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Transcription-replication conflicts: A regulated interface in genome maintenance

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Abstract

DNA replication and transcription operate on the same chromatin template and inevitably interfere with one another, giving rise to transcription-replication conflicts (TRCs), an important endogenous source of replication stress and genome instability. Although head-on (HO) conflicts are generally more disruptive than co-directional (CD) encounters, orientation alone does not determine the outcome. Gene architecture, transcriptional activity, replication timing, chromatin environment, DNA topology, and R-loop dynamics can all influence whether an approaching replication fork pauses, collapses, or traverses a transcribed region. Recent single-molecule, genomic, and biochemical studies further suggest that some CD encounters can be traversed under favorable topological and transcriptional conditions, although the mechanisms that permit such bypass and their broader biological significance remain incompletely understood. Here, we discuss TRC control within an operational framework comprising prevention, resolution, tolerance, and recovery. We examine how cells coordinate replication and transcription in space and time, regulate R-loop formation and turnover, protect or remodel challenged forks and chromatin, and modify or remove transcription complexes at conflict sites. We also consider how emerging single-molecule and multi-omic approaches can distinguish transient fork pausing from persistent pathological conflicts. Finally, we discuss the contributions of TRCs to cancer-associated chromosomal instability, cellular senescence, and neurodegenerative disease, and highlight the questions that must be resolved to understand how cells preserve genome integrity when replication and transcription compete for the same DNA template.

Keywords: Transcription–replication conflicts, replication stress, genome stability

1. Introduction

Genome stability depends on the faithful execution of both DNA replication and transcription. These two multi-megadalton machineries must operate on the same DNA template[1], making encounters between replisomes and RNA polymerases (RNAPs) unavoidable across all domains of life[2,3].

Early studies established that encounter orientation strongly influences the outcome of a transcription–replication conflict, although orientation alone is not deterministic[4]. In head-on (HO) conflicts, the replisome and transcription machinery approach one another from opposite directions, whereas in co-directional (CD) conflicts the replisome catches up with RNAPII from behind. HO encounters generally cause more pronounced fork slowing and genome instability, across bacterial, reporter, and reductionist systems, but they do not invariably cause fork collapse or double-strand breaks[4]. CD encounters are typically less disruptive, but they are not uniformly benign[4]. The outcome of either encounter is further shaped by gene length, transcriptional activity, replication timing, chromatin state, local DNA topology, and the experimental system[5,6].

Cells limit the impacts of transcription-replication conflicts (TRCs) at several levels. Genome organization and temporal coordination can reduce the probability of collisions, as illustrated by the leading-strand[7,8] bias of highly transcribed genes in bacteria and by coordination between replication origin usage[6] and transcriptional activity. Once a conflict occurs, cells can remove or remodel the transcriptional obstacle, process RNA-DNA hybrids[9], stabilize the replication fork[10], or promote fork restart and subsequent repair[11,12]. RNAPII displacement[13], R-loop processing[14], replisome bypass[15,16], and post-replicative repair act on different substrates and at different stages[17], providing mechanistically distinct means of limiting the consequences of TRCs.

Recent studies have sharpened the distinction between obstacle removal and conflict tolerance. A stalled fork may be protected, an RNA-DNA hybrid may be processed, a transcription complex may be remodeled, replication may restart downstream through

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repriming, or a post-replicative gap may be filled later. These outcomes are mechanistically distinct and do not all require removal of the original obstacle[18,19]. In human cells, the CUL3-KCTD10 E3 ligase is enriched at selected CD conflicts and promotes ubiquitination and removal of the transcription factor IIS (TFIIS)-family factor TCEA2[16]. This remodeling appears to make RNAPII more permissive to replisome bypass, although the structure of the bypass intermediate or the continuity of leading-strand synthesis remain unresolved[16].

Here, we consider the control of TRCs through four connected, non-exclusive components: prevention, resolution, tolerance, and recovery. We discuss how these processes intersect with conflict orientation, R-loop metabolism, fork remodeling and chromatin regulation. We also examine how DNAscent[20], biotinylated trimethylpsoralen-based sequencing (bTMP-seq)[21-24], and related approaches capture distinct features of TRCs, from single-molecule replication dynamics to population-level genomic signatures. Finally, we discuss the emerging links between TRCs and cancer vulnerabilities[25], ageing[26], and neurodegeneration[27], while emphasizing their context dependence[28,29].

2. The Molecular Anatomy of Conflicts: A Dynamic Spectrum

2.1 Orientation matters, but context adds complexity

TRC orientation strongly influences the collision outcomes, but it is not the only determinant. HO collisions are generally more deleterious than CD encounters in the systems examined to date[30,31]. In a purified, non-chromatinized single-molecule assay containing RNAPII elongation complexes, mechanical unzipping revealed a polar barrier: the force required to displace RNAPII depended on the direction from which it was encountered[30]. This finding points to an intrinsic polarity of the elongation complex that may contribute to the greater severity of HO conflicts. In cells, however, this behavior is likely modified by the full replisome[32], chromatin[33], topoisomerases[34], and fork-protection factors[35].

DNA topology provides another layer of asymmetry. According to the twin-supercoiling-domain model, both an advancing replisome and a transcribing RNAPII generate positive supercoiling ahead of their movement[36,37]. During an HO encounter, positive torsional stress can therefore accumulate between the two machineries and **impede** fork progression unless it is dissipated through topoisomerase activity, molecular rotation, or topological domain boundaries[34,36]. Biophysical studies have shown that torque can influence helicase translocation[34,38], but the magnitude and distribution of torsional stress generated during TRCs remain poorly defined in chromatinized eukaryotic replisomes. The contribution of topology is therefore likely to depend on the organism[39,40], template constraints[34], chromatin environment[41], and experimental setting[42]. The HO geometry and the resulting topological barrier are illustrated in Figure 1a.

