Biologia Futura (2024) 75:177–182
https://doi.org/10.1007/s42977-024-00213-7

REVIEW

Chromosomal R-loops: who R they?

Lóránt Székvölgyi[1]

Received: 19 November 2023 / Accepted: 14 February 2024 / Published online: 8 March 2024

Abstract
R-loops, composed of DNA–RNA hybrids and displaced single-stranded DNA, are known to pose a severe threat to genome integrity. Therefore, extensive research has focused on identifying regulatory proteins involved in controlling R-loop levels. These proteins play critical roles in preventing R-loop accumulation and associated genome instability. Herein I summarize recent knowledge on R-loop regulators affecting R-loop homeostasis, involving a wide array of R-loop screening methods that have enabled their characterization, from forward genetic and siRNA-based screens to proximity labeling and machine learning. These approaches not only deepen our understanding on R-loop formation processes, but also hold promise to find new targets in R-loop dysregulation associated with human pathologies.

Keywords R-loop screen · RNA-DNA hybrid · R-loop regulator · R-loop disorder

Introduction

R-loops are triple-stranded nucleic acid structures in the genome that consist of an RNA–DNA hybrid and a displaced single-stranded DNA (ssDNA) (Fig. 1). First identified in 1976 (Thomas et al. 1976), these structures were initially observed in living bacteria and have since been investigated across various organisms, underscoring their prevalence from yeast to humans. While originally regarded as transcriptional by-products, R-loops have gained attention in the past decade due to their involvement in diverse physiological and pathological processes.

Under normal conditions, R-loops regulate gene expression by inhibiting DNA methylation at CpG islands and recruiting noncoding RNAs near promoters (Arab et al. 2019; Boque-Sastre et al. 2015). Additionally, they serve as intermediates for the replication of the T4 bacteriophage, *Escherichia coli*, and mitochondrial genomes (Aguilera and García-Muse 2012) and contribute to the dynamics of chromosome telomeres (Balk et al. 2013). In B lymphocytes, the single-stranded DNA segment of R-loops induces mutagenic effects that stimulate immunoglobulin heavy chain isotype switching (Yu et al. 2003).

However, under pathological conditions, the accumulation of R-loops poses a severe threat to chromosome integrity, primarily due to transcription–replication conflicts, which can lead to genome instability and carcinogenesis. R-loops have also been associated with neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Aicardi–Goutières syndrome, Friedreich's ataxia, and Fragile X syndrome (Lim et al. 2015; Walker et al. 2017; Reddy et al. 2014; Kannan et al. 2019; Perego et al. 2018; Grunseich et al. 2018; Feró et al. 2024), highlighting their impact on nondividing (post-mitotic) cells.

The formation of R-loops can be attributed to mechanisms proposed by Lieber and Roy, known as the "threadback" and the "extended hybrid" models (Roy et al. 2010). In the "threadback" model, a single-stranded nascent RNA exits the RNA polymerase and promptly returns to the transcription bubble to hybridize with the DNA template, displacing the coding strand. The "extended hybrid" model, on the other hand, describes the formation of R-loops during abortive transcription due to RNA polymerase stalling. These structures, referred to as *cis* R-loops, are directly associated with ongoing transcription. Most R-loops are thought to form during transcription, and *cis* R-loops are promoted by guanine-rich regions, DNA single-stranded breaks (nicks) on the coding DNA strand, and supercoiled DNA (Roy et al. 2010).

✉ Lóránt Székvölgyi
lorantsz@med.unideb.hu

[1] MTA-DE Momentum, Genome Architecture and Recombination Research Group, Department of Molecular and Nanopharmaceutics, Faculty of Pharmacy, University of Debrecen, Debrecen 4032, Hungary

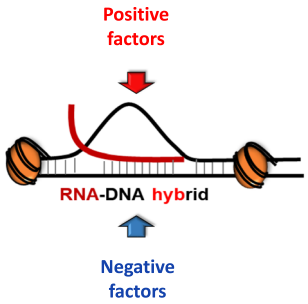

Fig. 1 Scheme of an R-loop. An R-loop is a three-stranded nucleic acid structure composed of an RNA–DNA hybrid and a displaced single-stranded DNA (ssDNA). Thermodynamic stability of the structure and its susceptibility to internal and external clastogenic signals nominate R-loops for hotspots of mutagenesis and genomic instability. Positive factors (red) refer to regulatory genes that directly or indirectly help the formation of the R-loop. Negative factors (blue) represent genes that prevent R-loop formation

