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Structure-forming repeats and their impact on genome stability

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Repetitive sequences throughout the genome are a major source of endogenous DNA damage, due to the propensity of many of them to form alternative non-B DNA structures that can interfere with replication, transcription, and DNA repair. These repetitive sequences are prone to breakage (fragility) and instability (changes in repeat number). Repeat fragility and expansions are linked to several diseases, including many cancers and neurodegenerative diseases, hence the importance of understanding the mechanisms that cause genome instability and contribute to these diseases. This review focuses on recent findings of mechanisms causing repeat fragility and instability, new associations between repeat expansions and genetic diseases, and potential therapeutic options to target repeat expansions.

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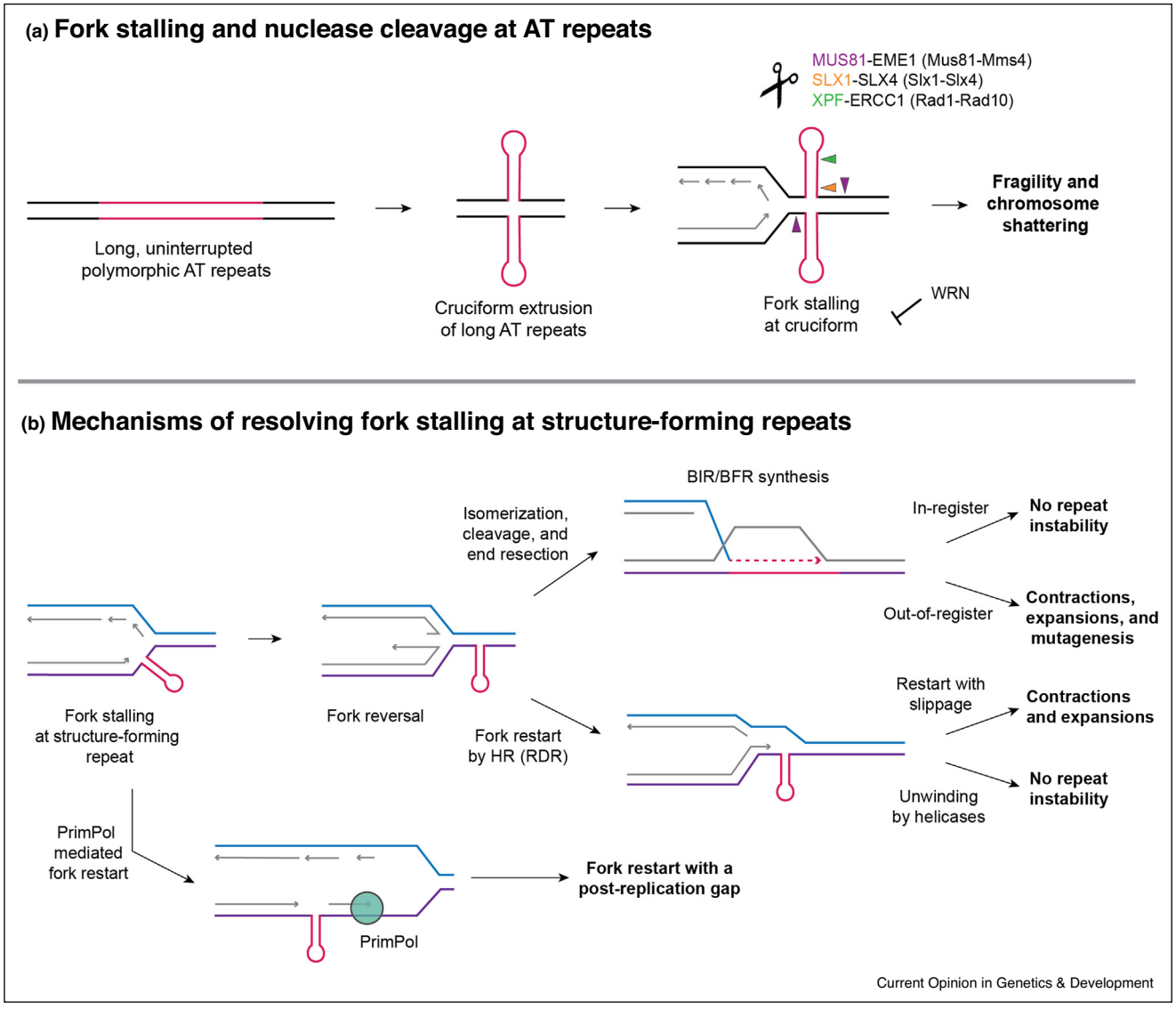
Introduction

Genome integrity is constantly under threat due to both endogenous and exogenous DNA damaging sources. One source of endogenous DNA damage is repetitive sequences that have the ability to form alternative secondary structures different from B-form DNA. These structure-forming repeats can interfere with various cellular processes including replication, transcription, and DNA repair. Structure-forming repeat sequences are prone to chromosome breakage and are enriched at breakpoints of genomic rearrangements in cancer cells [1]. Also, a still growing number of neurodegenerative diseases are caused by repeat expansions that occur in both coding and non-coding regions of the genome [2]. Many diseases linked to repeat instability do not have any successful treatment options, highlighting the importance of understanding mechanisms that cause these diseases and to develop potential therapeutic options in the future. In this review, we discuss mechanisms that contribute to repeat instability, focusing on the most recent advances, new associations between repeat expansions and genetic diseases, and potential therapies to contract expanded repeats.

