[Frontiers in Bioscience 12, 4788-4799, September 1, 2007]

Slipped strand DNA structures

Richard R. Sinden[1], Malgorzata J. Pytlos-Sinden[1], Vladimir N. Potaman[2]

[1]Laboratory of DNA Structure and Mutagenesis, Department of Biological Sciences, Florida Institute of Technology, 150 West University Blvd., Melbourne, FL 32901-6975, [2]Institute of Biosciences and Technology, Texas A and M University System Health Sciences Center, 2121 West Holcombe Blvd., Houston, TX 77030-3303

TABLE OF CONTENTS

1. ABSTRACT

Slipped strand DNA structures are formed when complementary strands comprising direct repeats pair in a misaligned, or slipped, fashion along the DNA helix axis. Although slipped strand DNA may form in almost any direct repeat, to date, these structures have only been detected in short DNA repeats, termed unstable DNA repeats, in which expansion is associated with many neurodegenerative diseases. This alternative DNA structure, or a similar slipped intermediate DNA that may form during DNA replication or repair, may be a causative factor in the instability of the DNA sequences that can form these structures.

2. INTRODUCTION

Slipped strand DNA structures can form in DNA sequences comprising direct repeat symmetry. To form a slipped strand DNA structure the direct repeat region must unwind and one strand of one copy of a direct repeat must pair with the complementary strand of another copy of a second direct repeat. Figure 1 shows the two possible isomers of slipped strand DNA that can be formed for a region comprising two direct repeats (Figure 1, A). One isomer has loops located at the 5' end of the direct repeats (Figure 1, B1) while the other has loops located at the 3' ends (Figure 1, B2).

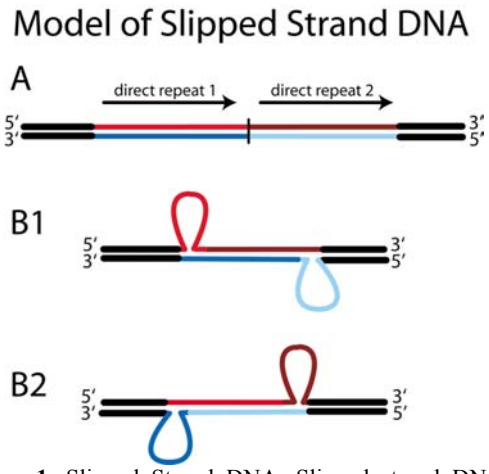

Figure 1. Slipped Strand DNA. Slipped strand DNA is formed from direct repeats (A) in which complementary strands of two adjacent direct repeats mispair such that one strand of one direct repeat pairs with the complementary strand of the other direct repeat. Structures B1 and B2 show the two possible different isomers of slipped strand DNA structures.

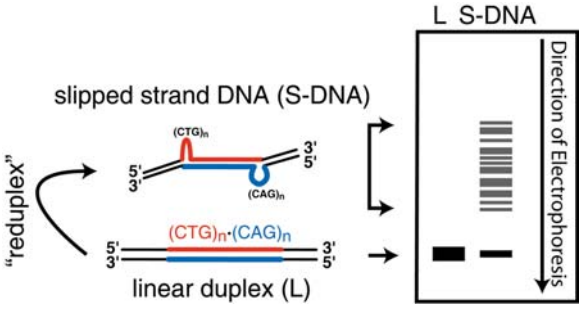

Figure 2. Assay for Slipped Strand DNA. Duplex DNA migrates with a characteristic mobility in polyacrylamide gels that is dependent on the length of the DNA (shown as a 'band' in the lane marked 'L" in the idealized gel at the right of the figure). Following "reduplexing," which involves denaturation followed by renaturation, slipped strand DNA is formed (shown in the left of the figure). Slipped strand DNA contains two bends introduced by the two three-way junctions. These bends make DNA migrate more slowly in polyacrylamide than non bent duplex DNA. As this shows from formation of slipped strand DNA within a $(CAG)_n$•$(CTG)_n$ repeat tract, multiple isomers are possible (as described in Figure 3) and slipped strand DNA actually migrates as a series of bands corresponding to multiple structural isomers as shown in the lane denoted S-DNA. (Note that while 'S-DNA' has been used to denote slipped strand DNA, it has also found favor with physicists to denote stretched-DNA (141)).

At first glance, slipped strand DNA structures would not appear to be stable. This is because a considerable loss of overall helix stability would be associated with slipped strand DNA formation from the loss of hydrogen bonding and base stacking interactions of DNA within the two loops, if the looped-out strands remain unpaired. Certain factors may compensate for the loss of hydrogen bonding and base stacking energy. It is possible for loop-loop interaction to occur as the looped-out strands in opposite DNA strands are complementary. In fact, structures with loop-loop interactions have been reported for various other DNA structures (1-6), and loop-loop interactions in slipped strand DNAs will be discussed below. Moreover, in all cases of DNA sequences known to form slipped strand DNA structures the sequences involved contain some degree of inverted repeat symmetry, in addition to direct repeat symmetry. This inverted repeat symmetry can result in the formation of DNA hairpins in the looped -out arms of slipped strand structures, and this base pairing likely contributes to the stability to these DNA structures. In addition, unwinding of the DNA double helix associated with slipped strand DNA formation results in the loss of DNA supercoils and, therefore, this process would be expected to drive the formation of slipped strand structures in supercoiled DNA.

3. SLIPPED STRAND DNA STRUCTURES AND SLIPPED INTERMEDIATE DNA

3.1.Slipped strand structures in (CTG)•(CAG) and (CGG)•(CCG) repeats

Although it was known for many years that slipped strand DNA structures should exist, the first characterization of these structures was reported for $(CTG)_n$•$(CAG)_n$ and $(CGG)_n$•$(CCG)_n$ repeats, associated with myotonic dystrophy and fragile X syndrome, respectively (7). Following denaturing and renaturing DNA molecules containing $(CTG)_n$•$(CAG)_n$ or $(CGG)_n$•$(CCG)_n$ repeats *in vitro*, a high proportion of the DNA fragments containing the DNA repeat migrated with very reduced electrophoretic mobility in polyacrylamide gels. The reduction in electrophoretic mobility is due to bends in the DNA that result from three way junctions associated with the looped-out strands, as illustrated in Figure 2. As also shown in Figure 2, a broad distribution of DNA bands corresponds to the large number of individual slipped strand isomers that results from a long simple repeat tract. The reason for the multiplicity of DNA isomers is because there are many possible locations for loop-outs to form due to the simple repeating nature of the trinucleotide repeat. This is illustrated in Figure 3.

Considerable work has been undertaken to understand the precise structure of slipped strand DNA structures (8-11). Biochemical, electron microscopy, and atomic force microscopy experiments mapped the site of the unusual structures within the triplet repeat region (8-10). As with many alternative DNA conformations, percentage of slipped strand DNA structures formed is proportional to the repeat tract length. Slipped strand structures do not readily form in repeat lengths associated with normal individuals (usually less than 30 (CTG)•(CAG) repeats), but they can form at levels of up to 80% in disease-associated repeat lengths of more than 50 repeats. Slipped strand structures are also remarkably stable and little conversion back to a correctly annealed duplex DNA is observed on prolonged incubation at 37°C (7). This stability presumably results from the combination of base pairing within potential hairpin stems (discussed

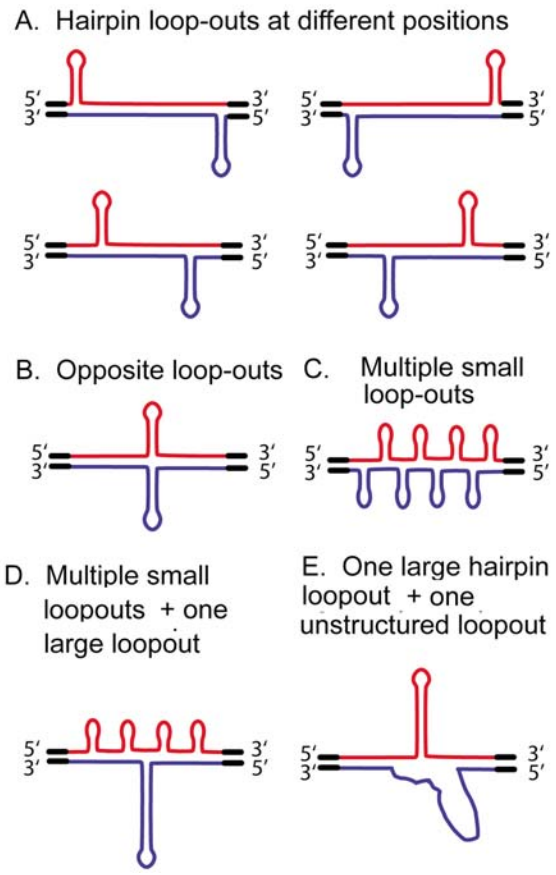

Figure 3. Heterogeneity in slipped strand DNA formation within a tract of simple repeats. This figure shows features possible for structural isomers formed from a long tract of a simple DNA repeat. The examples in this figure are based on our understanding of the characteristics of a $(CAG)_n$•$(CTG)_n$ repeat tract. A. Hairpin loop-outs can form at multiple different positions along the repeat tract. B. Theoretically the loop- outs can form opposite one another. In the absence of supercoiling, however they may tend to branch-migrate back into a linear duplex form. C. Multiple loop-outs, rather than a single loop-out (as in A) may form. D. Multiple loop-outs may form in one strand with a single loop-out in the opposite strand. E. One loop-out may form a hairpin, the $(CTG)_n$ strand in the case of a $(CAG)_n$•$(CTG)_n$ repeat tract, while the other strand may form an unpaired, or unstructured loop-out, as is the case for the $(CAG)_n$ strand.

