CLINICAL EPIGENETICS

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Is Friedreich ataxia an epigenetic disorder?

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Abstract

Friedreich ataxia (FRDA) is a debilitating and frequently fatal neurological disorder that is recessively inherited. It belongs to the group of genetic disorders known as the Repeat Expansion Diseases, in which pathology arises from the deleterious consequences of the inheritance of a tandem repeat array whose repeat number exceeds a critical threshold. In the case of FRDA, the repeat unit is the triplet GAA•TTC and the tandem array is located in the first intron of the frataxin (*FXN*) gene. Pathology arises because expanded alleles make lower than normal levels of mature *FXN* mRNA and thus reduced levels of frataxin, the *FXN* gene product. The repeats form a variety of unusual DNA structures that have the potential to affect gene expression in a number of ways. For example, triplex formation *in vitro* and in bacteria leads to the formation of persistent RNA:DNA hybrids that block transcription. In addition, these repeats have been shown to affect splicing in model systems. More recently, it has been shown that the region flanking the repeats in the *FXN* gene is enriched for epigenetic marks characteristic of transcriptionally repressed regions of the genome. However, exactly how repeats in an intron cause the *FXN* mRNA deficit in FRDA has been the subject of much debate. Identifying the mechanism or mechanisms responsible for the *FXN* mRNA deficit in FRDA is important for the development of treatments for this currently incurable disorder. This review discusses evidence for and against different models for the repeat-mediated mRNA deficit.

Keywords: Friedreich ataxia, heterochromatin, histone modifications, transcription, splicing, triplex

Introduction

Friedreich ataxia (FRDA) (OMIM 229300; http://www.omim.org/entry/229300), first described in 1863 by Nikolaus Friedreich, is a relentlessly progressive disorder caused by mutations in the frataxin (*FXN)* gene. It is the most common heritable ataxia in Caucasians [1]. The major pathological changes include loss of myelinated axons in peripheral neurons, particularly in the dorsal root ganglia, the degeneration of posterior columns of the spinal cord and the loss of peripheral sensory nerve fibers. Myocardial muscle fibers also degenerate and are replaced by macrophages and fibroblasts. The net result of these and other changes include not only limb and gait abnormalities, but also hypertrophic cardiomyopathy, limb muscle weakness, absent lower limb reflexes and a positive extensor plantar response (Babinski sign). Decreased vibration sense, skeletal abnormalities, dysarthria, and diabetes are common comorbid features. Many symptoms become apparent during adolescence. Loss of ambulation occurs roughly 15 years after disease onset with > 95% of patients becoming wheelchair bound by the age of 45. Early mortality due primarily to cardiac failure is not uncommon [2,3].

The most common FRDA mutation is an expansion of the GAA•TTC repeat tract in intron 1 of the frataxin gene

FRDA is inherited in an autosomal recessive fashion. The affected gene, frataxin (*FXN*) (OMIM 606829; http://omim.org/entry/606829), is located on chromosome 9q13 in humans [4]. The first intron contains a GAA•TTC repeat tract embedded in the central poly(A) tract of an AluSq element from which it probably arose [5]. The GAA•TTC repeat tract, which is located approximately 1.3 kb downstream of the major *FXN* transcription start site (TSS), is polymorphic in the human population (Figure 1). While normal alleles have between 8 to 33 repeats, most individuals with FRDA have 2 *FXN* alleles each with > 90 repeats, the majority having 600 to 900 repeats [4]. A minority of patients (approximately 4%) are compound heterozygotes, having one allele with > 90 repeats and a second allele with a small deletion or point mutation in the *FXN* open reading frame. No cases of individuals with deletions or point mutations in both alleles are known [4].

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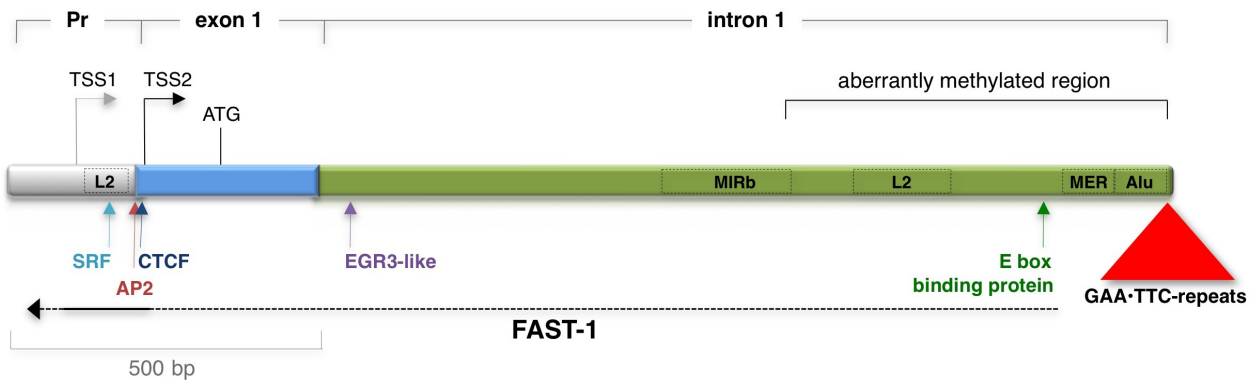