Experimental data also suggest that the RNA strand in the R-loop can originate from distant genomic regions (Wahba and Koshland 2013; Ariel et al. 2020), representing long noncoding RNAs (lncRNAs), circular RNAs (circRNAs), or repetitive RNAs. These RNA molecules hybridize *in trans* with complementary DNA segments distant from their transcription site. In some instances, both *cis* and *trans* R-loops can coexist in the same region, referred to as the "mixed" model. The resulting RNA–DNA hybrids are thermodynamically more stable than DNA–DNA duplexes, partially due to the intermediate nature of the RNA–DNA hybrid between the "A" form double-stranded RNA (dsRNA) and the "B" form double-stranded DNA (dsDNA). G quadruplex (G4) structures that often form on displaced ssDNA can also contribute to the extreme stability of the R-loop (Duquette et al. 2004).

In the context of chromatin, open and transcriptionally active chromatin regions tend to promote R-loop formation (Halász et al. 2017; Karányi et al. 2018, 2020; Hetey et al. 2017). Genes with R-loops are more likely to be expressed than those without R-loops (Sanz et al. 2016). However, R-loops can also form in repressive chromatin regions, such as repetitive elements, and telomeric and pericentromeric sections, and this formation depends partly on the activity of PRC1/2 complexes.

The formation of R-loops arises from complex interactions involving nucleotide sequence composition, DNA topology, transcription rate, and chromatin states. While the mechanistic details of these processes are not fully understood, several factors have been identified that either promote or prevent R-loop formation:

1 RNase H Enzymes**:** These enzymes cleave the RNA strand in the RNA–DNA hybrid in a sequence-independent manner. RNaseH1, found in both the nucleus and mitochondria, is essential for preventing chromosomal R-loops in vivo (Arudchandran et al. 2000). RNaseH2 can recognize and cleave single ribonucleotides misincorporated into the DNA duplex (Uehara et al. 2018).
2 RNA–DNA Hybrid Helicases**:** Proteins like senataxin, aquarius, Pif1, Srs1/BLM, UPF1, DHX9, DDX1, DDX19, DDX21, and DHX30 unwind R-loops, preventing R-loop-mediated DNA damage (Skourti-Stathaki et al. 2011; Groh et al. 2017; Cohen et al. 2018; Yun et al. 2017; Ngo et al. 2021; Chakraborty et al. 2018; Ribeiro de Almeida et al. 2018; Hodroj et al. 2017).
3 Topoisomerases**:** These enzymes alter DNA supercoiling to prevent uncontrolled R-loop formation during transcription and replication (Tuduri et al. 2009; Hegedüs et al. 2018; Székvölgyi et al. 2006, 2007). Topoisomerase I (Top1) removes co-transcriptional R-loops in human cells, preventing transcription pausing and collision with the replisome (Manzo et al. 2018).
4 RNA Biogenesis Factors**:** Proteins like ASF/SF2 (human) and the THO/TREX complex (yeast and human) play crucial roles in mRNA maturation/transport processes. They prevent nascent RNA from hybridizing back to the DNA duplex, thereby inhibiting R-loop formation and transcription–replication conflicts (Li and Manley 2005; Domínguez-Sánchez et al. 2011).
5 Recombinases and Anti-recombinases**:** Human proteins like Rad51, Srs2, UPF1, Rad52, BRCA1, BRCA2, and FANCM play roles in promoting or inhibiting R-loop formation. RAD51-associated protein 1 (RAD51AP1) stimulates R-loops in humans when transcription is active (Ouyang et al. 2021). Notably, RAD51AP1 is required for the formation of "DR-loops" that contain both DNA–RNA hybrids and DNA–DNA (D) loops, preferentially bound by RAD51. Thus, RAD51AP1 promotes the invasion of RNA transcripts into the donor DNA at DSBs in transcribed regions and stimulates recombination through DR loop intermediates. The yeast Rad51 also helps R-loop formation but may be recombinogenic when present in R-loop regions without DNA damage (Wahba et al. 2013). RECQ-like helicases Sgs1 (yeast) and BLM (human), on the other hand, prevent R-loop formation (Yun et al. 2017).

The regulation of R-loop structures is closely related to factors influencing genome integrity and mutagenesis. The displaced ssDNA strand in R-loops is sensitive to various DNA-damaging effects, such as spontaneous or cytidine deaminase (APOBEC)-induced dC → dU deamination, leading to mutagenesis and recombination (McCann et al. 2023). Repair of these lesions can insert faulty nucleotides or convert nicks into DSBs, increasing the potential for genome instability. Additionally, R-loops can promote non-mutagenic homology-directed DNA repair (HDD) through

the interaction of the MRN/MRX complex (Mre11, Rad50, Xrs2/Nbs1) with the RNA polymerase, resulting in the synthesis of an R-loop near the resected DNA end, an essential step in HDD. The recombinase Rad52 is required for this process (Yasuhara et al. 2018).

