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REVIEW

Spontaneous DNA-RNA hybrids: differential impacts throughout the cell cycle

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ABSTRACT

A large body of research supports that transcription plays a major role among the many sources of replicative stress contributing to genome instability. It is therefore not surprising that the DNA damage response has a role in the prevention of transcription-induced threatening events such as the formation of DNA-RNA hybrids, as we have recently found through an siRNA screening. Three major DDR pathways were defined to participate in the protection against DNA-RNA hybrids: ATM/CHK2, ATR/CHK1 and Postreplication Repair (PRR). Based on these observations, we envision different scenarios of DNA-RNA hybridization and their consequent DNA damage.

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During malignant transformation, cells acquire a specific set of features that transform a normal tissue into a tumor [1]. Tumorigenesis, cancer evolution and chemo-resistance are promoted by genetic instability, which is mainly induced by replicative stress [2]. A critical source of replicative stress and genome instability comes from the transcription process [3]. Seen as by-products of transcription, DNA-RNA hybrids are formed more frequently than previously foreseen, challenging replication fork progression and eventually leading to double-strand breaks (DSBs), which are among the most deleterious type of lesions [4]. The DNA damage response (DDR) coordinates DNA replication with damage sensing, repair and cell cycle progression thus protecting genome integrity during cell division. Accordingly, a large number of observations indicate that the DDR acts as an intrinsic barrier in the early phases of human tumorigenesis [5,6]. The DDR is based on signal transduction cascades in which apical kinases, such as ATR and ATM, are activated upon DNA damage and the signal is transferred to downstream kinases. Thus, the DDR network covers from sensing the damage to regulating the cellular processes involved in the repair of the lesion [7]. Hence, the DDR relies on DNA damage checkpoint (DDC) factors as well as on DNA damage repair factors and regulators.

The ATM/CHK2 checkpoint pathway is activated upon DSBs. ATR, by contrast, is activated by RPA-coated ssDNA, thus responding to hyper-resected DSBs and DNA lesions associated with DNA replication. Its main effector kinase, CHK1, triggers many cellular responses such as the prevention of fork collapse and the regulation of origin firing. Whereas DSB repair relies on two main and well-characterized pathways (non-homologous end joining – NHEJ – and homologous recombination – HR–), lesions encountered by DNA replication forks can be bypassed by several mechanisms that are collectively known as the DNA damage tolerance or Postreplication repair (PRR) pathways [8]. These mechanisms are mainly governed by the ubiquitylation state of PCNA and can leave bulky lesions behind the replication fork, leaving gaps in the leading or lagging strand in the sister chromatids. Recently, we have reported an increased DNA-RNA hybrid accumulation and DNA-RNA hybrid-dependent DNA damage in cells depleted of DDC and PRR factors, suggesting a role for the DDR in the prevention of DNA-RNA hybrid accumulation and its associated DNA damage [9] (Figure 1). However, not all of these DNA-RNA hybrids represent a threat to replication fork progression. Based on these results, we propose that DNA-RNA hybrids may be formed by different manners throughout the cell cycle with different consequences for replication and genome integrity.

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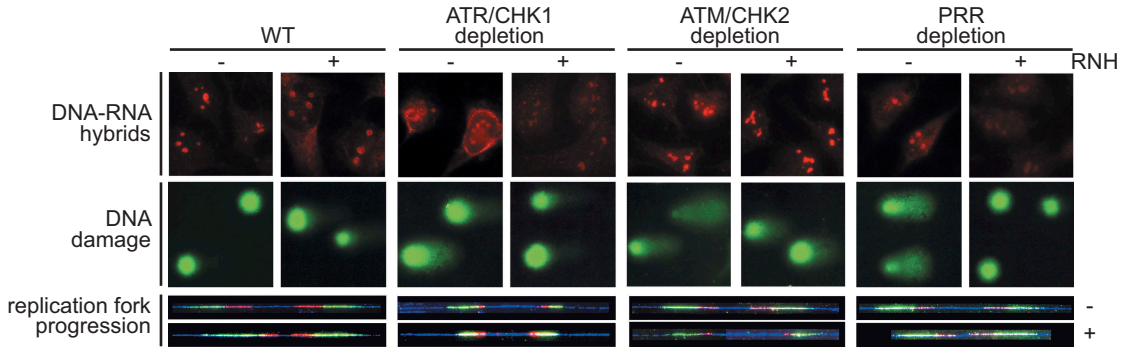

Figure 1. The effect of DDR depletion in DNA-RNA hybrid and its associated DNA damage and fork stalling.