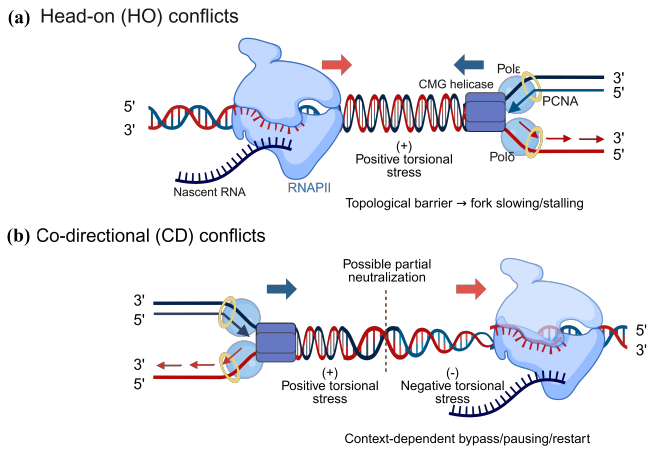


Figure 1. Geometry and topology of TRCs. (a) In HO conflicts, converging RNAPII and the replisome generate positive torsional stress in the intervening DNA, creating a topological barrier that can slow or stall fork progression; (b) In CD conflicts, positive torsional stress ahead of the replisome may partially counteract negative torsional stress behind RNAPII. Depending on the local context, the encounter may result in bypass, transient pausing or fork restart. DNA topology is shown schematically. Created in BioRender. Huang, J. (2026) https://BioRender.com/4z7z02a. CMG: CDC45-MCM2-7-GINS; TRCs: transcription-replication conflicts; RNAPII: RNA polymerase II; PCNA: proliferating cell nuclear antigen.

Fork slowing under these conditions does not necessarily imply that the CDC45-MCM2-7-GINS (CMG) helicase has dissociated from DNA. CMG can remain associated with the leading-strand template while DNA synthesis is slowed by torsional stress or a protein barrier. When helicase movement becomes uncoupled from polymerase progression, the resulting single-stranded DNA (ssDNA) can be coated by replication protein A (RPA) and engage ataxia-telangiectasia and Rad3-related (ATR) signaling through ATR-interacting protein (ATRIP)[43,44] and TOPBP1[45]. The extent and persistence of such uncoupling depend on the nature of the barrier and the mechanisms that protect and stabilize the fork[35,46].

Transcription-coupled RNA-DNA hybrids can further influence collision outcome. A paused transcription complex may favor hybrid persistence by prolonging exposure of the template strand and altering local DNA topology[30,47]. However, R-loops (three-stranded structures containing an RNA-DNA hybrid) formed in the vicinity of TRCs are heterogeneous in both structure and function[48]. Their stability is influenced by sequence composition[49], transcription rate[50], RNA processing[51], protein occupancy[52], topology[53], and chromatin state[54]. In some settings, an R-loop may reinforce a protein–nucleic-acid barrier and increase fork slowing or breakage[55], whereas in others it may be transient or regulatory[56]. R-loop formation alone is therefore insufficient to predict whether a paused fork will ultimately collapse[55,57].

CD conflicts present a different topological and structural context, as the replisome approaches RNAPII from behind. Negative supercoiling generated behind the transcription complex could, under some conditions, facilitate local DNA unwinding and fork progression, although the extent to which this occurs *in vivo* remains unclear[31]. Population-level topology maps such as bTMP-seq can identify underwound genomic regions associated with transcription and replication[23,24], but they do not directly capture an RNAPII-replisome encounter on the same DNA molecule[21]. Together with the orientation-dependent barrier observed in simplified single-molecule systems[30], these findings suggest that CD encounters can have several possible outcomes, including RNAPII displacement[58], R-loop removal[59], transcription-complex remodeling[57], replisome bypass[16], and subsequent fork recovery. Ultimately, collision orientation establishes a mechanistic bias rather than an invariant biological outcome, as either HO or CD geometry can manifest divergent fork fates shaped by local DNA topology, transcriptional activity, chromatin architecture, and the host cell's conflict-processing capacity. The contrasting CD geometry and its context-dependent outcomes are illustrated in Figure 1b.

2.2 The fate of the replication fork

A persistent transcriptional barrier can slow or stall replication forks[4,60], although the subsequent response is highly context dependent[60,61]. In mammalian cells and reconstituted fork-remodeling systems, a subset of stalled forks undergoes reversal[62,63], in which a three-way junction is converted into a four-way regressed structure[62,64]. Fork reversal therefore represents one protective remodeling response rather than an obligatory outcome of TRCs.

In mammalian cells, the DNA translocases SWI/SNF-related, matrix-associated, actin-dependent, regulator of chromatin, subfamily A-like 1 (SMARCAL1), helicase like transcription factor (HLTF), and zinc finger RANBP2-type containing 3 (ZRANB3) contribute to fork remodeling through distinct substrate preferences and regulatory inputs[65,66]. SMARCAL1 preferentially responds to RPA-coated ssDNA regions[67], the HIP116 and Rad5 N-terminal (HIRAN) domain of HLTF recognizes a nascent-DNA 3′ end[68], and ZRANB3 catalyzes fork remodeling and branch migration[64,66]. RAD51 can protect regressed fork arms[69] from nucleolytic degradation by factors such as MRE11 double strand break repair nuclease (MRE11)[70] and DNA2[71]. However, the contribution of individual remodeling factors depends on fork architecture, the nature of the blocking lesion, cell state, and assay[72,73].

Resumption of replication following fork pausing or remodeling represents a recovery process distinct from removal of the initiating transcriptional obstacle such as an R-loop. In bacteria, PriA-dependent primosome assembly can restore replication at selected stalled fork structures[74]. In eukaryotes, recovery may occur through the existing fork, dormant-origin firing, homologous-recombination-mediated restart, or completion by a converging replication fork[75,76]. These pathways provide alternative routes to restore genome duplication without necessarily resolving the original source conflict.

In mammalian cells, under-replicated regions at common fragile sites and other late-replicating loci can persist into mitosis[6,77]. Cyclin-dependent kinase 1 (CDK1)-dependent regulation of SLX4 and MUS81-EME1 promotes processing of selected replication intermediates[78,79], followed by POLD3-dependent mitotic DNA synthesis (MiDAS), which can complete DNA synthesis at a subset of these regions[79,80]. MiDAS is therefore best viewed as a late-stage recovery or salvage pathway[81]. Its activation reflects unresolved replication stress but is not specific to a single initiating lesion[82], and the requirement for cleavage, synthesis mode, and locus context varies among mammalian systems[79].

2.3 The fate of RNA polymerases

RNAPII removal represents one possible route for resolving TRCs. In bacteria, accessory helicases and translocases, including Rep[83], UvrD[84,85], PcrA, and Mfd[86], can displace or remodel stalled transcription complexes in defined biochemical and cellular contexts. In budding yeast, Rrm3 and Pif1-family helicases function at highly transcribed[87,88] and protein-bound genomic regions[89]. Although these factors share a common ability to modulate transcription-associated barriers, their substrates, recruitment mechanisms, and coupling to repair pathways differ substantially across organisms.