Identifying regulatory proteins that control R-loop levels

To understand the above R-loop-mediated processes, extensive studies have been conducted in a number of species to identify new regulatory proteins that control R-loop levels. Stirling et al. performed an R-loop screen in *Saccharomyces cerevisiae* that identified several mRNA cleavage and polyadenylation mutants (Stirling et al. 2012) that accumulated DNA–RNA hybrids and emphasized the role of these factors in suppressing R-loop formation. Cañas et al. examined the role of chromatin modifiers in R-loop homeostasis and associated genome instability (Cañas et al. 2022). Their specific screening revealed that the Rtt109 histone acetyltransferase prevents DNA–RNA hybrid accumulation and plays a role in repairing DNA breaks caused by R-loops. The study provides insights into the influence of chromatin context on DNA–RNA hybrid-associated DNA damage and repair. Penzo et al. investigated the intriguing relationship between R-loops and nuclear pore complexes (NPCs) (Penzo et al. 2023). Their screen uncovered an association between transcribed genes, NPCs, and the accumulation of R-loops. This relocation pathway mirrors mechanisms for sensing transcriptional and genotoxic stresses, shedding light on the convergence of these processes. In *Arabidopsis thaliana*, a forward mutagenesis screen identified the Nodulin homeobox (NDX) protein as an R-loop stabilizing factor within the *flowering locus C* (*FLC)* gene locus (Sun et al. 2013). However, subsequent research revealed that NDX primarily regulates heterochromatic small interfering RNA (het-siRNA) expression and heterochromatin homeostasis, rather than euchromatic R-loops (Karányi et al. 2022; Feró et al. 2023). In human cells, Cristini et al. utilized an affinity purification approach to define an RNA/DNA hybrid interactome (Cristini et al. 2018). The interactome comprises known R-loop-associated factors and previously uncharacterized interactors, including helicases, RNA processing, DNA repair, and chromatin factors. DHX9 helicase emerges as a top candidate for R-loop suppression, with interactions with PARP1 to prevent R-loop-associated DNA damage. Wang et al. performed a liquid chromatography/tandem mass spectrometry (LC–MS/MS) screen to identify human proteins bound to the RNA–DNA hybrids (Wang et al. 2018). A study by Kim et al. systematically explored the role of human bromodomain (BRD) proteins to genome stability and DNA double-stranded break repair (Kim et al. 2019). Using a siRNA screen, they identified BRD proteins, including BRD2 and BRD4, as negative regulators of transcription-associated RNA–DNA hybrids. Barroso et al. screened an siRNA library covering 240 human DNA damage response (DDR) genes to understand how cells counteract spontaneous RNA–DNA hybrids (Barroso et al. 2019). The study identified DDR factors involved in post-replicative repair and DNA damage checkpoint pathways. Wu et al. conducted a rigorous purification of proteins linked to R-loops in mouse embryonic stem cells, identifying 364 proteins strongly associated with R-loops. Notably, nucleolar proteins, particularly numerous DEAD-box family helicase proteins, were prominently enriched in this screen. In-depth analysis of selected DEAD-box helicases unveiled their involvement in post-transcriptional processes for generating mature rRNAs and their direct or indirect roles in regulating genes associated with differentiation. These discoveries highlighted an extensive network of R-loop-associated proteins crucial for maintaining stem cell homeostasis (Wu et al. 2021). Yan et al. employed a proximity-dependent labeling system to identify proteins that regulate R-loops (Yan et al. 2022). They discovered an unexpected enrichment of R-loop regulatory proteins containing zinc fingers and homeodomains, with implications for developmental disorders and cancer. Kumar et al. employed a machine learning approach to predict R-loop binding proteins (Kumar et al. 2022). They identified features enriched in R-loop binding proteins and created random forest classifiers. Known R-loop regulating pathways, including splicing, DNA damage repair, and chromatin remodeling, were highly enriched in their dataset, validating the predictive power of their in silico approach. Most recently, a screen by Camino et al. presented an intriguing discovery: DICER, the ribonuclease known for its role in RNA processing, acts as an R-loop resolvase (Camino et al. 2023). Through biochemical analysis, the study demonstrated that DICER cleaves the RNA strand within R-loops, thereby preventing their accumulation. This study not only expands our understanding of DICER's multifunctionality but also underscores the importance of RNA processing factors in maintaining genome integrity. Taken together, the above R-loop screens are well suited to understanding the regulation of R-loops and how R-loop formation influences RNA biogenesis, DNA repair and genome stability. We expect that R-loop screening approaches will seamlessly interface with the expanding range of functional genomic screens in human and other cell models, identifying many dozens of new R-loop regulators that control R-loop formation, stability, resolution, dissolution, and signaling.

