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When DNA topology turns deadly:

RNA polymerases dig in their R-loops to stand their ground: New positive and negative (super)twists in the replication-transcription conflict

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Abstract

Head-on replication-transcription conflict is especially bitter in bacterial chromosomes, explaining why actively-transcribed genes are always co-oriented with replication. Yet, the mechanism of this conflict remains unclear besides the anticipated accumulation of positive supercoils between head-on-conflicting polymerases. Unexpectedly, experiments in bacterial and human cells reveal that the head-on replication-transcription conflict induces R-loops, indicating hyper-negative supercoiling in the region, exactly the opposite of the assumed. Further, as a result of these R-loops, both replication and transcription in the affected region permanently stall, so the failure of R-loop removal in RNase H-deficient bacteria becomes lethal. How hyper-negative supercoiling emerges in the middle of a positively-supercoiled chromosomal domain is a mystery that requires re-thinking of topoisomerase action around polymerases. <115>

Keywords

replication-transcription conflicts; supercoiling; R-loops; RNase H; topoisomerases

Replication-transcription conflicts and the topoisomerases

In the bacterial chromosome, the slow and extremely stable transcription elongation complexes (TECs) clash with 10-fold faster replication forks independently of the gene orientation. Perhaps most graphically the reality of this conflict is illustrated by the associated mutagenesis [1], especially by the recent demonstration of the elaborate gradients of various types of orientation-dependent mutations [2]. However, the physiological consequences of the co-directional conflict are barely detectable [3]. This is in sharp contrast with the magnitude of the head-on conflict, long ago proposed to explain the co-orientation of heavily-expressed genes with replication [4], and then amply confirmed with physical and genetic experiments documenting serious replication problems [5–7]. Merrikh and

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colleagues now report unexpected conditions under which the head-on conflict in bacteria turns outright deadly [8].

Why would the two processes clash so bitterly in the head-on orientation? There are three aspects that conspire in the aggravation. First, in both bacterial and eukaryotic cells, replication factories [9–11] and transcription factories [12–14] (henceforth simply “factories”) are static, pulling through the mobile template DNA. Second, any such factory that not only pulls the DNA through processively, but also has to unwind the double helix for proper function, generates positive supercoiling ((+)sc, overtightening of the DNA helix) in the entering DNA and the same degree of negative supercoiling ((-)sc, undertightening of the DNA helix) in the exiting DNA [9, 12, 15, 16] (Fig. 1). Third, the two types of supercoiling, (+)sc versus (-)sc, appear to be relaxed (returned to normal duplex DNA coiling) in two distinct ways.

The (-)sc relaxation behind the factories is accomplished proximally, mostly by type I topoisomerases. In case of transcription, in both pro- and eukaryotes, type I topoisomerases are recruited by their RNA polymerase-interacting domains [17, 18]. In case of replication, most of the (-)sc relaxation should happen spontaneously, before maturation of Okazaki fragments (which in vivo form in both the leading and the lagging strands [19]). The recruitment mechanisms to relax the remaining negative supercoiling behind the replication factories are less clear: in bacteria like *E. coli*, Topo IV (type II topoisomerase) directly interacts with the nascent DNA-organizing protein SeqA [20]; in eukaryotes, topoisomerase recruitment may be a function of the replicative helicase [21].

In contrast to the factory-proximal targeted (-)sc relaxation, no mechanisms are known to target the (+)sc-relaxing topoisomerases (only type II in bacteria, and predominantly type II in eukaryotes) to the template DNA ahead of a replication fork or a TEC, to act on the template DNA being pulled into the factory. Type II enzymes may not need this factory recruitment, as their high affinity to (+)sc DNA [22, 23] should allow them to remove the bulk of (+)supercoils wherever they form in the chromosome, acting distributively in rapid bursts. At the same time, the complete removal of (+)sc and especially introduction of (-)sc is a harder task for type II enzymes and, to be processive, requires specific binding sites. Therefore, the overall population of Type II enzymes is split between those distributively-acting at replication bubbles and those processively acting at their strong binding sites [24]. Specifically, compared with (-)sc-relaxing Topo I in *E. coli* that acts at random sites [17], gyrase strongly prefers specific sites that function as “topological insulators” blocking (+)sc spread [25]. In general, type II topoisomerases are found associated with the chromosome scaffold that condenses DNA loops, both in eukaryotes [26–28] and in prokaryotes [29, 30]. In other words, chromosomal scaffold define boundaries of topological domains in chromosomal DNA by housing type II topoisomerases there (Fig. 1AB). Indeed, when the cells are treated with inhibitors of type II topoisomerases that disrupt enzymes’ breakage-reclosing cycle, chromosomes of both prokaryotes and eukaryotes release linear pieces (“chromatin loops”) corresponding in size to the expected distances between domain boundaries, indicating that type II topoisomerases act at the base of these loops [31, 32].