Repeats interfere with replication to cause genome instability

Repetitive sequences throughout the genome serve as potential barriers to replication that can result in fork stalling and collapse. Various regions throughout the genome termed fragile sites are prone to chromosome breakage, especially under replication stress. Several recent advances have confirmed that DNA structures play an important role in common fragile site (CFS) fragility. Sinai *et al.* inserted an AT-rich sequence predicted to form hairpin structures from CFS FRA16C into a normally non-fragile ectopic site and observed recurrent chromosome gaps, indicating that the inserted AT-rich sequences interfered with completion of replication [3•]. Another common fragile site, FRA16D, contains a polymorphic AT repeat (Flex1) that stalls replication and causes fragility when inserted into a yeast chromosome [4]. A recent study showed that Flex1 causes a length-dependent increase in fragility that is strongly correlated with the lengths that caused fork stalling and form cruciform structures *in vivo* [5•]. The AT repeat fragility was dependent on the Mus81–Mms4 nuclease complex working in the context of the Slx4 scaffold [5•], which is the same nuclease complex shown to be required for breaks at FRA16D and other CFSs in human cells (reviewed in Ref. [6]) (Figure 1a). It appears that AT repeat fragility may be a wide-spread phenomenon as a genome-wide study to identify sites of fork collapse upon inhibition of the ATR checkpoint kinase, detected through RPA-ChIP and BrITL (breaks identified by TdT labeling), identified AT-rich repeats as the most commonly represented sites in human cells [7•]. Interestingly, the same study showed that $(CAGAGG)_n$ and $(CACAG)_n$ repeats, which form quadruplex structures, were most commonly identified as sites of fork collapse in mouse cells, indicating that the most problematic repeats may vary by organism [7•]. Poly(dA:dT) tracts, that are unwinding elements but may also form triplex secondary structures, were demonstrated to be a causal factor of fork stalling and breakage under replication stress within early replicating fragile

Figure 1



Fork stalling at structure forming repeats results in repeat fragility and instability.
(a) Long, uninterrupted polymorphic AT repeats have the potential to form cruciform structures that serve as a barrier to replication and cause fork stalling and ATR activation. WRN (Werner Syndrome) helicase (a RecQ helicase) can be recruited to unwind the structure and prevent fork collapse. Loss of WRN results in chromosome shattering in MMR deficient MSI cancers with expanded AT repeats. **(b)** Pathways to resolve fork stalling at structure-forming repeats (a hairpin is shown but it could also be a G4 or triplex structure). Fork stalling can occur due to structure-forming repeats serving as a barrier to replication on either the leading or lagging strand and can result in fork reversal (a resected reversed fork is shown). Fork restart can occur through several pathways: (1) repriming past the structure, for example, by PrimPol (2) through recombination-dependent replication (RDR), using the displaced 3′ end from a reversed fork and template strand invasion, and (3) through a BIR-like pathway after fork cleavage and end resection (referred to as broken fork repair, BFR). These pathways can result in expansions or contractions if slippage or out-of-register invasion or structure bypass occurs. Alternatively, unwinding of the structure by helicases during restart can avoid repeat instability. Exposed ssDNA accumulating during BIR can result in repeat-induced mutagenesis (RIM).

sites in activated B cells isolated from mice [8]. Recent evidence also suggests that TG-repeats in stickleback fish contribute to recurrent deletions at the *Pel* locus due to their ability to form alternative secondary structures, driving evolution in these organisms [9]. Overall, these various findings indicate that replication fork stalling and subsequent breakage at structure-forming repeats are a significant source of genome instability across multiple organisms and conditions.

A new study provides a very important link between fragility at AT repeats and cancers caused by

microsatellite instability (MSI). Previous studies demonstrated that WRN (Werner Syndrome) helicase, a RecQ DNA helicase, is essential for survival in mismatch repair (MMR) deficient cells with MSI, and that loss of WRN resulted in increased chromatin bridges, chromosome fragmentation, and micronuclei (see Ref. [10••] and references therein). These studies identified WRN as a synthetic lethal target for potential MSI cancer therapeutics. The Nussenzweig lab followed up on this evidence to determine a mechanism through which WRN helicase acts in MSI cancers and why is it necessary for viability. They used END-seq to determine the genome-wide sites of double-strand breaks (DSBs) formed in MSI cancer cell lines upon depletion of WRN and observed that these breaks accumulate primarily at AT repeats [10••]. Interestingly, the repeats at the breakage sites were expanded compared to non-MSI control cell lines and were susceptible to MUS81-EME1-SLX4 nuclease cleavage, in agreement with the Kaushal *et al.* data described above [5•,10••]. Overall, the authors propose that in MSI cancers, MMR deficiencies contribute to AT repeat expansions which then can form cruciform-like secondary structures that stall replication forks [10••]. Fork stalling causes ATR activation and recruitment of WRN to aid in completion of DNA replication [10••]. Upon loss of WRN, MUS81-EME1 endonuclease cleaves at the expanded AT repeats, resulting in chromosome fragmentation and cell death [10••] (Figure 1a).

