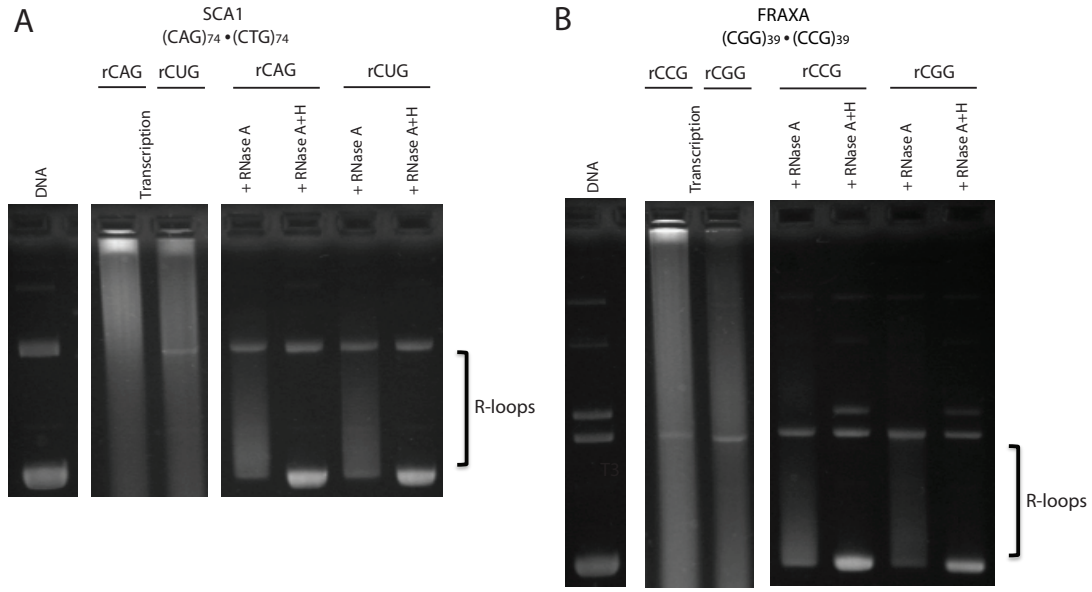
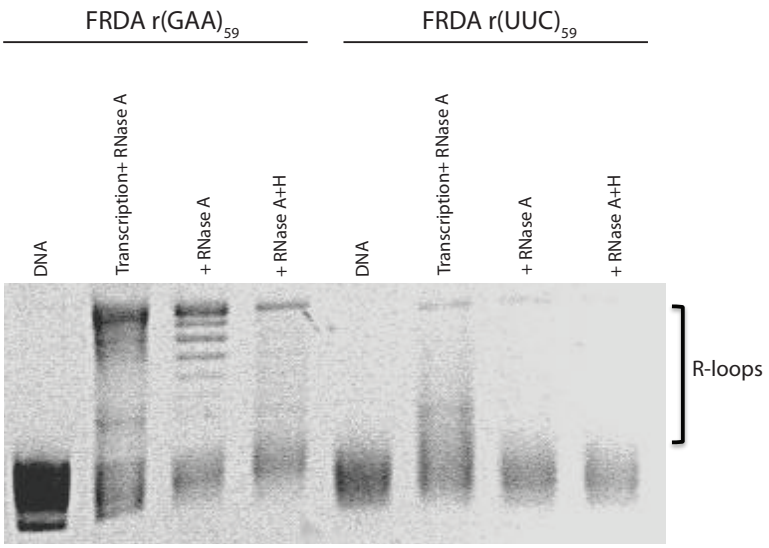
Supplementary Figure S1