under section 4) and base pairing in the DNA duplex between the loop-outs, which would have to unpair for the structure to convert back to the linear form (7). Seemingly there is little to prevent the dissolution of slipped strand DNAs by branch migration of the loops. However, base pairing interactions between the unpaired bases at the tips of the complementary hairpin-structured loop-outs (or between unpaired complementary loop-outs) forming folded slipped strand DNA, may be a major source of the stability of these structures. Folded slipped strand DNAs stabilized by loop-loop interactions between $(CTG)_n$ and $(CAG)_n$ hairpins have been demonstrated (Figure 4) (8). Both (CTG)•(CAG) and (CGG)•(CCG) repeats are inherently flexible (12-14) and this property may facilitate from formation of folded slipped strand structures.

The amount of slipped strand DNA structure formed is a function of the length of the repeat tract and the extent of repeat sequence heterogeneity, that is the number of sequence interruptions in the repeat tract (9, 15). In general, longer repeat tracts form more slipped strand DNA than shorter ones. Also, sequence interruptions reduce the overall amount and heterogeneity of the alternative DNA structure formed (7, 9, 10, 16).

For $(CTG)_n$•$(CAG)_n$ and $(CGG)_n$•$(CCG)_n$ repeats, the looped-out individual strands can form hairpins (for review see (17). Experiments by Pearson and coworkers, however, showed that for the $(CTG)_n$•$(CAG)_n$ slipped strand structure, the (CTG) strand forms a base-paired hairpin, while the (CAG) strand remains unpaired. This was demonstrated in slipped intermediate DNAs which are defined as DNA duplexes with different numbers of repeats in opposite strands, for example $(CTG)_{30}$•$(CAG)_{50}$ or $(CAG)_{30}$•$(CTG)_{50}$ (Figure 5). Slipped intermediate DNAs would occur during repeat expansion or deletion associated with replication, repair, or recombination, as discussed below. In the $(CTG)_{30}$•$(CAG)_{50}$ slipped intermediate the excess $(CAG)_{20}$ loop-out remains unpaired, while in the $(CAG)_{30}$•$(CTG)_{50}$ slipped intermediate the $(CTG)_{20}$ loop-out formed a hairpin (10).

3.2. Slipped strand structures in (CCTG)•(CAGG) repeats

Recent experiments have shown that the DM2 $(CCTG)_n$•$(CAGG)_n$ repeats can also form slipped strand DNA structures (Edwards and Sinden, unpublished). As with (CTG•(CAG) and (CCG)•(CGG) repeats a population of DNA species is formed following reduplexing, with preferred products giving prominent bands in addition to a heterogeneous distribution in polyacrylamide gels. A major and important difference found with $(CCTG)_n$•$(CAGG)_n$ repeats is that they form slipped strand DNA structures spontaneously in supercoiled DNA without heat or alkaline denaturation. This property had been expected for slipped strand DNA structures as the formation of slipped strand DNA will relax supercoils from duplex unwinding, but it had not been observed for (CTG•(CAG) and (CCG)•(CGG) repeats. The reasons for this difference are not understood at present, but it may reflect an easier initiation of structure formation or unwinding for CCTG repeats compared to CGG or CTG repeats.

4. HAIRPIN STRUCTURES IN SLIPPED STRAND DNA LOOP-OUTS

Many of the simple DNA repeats associated with neurodegenerative diseases contain some degree of inverted repeat, in addition to direct repeat, symmetry. Specifically, all repeats of the form $(CXG)_n$, where n = A, T, C, or G (which includes $(CTG)_n$•$(CAG)_n$ and $(CGG)_n$•$(CCG)_n$) can form hairpin stems stabilized by two C•G base pairs surrounding a T•T, A•A, C•C, or G•G mispair (Figure 6)

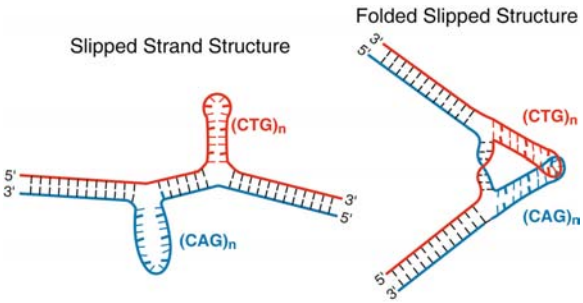

Figure 4. Folded slipped strand DNA. Since the loops, or loop-outs, of slipped strand DNAs are complementary, it is possible that loop-loop interactions involving complementary base pairing can occur. As described in the text, this has been observed for $(CAG)_n$•$(CTG)_n$ repeats. The pairing would necessarily be limited given the topology of the DNA, as extensive wrapping would not be possible. Folded slipped strand structures may be responsible for the unusual stability of slipped strand DNAs.

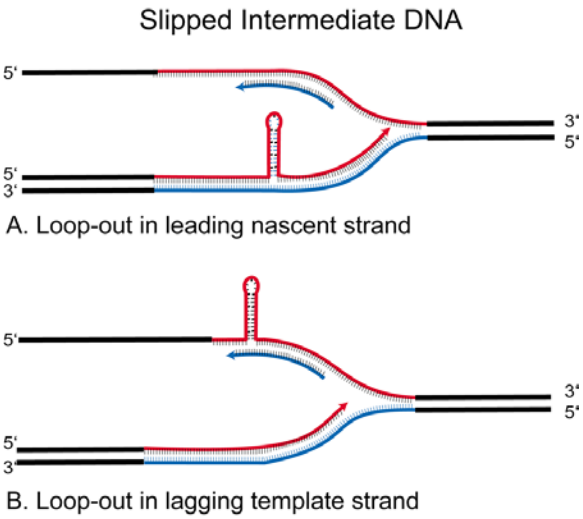

Figure 5. Slipped intermediate DNA. Slipped intermediate DNA has been defined as a $(CAG)_x$•$(CTG)_y$ repeat, where $x \neq y$. In this case one strand will contain an excess of repeats, either an excess of (CAG) or (CTG) repeats. This leads, in the case of a $(CTG)_n$ repeat, to a hairpin protruding from the duplex, forming a single three-way junction. These structures would result from primer-template misalignment followed by continued replication during DNA replication, as discussed below, and therefore represent replication intermediates in a pathway to repeat expansion or deletion.

(see for review (18-21)). CTG and CAG repeat tracts can form but a single hairpin structure with a T•T or A•A base pair mismatch flanking two C•G pairs, respectively (17-19). The hairpin formed by $(CTG)_n$ is more stable than that formed by $(CAG)_n$. This is because the smaller pyrimidine T•T mispairs stack better in the DNA helix than are larger purine A•A mispairs (18, 22, 23). In fact, single-stranded $(CAG)_{20}$ loop-outs in duplex DNA form an unstructured loop rather than a base-paired hairpin (10). Hairpin structures formed by CGG and CCG strands are more complex as each can fold into two different conformations while still containing a G•G or C•C mismatch, respectively. Hairpins formed by $(CGG)_n$ are more stable than those formed by $(CCG)_n$ (18). The CCG tract can form an unusual duplex called an e-motif in which C•C mispairs actually "kicked" out of the DNA helix and are extrahelical (24).

The $(CCTG)_n$•$(CAGG)_n$ repeats are also prone to hairpin formation with the CAGG strand forming a more stable hairpin than the CCTG strand (25, 26). The $(CAGG)_n$ hairpin is stabilized by two Watson-Crick G•C and two unusual G•A pairs per tetranucleotide repeat (Figure 7).