Could the gravity of the head-on replication-transcription conflict be a reflection of this difference between targeted and proximal (–)sc-relaxation and distributive or distal (+)sc-relaxation? Topological problems are not detected when a replication fork overcomes a transcription complex from the back, as the fast prokaryotic forks can apparently dislodge co-directional TECs [33], while the eukaryotic replication forks do not even have to slow down, having equal speed with transcription [34]. At the same time, the conspicuous head-on conflicts, whether natural or engineered [5, 6, 33, 35–37], suggest that the presumed accumulation of positive supercoiling between the two converging factories [4, 35, 36] is somehow not met by the adequate topoisomerase capacity of the cell (Fig. 1C). This is consistent with the model that the topoisomerases that can relax (+)sc *completely*, do not act distributively and are instead concentrated at topological domain boundaries (Fig. 1C), to which positive supercoils may need to be delivered.

Let us remember these suspected differences in the topoisomerase distribution around polymerases when we eventually consider the remarkable observation of Merrikh and colleagues [8]; but first — a primer on R-loops, to clarify one important confusion.

R-loops 101

RNA strand invasion into the corresponding duplex DNA displaces the identical DNA strand into a single-stranded “replacement” loop called “R-loop” [38]. Formation of R-loops is facilitated (somewhat) by the higher stability of some DNA-RNA hybrid sequences [39, 40] and (mostly) by negative superhelicity of target DNA [41–44] (Fig. 2A). On the other hand, formation of R-loops is prevented by any complexing of the nascent RNA strand (on itself in secondary structures, by specialized protein binding in eukaryotes [45], by translation in prokaryotes [46]), by the target DNA complexing into nucleosomes [47] and by target DNA relaxation [41]. Dissociation of R-loop is fast in the presence of the smallest amount of (+)sc [44] (Fig. 2A).

Therefore, the best chance for R-loops to form is behind transcribing RNA polymerases [43, 48] (Fig. 2B), in those rare instances when type I topoisomerases are looking away, translation fails to follow prokaryotic transcription closely (for example, during cold-shock [46]) or when histones are slow to redistribute to the DNA duplex in the wake of eukaryotic transcription complex [47]. However, R-loops are also thought to readily form independently of transcription (spontaneously, once the DNA-bound proteins are removed [43, 49], or catalyzed by strand-invasion activities [50]) or to persist after transcription termination [51]. For whatever reason, such protein-free R-loops are considered stable enough to stall replication forks [52–54].

In general, R-loops are implicated in a phenotype, if the effect: 1) depends on transcription, especially in particular orientation; 2) the effect is alleviated by overproduction of an RNase H endonuclease specific against RNA-DNA hybrids (see below); 3) RNA-DNA hybrids in the region are detected by specific antibodies [55]. Although all three assays are indirect (transcription may cause various effects; RNase H could degrade R-loops somewhere else; the antibodies actually detect any DNA-RNA hybrids, not only R-loops), the combination of the three criteria is taken to implicate R-loops in the problem.

“All R-loops are equal, but some R-loops are more equal than others”

In fact, failure to distinguish transcription-independent R-loops from transcription-associated R-loops creates unnecessary confusion, because of the huge difference in their stability. R-loops that are not associated with transcription elongation complexes, which we will refer to as “TEC-free R-loops”, are rather unstable in relaxed DNA [38, 41] and should be definitely expelled by overtightening (positive supercoiling) of the target DNA (Fig. 2A) [44], so would not be able to act as a replication forks barrier. Also, replicative helicases encountering DNA-RNA hybrids have no problem unwinding them, at least in vitro [56]. Finally, there are specialized helicases that remove R-loops, both in vitro and in vivo (for example, RecG [57, 58] and DinG [59, 60] in *E. coli*).

In contrast, R-loops behind transcribing RNA polymerases (Fig. 2B) must be extremely stable. Transcribing RNA polymerases by themselves are one of the most stable protein complexes on DNA [61]. In principle, attaching them to DNA via their own transcript forming a short R-loop (like an anchor with a cable) should stall their further progress, self-limiting the problem. However, the stalling cannot be efficient, partly because TECs are so robust, but also because, by continuing transcription, TECs are extending the very cable that secures them to the anchor. Remarkably, this continuation of transcription aggravates the problem: since the R-loop anchor effectively traps the generated negative supercoiling between the TEC and the anchor, all the newly-generated supercoils should quantitatively feed into elongation of the R-loop (Fig. 2B). Such a positive feedback further increases the strength of the overall complex. To distinguish them from unstable TEC-free R-loops, we will abbreviate R-loop-anchored transcription elongation complexes as R-loop-aTECs. In fact, formation of R-loop-aTECs is such a serious problem for the chromosome, that all cells have specialized RNases to remove these stable R-loop anchors.