Figure 1 The 5′ end of the frataxin (*FXN*) gene showing the minimal promoter, exon 1 and the promoter proximal end of intron 1. TSS1 and TSS2 refer to transcription start sites described in two different studies. TSS1 was identified based on a cDNA clone isolated from cardiac mRNA [4]. TSS2 is the major start site in lymphoblastoid cells identified by primer extension [52]. The positions of various interspersed repeated sequences are indicated by the rectangles outlined with black dashed lines. The dotted black arrow indicates the estimated extent of the FXN antisense transcript-1 (FAST-1) transcript based on nested PCR [25]. The solid black line superimposed on it is the region that could be amplified by a single round of PCR. The larger antisense transcript includes an open reading frame (ORF) with a non-canonical Kozak sequence whose significance is unknown. The ORF is intact in humans but truncated in closely related primates. Arrows indicate the location of the binding sites for serum response factor (SRF), activator protein 2 (AP2) [73], CCCTC-binding factor (CTCF) [25], an early growth response protein 3 (EGR3)-like factor [73] and an E-box binding protein [48] which have been shown to be positive regulators of *FXN* expression.

Since most FRDA patients have at least one allele that contains a large repeat expansion, FRDA is considered to belong to a group of approximately 20 human genetic disorders known as the Repeat Expansion Diseases. In this group of diseases pathology arises from the consequences of inheritance of alleles with repeat numbers above a critical pathological threshold, which in the case of FRDA is approximately 90 repeats. The basis of the underlying expansion mutation responsible for these disorders is unknown, and problems with DNA replication, recombination and repair have all been suggested as possible mechanisms [6].

FRDA results from a deficiency of *FXN* mRNA

Expansion results in *FXN* mRNA levels that are 4% to 29% of normal [7]. There is an inverse relationship between repeat number and the amount of *FXN* mRNA produced. The *FXN* gene product, frataxin, is a small, highly conserved, acidic protein that is essential for life [8]. It is highly expressed in the dorsal root ganglia, the granular layer of the cerebellum as well as the heart, pancreas, thymus, brown fat, muscle and liver. Although the protein is nuclear encoded, it functions in the mitochondria where it is thought to be involved in the biosynthesis of iron-sulfur clusters (ISCs) [9], the complexes that serve as prosthetic groups for a variety of enzymes involved in energy and iron metabolism, purine synthesis and DNA repair. However, its precise role is currently unknown.

In principle, an *FXN* mRNA deficit could arise via an effect of the intronic repeats on the efficiency of transcription or some post-transcriptional event. However, no difference has been seen in the decay rate for the mature transcripts produced from normal and FRDA alleles [10]. Thus, the *FXN* mRNA deficit presumably results from events occurring at the level of transcription, and/or pre-mRNA stability or processing.

The GAA•TTC repeats form an intrinsic block to transcription elongation in simple model systems

In vitro transcription of templates containing as few as 11 GAA•TTC repeats produces less full-length RNA than templates with no repeats [11]. The repeats form a variety of unusual secondary structures under the same conditions (Figure 2). These structures include purine:purine:pyrimidine and pyrimidine:purine:pyrimidine triplexes [11-15] and a related structure known as sticky DNA [16]. It has been suggested that triplex formation could affect transcription by sequestering transcription factors or RNA polymerase (RNAP) [17,18]. It has also been suggested that a pre-existing triplex or sticky DNA blocks RNAP by making it more difficult for the transcription complex to unwind the template [17].

However, whether the steady state levels of negative superhelicity present in mammalian chromosomes are high enough to allow the formation of such structures is unclear. β-Alanine-linked pyrrole-imidazole polyamides have been shown to bind GAA•TTC tracts with high

