石磊, 教授, 天津医科大学博士生导师, 教育部青年“长江学者”特聘教授。长期从事基因组稳定性和肿瘤发生发展的表观遗传机制研究。相关研究以独立或共同通讯作者身份发表在*Genome Biol*、*J Clin Invest*、*J Exp Med*、*Sci Adv*、*Nat Commun*和*Proc Natl Acad Sci USA*等杂志上。获得国家自然科学基金委优秀青年基金项目、天津市杰出青年基金项目和霍英东教育基金会高等院校青年教师基金资助, 作为第三完成人于2016年获得国家自然科学二等奖, 现为中国抗癌协会青年理事, 天津市抗癌协会理事, 中国细胞生物学学会染色质分会委员。

R环形成的调控机制及其生物学意义

李祎 石磊*

(天津医科大学基础医学院, 天津 300070)

摘要 R环(R-loop)是由一条DNA:RNA杂交链和一条被置换出的单链DNA组成的三链核酸结构, 通常在转录过程中形成。R环在基因调控、端粒稳定、DNA复制以及组蛋白修饰等方面都发挥着重要作用。越来越多的研究表明, 它们还是复制压力的重要来源, 过多的R环累积会造成DNA损伤以及基因组不稳定。此外, R环与许多人类疾病包括神经紊乱、癌症和自身免疫疾病等有关。鉴于R环的重要生理功能及其与疾病的潜在关系, 该文重点总结了R环的形成机制、生理功能及R环在基因转录调控和基因组不稳定性中的作用, 并讨论了R环调控异常与疾病之间的关系。

关键词 R环; DNA:RNA杂交链; DNA损伤; 基因组不稳定性; 癌症

The Regulation of R-Loop Formation and Its Biological Implications

LI Yi, SHI Lei*

(*School of Basic Medical Sciences, Tianjin Medical University, Tianjin 300070, China*)

Abstract R-loop is a three-stranded nucleic acid structure consisting of a DNA:RNA hybrid and a displaced single-stranded DNA. It is generally formed during transcription, and plays vital roles in regulating gene expression, DNA replication, DNA damage response, and genome stability. Although R-loop has been implicated in many biological processes, aberrant accumulation of R-loop is one of the major sources of replication stress that threatens genome integrity. Recent evidence suggests that R-loop is involved in many human diseases, including neurological disorders, cancer, and autoimmune diseases. Given the importance of R-loop in physiological and pathological processes, this review summarizes the mechanism of R-loop formation and its biological functions, and also discusses the relationship between R-loop dysregulation and human diseases.

Keywords R-loop; DNA:RNA hybrid; DNA damage; genome instability; cancer

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*通讯作者。Tel: 022-83336998, E-mail: shilei@tmu.edu.cn

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*Corresponding author. Tel: +86-22-83336998, E-mail: shilei@tmu.edu.cn

R环(R-loop)是一种由转录过程中新生的RNA链与模板DNA链结合形成的DNA:RNA杂交链, 以及游离的单链DNA(single-stranded DNA, ssDNA)构成的三链核酸复合物[1]。近年来, R环在基因转录调控和DNA损伤修复等过程中都发挥着重要作用因而备受关注[2-3]。一般来说, R环形成于RNA聚合酶的后侧, 其长度可超过1 Kb[4-5]。根据R环在体内的功能可分为两类, 即“生理性”和“病理性”R环。“生理性”R环通常需要特定的因素诱导形成, 并在其发挥功能的特定区域进一步增多, 主要过程包括B细胞中的体细胞超突变、免疫球蛋白类转换重组、DNA复制、CRISPR-Cas9活性、转录起始和终止的调控、端粒稳态等[6-7]。然而, 过多R环在体内累积可能会干扰DNA复制、转录和修复, 从而破坏基因组的完整性并与疾病发生发展相关, 这称为“病理性”R环。机体存在着不同的机制来防止或消除此类R环。

1 R环的形成与调控

R环的形成受基因组DNA序列特征或结构特征等因素的调节, 这些因素包括: 有R环倾向的DNA序列[8-11], 非模板DNA链发生断裂[12-13], 负超螺旋结构[14], 非典型的DNA结构[15-17]。它们独立性或协同性地促进新生RNA和DNA模板链的结合。若RNA的5′端附近含有四个及以上连续的鸟嘌呤(guanine, G), R环的形成率会显著提高[18]。此外, 其他因素也可以影响R环的形成, 例如在转录泡后面出现的负超螺旋结构也会增加新生RNA和模板链结合的概率[19]。有趣的是, 距离转录起始位点越远, R环出现的概率就越低[12]。最有可能的原因是, 较长的RNA“尾巴”形成了高级结构或者被蛋白质复合物所保护, 在空间上阻碍RNA侵入DNA双链。尽管已经有大量的相关研究, 但转录过程中R环形成的实际概率和调控机理还有待深入研究。