Replication fork stalling occurs at telomeres due to the G-quadruplex (G4) structures formed by telomeric sequences, and telomeres display features of fragile sites (reviewed in Ref. [11]). Internal G4 structures can also cause fork stalling, which can be overcome by repriming by PrimPol (reviewed in Ref. [12]) (Figure 1b). Recent studies demonstrate that the Exo1 exonuclease is important for preventing telomere length instability, chromosomal aberrations, and cell death, especially when cells are treated with a G4-stabilizer [13,14]. Exo1 was proposed to process forks stalled within the telomere and mediate repair by a recombination-based repair mechanism [13]. The Warsaw Breakage Syndrome associated DDX11 helicase was also recently found to resolve G4 structures and protect cells from DNA damage during replication and improper sister chromatid cohesion [15].

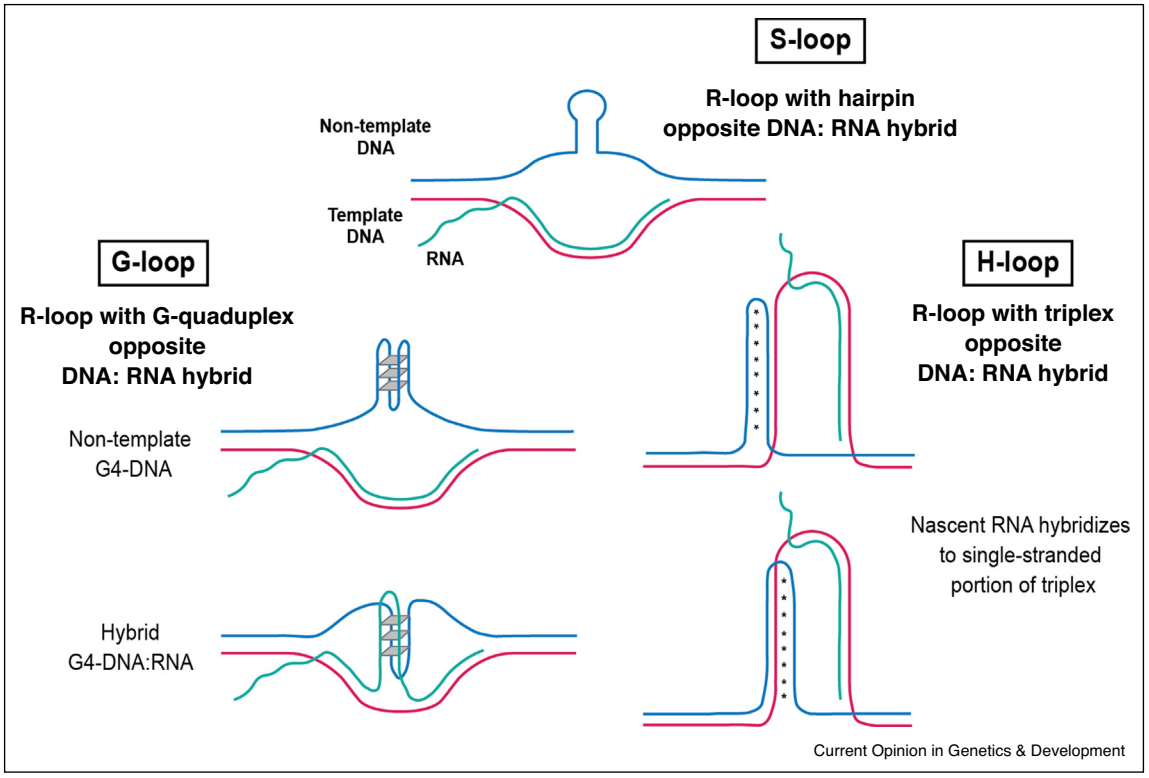
In addition to fragility, replication problems at structure-forming repeats can also lead to repeat expansions and contractions (reviewed in Refs. [16,17]). One ended breaks, for example caused by fork collapse, can trigger break-induced replication (BIR). Large-scale expansions at CAG/CTG, CGG/CCG, and GAA/TTC repeats are dependent on proteins known be involved in BIR, such as Pol32, Pif1, and HR proteins Rad51 and Rad52 [18,19,20•] (Figure 1b). It is not yet clear if the BIR mechanism involves fork restart in S phase using the as yet unreplicated chromosome ahead of the stall as a template (broken fork repair, BFR), or whether it occurs in G2, using the replicated sister chromatid or another chromosome as the template (Figure 1b). Recent results show that a late S-phase event that is likely fork restart happens after fork collapse at an expanded CAG/CTG repeat [21,22].