Single-stranded tracts of GAA repeats can also fold into hairpins comprised of G•A and A•A mispairs (27, 28) (Figure 7). Short GAA repeat tracts (n = 15) are only stable at very low temperatures, however, longer $(GAA)_n$ and $(TTC)_n$ repeat tracts may exist as hairpins at physiological salt concentrations and temperatures (28). However, the Friedreich ataxia (GAA)•(TTC) repeat forms a Py•Pu•Py intramolecular triplex rather than slipped strand DNA (29).

5. DNA DIRECT REPEATS, SLIPPED STRAND DNA STRUCTURES AND GENOMIC INSTABILITY

5.1. Polymerase misbehavior and DNA misdirection

Direct repeat DNA sequences, which can form slipped stand DNA structures, are associated with DNA mutations that can cause disease. In 1966, Streisinger et al. proposed a DNA slippage model to explain frameshift mutations within runs of a simple DNA repeats of a single base, or a few bases. The mechanism proposed for frameshift mutagenesis within a simple repeating sequence involves unpairing the newly synthesized (nascent) DNA strand from its template during the process of DNA replication, followed by the reformation of hydrogen bonds with a different set of complementary bases. This slippage of base pairing between the template and nascent strand will result in the formation of extrahelical bases in either the template or nascent DNA strand, resulting in duplication or deletion mutations, respectively when replication continues (Figure 8).

Large deletions and duplications can also occur between direct repeats. These direct repeats can be separated hundreds of base pairs, or they can be adjacent (30-32). If the DNA sequence between the direct repeats contains inverted repeat symmetry, the formation of a hairpin within the inverted repeat can increase the frequency of deletion by bringing the direct repeats into close proximity (33-38). Misalignment between nonadjacent regions of a tract of short direct repeats will result in the formation of slipped intermediate structures, as shown in Figure 5.

Mutations associated with primer-template misalignment have been established in many model systems (39-46). Misalignment resulting in mutations can occur within runs of repeats (47-50) or between distant direct repeats (31, 52).

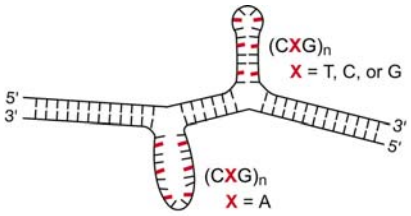

Figure 6. Hairpin structures in $(CXG)_n$ repeats. All repeats of the form $(CXG)_n$, where X = A, T, C, or G can form a hairpin stem containing two C•G base pairs and a T•T, C•C, A•A, or G•G mispair. In the case of $(CAG)_n$•$(CTG)_n$, the $(CAG)_n$ strand forms an unpaired loop, while the $(CTG)_n$ repeat tract forms a paired hairpin stem containing T•T mismatches, as shown above.

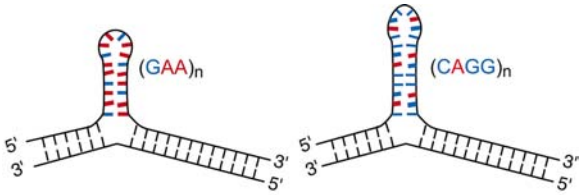

Figure 7. Hairpins in $(GAA)_n$ and $(CAGG)_n$ repeats. Hairpins have been reported to form in $(GAA)_n$ and $(CAGG)_n$ repeat strands as shown above, and as described in the text.

5.2. Repeat expansion and deletion associated with human neurodegenerative disease

Since 1991, many different genetic neurodegenerative diseases and chromosomal fragile sites have been associated with the expansion of $(CTG)_n$•$(CAG)_n$, $(CGG)_n$•$(CCG)_n$, $(GAA)_n$•$(TTC)_n$, $(CCTG)_n$•$(CAGG)_n$, $(ATTCT)_n$•$(AGAAT)_n$, or $(CCCCGCCCCGCG)_n$•$(CGCGGGGCGGGG)_n$ repeats (for review see (8, 53-62)). Both small and large changes in repeat lengths are associated with human neurodegenerative diseases. While repeats shorter than about 30 copies are stable, large intergenerational changes in repeat tract length, from a length of typically 30 -100 repeats to more than 1000 copies, have been identified in several neurodegenerative diseases including myotonic dystrophy types 1 and 2 (DM1 and DM2), fragile X syndrome, Friedreich ataxia, and spinocerebellar ataxia type 10 (SCA10). In SCA10 and DM2, expansion occurs to 4,500 and 11,000 repeats, respectively. These repeat expansions may occur during germ cell development (see for review (57, 58)). In patients with some neurodegenerative diseases, small repeat length changes are observed in somatic cells throughout the life of an individual (63, 64). These changes could occur via primer-template misalignment during normal DNA replication. Repeat length changes may even occur in nondividing cells where mismatch or other DNA repair activity may be responsible (58, 65-67).

The mechanism(s) that explain expansion from 30-100 repeats to lengths of 1000, or even 4,000-11,000 repeats remains unknown, and we have recently reviewed several possibilities (53, 68). The expansion of these repeats may involve the formation of alternative structures in the DNA. These structures include slipped intermediate DNA, slipped strand DNA, triplex DNA, quadruplex DNA, parallel strand DNA, or unwound DNA. The roles that alternative DNA structures may play in both short and long repeat expansions will be discussed below, as will models for repeat deletion. Understanding deletion mutation (also called contractions) is also important since, for expanded alleles associated with Friedreich ataxia and SCA10, deletions may be the predominant event in patients (69-73).

6. BIOLOGICAL CONSEQUENCES OF ALTERNATIVE DNA CONFORMATIONS

6.1. Replication blockage

Alternative structures can have important roles in the functioning of a cell. For example, alternative DNA structures are involved in DNA replication or transcription (74). All too often, however, associations are observed between DNA sequences that can form alternative DNA structures and mutations in genes that cause human disease (75-77). Stable alternative DNA structures, including hairpins, triplex, quadruplex, and slipped strand DNA structures act to block DNA polymerase leading to the stalling or termination of a replication fork. Many different DNA polymerases either pause or can be completely inhibited by DNA hairpins (78-81). Dissociation from the template during replication of a hairpin may lead to primer-template misalignment resulting in mutations (42, 82-85). Following blockage, replication restart may provide a window for repeat expansion as discussed previously (53).

DNA polymerase pausing has been observed at $(CGG)_n$ hairpins *in vitro* (86), and at $(CGG)_n$•$(CCG)_n$ and $(CTG)_n$•$(CAG)_n$ repeats in bacteria and yeast (87-89). Replication fork stalling at these repeats in cells is dependent on repeat length and orientation relative to a replication origin. The formation of unusual DNA structures in these trinucleotide repeats may cause the replication blockage.

$(CGG)_n$, $(AGG)_n$, and $(TGG)_n$ repeats can form DNA quadruplex structures that may also be responsible for blockage of bacterial, bacteriophage, and various eukaryotic DNA polymerases (86, 90, 91).

Py•Pu tracts with mirror repeat symmetry have long been known to block the DNA replication fork in cells (92, 93). Replication fork blockage may result from the folding of a single strand DNA template back into the major groove of the nascent duplex in part of the Py•Pu tract, resulting in the formation of triplex DNA (93-95). Strong blocks to DNA replication have also been observed when triplex structures are formed prior to polymerization (96).

$(GAA)_n$•$(TTC)_n$ repeats can also form triplex DNA (29, 97-102) and pause DNA replication *in vitro* within the repeat tract (101), as observed for other triplex-forming DNA sequences (93-95). The formation of a $[(GAA)_n$•$(dGAA)_n$•$(dTTC)_n]$ RNA-DNA triplex during transcription of the $(GAA)_n$•$(TTC)_n$ repeat in the *frataxin* gene has also been proposed as a mechanism of gene