R环通常富集于基因启动子区未甲基化修饰的CpG岛和转录终止区域[20]。细胞内存在多种蛋白质机器负责消除异常积累的R环, 进而维持转录泡的完整性和转录的保真性[21-23]。如: RNA酶RNase H1和RNase H2利用其核酸内切酶活性将DNA:RNA杂交链中的RNA水解[24]。除RNase H1/2外, 多种解旋酶如DHX9[25]、AQR[26]、和SETX[26-27]及染色质重塑复合物(表1)等, 也可以参与DNA:RNA杂交链解旋。此外, 哺乳动物的加帽酶会结合磷酸化的RNAPII, 从而促进R环的形成[28]。

2 R环的生理功能

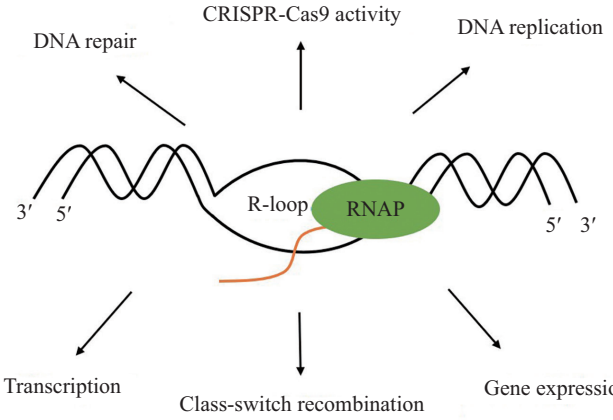
目前研究揭示, R环可以调控多种生理活动(图1), 包括: 免疫球蛋白抗体类型重组(immunoglobulin class switch recombination, CSR)[10], CRISPR-Cas9活性[44], 在线粒体DNA、细菌质粒和噬菌体中调控DNA复制过程[6]。DDX1解旋酶可以结合在免疫球蛋白重链(immunoglobulin heavy, IgH)可变(switch, S)区转录产物的G四链体(G-quadruplex, G4)结构上, 促进RNA与DNA的杂交, 为胞嘧啶核苷脱氨酶(activation-induced cytidine deaminase, AID)提供可靶向的ssDNA底物, 进而促进CSR[39]。

在基因转录调控过程中, 长链非编码RNA(long non-coding RNA, lncRNA)可以通过促进R环的形成来诱导转录[45]。在芽殖酵母(*S. cerevisiae*)中的研究发现, *GAL*基因簇相关的lncRNA(GAL lncRNA)会在*GAL*基因簇中形成R环, 其中DEAD-box RNA解旋酶Dbp2可以通过调控该DNA:lncRNA杂交链, 提高基因转录活性。参与调控细胞和组织完整性的*VIM*(vimentin)基因在多种癌症中表达上调, 有研究

表1 DNA:RNA杂交链调控因子

Table 1 Factors involved in DNA:RNA hybrid

相关因子 Factors	功能 Function
SETX[26], THO complex[5], capping enzyme[28], WDR33[29], XRN2[30]	Transcription and mRNA processing
Fanconi anemia pathway (FANCM, FANCD2)[17], BRCA1[31], BRCA2[31], XPG[32], XPF[32], CtIP[33]	DNA repair
FACT complex[34], SIN3A[35], SNF2[36]	Chromatin remodelers
RNase H1[24], RNase H2[24]	Ribonucleases
DHX9[25], SETX[26], AQR[26], DDX23[37], DDX19[38], DDX1[39], DDX21[40], BLM[41], RECQL5[42]	Helicases
TOP1[43], TOP2[43]	Topoisomerases



RNAP: RNA聚合酶。
RNAP: RNA polymerase.