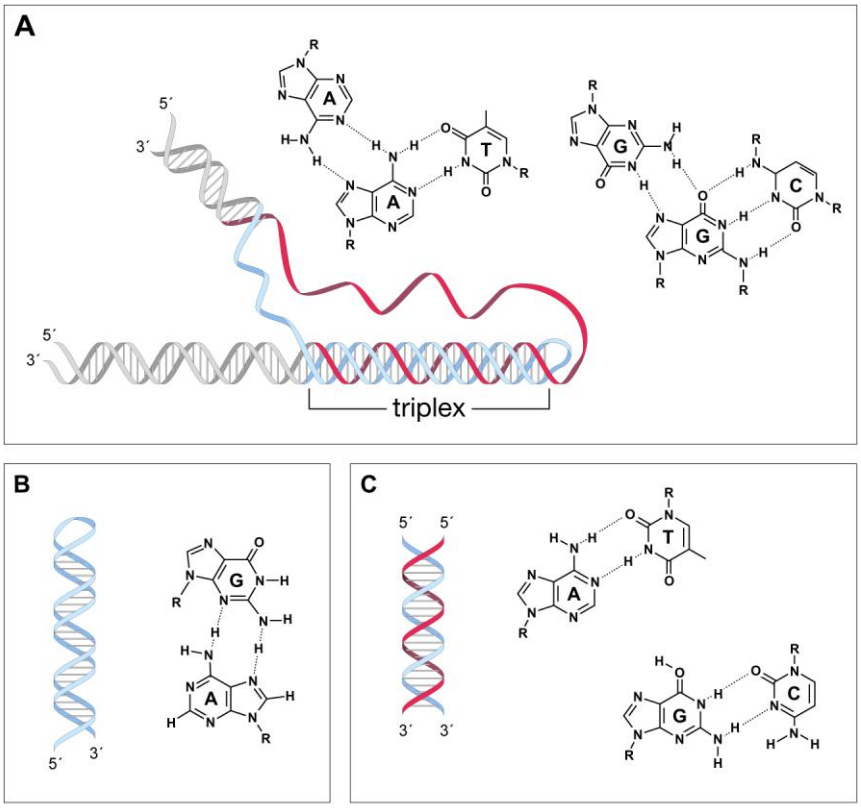

Figure 2 Examples of different structures formed by GAA•TTC repeats showing their constituent hydrogen bonding schemes. **(A)** A purine:purine:pyrimidine triplex [11,12]. This triplex involves Hoogsteen hydrogen bonding between a purine already in a Watson-Crick base pair with a pyrimidine, and a purine from a different region of the repeat. **(B)** A GAA hairpin [61]. Various hydrogen bonding schemes involving G•G, G•A and A•A base pairs have been proposed [74] including the one shown. However, the precise molecular details of the GAA hairpin are unknown. **(C)** A parallel duplex in which, unlike the antiparallel configuration of normal Watson-Crick duplex, the polarity of the two base-paired strands is the same [75]. In this configuration the base pairs involve a reverse Watson-Crick orientation and two, rather than three, hydrogen bonds.

affinity, to block sticky DNA formation and to increase *FXN* expression in cells from individuals with FRDA [19]. This would be consistent with a role for sticky DNA in FRDA. However, the specificity of these polyamides is uncertain and thus, the molecular basis of their effect is unclear.

In addition to preformed triplexes, there is also evidence to suggest that triplexes formed during transcription *in vitro* lead to the formation of an RNA:DNA hybrid as illustrated in Figure 3[11,12,20]. This results in a block to transcription and trapping of RNAP on the template at the end of the repeat. Other sequences or conditions that favor the formation of R-loops also impede transcription [21,22]. Thus it is reasonable to think that an R-loop on a FRDA allele, however it is formed, could cause a block to transcription elongation. Single-stranded nicks in the template, perhaps arising from attempts to repair one of the structures formed by the repeat, can also increase the likelihood that R-loops will form during subsequent rounds of transcription [23]. Furthermore, work *in vitro* suggests that R-loops could also arise via bidirectional transcription through the repeat [24]. An antisense transcript, FXN antisense transcript-1 (FAST-1), has been identified in the *FXN* gene that could potentially contribute to such hybrids (see Figure 1). However, its 5' end has not been mapped, its concentration is low and it is unclear at this time whether it includes the repeat [25].

While direct proof of the formation of an R-loop by the FRDA GAA•TTC repeats in mammalian cells is lacking, other purine-rich repeats are known to do so [26]. In addition, the promoter distal end of the repeat in human induced pluripotent cells generated from patient cells is known to bind the mismatch repair proteins MSH2 and MSH3 [27], which would be consistent with the formation of an unusual DNA conformation of some sort at this locus.

While a consistent inhibition of transcription elongation is seen with different RNAPs on naked DNA templates *in vitro* [11,12,16,28,29], conflicting results have been seen with mammalian nuclear extracts and episomes in yeast and mammalian cells [16,28,30-32]. Integrated

