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Review

Gene-based approaches toward Friedreich ataxia therapeutics

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Abstract. Friedreich ataxia is an autosomal recessive trinucleotide-repeat disease caused by expanded GAA repeats in the first intron of the FRDA gene. These GAA repeats are suspected to form unusual non-B DNA conformations that decrease transcription and subsequently reduce levels of the encoded protein, frataxin. GAA repeats also induce heterochromatin formation and silencing of the frataxin gene locus. Frataxin plays a crucial role in iron metabolism and detoxification and interacts with electron transport chain proteins. There is no effective therapy for Friedreich ataxia, but antioxidant therapy has shown promise and is currently in clinical trials. In this review we focus on the mechanisms by which expanded GAA repeats reduce transcription and discuss how these findings have lead to gene-based approaches that may be effective in treating Friedreich ataxia.

Keywords. Triplet repeat, neurodegenerative, HDAC inhibitor, DNA binding, frataxin.

Introduction

Loss-of-function mutations usually result in recessive phenotypes and can be caused by a variety of genetic lesions. Expanded trinucleotide repeats, for example, cause fragile X syndrome, fragile XE syndrome and Friedreich ataxia (FRDA). These disorders are distinct from dominant gain-of-function disorders, such as Huntington disease and Machado-Joseph disease, in which encoded CAG repeats generate deleterious proteins with polyglutamine tracts. Rather, loss-of-function trinucleotide repeat disorders are associated with decreased gene expression. For example, fragile X syndrome is caused by a pathogenic expansion of CGG repeats in the 5′ untranslated region of the *FMR1* gene. The repeat expansion promotes silencing of *FMR1* expression by altering the methylation of a regulatory CpG island and decreasing histone acetylation at the 5′ end of the gene [1]. Consequently, patients have reduced levels of the encoded protein, FMRP. In FRDA, the majority of patients have two alleles that contain expanded GAA repeats in the first intron of the *FRDA* gene (also known as *FXN* or *X25*). A few FRDA patients are compound heterozygotes and have one expanded allele and a point mutation in the second allele. The pathogenic repeat size can range from around 200 to 1700 repeats. These repeats disrupt *FRDA* transcription and result in pathogenically low levels of the encoded protein, frataxin [1]. Longer repeats correlate with decreasing frataxin levels, earlier onset and increased severity. In this review, we will explore various strategies to develop effective therapies for FRDA, with an emphasis on gene-based approaches.

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Frataxin reduction causes FRDA

FRDA is progressively lethal and is the most prevalent genetic cause of ataxia, affecting approximately 1 in 30000 individuals [2]. Cardinal clinical features include progressive gait and limb ataxia, lower-limb muscle weakness and dysarthria [2, 3]. Other symptoms include vision and hearing problems, diabetes and hypertrophic cardiomyopathy [2, 3]. In the years since the causative gene was identified [4], a wealth of information has been obtained regarding the function of frataxin and the mechanisms by which GAA repeats decrease mRNA production. Even before the identification of frataxin as a mitochondrial protein [5–8], the shared clinical features of FRDA with some mitochondrial diseases suggested that the activity of this vital organelle was affected. Numerous studies have indeed demonstrated that frataxin plays a crucial role in many aspects of mitochondrial function relating to iron usage. Specifically, frataxin reduction leads to the accumulation of mitochondrial iron, a decrease in iron-sulfur clusters and an increased susceptibility to oxidative stress [3, 9–11]. Thus low levels of frataxin in FRDA reduce iron-sulfur-containing proteins such as aconitase, which is involved in the citric acid cycle, and respiratory transport complexes I–III. Additionally, since there are two iron-sulfur-containing proteins in the heme biosynthetic pathway (ferrochelatase and adrenodoxin), reductions in frataxin would be expected to impact the activity of enzymes that contain this prosthetic group, and this has been observed [12–16]. Yeast frataxin interacts with electron transport chain proteins, suggesting that oxidative phosphorylation may be impaired in FRDA [17]. Other studies have shown that the targeted disruption of hepatic frataxin in mice leads to liver tumor growth, indicating that frataxin may act indirectly as a tumor suppressor protein [18]. While there is still much to be learned about frataxin function, the information garnered thus far has rendered several therapeutic targets. For example, given the potential for the formation of free radicals and oxidative damage when frataxin levels are reduced, antioxidants are being investigated as a potential FRDA therapy. Two such compounds are idebenone, a short-chain analogue of coenzyme Q10 (ubiquinone), and mitoquinone (MitoQ), a derivative of ubiquinone that is highly concentrated in mitochondria [11, 19–21]. These compounds have been shown to be effective in reducing oxidative stress and are currently in phase I (MitoQ) and phase II (idebenone) clinical trials in patients with FRDA. In particular, idebenone has been shown to reduce cardiac hypertrophy [19]. Other therapeutic options at the protein level include, but are not limited to, mitochondrial iron chelators [22] and exogenous heme administration [16]. Interestingly, erythropoietin, a known stimulant of heme synthesis, can rescue defects in frataxin-deficient cells [23]. At present, the mechanism(s) by which erythropoietin increases frataxin levels is unknown. Further support for a link between heme and frataxin comes from studies (detailed below) in which hemin was found to induce frataxin expression [24].