图1 R环的生理作用(根据参考文献[22]修改)
Fig.1 Physiological roles of R-loops (modified from reference [22])

表明*VIM*的表达受制于反义lncRNA VIM-AS1(VIM antisense RNA 1)在*VIM*启动子区域和转录起始位点(transcription start site, TSS)形成的R环[46]。此外, 反义lncRNA TARID(TCF21 antisense RNA inducing demethylation)与抑癌基因*TCF21*的启动子区DNA形成R环, 应激反应蛋白GADD45A与之结合后招募甲基胞嘧啶双加氧酶TET1, 诱导局部DNA去甲基化进而活化*TCF21*的表达[47]。

R环不仅可以在基因启动子区调控转录活化, 还可以于G-rich区域富集终止RNA聚合酶II(RNA polymerase II, RNAPII)的延伸。在该过程中, 解旋酶SETX作用于poly(A)下游的R环[48]并与Tudor结构域蛋白SMN相互作用, 允许Xrn2核酸外切酶和终止因子的进入, 从而去除这些R环[49]。SMN通过识别RNAPII的C末端结构域(carboxy-terminal domain, CTD)的精氨酸二甲基化修饰进而募集SETX[50]。然而, 这种机制的普适性如何, 尚不清楚。G-rich终止区上的R环也会诱导反义转录, 形成双链RNA并募集核酸酶DICER、AGO1和AGO2等RNA干扰(RNAi)因子, 导致转录终止[51]。

3 R环与基因组稳定性

3.1 R环阻碍复制叉进程

由于转录和复制共享相同的DNA模板, 当复制复合体遇到转录机器时, 会导致转录–复制冲撞(transcription-replication collisions, TRCs)。当复制和转录对向进行时, 产生的对向转录–复制冲撞(head-on transcription-replication collisions, HO TRCs)可以诱导R环生成, 阻碍复制叉前进, 导致复制压力和DNA损伤[52-53]。过表达RNase H可以显著减少TRCs引起的DNA损伤, 并恢复复制叉进程[54-55], 这表明R环干扰了复制过程。范可尼贫血信号通路中的FANCA和FANCD2通常作用于复制叉来调控链内交联, 可以抑制R环的形成进而减轻TRCs造成的损伤。此外, FANCM可以利用其转位酶活性直接消除R环[56-57]。相反, 当复制和转录同向进行时, 共向的转录–复制冲撞(co-directional transcription-replication collisions, CD TRCs)则会减少R环的累积, 基因组对这种冲撞具有更大的承受性[58-59], 表明机体对不同方向TRCs的调控机制存在差异。

3.2 R环与转录调控

基因启动子区域的R环可以促进部分基因转录, 但RNA聚合酶的过度停滞或回溯引发的R环, 可能延缓转录从而引起细胞损伤[60]。这种转录应激不良状态伴生的R环, 在细胞中存在时间较短, 没有持续足够长的时间来阻碍RNA聚合酶。但是, 它们可能会建立瞬时招募平台, 通过下游效应因子引发转录应激[7]。在不同染色质环境下, R环差异性调节转录活性的分子机制还有待进一步探究。

目前有研究发现, 同源重组修复因子BRCA1和BRCA2也参与调控转录过程中生成的R环[61-63]。BRCA1通过将解旋酶SETX招募到终止位点来消除R环, 从而防止DNA损伤和突变。反之, R环的形成也会使RNAPII停滞从而阻隔BRCA1和BRCA2, 导

致R环进一步积累造成DNA损伤[64]。这提示, BRCA参与转录相关R环的识别和消除可能存在精密的负反馈调控机制。

转录阻断诱发的DNA损伤, 可以激活转录偶联核苷酸切除修复(transcription-coupled nucleotide excision repair, TC-NER)途径。但由于R环比典型的阻断损伤更大, TC-NER中的核酸酶XPG和XPF切除阻碍转录的R环, 留下一个ssDNA缺口, 该缺口可进一步发展为DNA双链断裂(double strand break, DSB)[22,26,32,63]。近期研究发现, XPG和XPF可以通过这种机制消除酵母细胞和哺乳动物中的R环[32,65]。虽然该切除方式可能会导致DNA损伤, 但它仍是调控R环和修复DNA损伤的有效方法。