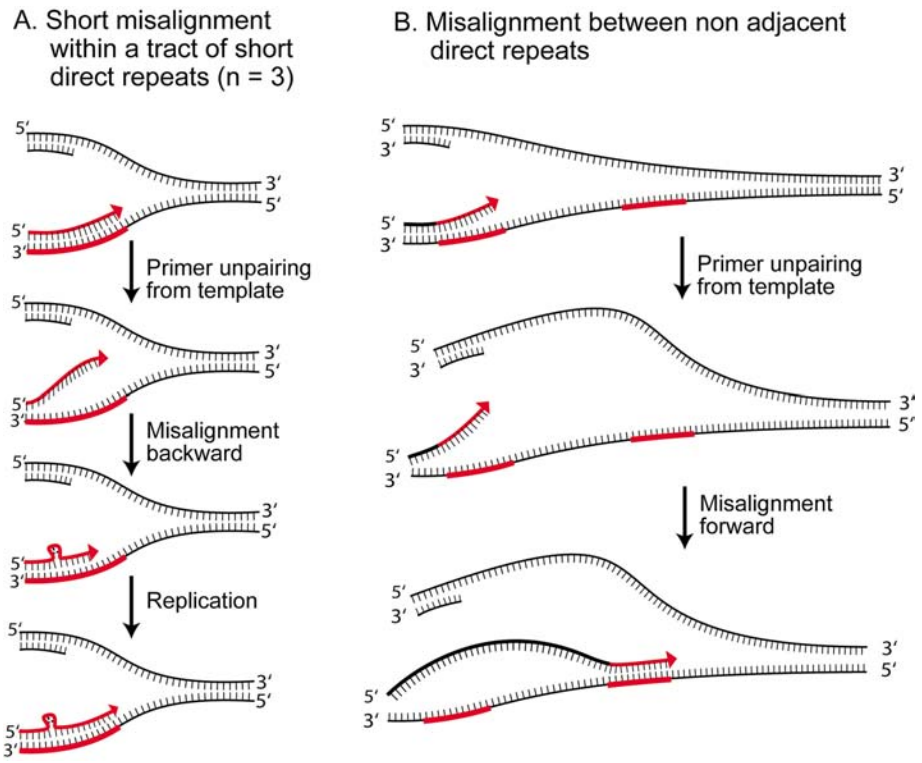

Figure 8. Primer template misalignment during DNA replication. Primer-template misalignment is responsible for many mutations. It can explain expansions and deletions within runs of DNA repeats. A. In the case of triplet repeats, backward slippage of the nascent leading strand would result in a 3-nt loop-out. Continued replication would result in a 3 bp duplication (or addition). B. Misalignment can also occur between distant direct repeats. This figure shows misalignment of one repeat in the nascent leading strand with a 'downstream' second copy of the repeat. This results in the formation of an intervening DNA loop (of nonrepetitive DNA in the figure shown above). When this occurs within a long simple direct repeat tract, such as $(CAG)_n$•$(CTG)_n$ repeats, slippage and replication would result in the formation of slipped intermediate DNA, as shown in Figure 5, A.

downregulation. This would result from the $(GAA)_n$ transcript becoming trapped in the RNA-DNA triplex-duplex combination (99, 104), or between the $(GAA)_n$•$(TTC)_n$ duplex and the nontranscribed $(GAA)_n$ single strand of the transcription bubble (105).

In summary, the data for replication pausing at slipped strand DNA structures is less strong than for other alternative DNA structures formed by expandable disease-associated repeats. However, the important point to be made is that all unstable repeats can form one or more alternative structures that may influence the processes of replication or repair. In fact, in contrast to all other unstable repeats, the SCA10 (ATTCT)•(AGAAT) repeats that expand to 4,500 copies do not form a structure that blocks DNA replication. Rather, they form an unwound DNA structure (106), and models for expansion and deletion resulting from aberrant replication origin activity have been described (68, 106)

6.2. Does replication slippage occur at DNA repeats?

While the formation of slipped strand DNA may promote replication errors, the process of replication slippage would result in the formation of slipped intermediate structures, which are structural intermediates in the path of mutagenesis. Primer-template slippage by bacterial and mammalian enzymes occurs *in vitro* at disease-associated repeats (25, 28, 107-109). Evidence for primer-template slippage and the formation of slipped intermediate DNA in living cells abounds. Changes in repeat length are expected to reflect the formation of slipped strands during DNA replication, according to the simple models described in Figure 7. Repeat heterogeneity in *E. coli*, especially in mismatch repair deficient strains, is consistent with slipped misalignment during replication of repeats in bacteria (110-112). Thus, slipped misalignment may be the simplest mechanism for repeat instability and it could be operable for all repeats.

Moreover, since hairpins formed from opposite strands can have different thermal stabilities, one may reasonably expect asymmetries in slippage during replication of the leading or lagging DNA strands. This is a results that would also point to the involvement of replication slippage and slipped strand DNA formation during replication. Consistent with these predictions, repeat instability that is dependent on the orientation of the repeat with respect to the approach of a replication fork is observed in *E. coli* (113), yeast (114-118), and mammalian model systems (119, 120).

7. FATE OF SLIPPED STRAND AND SLIPPED INTERMEDIATE DNA IN CELLS

7.1. Role of DNA repair proteins

The repair of loop-outs in *E. coli* may involve excision repair proteins UvrA, UvrB, and SbcC (121, 122). The UvrA protein, which is generally thought to recognize DNA damages in cells, binds *in vitro* to DNA structures containing $(CAG)_n$ repeat loop-outs where the loops comprise 1, 2, or 17 repeats (121). Significantly, UvrA and other excision repair proteins presumably bind $(CTG)_n$ and $(CAG)_n$ loop-outs in cells since these loops are effectively excised from plasmids containing either a $(CTG)_{23}$ or a $(CAG)_{23}$ heteroduplex loop when introduced into *E. coli* cells in cells containing functional UvrA. In cells defective in UvrA, loops were effectively excised (121).

Mammalian cell mismatch repair proteins also bind to DNA structures containing $(CTG)_n$ or $(CAG)_n$ loops in slipped strand DNA (10, 123). The $(CAG)_n$ loop-out is preferentially recognized by MSH2, a human mismatch repair protein, compared with recognition of the $(CTG)_n$ loop-out (10, 123). In addition, a MSH2-MSH3 heterodimer binds to a $(CAG)_n$ loop-out (124). In mice, short expansions seen in ectopic repeat tracts depend on the presence of functional mismatch repair proteins MSH2, MSH3, MSH6, or PMS2 (65, 67, 125-130).

Pearson and coworkers have also characterized the repair of slipped strand DNA in mammalian cell extracts. Plasmid DNA containing a slipped intermediate DNAs with either a $(CAG)_n$ loop-out or $(CTG)_n$ hairpin in a continuous template or nicked nascent strand (131). These templates, which mimic products of replication slippage or strand exchange during replication restart or double-strand break repair, are repaired with very different efficiencies (131). These results suggest that different slipped intermediate DNA structures have very different biological consequences and fates in mammalian cells.

7.2. Role of DNA replication proteins

FEN-1 is a human flap endonuclease, which is responsible for digesting the RNA primer from the 5' end of an Okazaki fragment during lagging strand DNA replication (132). However, a triplet repeat tract at the 5' end of an Okazaki fragment can form a hairpin that is refractory to FEN-1 digestion (101, 133, 134). This could result in DNA expansion from ligation of repeat containing the hairpin near the 5' terminus, resulting in the formation of a slipped intermediate DNA structure (135, 136). Supporting evidence for this scenario comes from *in vitro* replication studies with human pol beta, in which large $(GAA)_n$ expansions are generated during replication of repeat tract when FEN-1 is omitted from the reaction. The addition of FEN-1 prevented GAA repeat expansion (109).

In *E. coli*, replication restart proteins PriA, which is involved in restarting replication on the lagging strand following fork pausing and collapse, and RecG, which drives fork reversal forming a four-stranded DNA structure, both bind to $(CTG)_n$ and $(CAG)_n$ hairpins. They also bind to single-stranded and duplex DNA molecules containing $(CTG)_n$ and/or $(CAG)_n$ loop-outs (137). Moreover, both PriA and RecG bound to forked DNA substrates containing a $(CTG)_7$ or $(CAG)_7$ loop-out in a model lagging template strand, but they did not bind when the loop-out was contained in the leading template strand.

7.3. Role of replication restart in repeat instability

Repeat deletions as well as repeat expansions may result from errors occurring during replication restart following the collapse of the replication fork during synthesis of the repeats (50, 137, 138). Hairpins, slipped strand DNA, or other secondary structures may block the progression of a replication fork in $(CTG)_n$•$(CAG)_n$ or $(CGG)_n$•$(CCG)_n$ repeats (28, 86, 90, 107, 139, 140).

Several pathways are available for restarting a collapsed or paused fork, as we have described previously (53, 137). For the purposes here, it is important to mention that significant potential for slipped intermediate or slipped strand DNA formation exists during the replication restart process. The potential for hairpin formation when the CTG strand is single-stranded during a RecA and RecBC dependent restart pathway may explain the generally observed orientation bias for deletions in *E. coli* (137, 138). Also, following fork collapse, a hairpin may form in a single-stranded lagging strand upstream of the fork. Reannealing of the template strands would result in the formation of slipped strand DNA. Therefore, ample opportunity exists for formation of slipped strand DNA or slipped intermediate DNA during replication restart, resulting in the structure directed mutations.