These are RNase H endonucleases, attacking the RNA part of long RNA-DNA hybrids [62, 63]. Other classes of RNase H enzymes exist, some incising single ribonucleotides in DNA, others being exoribonuclease activities of reverse transcriptases [62, 63]. However, it is the RNase H endonucleases attacking long RNA-DNA hybrids, which are critically important for the cell, attested by a noticeable growth defect due to their inactivation in bacteria and their essentiality in higher eukaryotes. For the rest of the paper, when we mention “RNase H”, we will mean only the R-loop-attacking enzymes.

The unexpected and perplexing finding

Now that we know how to better distinguish the innocuous TEC-free R-loops from the dangerous R-loop-aTECs that need RNase H attention, we can fully appreciate the significance of the findings of Merrikh and colleagues [8]. They found that a highly-transcribed gene in the chromosome of growing *Bacillus subtilis* stimulates formation of DNA:RNA hybrids in the region, 4–5-fold over the background, but only if the gene is oriented head-on to replication, and only in the *rnhC* mutant, deficient in the main RNase H enzyme, suggesting significant R-loops accumulation in the replicating head-on construct, and their removal by RNase H (Fig. 1 in [8]). This was paradoxical, as the arrival of a replication fork in the region is expected to generate so much positive supercoiling, that any

negative supercoiling from any source should be cancelled out. They also detected significant dwelling of the replicative polymerase and accumulation of replication forks in the region when the conflict was head-on, especially in the *rnhC* mutant. They verified this by the replication profile of the whole chromosome, which strikingly confirmed massive replication forks stalling in the 20 kb region downstream of the induced head-on transcription unit in the absence of RNase H (Fig. 2 in [8]). Perhaps not surprisingly then, the fully-induced head-on transcription killed the *rnhC* mutant cells, while RNase H production revitalized the mutants (Fig. 3 in [8]).

Even though this is less than half of all their results, we need a pause here, to appreciate the topological paradox of these observations. First of all, these results confirm an earlier surprising report of R-loops formed at the site of a head-on replication-transcription conflict in *E. coli* [60]. Formation of R-loops associated with transcription complexes literally next door to a supposedly massive pile-up of positive supercoils indicates that factories form topologically-insulated domains of their own. Moreover, complete replication inhibition, supposedly indicative of the positive supercoils piling up between the converging factories, suggests that (+)sc-relaxing type II topoisomerases either cannot reach their substrates or cannot remove (+)sc completely. In other words, 1) factories are not associated with the topoisomerase-housing chromosomal scaffold; 2) factories do not recruit (+)sc-relaxing topoisomerases. A similar observation of R-loops due to head-on replication-transcription conflict in human cells indicates the trans-kingdom nature of these unusual polymerase-topoisomerase phenomena [64].

But let us continue with the results of Merrikh and colleagues. Turning their attention to transcription now, the authors measured transcription levels of the two constructs and found that transcription of the head-on construct in the *rnhC* mutants is significantly lower than the one in co-directional construct, but only in the replicating cells (Fig. 5 in [8]). In the nonreplicating cells, transcription in both orientations was fine, suggesting that R-loop-aTECs are not formed in the absence of replication. Even more surprisingly, according to the authors' personal communication, a modest inhibition of type II topoisomerases relieves the inviability of the head-on conflict in the *rnhC* mutants, suggesting that part of the problem is accumulation of hypernegative supercoiling, most likely behind the conflicting TECs. In other words, (i) positive supercoiling between the two converging factories is in fact communicated to the nearest (+)sc-relaxing station; (ii) the station is taking the appropriate action, as R-loop formation indicates hypernegative supercoiling accumulating behind TECs; (iii) yet this action fails to alleviate the conflict between the two converging polymerases. The TEC- based boundary between the two neighboring topological domains in this case apparently prevents (+)sc and (–)sc from cancelling each other.