The lagging strand, which has single-stranded stretches exposed during replication, is particularly prone to allowing DNA structure formation. Khristich *et al.* found that large-scale contractions of GAA repeats occurred primarily during lagging strand replication and were dependent on their ability to form a H-DNA triplex structure [23•]. Contractions are proposed to occur by bypass of the template structure by Pol δ, and are exacerbated by mutations that affect Pol δ processivity [23•]. Another response to lagging strand hairpins can be a template switch to copy from the sister chromatid, which has been shown by multiple groups to cause repeat expansions (see Ref. [17] for review). A new appreciation is that histone modifications are required to facilitate efficient D-loop extension during sister-chromatid recombination and prevent CAG repeat expansions in a yeast model. These include histone H4 acetylation on lysines 12 and 16 and histone H2A.1 phosphorylation on threonine 126 [24,25]. In human cells the histone deacetylase HDAC3 stimulates CAG repeat expansions [26]. The Lahue group recently identified that, MSH3 previously identified to be required for CAG expansions, is the target of HDAC3 deacetylation, which is required for MutSβ nuclear localization [27]. HDAC2 also mildly enhances CAG expansions at the HD locus, perhaps by altering chromatin structure of the locus [28].

Transcription-induced DNA structures

Transcription can also pose a threat to genome integrity since it involves unwinding of the DNA double helix. One byproduct of transcription that is particularly relevant for structure-forming repeats is R-loops [29,30]. When the transcribed strand is engaged in an DNA:RNA hybrid, it can allow DNA structure formation on the exposed single-stranded non-transcribed strand, and this is particularly likely in G-rich sequences such as expandable CGG, CAG, or G_4C_2 repeats (see Refs. [2,31] for review). An R-loop with a G4 structure is referred to as a G-loop, and with a slipped-out hairpin an S-loop (Figure 2). R-loops at the FMR1 and C9orf72 gene loci that can contain expanded CGG and G_4C_2 repeats, respectively, were recently mapped at the nucleotide level using bisulfite footprinting and deep sequencing, confirming that their presence allows structure formation on the non-transcribed strand *in vivo* and showing that even non-expanded alleles form unusually long, stable R-loops of ~500−800 bp at these loci [32]. These results are consistent with earlier models suggesting that G-loops can also form as a hybrid between the ssDNA and

Figure 2



Predicted models of R-loop formation at structure-forming repeats.
S-loops contain a hairpin structure formed on the non-template displaced single DNA strand opposite a DNA:RNA hybrid. G-loops contain a G-quadruplex opposite a DNA:RNA hybrid; either the displaced non-template single DNA strand can form a G-quadruplex (top) or a hybrid DNA:RNA G-quadruplex can form in the context of an R-loop (bottom). H-loops contain a triplex or H-DNA structure opposite a DNA:RNA hybrid. Nascent RNA can bind the single-stranded portion of the triplex structure and the triplex can form in two orientations, in which Hoogsteen bonding occurs either between two purine strands (top) or a purine and pyrimidine strand (bottom).

ssRNA structures [33] (Figure 2). Su and Freudenreich investigated how R-loops at expanded CAG repeats cause repeat instability and fragility, and demonstrated that expanded CAG repeats engaged in R-loops are prone to cytosine deamination, which recruits the base excision repair (BER) pathway to cause repeat contractions [34•]. In addition, cleavage by the MutLγ (Mlh1–Mlh3) nuclease was R-loop dependent, providing a possible mechanism for how this nuclease acts inappropriately on CAG or CTG hairpins to cause repeat expansions (see Ref. [31] for review).

Even though they aren't GC rich, expanded GAA repeats have also been shown to form R-loops. Neil *et al.* examined the role of DNA:RNA hybrids in GAA repeat stability and found that loss of RNase H (which cleaves the RNA of DNA:RNA hybrids) resulted in an increase in GAA expansions [20•]. However, unlike canonical R-loops, GAA repeats may form a novel type of structure they termed an H-loop, which is a combination of an R-loop and triplex H-DNA [20•] (Figure 2). There is evidence that PrimPol repriming activity may prevent unscheduled R-loop formation at GAA repeats by helping to restart stalled replication and preventing ssDNA from accumulating [35].

H3K9 methylation has recently been shown to suppress DNA:RNA hybrid-induced instability of satellite repeats in *Caenorhabditis elegans* [36,37]. More specifically, *C. elegans* with loss of the MET-2 (SETDB1 homolog) H3K9 methyltransferase accumulate satellite repeat transcripts, sequences that are normally marked by H3K9me2 and are repressed. These transcripts can bind to simple and satellite repeat sequences, resulting in the formation of DNA:RNA hybrids [36,37]. Simple repetitive transcripts may be even more dangerous to genomic integrity than unique sequences as they lack signals for RNA processing and can form structures that may stall replication. A genome-wide screen examining synthetic lethality in *met-2* mutants identified RNA processing, nuclear RNA degradation, and DNA repair and replication fork stability factors [37]. For example the BRCA1/BARD1 complex was found to be partially redundant with MET-2, as it

prevents the accumulation of satellite repeat transcripts and DNA:RNA hybrid formation that contribute to genomic instability and germline lethality in *C. elegans* [37].

