Published in final edited form as:

Curr Opin Genet Dev. 2013 June ; 23(3): 280–288. doi:10.1016/j.gde.2013.04.009.

The Balancing Act of DNA Repeat Expansions

Jane C. Kim¹ and Sergei M. Mirkin^{1,*}

¹Department of Biology, Tufts University, Medford, MA 02155

Abstract

Expansions of microsatellite DNA repeats contribute to the inheritance of nearly 30 developmental and neurological disorders. Significant progress has been made in elucidating the molecular mechanisms of repeat expansions using various model organisms and mammalian cell culture, and models implicating nearly all DNA transactions such as replication, repair, recombination, and transcription have been proposed. It is likely that different models of repeat expansions are not mutually exclusive and may explain repeat instability for different developmental stages and tissues. This review focuses on the contributions from studies in budding yeast toward unraveling the mechanisms and genetic control of repeat expansions, highlighting similarities and differences of replication models and describing a balancing act hypothesis to account for apparent discrepancies.

Introduction

Nearly thirty hereditary neurological, neurodegenerative, or developmental diseases in humans are caused by expansions of microsatellite DNA repeats (reviewed in [1-3]). These repeats can have anywhere from 3 to 12 nucleotides in their repetitive unit, albeit the majority of expansion diseases are linked to trinucleotide repeats. Most expandable DNA repeats form unusual secondary structures, including imperfect hairpins formed by (CNG)_n and (CCTG)_n repeats, triplexes formed by (GAA)_n repeats, and G-quadruplexes formed by (CGG)_n and (C₄GC₄GCG)_n repeats (reviewed in [3]). However, some repeats that are exceptionally prone to expansions, such as the (ATTCT)_n repeats causing spinocerebellar ataxia 10, do not form intrastand or multistrand secondary structures but instead comprise a DNA unwinding element [4]. Repeats become unstable when the perfect repetitive run surpasses a threshold length varying from ~60 to 150 base pairs (bps) depending on the repeat, after which multiple extra copies can be added during a single intergenerational transmission [5,6] accounting for a progressively more severe disease phenotype in a phenomenon called genetic anticipation.

The molecular mechanisms of repeat expansion are being intensively studied in many experimental model systems (reviewed in [7]). These studies implicate various DNA transactions, including replication [8,9], transcription [10], repair [5,11] and recombination [12] in the process. There are substantial differences in the data and their interpretation for different repeats and experimental systems. However, there need not be a single uniform mechanism that describes the expansion of every microsatellite sequence. Rather, the

© 2013 Elsevier Ltd. All rights reserved.

* The author to whom all correspondence should be addressed. Phone: (617) 627-4794 FAX: (617) 627-3805 sergei.mirkin@tufts.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

mechanisms of repeat expansion may differ depending on the sequence and length of the repeat, the scale of the expansion, and whether the cells are dividing or post-mitotic. This review concentrates on recent data obtained in the budding yeast *S. cerevisiae*, where DNA replication is a major contributor to repeat instability. That said, there are significant differences in the details and genetic control of the expansion process for various repeats. Here we propose a balancing act hypothesis to account for these differences.

Replication models and the genetic control of repeat expansion

Almost immediately after trinucleotide repeat expansions were found to be the causative mutation of diseases such as Huntington's disease (CAG/CTG), fragile X syndrome (CGG/CCG) and Friedrich's ataxia (GAA/TTC), investigators began to study the properties of these repeats and their propensity to expand in various model organisms. Due to the ease of creating gene knock-outs and introducing DNA cassettes for selectively identifying expansion events, budding yeast has been a particularly useful model system to study the repeat expansion process. Besides the classical candidate gene approach, genome-wide analyses have recently been performed. In sum, some genes appeared to have similar effects on expandable repeats, while other genes had conflicting results for different repeats (Table 1).

Here we describe the predominant replication models for repeat expansion, emphasizing their relevance with respect to various repeat sequences, starting lengths of the repetitive tract, and the step of the expansion size. We distinguish between small- and large-scale expansions based on the absolute number of added DNA repeats, rather than the proportion of added DNA relative to the starting sequence: small-scale expansions refer to the addition of 1-20 repeats whereas large expansions is the addition of more than 20 repeats. Due to space limitations, we are not discussing DNA repair or transcription models of repeat expansions, which are discussed in depth in [5,11,13].

5' flap

Because deletion of *RAD27* resulted in repeat addition at dinucleotide array tracts as well as other microsatellite sequences [14,15], it was proposed that repeats might form a stable hairpin structure within a 5' flap, which could lead to enhanced expansion in the absence of *RAD27* [16]. In the 5' flap model, ligation of this hairpin to the next Okazaki fragment could then lead to a small expansion (Figure 1A). Additionally, 5' DNA flaps can similarly be formed and processed by Rad27/Fen1 during long-patch base excision repair [17].