In human cells, a recent study identified CUL3-KCTD10 as an example of remodeling-enabled bypass at selected CD conflicts[16]. KCTD10 associates with proliferating cell nuclear antigen (PCNA) and RNAPII machinery and recruits the CUL3 ubiquitin ligase complex, promoting ubiquitination and removal of TCEA2. TCEA2 is a TFIIS-family elongation factor involved in transcript cleavage during RNAPII backtracking[16,90]. Current evidence supports a model in which TCEA2 depletion alters RNAPII elongation-complex dynamics and increases the accessibility of the transcription machinery to replisome passage[16]. However, whether RNAPII remains

continuously DNA-associated, undergoes transient remodeling, or is partially displaced during this process remains unresolved. Likewise, the extent to which leading-strand synthesis is maintained or followed by repriming and gap repair remains an open question.

Loss of KCTD10 leads to persistence of unresolved CD-TRCs, resulting in increased DNA damage signals, elevated CHK2 phosphorylation, and selective sensitivity to ATM serine/threonine kinase (ATM) or checkpoint kinase 2 (CHK2) inhibition in cell-based assays. These findings are consistent with the observation that CD-TRCs preferentially engage the ATM-CHK2 checkpoint pathway, rather than the ATR-CHK1 response typically associated with other forms of replication stress[4]. Thus, the increased checkpoint dependency observed after KCTD10 loss likely reflects the accumulation of conflict-associated lesions rather than direct regulation of ATM-CHK2 signaling by KCTD10[16].

Recent cancer dependency analyses suggest that KCTD10 represents a context-dependent vulnerability rather than a universal requirement of TRC-high tumors. KCTD10 dependency is enriched in tumor states associated with oncogenic transcriptional programs, including HER2- and MYC-driven contexts, where elevated transcriptional activity may increase the burden of co-directional transcription-replication conflicts. These observations support a model in which KCTD10 becomes critical when conflict-associated stress exceeds the capacity of compensatory genome-maintenance pathways[91].

2.4 The chromatin barrier: Epigenetic scars and active remodeling

Chromatin is not merely a passive substrate for TRCs but can influence both the formation and outcome of conflicts. In an inducible TRC reporter system, transcription-replication collisions were associated with R-loop-dependent nucleosome eviction and replication fork arrest[33]. The same study identified increased DOT1L-dependent H3K79 methylation following collision and showed that DOT1L activity contributed to transcriptional recovery[33]. These findings suggest that local chromatin remodeling may provide a transient record of conflict, although the prevalence and persistence of such chromatin states at endogenous loci remain unclear.

Chromatin alterations can also influence susceptibility to TRCs. In MYC-deregulated triple-negative breast cancer (TNBC) models, ANP32E overexpression increased H2A.Z turnover and altered the distribution of RNAPII: promoter-proximal pausing was reduced, whereas elongating RNAPII stalled more frequently within gene bodies. These changes coincided with longer R-loops, more frequent TRCs, and activation of an ATR-dependent DNA damage response. Accordingly, ANP32E-overexpressing cells were more sensitive to ATR inhibition[48]. However, this dependency has so far been demonstrated only in the MYC-ANP32E-deregulated TNBC models examined, and it remains unclear whether ANP32E expression alone can predict ATR inhibitor sensitivity in other tumor settings.

3. R-Loops as Modulators of TRC Outcomes

3.1 Formation and heterogeneity of conflict-associated R-loops

R-loops are defined strictly as canonical three-stranded nucleic-acid structures comprising an RNA-DNA hybrid and a displaced ssDNA. All R-loops contain an RNA-DNA hybrid, but not all RNA-DNA hybrids are bona fide R-loops; short primer-derived hybrids, resected intermediates and cytosolic RNA-DNA hybrids may lack the displaced ssDNA that defines the canonical structure[92]. Their formation and persistence are influenced by DNA sequence, transcription kinetics, RNA processing, DNA topology, chromatin state, and proteins associated with the RNA, hybrid, or displaced DNA strand[93,94]. RPA can protect exposed ssDNA, and *in vitro* observations suggest that RPA-RNA interactions may influence early R-loop nucleation under selected biochemical conditions; the contribution of this mechanism *in vivo* remains unclear[9,95]. Conflict-associated R-loops should therefore be treated as a heterogeneous group of three-stranded structures rather than as a synonym for all RNA-DNA hybrids.

Genome-wide mapping methods provide complementary but non-identical views of these structures. S9.6-based DNA:RNA immunoprecipitation (DRIP) approaches, catalytically inactive RNase-H-based methods such as R-ChIP[96], and nuclease-tethering strategies such as MapR[97], primarily detect RNA-DNA hybrids; they do not, by themselves, demonstrate the displaced ssDNA strand required for a bona fide R-loop. Their signals therefore should be interpreted as assay-dependent hybrid maps unless supported by structure-sensitive evidence.

Encounter orientation can bias hybrid accumulation, but the magnitude and direction of this effect depend on organism[4,39], locus, transcriptional activity, and experimental system[98]. HO encounters are associated with increased hybrid accumulation and damage in several bacterial and engineered reporter systems. In eukaryotes, convergence between CMG and RNAPII may constrain transcription-bubble dynamics and increase opportunities for nascent RNA reannealing[99,100]. Nevertheless, detection of a hybrid or R-loop alone does not predict whether a fork will slow, uncouple, restart, or collapse.

3.2 Topological regulation of R-loops

R-loop homeostasis is closely coupled to torsional stress generated during transcription and replication[21,101]. The twin-supercoiling-domain model predicts positive supercoiling ahead of a tracking polymerase and negative supercoiling behind it[24]. These topological states influence DNA strand separation, RNA reannealing, and hybrid stability. The enzymes that modulate them differ across organisms: eukaryotic topoisomerase 1 (TOP1) and TOP2 act within chromatinized genomes[102,103], whereas bacterial DNA gyrase

actively introduces negative supercoils and has no direct eukaryotic counterpart[104]. Cross-species comparisons are therefore most informative when framed around the shared topological problem rather than presumed enzymatic equivalence.

In *Bacillus subtilis* reporter and genomic systems, HO conflicts have been associated with gyrase-dependent changes in local supercoiling and increased R-loop formation[31,39]. One model proposes that gyrase first relieves positive supercoils and subsequently generates an underwound domain that favors RNA reannealing. The extent to which gyrase redistributes torsional stress at individual conflicts remains difficult to measure directly[31]. In chromatinized eukaryotic genomes, nucleosomes[105], TOP1 and TOP2 activity[106], chromosome architecture[107], and topological domain[108] boundaries are likely to impose a different set of constraints.