8. REFERENCES

1. L.W.Coggins, M.O'Prey, S.Akhter: Intrahelical pseudoknots and interhelical associations mediated by mispaired human minisatellite DNA sequences in vitro. *Gene.* 121, 279-285 (1992)
2. L.W.Coggins, M.O'Prey: DNA tertiary structures formed in vitro by misaligned hybridization of multiple tandem repeat sequences. *Nucleic Acids Res.* 17, 7417-7426 (1989)
3. M.Kato, K.Matsunaga, N.Shimizu: A novel unusual DNA structure formed in an inverted repeat sequence. *Biochem Biophys Res Commun* 246, 532-534 (1998)
4. M.Kato, S.Hokabe, S.Itakura, S.Minoshima, Y.L.Lyubchenko, T.D.Gurkov, H.Okawara, K.Nagayama, N.Shimizu: Interarm interaction of DNA cruciform forming at a short inverted repeat sequence. *Biophys. J.* 85, 402-408 (2003)
5. T.Ohta, S.Nettikadan, F.Tokumasu, H.Ideno, Y.Abe, M.Kuroda, H.Hayashi, K.Takeyasu: Atomic force microscopy proposes a novel model for stem-loop structure that binds a heat shock protein in the Staphylococcus aureus HSP70 operon. *Biochem. Biophys. Res. Commun.* 226, 730-734 (1996)
6. Y.L.Lyubchenko, L.S.Shlyakhtenko, M.Binus, C.Gaillard, F.Strauss: Visualization of hemiknot DNA structure with an atomic force microscope. *Nucleic Acids Res.* 30, 4902-4909 (2002)
7. C.E.Pearson, R.R.Sinden: Alternative structures in duplex DNA formed within the trinucleotide repeats of the myotonic dystrophy and fragile X loci. *Biochemistry* 35, 5041-5053 (1996)

8. R.R.Sinden, V.N.Potaman, E.A.Oussatcheva, C.E.Pearson, Y.L.Lyubchenko, L.S.Shlyakhtenko: Triplet repeat DNA structures and human genetic disease: dynamic mutations from dynamic DNA. *J. Biosci.* 27, 53-65 (2002)
9. C.E.Pearson, R.R.Sinden. Slipped strand DNA, dynamic mutations, and human disease, in: R.D.Wells and S.T.Warren (Eds.), Genetic Instabilities and Hereditary Neurological Disorders, *Academic Press*, San Diego, pp. 585-621 (1998)
10. C.E.Pearson, M.Tam, Y.H.Wang, S.E.Montgomery, A.C.Dar, J.D.Cleary, K.Nichol: Slipped-strand DNAs formed by long (CAG)•(CTG) repeats: slipped-out repeats and slip-out junctions. *Nucleic Acids Res.* 30, 4534-4547 (2002)
11. M.Tam, M.S.Erin, M.Kekis, S.B.David, G.B.Price, C.E.Pearson: Slipped (CTG)•(CAG) repeats of the myotonic dystrophy locus: surface probing with anti-DNA antibodies. *J. Mol. Biol.* 332, 585-600 (2003)
12. P.D.Chastain, E.E.Eichler, S.Kang, D.L.Nelson, S.D.Levene, R.R.Sinden: Anomalous rapid electrophoretic mobility of DNA containing triplet repeats associated with human disease genes. *Biochemistry* 34, 16125-16131 (1995)
13. P.D.Chastain, R.R.Sinden: CTG repeats associated with human genetic disease are inherently flexible. *J Mol Biol* 275, 405-411 (1998)
14. A.Bacolla, R.Gellibolian, M.Shimizu, S.Amirhaeri, S.Kang, K.Ohshima, J.E.Larson, S.C.Harvey, B.D.Stollar, R.D.Wells: Flexible DNA - genetically unstable CTG•CAG and CGG•CCG from human hereditary neuromuscular disease genes. *J. Biol. Chem.* 272, 16783-16792 (1997)
15. C.E.Pearson, E.E.Eichler, D.Lorenzetti, S.F.Kramer, H.Y.Zoghbi, D.L.Nelson, R.R.Sinden: Interruptions in the triplet repeats of SCA1 and FRAXA reduce the propensity and complexity of slipped strand DNA (S-DNA) formation. *Biochemistry* 37, 2701-2708 (1998)
16. C.E.Pearson, Y.H.Wang, J.D.Griffith, R.R.Sinden: Structural analysis of slipped-strand DNA (S-DNA) formed in $(CTG)_n$·$(CAG)_n$ repeats from the myotonic dystrophy locus. *Nucleic Acids Res.* 26, 816-823 (1998)
17. C.E.Pearson, R.R.Sinden: Trinucleotide repeat DNA structures: Dynamic mutations from dynamic DNA. *Curr. Opin. Struct. Biol.* 8, 321-330 (1998)
18. M.Mitas: Trinucleotide repeats associated with human disease. *Nucleic Acids Res.* 25, 2245-2253 (1997)
19. J.M.Darlow, D.F.Leach: Evidence for two preferred hairpin folding patterns in d(CGG)•d(CCG) repeat tracts *in vivo*. *J. Mol. Biol.* 275, 17-23 (1998)
21. J.M.Darlow, D.R.F.Leach: Secondary structures in d(CGG) and d(CCG) repeat tracts. *J. Mol. Biol.* 275, 3-16 (1998)
22. J.Petruska, N.Arnheim, M.F.Goodman: Stability of intrastrand hairpin structures formed by the CAG/CTG class of DNA triplet repeats associated with neurological diseases. *Nucleic Acids Res.* 24, 1992-1998 (1996)
23. A.M.Gacy, C.T.McMurray: Influence of hairpins on template reannealing at trinucleotide repeat duplexes: a model for slipped DNA. *Biochemistry* 37, 9426-9434 (1998)
24. X.Gao, X.Huang, G.K.Smith, M.Zheng, H.Liu: A new antiparallel duplex motif of DNA CCG repeats that is stabilized by extrahelical bases symetrically localized in the minor groove. *J. Am. Chem. Soc.* 117, 8883-8884 (1995)
25. B.L.Heidenfelder, M.D.Topal: Effects of sequence on repeat expansion during DNA replication. *Nucleic Acids Res.* 31, 7159-7164 (2003)
26. R.Dere, M.Napierala, L.P.Ranum, R.D.Wells: Hairpin structure-forming propensity of the (CCTG.CAGG) tetranucleotide repeats contributes to the genetic instability associated with myotonic dystrophy type 2. *J Biol. Chem.* 279, 41715-41726 (2004)
27. I.S.Suen, J.N.Rhodes, M.Christy, B.McEwen, D.M.Gray, M.Mitas: Structural properties of Friedreich's ataxia d(GAA) repeats. *Biochim. Biophys. Acta* 1444, 14-24 (1999)
28. B.L.Heidenfelder, A.M.Makhov, M.D.Topal: Hairpin formation in Friedreich's ataxia triplet repeat expansion. *J. Biol. Chem.* 278, 2425-2431 (2003)
29. V.N.Potaman, E.A.Oussatcheva, Y.L.Lyubchenko, L.S.Shlyakhtenko, S.I.Bidichandani, T.Ashizawa, R.R.Sinden: Length-dependent structure formation in Friedreich ataxia $(GAA)_n$•$(TTC)_n$ repeats at neutral pH. *Nucleic Acids Res.* 32, 1224-1231 (2004)
30. A.M.Albertini, M.Hofer, M.P.Calos, J.H.Miller: On the formation of spontaneous mutations: the importance of short sequence homologies in generation of large deletion. *Cell* 29, 319-328 (1982)
31. J.W.Drake, B.W.Glickman, L.S.Ripley: Updating the theory of mutation. *Am. Scientist* 71, 621-630 (1983)
32. L.S.Ripley: Frameshift mutation: Determinants of specificity. *Annu. Rev. Genet.* 24, 189-213 (1990)
33. R.R.Sinden, G.Zheng, R.G.Brankamp, K.N.Allen: On the deletion of inverted repeated DNA in *Escherichia coli*: Effects of length, thermal stability, and cruciform formation i*n vivo*. *Genetics* 129, 991-1005 (1991)
34. T.Q.Trinh, R.R.Sinden: Preferential DNA secondary structure mutagenesis in the lagging strand of replication in *E. coli*. *Nature* 352, 544-547 (1991)
35. T.Q.Trinh, R.R.Sinden: The influence of primary and secondary DNA structure in deletion and duplication between direct repeats in *Escherichia coli*. *Genetics* 134, 409-422 (1993)
36. U.Dasgupta, K.Weston-Hafer, D.E.Berg: Local DNA sequence control of deletion formation in *Escherichia coli* plasmid pBR322. *Genetics* 115, 41-49 (1987)
37. K.Weston-Hafer, D.E.Berg: Palindromy and the location of deletion endpoints in *Escherichia coli*. *Genetics* 121, 651-658 (1989)
38. W.L.Williams, U.R.Muller: Effects of palindrome size and sequence on genetic stability in the bacteriophage ΦX174 genome. *J. Mol. Biol.* 196, 743-755 (1987)
39. K.Bebenek, T.A.Kunkel: Streisinger revisited: DNA synthesis errors mediated by substrate misalignments. *Cold Spring Harb. Symp. Quant. Biol* 65, 81-91 (2000)
40. T.A.Kunkel, A.Soni: Mutagenesis by transient misalignment. *J. Biol Chem.* 263, 14784-14789 (1988)
41. T.A.Kunkel: Misalignment-mediated DNA synthesis errors. *Biochemistry* 29, 8003-8011 (1990)
42. W.A.Rosche, L.S.Ripley, R.R.Sinden: Primer-template misalignments during leading strand DNA synthesis account for the most frequent spontaneous mutations in a quasipalindromic region in Escherichia coli. *J. Mol. Biol.* 284, 633-646 (1998)
43. C.Papanicolaou, L.S.Ripley: An in vitro approach to identifying specificity determinants of mutagenesis