Indeed, *RAD27/FEN1* has been found to affect repeat expansion across a range of repeat sequences and starting lengths. The frequencies of CAG/CTG repeat expansions were elevated in the absence of Rad27 whether CAG was on the leading or lagging strand template and for a variety of starting lengths [18-21]. With the development of a system in which the addition of as few as five repeats to a starting length of (CTG)₁₃ could be quantitatively measured (Figure 2A), Daee *et al.* found that deletion of *RAD27* elevated the expansion rate of the (CTG)₁₃ tract by nearly two orders of magnitude [22]. Similarly, the absence of Rad27 was found to increase the frequency of CGG/CCG repeat expansions [23]. Genetic analysis with other mutants involved in lagging strand DNA replication (i.e. DNA ligase I) also exhibited elevated frequencies of CAG/CTG repeat expansion [20,24], demonstrating that the effects of Okazaki fragment processing on repeat expansion are not limited to Rad27. Combined, these data support the idea that the 5' flap model would apply to any starting tract length so long as the sequence was capable of hairpin formation. However, the upper limit for a single expansion event would be the number of base pairs contained in the hairpin, which is constrained by the length of the flap (i.e. 20-30 bps for long flaps [25]). To explain large-scale expansions, 5' flap expansion would need to occur

iteratively during replication cycles. Supporting this possibility, (CAG)₇₀ repeats showed a mean expansion size of 35 repeats in *rad27Δ* mutants [20].

The 5' flap model typically points to hairpin formation as the source of expanded DNA sequence. Whereas CAG/CTG and CGG/CCG readily form hairpin structures *in vitro* [26,27], GAA/TTC sequences are not predicted to self-anneal, though there are reports of hairpin formation [28]. They readily form triplex structures instead [29,30], which could block Rad27 cleavage and lead to expansion. A *RAD27* knock-out was indeed shown to elevate the rate of large-scale expansions of (GAA)₁₀₀ by an order of magnitude [31]. Note that Rad27 may also play an additional role in replication fork dynamics contributing to GAA expansion via another mechanism (see "Fork reversal / Template switching" below).

3' replication slippage

During DNA synthesis, the misalignment of template and daughter strands could lead to small contractions or expansions. For expansions, polymerase slippage at the 3' end of the nascent strand could result in the formation of a hairpin, and the looped-out DNA would then contribute to an expansion in the next round of replication (Figure 1B) [32]. Although slippage could occur on either the leading or lagging strand, the complementary strands of expandable repeat sequences have different thermodynamics of structure formation and thus may dictate which nascent strand preferentially undergoes slippage. As with the 5' flap model, expansion would be independent of starting tract length as long as the sequence was capable of hairpin formation but should likewise result in small-scale expansions for thermodynamic reasons [33,34]. The model would apply best to repeats that are capable of hairpin formation.

In agreement with 3' replication slippage as a model for repeat expansion, several yeast mutants that impair DNA synthesis have been implicated in expansion of different trinucleotide repeats. The affected proteins include DNA primase and various DNA polymerase mutants for CAG/CTG [20,35] and GAA/TTC [36] repeats, proliferating cell nuclear antigen for CAG/CTG repeats [35], the large subunit (Rfc1) of the clamp-loading complex for CGG/CCG repeats [37], and replication fork stabilizers such as Mrc1 [38,39] and Ctf18 for CAG/CTG repeats [40].

A more molecularly defined 3' replication slippage model of repeat expansion comes from genetic analysis of the helicase Srs2 in yeast. *SRS2* was identified in a screen for genes that would increase expansions of a (CTG)₁₃ reporter (Figure 2A) [41]. Importantly, the role of Srs2 was specific to the two trinucleotide repeats tested, CAG/CTG and CGG/CCG. In contrast to *rad27* mutants, which destabilized all microsatellites and minisatellites genome-wide [14,15], *srs2* mutants had no effect on dinucleotide repeats, unique sequences, or trinucleotide repeat contractions [41]. It was proposed that Srs2 normally inhibits repeat expansions, possibly by unwinding trinucleotide repeat hairpins formed at the 3' end of the newly replicated DNA strand [41]. In addition, epistasis analysis indicated that Srs2 inhibits repeat expansion via post-replication repair (PRR) since mutations in various components of the PRR pathway, such as *RAD5*, *RAD18*, and deficient ubiquitination of PCNA, increased the expansion rates of short CAG/CTG tracts [22]. According to this model, replication through a trinucleotide repeat would result in hairpin formation and polymerase stalling. This event, analogous to spontaneous lesion bypass, would signal the recruitment of Srs2 to unwind the hairpin and PRR to protect against repeat expansion [22]. Furthermore, the authors of this study analyzed expansion rates and spectra for single and double mutants of *RAD27* and PRR components, proposing that the 3' replication slippage pathway functions independent of, and possibly synergistic with, Okazaki fragment 5' flap processing. Srs2 has also been implicated in maintaining longer repeats, specifically in the error-free branch of PRR (sister chromatid recombination or SCR) [42]. In this case, the expansions occurring in

the absence of Srs2 were dependent on Rad51, and a model based on increased 3' slippage during an SCR event was proposed [42].