3.3 R-loop processing and genome maintenance

Cells use partially overlapping pathways to limit R-loop-associated instability. ribonuclease H1 (RNase H1) directly removes hybrids by hydrolysing their RNA component[9,109]. RNase H2 is well known for initiating ribonucleotide-excision repair at single embedded ribonucleotides, but it can also process selected RNA-DNA hybrid substrates[56]. The two enzymes therefore contribute to R-loop control through distinct, partly overlapping biochemical activities.

RNA-DNA helicases and translocases provide additional, context-dependent routes for hybrid remodeling. Sen1/senataxin (SETX)[12], Pif1-family proteins and bacterial DinG[85] act on selected hybrid-associated substrates. In mammalian cells, DDX19[59] can resolve nuclear R-loops after replication stress or DNA damage, whereas DDX39A resolves replication-fork-associated RNA-DNA hybrids and regulates their processing by DNA2[71]. Conversely, DDX1 can promote R-loop formation from RNA G-quadruplexes[110], and DHX9 can promote R-loop formation when RNA splicing is impaired[111]. DDX and DHX therefore denote diverse helicase families, not uniform R-loop-resolving activities; each protein must be interpreted according to its substrate, locus, and cellular context.

Additional pathways operate in more restricted settings. In RNase H-deficient fission yeast, Dcr1 has been implicated in processing R-loop-associated transcriptional blocks at gene boundaries, whereas Ago1 has an opposing effect on genome stability[112]. These findings define an RNAi-linked response in a specific species and genotype rather than a conserved mammalian pathway.

In mammalian cells, studies also suggest an epitranscriptomic layer of R-loop regulation. METTL3-METTL14-dependent N6-methyladenosine (m^6A) deposition and YTH-domain readers can influence hybrid turnover[113], and YT521-B homology domain family (YTHDF)2-associated RNA decay has been linked to CCR4-NOT and XRN1 in selected contexts[114,115]. The relevant RNA substrates, genomic locations, and relationship to RNase-H- or helicase-mediated processing remain to be defined.

When hybrid-processing pathways are compromised, structure-specific nucleases including XPG, XPF, and MUS81 can process R-loop-associated DNA structures in selected cell systems[28,116]. Such processing can generate DNA damage and cytosol-exposed nucleic acids, but excised RNA-DNA hybrids are only one possible class of ligand; micronuclei, chromatin fragments, and other damage-derived DNA can also activate cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING)[29]. Links from R-loop accumulation to type I interferon signaling[28,117], the senescence-associated secretory phenotype, or senescence therefore depend on the lesion and experimental context[29,118].

4. The Conflict Management Arsenal

Cells manage TRCs through four connected, non-exclusive components: prevention, resolution, tolerance and recovery. Prevention lowers encounter probability; resolution removes or neutralizes an immediate obstacle; tolerance sustains replication or viability when complete resolution is delayed; and recovery restores replication continuity after fork perturbation. Bypass and repriming are mechanisms of tolerance, whereas recovery is defined by its downstream timing. Depending on the lesion and cellular context, these components may operate sequentially or in parallel.

Throughout this Review, prevention refers to mechanisms that lower the probability of an encounter before collision. Resolution removes or neutralizes the transcriptional complex or hybrid-containing structure that constitutes the immediate obstacle. Bypass denotes physical passage of the replisome past an obstacle that may remain DNA-associated or only partly remodeled. Tolerance is the broader outcome of maintaining DNA synthesis or viability despite incomplete immediate resolution and can include fork protection, bypass, repriming, gap formation and filling, or completion by a converging fork. Recovery encompasses events after fork slowing, reversal, or discontinuous synthesis, including restart, dormant-origin firing, homologous-recombination-mediated restart, gap repair and MiDAS, that restore replication continuity. Crucially, bypass does not necessarily clear the initiating obstacle, and recovery may occur downstream of an unresolved or only partially remodeled conflict. Figure 2 summarizes this hierarchy, and Table 1 assigns representative processes and factors to each component.

4.1 Prevention: Spatial and temporal coordination

Prevention lowers the probability that active replication and transcription occupy the same DNA segment at the same time. Transcription can redistribute minichromosome maintenance (MCM) complexes and influence origin usage, thereby changing both encounter probability and direction[119,132]. This coordination reduces risk but does not make CD geometry intrinsically benign. Nuclear positioning and checkpoint-dependent relocalization can further alter conflict probability in specific yeast and mammalian

systems. In human cells, CFAP20 supports promoter-proximal RNAPII progression under high Mediator-driven transcription and limits cis CD-conflict signals[133]; the accompanying effects on fork speed, gaps, and origin firing define the consequences of that perturbation rather than a general prevention pathway[133].

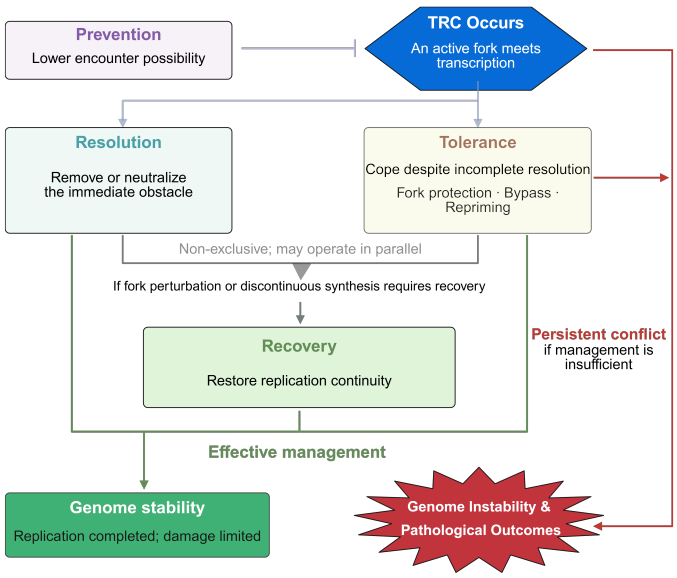


Figure 2. An operational framework for TRC management and genome maintenance. Prevention reduces the probability of encounters between active replication forks and transcription machinery. Once a TRC occurs, resolution removes or neutralizes the immediate obstacle, whereas tolerance preserves fork integrity or sustains replication despite incomplete obstacle removal. Fork protection, bypass and repriming are mechanisms of tolerance; bypass does not necessarily resolve the initiating obstacle. Resolution and tolerance are non-exclusive responses that may overlap or operate in parallel. When fork perturbation or discontinuous synthesis requires recovery, downstream mechanisms restore replication continuity. Recovery may therefore follow an unresolved or only partially remodeled conflict and is not obligatory for every encounter. Green paths indicate contributions of effective conflict management to replication completion and genome stability. The red path indicates that persistent conflicts under insufficient management can lead to DNA damage and genome instability. Arrows represent possible routes rather than an obligatory sequence. TRC: transcription-replication conflict.