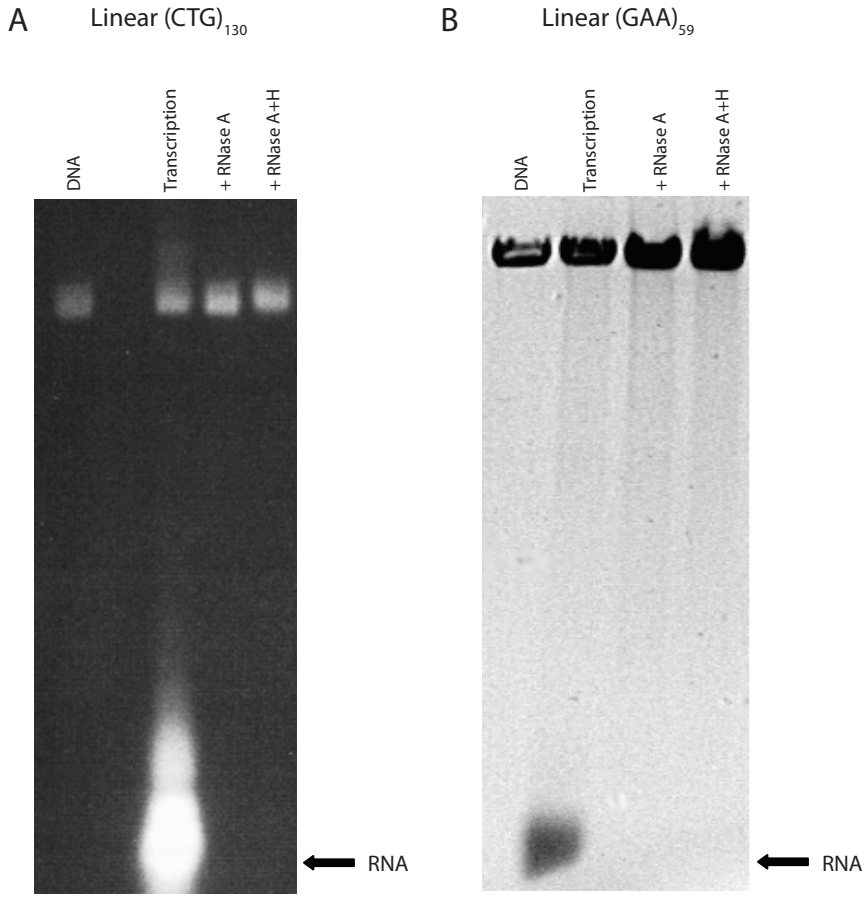
Supplementary Figure S1. Phenol/chloroform extraction after *in vitro* transcription and after RNAse treatments. (A) *In vitro* transcription of SCA1 templates (CAG)74•(CTG)74 in either direction using T3 or T7 RNA polymerase. The RNA produced and bound in the hybrid is indicated below the template. Samples following *in vitro* transcription are phenol and then chloroform extracted, subsequently treated with either RNase A or A+H as indicated then extracted again with phenol and chloroform to observe R-loop formation (as indicated). (B) Exact same reactions and gel conditions as in (A) but transcription is performed on FRAXA (CGG)39•(CCG)39 templates. All samples from (A) and (B) were run on the same gel but re-organized for clarity.