Future directions for R-loop research

An important issue in the field involves distinguishing between physiologically relevant regulatory R-loops and co-transcriptional by-products. The formation of R-loops by RNA polymerases, whether acting at the transcription site

(*cis*) or away from it (*trans*), demands complex approaches for comprehensive understanding. Quantitative changes in R-loop levels, observed in DRIP-seq (or similar genome-wide) experiments, require cautious interpretation due to their potential diverse sources. Factors such as transcriptional perturbations with agents like actinomycin D or α-amanitin, or conditional RNA polymerase mutations, can significantly impact R-loop levels, emphasizing the need for parallel measurements of nascent transcription, as demonstrated by GRO-seq. To compare and quantify R-loop profiles in different samples, it is desirable to use a spike-in control.

Careful evaluation of the effects of genetic perturbations on R-loop levels is also crucial. Merely observing an increase in R-loop levels upon deletion or silencing of a specific gene does not conclusively establish that the gene affects R-loop formation. Cell cycle variability further complicates the picture, with R-loop levels fluctuating throughout the G1/S/G2/M phases.

The relationship between R-loops and DSB repair, demonstrated by Rad52 performing inverse strand exchange using ssRNA, raises intriguing possibilities that need to be explored. However, the controversies on the role of de novo transcription in R-loop-mediated repair processes need to be clarified. Particularly, co-transcriptional R-loops with a regulatory role can be created by an RNA polymerase that "runs into" a DNA break and turns back from the break site to synthesize an R-loop (Lim et al. 2023). This R-loop is then extended in one direction from the break to the transcription start site to prevent transcription in the vicinity of the DNA lesion. These observations add to the complexity of R-loops and remain a challenging frontier.

In addition to *cis* R-loops, much less is known about the generation and abundance of *trans* R-loops, which exert their effects by hybridizing to distant genomic regions. A recent study demonstrated the role of *APOLO* lncRNA in the establishment of transchromosomal R-loops that regulate the activity of the Polycomb repressive complex 1 (PRC1) complex in *Arabidopsis* (Ariel et al. 2020). In yeast, the role of Rad51 in the formation of *trans* R-loops was demonstrated (Wahba et al. 2013), which was later refuted by another group (Lafuente-Barquero et al. 2020). These contradictions also prove that many aspects of R-loop biology are unexplored, and its relationship with transcription and DNA repair awaits further explanation.

Conclusions for future biology

R-loops, initially perceived as mere transcriptional by-products, have emerged as pivotal players in a range of cellular processes. Their role in regulating gene expression under normal conditions and their potential to cause genome instability under pathological conditions make them a topic of intense research. The identification new regulatory factors that either promote or prevent R-loop formation has added depth to our understanding of these structures. Recent studies across different species have revealed new insights into the regulation of R-loop levels, highlighting the importance of chromatin modifiers, RNA processing factors, and resolvases. Thee powerful R-loop screening methods not only broaden our knowledge on R-loop homeostasis but also provide potential new targets for therapeutic interventions in diseases linked to R-loop dysregulation.

Acknowledgements This study was supported by E-RARE-3 2018 Repetomics, NKFIH-K-142137, NKFIH-NNE-130913, and the Thematic Excellence Programme TKP2021-EGA-18 that has been implemented with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-EGA and TKP2021-EGA funding schemes. L.Sz. was supported by the Bolyai Janos fellowship of the Hungarian Academy of Sciences.

Funding Open access funding provided by University of Debrecen.

Declarations

Competing interests The author declares no conflict of interest.