Model: abrupt replication fork regression spawns R-loop-aTECs

However, if the boundary is strong, then how does the “relaxation station” know there is positive supercoiling in the region? A more realistic assumption is that some positive supercoiling does seep through the TEC barrier making gyrases work harder, but not hard enough to remove (+)sc completely. A generic scenario of what happens next envisions that accumulation of positive supercoiling ahead of a replication fork will not only stall the fork

(Fig. 3B, Key Figure), but will eventually trigger sudden removal of the bulk of (+)sc (Fig. 3C, Key Figure). According to this scenario, the accumulation of positive supercoiling between the two factories partially compensates for the negative supercoiling behind the RNA polymerase, preventing R-loop formation when both complexes still progress (Fig. 3AB, Key Figure). The translating ribosomes covering the nascent transcript also block R-loop formation. The sudden disappearance of the bulk of (+)sc “jump-starts” rapid progress of RNA polymerase, which synthesizes a stretch of mRNA that is not covered by ribosomes (Fig. 3C, Key Figure). But even more importantly, in the absence of the compensating positive supercoiling, there is now a surge of hyper-negative supercoiling in the region (Fig. 3C, Key Figure) due to the hyperaction of (+)SC-relaxing topoisomerases at domain boundaries, which, in combination with the empty transcript, spawns formation of R-loop-aTEC (Fig. 3D, Key Figure). Eventually, the RNA polymerase also grinds to a halt, unable to reach the end of the gene, being securely anchored by the ever-growing R-loop. Such an extended R-loop-aTEC, in the absence of RNase H activity, may become an impenetrable barrier for subsequent DNA replication through the region, killing the cell.

What could cause the proposed sudden drop in (+)sc between the two converging factories? Typical suspects [65] include replication fork rotation (Fig. 4B-1), for example [66], and replication fork regression (Fig. 4B-2), for example [6]. One more obvious possibility is the distributive and incomplete action of (+)sc relaxases (Fig. 4B-3). Intriguingly, all three scenarios predict differences in the position of the replisome relative the replication fork: the replication fork rotation idea predicts complete replisome displacement (Fig. 4B-1), the replication fork regression idea predicts replisome association with the regions upstream of the replication point (Fig. 4B-2), while the partial (+)sc removal idea predicts that the replisome stays put at the replication point (Fig. 4B-3). These might be distinguishable by ChIP-based approaches.

Conclusion: Accumulation of positive supercoiling as a recently recognized challenge for the cell

Accumulation of positive supercoiling was never considered to be a problem because of the rarity of strong head-on conflicts, the high efficiency of type II topoisomerases and their presumed distributive action. Some of these assumptions regarding chromosomal topology in vivo need reevaluation in the light of the work by Merrikh and colleagues, who show that a lot of stress-response genes in *Bacillus subtilis* repressed during normal growth, are oriented head-on to replication and thus mount strong replication-transcription conflict during the corresponding stress (Fig. 6 in [8]). There are other indications of the actual severity of the problem of positive supercoil accumulation and of the proactive approach cells are taking against it. The mysterious SMC5/6 complex of higher eukaryotes is loaded between replication forks and head-on oriented genes [67], and at the same time binds Topo II [68], suggesting that this overlooked cohesion/condensin relative recognizes and associates with the loops of positive supercoils to bring them to the attention of type II topoisomerases.

It is likely that the impact of positive supercoiling was underappreciated because detection of transient and local supercoiling in DNA is non-trivial. Also, high degree of positive supercoiling between two DNA machineries may be simply impossible if one of them is a replication fork, as forks may respond to accumulating positive supercoils ahead of them by rotation [69] or by regression, turning into Holliday junctions [70, 71]. In other words, replication fork inactivation may be the simplest indication of accumulating positive supercoiling.

It looks like there is a real problem that some positive supercoils could be shielded from nearby relaxation stations by formation of independent transcription-induced topological domains with the help of R-loop-aTECs. In addition to destroying R-loop-aTECs with a robust RNase H activity, thereby disrupting the spurious topological domains, the cell may employ an additional strategy against this problem, by actively chaperoning (or reporting) positive supercoils to the nearby relaxation stations. How R-loop-aTECs induce independent topological domains and how cells deal with the problem may define a new area of exciting research (see “Outstanding Questions”). In general, the chromosomal topology around factories, especially between colliding replication and transcription factories, seems to be more “twisted” than previously assumed and requires further studies. <2,889>

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Glossary

(+)sc	positive supercoiling
(-)sc	negative supercoiling
TEC	transcription elongation complex
TEC-free R-loop	transcription elongation complex-free R-loop
R-loop-aTEC	R-loop-anchored transcription elongation complex