Fork reversal / Template switching

Expandable DNA repeats have been shown to stall the replication fork in all systems where they have been studied. Typically, pronounced replication stalling becomes evident when the length of the repetitive run approaches or exceeds the expansion threshold (reviewed in [8]). Based on these observations, a model involving replication fork stalling, fork reversal, and replication restart was proposed (Figure 1C) [3,8]. Replication stalling may lead to fork reversal and the formation of a four-way junction or “chicken foot” structure. The 3' end of the leading strand could fold into a secondary structure such as a hairpin or loop-out and continue synthesis after the fold-back. If the secondary structure persists after the reversed fork continues past the repetitive tract, extra repeats will be added after the next round of replication. This model receives support from *in vitro* data [43] as well as *in vivo* data identifying replication intermediates characteristic of reversed forks in yeast [42] and most recently in human cell culture [44].

A unique yeast system to study large-scale expansions of GAA/TTC repeats has been described (Figure 2B). Although large expansions (i.e. 50 to hundreds of repeats from a starting (GAA)₁₀₀ tract) occurred with roughly equivalent rates ($\sim 10^{-5}$ per replication) whether GAA or TTC was located on the lagging strand template, profound replication fork stalling was detectable only when GAA repeats were on the lagging strand template [45]. This difference between infrequent and orientation-independent repeat expansions versus strong, orientation-dependent fork stalling, combined with candidate gene analysis data, led us to propose a template switching model for large-scale repeat expansion (Figure 1D) [45] loosely based on a template switching model for expansion of tandem repeats [46]. Note that while the template switching and fork reversal models are distinct, they share structural similarities and can both explain large expansions beyond the hairpin size limit.

The template switching model suggests that during replication of a repetitive DNA tract, the leading strand DNA polymerase can switch templates and continue synthesis using the nascent lagging strand as template. Upon reaching the end of the Okazaki fragment, the polymerase would switch back to the leading strand template, resulting in an expansion in length roughly equivalent to the size of an Okazaki fragment. In this system, large-scale expansions were elevated when genes blocking template switching (i.e. encoding components of the Tof1/Csm3/Mrc1 replication fork pausing complex) were deleted and reduced when genes that promote template switching (i.e. *RAD5* and *RAD6*) were deleted [45]. In contrast, eliminating genes involved in homologous recombination and double strand break repair or mismatch repair had no effect on large-scale expansion. Interestingly, point mutants of Pol α /Primase showed an increased primary step of expansion, confirming a link between the scale of expansions and Okazaki fragment size [36].

It is likely that the initiating template switching event occurs infrequently and is thus undetectable using 2-D gels to analyze replication intermediates. In contrast, replication fork stalling is orientation dependent and may be more relevant to chromosomal breakage (see “Double strand break formation and aberrant repair” below). In support of this, chromosomal fragility at GAA repeat tracts is significantly elevated when GAA is on the lagging strand template, the same orientation where fork stalling can be detected [47].

The template switching model describes how large-scale expansions could occur in a single step. For example, the primary initial step of expansion for GAA and ATTCT repeats was 160-to-200 bps [36,48]. Furthermore, (GAA)_n repeats shorter than the length of an average Okazaki fragment did not undergo large-scale expansions [36,45]. Thus, this model seems to

apply only when the starting repeat tract is sufficiently long to undergo “productive” template switching.

Double strand break formation and aberrant repair

Long microsatellite tracts are prone to DNA breakage. This fragility is evident from the cytological analysis of long CGG repeats in individuals with fragile X syndrome [49]. Additionally, long CAG/CTG, CGG/CTG, and GAA/TTC repeats showed elevated levels of double strand breaks (DSBs) in both yeast and mammalian cell cultures (Figure 2C) [18,47,50]. The origin of these DSBs is most likely replication fork stalling and reversal, albeit replication-independent breakage has also been detected in G1 [51] and stationary phase cells [31]. DSBs at the repetitive sequence may be repaired by homologous recombination, in the course of which out-of-register synthesis from the sister chromatid could lead to repeat expansions. In bacteria, yeast, and mammalian cell culture, various DNA repeats have indeed been shown to elevate recombination, resulting in increased expansions and contractions and gross chromosomal rearrangements (reviewed in [3]). The breakage and repair model of repeat expansion is length dependent, as longer repeat tracts are more susceptible to breakage. In support of this, instability of long CAG repeats is exacerbated by loss of recombination proteins Rad51 and Rad52 [52], while short repeats are not affected by their loss [41,53].

When a DSB was repaired from a separate template containing a (CAG)₉₈ tract, it expanded by adding 25 repeats on average; this expansion step further increased by overexpression of Mre11 and Rad50 protein [54]. Thus, repair of DSBs via synthesis-dependent strand annealing could also lead to large-scale expansions [55].