In addition to DNA:RNA hybrids, transcription itself can cause repeat instability through unwinding of DNA and introduction of negative supercoils behind RNA Polymerase II (RNAPII) (see Ref. [38] for review). Koch *et al.* demonstrated that defects in chromatin remodeling upon loss of Isw1 causes an increase in CAG repeat expansions and this was dependent on both transcription through the CAG repeat tract and nucleotide excision repair (NER) and BER proteins [39]. Isw1 is known to be important for nucleosome spacing, which was altered in the mutant cells following transcription. The authors proposed that improper establishment of nucleosomes after RNAPII passage in cells lacking Isw1 leads to CAG/CTG hairpin formation, which triggers BER and NER to cause CAG instability [39].

Interference with repair causes repeat instability

DNA repair pathways are a double-edged sword. Though they are meant to protect the genome and maintain genome integrity, in the context of repetitive DNA they can lead to inappropriate repair, repeat instability, or genome rearrangements. Some recent advances have shed light on the players and what can go wrong.

Multiple NER nucleases have recently been shown to target structure-forming repetitive sequences. XPF-ERCC1 (Rad1-Rad10 in yeast) causes deletions and translocations due to cleavage of cruciform, inverted repeat, and H-DNA triplex structures, resulting in genome instability [5•,40,41]. Additionally, the MutSβ (MSH2–MSH3) complex recognizes Z-DNA as damage, resulting in recruitment of and cleavage by XPF-ERCC1 to cause deletions and translocations [42•]. These nucleases act in both replication-dependent and replication-independent pathways to cause repeat fragility.

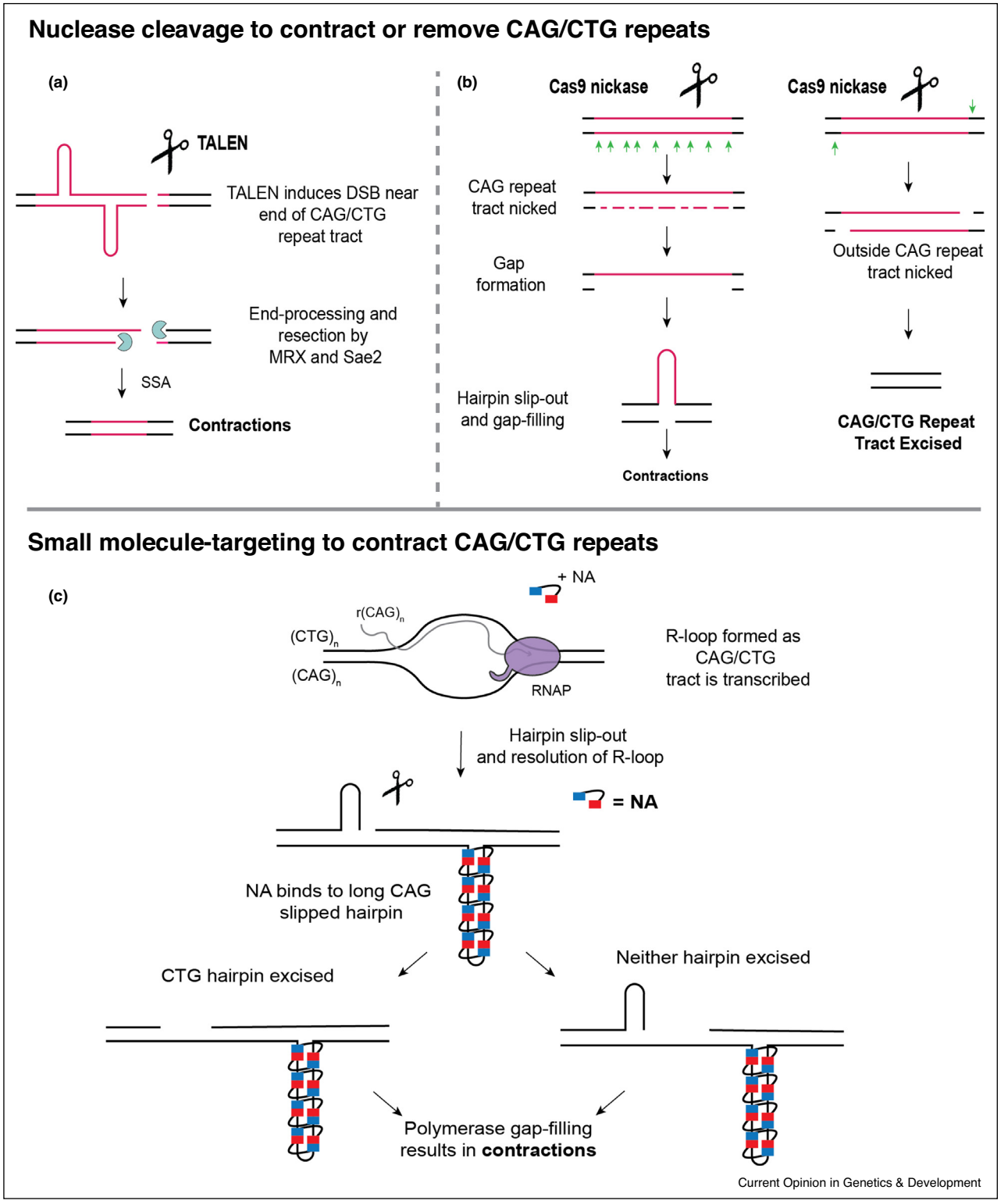
In contrast, other nucleases have been found to be protective of repeat instability. A new player on the scene is FANCD2 and FANCI-associated nuclease 1 (FAN1), which was identified in a GWA study as a modifier of Huntington's disease (HD) onset [43,44••,45]. FAN1 protects against CAG repeat expansions in HD cell lines in a dose-dependent and nuclease-independent manner, and knockout of FAN1 increases CAG repeat expansion in HD induced pluripotent stem cells (iPSCs) [45,46]. FAN1 also protects against somatic CGG repeat expansions in a Fragile X mouse model [47•]. Interestingly, it was recently shown that Fan1-dependent somatic CAG expansions in HD knock-in mice are dependent on the presence of MLH1, indicating that it acts downstream of a MutL-dependent process [48].