References

1. Merrikh H. Spatial and Temporal Control of Evolution through Replication-Transcription Conflicts. *Trends Microbiol.* 2017; 25:515–521. [PubMed: 28216294]
2. Sankar TS, et al. The nature of mutations induced by replication–transcription collisions. *Nature.* 2016; 535:178–181. [PubMed: 27362223]
3. Merrikh H, et al. Co-directional replication-transcription conflicts lead to replication restart. *Nature.* 2011; 470:554–557. [PubMed: 21350489]
4. Brewer BJ. When polymerases collide: replication and the transcriptional organization of the *E. coli* chromosome. *Cell.* 1988; 53:679–686. [PubMed: 3286014]
5. Wang JD, Berkmen MB, Grossman AD. Genome-wide coorientation of replication and transcription reduces adverse effects on replication in *Bacillus subtilis*. *Proc Nat Acad Sci USA.* 2007; 104:5608–5613. [PubMed: 17372224]
6. De Septenville AL, et al. Replication fork reversal after replication-transcription collision. *PLoS Genet.* 2012; 8:e1002622. [PubMed: 22496668]

7. Mangiameli SM, et al. Transcription leads to pervasive replisome instability in bacteria. *Elife*. 2017; 6:e19848. [PubMed: 28092263]
8. Lang KS, et al. Replication-Transcription Conflicts Generate R-Loops that Orchestrate Bacterial Stress Survival and Pathogenesis. *Cell*. 2017; 170:787–799. [PubMed: 28802046]
9. Cook PR. The nucleoskeleton and the topology of replication. *Cell*. 1991; 66:627–635. [PubMed: 1652367]
10. Lemon KP, Grossman AD. The extrusion-capture model for chromosome partitioning in bacteria. *Genes Dev*. 2001; 15:2031–2041. [PubMed: 11511534]
11. Mangiameli SM, et al. The Replisomes Remain Spatially Proximal throughout the Cell Cycle in Bacteria. *PLoS Genet*. 2017; 13:e1006582. [PubMed: 28114307]
12. Cook PR. The organization of replication and transcription. *Science*. 1999; 284:1790–1795. [PubMed: 10364545]
13. Papantonis A, Cook PR. Transcription factories: genome organization and gene regulation. *Chem Rev*. 2013; 113:8683–8705. [PubMed: 23597155]
14. Jin DJ, et al. Nucleolus-like compartmentalization of the transcription machinery in fast-growing bacterial cells. *Crit Rev Biochem Mol Biol*. 2017; 52:96–106. [PubMed: 28006965]
15. Liu LF, Wang JC. Supercoiling of the DNA template during transcription. *Proc Natl Acad Sci U S A*. 1987; 84:7024–7027. [PubMed: 2823250]
16. Peter BJ, et al. The structure of supercoiled intermediates in DNA replication. *Cell*. 1998; 94:819–827. [PubMed: 9753328]
17. Cheng B, et al. Direct interaction between *Escherichia coli* RNA polymerase and the zinc ribbon domains of DNA topoisomerase I. *J Biol Chem*. 2003; 278(33):30705–30710. [PubMed: 12788950]
18. Shaiu WL, Hsieh TS. Targeting to transcriptionally active loci by the hydrophilic N-terminal domain of *Drosophila* DNA topoisomerase I. *Mol Cell Biol*. 1998; 18:4358–4367. [PubMed: 9632819]
19. Amado L, Kuzminov A. Low-Molecular-Weight DNA Replication Intermediates in *Escherichia coli*: Mechanism of Formation and Strand Specificity. *J Mol Biol*. 2013; 425:4177–4191. [PubMed: 23876705]
20. Kang S, et al. SeqA protein stimulates the relaxing and decatenating activities of topoisomerase IV. *J Biol Chem*. 2003; 278:48779–48785. [PubMed: 14512422]
21. Vijayraghavan S, Tsai FL, Schwacha A. A Checkpoint-Related Function of the MCM Replicative Helicase Is Required to Avert Accumulation of RNA:DNA Hybrids during S-phase and Ensuing DSBs during G2/M. *PLoS Genet*. 2016; 12:e1006277. [PubMed: 27556397]
22. Crisona NJ, et al. Preferential relaxation of positively supercoiled DNA by *E. coli* topoisomerase IV in single-molecule and ensemble measurements. *Genes Dev*. 2000; 14:2881–2892. [PubMed: 11090135]
23. Ashley RE, et al. Activities of gyrase and topoisomerase IV on positively supercoiled DNA. *Nucleic Acids Res*. 2017; 45:9611–9624. [PubMed: 28934496]
24. Bermejo R, et al. Top1- and Top2-mediated topological transitions at replication forks ensure fork progression and stability and prevent DNA damage checkpoint activation. *Genes Dev*. 2007; 21:1921–1936. [PubMed: 17671091]
25. Moulin L, Rahmouni AR, Boccard F. Topological insulators inhibit diffusion of transcription-induced positive supercoils in the chromosome of *Escherichia coli*. *Mol Microbiol*. 2005; 55:601–610. [PubMed: 15659173]
26. Taylor JH. Chromosome reproduction: units of DNA for segregation. *BioEssays*. 1990; 12:289–296. [PubMed: 2201295]
27. Saitoh N, Goldberg I, Earnshaw WC. The SMC proteins and the coming of age of the chromosome scaffold hypothesis. *BioEssays*. 1995; 17:759–766. [PubMed: 8763828]
28. Maeshima K, Laemmli UK. A two-step scaffolding model for mitotic chromosome assembly. *Dev Cell*. 2003; 4:467–480. [PubMed: 12689587]