Temporal coordination provides an additional preventive layer. In eukaryotes, replication timing regulatory factor 1 (RIF1)–protein phosphatase 1 (PP1) complex controls replication timing[134,135] by opposing phosphorylation of MCM and origin-firing factors[121,122]. Replication timing correlates with chromatin state and transcriptional activity, but early replication of active domains does not fully separate replication from transcription. Its protective contribution is therefore better understood as a change in encounter probability and locus context than as complete temporal segregation[135,136].

Bacterial genome organization provides a related preventive strategy. Essential and highly transcribed operons, including many ribosomal RNA loci, are frequently biased towards the leading strand[7]. The strength of this bias varies among species and reflects several selective pressures, but it is consistent with reduced exposure of selected genes to deleterious HO encounters.

4.2 Resolution: Removing or neutralizing immediate obstacles

Resolution acts on the immediate conflict-generating obstacle. Physical RNAPII displacement by helicases or translocases and direct cleavage or unwinding of an RNA-DNA hybrid are forms of resolution[85,87]. Transcription-complex processing may also neutralize the barrier without removing every component; this should be distinguished from bypass when the replisome passes an obstacle that remains DNA-associated or only partly remodeled.

The evidence for these responses is distributed across distinct experimental systems. In bacteria, Rep[85], UvrD[84,85], PcrA[123], and Mfd[86] provide examples of RNAP displacement or transcription-coupled processing[98,100]. In budding yeast, Pif1-family helicases act at highly transcribed loci, whereas mammalian studies implicate RECQL5, Cockayne syndrome B (CSB), RNase H1 and SETX in transcription-complex or hybrid processing[9,12,137]. These activities are not one-to-one equivalents across species. CUL3-KCTD10-dependent TCEA2 removal is classified below as remodeling-enabled tolerance because current evidence supports replisome passage after remodeling, rather than demonstrated clearance of the entire RNAPII complex[16].

Transcription-complex remodeling can instead enable bypass at selected human-cell CD conflicts. Fork protection by factors such as FANCD2-FANCI does not remove the initiating obstacle; it preserves fork integrity and supports subsequent recovery[10]. RECQL5 can reduce conflict formation by associating with RNAPII and modulating elongation, but its contribution depends on genomic and cellular context[124].

Table 1. Operational hierarchy of transcription-replication conflict management.

Layer/process	Representative mechanisms and factors	Main action	Key caveat/context
Prevention Spatial and temporal coordination	Transcription-dependent MCM redistribution[119]; promoter-proximal or intergenic origin usage[120]; Rif1-PP1[121,122]; leading-strand bias of highly transcribed bacterial genes[7,8]	Lowers encounter probability before collision	Changes encounter probability and locus context; does not fully separate replication from transcription.
Resolution Transcription-complex processing	Rep, UvrD, PcrA, Mfd (bacteria)[85,86,123]; Rrm3/Pif1 (yeast)[89]; RECQL5, CSB (mammals)[124]	Displaces or neutralizes the immediate transcriptional obstacle	Functions are system-specific, not direct cross-species equivalents.
Resolution RNA-DNA-hybrid/R-loop processing	RNase H1[9]; RNase H2[125]; SETX[126], AQR[127], DDX19[59]; DDX39A[71]; Sen1/Pif1-family[87]; DinG[85]	Cleaves RNA within a hybrid or remodels a hybrid-containing three-stranded structure	Effects depend on substrate and context; DDX1[110] and DHX9[111] can promote R-loop formation in selected settings
Tolerance Fork protection	RAD51[46], BRCA1[11], BRCA2[62], FANCD2-FANCI[128]	Preserves challenged or remodeled forks and limits nucleolytic degradation	Protects fork integrity but does not remove the initiating obstacle.
Tolerance Bypass/repriming/converging-fork completion	PrimPol[129]; CUL3-KCTD10-TCEA2 axis[16]; converging-fork completion[75]	Maintains DNA synthesis or viability despite incomplete immediate resolution	Bypass is one route to tolerance; selected CD-conflict pathways remain model-dependent.
Recovery Restart and post-replicative completion	SMARCAL1, HLTF, ZRANB3[64-66]; dormant-origin firing; HR-mediated restart[75]; PriA[74]; SLX4-MUS81-EME1[78,79]; POLD3-dependent MiDAS[79]	Restores replication after slowing, reversal, or under-replication	MiDAS is a late salvage pathway and not evidence that all lesions were TRCs.
Cross-cutting consequences Checkpoint/outcomes	ATR-CHK1[116]; ATM-CHK2[130]; p53[131]	Coordinates cell-cycle response, survival, arrest or inflammatory signaling	Outcomes depend on cell state, lesion burden, and signaling context.

The first term in each row identifies the framework component—prevention, resolution, tolerance or recovery—and the second identifies the process assigned to that component. Checkpoint signaling and pathological outcomes are shown as cross-cutting consequences rather than as a fifth management component. Assignments reflect the principal role emphasized in this Review and do not exclude additional functions. Factors from bacteria, yeast and mammals are not intended as one-to-one equivalents. MiDAS is classified as late recovery or salvage and is not, by itself, evidence that the initiating lesion was a TRC. MCM: minichromosome maintenance; MiDAS: mitotic DNA synthesis; TRC: transcription-replication conflict; ATR-CHK1: ataxia-telangiectasia and Rad3-related-checkpoint kinase 1; ATM-CHK2: ataxia-telangiectasia mutated-checkpoint kinase 2; SMARCAL1: SWI/SNF-related, matrix-associated, actin-dependent, regulator of chromatin, subfamily A-like 1; HLTF: helicase like transcription factor; ZRANB3: zinc finger RANBP2-type containing 3; CD: co-directional; SETX: senataxin; RNase H: ribonuclease H; CSB: Cockayne syndrome B; Rif1-PP1: Rap1-interacting-factor-1-protein phosphatase 1.

4.3 Tolerance: Continued replication without complete resolution

Tolerance describes maintenance of replication or cell survival despite incomplete immediate removal of the obstacle. Fork protection by FANCD2-FANCI and related factors preserves challenged-fork integrity without resolving the initiating barrier[10,94].

Bypass is one route to tolerance but is not synonymous with it: at selected CD conflicts, CUL3-KCTD10-dependent TCEA2 removal remodels RNAPII to facilitate replisome passage[16]. Tolerance can also involve downstream repriming, ssDNA-gap formation and filling, or completion by a converging fork. Reports of altered or faster fork progression at selected highly transcribed loci therefore identify context-dependent relationships, not a universal tolerance mechanism[133,137].