mediated by DNA misalignments. *J. Mol Biol* 221, 805-821 (1991)
44. L.S.Ripley: Frameshift mutation: Determinants of specificity. *Annu. Rev. Genet.* 24, 189-213 (1990)
45. V.van Noort, P.Worning, D.W.Ussery, W.A.Rosche, R.R.Sinden: Strand misalignments lead to quasipalindrome correction. *Trends Genet.* 19, 365-369 (2003)
46. R.R.Sinden, V.I.Hashem, W.A.Rosche: DNA-directed mutations: Leading and lagging strand specificity. *Ann N Y Acad Sci* 870, 173-189 (1999)
47. G.Streisinger, Y.Okada, J.Emrich, J.Newton, A.Tsugita, E.Terzaghi, M.Inouye: Frameshift mutations and the genetic code. *Cold Spring Harb. Symp. Quant. Biol.* 31, 77-84 (1966)
48. L.C.Kroutil, T.A.Kunkel: Deletion errors generated during replication of CAG repeats. *Nucleic Acids Res.* 27, 3481-3486 (1999)
49. M.Wierdl, M.Dominska, T.D.Petes: Microsatellite instability in yeast - dependence on the length of the microsatellite. *Genetics* 146, 769-779 (1997)
50. V.I.Hashem, W.A.Rosche, R.R.Sinden: Genetic assays for measuring rates of (CAG)•(CTG) repeat instability in *Escherichia coli*. *Mutat. Res.* 502, 25-37 (2002)
52. L.S.Ripley, A.Clark, J.G.deBoer: Spectrum of spontaneous frameshift mutations. Sequences of bacteriophage T4 rII gene frameshifts. *J. Mol. Biol.* 191, 601-613 (1986)
53. R.R.Sinden, M.J.Pytlos, V.N.Potaman. Mechanisms of DNA Repeat Expansion, in: M.Fry and K.Usdin (Eds.), *Human Nucleotide Expansion Disorders*, *Springer*, Berlin, pp. 3-53 (2006)
54. C.J.Cummings, H.Y.Zoghbi: Fourteen and counting: unraveling trinucleotide repeat diseases. *Hum. Mol. Genet.* 9, 909-916 (2000)
55. P.Parniewski, P.Staczek: Molecular mechanisms of TRS instability. *Adv. Exp. Med. Biol.* 516., 1-25 (2002)
56. B.A.Lenzmeier, C.H.Freudenreich: Trinucleotide repeat instability: a hairpin curve at the crossroads of replication, recombination, and repair. *Cytogenet. Genome Res.* 100, 7-24 (2003)
57. J.D.Cleary, C.E.Pearson: The contribution of CIS-elements to disease-associated repeat instability: clinical and experimental evidence. *Cytogenet. Genome Res.* 100, 25-55 (2003)
58. C.E.Pearson: Slipping while sleeping? Trinucleotide repeat expansions in germ cells. *Trends Mol. Med.* 9, 490-495 (2003)
59. L.Y.Brown, S.A.Brown: Alanine tracts: the expanding story of human illness and trinucleotide repeats. *Trends Genet.* 20, 51-58 (2004)
60. L.P.Ranum, J.W.Day: Myotonic dystrophy: RNA pathogenesis comes into focus. *Am. J. Hum. Genet.* 74, 793-804 (2004)
61. J.D.Cleary, C.E.Pearson: Replication fork dynamics and dynamic mutations: the fork-shift model of repeat instability. *Trends Genet.* 21, 272-280 (2005)
62. S.M.Mirkin: Molecular Models for Repeat Expansion. *Chemtracts Biochem. Mol. Biol.* 17, 639-662 (2004)
63. L.-J.C.Wong, T.Ashizawa, D.G.Monckton, C.T.Caskey, C.S.Richards: Somatic heterogeneity of the CTG repeat in myotonic dystrophy is age and size dependent. *Am. J. Hum. Genet.* 56, 114-122 (1995)
64. L.Martorell, D.G.Monckton, J.Gamez, K.J.Johnson, I.Gich, A.L.de Munain, M.Baiget: Progression of somatic CTG repeat length heterogeneity in the blood cells of myotonic dystrophy patients. *Hum. Mol Genet.* 7, 307-312 (1998)
65. I.V.Kovtun, C.T.McMurray: Trinucleotide expansion in haploid germ cells by gap repair. *Nat. Genet.* 27, 407-411 (2001)
66. C.T.McMurray, I.V.Kortun: Repair in haploid male germ cells occurs late in differentiation as chromatin is condensing. *Chromosoma* 111, 505-508 (2003)
67. M.Gomes-Pereira, M.T.Fortune, L.Ingram, J.P.McAbney, D.G.Monckton: Pms2 is a genetic enhancer of trinucleotide CAG•CTG repeat somatic mosaicism: implications for the mechanism of triplet repeat expansion. *Hum Mol. Genet* 13, 1815-1825 (2004)
68. V.N.Potaman, M.J.Pytlos, V.I.Hashem, J.J.Bissler, M.Leffak, R.R.Sinden. DNA structures and genetic instabilities associated with SCA10 $(ATTCT)_n$•$(AGAAT)_n$ repeats suggest a DNA amplification model for repeat expansion, in: R.D.Wells and T.Ashizawa (Eds.), Genetic Instabilities and Neurological Diseases, *Academic Press*, pp. 447-460 (2006)
69. L.M.Pollard, R.Sharma, M.Gomez, S.Shah, M.B.Delatycki, L.Pianese, A.Monticelli, B.J.Keats, S.I.Bidichandani: Replication-mediated instability of the GAA triplet repeat mutation in Friedreich ataxia. *Nucleic Acids Res.* 32, 5962-5971 (2004)
70. R.Sharma, S.Bhatti, M.Gomez, R.M.Clark, C.Murray, T.Ashizawa, S.I.Bidichandani: The GAA triplet-repeat sequence in Friedreich ataxia shows a high level of somatic instability in vivo, with a significant predilection for large contractions. *Hum. Mol Genet.* 11, 2175-2187 (2002)
71. S.I.Bidichandani, S.M.Purandare, E.E.Taylor, G.Gumin, H.Machkhas, Y.Harati, R.A.Gibbs, T.Ashizawa, P.I.Patel: Somatic sequence variation at the Friedreich ataxia locus includes complete contraction of the expanded GAA triplet repeat, significant length variation in serially passaged lymphoblasts and enhanced mutagenesis in the flanking sequence. *Hum Mol Genet* 8, 2425-2436 (1999)
72. T.Matsuura, P.Fang, X.Lin, M.Khajavi, K.Tsuji, A.Rasmussen, R.P.Grewal, M.Achari, M.E.Alonso, S.M.Pulst, H.Y.Zoghbi, D.L.Nelson, B.B.Roa, T.Ashizawa: Somatic and germline instability of the ATTCT repeat in spinocerebellar ataxia type 10. *Am. J. Hum. Genet.* 74, 1216-1224 (2004)
73. T.Matsuura, T.Yamagata, D.L.Burgess, A.Rasmussen, R.P.Grewal, K.Watase, M.Khajavi, A.E.McCall, C.F.Davis, L.Zu, M.Achari, S.M.Pulst, E.Alonso, J.L.Noebels, D.L.Nelson, H.Y.Zoghbi, T.Ashizawa: Large expansion of the ATTCT pentanucleotide repeat in spinocerebellar ataxia type 10. *Nat. Genet.* 26, 191-194 (2000)
74. R.R.Sinden. DNA Structure and Function, *Academic Press*, San Diego, (1994).
75. D.N.Cooper, M.Krawczak. Human Gene Mutation, *Bios Scientific Publishers*, Oxford, U.K., (1993).
76. R.R.Sinden, R.D.Wells: DNA structure, mutations, and human genetic disease. *Curr Opin Biotechnol* 3, 612-622 (1992)
77. J.J.Bissler: DNA inverted repeats and human disease. *Front Biosci* 3, d408-d418 (1998)