3.3 R环与DNA损伤应答

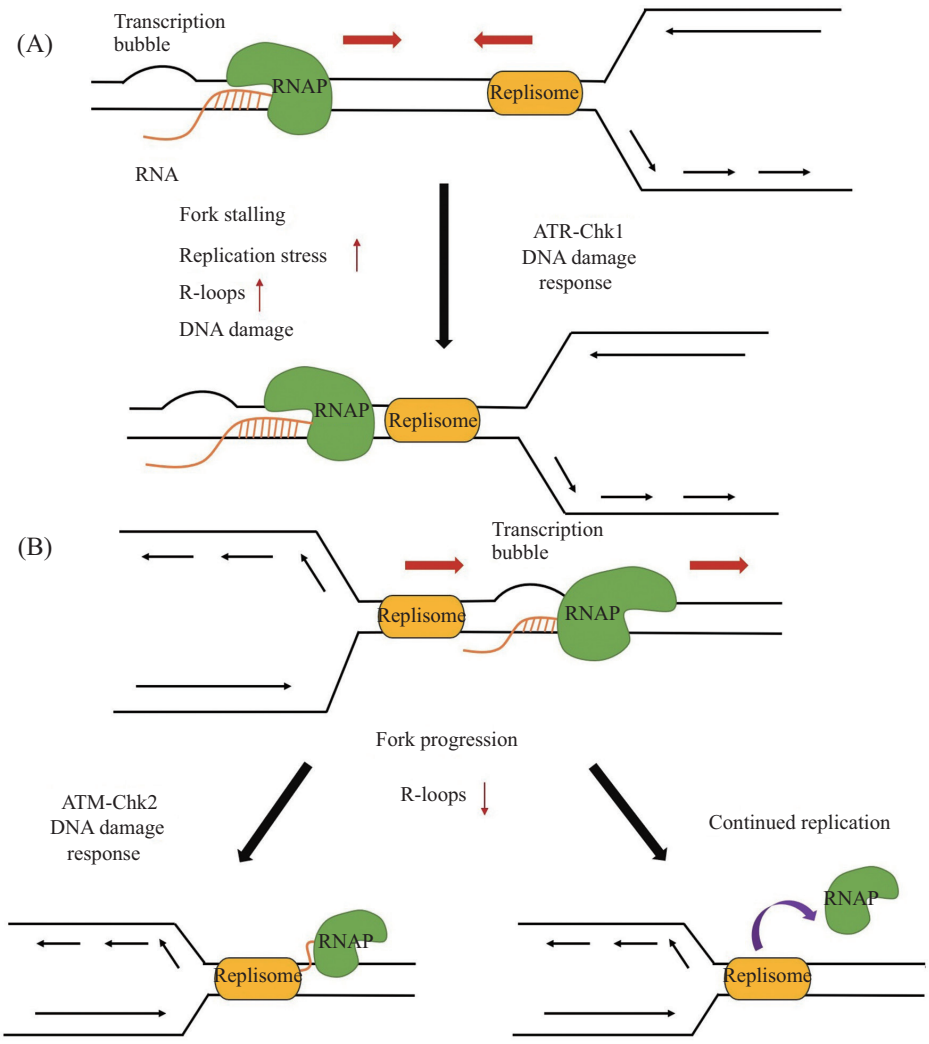
虽然R环具有多种生理作用, 但R环也是造成DNA损伤的一个主要的来源, 大多数损伤来自于体内异常积累的R环[66]。例如, 在酵母中HO TRCs是一种导致DNA损伤的主要R环形式[67]。在人类细胞中, HO TRCs也已被证明会促进R环进一步累积, 进而破坏复制进程, 严重威胁基因组稳定性[58]。细胞自身接受R环的量可能存在一个临界阈值, 当细胞内R环的量达到该阈值时, 体内消除R环的途径会达到饱和状态[7]。这可能会导致R环在体内异常持续增加并影响复制或转录过程, 进而影响基因组稳定性。

ATR和ATM蛋白激酶作为DNA损伤应答(DNA damage response, DDR)和维持基因组稳定性的关键激酶, 分别参与调控复制叉停滞产生的复制压力和DSB[68-69]。复制叉停滞可以激活ATR信号通路, 复制叉崩溃产生的DSB则进一步活化ATM信号通路[22,69]。然而, 并不是所有的R环诱导的复制压力都可以同时激活ATM和ATR。例如, 由剪接因子突变引起的R环累积造成的复制压力仅激活ATR信号通路[70]。此外, 最新研究表明, HO TRCs可以特异性激活ATR(图2A), 而CD TRCs则特异性激活ATM[58](图2B)。不同情况下, R环选择性激活ATR或ATM信号通路的机制有待进一步研究。ATM的激活可能发生在R环异常积累产生DSB时, 或者是在通过移位的ssDNA缺口进行复制时。在细菌中, 同向碰撞时RNA聚合酶的回溯会导致R环介导的DSB[71], 真核生物中也可能存在类似的机制[58]。R环上停滞的复制叉可以通过将复制蛋白A(replication protein A, RPA)招募到复制叉处暴露的ssDNA上从而激活ATR[7]。然而, 其他ATR激活途径也可能存在。例如, R环中游离的ssDNA, 也可以被RPA包被[72]。近期研究发现, 在有丝分裂过程中, ATR由R环激活, 而不受着丝粒DNA损伤的影响, 从而促进染色体分离[73]。此外, ATR和ATM的激活可以促进解旋酶SETX向TRCs的募集[74]; ATR的激活会使解旋酶DDX19入核, 在核内解开DNA:RNA杂交链, 以减轻TRCs[38]。