Illustrative pictures depicting the effects of ATR/CHK1, ATM/CHK2 or PRR depletion on DNA-RNA hybrid accumulation in HeLa cells (immunofluorescence with S9.6 antibody), DNA damage (as determined by single cell electrophoresis assay) and replication fork progression (as determined by DNA combing) and with or without RNase H overexpression (RNH). For DNA-RNA hybrid detection, cells were fixed 72 hours after siRNA transfection and immunostained with the mouse S9.6 antibody (hybridoma cell line HB-8730) and chicken α-mouse secondary antibody conjugated with Alexa fluor 594. For DNA damage, single-cell alkaline gel electrophoresis was performed 72 hours after siRNA transfection with CometAssay kit (Trevigen) following manufacturer's instructions. For replication fork progression, DNA combing was performed 72 hours after siRNA transfection essentially as described [52] but after sequential addition of iododeoxyuridine (IdU) and chlorodeoxyuridine (CldU) labels for 20 min each. Anti-ssDNA from Developmental Studies Hybridoma Bank (DSHB) was used instead of the one described. ssDNA is shown in blue, IdU tracks in red and CldU tracks in green. In all panels, either pEGFP-C1 (RNH-) or pEGFP-M27 (RNH+) to overexpress RNase H (RNH) were used in addition to the indicated siRNAs. All images were acquired with a Leica DM6000 microscope equipped with a DFC390 camera (Leica) at x63 magnification for S9.6 immunofluorescence, x10 magnification for single-cell alkaline gel electrophoresis and x40 for DNA combing.

Spontaneous DNA-RNA hybrids accumulate in the absence of the ATR/CHK1 pathway

The ability of the nascent mRNA to thread back to its DNA template is prevented by its rapid coating and maturation into the messenger ribonucleoprotein particle (mRNP), as originally described in yeast THO mutants [10]. Although there is not yet an accurate estimation either of the frequency at which DNA-RNA hybrids form at the different genomic regions or of their stability once formed, the fact that several mechanisms have evolved to deal with them highlights not only their importance in threatening genome integrity but also the heterogeneity in nature of such hybrids [4]. In agreement with their relevance as a source of DNA damage during replication, ATR depletion by siRNA as well as ATR inhibition causes increased DNA-RNA hybrid accumulation [9] (Figures 1 and 2). We have recently reported that a significant fraction of the fork stalling and DNA damage observed after depletion of the ATR/CHK1 pathway is dependent on DNA-RNA hybrids, as evidenced by the observation that overexpression of RNase H, which degrades the RNA moiety of DNA-RNA hybrids, significantly reduced such phenotypes [9] (Figure 1). These

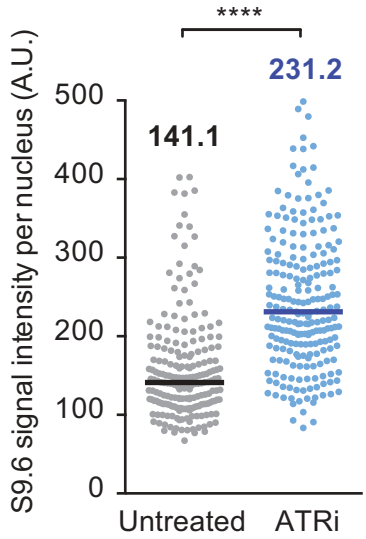

Figure 2. ATR inhibition causes enhanced DNA-RNA hybrid accumulation.