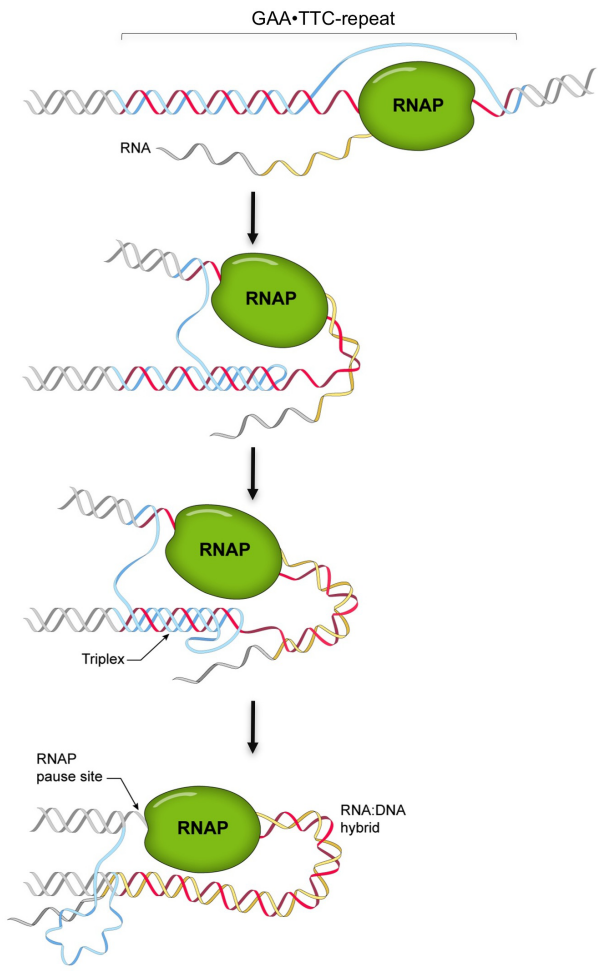

Figure 3 A triplex/RNA:DNA hybrid model for Friedreich ataxia (FRDA) [11,12,20]. Transcription through the repeat leaves the non-template purine-rich strand transiently unpaired. This strand can then fold back and interact with the duplex that has already reannealed behind the RNA polymerase (RNAP), thereby forming a triplex. Triplex formation, in turn, leaves the pyrimidine-rich strand in the second half of the repeat free to form a hybrid with the nascent RNA. This may be facilitated by the particular stability of an RNA:DNA hybrid containing a purine-rich RNA strand [76]. Evidence suggests that nucleation of this hybrid leads to unwinding of the triplex and the formation of a long persistent RNA:DNA hybrid that involves the whole repeat. The net result is the formation of a stable R loop in which the pyrimidine strand of the repeat is hybridized to the nascent transcript leaving the purine-rich strand unpaired. The RNAP becomes trapped on the template at the 3′ end of the repeat.

constructs do show a consistent mRNA decrease in cells and mouse models [33-35], but whether this effect is mediated at the level of transcription elongation is unclear.

The GAA•TTC repeats affect splicing in model systems

A so-called frataxin minigene construct, containing a CMV promoter, the *FXN* exon 1, part of intron 1 and all of exon 2, that was transfected into mammalian cells, showed a decreased splicing efficiency when the GAA-rich strand was transcribed but not the TTC-strand [30]. It was thus suggested that the deficit of mature *FXN* mRNA in FRDA results from aberrant mRNA splicing in which intron 1 is retained. The aberrant splicing seen with the minigene was attributed to the ability of the repeats to bind splicing factors such as the serine/arginine (SR)-rich protein family and the proteins heterogeneous nuclear ribonucleoprotein (hnRNP) A1 and hnRNP A2 as outlined in Figure 4, although how binding of these factors would lead to intron retention is unclear.

However, since total *FXN* mRNA abundance is reduced in FRDA cells, any mis-spliced transcript would have to be rapidly degraded for aberrant splicing to account for the mRNA deficit, which was not the case with the minigene tested [30]. In addition, the splicing abnormalities in the frataxin minigene were context and position dependent. This is important since in this construct both the repeat context and position differed from what is seen in the *FXN* locus.

The FRDA GAA•TTC repeats have also been shown to reduce splicing in yeast [36]. This effect was attributed to the increased length of the intron rather than any specific effects of the repeat *per se*. In yeast the largest known intron is < 1 Kb and in these organisms splicing efficiency is related to intron length [37]. However, many efficiently spliced human introns are much longer, with the human genome containing > 3000 genes with introns > 50 Kb. Since the *FXN* intron 1 of normal alleles is already 11 Kb and cases of FRDA are apparent with as few as 90 repeats, it seems unlikely that a change in intron length *per se*, is responsible for the reduced *FXN* expression in FRDA.

Furthermore, studies of transcripts produced from the intact *FXN* gene did not detect any splicing abnormalities in FRDA cells [10,28]. However, since the existence of a very unstable splice isoform is difficult to definitively exclude, this issue is still unresolved.

Expansion of the FRDA GAA•TTC repeat tract also causes epigenetic changes

While it has been known for some time that a subset of Repeat Expansion Diseases are associated with heterochromatin formation, notably those disorders arising from CGG•CCG repeat expansion such as fragile X syndrome (FXS) [38], the idea that the FRDA GAA•TTC repeats produce aberrant epigenetic modifications has only recently been appreciated. In part, the possibility that FRDA could be an epigenetic disorder was not initially entertained since unlike the affected gene in FXS, significant transcription still occurs from most FRDA alleles and early thinking in the field was that DNA methylation was required for epigenetic silencing