3.4 R环与DNA双链断裂

虽然R环是造成DNA损伤的一个潜在原因, 但也有研究表明, DNA:RNA杂交链可以在DNA损伤后形成。这些DNA:RNA杂交链既存在于转录起始位点[75], 也可以由DNA末端剪切后的ssDNA产物和新生RNA杂交形成[76]。DNA:RNA杂交链能够以多种方式影响DSB修复, 例如该结构可能阻碍损伤修复因子在DSB位点的募集, 或影响DSB附近染色质结构从而抑制修复[24,77]。相反, R环也可能促进DSB修复[75], R环的过度去除会降低DSB修复的两条主要途径即同源重组(homologous recombination, HR)和非同源末端连接(non-homologous end joining, NHEJ)的修复效率[7,78-79]。

R环调控DSB修复的方式之一是影响DNA末端切除效率。在酵母中, R环的形成防止了DSB末端过度切除, R环的去除需要DSB应答过程中ssDNA有效地与RPA结合[75]。此外, 酵母中末端剪切因子SAE2及其同源基因*CtIP*被证明可以促进R环的解开[33]。相反, 人类细胞中的DNA:RNA杂交链可以增强切除能力[78]。有趣的是, R环会促进一种特殊形式的HR: 转录相关的同源重组修复(transcription-associated homologous recombination repair, TA-HRR)。在TA-HRR过程中, DNA:RNA杂交链可以在DSB位点招募Rad52, 促进XPG介导的R环消除进而启动后续的HR修复过程[32]; 若TA-HRR活性降低, DSB修复则倾向于选择NHEJ途径, 导致基因组稳定性降低。一种更为普遍的调节机制是, DSB附近的转录产物与HR过程中的重要三链DNA结构D-loop(displacement loop)形成由重组因子RAD51相互作用蛋白1(RAD51 associated protein 1, RAD51AP1)所驱动的DR-loop, 该结构可以促进RAD51的重组活性, 提高HR修复效率[79]。DNA:RNA杂交链也可能有助于另一种以RNA为替代模板形式的HR过程。在此过程, 同源RNA分子被用来代替DNA作为DSB修复的模板[80-81]。以上研



A: 当复制与转录方向相向时, 会发生对向转录–复制冲撞(HO TRCs), R环积累产生复制压力并激活ATR-Chk1信号通路。B: 当复制与转录方向相同时, 会发生共向转录–复制冲撞(CD TRCs), R环水平降低并激活ATM-Chk2信号通路。向上的红色箭头: 增加; 向下的红色箭头: 减少。

A: the ATR-Chk1 DNA damage signaling pathway is activated by HO TRCs (head-on transcription-replication collisions). B: co-directional collisions trigger the ATM-Chk2 DNA damage checkpoint. Upward red arrow: increase; downward red arrow: reduce.

图2 对向和共向转录–复制冲撞调控R-环的模型(根据参考文献[58]修改)

Fig.2 Model for head-on and co-directional transcription-replication conflicts regulating R-loop (modified from reference [58])

究提示, R环可以通过不同的机制调控HR修复, 但调控方式的选择性和特异性还需深入探索。

4 R环与疾病

4.1 癌症

癌症是一种复杂且多样化的疾病, 许多癌症表现出高水平的DNA突变和DNA损伤[82]。R环在转录过程中形成, 在调控异常时会导致DNA损伤, 影响基因组稳定性, 这提示了癌症与R环之间的潜在联系。在高水平雌激素刺激的乳腺癌细胞中, R环积累并驱动了DNA损伤的产生[55]。RAS原癌基因的突变同样会导致R环的积累, 并造成DNA损伤和复制压力[54]。