As a loss-of-function disorder, gene-based strategies designed to increase frataxin levels would be an ideal therapy for FRDA. Given that healthy FRDA carriers can have around 40% of the normal frataxin mRNA level [25], only a modest increase in frataxin levels may be sufficient to moderate the symptoms of FRDA. Like other recessive disorders, gene therapy is a viable option for FRDA. Recombinant adeno-associated viral and lentiviral vectors expressing frataxin cDNA have been shown to partially correct sensitivity to oxidative stress in FRDA primary fibroblasts [26]. Two recent reports have demonstrated in both cellular and animal models that herpes simplex virus type 1 (HSV-1) amplicon vectors expressing either the entire *FRDA* genomic locus [27] or frataxin cDNA [28] can successfully restore normal phenotypes. Specifically, Gomez-Sebastian et al. [27] found that FRDA patient primary fibroblasts transduced with HSV-1:*FRDA* have a restored response to oxidative stress. A neurotropic herpes vector was used in both studies given the need to correct neurological degeneration found in FRDA. Towards this end, Lim et al. [28] have shown that mice with a localized frataxin reduction in the brainstem display rotarod deficits that can be corrected by exposure to HSV-1:frataxin cDNA. Collectively, these studies demonstrate that a gene-based therapeutic approach to treat FRDA shows promise in treating the range of symptoms found in this disease.

Another gene-based approach to treat FRDA involves the identification of compounds that increase *FRDA* gene transcription. To appreciate the various strategies that are currently being investigated, an in-depth analysis of the mechanisms by which expanded GAA repeats reduce *FRDA* gene transcription is necessary. Table 1 lists papers that either specifically address the role of GAA repeats in FRDA or describe methods that increase frataxin levels or transcription through GAA repeats. Collectively, the data provided by these papers demonstrate that there are two distinct, but inter-related, characteristics of expanded GAA repeats that conspire to reduce frataxin mRNA levels.

Table 1. Studies that explore the role of the GAA repeat in FRDA or identify methods to increase frataxin production.