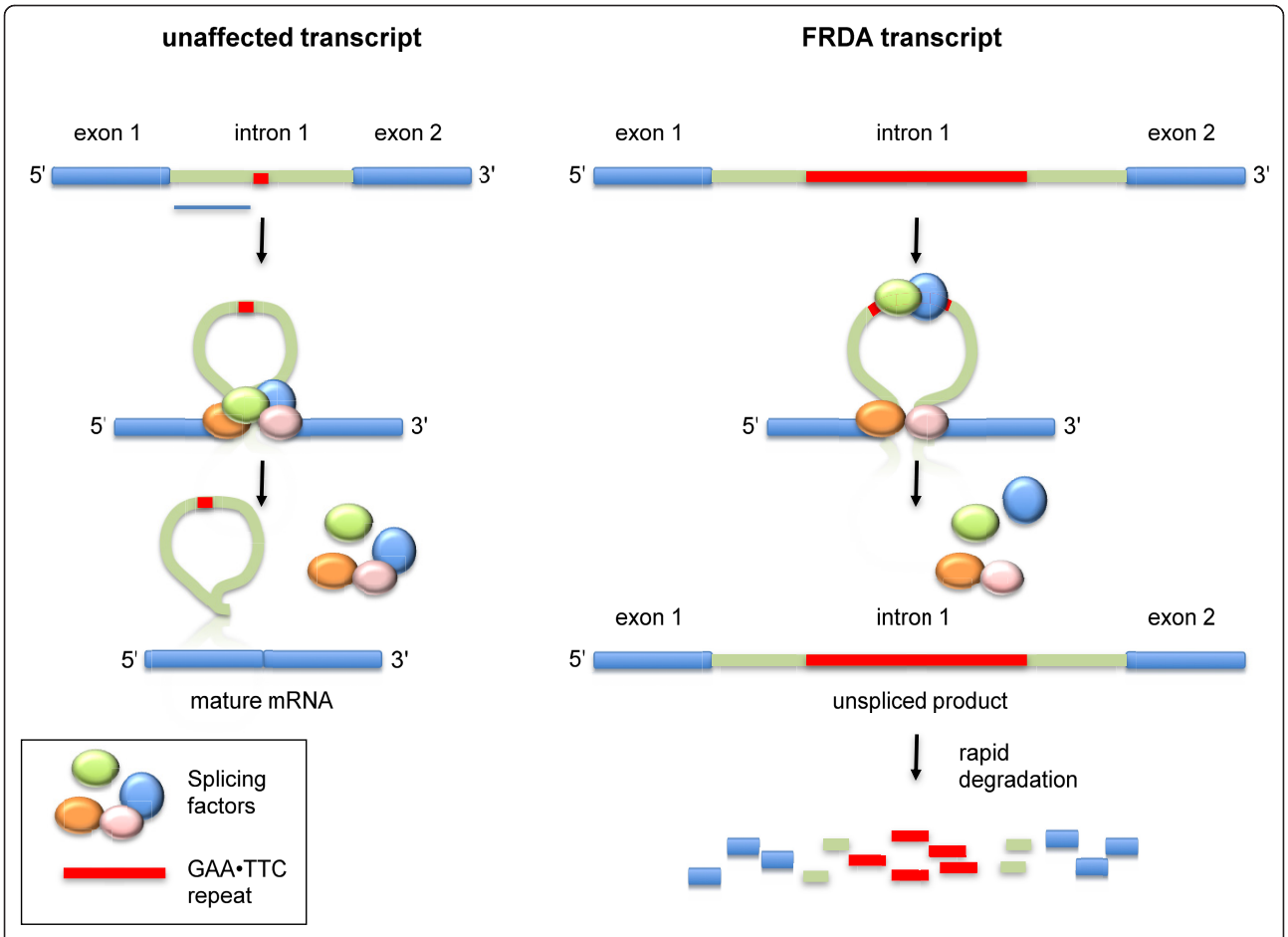

Figure 4 Altered splicing model for Friedreich ataxia (FRDA) [30]. On unaffected frataxin (*FXN*) alleles the repeat is too short to significantly impact splicing. However, once the repeat number exceeds 90, splicing factors that are normally involved in the proper splicing of the *FXN* gene become mislocalized such that normal splicing is prevented. This could be the result of binding of splicing factors to the repeat, preventing their normal assembly at the splice junctions. Alternatively, the unrestrained spread of these or other repeat-binding proteins, such as heterogeneous nuclear ribonucleoprotein (hnRNP) A1 [77], could block access of factors needed for proper splicing analogous to what has been proposed for HIV [78]. It may also be that the repeat sequesters serine/arginine (SR) proteins such as alternative splicing factor/splicing factor 2 (ASF/SF2). Since these proteins are is required for 5′ splice site selection and cleavage [79], this could lead to a local deficiency at the splice site and thus the failure to efficiently remove intron 1.

[39-43]. Since the FRDA repeat contains no CpG residues, the only dinucleotide subject to significant methylation in mammals, non-epigenetic mechanisms, like those described earlier, initially received more attention.

However, it is now appreciated that even in those repeat expansion diseases where the repeat has a high density of CpG residues, such as FXS, DNA methylation is probably not the first step in heterochromatinization [44,45]. Furthermore, the expanded CTG•CAG repeats in myotonic dystrophy type 1 (DM1) are associated with heterochromatin despite their lack of CpG residues [46]. In addition, work with transgenic mice containing GAA•TTC repeats or CAG•CTG repeats showed that the repeats conferred variegation in the expression of a linked transgene, analogous to position effect variegation (PEV) in *Drosophila* [47]. These observations suggested that, despite the absence of methylatable residues, the FRDA repeats might trigger the formation of heterochromatin that could spread to adjacent sequences.

While the repeat itself cannot be methylated, DNA methylation could potentially occur secondarily to other chromatin changes in the region flanking the repeat. Consistent with that idea, we have shown that while DNA methylation is seen in the region flanking the repeat on normal alleles, perhaps due to spreading from adjacent Alu elements, more extensive DNA methylation is seen in this region in patient cells [33,48]. A direct relationship between repeat length and the extent of DNA methylation has also been found in patient cells [49]. Since disease severity is related to repeat length, a direct relationship between disease severity and DNA methylation thus also exists.