MMR proteins have long been known to play a role in repeat instability, and some new data sheds light on possible mechanisms. It was observed earlier that CAG expansions in an HD mouse model, as well as all germ line and somatic CGG expansions in a Fragile X mouse, were dependent on MutLγ (MLH1–MLH3) [49,50]. MutLα and MutLβ were also found to prevent CGG expansions in mouse embryonic stem cells, as PMS1 and PMS2 prevented expansions similarly to MLH1 and MLH3 [51]. MLH3 nuclease activity is required for CGG expansions in a mouse stem cell model, consistent with evidence from a yeast model that Mlh3 nuclease activity causes CAG repeat fragility and instability [34•,52]. Therefore, MutLγ cleavage activity is implicated as a key component of its inappropriate action at repeats and its target is likely a conserved feature of hairpin-forming sequences. However EXO1, which normally acts downstream of MutLγ in meiosis, protects against somatic CGG repeat expansions in a Fragile X mouse model [50]. Therefore, EXO1 may process a MutLγ cleaved structure to prevent expansions. There is also evidence of crosstalk between the MMR and BER machinery: MutSβ stimulates Polβ to copy through DNA structures on the template strand and displace a 5′ flap during BER, which promotes CAG and GAA trinucleotide expansion [53]. In the absence of MutSβ, Polβ bypasses the loop structure, resulting in repeat deletions [53]. Overall, the evidence shows that various repair pathways act inappropriately in the context of DNA structures to contribute to repeat instability.

Repeats traveling to specific nuclear domains for repair

There is mounting evidence that several types of DNA damage relocate within the nucleus for repair, including persistent DSBs, DSBs within rDNA and heterochromatin, and collapsed forks due to severe replication stress or replication fork barriers (RFBs), including structure-forming repeats (see Ref. [54] for review). In yeast, persistent DSBs can relocate to either the NPC in all cell cycle phases or the SUN domain protein Mps3 in the inner nuclear membrane during S and G2 phases [55]. While relocation to the NPC can promote BIR or other events requiring strand invasion, association with Mps3 prevents aberrant recombination from occurring, indicating that different destinations can control repair outcome ([56] and reviewed in Refs. [57,58]). However, when relocation fails, genome instability occurs. Recent evidence demonstrates that collapsed forks that encounter a CAG repeat replication barrier in yeast relocate to the nuclear pore complex (NPC) through a sumoylation-mediated mechanism that prevents chromosomal breaks and end loss events [22]. This relocation also suppresses Rad52-mediated repeat instability [21]. Interestingly, Rad51 was excluded from the CAG repeat when it was in the nuclear interior, only associating after movement to the NPC [22]. In a recent study of eroded telomeres, it

Figure 3



Recent advances in contracting or removing expanded CAG/CTG repeat tracts.
(a) A TALEN (transcription-activator like effector nuclease) targeting expanded CAG/CTG repeats induces a DSB near the end of the repeat tract. The DSB is processed and resected by the MRX (Mre11-Rad50-Xrs2) endonuclease complex stimulated by Sae2. Repair of the gap created by end-processing occurs through single-strand annealing (SSA), resulting in contractions [70]. **(b)** Use of the Cas9 D10A nickase to contract or remove an expanded CAG repeat tract. Left, Cinesi *et al.* introduced nicks throughout the CAG repeat tract, resulting in contractions [71]. Right,

was found that mutating the NPC basket protein Nup1 impairs relocalization of both telomeric and expanded CAG repeats [59]. In both cases, the Nup1 defect led to altered repair: either increased Rad52-dependent CAG repeat contractions or increased sister chromatid recombination at telomeres, providing evidence that relocation is important for suppressing inappropriate HR at repetitive DNA [59]. Related, Maestroni *et al.* showed that loss of telomerase and Bqt4, a protein involved in anchoring telomeres to the nuclear envelope, resulted in enhanced subtelomeric recombination [60].