Transcription generates negative supercoiling behind advancing RNAPII[138,139]. During a CD encounter, a trailing fork may enter this underwound domain, potentially lowering the energetic cost of duplex opening[137,140]. This remains a biophysical model because local superhelical density, RNAPII position, CMG movement, and adenosine triphosphate (ATP) consumption have not been measured simultaneously on a chromatinized, transcribing template. Topological facilitation is therefore plausible under selected conditions but is not an established universal property of CD encounters.

Any contribution of transcription-generated topology is expected to depend on transcription-unit length[141], RNAPII-fork spacing[30], topoisomerase activity[142], domain boundaries[21,24,103], and chromatin constraints[34] on torsional diffusion. Existing observations are compatible with facilitation at selected loci, but origin placement, fork stabilization, sampling, and selection of conflict-resistant molecules remain alternative explanations[60,75]. Matched perturbations and direct single-molecule measurements will be needed to define the relevant topological range and any required replisome-stabilizing factors[16].

4.4 Recovery: Restoring replication after fork perturbation

Recovery is temporally downstream of fork perturbation or discontinuous synthesis. Depending on the intermediate, continuity can be restored by remodeling and restart of the existing fork, dormant-origin firing, homologous-recombination-mediated restart, converging-fork completion or post-replicative gap repair[65-67,76]. Under-replicated loci that persist into mitosis may undergo SLX4-MUS81-EME1-dependent processing and POLD3-dependent MiDAS[78-82]. These routes may follow resolution, tolerance or a persistent conflict, and several can operate in parallel; their activation does not by itself identify the initiating lesion as a TRC.

5. Technological Advances: Resolving Conflict Dynamics

5.1 Single-molecule fibers and acute protein degradation

Population-averaged approaches provided the first maps of replication intermediates and protein occupancy at conflict-prone loci, but they obscure the transient and heterogeneous behavior of individual encounters in asynchronous populations[4,143]. Single-molecule and acute-perturbation methods now make it possible to separate fork phenotypes that were previously conflated. A comparison of the principal experimental platforms used to study TRCs, including their major strengths and limitations, is provided in Table 2.

DNA-fiber assays[154] measure distributions of fork speed, asymmetry, stalling, and restart on individual labelled DNA molecules[144,155]. Sequential pulses of thymidine analogues such as 5-chloro-2′-deoxyuridine (CldU) and 5-iodo-2′-deoxyuridine (IdU) yield tract lengths from which average fork velocities and changes during defined labelling windows can be estimated. Because DNA is analyzed after labelling and spreading, conventional fiber assays are not real-time movies of replisome movement and can miss pauses below their spatial or temporal resolution.

Pairing fiber assays with rapid targeted degradation, including degradation tag (dTAG) and auxin-inducible degron systems, can distinguish immediate fork phenotypes from adaptations caused by chronic depletion[63,145-147]. This is particularly useful for essential or pleiotropic TRC factors. Acute degradation nevertheless requires controls for degradation kinetics, cell-cycle position, destabilization of associated complexes, and early checkpoint responses[16,146].

5.2 Sequence-resolved nanopore replication mapping

Optical DNA-fiber assays preserve single-fiber replication tracks but generally lack native sequence coordinates. Nanopore-based methods including DNAscent[20], NanoForkSpeed[148], and FORK-seq[149] detect incorporated thymidine analogues on long, sequence-mapped DNA molecules and infer origin firing, fork direction, progression, termination, velocity, and selected pausing signatures. Cas9-guided targeted nanopore sequencing is an enrichment strategy rather than a replication readout[156]. These methods report pulse-labelled DNA synthesis; DNAscent does not natively measure transcription, RNAPII occupancy, or the geometry of an RNAPII-replisome encounter on the same molecule.

Nanopore replication calls can be compared with independently generated RNA-seq, precision run-on sequencing (PRO-seq), RNAPII occupancy, chromatin, or R-loop maps[96,157]. Such integration associates fork behavior with transcriptional context at shared genomic coordinates. Unless both processes are measured on the same physical molecule, however, overlap remains a multi-omic association rather than direct observation of a collision. Altered fork speed in highly transcribed regions may reflect topology, chromatin, origin placement, fork stabilization, or sampling and does not by itself show that RNAPII density directly accelerates CMG[61,133,158].

5.3 Genome-wide topology mapping and multi-omic integration

Genome-wide topology assays provide conformation-sensitive proxies rather than calibrated measurements of torsional stress[150]. bTMP-seq[24] exploits preferential psoralen intercalation and crosslinking in underwound, accessible DNA, followed by enrichment of biotinylated fragments[22,23]. Its signal is also influenced by chromatin accessibility, nucleosome occupancy, crosslinking chemistry, and sample processing; it therefore cannot be interpreted as an absolute torque map or direct visualization of a transcriptional wake.

Table 2. Experimental platforms used to study transcription-replication conflicts.

Method	What it measures	Major strengths	Key limitations/caveats	Ref.
DNA-fiber assay (with or without acute degrons)	Single-fiber tract lengths, fork speed distributions, sister-fork asymmetry, stalling and restart phenotypes	Direct single-molecule readout of labeled DNA synthesis; can be paired with rapid perturbation	Endpoint assay, not real-time imaging; mechanistic interpretation needs orthogonal validation.	[63,144-147]
DNAscent/NanoForkSpeed/FORK-seq	Long-read sequence-mapped replication features such as origin firing, direction, termination, velocity, and candidate pausing signatures	Connects replication behavior to genomic coordinates	Does not directly measure RNAPII occupancy or same-molecule collisions.	[20,148,149]
bTMP-seq	Relative enrichment of underwound, accessible DNA consistent with negative supercoiling	Genome-wide proxy for underwound, accessible DNA	Influenced by chromatin accessibility and chemistry; not an absolute torque measurement.	[21,24]
GapR-seq	Relative enrichment of overtwisted DNA associated with positive supercoiling	Complements bTMP-based maps	Signal depends on GapR binding, chromatin accessibility, and protein occupancy.	[150]
DRIP-seq	S9.6-based immunoprecipitation of RNA-DNA hybrids	Widely used hybrid-mapping approach in R-loop studies	Does not directly detect the displaced ssDNA strand; affected by substrate preference, dsRNA cross-reactivity and sample processing.	[93]
R-ChIP	Catalytically inactive RNase H1-based chromatin capture of RNA-DNA hybrids	Higher specificity for RNase-H1-bound hybrids	Requires engineered reagent expression and captures a method-dependent subset.	[96]
MapR	Catalytically inactive RNase H-MNase local cleavage near RNA-DNA hybrids	Antibody-independent mapping with relatively high local resolution	Method-dependent substrate capture and processing biases; no direct displaced-ssDNA readout.	[97]
SMRF-seq	Non-denaturing bisulfite footprinting of displaced ssDNA within intact R-loop structures	Single-molecule structural evidence for the displaced strand of a bona fide R-loop	Lower throughput and specialized analysis.	[151]
Integrated multi-omics	Comparison of fork maps with transcription, chromatin, topology, and R-loop data	Helps prioritize loci and distinguish competing mechanistic models	Usually association-based, not direct same-molecule proof of collisions.	[152,153]