78. L.S.Kaguni, D.A.Clayton: Template-directed pausing in in vitro DNA synthesis by DNA polymerase a from *Drosophila melanogaster* embryos. *Proc. Natl. Acad. Sci. USA* 79, 983-987 (1982)
79. D.T.Weaver, M.L.DePamphilis: Specific sequences in native DNA that arrest synthesis by DNA polymerase alpha. *J. Biol Chem.* 257, 2075-2086 (1982)
80. K.J.Hacker, B.M.Alberts: The rapid dissociation of the T4 DNA polymerase holoenzyme when stopped by a DNA hairpin helix. A model for polymerase release following the termination of each Okazaki fragment. *J. Biol. Chem.* 269, 24221-24228 (1994)
81. Z.Suo, K.A.Johnson: DNA secondary structure effects on DNA synthesis catalyzed by HIV-1 reverse transcriptase. *J. Biol Chem.* 273, 27259-27267 (1998)
82. D.A.Gordenin, K.S.Lobachev, N.P.Degtyareva, A.L.Malkova, E.Perkins, M.A.Resnick: Inverted DNA repeats: A source of eukaryotic genomic instability. *Mol. Cell. Biol.* 13, 5315-5322 (1993)
83. W.A.Rosche, T.Q.Trinh, R.R.Sinden: Leading strand specific spontaneous mutation corrects a quasipalindrome by an intermolecular strand switch mechanism. *J. Mol. Biol.* 269, 176-187 (1997)
84. M.Viswanathan, J.J.Lacirignola, R.L.Hurley, S.T.Lovett: A novel mutational hotspot in a natural quasipalindrome in *Escherichia coli*. *J. Mol. Biol.* 302, 553-564 (2000)
85. K.Yoshiyama, H.Maki: Spontaneous hotspot mutations resistant to mismatch correction in Escherichia coli: transcription-dependent mutagenesis involving template-switching mechanisms. *J Mol. Biol.* 327, 7-18 (2003)
86. A.S.Kamath-Loeb, L.A.Loeb, E.Johansson, P.M.Burgers, M.Fry: Interactions between the Werner syndrome helicase and DNA polymerase delta specifically facilitate copying of tetraplex and hairpin structures of the d(CGG)n trinucleotide repeat sequence. *J. Biol. Chem.* 276, 16439-16446 (2001)
87. G.M.Samadashwily, G.Raca, S.M.Mirkin: Trinucleotide repeats affect DNA replication *in vivo*. *Nature Genet.* 17, 298-304 (1997)
88. M.M.Krasilnikova, S.M.Mirkin: Replication stalling at Friedreich's ataxia $(GAA)_n$ repeats in vivo. *Mol. Cell. Biol.* 24, 2286-2295 (2004)
89. R.Pelletier, M.M.Krasilnikova, G.M.Samadashwily, R.Lahue, S.M.Mirkin: Replication and expansion of trinucleotide repeats in yeast. *Mol. Cell. Biol.* 23, 1349-1357 (2003)
90. K.Usdin, K.J.Woodford: CGG repeats associated with DNA instability and chromosome fragility form structures that block DNA synthesis *in vitro*. *Nucleic Acids Res.* 23, 4202-4209 (1995)
91. K.Usdin: NGG-triplet repeats form similar intrastrand structures: implications for the triplet expansion diseases. *Nucleic Acids Res.* 26, 4078-4085 (1998)
92. B.S.Rao, H.Manor, R.G.Martin: Pausing in simian virus 40 DNA replication by a sequence containing $(dG\text{-}dA)_{27}\bullet(dT\text{-}dC)_{27}$. *Nucleic Acids Res.* 16, 8077-8094 (1988)
93. N.Baran, A.Lapidot, H.Manor: Formation of DNA triplexes accounts for arrests of DNA synthesis at $d(TC)_n$ and $d(GA)_n$ tracts. *Proc. Natl. Acad. Sci. USA* 88, 507-511 (1991)
94. A.S.Krasilnikov, I.G.Panyutin, G.M.Samadashwily, R.Cox, Y.S.Lazurkin, S.M.Mirkin: Mechanisms of triplex-caused polymerization arrest. *Nucleic Acids Res.* 25, 1339-1346 (1997)
95. V.N.Potaman, J.J.Bissler: Overcoming a barrier for DNA polymerization in triplex-forming sequences. *Nucleic Acids Res.* 27, e5 (1999)
96. A.Dayn, G.M.Samadashwily, S.M.Mirkin: Intramolecular DNA triplexes: Unusual sequence requirements and influence on DNA polymerization. *Proc. Natl. Acad. Sci. USA* 89, 11406-11410 (1992)
97. J.C.Hanvey, M.Shimizu, R.D.Wells: Intramolecular DNA triplexes in supercoiled plasmids. *Proc. Natl. Acad. Sci. USA* 85, 6292-6296 (1988)
98. K.Ohshima, S.Kang, J.E.Larson, R.D.Wells: TTA·TAA triplet repeats in plasmids form a non-H bonded structure. *J. Biol. Chem.* 271, 16784-16791 (1996)
99. S.I.Bidichandani, T.Ashizawa, P.I.Patel: The GAA triplet-repeat expansion in Friedreich ataxia interferes with transcription and may be associated with an unusual DNA structure. *Am. J. Hum. Genet.* 62, 111-121 (1998)
100. N.Sakamoto, P.D.Chastain, P.Parniewski, K.Ohshima, M.Pandolfo, J.D.Griffith, R.D.Wells: Sticky DNA: self-association properties of long GAA•TTC repeats in R•R•Y triplex structures from Friedreich's ataxia. *Mol. Cell* 3, 465-475 (1999)
101. C.Spiro, R.Pelletier, M.L.Rolfsmeier, M.J.Dixon, R.S.Lahue, G.Gupta, M.S.Park, X.Chen, S.V.Mariappan, C.T.McMurray: Inhibition of FEN-1 processing by DNA secondary structure at trinucleotide repeats. *Mol Cell* 4, 1079-1085 (1999)
102. A.A.Vetcher, M.Napierala, R.R.Iyer, P.D.Chastain, J.D.Griffith, R.D.Wells: Sticky DNA, a long GAA.GAA.TTC triplex that is formed intramolecularly, in the sequence of intron 1 of the frataxin gene. *J Biol. Chem.* 277, 39217-39227 (2002)
104. K.Ohshima, L.Montermini, R.D.Wells, M.Pandolfo: Inhibitory effects of expanded GAA•TTC triplet repeats from intron I of the Friedreich ataxia gene on transcription and replication *in vivo*. *J. Biol. Chem.* 273, 14588-14595 (1998)
105. E.Grabczyk, K.Usdin: The GAA•TTC triplet repeat expanded in Friedreich's ataxia impedes transcription elongation by T7 RNA polymerase in a length and supercoil dependent manner. *Nucleic Acids Res.* 28, 2815-2822 (2000)
106. V.N.Potaman, J.J.Bissler, V.I.Hashem, E.A.Oussatcheva, L.Lu, L.S.Shlyakhtenko, Y.L.Lyubchenko, T.Matsuura, T.Ashizawa, M.Leffak, C.J.Benham, R.R.Sinden: Unpaired structures in SCA10 $(ATTCT)_n\bullet(AGAAT)_n$ repeats. *J. Mol. Biol.* 326, 1095-1111 (2003)
107. M.J.Hartenstine, M.F.Goodman, J.Petruska: Base stacking and even/odd behavior of hairpin loops in DNA triplet repeat slippage and expansion with DNA polymerase. *J. Biol. Chem.* 275, 18382-18390 (2000)
108. J.Petruska, M.J.Hartenstine, M.F.Goodman: Analysis of strand slippage in DNA polymerase expansions of CAG/CTG triplet repeats associated with neurodegenerative disease. *J. Biol. Chem.* 273, 5204-5210 (1998)