29. Hayama R, Mariani KJ. Physical and functional interaction between the condensin MukB and the decatenase topoisomerase IV in *Escherichia coli*. *Proc Natl Acad Sci U S A*. 2010; 107:18826–18831. [PubMed: 20696938]
30. Li Y, et al. *Escherichia coli* condensin MukB stimulates topoisomerase IV activity by a direct physical interaction. *Proc Natl Acad Sci U S A*. 2010; 107:18832–18837. [PubMed: 20921377]
31. Hsu YH, Chung MW, Li TK. Distribution of gyrase and topoisomerase IV on bacterial nucleoid: implications for nucleoid organization. *Nucleic Acids Res*. 2006; 34:3128–3138. [PubMed: 16757578]
32. Kantidze OL, Razin SV. Chromatin loops, illegitimate recombination, and genome evolution. *BioEssays*. 2009; 31:278–286. [PubMed: 19260023]
33. French S. Consequences of replication fork movement through transcription units in vivo. *Science*. 1992; 258:1362–1365. [PubMed: 1455232]
34. Kuzminov A. The Precarious Prokaryotic Chromosome. *J Bacteriol*. 2014; 196:1793–1806. [PubMed: 24633873]
35. Deshpande AM, Newlon CS. DNA replication fork pause sites dependent on transcription. *Science*. 1996; 272:1030–1033. [PubMed: 8638128]
36. Olavarrieta L, et al. DNA knotting caused by head-on collision of transcription and replication. *J Mol Biol*. 2002; 322:1–6. [PubMed: 12215409]
37. Mirkin EV, Mirkin SM. Mechanisms of transcription-replication collisions in bacteria. *Mol Cell Biol*. 2005; 25(3):888–895. [PubMed: 15657418]
38. Thomas M, White RL, Davis RW. Hybridization of RNA to double-stranded DNA: formation of R-loops. *Proc Natl Acad Sci USA*. 1976; 73:2294–2298. [PubMed: 781674]
39. Ratmeyer L, et al. Sequence specific thermodynamic and structural properties for DNA-RNA duplexes. *Biochemistry*. 1994; 33:5298–5304. [PubMed: 7513557]
40. Roberts RW, Crothers DM. Stability and properties of double and triple helices: dramatic effects of RNA or DNA backbone composition. *Science*. 1992; 258:1463–1466. [PubMed: 1279808]
41. Reddy K, et al. Determinants of R-loop formation at convergent bidirectionally transcribed trinucleotide repeats. *Nucleic Acids Res*. 2011; 39:1749–1762. [PubMed: 21051337]
42. Phoenix P, et al. Roles of DNA topoisomerases in the regulation of R-loop formation in vitro. *J Biol Chem*. 1997; 272:1473–1479. [PubMed: 8999816]
43. Richardson JP. Attachment of nascent RNA molecules to superhelical DNA. *J Mol Biol*. 1975; 98:565–579. [PubMed: 811809]
44. Szczelkun MD, et al. Direct observation of R-loop formation by single RNA-guided Cas9 and Cascade effector complexes. *Proc Natl Acad Sci U S A*. 2014; 111:9798–9803. [PubMed: 24912165]
45. Huertas P, Aguilera A. Cotranscriptionally formed DNA:RNA hybrids mediate transcription elongation impairment and transcription-associated recombination. *Mol Cell*. 2003; 12:711–721. [PubMed: 14527416]
46. Gowrishankar J, Harinarayanan R. Why is transcription coupled to translation in bacteria? *Mol Microbiol*. 2004; 54:598–603. [PubMed: 15491353]
47. Herrera-Moyano E, et al. The yeast and human FACT chromatin-reorganizing complexes solve R-loop-mediated transcription-replication conflicts. *Genes Dev*. 2014; 28:735–748. [PubMed: 24636987]
48. Drolet M, Bi X, Liu LF. Hypernegative supercoiling of the DNA template during transcription elongation in vitro. *J Biol Chem*. 1994; 269:2068–2074. [PubMed: 8294458]
49. Chatteraj DK, Stahl FW. Evidence of RNA in D loops of intracellular lambda DNA. *Proc Natl Acad Sci U S A*. 1980; 77(4):2153–7. [PubMed: 6445565]
50. Wahba L, Gore SK, Koshland D. The homologous recombination machinery modulates the formation of RNA-DNA hybrids and associated chromosome instability. *Elife*. 2013; 2:e00505. [PubMed: 23795288]
51. Gowrishankar J, Leela JK, Anupama K. R-loops in bacterial transcription: their causes and consequences. *Transcription*. 2013; 4:153–157. [PubMed: 23756343]