Balancing act hypothesis for replication models of repeat expansions

For expansions that occur during genomic DNA replication, what factors determine which expansion mechanism predominates for a given sequence and length of microsatellite DNA? Well-characterized yeast systems support several models of repeat expansion: two that are specific to replication of the individual DNA strand (5′ flap and 3′ replication slippage) and two that generally implicate replication fork indiscretions (i.e. fork reversal and template switching). When is each of these mechanistic models relevant? One discriminating factor is the starting length of the repeat tract. In one selectable system (Figure 2A), the starting repeat length is 25 (or even fewer) triplets, and it expands modestly, resembling the characteristics of polyglutamine or polyalanine diseases where even small-scale expansions can result in an individual inheriting the disease [53]. In the other system (Figure 2B), only repeats longer than 70 triplets were found to expand [36,45] by adding 50 to hundreds of repeats at a step, which resembles diseases of repeat expansions in the non-coding parts of the genes (i.e. where hundreds to thousands of repeats can be added in a single generation once the threshold repeat length has been passed). Importantly, however, starting repeat length is not the sole determinant of the expansion mechanism because in the system tuned to detect the addition of as few as five repeats (Figure 2A), small-scale expansions of GAA repeats were never detected [56].

To account for the differences between yeast experimental systems, we propose the balancing act hypothesis. It states that each DNA repeat can expand via either a DNA strand specific mechanism, such as 3′ replication slippage, or a fork deviation mechanism, such as template switching (Figure 3). During genomic DNA replication and synthesis through a microsatellite repeat tract, one of two scenarios may occur. Small-scale expansions occasionally arise if Srs2 and/or PRR fail to unwind the hairpin. They become more apparent in cells lacking Srs2 or PRR components. Alternatively, large-scale expansions arise if the replication fork undergoes reversal or if template switching occurs while it

proceeds through the long repetitive run. The delicate balancing act discriminating between these two pathways is determined by the propensity of a given DNA repeat to form secondary structure, specifically a very stable DNA hairpin.

CAG/CTG and CGG/CCG repeats form very stable hairpins, so we propose that these repeats would expand preferentially via models involving the individual DNA strand (5' flap or 3' replication slippage). In support of this, we have not observed large-scale CAG/CTG or CGG/CCG expansions in the system designed to recover large expansions (Figure 2B), though small-scale instability is observed even without selection (Kim *et al.*, unpublished results). In contrast, since GAA and ATTCT repeats do not form hairpin structures, we propose that they are likely to have a greater propensity to expand via template switching/fork reversal. In support of this, we and others were never able to detect small-scale expansions of short GAA repeats [36,45,56].

The balancing act hypothesis also explains conflicting results between genetic analyses in yeast experimental systems (Table 1). For 3' replication slippage, components of the PRR pathway protect against repeat expansion [22], explaining why inactivation of these genes would result in elevated rates of CAG/CTG expansion. In contrast, PRR components promote expansion via template switching for sequences less prone to hairpin formation, such as GAA and ATTCT, resulting in reduced rates of large-scale expansion in their absence [45,48]. Additionally, inactivation of Srs2 helicase increases expansion rates in the 3' replication slippage model whereas disruption of the Sgs1 helicase is specific to the template switching model [41,45]. In all cases, inactivation of *TOF1* led to a significant increase in the expansion rate of CAG/CTG, GAA, and ATTCT repeats of different starting lengths [38,45,48] suggesting that some proteins universally oppose small- and large-scale expansions of various microsatellite repeats.

Conclusions

Studying repeat instability in yeast revealed fine molecular mechanisms of the expansion process and their genetic control. It became clear that repeat-mediated instability in yeast primarily originates in the course of DNA replication. Two modes of instability were observed that differ in the threshold length of the repeat, its expansion scale, and genetic control of the event. We propose a balancing act hypothesis, which states that a repeat can undergo expansions either in the course of individual DNA strand slippages or upon fork deviation as a whole, such as fork reversal or template switching. We believe that this hypothesis accounts for the bulk of the experimental data obtained in yeast. When it comes to human disease, individual DNA strand slippages may be the predominant pathway of repeat instability near the expansion threshold length, which is around 30 to 50 repeats (i.e. shorter than an Okazaki fragment). In this way, the variance in threshold length for different repeats might simply depend on the differential stabilities of repetitive hairpins. Template switching/fork reversal models seem to be more applicable for the subsequent larger-scale expansion of longer repeat tracts. Further experimental support for the balancing act hypothesis in humans remains to be ascertained.

Acknowledgments

Research in the lab of S.M.M. is supported by NIH grant GM60987 and generous contribution from the White family. J.C.K. is supported by the NIH Training in Education and Critical Research Skills postdoctoral program (K12GM074869). We thank Catherine Freudenreich, Bob Lahue, and Jared Nordman for critical comments to the manuscript.