References

Aguilera A, García-Muse T (2012) R loops: from transcription byproducts to threats to genome stability. Mol Cell 46:115–124. https://doi.org/10.1016/j.molcel.2012.04.009

Arab K, Karaulanov E, Musheev M, Trnka P, Schäfer A, Grummt I, Niehrs C (2019) GADD45A binds R-loops and recruits TET1 to CpG island promoters. Nat Genet. https://doi.org/10.1038/s41588-018-0306-6

Ariel F, Lucero L, Christ A, Mammarella MF, Jegu T, Veluchamy A, Mariappan K, Latrasse D, Blein T, Liu C, Benhamed M, Crespi M (2020) R-Loop Mediated trans action of the APOLO long noncoding RNA. Mol Cell 77:1055-1065.e4. https://doi.org/10.1016/j.molcel.2019.12.015

Arudchandran A, Cerritelli S, Narimatsu S, Itaya M, Shin DY, Shimada Y, Crouch RJ (2000) The absence of ribonuclease H1 or H2 alters the sensitivity of *Saccharomyces cerevisiae* to hydroxyurea, caffeine and ethyl methanesulphonate: implications for roles of RNases H in DNA replication and repair. Genes Cells Devoted Mol. Cell. Mech. 5:789–802

Balk B, Maicher A, Dees M, Klermund J, Luke-Glaser S, Bender K, Luke B (2013) Telomeric RNA–DNA hybrids affect telomere-length dynamics and senescence. Nat Struct Mol Biol 20:1199–1206. https://doi.org/10.1038/nsmb.2662

Barroso S, Herrera-Moyano E, Muñoz S, García-Rubio M, Gómez-González B, Aguilera A (2019) The DNA damage response acts as a safeguard against harmful DNA–RNA hybrids of different origins. EMBO Rep. https://doi.org/10.15252/embr.201847250

Boque-Sastre R, Soler M, Oliveira-Mateos C, Portela A, Moutinho C, Sayols S, Villanueva A, Esteller M, Guil S (2015) Head-to-head antisense transcription and R-loop formation promotes transcriptional activation. Proc Natl Acad Sci 112:201421197. https://doi.org/10.1073/pnas.1421197112

Camino LP, Dutta A, Barroso S, Pérez-Calero C, Katz JN, García-Rubio M, Sung P, Gómez-González B, Aguilera A (2023) DICER ribonuclease removes harmful R-loops. Mol Cell. https://doi.org/10.1016/j.molcel.2023.09.021

Cañas JC, García-Rubio ML, García A, Antequera F, Gómez-González B, Aguilera A (2022) A role for the *Saccharomyces cerevisiae* Rtt109 histone acetyltransferase in R-loop homeostasis and associated genome instability. Genetics. https://doi.org/10.1093/genetics/iyac108

Chakraborty P, Huang JTJ, Hiom K (2018) DHX9 helicase promotes R-loop formation in cells with impaired RNA splicing. Nat Commun 9:4346. https://doi.org/10.1038/s41467-018-06677-1

Cohen S, Puget N, Lin Y-L, Clouaire T, Aguirrebengoa M, Rocher V, Pasero P, Canitrot Y, Legube G (2018) Senataxin resolves RNA:DNA hybrids forming at DNA double-strand breaks to prevent translocations. Nat Commun 9:533. https://doi.org/10.1038/s41467-018-02894-w

Cristini A, Groh M, Kristiansen MS, Gromak N (2018) RNA/DNA Hybrid interactome identifies DXH9 as a molecular player in transcriptional termination and R-loop-associated DNA damage. Cell Rep 23:1891–1905. https://doi.org/10.1016/j.celrep.2018.04.025

Domínguez-Sánchez MS, Barroso S, Gómez-González B, Luna R, Aguilera A (2011) Genome instability and transcription elongation impairment in human cells depleted of THO/TREX. PLoS Genet 7:e1002386. https://doi.org/10.1371/journal.pgen.1002386

Duquette ML, Handa P, J. a Vincent, A.F. Taylor, and N. Maizels. (2004) Intracellular transcription of G-rich DNAs induces formation of G-loops, novel structures containing G4 DNA. Genes Dev 18:1618–1629. https://doi.org/10.1101/gad.1200804

Feró O, Karányi Z, Nagy É, Mosolygó-L Á, Szaker HM, Csorba T, Székvölgyi L (2023) Coding and noncoding transcriptomes of NODULIN HOMEOBOX (NDX)-deficient Arabidopsis inflorescence. Sci Data 10:364. https://doi.org/10.1038/s41597-023-02279-9

Feró O, Varga D, Nagy É, Karányi Z, Sipos É, Engelhardt J, Török N, Balogh I, Vető B, Likó I, Fóthi Á, Szabó Z, Halmos G, Vécsei L, Arányi T, Székvölgyi L (2024) DNA methylome, R-loop and clinical exome profiling of patients with sporadic amyotrophic lateral sclerosis. Sci Data 11:123. https://doi.org/10.1038/s41597-024-02985-y

Groh M, Albulescu LO, Cristini A, Gromak N (2017) Senataxin: Genome guardian at the interface of transcription and neurodegeneration. J Mol Biol 429:3181–3195. https://doi.org/10.1016/j.jmb.2016.10.021