The table summarizes the principal readout, strengths and limitations of DNA-fiber assays, nanopore-based replication mapping, topology-sensitive bTMP-seq and GapR-seq, RNA-DNA-hybrid mapping by DRIP-seq, R-ChIP and MapR, structure-sensitive R-loop analysis by SMRF-seq, and integrated multi-omic analysis. Hybrid-detection methods do not, by themselves, demonstrate the displaced ssDNA strand of a bona fide R-loop. Likewise, overlap between independently generated replication, transcription, chromatin and hybrid/R-loop maps is associative unless the relevant features are measured on the same physical molecule. RNAPII: RNA polymerase II; ssDNA: single-stranded DNA; DRIP-seq: DNA:RNA immunoprecipitation sequencing; ChIP: chromatin immunoprecipitation; bTMP-seq: biotinylated trimethylpsoralen-based sequencing; RNase H: ribonuclease H.

GapR-based mapping uses the preference of the bacterial GapR protein for particular overtwisted DNA conformations associated with positive supercoiling[22,150]. Enrichment can identify candidate overtwisted or topologically compressed regions, but it remains sensitive to protein binding, occupancy, sequence, and chromatin context. GapR signal neither measures force directly nor identifies the machineries that generated a topological state.

bTMP-seq[24] and GapR-seq[150] thus provide complementary, assay-dependent proxies for underwound and overwound DNA[22]. Integration with hybrid/R-loop maps, replication timing, transcription, and accessibility data can prioritize candidate conflict zones, but overlap among population-averaged tracks does not establish that replication and transcription collided on the same molecule. Locus-specific perturbations and genuinely joint single-molecule measurements are required to test that inference.

Integrating replication, topology, RNA-DNA-hybrid/R-loop, transcription, and chromatin data can distinguish competing models, provided the biochemical and spatial biases of each layer are considered. Repli-seq profiles population replication timing[159]; assay for transposase-accessible chromatin using sequencing (ATAC)-seq measures accessibility[160]; RNA-seq[161] and PRO-seq[162] capture different aspects of transcription; and DRIP-seq[163], R-ChIP[164], MapR[165], and single-molecule assays[61] detect non-identical hybrid populations. Concordant signals can support a mechanism but cannot predict the fate of an individual encounter. Causal inference requires timed perturbations, matched controls, and direct validation in a defined organism and experimental system. In summary, population-level spatial overlap across replication, transcription, R-loop, chromatin, and topological datasets merely highlights potential collision zones and cannot, by itself, demonstrate a bona fide physical collision occurring synchronously on the same DNA molecule.

6. Pathological and Therapeutic Implications

6.1 TRCs in cancer: Oncogene-induced stress and context-specific vulnerabilities

Disrupted coordination between transcription and replication contributes to oncogene-associated replication stress in selected models. MYC[166], KRAS[167,168], and cyclin E[169] can alter transcriptional output, origin usage, cell-cycle timing, nucleotide metabolism, and redox state. MYC-driven licensing changes and shortened G1 have been linked in some systems to intragenic initiation and an increased probability of encounters with active RNAPII. The proportion of oncogene-associated damage caused directly by TRCs, rather than nucleotide limitation, oxidative stress, excess origin firing, or other barriers, remains unresolved.

Metabolic rewiring can further increase replication stress[25,168]. In KRAS-driven malignancies, altered glucose and glutamine metabolism can constrain nucleotide production or redox balance[167,168]. Reduced deoxyribonucleoside triphosphate (dNTP) availability slows DNA synthesis, promotes helicase–polymerase uncoupling and RPA-coated ssDNA, and can prolong fork exposure to transcriptional barriers[25,170]. Basal TRC burden nevertheless varies with genotype, cell state, and assay, precluding broad quantitative comparisons without matched measurements.

TRC-associated stress can create dependencies on checkpoint and recovery pathways in selected preclinical models[4,41]. ATR-CHK1 signaling restrains excess origin firing and supports challenged forks, providing a rationale for molecularly selected studies of ATR or CHK1 inhibition[170,171]. Other proposed interventions act through distinct mechanisms: AOH1996 targets PCNA-associated processes[172], BBI-2779 exploits CHK1 dependence in ecDNA-positive models[173], and CDK9 inhibition with AZD4573 perturbs transcription elongation[174]. Their therapeutic activity therefore cannot be considered TRC-specific unless conflict burden is measured directly and shown to be causally linked to treatment response. These studies nominate testable vulnerabilities but do not establish a common TRC-specific biomarker or therapeutic window.

KCTD10-dependent remodeling represents another context-dependent vulnerability. DepMap analysis identified KCTD10 dependency in 51 of 1,100 cancer cell lines, with enrichment in breast invasive carcinoma, diffuse glioma, and ovarian epithelial tumor models; KCTD10-dependent states were also associated with HER2 activation, damaging *TP53* mutations, and AKT-, ERBB2-, MYC-, and MEK-related transcriptional programs[91]. These associations support a model in which KCTD10 becomes important when CD-conflict stress exceeds the capacity of parallel genome-maintenance pathways[16]. They do not yet establish KCTD10 as a drug target: targetability, predictive biomarkers, and tolerability in normal proliferating tissues remain to be tested.

6.2 TRCs in cellular senescence and ageing

Unresolved TRCs may contribute to cellular senescence in defined proliferative models[4,102]. Persistent fork stalling or incomplete replication can generate lesions and sustained ATM- or ATR-dependent signaling that engages p53-p21 and RB-dependent arrest[175,176]. The extent to which TRCs contribute to stem-cell exhaustion or tissue degeneration relative to other sources of replication stress and inflammation remains uncertain and will require *in vivo* testing.