In higher eukaryotes including Drosophila and mammalian cells repair within repetitive heterochromatic DNA is also controlled by nuclear position (see Ref. [61] for review). DSBs within pericentromeric heterochromatin of the *Drosophila* genome, mainly consisting of satellite repeats, relocalize to the nuclear periphery (see Ref. [62] for review). In a recent development, this was suggested to occur by directed motion along nuclear actin filaments [63]. Impairment of relocalization resulted in genomic instability including chromosome fusions, aneuploidy, and abnormal satellite DNA copy number [63]. In mammalian cells, the Soutoglou lab showed that breaks within heterochromatic satellite DNA move to the periphery of the heterochromatin domain in S/G2 in a manner dependent on chromatin relaxation [64]. In both systems, Rad51 is excluded from the heterochromatin domain, only becoming associated after the movement, though interestingly this was not the case for mammalian centromeric repeats [64]. Though the rDNA is an actively transcribed area it contains many tandemly repeated genes and is prone to accumulating deletions. In both yeast and mammalian cells, DSBs within the rDNA move to the periphery of the nucleolus for repair and defects in this process lead to rDNA hyperrecombination and genome instability (see Refs. [58,61] for review). A recent study of DSB mobility in the rDNA of human cells showed that movement to the nucleolar periphery is an active process that involves actin as well as the nuclear envelope-associated LINC complex [65].

In addition to relocation for repair, 3D genome organization appears to play a more constitutive role in preventing repeat expansions. Disease-associated loci containing short tandem repeats, including FMR1, HTT, DMPK, FXN, C9orf72, and ATXN1, localize to TAD (topologically associated domain) boundaries [66•]. The authors tested cells from Fragile X Syndrome (FXS) patients and healthy siblings and found that at the FMR1 locus, FXS patients with expanded repeats (>600 CGG repeats) exhibited disrupted TAD boundaries and CCCTC-binding factor (CTCF) binding that correlated to FMR1 silencing, compared to healthy siblings that did not exhibit these phenotypes [66•]. However, another study found that CTCF binding and chromatin interactions were unchanged upon CAG/CTG repeat expansions at the DMPK and HTT loci [67]. These studies highlight that chromatin interactions may have different effects at different loci.

New repeat expansion diseases and potential therapies

The Genetic Modifiers of Huntington's Disease Consortium (GeM-HD) has been working to identify factors that alter age at onset of motor symptoms and progression of HD. The 2019 GeM-HD study clearly distinguished for the first time that uninterrupted CAG repeat length and not polyglutamine length is the driving factor in timing of age at onset of HD motor symptoms [44••]. This highlights the extreme importance of repeat instability in driving HD and likely other neurodegenerative diseases. Previously, it had been demonstrated that somatic instability of CAG repeats is associated with age at onset of HD symptoms, specifically within tissues in the brain that are most affected (striatum and cortex), suggesting that factors that impact somatic instability may be modifiers of the disease [68]. Sure enough, genome-wide association (GWA) analysis revealed genetic modifiers of HD are genes involved in DNA repair: MLH1, FAN1, PMS1, MSH3, DHFR, PMS2, and LIG1, and polymorphisms that either increase or decrease expression of these factors can either delay or advance age at onset of HD [44••].

Since uninterrupted CAG repeat length is a major determinant of HD motor symptoms, it has become a central target in therapeutics for HD. As use of endonucleases, such as ZFNs (zinc-finger nucleases), TALENs (transcription-activator like effector nucleases), and CRISPR-Cas9, have become popular gene editing tools, these have been used to target trinucleotide repeats to shorten their length as a potential therapy for repeat expansion diseases [69]. Mosbach *et al.* showed that a TALEN targeting expanded CAG repeats can contract them below pathological length and the induced DSB is repaired through single-strand annealing (SSA) [70] (Figure 3a). Cinesi *et al.* demonstrated that use of CRISPR-Cas9 D10A nickase promotes CAG contractions by activating nick repair within the repeat tract [71] (Figure 3b). The use of Cas9 nickases is a potentially promising therapeutic, as inducing nicks rather than DSBs likely helps to avoid expansions from occurring [71]. A follow up study excised