Grunseich C, Wang IX, Watts JA, Crain B, Fischbeck KH, Cheung VG (2018) Senataxin mutation reveals how R-loops promote transcription by blocking DNA methylation at gene promoters. Mol Cell 69:426-437.e7. https://doi.org/10.1016/j.molcel.2017.12.030

Halász L, Karányi Z, Boros-oláh B, Kuik-rózsa T, Sipos É, Nagy É, Mosolygó-l Á, Mázló A, Rajnavölgyi É, Halmos G, Székvölgyi L (2017) RNA–DNA hybrid (R-loop) immunoprecipitation mapping: an analytical workflow to evaluate inherent biases. Genome Res 27:1063–1073. https://doi.org/10.1101/gr.219394

Hegedüs É, Kókai E, Nánási P, Imre L, Halász L, Jossé R, Antunovics Z, Webb MR, El Hage A, Pommier Y, Székvölgyi L, Dombrádi V, Szabó G (2018) Endogenous single-strand DNA breaks at RNA polymerase II promoters in *Saccharomyces cerevisiae*. Nucleic Acids Res 46:10649–10668. https://doi.org/10.1093/nar/gky743

Hetey S, Boros-Oláh B, Kuik-rózsa T, Li Q, Karányi Z, Szabó Z, Roszik J, Szalóki N, Vámosi G, Tóth K, Székvölgyi L (2017) Biophysical characterization of histone H3.3 K27 M point mutation. Biochem Biophys Res Commun 490:868–875. https://doi.org/10.1016/j.bbrc.2017.06.133

Hodroj D, Recolin B, Serhal K, Martinez S, Tsanov N, Abou Merhi R, Maiorano D (2017) An ATR-dependent function for the Ddx19 RNA helicase in nuclear R-loop metabolism. EMBO J 36:1182–1198. https://doi.org/10.15252/embj.201695131

Kannan A, Jiang ÃX, He L, Ahmad S, Gangwani L (2019) ZPR1 prevents R-loop accumulation, upregulates SMN2 expression and rescues spinal muscular atrophy. Brain. https://doi.org/10.1093/brain/awz373

Karányi Z, Halász L, Acquaviva L, Jónás D, Hetey S, Boros-Oláh B, Peng F, Chen D, Klein F, Géli V, Székvölgyi L (2018) Nuclear dynamics of the Set1C subunit Spp1 prepares meiotic recombination sites for break formation. J Cell Biol 217:3398–3415. https://doi.org/10.1083/jcb.201712122

Karányi Z, Hornyák L, Székvölgyi L (2020) Histone H3 Lysine 56 Acetylation is required for formation of normal levels of meiotic DNA breaks in S. *cerevisiae*. Front. Cell Dev. Biol. 7:364. https://doi.org/10.3389/fcell.2019.00364

Karányi Z, Mosolygó-l Á, Feró O, Horváth A, Boros-oláh B, Nagy É, Hetey S, Holb I, Szaker HM, Miskei M, Csorba T, Székvölgyi L (2022) NODULIN HOMEOBOX is required for heterochromatin homeostasis in Arabidopsis. Nat Commun 13:5058. https://doi.org/10.1038/s41467-022-32709-y

Kim JJ, Lee SY, Gong F, Battenhouse AM, Boutz DR, Bashyal A, Refvik ST, Chiang C, Xhemalce B, Paull TT, Brodbelt JS, Marcotte EM, Miller KM (2019) Systematic bromodomain protein screens identify homologous recombination and R-loop suppression pathways involved in genome integrity. Genes Dev. https://doi.org/10.1101/gad.331231.119

Kumar A, Fournier L-A, Stirling PC (2022) Integrative analysis and prediction of human R-loop binding proteins. G3 Bethesda. https://doi.org/10.1093/g3journal/jkac142

Lafuente-Barquero J, García-Rubio ML, Martin-Alonso MS, Gómez-González B, Aguilera A (2020) Harmful DNA:RNA hybrids are formed in cis and in a Rad51-independent manner. Elife 9:e56674. https://doi.org/10.7554/eLife.56674

Li X, Manley JL (2005) Inactivation of the SR protein splicing factor ASF/SF2 results in genomic instability. Cell 122:365–378. https://doi.org/10.1016/j.cell.2005.06.008

Lim YW, Sanz LA, Xu X, Hartono SR, Chédin F (2015) Genome-wide DNA hypomethylation and RNA:DNA hybrid accumulation in Aicardi–Goutières syndrome. Elife 4:e08007. https://doi.org/10.7554/eLife.08007