Not only is DNA methylation more extensive on FRDA alleles, but the methylation protection of 3 CpG residues that is seen upstream of the repeat on unaffected alleles is also lost [48]. One of these residues is within an E-box site that is important for maximal promoter activity in reporter assays in mouse myoblast cells. However, plasmids that are specifically methylated at this site do not show reduced transcription [48]. This suggests that loss of factor binding does not occur secondarily to DNA methylation, but rather that protein binding normally protects those CpG residues from methylation. Thus, the loss of the normal methylation 'footprint' in FRDA cells likely reflects chromatin changes that restrict access of these factors to their normal binding sites. Consistent with this view, FRDA patient alleles have been shown to be enriched for a variety of histone modifications characteristic of silenced genes including hypoacetylated H3 and H4 and dimethylation and trimethylation of histone H3 lysine 9 (H3K9) [48,50]. These histone modifications are highest in the regions flanking the repeat [50-52].

Aberrant DNA methylation does not extend as far as the promoter in any of the patient cell lines that have been tested thus far. However, whether histone modifications extend into the promoter is still controversial. The wide variation in the level of histone modifications seen in normal cells, the use of FRDA cell lines with very different repeat numbers and mRNA levels and differences in the experimental design and data analysis have added to the difficulty in reaching a consensus.

However, to date there have been a number of reports of a histone profile typical of transcriptionally repressed genes on the affected *FXN* promoter in lymphoblastoid cells [25,52], the brains of affected individuals [33] and in a cell culture model [35]. Enrichment of repressive chromatin marks on the *FXN* promoter has also been reported in the brain and heart in transgenic mice models of the disorder [33]. In addition, enrichment of the α and γ isoforms of heterochromatin protein 1 (HP1), a non-chromosomal protein associated with heterochromatin, on the promoter and the loss of CCCTC-binding factor (CTCF) binding to the promoter region in patient cells lends support to the idea that epigenetic changes originating in the repeat can spread to the 5' end of the *FXN* gene [25].

What is the basis of the epigenetic changes?

It has been suggested that the loss of CTCF binding is responsible for the observed histone changes on FRDA alleles [25]. However, this raises the question of what leads to the loss of CTCF binding. Since heterochromatin can be generated by the repeats embedded in a completely different sequence context [35,47] and levels of the repressive histone modifications are highest in the region of the *FXN* gene that includes the repeat [50-52], it may be that the trigger for these epigenetic modifications is specifically related to some intrinsic property of the repeat itself as has been suggested for FXS [53]. This effect may be at the DNA level perhaps via the ability of the repeat to bind proteins that then recruit silencing factors [54]. It could also be a consequence of the repair of DNA damage occurring in the repeat [55-57]. An unusual structure formed by the FRDA repeat may contribute to this process if it were trigger the DNA damage response. The binding of MSH2/MSH3 complexes to the region containing the repeat in patient cells lends weight to the idea that some sort of structure formed by the repeat is recognized by the cell as a site of DNA damage [27]. It is also possible that reduced transcription, resulting perhaps from a triplex/RNA:DNA hybrid formation, leads to heterochromatic changes, as it does in some plant genes by favoring the recruitment of H3K27 trimethylation (H3K27Me3) [58].

It could also be that heterochromatinization is RNA dependent perhaps involving a long non-coding RNA (lncRNA), as has been described for HOX genes and the lncRNA HOTAIR [59]. The non-coding RNA could be generated *in cis* or *trans*. Recent work has shown that the formation of a DNA:RNA triplex between a chromosomally located gene and ectopic RNA leads to enrichment of the DNA with H4K20Me3 and subsequent gene silencing [60]. Formation of such a triplex by the GAA•TTC repeat and either the repeat region in the sense or antisense transcript could thus lead to heterochromatin initiation within the repeat. Alternatively, if RNA containing a large number of GAA repeats can form hairpin-like GAA repeats in DNA [61], they may be source of double-stranded (ds)RNA for the RNA interference (RNAi) pathway. Transcripts containing the repeats may thus enter the RNAi pathway as has been demonstrated for the repeats responsible for FXS and DM1 [62,63].

How could these chromatin changes affect *FXN* transcription?

Since the repeat-associated chromatin changes are located in both the transcriptional unit and in the promoter of at least some patient cells, they have, in principle, the potential to affect expression of the *FXN* gene in a number of different ways. This effect could be exerted close to the start of transcription mediated by chromatin changes on the promoter. In addition, CTCF binding has been shown to play an important role in *FXN* expression [25]. So, simply the loss of this factor from patient alleles could lead to reduced rates of transcription. Furthermore, even in the absence of altered promoter chromatin, histone and DNA methylation changes in the intron that lead to loss of binding of important regulatory factors may affect transcription initiation or early steps in elongation. Since DNA

methylation in the body of a gene can affect the efficiency of transcription elongation [64], an effect on transcription through the intron is also possible.

Do epigenetic changes account for the *FXN* mRNA deficit?

The role of chromatin changes in causing the *FXN* mRNA deficit in FRDA is currently the subject of much debate. Histone deacetylase inhibitors have been shown to increase *FXN* expression in FRDA primary lymphocytes and the brain and heart of a knock-in mouse model of the disorder [34,50]. The histone deacetylase, HDAC3, has been identified as an important target of these drugs [65]. The increase in *FXN* expression is accompanied by an increase in histone acetylation on FRDA alleles. However, it has been reported that while the histone methyltransferase inhibitor BIX-01294 reduced the levels of H3K9 dimethylation and trimethylation on FRDA alleles, no accompanying increase in *FXN* transcription was seen. This has led to the suggestion that epigenetic changes are not responsible for the *FXN* deficit and that repeat expansion causes FRDA by forming a structural block to transcription elongation [10].