(Figure 3 Legend Continued) Dabrowska *et al.* excised the CAG repeat tract by nicking outside of the tract [72]. **(c)** Nakamori *et al.* used a small molecule, NA (naphthyridine-azaquinolone) to target slipped-out CAG repeats to promote contractions [74••]. NA binds only to long CAG slip-outs, for example that could form upon resolution of R-loops. Though the NA-bound CAG tract is resistant to repair, CTG hairpins on the opposite strand promote nicking or hairpin excision, and polymerase fill-in of the resulting gap will result in a bias towards contractions.

the CAG tract from the HTT gene in HD patient-derived fibroblasts and observed a reduction in huntingtin protein level upon excision by Cas9 [72] (Figure 3b). However, use of CRISPR-Cas9 to induce a DSB within an expanded CAG/CTG tract led to frequent chromosomal deletions in yeast, demonstrating some downsides of using this system to promote repeat contractions [73]. Studies excising other expanded repeats, such as CGG from FMR1 and GAA from FXN, resulted in reactivation of expression of genes that are silenced upon repeat expansion (reviewed in Ref. [69]).

A recent study tested a small molecule, called NA (naphthyridine-azaquinolone), that specifically binds slipped CAG repeats as a potential therapeutic for promoting contractions of expanded CAG repeats [74••] (Figure 3c). This molecule was found to be highly specific, only binding to long slip-outs, and blocked their repair in a manner dependent on transcription [74••]. Additionally, NA was able to contract expanded CAG repeats in striatum tissue when injected into the brain of R6/2 HD mice [74••]. This is a promising approach and the hope is that this molecule can be optimized to be used as a potential therapy in the future. In another promising approach, treatment of an HD mouse model with the HDAC3-selective inhibitor RGFP966 suppressed CAG expansions in the striatum and prevented cognitive decline [26].

In the last 2 years several new repeat expansions linked to various genetic diseases have been identified through repeat-primed PCR and long-read sequencing. A biallelic pentanucleotide repeat expansion was identified in CANVAS (cerebellar ataxia, neuropathy, and vestibular areflexia syndrome) patients within intron 2 of the RFC1 (replication factor C subunit 1) gene (reviewed in Ref. [2]). There is a range of potential genotypes present at this locus in healthy and CANVAS individuals, including $(AAAAG)_{11}$ in healthy individuals, and $(AAAAG)_{exp}$, $(AAAGG)_{exp}$, and $(AAGGG)_{exp}$ in the disease state. The sequence from normal to disease-causing appears not only to expand, but the nucleotide content of the repeat unit changes, gaining more guanines and losing adenines. Additionally, intronic ATTTT repeat expansions containing an $(ATTTC)_{exp}$ interruption were identified in several loci, including STARD7, MARCH6, SAMD12, TNRC6A, and RAPGEF2, linked to various types of familial adult myoclonic epilepsy (FAME) (reviewed in Ref. [2]). A study examining the evolution of a similar repeat expansion in the DAB1 locus that is linked to spinocerebellar ataxia type 37 (SCA37) revealed a potential mechanism in which the ATTTT allele underwent a T-to-C mutation to create the ATTTC interruption that is present in affected individuals [75]. Also through repeat-primed PCR and long-read sequencing, a $(GGC)_n$ expansion was identified in patients with NIID (neuronal intranuclear inclusion disease) within the NOTCH2NLC gene [76]. Most recently, through genome-wide interrogation, gene-associated rare tandem repeat expansions were linked to autism [77•]. Future studies are expected to determine mechanisms of repeat expansions at these loci to elucidate potential genetic causes of these diseases.

Conflict of interest statement

Nothing declared.

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- • of special interest
- •• of outstanding interest

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This study detected sites of fork collapse genome-wide upon ATR inhibition, using RPA-ChIP and BrITL (breaks identified by TdT labeling). In human cells, AT repeats were most commonly identified, whereas $(CAGAGG)_n$ and $(CACAG)_n$ repeats, which form quadruplex structures, were most commonly identified in mouse cells. Tubbs *et al.* [8] did a similar study using HU and mouse B cells and the END-seq method, and identified poly(dA:dT) tracts as the most common repeat at early replicating fragile sites.

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Through 5C experiments to examine 3D genome and chromatin interactions, the authors found that disease-associated short tandem repeats (daSTRs) at FMR1, HTT, DMPK, FXN, C9orf72, and ATXN1 loci localized to TAD boundaries. Additionally, at the FMR1 locus, expansion of CGG repeats ablated chromatin interactions and TAD organization. This indicates that overall genome organization may contribute to disease phenotypes of repeat expansion diseases.

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The authors used the small molecule NA (naphthyridine-azaquinolone) to stabilize long-slipped CAG repeats and promote CAG contractions. They show that the compound works *in vivo* in an HD mouse model to cause contractions. This is a promising potential future therapeutic option for HD and other genetic diseases caused by CAG repeat expansions.

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Through genome-wide interrogation, the authors identified tandem repeat expansions that are linked to autism within both known and novel loci. Tandem repeat motifs were identified at different genetic loci, indicating that multiple expansion-prone motifs may contribute. Although the effect is small (2.6% contribution), these repeat expansions are a potential cause of autism in some children.