Year	Paper	Comment
1996	Campuzano et al. [4]	identified *FRDA* gene and described expanded GAA mutation in intron 1
	Ohshima et al. [29]	proposed model for how GAA repeats reduce transcription
	Filla et al. [30]	established relationship between repeat length and clinical features
1997	Campuzano et al. [7]	demonstrated that frataxin is reduced in patients with FRDA
1998	Bidichandani et al. [31]	showed that GAA repeats decrease transcription in vitro
	Ohshima et al. [32]	found that GAA repeats decrease transcription and replication in vivo
1999	Mariappan et al. [55]	used high resolution NMR to show that GAA repeats form triplexes.
	Sakamoto et al. [33]	established that large GAA repeats (> 60 repeats) form sticky DNA
2000	Grabczyk and Usdin [35]	showed evidence that GAA repeats form an intramolecular triplex
	Grabczyk and Usdin [36]	utilized oligodeoxyribonucleotides to increase GAA transcription
2001	Sakamoto et al. [34]	demonstrated that sticky DNA may sequester transcription factors
	Sakamoto et al. [37]	found that GAA repeats interrupted by GGA reduce sticky DNA formation
2002	Miranda et al. [53]	generated mouse model of FRDA with GAA repeats
	Vetcher et al. [38]	visualized sticky DNA by electron microscopy and clarified how it forms
	Sharma et al. [56]	described that GAA repeats show somatic instability in vivo
2003	Heidenfelder et al. [39]	further characterized GAA triplex formation
	Sarsero et al. [24]	identified that hemin and butyric acid increase frataxin expression
	Saveliev et al. [45]	demonstrated that GAA repeats can mediate heterochromatic gene silencing
	Ghazizadeh [50]	found that cisplatin may induce frataxin expression
	Turano et al. [51]	showed that 3-nitropropionic acid induces frataxin expression
2004	Vetcher and Wells [40]	demonstrated that sticky DNA alters the plasmid dimer/monomer ratio
	Napierala et al. [41]	identified structure-dependent recombination hot spots of GAA repeats
	Krasilnikova and Mirkin [42]	found in yeast that GAA repeats cause replication stalling
	Pianese et al. [25]	described frataxin mRNA levels in patients and carriers
	Miranda et al. [60]	showed in mouse model that overexpression of frataxin is harmless
2005	Greene et al. [57]	defined the minimal frataxin promoter in humans
	Sarsero et al. [58]	generated wild-type *FRDA*:GFP mouse model
	Sturm et al. [23]	demonstrated that erythropoietin increases frataxin expression
	Fleming et al. [26]	used viral vector to partially correct frataxin deficiency in patient cells
2006	Son et al. [61]	further characterized the functional consequences of sticky DNA
	Burnett et al. [43]	identified polyamides that bind GAA repeats and increase frataxin expression
	Al-Mahdawi et al. [54]	characterized mouse model of FRDA with GAA repeats; mice exhibit symptoms of FRDA
	Herman et al. [46]	found that certain HDAC inhibitors increase frataxin expression
	Grant et al. [52]	identified several compounds that increase transcription through GAA repeats
2007	Gomez-Sebastian et al. [27]	used viral vector to complement frataxin deficiency in patient cells
	Krasilnikova et al. [44]	explored role of GAA repeats in reducing transcription
	Lim et al. [28]	used viral vector to correct neurological function in FRDA mouse model
	Greene et al. [47]	clarified gene silencing at the mutant, GAA-expanded, *FRDA* gene locus

NMR, nuclear magnetic resonance; GFP, green fluorescent protein.

Two B or not Two B

The first and most extensively studied of these characteristics is that GAA repeats can form non B-DNA structures such as triplex and sticky DNA. Taking advantage of their prior work investigating alternative DNA structures found in other triplet repeat diseases, the Wells laboratory was well positioned to examine expanded GAA repeats when they were found to be the causative mutation in FRDA. Indeed, less than 4 months after the publication which described the GAA repeat mutation in FRDA [4], Wells and colleagues published a model whereby GAA repeats could form a DNA:RNA triplex

structure that was predicted to reduce transcription [29]. The role of GAA repeats in FRDA was further vindicated by studies that found a significantly higher mean allele length in younger patients with a more severe clinical phenotype [30]. With the development of a suitable antibody, frataxin protein levels were found to be significantly reduced in FRDA patients, and an inverse correlation was observed between frataxin levels and repeat length [7]. Consequently, within two years from the initial gene discovery, it was known that the majority of FRDA patients contain two alleles with GAA expansions thought to generate some type of alternative DNA structure that perturbs transcription and subsequently reduces frataxin to pathogenically low levels.