Relative S9.6 signal intensity per nucleus after nucleolus signal removal in HeLa cells treated with the ATR inhibitor developed in a previously reported chemical screen [53] at a 5 mM concentration during 2 hours. After fixation, cells were immunostained with the mouse S9.6 (hybridoma cell line HB-8730) and chicken α-mouse secondary antibody conjugated with Alexa fluor 594. More than 150 total cells were considered. The median of each population is shown. ***$p < 0.001$ (Mann-Whitney U test).

data strongly suggest that DNA-RNA hybrids form spontaneously in a substantial manner, and are present in the genome of cells entering S phase.

Indeed, it has been estimated that 5% of the human genome is covered with DNA-RNA hybrids [11] and numerous reports have claimed that replication fork progression can be hampered by their presence [12–18], constituting one of the best studied cases of transcription-replication conflicts [3,4]. Furthermore, DNA-RNA hybrids are confronted by replication-associated repair factors such as FACT [19] or the Fanconi anemia (FA) pathway [20,21], also fished in our DDR screening [9], reinforcing the idea of DNA-RNA hybrids impeding fork progression.

Whereas it is possible that there are specific DDC targets (such as RNA helicases) to deal with DNA-RNA hybrid structures to prevent them to lead to stable DNA damage, it is also plausible that a general and unspecific function of the DDR tackles them. For instance, the DDR might just aid replication fork progression through DNA-RNA hybrid-containing regions leading to their dissolution. In this regard, it has been proposed that the passage of replication forks through co-transcriptionally transcribed units can disassemble DNA-RNA hybrids in yeast and human cells preventing DNA damage [14,22], what could rely on ATR/CHK1. Of note, replicative helicases themselves can unwind DNA-RNA hybrids [23] but the situation might change *in vivo*, where additional factors or DDR regulation might be required. Bizarrely, ATM, but not ATR activation, has been detected in human cells after co-transcriptional encounters with DNA-RNA hybrids, although the reasons for such activation are unexplained [14]. In agreement with DNA-RNA hybrids being a substantial source of replication fork stalling, spontaneous checkpoint activation was reported in yeast cells accumulating DNA-RNA hybrids [24] and ATR activation was observed after head-on transcription-replication conflicts [14].

DNA-RNA hybrids at unrepaired DNA breaks

The state of the DNA itself can also restrain or stimulate RNA invasion and this is evidenced by the fact that DNA-RNA hybrid formation is favored by negative supercoiling and by DNA breakage. The latter likely helps DNA-RNA hybridization through relieving the torsional stress imposed by the annealing of the invading RNA [25]. Accordingly, the presence of DNA-RNA hybrids at break sites was directly demonstrated by DNA-RNA immunoprecipitation after *in vivo* DSB induction in *S. pombe* [26] or human cells [27,28]. In agreement with the role of mRNA coating and supercoiling in the prevention of DNA-RNA hybridization, co-transcriptional DNA-RNA hybrids have been reported in the absence of several mRNP processing factors and in topoisomerase mutants [4]. Along this line, it is reasonable to envision that DNA-RNA hybrids would also be enriched if DNA break repair were prevented. The loss of the ATM/CHK2 kinase pathway likely reflects this situation in which DNA breaks are not efficiently handled by the cellular DNA repair machineries and remain unrepaired [29] leading to DNA-RNA hybrid accumulation [9].

In contrast to what has been reproducibly reported for DNA-RNA hybrids formed at non-break sites, which challenge DNA replication [12–18], hybrids accumulated at DNA breaks are not inducing any further damage or replication problems [9] (Figure 1) and therefore may not contribute to replicative stress. Other factors with a defined role in DNA repair have been reported to prevent the accumulation of DNA-RNA hybrids. This is exemplified by BRCA1 [30], BRCA2 [31] and other FA proteins, such as FANCA, FANCD2 or FANCM [20,21]. However, the possibility that DNA-RNA hybrid could be removed during replication was suggested in these cases given their function as replication-associated repair factors (see [32] and references therein). Indeed, FANCD2 aids replication through fragile sites both by influencing dormant origin firing and by ameliorating DNA-RNA hybrid accumulation [33]. It is therefore possible that in this case, hybrid removal is a consequence of the facilitated replication fork progression through the hybrid-containing region, rather than such factors having a direct role in hybrid resolution. DNA-RNA hybrid accumulation has also been reported in cells depleted for other factors involved in DSB repair such as CtIP [34] or BLM [35]. Identifying how much of the DNA-RNA hybrid accumulation reported after the depletion of all such factors is also due to the accumulation of unrepaired DNA breaks represents an important question for the future.