Lim G, Hwang S, Yu K, Kang JY, Kang C, Hohng S (2023) Translocating RNA polymerase generates R-loops at DNA double-strand breaks without any additional factors. Nucleic Acids Res. https://doi.org/10.1093/nar/gkad689

Manzo SG, Hartono SR, Sanz LA, De S, Cossarizza A, Capranico G, Chedin F (2018) DNA Topoisomerase I differentially modulates R-loops across the human genome. Genome Biol 19:1–18. https://doi.org/10.1186/s13059-018-1478-1

McCann JL, Cristini A, Law EK, Lee SY, Tellier M, Carpenter MA, Beghè C, Kim JJ, Sanchez A, Jarvis MC, Stefanovska B, Temiz NA, Bergstrom EN, Salamango DJ, Brown MR, Murphy

S, Alexandrov LB, Miller KM, Gromak N, Harris RS (2023) APOBEC3B regulates R-loops and promotes transcription-associated mutagenesis in cancer. Nat Genet. https://doi.org/10.1038/s41588-023-01504-w

Ngo GHP, Grimstead JW, Baird DM (2021) UPF1 promotes the formation of R loops to stimulate DNA double-strand break repair. Nat Commun 12:1–15. https://doi.org/10.1038/s41467-021-24201-w

Ouyang J, Yadav T, Zhang J-M, Yang H, Rheinbay E, Guo H, Haber DA, Lan L, Zou L (2021) RNA transcripts stimulate homologous recombination by forming DR-loops. Nature 594:283–288. https://doi.org/10.1038/s41586-021-03538-8

Penzo A, Dubarry M, Brocas C, Zheng M, Mangione RM, Rougemaille M, Goncalves C, Lautier O, Libri D, Simon M-N, Géli V, Dubrana K, Palancade B (2023) A R-loop sensing pathway mediates the relocation of transcribed genes to nuclear pore complexes. Nat Commun 14:5606. https://doi.org/10.1038/s41467-023-41345-z

Perego MGL, Taiana M, Bresolin N, Comi GP, Corti S (2018) R-Loops in motor neuron diseases. Mol Neurobiol. https://doi.org/10.1007/s12035-018-1246-y

Reddy K, Schmidt MHM, Geist JM, Thakkar NP, Panigrahi B, Wang Y, Pearson CE (2014) Processing of double-R-loops in (CAG) · (CTG) and C9orf72 (GGGGCC) · (GGCCCC) repeats causes instability. Nucleic Acids Res 42:10473–10487. https://doi.org/10.1093/nar/gku658

Ribeiro de Almeida C, Dhir S, Dhir A, Moghaddam AE, Sattentau Q, Meinhart A, Proudfoot NJ (2018) RNA helicase DDX1 converts RNA G-quadruplex structures into R-loops to promote IgH class switch recombination. Mol Cell 70:650-662.e8. https://doi.org/10.1016/j.molcel.2018.04.001

Roy D, Zhang Z, Lu Z, Hsieh C-L, Lieber MR (2010) Competition between the RNA transcript and the nontemplate DNA strand during R-loop formation in vitro: a nick can serve as a strong R-loop initiation site. Mol Cell Biol 30:146–159. https://doi.org/10.1128/MCB.00897-09

Sanz LA, Hartono SR, Lim YW, Ginno PA, Sanz LA, Hartono SR, Lim YW, Steyaert S, Rajpurkar A, Ginno PA, Xu X, Chédin F (2016) Prevalent, dynamic, and conserved R-loop structures associate with specific epigenomic signatures in mammals. Mol Cell 63:167–178. https://doi.org/10.1016/j.molcel.2016.05.032

Skourti-Stathaki K, Proudfoot NJ, Gromak N (2011) Human senataxin resolves RNA/DNA hybrids formed at transcriptional pause sites to promote Xrn2-dependent termination. Mol Cell 42:794–805. https://doi.org/10.1016/j.molcel.2011.04.026

Stirling PC, Chan Ya, Minaker SW, Aristizabal MJ, Barrett I, Sipahimalani P, Kobor MS, Hieter P (2012) R-loop-mediated genome instability in mRNA cleavage and polyadenylation mutants. Genes Dev 26:163–175. https://doi.org/10.1101/gad.179721.111

Sun Q, Csorba T, Skourti-Stathaki K, Proudfoot NJ, Dean C (2013) R-loop stabilization represses antisense transcription at the Arabidopsis FLC locus. Science 340:619–621. https://doi.org/10.1126/science.1234848