109. B.L.Ruggiero, M.D.Topal: Triplet repeat expansion generated by DNA slippage is suppressed by human flap endonuclease 1. *J. Biol. Chem.* 279, 23088-23097 (2004)
110. K.H.Schmidt, C.M.Abbott, D.R.Leach: Two opposing effects of mismatch repair on CTG repeat instability in *Escherichia coli*. *Mol. Microbiol.* 35, 463-471 (2000)
111. S.Schumacher, R.P.P.Fuchs, M.Bichara: Expansion of CTG repeats from human disease genes is dependent upon replication mechanisms in *Escherichia coli* - The effect of long patch mismatch repair revisited. *J. Mol. Biol.* 279, 1101-1110 (1998)
112. P.Parniewski, A.Jaworski, R.D.Wells, R.P.Bowater: Length of CTG•CAG repeats determines the influence of mismatch repair on genetic instability. *J. Mol Biol.* 299, 865-874 (2000)
113. S.Kang, A.Jaworski, K.Ohshima, R.D.Wells: Expansion and deletion of CTG triplet repeats from human disease genes are determined by the direction of replication in *E. coli*. *Nat. Genet.* 10, 213-218 (1995)
114. C.H.Freudenreich, J.B.Stavenhagen, V.A.Zakian: Stability of a CTG/CAG trinucleotide repeat in yeast is dependent on its orientation in the genome. *Mol. Cell. Biol.* 17, 2090-2098 (1997)
115. C.H.Freudenreich, S.M.Kantrow, V.A.Zakian: Expansion and length-dependent fragility of CTG repeats in yeast. *Science* 279, 853-856 (1998)
116. J.J.Miret, L.Pessoabrandao, R.S.Lahue: Instability of CAG and CTG trinucleotide repeats in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 17, 3382-3387 (1997)
117. J.J.Miret, L.Pessoa-Brandao, R.S.Lahue: Orientation-dependent and sequence-specific expansions of CTG/CAG trinucleotide repeats in Saccharomyces cerevisiae. *Proc. Natl. Acad. Sci. USA* 95, 12438-12443 (1998)
118. D.J.Maurer, B.L.O'Callaghan, D.M.Livingston: Orientation dependence of trinucleotide CAG repeat instability in *Saccharomyces cerevisiae. Mol. Cell. Biol.* 16, 6617-6622 (1996)
119. G.B.Panigrahi, J.D.Cleary, C.E.Pearson: In vitro (CTG)•(CAG) expansions and deletions by human cell extracts. *J. Biol. Chem.* 277, 13926-13934 (2002)
120. J.D.Cleary, K.Nichol, Y.H.Wang, C.E.Pearson: Evidence of cis-acting factors in replication-mediated trinucleotide repeat instability in primate cells. *Nat. Genet.* 31, 37-46 (2002)
121. E.A.Oussatcheva, V.I.Hashem, Y.Zou, R.R.Sinden, V.N.Potaman: Involvement of the nucleotide excision repair protein UvrA in instability of (CAG)•(CTG) repeat sequences in *E. coli*. *J. Biol. Chem.* 276, 30878-30884 (2001)
122. P.Parniewski, A.Bacolla, A.Jaworski, R.D.Wells: Nucleotide excision repair affects the stability of long transcribed (CTG)•(CAG) tracts in an orientation-dependent manner in *Escherichia coli. Nucleic Acids Res.* 27, 616-623 (1999)
123. C.E.Pearson, A.Ewel, S.Acharya, R.A.Fishel, R.R.Sinden: Human MSH2 binds to trinucleotide repeat DNA structures associated with neurodegenerative diseases. *Hum. Mol. Genet.* 6, 1117-1123 (1997)
124. B.A.Owen, Z.Yang, M.Lai, M.Gajek, J.D.Badger, J.J.Hayes, W.Edelmann, R.Kucherlapati, T.M.Wilson, C.T.McMurray: (CAG)(n)-hairpin DNA binds to Msh2-Msh3 and changes properties of mismatch recognition. *Nat. Struct. Mol Biol* ., (2005)
125. V.C.Wheeler, L.A.Lebel, V.Vrbanac, A.Teed, H.te Riele, M.E.MacDonald: Mismatch repair gene Msh2 modifies the timing of early disease in Hdh(Q111) striatum. *Hum. Mol Genet.* 12, 273-281 (2003)
126. K.Manley, T.L.Shirley, L.Flaherty, A.Messer: Msh2 deficiency prevents in vivo somatic instability of the CAG repeat in Huntington disease transgenic mice. *Nat. Genet.* 23, 471-473 (1999)
127. C.Savouret, C.Garcia-Cordier, J.Megret, H.te Riele, C.Junien, G.Gourdon: MSH2-dependent germinal CTG repeat expansions are produced continuously in spermatogonia from DM1 transgenic mice. *Mol. Cell Biol.* 24, 629-637 (2004)
128. C.Savouret, E.Brisson, J.Essers, R.Kanaar, A.Pastink, H.te Riele, C.Junien, G.Gourdon: CTG repeat instability and size variation timing in DNA repair-deficient mice. *EMBO J.* 22, 2264-2273 (2003)
129. W.J.van Den Broek, M.R.Nelen, D.G.Wansink, M.M.Coerwinkel, te Riele H., P.J.Groenen, B.Wieringa: Somatic expansion behaviour of the (CTG)(n) repeat in myotonic dystrophy knock-in mice is differentially affected by Msh3 and Msh6 mismatch-repair proteins. *Hum. Mol Genet.* 11, 191-198 (2002)
130. K.Watase, K.J.Venken, Y.Sun, H.T.Orr, H.Y.Zoghbi: Regional differences of somatic CAG repeat instability do not account for selective neuronal vulnerability in a knock-in mouse model of SCA1. *Hum. Mol Genet.* 12, 2789-2795 (2003)
131. G.B.Panigrahi, R.Lau, S.E.Montgomery, M.R.Leonard, C.E.Pearson: Slipped (CTG)•(CAG) repeats can be correctly repaired, escape repair or undergo error-prone repair. *Nat. Struct. Mol Biol* 12, 654-662 (2005)
132. Y.Liu, H.I.Kao, R.A.Bambara: Flap endonuclease 1: a central component of DNA metabolism. *Annu. Rev. Biochem.* 73,, 589-615 (2004)
133. S.Lee, M.S.Park: Human FEN-1 can process the 5'-flap DNA of CTG/CAG triplet repeat derived from human genetic diseases by length and sequence dependent manner. *Exp. Mol. Med.* 34, 313-317 (2002)
134. L.A.Henricksen, J.Veeraraghavan, D.R.Chafin, R.A.Bambara: DNA ligase I competes with FEN1 to expand repetitive DNA sequences in vitro. *J. Biol Chem.* 277, 22361-22369 (2002)
135. D.A.Gordenin, T.A.Kunkel, M.A.Resnick: Repeat expansion--all in a flap? *Nature Genet.* 16, 116-118 (1997)
136. J.Veeraraghavan, M.L.Rossi, R.A.Bambara: Analysis of DNA replication intermediates suggests mechanisms of repeat sequence expansion. *J. Biol Chem.* 278, 42854-42866 (2003)
137. S.H.Kim, M.J.Pytlos, R.R.Sinden: Replication restart: a pathway for (CTG).(CAG) repeat deletion in Escherichia coli. *Mutat. Res.* 595, 5-22 (2006)
138. V.I.Hashem, W.A.Rosche, R.R.Sinden: Genetic recombination destabilizes $(CTG)_n(CAG)_n$ repeats in *E. coli*. *Mutat. Res.* 554, 95-109 (2004)
139. S.Kang, K.Ohshima, M.Shimizu, S.Amirhaeri, R.D.Wells: Pausing of DNA synthesis in vitro at specific loci in CTG and CGG triplet repeats from human hereditary disease genes. *J. Biol. Chem.* 270, 27014-27021 (1995)

140. R.R.Sinden: Biological implications of the DNA structures associated with disease-causing triplet repeats. *Am. J. Hum. Genet.* 64, 346-353 (1999)
141. P.Cluzel, A.Lebrun, C.Heller, R.Lavery, J.L.Viovy, D.Chatenay, F.Caron: DNA: an extensible molecule. *Science.* 271, 792-794 (1996)

Key Words: Slipped Strand DNA, DNA Repeats, Triplet Repeats, Unstable DNA repeats, DNA structure, Alternative DNA Conformations, Neurodegenerative Diseases, Review

Send correspondence to: Richard R. Sinden, Ph.D., Laboratory of DNA Structure and Mutagenesis, Department of Biological Sciences, Florida Institute of Technology, 150 West University Blvd., Melbourne, FL 32901-6975, Tel: 321-674-8576, Fax: 321-674-7238, E-mail rsinden@fit.edu

http://www.bioscience.org/current/vol12.htm