References Cited

1. Lopez Castel A, Cleary JD, Pearson CE. Repeat instability as the basis for human diseases and as a potential target for therapy. *Nature Reviews. Molecular Cell Biology*. 2010; 11:165–170.
2. Orr HT, Zoghbi HY. Trinucleotide repeat disorders. *Annual Review of Neuroscience*. 2007; 30:575–621.
3. Mirkin SM. Expandable DNA repeats and human disease. *Nature*. 2007; 447:932–940. [PubMed: 17581576]
4. Potaman VN, Bissler JJ, Hashem VI, Oussatcheva EA, Lu L, Shlyakhtenko LS, Lyubchenko YL, Matsuura T, Ashizawa T, Leffak M, et al. Unpaired structures in SCA10 (ATTCT)_n.(AGAAT)_n repeats. *J Mol Biol*. 2003; 326:1095–1111. [PubMed: 12589756] • A thorough study using electrophoretic and AFM approaches to show that the SCA10 repeat is a DNA unwinding element (DUE).
5. McMurray CT. Mechanisms of trinucleotide repeat instability during human development. *Nature Reviews. Genetics*. 2010; 11:786–799.
6. Ashley C Jr, Warren ST. Trinucleotide repeat expansion and human disease. *Annual Review of Genetics*. 1995; 29:703–728.
7. Kovtun IV, McMurray CT. Features of trinucleotide repeat instability *in vivo*. *Cell Res*. 2008; 18:198–213. [PubMed: 18166978]
8. Mirkin SM. DNA structures, repeat expansions and human hereditary disorders. *Curr Opin Struct Biol*. 2006; 16:351–358. [PubMed: 16713248]
9. Cleary JD, Pearson CE. Replication fork dynamics and dynamic mutations: The fork-shift model of repeat instability. *Trends in Genetics : TIG*. 2005; 21:272–280. [PubMed: 15851063]
10. Dion V, Wilson JH. Instability and chromatin structure of expanded trinucleotide repeats. *Trends in Genetics : TIG*. 2009; 25:288–297. [PubMed: 19540013]
11. McMurray CT. Hijacking of the mismatch repair system to cause CAG expansion and cell death in neurodegenerative disease. *DNA Repair*. 2008; 7:1121–1134. [PubMed: 18472310]
12. Wells RD, Dere R, Hebert ML, Napierala M, Son LS. Advances in mechanisms of genetic instability related to hereditary neurological diseases. *Nucleic Acids Res*. 2005; 33:3785–3798. [PubMed: 16006624]
13. Lin Y, Hubert L Jr, Wilson JH. Transcription destabilizes triplet repeats. *Mol Carcinog*. 2009; 48:350–361. [PubMed: 18973172]
14. Johnson RE, Kovvali GK, Prakash L, Prakash S. Role of yeast Rth1 nuclease and its homologs in mutation avoidance, DNA repair, and DNA replication. *Curr. Genet*. 1998; 34:21–29. [PubMed: 9683672]
15. Kokoska RJ, Stefanovic L, Tran HT, Resnick MA, Gordenin DA, Petes TD. Destabilization of yeast micro- and minisatellite DNA sequences by mutations affecting a nuclease involved in Okazaki fragment processing (rad 27) and DNA polymerase delta (pol3-t). *Molecular and Cellular Biology*. 1998; 18:2779–2788. [PubMed: 9566897] • The first paper that provided experimental support for the 5′ flap model of expansions.
16. Gordenin DA, Kunkel TA, Resnick MA. Repeat expansion--all in a flap? *Nature Genetics*. 1997; 16:116–118. [PubMed: 9171819] •• A prescient case of a News and Views piece that postulated a new, 5′ flap model for repeat expansions.
17. Balakrishnan L, Bambara RA. Okazaki fragment metabolism. *Cold Spring Harb Perspect Biol*. 2013; 5
18. Freudenreich CH, Kantrow SM, Zakian VA. Expansion and length-dependent fragility of CTG repeats in yeast. *Science*. 1998; 279:853–856. [PubMed: 9452383] •• A first-of-a-kind study showing repeat-mediated chromosomal fragility in yeast.
19. Schweitzer JK, Livingston DM. Expansions of CAG repeat tracts are frequent in a yeast mutant defective in Okazaki fragment maturation. *Hum. Mol. Genet*. 1998; 7:69–74. [PubMed: 9384605]
20. Callahan JL, Andrews KJ, Zakian VA, Freudenreich CH. Mutations in yeast replication proteins that increase CAG/CTG expansions also increase repeat fragility. *Mol Cell Biol*. 2003; 23:7849–7860. [PubMed: 14560028] • The first paper establishing a YAC experimental system to study repeat-mediated instability.