52. Santos-Pereira JM, Aguilera A. R loops: new modulators of genome dynamics and function. *Nat Rev Genet.* 2015; 16:583–597. [PubMed: 26370899]
53. Sollier J, Cimprich KA. Breaking bad: R-loops and genome integrity. *Trends Cell Biol.* 2015; 25:514–522. [PubMed: 26045257]
54. Costantino L, Koshland D. The Yin and Yang of R-loop biology. *Curr Opin Cell Biol.* 2015; 34:39–45. [PubMed: 25938907]
55. Aguilera A, García-Muse T. R loops: from transcription byproducts to threats to genome stability. *Mol Cell.* 2012; 46:115–124. [PubMed: 22541554]
56. Shin JH, Kelman Z. The replicative helicases of bacteria, archaea, and eukarya can unwind RNA-DNA hybrid substrates. *J Biol Chem.* 2006; 281:26914–26921. [PubMed: 16829518]
57. Vincent SD, Mahdi AA, Lloyd RG. The RecG branch migration protein of *Escherichia coli* dissociates R-loops. *J Mol Biol.* 1996; 264:713–721. [PubMed: 8980680]
58. Hong X, Cadwell GW, Kogoma T. *Escherichia coli* RecG and RecA proteins in R-loop formation. *EMBO J.* 1995; 14(10):2385–2392. [PubMed: 7774596]
59. Voloshin ON, Camerini-Otero RD. The DinG protein from *Escherichia coli* is a structure-specific helicase. *J Biol Chem.* 2007; 282:18437–18447. [PubMed: 17416902]
60. Boubakri H, et al. The helicases DinG, Rep and UvrD cooperate to promote replication across transcription units in vivo. *EMBO J.* 2010; 29:145–157. [PubMed: 19851282]
61. von Hippel PH, et al. Specificity mechanisms in the control of transcription. *Biophys Chem.* 1996; 59:231–246. [PubMed: 8672714]
62. Cerritelli SM, Crouch RJ. Ribonuclease H: the enzymes in eukaryotes. *FEBS J.* 2009; 276:1494–1505. [PubMed: 19228196]
63. Tadokoro T, Kanaya S, Ribonuclease H. molecular diversities, substrate binding domains, and catalytic mechanism of the prokaryotic enzymes. *FEBS J.* 2009; 276:1482–1493. [PubMed: 19228197]
64. Hamperl S, et al. Transcription-Replication Conflict Orientation Modulates R-Loop Levels and Activates Distinct DNA Damage Responses. *Cell.* 2017; 170:774–786. [PubMed: 28802045]
65. Keszthelyi A, Minchell NE, Baxter J. The Causes and Consequences of Topological Stress during DNA Replication. *Genes (Basel).* 2016; 7:E134. [PubMed: 28009828]
66. Grompone G, Ehrlich SD, Michel B. Replication restart in *gyrB* *Escherichia coli* mutants. *Mol Microbiol.* 2003; 48(3):845–854. [PubMed: 12694626]
67. Jeppsson K, et al. The chromosomal association of the Smc5/6 complex depends on cohesion and predicts the level of sister chromatid entanglement. *PLoS Genet.* 2014; 10:e1004680. [PubMed: 25329383]
68. Verver DE, et al. Non-SMC Element 2 (NSMCE2) of the SMC5/6 Complex Helps to Resolve Topological Stress. *Int J Mol Sci.* 2016; 26:1782.
69. Postow L, Peter BJ, Cozzarelli NR. Knot what we thought before: the twisted story of replication. *BioEssays.* 1999; 21:805–808. [PubMed: 10497329]
70. Postow L, et al. Positive torsional strain causes the formation of a four-way junction at replication forks. *J Biol Chem.* 2001; 276(4):2790–2796. [PubMed: 11056156]
71. Olavarrieta L, et al. Supercoiling, knotting and replication fork reversal in partially replicated plasmids. *Nucleic Acids Res.* 2002; 30(3):656–666. [PubMed: 11809877]

Trends Box

The (–)sc behind factories is relaxed targeted specialized topoisomerases, but no such targeted relaxation is known for the (+)sc ahead of the factories. I propose that (+)sc is mostly relaxed at the domain boundaries, far from the factories that generate (+)sc.