DNA-RNA hybrids at postreplicative ssDNA gaps

Our recent observation that PRR factors prevent DNA-RNA hybrids from causing DNA damage [9] suggests that ssDNA gaps facilitates the formation of potentially harmful hybrids (Figure 3). Notably, we did not detect any increase in replication fork asymmetry in cells defective in PRR [9] (Figure 1), implying that DNA-RNA hybrids at ssDNA gaps would cause DNA damage in a replication-independent manner. The mechanism for this replication-independent instability induced by DNA-RNA hybrids remains to be elucidated. One possibility is that these hybrids are directly processed by nucleases, leading to breaks, such as XPG, XPF and FEN1 [36,37]. However, we envision that, in the absence of PRR, the RNA might somehow interfere with gap filling and thus enhance the probability of cells entering mitosis with under-replicated DNA. Under-replication is a potential cause for DNA breaks, as it is believed for fragile sites that are intrinsically difficult to replicate regions frequently co-localizing with the sites for chromosome rearrangements observed in tumor cells [38,39]. Remarkably, DNA-RNA hybrids contribute to the fragility of common fragile sites [33] and can also form in other regions of the genome that are intrinsically difficult to replicate, such as the rDNA [40] or telomeres [41–44]. Furthermore, it has been already proposed that DNA-RNA hybrids might be involved in the origin of mitotic DNA repair synthesis, a process that enables the completion of DNA replication in mitosis (MiDAS) [45]. One prediction from our model is that the DNA-RNA hybrids formed in the absence of PRR would require the action of RNases or DNA-RNA helicases prior to gap filling. In accordance, it was reported in yeast that a negative genetic interaction exists between PRR factors and RNase H leading to increased genetic instability, as measured by the rates of gross chromosomal