This idea would appear to be supported by the observation that phosphorylation of serine 5, a mark characteristic of the initiating form of RNA polymerase II (Pol II Ser5-P), is present at similar levels at TSS1 [10], a transcription start site identified in early studies [4]. However, recent work has shown that the major TSS (TSS2) used in lymphoblastoid cells, the cell type used for these studies, is closer to the start of the *FXN* open reading frame than previously thought [52]. This is relevant since the initiating form of Pol II is typically found to have a narrow distribution at or downstream of the TSS [66]. When a region immediately downstream of TSS2 was examined, reduced levels of the initiating form of Pol II [52] as well as total Pol II [51] were seen in FRDA patient cells. A reduced level of H3K4 trimethylation (H3K4Me3) was also seen the region in the region immediately downstream of TSS2 in patient cells [52]. Deposition of this histone mark occurs early in the transcription cycle primarily on the first nucleosome [67,68]. Trimethylation of H3K4 is thought to be required for both recruitment of the basal transcription machinery and for transcription initiation on genes that, like *FXN*, lack a TATA box [69]. In other genes, deposition of this histone mark is thought to occur immediately downstream of the promoter in a manner dependent on the levels of the initiating form of Pol II [69,70]. In either event, the reduced level of H3K4Me3 seen on patient alleles suggests that a problem with transcription from FRDA templates is apparent very early in the transcription cycle, perhaps at the level of polymerase recruitment or transcription initiation.

More recently it has been suggested that the reduced levels of Pol II are not due to reduced initiation but to reduced promoter proximal pausing [51]. This conclusion was based on the fact that no difference was seen in H3K4Me3 levels on unaffected and affected alleles at the 5' end of the gene. However, in this study the region examined was upstream of what we now know to be the major TSS, in a part of the promoter that also did not show differences between affected and unaffected alleles in earlier reports [10,52]. Since H3K4Me3 is highest on nucleosomes immediately downstream of the TSS, the lower levels of H3K4Me3 that were seen on patient alleles just upstream of the repeat in the study of Kim *et al.* [51], in fact lend support to the idea that early events in transcription occurring prior to or during H3K4 trimethylation are abnormal in FRDA. However, further work is needed to establish precisely what step or steps are affected.

Whatever the cause of the reduced levels of Pol II on FRDA alleles, the lower levels of H3K36 trimethylation, a histone mark associated with transcription elongation, in the promoter proximal region [10,51,52], supports the idea that there is an effect of the repeat on transcription very close to the TSS more than 1 kb upstream of the repeat. Furthermore, the reduced levels of H3K79Me2, another mark of transcription elongation, found upstream of the repeat in patient cells [51], further strengthens the idea that there is reduced transcription in the region preceding the repeat.

This is not to say that there is not a problem with transcription closer to the repeat as well. An additional effect of repeat expansion on Pol II elongation is suggested by the reduced accumulation of H3K36Me3 downstream of the repeat on FRDA alleles [10,51,52]. Whether this represents an effect of the histone changes and DNA hypermethylation in the vicinity of the repeat in patient cells or a chromatin-independent process remains to be seen.

The relationship between GAA repeat number and the extent of intron DNA methylation raises the possibility that the epigenetic changes on smaller alleles may be smaller than on larger alleles and less likely to extend into the promoter. Thus the relative contribution of promoter-proximal and promoter-distal events may vary with repeat number.

Conclusions

An effect of the GAA•TTC repeat on events occurring > 1 kb away at the *FXN* promoter is difficult to reconcile with an effect of aberrant splicing. It is also difficult to reconcile with a direct effect of the formation of a triplex/R-loop unless problems occurring in the repeat lead to the buildup of stalled polymerases that stretches back to the promoter. Therefore, perhaps the most likely

explanation for the promoter proximal effects is that the repeat-mediated epigenetic changes generate a chromatin configuration that is less permissive for early steps in transcription as illustrated in Figure 5. That is that FRDA is, at least in part, a disorder of epigenetic dysregulation. The lack of an effect of BIX-01294 on *FXN* mRNA yield can be reconciled with this idea, if histone marks other than H3K9 methylation need to be removed before a chromatin conformation permissive for transcription is reestablished, as has been suggested for a number of other repressed genes [71,72]. If this is the case, it would suggest that histone deacetylase inhibitors, which are currently in clinical trials for treating FRDA, are probably acting on one of the direct causes of the transcription deficit. Such a mechanism would not necessarily preclude a role for triplexes/R-loops in events occurring at the promoter if, as discussed earlier, such structures contribute in some way to the formation of heterochromatin.