In 1998, both *in vitro* and *in vivo* studies demonstrated that GAA repeats do indeed reduce transcription [31, 32]. For example, Ohshima et al. [32] employed a β-galactosidase reporter system that contained an intronic multiple cloning site. Different GAA repeat fragments, ranging in size from 9 to 230 repeats, were cloned into the intronic site. As negative controls, two additional plasmids were generated that contained 270 TTC repeats or a luciferase gene fragment cloned in the antisense orientation. The plasmids were transfected into COS-7 cells and β-galactosidase activity was measured. As expected, there was a significant decrease in the level of β-galactosidase from cells transfected with constructs harboring larger GAA repeats compared to those with smaller repeats. Moreover, β-galactosidase activity from cells transfected with the $GAA_{(230)}$ construct was significantly less than that observed from cells which contained $TTC_{(270)}$ or the antisense luciferase gene fragment. During these studies, it was found that linearized plasmids containing long GAA tracts had a portion that migrated anomalously on agarose gels. Specifically, GAA-repeat-containing plasmids generated bands on agarose gels with retarded mobilities. Plasmids with larger GAA repeats displayed a greater propensity to form the retarded band. This aberrantly migrating band was found to contain self-associating DNA, termed sticky DNA, which could form under physiological conditions [33]. The transcription through gel-isolated sticky DNA was found to be reduced compared to linear DNA [34]. Therefore, sticky DNA can, along with triplex DNA, represent alternative non-B-form DNA structures that contribute to the etiology of FRDA.

To more fully understand the molecular mechanism by which GAA repeats reduce transcription, Grabczyk and Usdin [35] conducted *in vitro* studies using T7 RNA polymerase. Their results provided additional support for a model in which RNA polymerase is trapped in an R●R●Y intramolecular triplex (Fig. 1). In a follow-up paper, Grabczyk and Usdin [36] demonstrated that oligodeoxyribonucleotides specifically designed to block triplex structures (Fig. 1) are capable of increasing transcription through GAA repeats. This is the first demonstration of a method by which the relative rate of transcription through GAA repeats is improved, thus providing proof of concept for the development of therapeutic agents that target triplex formation. In theory, such a strategy should benefit patients with FRDA. Numerous other studies have provided more proof that GAA repeats can form triplex and sticky DNA configurations, increasing our knowledge into how these alternative DNA structures form [37–44]. Hence, one characteristic of expanded GAA repeats found in FRDA is an inherent ability to reduce transcription by the formation of non B-DNA structures.

Gene silencing

In addition to alternative DNA structures, the second characteristic of expanded GAA repeats that results in decreased frataxin is heterochromatin formation, or gene silencing. While it has been known that tandem repeats are associated with heterochromatin formation in normal human centromeres, recent work has shown that certain triplet-repeat diseases possess some of the hallmarks of silent chromatin. For example, fragile X syndrome is caused by CGG repeats in the 5′ untranslated region of the *FMR1* gene that result in heterochromatic features such as CpG island hypermethylation and histone hypoacetylation [1]. Like fragile X syndrome, myotonic dystrophy and FRDA are caused by non-coding triplet repeats. To determine whether CTG repeats found in myotonic dystrophy or GAA repeats found in FRDA could silence genes by inducing heterochromatin, Festenstein and colleagues generated transgenic mice with a heterochromatin-sensitive cell surface marker protein (CD2) as a reporter. They found that both $CTG_{(192)}$ and $GAA_{(200)}$ repeats could exert position effect variegation (PEV) on the expression of the CD2 reporter gene [45]. PEV occurs when a gene lies in close proximity to heterochromatin, and results in the silencing of the affected gene in a subset of cells. Overexpression of HP1, which binds the histone H3 tail containing methylated lysine 9 (H3K9), was found to enhance silencing. Importantly, PEV induced by CTG or GAA repeats occurred whether the transgene was located in euchromatic or heterochromatic chromosomal locations.

With this knowledge in mind, two groups went the next step by examining if the GAA-expanded *FRDA* gene did in fact show characteristics of heterochro-