同源重组修复因子BRCA1和BRCA2驱动的信号通路有缺陷时也会诱导R环介导的DNA损伤, 这些R环的积累被发现与小鼠乳腺肿瘤的发生有关[83]。一些癌症诱发因素也会导致BRCA功能不全, 从而导致R环调控异常, 且R环自身也会干扰BRCA的功能。在尤文氏肉瘤患者细胞中, EWSFLI融合蛋白诱导形成的R环阻断了BRCA1的功能, 使这些癌细胞中BRCA1单倍剂量不足, DNA修复无法正常进行。

R环可以通过诱导DNA损伤的方式对癌细胞施加选择压力。在对白血病骨髓增生异常综合征的研究中发现, 许多剪接因子的突变都可以诱导R环的

形成, 这些R环会激活ATR并影响细胞增殖, 在这种压力下仍能增殖的细胞最终可能会发生癌变[70]。针对癌症中R环的调控异常进行特定治疗, 可能是治疗某些难治性肿瘤的一种有效方法。例如, 在滑膜肉瘤细胞中, 抑制ATR可使肿瘤细胞中R环积累, 增加其对化疗的敏感性[84]。

最近有研究表明, 基因组不稳定性和DNA损伤也会触发先天免疫和炎症反应通路, 特别是通过激活cGAS-STING途径[85]。由致癌刺激导致的R环是诱发DNA损伤的主要来源之一, 癌细胞中R环的形成可能直接激活固有免疫应答[85]。cGAS-STING的激活对抗肿瘤免疫至关重要。然而, cGAS-STING介导的炎症反应也可能会促进某些肿瘤的生长和转移[85]。因此, R环是如何影响cGAS-STING和其他先天免疫信号通路的, 同样需要进一步探究。

4.2 神经紊乱性疾病

R环还与一些神经系统疾病相关, 在扩展的三核苷酸DNA重复序列上形成的R环与某些神经疾病相关基因的转录抑制有关, 包括弗里德赖希共济失调(Friedreich's ataxia)和脆性X综合征(fragile X syndrome)[86-87]。在这种类型的疾病中, R环的形成仅限于扩展的三核苷酸, 并主要影响含重复序列的基因[88]。解旋酶SETX的突变存在于多种神经系统疾病中, 包括II型精神性视觉失明共济失调(ataxia-ocular apraxia type 2, AOA2)和IV型肌萎缩性(脊髓)侧索硬化(amyotrophic lateral sclerosis type 4, ALS4)。AOA2患者细胞中, R环水平升高[89], 但这与SETX的功能是否相关还有待研究。虽然R环水平的升高通常与疾病相关, 但R环水平降低也可能是病理性的。在ALS4患者细胞中发现, SETX解旋酶功能的增强使负调控TGF-β的基因启动子处R环水平降低、甲基化增加, 进而导致了ALS4患者细胞TGF-β信号异常, 最终诱发神经元功能障碍和死亡[90]。

4.3 自体免疫性疾病

R环失调在自身免疫性疾病中也发挥着重要作用。Aicardi Goutieres综合征(Aicardi Goutieres syndrome, AGS)是一种罕见的炎症性疾病, 通常由核酸酶TREX1、SAMHD1或RNase H2突变引起。这些核酸酶的突变, 可导致核酸在细胞质内积累、激活cGAS-STING和干扰素应答通路, 引发机体发生炎性反应[91]。一方面, AGS细胞中的R环水平升高, 可能改变了基因表达或重新激活了逆转录因子[92]; 另一方面, AGS细胞中R环诱导的停滞复制叉处产生的DNA片段可能被释放到细胞质中。两者共同参与了cGAS-STING和干扰素应答通路的激活[93-94], 最终导致AGS的发生。

5 总结与展望

大量实验证据表明, R环的生成和消除依赖于蛋白质机器的协同性和精密性调节。RNase H作为核酸内切酶广泛参与了R环的消除, 我们非常好奇细胞内是否存在核酸外切酶可以降解R环中的RNA。R环在许多基于染色质的生理活动中发挥着重要的功能, 同时它们也是DNA损伤和基因组不稳定性的来源。当前, R环形成的分子机制及对基因组稳定性的影响已引起广泛关注。尽管关于R环与复制压力应答的关系已经相对清楚, 但R环是否可以直接影响ATR激活或在空间上与复制压力应答机器产生联系, 还有待深入研究。围绕这些科学问题的研究可以更好地理解R环的生理和病理功能, 加深我们对相关疾病机理的认识。

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