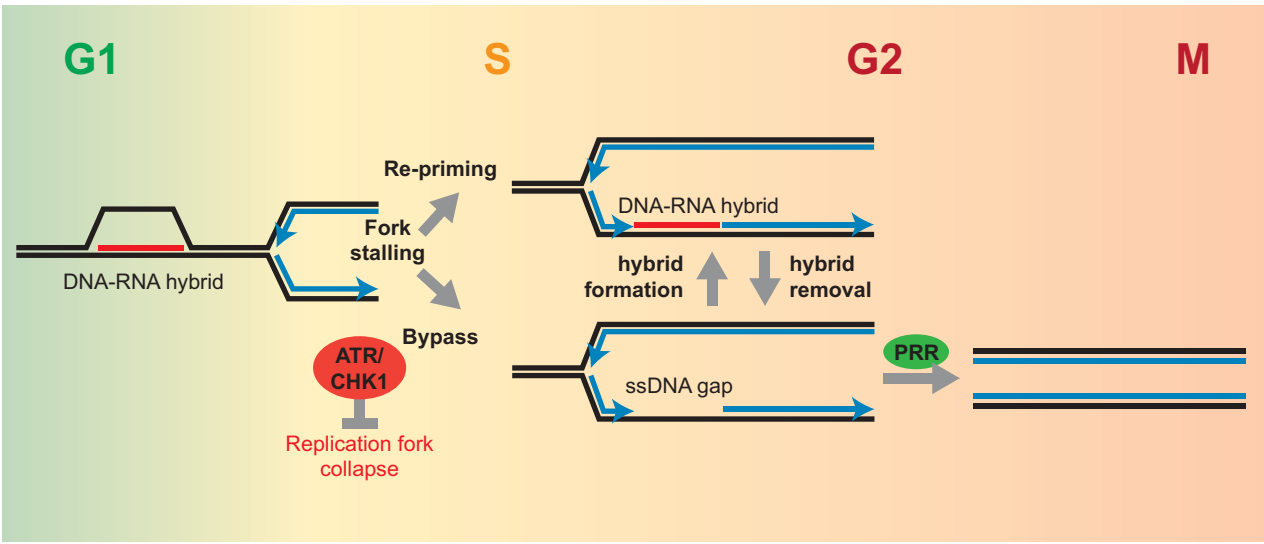

Figure 3. A model for DNA-RNA hybrids and their consequences for replication throughout the cell cycle.

Spontaneous DNA-RNA hybridization might be favored by different conditions at any moment of the cell cycle, including insufficient RNA coating, negative supercoiling and DNA breaks or ssDNA gaps. This is counteracted by the action of multiple mechanisms including DNA-RNA helicases. The ssDNA displaced by the DNA-RNA hybrid entails a major susceptibility to DNA damaging agents. Moreover, DNA-RNA hybrids can be processed by nucleases leading to breakage all over the cell cycle. Upon encountering by the replication forks, DNA-RNA hybrids could be tolerated by directly re-priming downstream of the lesion, leading to DNA-RNA hybrids at un-replicated regions in S phase. Alternatively, DNA-RNA hybrids, as well as other types of lesions, can induce fork stalling and eventually fork collapse in S phase. This would be prevented by the ATR/CHK1 DDC pathway. Stalled forks may trigger the formation of postreplicative ssDNA gaps, which are a substrate for spontaneous RNA hybridization. In the absence of PRR, the extensive accumulation of ssDNA gaps would obstacle further processing, possibly delaying the full completion of DNA synthesis and prompting mitotic failures.

rearrangements [46]. Exploring the actual role of DNA-RNA hybrids in mitotic failures and aberrations will surely shed light on this so far speculative model.

Interestingly, experiments in *E. coli* performed with an engineered synthetic protein that traps Holliday junctions have revealed that most spontaneous recombination events arise from the repair of ssDNA gaps originated during DNA replication rather than from the repair of DSBs [47]. This is likely the case in other organisms, what points to ssDNA gaps caused by replication as a major source for tumorigenesis [48], and hence, opens the possibility that DNA-RNA hybridization at such gaps has a role in cancer origin. The initial lesions leading to such ssDNA gaps could be different sorts of DNA damage or replication stalling agents, among which DNA-RNA hybrids themselves are included (Figure 3). Based on the fact that a significant amount of the DNA damage and fork asymmetry observed upon depletion of ATR/CHK1 proteins was reduced by the overexpression of RNase H [9] (Figure 1), we propose that DNA-RNA hybrids are a significant sources of fork stalling that require the PRR machinery and can ultimately lead to DNA-RNA hybridization at the unrepaired ssDNA gaps (Figure 3). Notwithstanding, our observation that DNA-RNA hybrid accumulation rises along the cell cycle, with S9.6 signal detection increasing from G1 to S and from S to G2 at levels that would not be easily explained by genome duplication and primers at the Okazaki fragments [9], is in agreement with DNA-RNA hybrids occurring or remaining at post-replicative ssDNA gaps. In addition, it also suggests that there is an additional source of DNA-RNA hybrids in G2 beyond those formed during G1, further arguing that other lesions causing fork stalling can also contribute to the formation of postreplicative ssDNA gaps.

Moreover, it is currently known that DNA lesions encountered by the replication fork can be re-primed by PrimPol, a specific DNA primase reinitiating downstream of lesions [49,50]. Interestingly, PrimPol depletion has been reported to enhance DNA-RNA hybrid formation as shown for repetitive sequences in chicken DT40 cells [51]. Thus, it would be interesting to address whether re-priming after hybrid-containing regions would leave ssDNA gaps behind the fork. In this case, ssDNA gaps would represent a scar of the original DNA-RNA hybrid, and might as well require proper PRR machinery for their timely dissolution before mitosis.