Persistent TRCs and R-loops have also been linked to innate immune signaling in defined settings[28,51]. Processing of unresolved hybrids or associated lesions can generate cytosol-exposed nucleic acids, including RNA-DNA hybrids, that engage cGAS-STING[29,177]. Downstream TBK1-IRF3 signaling can promote type I interferon responses and components of the senescence-associated secretory phenotype[178,179]. The relative contributions of excised hybrids, micronuclei, and other damage-derived ligands remain unresolved.

These pathways may support cell-autonomous and paracrine inflammation, but a direct route from individual TRCs to organismal inflammageing has not been established.

6.3 From bacterial evolution to neurodegeneration

TRCs can provide a context-dependent source of mutation and selection, particularly in bacteria. Many prokaryotic genomes bias essential and highly transcribed genes towards the leading strand, consistent with selection against deleterious HO encounters[7,8,180]. Lagging-strand genes show increased conflict-associated mutagenesis in some species and growth conditions, but this is not a universal feature of virulence or environmentally responsive genes[7]. PcrA and related bacterial factors are therefore candidate antimicrobial targets, although target validity, selectivity, and the consequences for resistance evolution remain to be established.

Failure to control transcription-associated DNA structures is linked to neurological disease. *SETX* mutations cause ataxia with oculomotor apraxia type 2 and amyotrophic lateral sclerosis type 4 (ALS4)[181,182]. A patient-derived iPSC study reported as a preprint links ALS4-associated *SETX* variants to RNA dysregulation and loss of motor-neuron identity[183], while acute *SETX* depletion perturbs RNAPII elongation and backtracking beyond termination sites[184]. SETX also resolves R-loops at termination regions[12] and other transcription-associated loci[184,185]. Canonical replication-transcription conflicts are most relevant to cycling neural progenitors; in post-mitotic neurons, SETX-associated pathology is better considered within a broader framework of transcription-associated DNA damage. The fraction of disease pathology attributable specifically to replication–transcription collisions remains unknown.

7. Conclusions and Future Perspectives

TRCs are context-dependent encounters whose outcomes are shaped by orientation, topology, transcription, chromatin, and the availability of prevention, resolution, bypass, tolerance, and recovery pathways. Studies of RNAPII polarity[30], sequence-resolved single-molecule replication[20,61,133], and CUL3-KCTD10-dependent remodelling[16] have expanded the mechanistic landscape without revealing a single route through transcribed chromatin[60,186]. A central challenge is to connect defined biochemical intermediates to locus-specific outcomes while keeping direct observation, multi-omic association, and mechanistic inference distinct.

7.1 How is encounter direction recognized?

How cells distinguish HO from CD encounters remains unresolved. Recent findings support a model in which KCTD10 bridges PCNA-associated replication machinery and RNAPII components at selected CD conflicts[16], although its designation as a sensor does not yet imply a defined molecular recognition mechanism. CFAP20 provides a complementary but mechanistically distinct example. Rather than directly sensing encounter orientation, CFAP20 promotes the productive elongation or clearance of slowly elongating, R-loop-engaged RNAPII in promoter-proximal regions, thereby keeping transcription complexes ahead of replisomes that frequently initiate nearby and progress co-directionally[133]. Its apparent CD selectivity may therefore arise from promoter–origin organization and transcription-unit orientation, rather than from direct recognition of CD geometry. Possible routes to orientation-specific recognition include geometry-dependent protein interactions, responses to topological compression[24,187], and indirect sensing of ssDNA or checkpoint-signaling architectures[32,99]. These mechanisms are not mutually exclusive.

7.2 The molecular basis of tolerance

The determinants of transcription-associated changes in fork speed remain unresolved. Negative supercoiling behind RNAPII could lower the energetic barrier to duplex unwinding[137,140]. Rapid progression through transcribed chromatin may also require active fork stabilization by factors such as AND-1[188] or TIMELESS-TIPIN[189]. Reconstituted replication–transcription systems on topologically defined, chromatinized templates will be needed to determine whether topology is sufficient and to define the contribution of replisome-stabilizing factors[39].

7.3 Molecular consequences of bypass

If KCTD10-dependent remodeling enables replisome bypass, the consequences for RNAPII occupancy and leading-strand synthesis remain unknown[16]. A DNA-bound transcription complex could promote transient helicase–polymerase uncoupling or discontinuous synthesis, but extensive ssDNA gaps have not been shown to be an obligatory product of this pathway[61,190]. PrimPol-dependent repriming and translesion-polymerase-mediated gap filling are testable recovery models rather than demonstrated requirements[129]. Resolving these possibilities will require matched genetic perturbations and orthogonal gap-mapping methods that distinguish gaps from breaks and other sources of replication stress[191].

When an RNAPII complex is displaced, the fate of its nascent transcript is also unclear[98,100]. Prematurely terminated RNAs may be substrates for nuclear RNA-quality-control pathways, including the *Tr*f4p/*A*ir2p/*M*tr4p *p*olyadenylation (TRAMP)[192] or Nuclear Exosome Targeting (NEXT)[193] exosome complexes, but recruitment of these pathways to collision sites has not been demonstrated. Testing this possibility would connect TRC processing to transcriptional surveillance and RNA homeostasis.

7.4 Do condensates organize TRCs response?

RNA-driven phase separation of DNA-damage-response factors has been reported at double-strand breaks[194], but analogous

condensate-like compartments have not been demonstrated at endogenous TRCs. Puncta, colocalization, or fluorescence recovery alone do not establish liquid–liquid phase separation. Any proposed TRC-associated condensate must also be distinguished from a secondary DNA-damage-response assembly that forms only after fork collapse or DNA breakage. Establishing a direct role in conflict processing will require temporal ordering before overt damage, locus-specific recruitment, perturbation of multivalent interactions, quantitative analysis of material properties and evidence that disrupting the assembly changes a defined conflict outcome.

7.5 Translational outlook

Replication stress is common in proliferating, oncogene-driven tumors[158,195], but dependence on individual TRC-response pathways is likely to be highly context specific. Translation will require a therapeutic window between selected TRC-burdened tumors and normal proliferating tissues. Composite biomarkers may integrate oncogene or ecDNA status[173], replication-stress markers[196], pathway genotype or expression[196], and functional R-loop measurements[164]; none is currently validated as a stand-alone clinical TRC biomarker. Structural analysis of complexes such as KCTD10-PCNA-RNAPII may identify selective intervention points[16], but targetability and normal-tissue tolerability must precede clinical development.

Declarations

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Authors contribution

Wang Q, He J: Conceptualization, investigation, writing-original draft, visualization.
Duan Z: Investigation, writing-review & editing.
Huang J: Conceptualization, supervision, project administration, funding acquisition, writing-review & editing.
All authors read and approved the final manuscript.

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