention	Maintain proteostasis → prevent polyglutamine aggregation in neurons	Chaperone enhancement strategies

5.3 Limitations and Alternative Interpretations

Several caveats and alternative interpretations merit acknowledgment. First, the correlation between G4-forming potential and regulatory function does not definitively establish causation in all cases. Some promoter G4 motifs may be evolutionary remnants without current function, or their regulatory effects may be indirect. Second, the helicase-aging connection admits alternative explanations: these enzymes participate in pathways beyond structure resolution, including DNA repair, replication restart, and telomere maintenance. The accelerated aging in helicase deficiency syndromes likely reflects multiple mechanism failures rather than structure dysregulation alone. Third, not all non-B structures correlate with mutational hotspots—some G4-forming sequences show no evidence of elevated mutation rates, suggesting that context determines whether structural potential translates to mutagenic outcome.

Additionally, the relative contribution of structure dysregulation to normal aging versus other aging mechanisms—mitochondrial dysfunction, proteostatic collapse, epigenetic drift—remains to be determined. This framework proposes that structure maintenance failure is one convergent mechanism contributing to aging, not that it is the sole or primary driver. The experimental tests outlined below will help establish the magnitude of this contribution.

5.4 Open Questions and Testable Predictions

This framework generates predictions accessible to experimental test. If non-B DNA structures function as evolvability platforms, their distribution should correlate with evolutionary rates—genes requiring regulatory flexibility should be enriched for structure-forming sequences compared to genes under purifying selection. If aging represents maintenance failure, helicase expression and activity should decline measurably with age across tissues, and helicase enhancement should delay aging phenotypes in animal models. If CHIP represents selection at evolvability platforms, the chromatin accessibility landscape should differ systematically between mutant and normal HSCs, with alterations concentrated at non-B DNA regulatory elements.

Methodological advances now enable direct testing of these predictions. G4-seq maps G4 structures genome-wide with nucleotide resolution (Chambers et al., 2015). DRIP-seq and variants map R-loops across cell types and conditions (Petermann et al., 2022). Single-cell whole-genome sequencing can track somatic mutations at specific genomic loci across aging (Zhang et al., 2019). CRISPR-based structure ablation, as demonstrated for the MYC G4, enables direct testing of structure-function relationships (Esain-Garcia et al., 2024). The tools exist to test whether aging is indeed, as I propose, the progressive dysregulation of the genome's evolvability layer.

Conclusions

Genomes encode not only the sequence of genes but also the capacity for their own evolution. Non-B DNA structures—G-quadruplexes, R-loops, Z-DNA, cruciforms—constitute evolvability platforms specifying where genetic change preferentially occurs. These structures cluster at regulatory domains controlling proliferation, enabling expression modulation without functional destruction.

This structural layer represents a second genomic code, though one that operates differently from the deterministic genetic code. Where codons map invariably to amino acids, structural potential maps probabilistically to regulatory outcomes—and this context-dependence enables the dynamic regulation that makes evolvability controllable.

Cancer co-opts evolvability mechanisms for malignant adaptation. The TERT promoter mutations—the most common non-coding drivers—work through G4 structure modulation. APOBEC mutational signatures cluster at structure-forming sequences where single-stranded DNA substrates become available. MSI cancers become dependent on helicases processing expanded non-B DNA.

Aging represents the failure of structure maintenance. R-loop accumulation in aged hematopoietic stem cells—reversible through Alyref restoration—provides direct evidence. Werner syndrome, Bloom syndrome, and ATRX deficiency establish that helicase dysfunction accelerates aging through mechanisms that include, though are not limited to, structure processing failure. Telomeric G4s fall prey to oxidative damage that hastens telomere loss and cellular senescence.

This unified framework connects evolutionary theory to molecular medicine. Understanding how genomes encode their own evolvability illuminates both how adaptation occurs and what transpires when evolvability mechanisms become dysregulated. The therapeutic implications span oncology and gerontology, revealing shared targets for distinct goals: disrupting malignant evolvability in cancer, restoring regulated evolvability in aging. If this framework proves correct, interventions that maintain structure homeostasis could simultaneously reduce cancer risk and extend healthy lifespan—a convergence with profound implications for human health.

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