Whether problems with Pol II elongation in the vicinity of the repeat are epigenetically mediated or arise from a physical block to elongation like that formed by triplex/R-loops also remains an open question, with some data supporting a role for chromatin-mediated events and some data favoring a chromatin-independent mechanism. It may be that both mechanisms contribute to the *FXN* mRNA deficit in some way and further work will be necessary to understand the relative

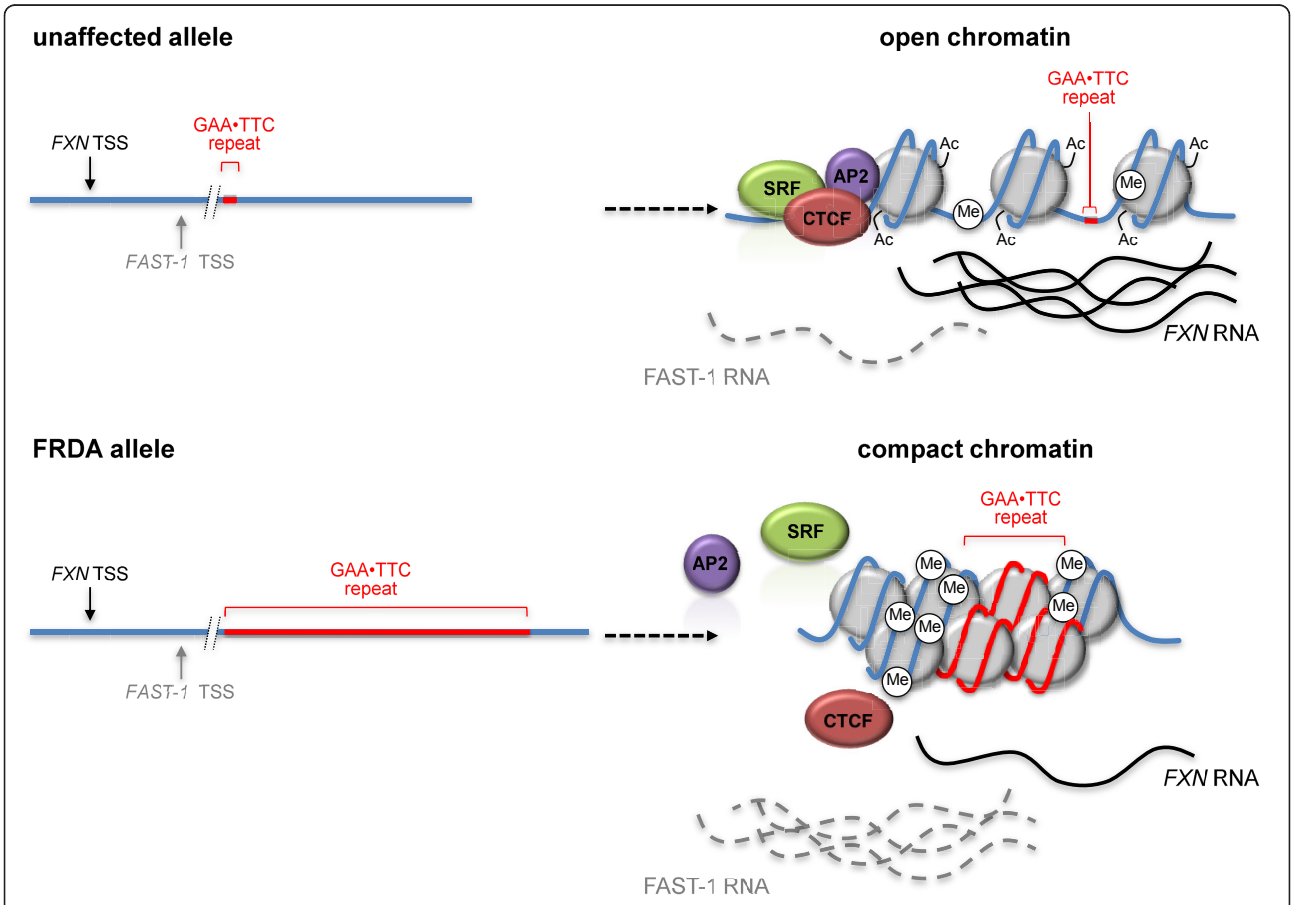

Figure 5 Diagrammatic representation of an epigenetic model for Friedreich ataxia (FRDA). Not shown to scale. Unaffected alleles are aberrantly methylated in the region flanking the repeat. Nonetheless, the 5′ end of the gene is associated with histones that are enriched for marks of active chromatin. In particular, acetylation of histone H3 and H4 is high. The net result is that the chromatin is open and permissive for transcription. Transcription factors including serum response factor (SRF), activator protein 2 (AP2) [73] and CCCTC-binding factor (CTCF) [25] associate with the 5′ end of the gene. An early growth response protein 3 (EGR3)-like factor binds to the 5′ end of intron 1 [73] and an E-box binding protein [48] bind to the region immediately upstream of the repeat. Under these conditions transcription initiation and elongation takes place normally. In contrast, FRDA alleles become associated with histones that are hypoacetylated and show more extensive DNA methylation in the region flanking the repeat. The net effect of these and other histone changes is the formation of a compact chromatin configuration. This reduces binding of transcription factors and both frataxin (*FXN*) transcription initiation and elongation are reduced. Loss of CTCF binding is correlated with an increase in the amount of FXN antisense transcript-1 (FAST-1) RNA that is transcribed antisense to *FXN*, but how this relates to silencing is unclear. TSS: transcription start site.

contribution of these mechanisms to the *FXN* mRNA deficit responsible for FRDA.

Authors' contributions

Both DK and KU contributed equally to the writing of this manuscript. Both authors read and approved the final manuscript.

Competing interests

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