21. Spiro C, Pelletier R, Rolfsmeier ML, Dixon MJ, Lahue RS, Gupta G, Park MS, Chen X, Mariappan SVS, McMurray CT. Inhibition of FEN-1 processing by DNA secondary structure at trinucleotide repeats. *Mol Cell*. 1999; 4:1079–1085. [PubMed: 10635332] • The first study to show inhibition of FEN1 by secondary structures of the repetitive flap.
22. Daee DL, Mertz T, Collins N, Lahue RS. Post-replication repair inhibits CAG/CTG repeat expansions in *Saccharomyces cerevisiae*. *Mol Cell Biol*. 2007; 27:102–110. [PubMed: 17060452] • This study convincingly demonstrated the role of PRR in CAG/CTG repeat expansions.
23. White PJ, Borts RH, Hirst MC. Stability of the human fragile X (CGG)_n triplet repeat array in *Saccharomyces cerevisiae* deficient in aspects of DNA metabolism. *Mol. Cell. Biol*. 1999; 19:5675–5684. [PubMed: 10409756]
24. Ireland MJ, Reinke SS, Livingston DM. The impact of lagging strand replication mutations on the stability of CAG repeat tracts in yeast. *Genetics*. 2000; 155:1657–1665. [PubMed: 10924464]
25. Rossi ML, Purohit V, Brandt PD, Bambara RA. Lagging strand replication proteins in genome stability and DNA repair. *Chem Rev*. 2006; 106:453–473. [PubMed: 16464014]
26. Gacy AM, Goellner G, Juranic N, Macura S, McMurray CT. Trinucleotide repeats that expand in human disease form hairpin structures *in vitro*. *Cell*. 1995; 81:533–540. [PubMed: 7758107] • A pivotal paper showing formation of hairpin-like DNA structures by various expandable repeats.
27. Mitás M, Yu A, Dill J, Haworth IS. The trinucleotide repeat sequence d(CGG)₁₅ forms a heat-stable hairpin containing Gsyn.Ganti base pairs. *Biochemistry*. 1995; 34:12803–12811. [PubMed: 7548035]
28. Heidenfelder BL, Makhov AM, Topal MD. Hairpin formation in Friedreich's ataxia triplet repeat expansion. *J Biol Chem*. 2003; 278:2425–2431. [PubMed: 12441336]
29. Sakamoto N, Chastain PD, Parniewski P, Oshima K, Pandolfo M, Griffith JD, Wells RD. Sticky DNA: self association properties of long GAA/TTC repeats in R.R.Y triplex structures from Friedreich's ataxia. *Mol Cell*. 1999; 3:465–475. [PubMed: 10230399] • A paper proposing a new multi-strand DNA structure, called sticky DNA, by FRDA DNA repeats.
30. Gacy AM, Goellner GM, Spiro C, Chen X, Gupta G, Bradbury EM, Dyer RB, Mikesell MJ, Yao JZ, Johnson AJ, et al. GAA instability in Friedreich's ataxia shares a common, DNA-directed and intraallelic mechanism with other trinucleotide diseases. *Mol Cell*. 1998; 1:583–593. [PubMed: 9660942] • First data on DNA polymerization problems caused by expanded GAA repeats.
31. Zhang Y, Shishkin AA, Nishida Y, Marcinkowski-Desmond D, Saini N, Volkov KV, Mirkin SM, Lobachev KS. Genome-wide screen identifies pathways that govern GAA/TTC repeat fragility and expansions in dividing and nondividing yeast cells. *Mol Cell*. 2012; 48:254–265. [PubMed: 22959270] • Comprehensive analysis of the genetic control of instability of GAA repeats in yeast at the genome-wide level.
32. Kang S, Jaworski A, Ohshima K, Wells RD. Expansion and deletion of CTG repeats from human disease genes are determined by the direction of replication in *E.coli*. *Nat Genet*. 1995; 10:213–218. [PubMed: 7663518] • The first paper showing 3' replication slippage on the nascent lagging strand as a source of repeat expansions in a bacterial experimental system.
33. Petruska J, Hartenstine MJ, Goodman MF. Analysis of strand slippage in DNA polymerase expansions of CAG/CTG triplet repeats associated with neurodegenerative disease. *The Journal of Biological Chemistry*. 1998; 273:5204–5210. [PubMed: 9478975]
34. Hartenstine MJ, Goodman MF, Petruska J. Base stacking and even/odd behavior of hairpin loops in DNA triplet repeat slippage and expansion with DNA polymerase. *The Journal of Biological Chemistry*. 2000; 275:18382–18390. [PubMed: 10849445]
35. Schweitzer JK, Livingston DM. The effect of DNA replication mutations on CAG tract stability in yeast. *Genetics*. 1999; 152:953–963. [PubMed: 10388815]
36. Shah KA, Shishkin AA, Voineagu I, Pavlov YI, Shcherbakova PV, Mirkin SM. Role of DNA polymerases in repeat-mediated genome instability. *Cell Rep*. 2012; 2:1088–1095. [PubMed: 23142667] • This paper describes the phenomenon of repeat-mediated mutagenesis, resulting from replication blockage caused by GAA repeats.
37. Pelletier R, Krasilnikova MM, Samadashwily GM, Lahue RS, Mirkin SM. Replication and expansion of trinucleotide repeats in yeast. *Mol Cell Biol*. 2003; 23:1349–1357. [PubMed:

- 12556494] • The first paper to state the link between repeat replication and their expansions in yeast combining electrophoreses of replication intermediates and candidate gene analysis.
38. Razidlo DF, Lahue RS. Mrc1, Tof1 and Csm3 inhibit CAG-CTG repeat instability by at least two mechanisms. *DNA Repair*. 2008; 7:633–640. [PubMed: 18321795] • The first paper to show the role of the fork stabilization complex in repeat instability.
 39. Freudenreich CH, Lahiri M. Structure-forming CAG/CTG repeat sequences are sensitive to breakage in the absence of Mrc1 checkpoint function and S-phase checkpoint signaling: implications for trinucleotide repeat expansion diseases. *Cell Cycle*. 2004; 3:1370–1374. [PubMed: 15483399]
 40. Gellon L, Razidlo DF, Gleeson O, Verra L, Schulz D, Lahue RS, Freudenreich CH. New functions of Ctf18-RFC in preserving genome stability outside its role in sister chromatid cohesion. *PLoS Genetics*. 2011; 7:e1001298. [PubMed: 21347277]
 41. Bhattacharyya S, Lahue RS. *Saccharomyces cerevisiae* Srs2 DNA helicase selectively blocks expansions of trinucleotide repeats. *Molecular and Cellular Biology*. 2004; 24:7324–7330. [PubMed: 15314145] • Yeast genetic screen that demonstrated the role of Srs2 DNA helicase as a guardian against repeat expansions.
 42. Kerrest A, Anand RP, Sundararajan R, Bermejo R, Liberi G, Dujon B, Freudenreich CH, Richard GF. SRS2 and SGS1 prevent chromosomal breaks and stabilize triplet repeats by restraining recombination. *Nat Struct Molec Biol*. 2009; 16:159–167. [PubMed: 19136956] • Direct demonstration for the role of fork reversal in repeat expansions combining electrophoretic analysis of replication intermediates with genetic analysis.
 43. Fouche N, Ozgur S, Roy D, Griffith JD. Replication fork regression in repetitive DNA's. *Nucleic Acids Res*. 2006; 34 • The first study demonstrating fork reversal at expandable DNA repeats *in vitro*.
 44. Follonier C, Oehler J, Herrador R, Lopes M. Friedreich's ataxia-associated GAA repeats induce replication-fork reversal and unusual molecular junctions. *Nature Structural & Molecular Biology*. 2013 • A terrific study confirming replication fork reversal at (GAA)_n repeats in mammalian cells.
 45. Shishkin AA, Voineagu I, Matera R, Cherg N, Chernet BT, Krasilnikova MM, Narayanan V, Lobachev KS, Mirkin SM. Large-scale expansions of Friedreich's ataxia GAA repeats in yeast. *Mol Cell*. 2009; 35:82–92. [PubMed: 19595718] • This paper describes a one-of-a-kind system to analyze large-scale repeat expansions in yeast.
 46. Goldfless SJ, Morag AS, Belisle KA, Sutera VA, Lovett ST. DNA repeat rearrangements mediated by DnaK-dependent replication fork repair. *Mol Cell*. 2006; 21:595–604. [PubMed: 16507358] • The first paper proposing a template switching model for direct tandem repeats.
 47. Kim HM, Narayanan V, Mieczkowski PA, Petes TD, Krasilnikova MM, Mirkin SM, Lobachev KS. Chromosome fragility at GAA tracts in yeast depends on repeat orientation and requires mismatch repair. *EMBO J*. 2008; 27:2896–2906. [PubMed: 18833189] • A principal study confirming the link between replication fork stalling at an expandable repeat and chromosomal fragility in yeast.
 48. Cherg N, Shishkin AA, Schlager LI, Tuck RH, Sloan L, Matera R, Sarkar PS, Ashizawa T, Freudenreich CH, Mirkin SM. Expansions, contractions, and fragility of the spinocerebellar ataxia type 10 pentanucleotide repeat in yeast. *Proc Natl Acad Sci USA*. 2011; 108:2843–2848. [PubMed: 21282659]
 49. Sutherland GR, Baker E, Richards RI. Fragile sites still breaking. *TIGS*. 1998; 14:501–506.
 50. Balakumaran BS, Freudenreich CH, Zakian VA. CGG/CCG repeats exhibit orientation-dependent instability and orientation-independent fragility in *Saccharomyces cerevisiae*. *Hum Mol Genet*. 2000; 9:93–100. [PubMed: 10587583]
 51. Tang W, Dominska M, Greenwell PW, Harvanek Z, Lobachev KS, Kim HM, Narayanan V, Mirkin SM, Petes TD. Friedreich's ataxia (GAA)_n*(TTC)_n repeats strongly stimulate mitotic crossovers in *Saccharomyces cerevisiae*. *PLoS Genetics*. 2011; 7:e1001270. [PubMed: 21249181] • This study shows that expandable DNA repeats can break during the G1 phase of the cell cycle stimulating mitotic crossingover.
 52. Sundararajan R, Gellon L, Zunder RM, Freudenreich CH. Double-strand break repair pathways protect against CAG/CTG repeat expansions, contractions and repeat-mediated chromosomal fragility in *Saccharomyces cerevisiae*. *Genetics*. 2010; 184:65–77. [PubMed: 19901069]