Concluding remarks

Based on a large number of reports, our current model envisages that DNA-RNA hybrids might be formed by different reasons, including insufficient RNA coating, negative supercoiling and DNA breaks or ssDNA gaps. Importantly, they seem to represent a natural source of DNA damage throughout the entire cell cycle, constituting a major cause of replicative stress and genetic instability. The DDR, thus, seems to work to efficiently prevent and minimize their deleterious consequences throughout the entire cell cycle (Figure 3). All over the cell cycle, the ssDNA displaced by the DNA-RNA hybrid entails a major susceptibility to metabolites, ROS, DNA modifying enzymes such as AID or APOBEC, or nucleases. In S phase, in addition to this damage, DNA-RNA hybrids challenge replication fork progression. At this stage, hybrid bypass might directly lead to un-replicated regions containing the DNA-RNA hybrid that would require further hybrid removal and PRR. Alternatively, such conflicts with replication forks may trigger the formation of ssDNA gaps or even collapse into DSBs. RNA hybridization at ssDNA gaps in S and G2 might obstacle further processing, delaying the full completion of DNA synthesis and leading to mitotic failures. Important questions that remain to be addressed include determining the abundance and stability of spontaneous DNA-RNA hybrids genome-wide along different stages of the cell cycle as well as deepening into the likely distinct mechanisms by which they end up imposing a threat to genome stability at the different cell cycle stages. Future experiments will likely help us acquire a neat vision of the real contribution of DNA-RNA hybrids in the total DNA damage that cells face and their possible involvement in tumorigenesis. Even though, the data support the conclusion that unscheduled DNA-RNA hybrids form sporadically in eukaryotic cells, being a natural source

of genome instability that is efficiently counteracted by the DDR machinery.

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Disclosure statement

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References

[1] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646–674.
[2] Hills SA, Diffley JF. DNA replication and oncogene-induced replicative stress. Curr Biol. 2014;24:R435–444.
[3] Gomez-Gonzalez B, Aguilera A. Transcription-mediated replication hindrance: a major driver of genome instability. Genes Dev. 2019;33:1008–1026.
[4] Garcia-Muse T, Aguilera A. R-loops: from physiological to pathological roles. Cell. 2019;179: 604–618. *in press*.
[5] Bartkova J, Horejsi Z, Koed K, *et al*. DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. Nature. 2005;434:864–870.
[6] Gorgoulis VG, Vassiliou LV, Karakaidos P, *et al*. Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature. 2005;434:907–913.
[7] Lanz MC, Dibitetto D, Smolka MB. DNA damage kinase signaling: checkpoint and repair at 30 years. Embo J. 2019;38:e101801.
[8] Branzei D, Foiani M. Maintaining genome stability at the replication fork. Nat Rev. 2010;11:208–219.
[9] Barroso S, Herrera-Moyano E, Munoz S, et al. The DNA damage response acts as a safeguard against harmful DNA-RNA hybrids of different origins. EMBO Rep. 2019;20:e47250.
[10] Huertas P, Aguilera A. Cotranscriptionally formed DNA: RNA hybrids mediate transcription elongation impairment and transcription-associated recombination. Mol Cell. 2003;12:711–721.
[11] Sanz LA, Hartono SR, Lim YW, et al. Prevalent, Dynamic, and Conserved R-Loop Structures Associate with Specific Epigenomic Signatures in Mammals. Mol Cell. 2016;63:167–178.
[12] Castellano-Pozo M, Garcia-Muse T, Aguilera A. R-loops cause replication impairment and genome instability during meiosis. EMBO Rep. 2012;13:923–929.
[13] Gan W, Guan Z, Liu J, et al. R-loop-mediated genomic instability is caused by impairment of replication fork progression. Genes Dev. 2011;25:2041–2056.
[14] Hamperl S, Bocek MJ, Saldivar JC, et al. Transcription-Replication Conflict Orientation Modulates R-Loop Levels and Activates Distinct DNA Damage Responses. Cell. 2017;170:774–786 e719.
[15] Lang KS, Hall AN, Merrikh CN, et al. Replication-Transcription Conflicts Generate R-Loops that Orchestrate Bacterial Stress Survival and Pathogenesis. Cell. 2017;170:787–799 e718.
[16] Salas-Armenteros I, Perez-Calero C, Bayona-Feliu A, et al. Human THO-Sin3A interaction reveals new mechanisms to prevent R-loops that cause genome instability. Embo J. 2017;36:3532–3547.
[17] Tuduri S, Crabbe L, Conti C, *et al*. Topoisomerase I suppresses genomic instability by preventing interference between replication and transcription. Nat Cell Biol. 2009;11:1315–1324.
[18] Wellinger RE, Prado F, Aguilera A. Replication fork progression is impaired by transcription in hyperrecombinant yeast cells lacking a functional THO complex. Mol Cell Biol. 2006;26:3327–3334.
[19] Herrera-Moyano E, Mergui X, Garcia-Rubio ML, et al. The yeast and human FACT chromatin-reorganizing complexes solve R-loop-mediated transcription-replication conflicts. Genes Dev. 2014;28:735–748.
[20] Garcia-Rubio ML, Perez-Calero C, Barroso SI, et al. The Fanconi Anemia Pathway Protects Genome Integrity from R-loops. PLoS Genet. 2015;11:e1005674.
[21] Schwab RA, Nieminuszczy J, Shah F, et al. The Fanconi Anemia Pathway Maintains Genome Stability by Coordinating Replication and Transcription. Mol Cell. 2015;60:351–361.
[22] Garcia-Rubio M, Aguilera P, Lafuente-Barquero J, et al. Yra1-bound RNA-DNA hybrids cause orientation-independent transcription-replication collisions and telomere instability. Genes Dev. 2018;32:965–977.
[23] 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.