Supplementary Figure S2



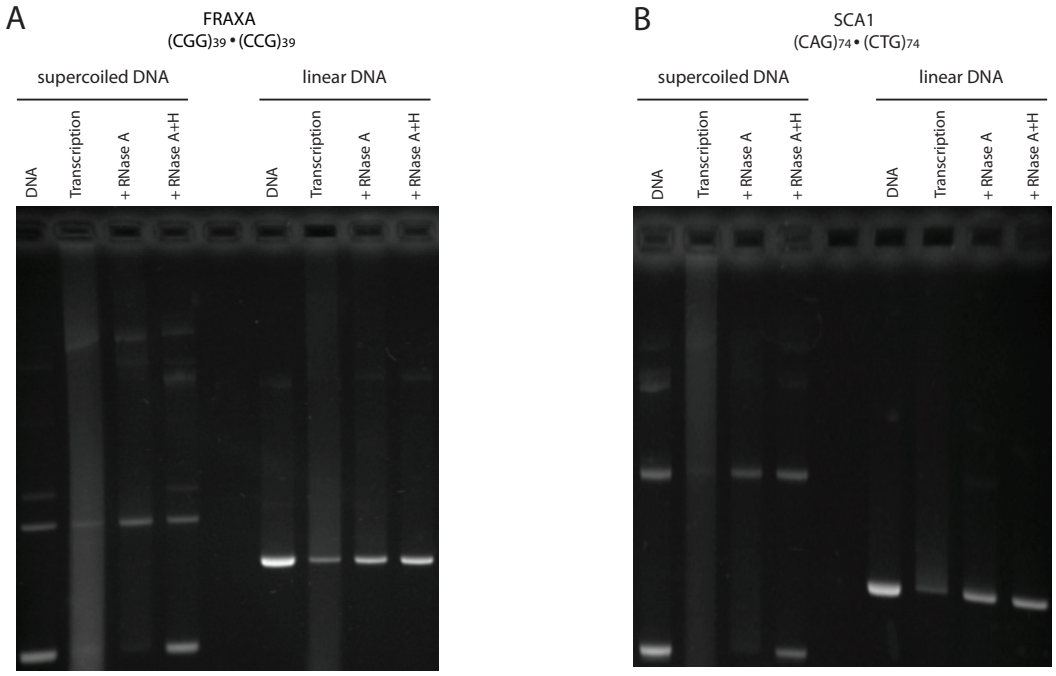
Supplementary Figure S2. RNA:DNA hybrid formation from FRDA $(GAA)_{59}$•$(TTC)_{59}$ template. Transcription from the $(GAA)_{59}$•$(TTC)_{59}$ template to produce rGAA- containing transcript in the presence of RNase A removes all single-stranded RNA but RNA:DNA hybrid formation is visible as altered migration of the template DNA owing to the presence of the rGAA RNA base-paired to it. Subsequent treatment with additional RNase A is not able to ablate this altered migration but treatment of hybrid structures with RNA:DNA-specific RNase H removes the RNA in the hybrids and returns the template DNA to normal migration. When transcription is performed in the opposite direction to produce rUUC-containing RNA, there is no hybrid formation evident.

Supplementary Figure S3



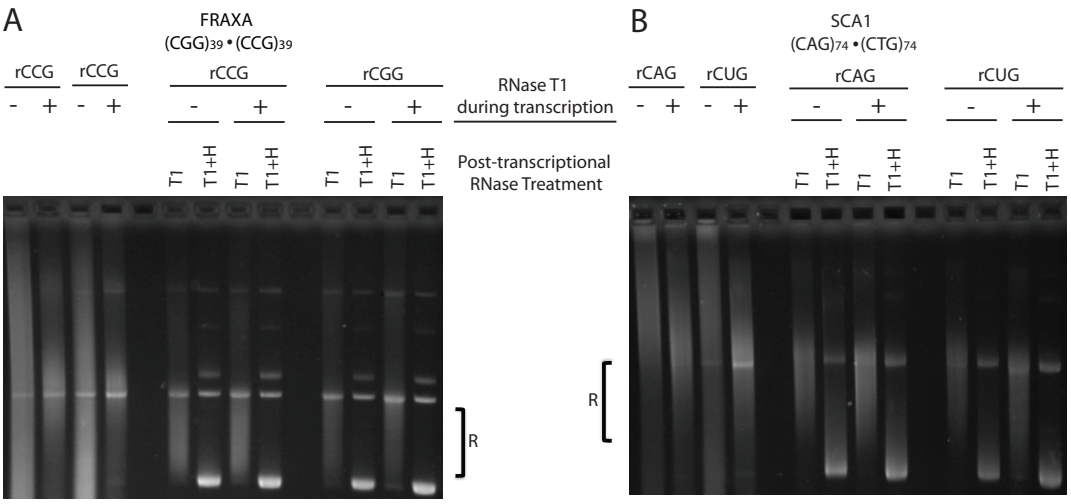
Supplementary Figure S3. *In vitro* transcription from linear templates. (A) Transcription from linear DM1 (CTG)130 template . When *in vitro* transcription was performed to produce an rCUG-containing RNA, there was a product of defined size as indicated onthe gel. However there was no hybrid formation as the template DNA did not exhibit altered migration following transcription and RNAse A treatment. (B) Transcription from linear FRDA (GAA)59. *In vitro* transcription producing an rGAA-containing transcript, followed by RNase A treatment did not reveal altered migration in the template DNA hence there was no hybrid formation.