53. Miret JJ, Pessoa-Brandao L, Lahue RS. Orientation-dependent and sequence-specific expansions of CTG/CAG trinucleotide repeats in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA*. 1998; 95:12438–12443. [PubMed: 9770504] •• This paper describes the first selectable system to analyze repeat expansions in yeast.
54. Richard G-F, Goellner GM, McMurray CT, Haber JE. Recombination-induced CAG trinucleotide repeat expansions in yeast involve the MRE11-RAD50-XRS2 complex. *EMBO J*. 2000; 19:2381–2390. [PubMed: 10811629] • This is the first study to show the role of homologous recombination in repeat expansions in yeast.
55. Richard GF, Cyncynatus C, Dujon B. Contractions and expansions of CAG/CTG trinucleotide repeats occur during ectopic gene conversion in yeast, by a MUS81-independent mechanism. *J Mol Biol*. 2003; 326:769–782. [PubMed: 12581639]
56. Rolfsmeier ML, Dixon MJ, Pessoa-Brandao L, Pelletier R, Miret JJ, Lahue RS. Cis-elements governing trinucleotide repeat instability in *Saccharomyces cerevisiae*. *Genetics*. 2001; 157:1569–1579. [PubMed: 11290713]

Fig. 1.

Replication-based models for repeat expansions. **(A)** and **(B)** are DNA strand specific models. **(C)** and **(D)** are fork deviation models. Closed circles denote 5' end of template strand, and closed arrows denote 3' end of nascent strand. **(A)** 5' flap: During Okazaki fragment maturation, expandable repeats within the flap can form a stable hairpin and impair its normal processing. Ligation of this hairpin to the 5'-most Okazaki fragment could then lead to a small expansion after the next replication round. A similar event could happen in the course of long-patch base excision repair of repetitive DNA. **(B)** 3' replication slippage: Polymerase slippage at the 3' end of the nascent DNA strand could result in the formation of a hairpin, which would then result in repeat expansion in the next round of replication. This event can occur more than once during replication of long DNA repeats. **(C)** Replication fork stalling, reversal and restart: The replication fork stalls at a repetitive DNA tract, potentially due to secondary structure formation in the lagging strand template. Fork reversal generates a four-way junction (also called "chicken foot" structure) (marked by *) with a single-stranded, repetitive 3' end that can fold back on itself. Replication restart upon flipping back this loop-out primer (marked by #) results in a longer repetitive run in the leading strand. **(D)** Template switching: During replication of a repetitive DNA tract, the leading strand DNA polymerase can switch templates and continue synthesis using the nascent lagging strand as template. Upon reaching the end of the Okazaki fragment, the polymerase has to switch back to the leading strand template for replication to continue. (Note that a repetitive Okazaki fragment is removed in this process.) This results in a repeat expansion, the step of which is roughly equivalent to the size of an Okazaki fragment.

Fig. 2.

Yeast experimental systems to study repeat expansions. **(A)** In this system, the addition of as few as five repeats can be detected, since expansions move the transcription start site upstream and result in translation initiating from the upstream, out-of-frame start codon (adapted from [53]). Yeast cells grow in the presence of the drug 5-FOA when *URA3* expression is OFF. **(B)** This system relies on the inability of budding yeast to splice introns longer than ~1 kb. DNA repeats were inserted into the artificially split (black bars) *URA3* gene, and their large-scale expansions inhibit splicing, allowing cells to grow on 5-FOA (adapted from [45]). The precisely mapped position of the early firing and efficient replication origin *ARS306* allows one to unambiguously determine the effects of replication direction on repeat-induced fragility and expansions. **(C)** This YAC based system [20], or its conceptually similar chromosomal analogs [47], assay chromosomal instability. Breaks occurring in the repeat sequence may result in the resection of DNA to a telomere seed sequence (G_4T_4) and recovery of the YAC without the *URA3* marker, permitting growth on 5-FOA. This system was also used to assay instability of the repeats without selection. In all cases, the lengths of repetitive tracts were determined by PCR and/or DNA sequencing.

Fig. 3.

Balancing act hypothesis for the replication models of repeat expansions. The delicate balancing act discriminates between individual slipped-strand and replication fork models of repeat expansions. If a given DNA repeat (such as CAG/CTG or CGG/CCG) has a propensity to form stable hairpin structures it will expand preferentially via 5' flap or 3' replication slippage models, whereas a DNA repeat that does not form stable intra-molecular structures (such as GAA/TTC and ATTCT/AGAAT) would expand via fork reversal or template switching, thus, adding more repeats in a single expansion step.

Table 1

Comparative genetic analysis of expansions of different DNA repeats in budding yeast

	CAG/CTG	CGG/CCG	GAA/TTC	ATTCT
5' flap processing				
<i>rad27Δ</i>	↑ [18-22]	↑ [23]	↑ [31]	
Replication pausing				
<i>tof1Δ</i>	↑ [38]		↑ [45]	↑ [48]
<i>csm3Δ</i>	↑ [38]		↑ [45]	
Helicase activity				
<i>srs2Δ</i>	↑ [41, 42]	↑ [41]	No effect [45]	
<i>sgs1Δ</i>	No effect [41, 42]		↓ [45]	
Post-replication repair				
<i>rad5Δ</i>	↑ [22]		↓ [45]	↓ [48]
<i>pol30-K164R</i>	↑ [22]		No effect [36]	
Homologous recombination				
<i>rad52Δ</i>	No effect [53] ↑ [52]		No effect [45]	↓/No effect [48]