[24] Gomez-Gonzalez B, Felipe-Abrio I, Aguilera A. The S-phase checkpoint is required to respond to R-loops accumulated in THO mutants. Mol Cell Biol. 2009;29:5203–5213.
[25] Aguilera A, Gomez-Gonzalez B. DNA-RNA hybrids: the risks of DNA breakage during transcription. Nat Struct Mol Biol. 2017;24:439–443.
[26] Ohle C, Tesorero R, Schermann G, et al. Transient RNA-DNA Hybrids Are Required for Efficient Double-Strand Break Repair. Cell. 2016;167:1001–1013 e1007.
[27] Cohen S, Puget N, Lin YL, et al. Senataxin resolves RNA:DNA hybrids forming at DNA double-strand breaks to prevent translocations. Nat Commun. 2018;9:533.
[28] Li L, Germain DR, Poon HY, et al. DEAD Box 1 Facilitates Removal of RNA and Homologous Recombination at DNA Double-Strand Breaks. Mol Cell Biol. 2016;36:2794–2810.
[29] Morrison C, Sonoda E, Takao N, et al. The controlling role of ATM in homologous recombinational repair of DNA damage. Embo J. 2000;19:463–471.
[30] Hatchi E, Skourti-Stathaki K, Ventz S, *et al*. BRCA1 recruitment to transcriptional pause sites is required for R-loop-driven DNA damage repair. Mol Cell. 2015;57:636–647.
[31] Bhatia V, Barroso SI, Garcia-Rubio ML, et al. BRCA2 prevents R-loop accumulation and associates with TREX-2 mRNA export factor PCID2. Nature. 2014;511:362–365.
[32] Bhatia V, Herrera-Moyano E, Aguilera A, et al. The Role of Replication-Associated Repair Factors on R-Loops. Genes (Basel). 2017;8:e171.
[33] Madireddy A, Kosiyatrakul ST, Boisvert RA, *et al*. FANCD2 Facilitates Replication through Common Fragile Sites. Mol Cell. 2016;64:388–404.
[34] Makharashvili N, Arora S, Yin Y, et al. Sae2/CtIP prevents R-loop accumulation in eukaryotic cells. Elife. 2018;7:e42733.
[35] Chang EY, Novoa CA, Aristizabal MJ, *et al*. RECQ-like helicases Sgs1 and BLM regulate R-loop-associated genome instability. J Cell Biol. 2017;216:3991–4005.
[36] Cristini A, Ricci G, Britton S, *et al*. Dual Processing of R-Loops and Topoisomerase I Induces Transcription-Dependent DNA Double-Strand Breaks. Cell Rep. 2019;28:3167–3181 e3166.
[37] Sollier J, Stork CT, Garcia-Rubio ML, et al. Transcription-coupled nucleotide excision repair factors promote R-loop-induced genome instability. Mol Cell. 2014;56:777–785.
[38] Durkin SG, Glover TW. Chromosome Fragile Sites. Annual Review of Genetics. 2007;41:169–192.