When two factories are in the head-on orientation in the same topological domain, not only (+)sc between them is generated at the combined rate, but there may be no easy way to relieve this (+)sc completely, explaining the disrupting power of the head-on conflict.

Formation of R-loops signals high (–)sc in the region. Replication conflict with a highly-expressed gene in head-on orientation generates R-loops in the gene, suggesting unexpected formation of (–)sc in the middle of massive (+)sc around.

In the head-on conflict, both replication and transcription are permanently stalled, unless RNase H enzyme removes the R-loops.

Outstanding Questions Box

- Are indeed the topoisomerases that *introduce* (–)sc stationed mostly at the topological domain boundaries, in contrast to both (+)sc and (–)sc relaxing topoisomerases, that work distributively and, therefore, tend to act locally?
- Does (+)sc accumulate at combined rates between converging factories (especially between TECs)?
- What is the stability of TEC-free R-loops, compared to R-loop-aTECs? In particular, could Tec-free R-loops stall replication forks?
- Do factories form topological domains of their own? Do factories operate independently of the chromosome scaffold?
- Do replication fork regress as a result of conflict with an intense head-on transcription unit? Do they lose contact with the replication factory and rotate as a result of this?
- Are there specialized proteins in the cell, besides topoisomerases, that specifically recognize positively-supercoiled DNA?
- Do some of these (+)sc-recognizing proteins indeed chaperone or report positive supercoils to the nearby relaxation stations?

Fig. 1. The twin-domain topological nature of DNA replication and transcription

Duplex DNA is shown by double blue lines, RNA is shown as orange lines. The 3'-ends are identified by arrowheads. Position of relaxation of negative and positive supercoils are shown by colored arrows.

A. An independent topological domain with a replication factory (green hexagon) that pulls template DNA through (from right to left here), generating positive supercoiling in the template DNA, while releasing negatively supercoiled daughter duplexes.

B. An independent topological domain with a transcription factory (green circle), that pulls the template DNA through (from left to right here), generating positive supercoiling ahead of itself, while negative supercoiling behind.

C. An independent topological domain with both a replication fork and a head-on transcription unit. Due to the possible distal nature of processive (+)sc relaxation, accumulation of hyper(+)sc between the two factories is suspected.

Fig. 2. The two types of R-loops

A. TEC-free R-loop.

B. R-loop-aTEC.

Fig. 3. How replication-transcription conflict could spawn R-loops behind RNA polymerases
 The replisome is shown as a hexagon surrounding the replication fork or a Holiday junction. The TEC is shown as a circle surrounding the transcription loop. Blue lines, DNA strands; orange lines, RNA strands. Big blue circles, ribosomes; chains of small circles, nascent polypeptide chains.

- A.** Converging replication and transcription factories find themselves in the head-on conflict.
B. Accumulation of positive supercoils ((+)sc) between the two factories stalls both.
C. An unspecified event suddenly drops the level of (+)sc in the region, which allows the transcription factory to resume pulling in the template DNA and releasing it with hyper(-)sc behind.
D. While the hyper(-)sc behind the TEC, in combination with a slow ribosome restart, invites formation of an R-loop-aTEC, the replication fork remains stalled at the original position due to accumulated (+sc).

Fig. 4. Possible scenarios of the sudden drop in (+)sc between two converging factories

A. and **C.** These two panels corresponds to Fig. 3B and D, with ribosomes omitted for clarity.

B-1. Replication factory loses the contact with the fork. The fork freely rotates as a result, redistributing the (+)sc in the unreplicated DNA as precatenanes behind the fork.

B-2. Replication factory malfunctions and allows the replication fork to regress, still holding on to the resulting Holliday junction.

B-3. Massive (+)sc attracts distributive topo II enzymes, which remove the bulk of (+)sc, but dissociate due to their low affinity to random DNA, leaving behind enough (+)sc to inhibit the replisome, but not enough to inhibit the TEC.