Supplementary Figure S4



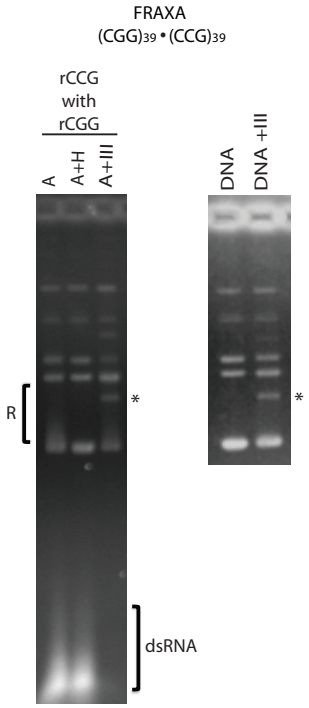
Supplementary Figure S4. Effect of the loss of superhelical tension on R-loop retention. (A) FRAXA plasmid $(CGG)_{39}$•$(CCG)_{39}$ was transcribed with T7 RNA polymerase and then treated with either TE (Transcription control), RNase A or RNase A+H. Samples were then divided into 2 and one set was treated with SacI restriction endonuclease to linearize the DNA downstream of the transcription start site and repeat tract: 'linear DNA'. (B) Same experiment was performed with SCA1 plasmid $(CAG)_{74}$•$(CTG)_{74}$ which was linearized with StuI restriction endonuclease downstream of the transcription start site and repeat tract.

Supplementary Figure S5



Supplementary Figure S5. Mechanism of RNA:DNA hybrid formation during *in vitro* transcription. FRAXA plasmid (CGG)39•(CCG)39 was transcribed in either direction in the absence (-) or presence (+) of RNase T_1 during the transcription reaction. All transcription reactions were subjected to further RNase T_1 or T_1+H treatment to analyze hybrid formation. (B) Same experiment as in (A) performed with SCA1 plasmid containing a (CAG)74•(CTG)74 repeat tract.