[39] Le Tallec B, Koundrioukoff S, Wilhelm T, et al. Updating the mechanisms of common fragile site instability: how to reconcile the different views? Cell Mol Life Sci. 2014;71:4489–4494.
[40] El Hage A, French SL, Beyer AL, et al. Loss of Topoisomerase I leads to R-loop-mediated transcriptional blocks during ribosomal RNA synthesis. Genes Dev. 2010;24:1546–1558.
[41] Arora R, Lee Y, Wischnewski H, et al. RNaseH1 regulates TERRA-telomeric DNA hybrids and telomere maintenance in ALT tumour cells. Nat Commun. 2014;5:5220.
[42] Balk B, Maicher A, Dees M, et al. Telomeric RNA-DNA hybrids affect telomere-length dynamics and senescence. Nat Struct Mol Biol. 2013;20:1199–1205.
[43] Pfeiffer V, Crittin J, Grolimund L, et al. The THO complex component Thp2 counteracts telomeric R-loops and telomere shortening. Embo J. 2013;32:2861–2871.
[44] Yu TY, Kao YW, Lin JJ. Telomeric transcripts stimulate telomere recombination to suppress senescence in cells lacking telomerase. Proc Natl Acad Sci U S A. 2014;111:3377–3382.
[45] Ozer O, Hickson ID. Pathways for maintenance of telomeres and common fragile sites during DNA replication stress. Open Biol. 2018;8:180018.
[46] Allen-Soltero S, Martinez SL, Putnam CD, et al. A saccharomyces cerevisiae RNase H2 interaction network functions to suppress genome instability. Mol Cell Biol. 2014;34:1521–1534.
[47] Xia J, Chen LT, Mei Q, *et al*. Holliday junction trap shows how cells use recombination and a junction-guardian role of RecQ helicase. Sci Adv. 2016;2:e1601605.
[48] Xia J, Mei Q, Rosenberg SM. Tools To Live By: bacterial DNA Structures Illuminate Cancer. Trends Genet. 2019;35:383–395.
[49] Garcia-Gomez S, Reyes A, Martinez-Jimenez MI, *et al*. PrimPol, an archaic primase/polymerase operating in human cells. Mol Cell. 2013;52:541–553.
[50] Mouron S, Rodriguez-Acebes S, Martinez-Jimenez MI, et al. Repriming of DNA synthesis at stalled replication forks by human PrimPol. Nat Struct Mol Biol. 2013;20:1383–1389.
[51] Svikovic S, Crisp A, Tan-Wong SM, et al. R-loop formation during S phase is restricted by PrimPol-mediated repriming. Embo J. 2018:e99793.
[52] Bianco JN, Poli J, Saksouk J, et al. Analysis of DNA replication profiles in budding yeast and mammalian cells using DNA combing. Methods. 2012;57:149–157.
[53] Toledo LI, Murga M, Zur R, et al. A cell-based screen identifies ATR inhibitors with synthetic lethal properties for cancer-associated mutations. Nat Struct Mol Biol. 2011;18:721–727.