Székvölgyi L, Hegedüs E, Molnár M, Bacsó Z, Szarka K, Beck Z, Dombrádi V, Austin C, Szabó G (2006) Nick-forming sequences may be involved in the organization of eukaryotic chromatin into approximately 50 kbp loops. Histochem Cell Biol 125:63–73. https://doi.org/10.1007/s00418-005-0073-1

Székvölgyi L, Rákosy Z, Bálint BL, Kókai E, Imre L, Vereb G, Bacsó Z, Goda K, Varga S, Balázs M, Dombrádi V, Nagy L, Szabó G (2007) Ribonucleoprotein-masked nicks at 50-kbp intervals in the eukaryotic genomic DNA. Proc Natl Acad Sci U S A 104:14964–14969. https://doi.org/10.1073/pnas.0702269104

Thomas M, White RL, Davis RW (1976) Hybridization of RNA to double stranded DNA: formation of R loops. Proc Natl Acad Sci U S A 73:2294–2298. https://doi.org/10.1073/pnas.73.7.2294

Tuduri S, Crabbé L, Conti C, Tourrière H, Holtgreve-Grez H, Jauch A, Pantesco V, De Vos J, Thomas A, Theillet C, Pommier Y, Tazi J, Coquelle A, Pasero P (2009) Topoisomerase I suppresses genomic instability by preventing interference between replication and transcription. Nat Cell Biol 11:1315–1324. https://doi.org/10.1038/ncb1984

Uehara R, Cerritelli SM, Hasin N, Sakhuja K, London M, Iranzo J, Chon H, Grinberg A, Crouch RJ (2018) Two RNase H2 mutants with differential rNMP processing activity reveal a threshold of ribonucleotide tolerance for embryonic development. Cell Rep 25:1135-1145.e5. https://doi.org/10.1016/j.celrep.2018.10.019

Wahba L, Koshland D (2013) The Rs of biology: R-loops and the regulation of regulators. Mol Cell 50:611–612. https://doi.org/10.1016/j.molcel.2013.05.024

Wahba L, Gore SK, Koshland D (2013) The homologous recombination machinery modulates the formation of RNA–DNA hybrids and associated chromosome instability. Elife. https://doi.org/10.7554/eLife.00505

Walker C, Herranz-Martin S, Karyka E, Liao C, Lewis K, Elsayed W, Lukashchuk V, Chiang S-C, Ray S, Mulcahy PJ, Jurga M, Tsagakis I, Iannitti T, Chandran J, Coldicott I, De Vos KJ, Hassan MK, Higginbottom A, Shaw PJ, Hautbergue GM, Azzouz M, El-Khamisy SF (2017) C9orf72 expansion disrupts ATM-mediated chromosomal break repair. Nat Neurosci. https://doi.org/10.1038/nn.4604

Wang IX, Grunseich C, Fox J, Burdick J, Zhu Z, Ravazian N, Hafner M, Cheung VG (2018) Human proteins that interact with RNA/DNA hybrids. Genome Res. https://doi.org/10.1101/gr.237362.118

Wu T, Nance J, Chu F, Fazzio TG (2021) Characterization of R-loop–interacting proteins in embryonic stem cells reveals roles in rRNA processing and gene expression. Mol Cell Proteomics 20:100142. https://doi.org/10.1016/j.mcpro.2021.100142

Yan Q, Wulfridge P, Doherty J, Tang H, Sarma K (2022) Proximity labeling identifies a repertoire of site-specific R-loop modulators. Commun Nat. https://doi.org/10.1038/s41467-021-27722-6

Yasuhara T, Kato R, Hagiwara Y, Shiotani B, Yamauchi M, Nakada S, Shibata A, Miyagawa K (2018) Human Rad52 promotes XPG-mediated R-loop processing to initiate transcription-associated homologous recombination repair. Cell 175:558-570.e11. https://doi.org/10.1016/j.cell.2018.08.056

Yu K, Chedin F, Hsieh C-L, Wilson TE, Lieber MR (2003) R-loops at immunoglobulin class switch regions in the chromosomes of stimulated B cells. Nat Immunol 4:442–451. https://doi.org/10.1038/ni919

Yun E, Chang C, Novoa CA, Aristizabal MJ, Coulombe Y, Segovia R, Chaturvedi R, Shen Y, Keong C, Tam AS, Jones SJM, Masson JY, Kobor MS, Stirling PC (2017) RECQ-like helicases Sgs1 and BLM regulate R-loop–associated genome instability. J Cell Biol 216:3991–4005. https://doi.org/10.1083/jcb.201703168