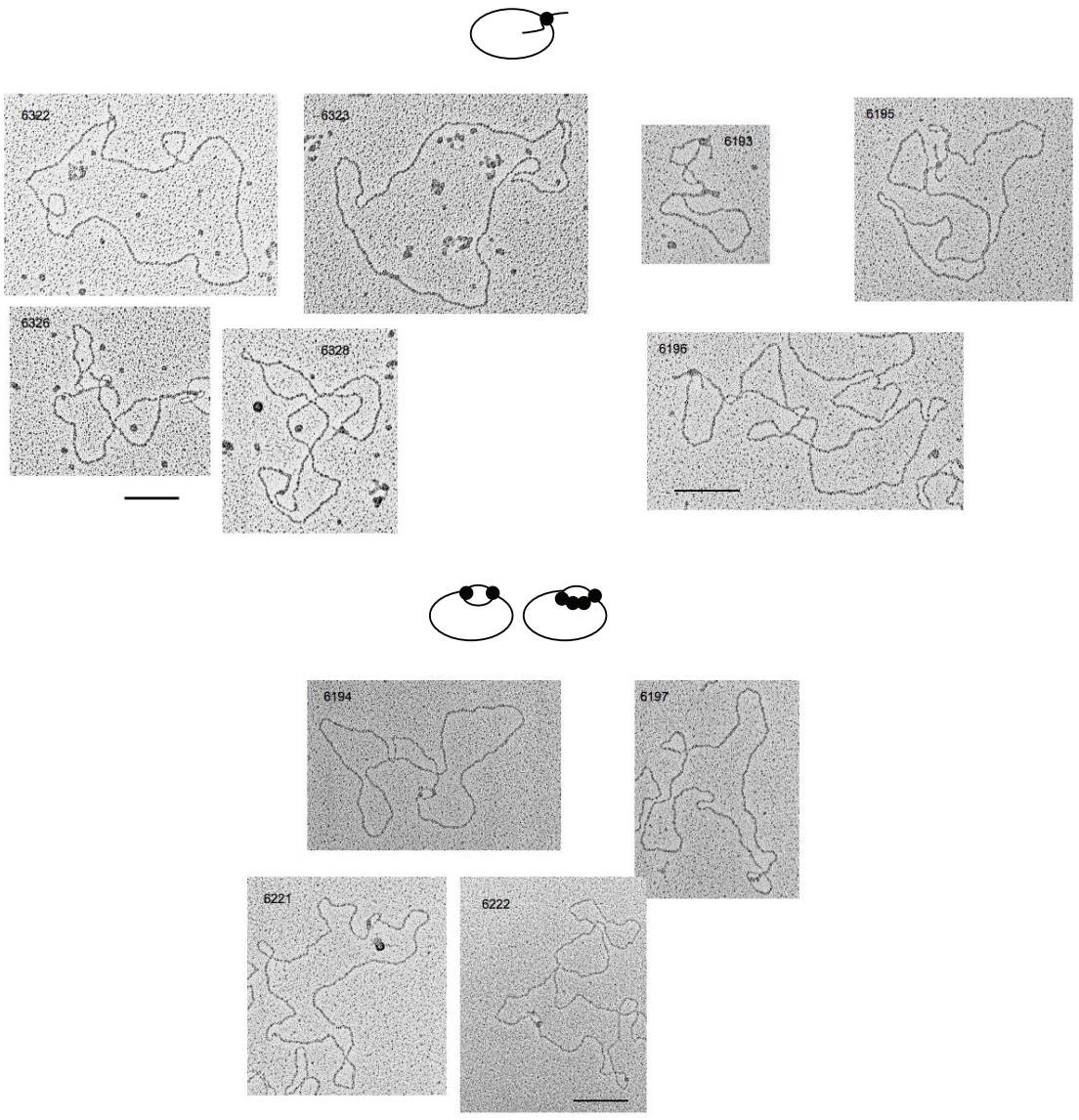
Supplementary Figure 6



Supplementary Figure S6. Cleavage of double stranded RNA generated from convergent bidirectional transcription by RNase III. Following convergent transcription (either bidirectionally as shown here or serially (see Figure7) complementary RNA is generated that can potentially form double stranded species. To ensure that the fast migrating products we observe were double stranded RNA, these reactions were treated with the double stranded RNA-specific endoribonuclease RNase III which specifically digested these products without digesting RNA:DNA hybrids. *There is some non-specific cleavage of supercoiled DNA by the RNase III prep (New England Biolabs). To ensure this cleavage was not occurring due to R-loops, we incubated RNase III with supercoiled DNA that had not been transcribed and observed the same non-specific cleavage.

Supplementary Figure S7

Structures derived from convergent bidirectional transcription of a (CAG)74 • (CTG)74



Supplementary Figure S7. Electron microscopy (EM) analysis of convergent transcription reaction products from DM1 (CAG)79 • (CTG)79 templates. Samples were transcribed convergently using T3 and T7 RNA polymerase promoters simultaneously then the products were treated with RNAse A and prepared for EM as described in the 'Materials and Methods' section. Schematic above the EM images indicates the type of molecules shown. Each scale bar represents 100nm.