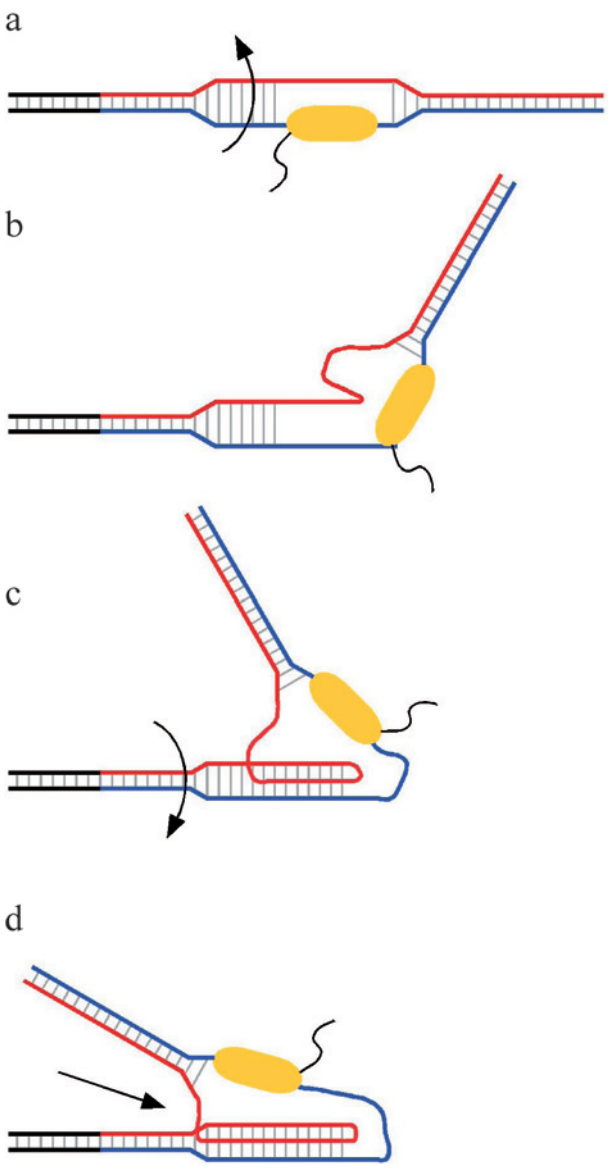

Figure 1. A model of GAA-repeat-induced transcription inhibition. The GAA repeat strand is red. The complementary CTT repeat strand is blue and flanking DNA is black. Transcribing RNA polymerase is yellow. (*a*) RNA polymerase induces negative supercoiling (arrow) as it enters the GAA repeat region, which widens the helix. (*b*) As transcription proceeds, the GAA non-template strand can begin to fold back, forming an intramolecular triplex structure. (*c*) More extensive triplex formation is followed by the relaxation of negative supercoiling (arrow). This facilitates triplex formation. (*d*) When the triplex structure reaches a certain length, RNA polymerase is stalled (arrow). Grabczyk and Usdin [35].

matin. By employing chromatin immunoprecipitation (ChIP) with antibodies to acetylated histones H3 and H4, Gottesfeld and colleagues observed that lymphoblastoid cells from an FRDA-affected individual had reduced histone modifications compared to the profile obtained from an unaffected sibling [46]. Specifically, histones H3K9, H3K14, H4K5, H4K8, H4K12 and H4K16 were all significantly hypoacetylated in FRDA cells in chromatin regions upstream and downstream of the GAA repeat. In contrast, the level of histone modification between FRDA-affected and normal cells was not different in chromatin from the promoter region of the *FRDA* gene. In addition, this study observed that H3K9 methylation, which is a hallmark of heterochromain, is increased in the FRDA cell line. Increased H3K9 methylation was also seen using different FRDA lymphoblastoid cell lines by Greene et al. [47]. Interestingly, these investigators found that intron 1 of the *FRDA* gene contributes to this gene's promoter activity. Detailed analysis of the DNA at the 5′ end of intron 1 that is directly upstream of the GAA repeats revealed that there are a number of methylatable CpG residues. By comparing the level of CpG methylation in lymphoblastoid cells from unaffected and FRDA-affected individuals, it was found that affected individuals had, in general, higher rates of methylation. Strikingly, three residues (termed 3, 6 and 13) showed almost no methylation in unaffected cells but were almost always methylated in affected cells. Based on these findings, Greene et al. [47] proposed that the methylation of certain DNA residues in intron 1 might disrupt transcription factor binding and lead to a reduction in frataxin message. Collectively, Herman et al. [46] and Greene et al. [47] support data provided by Saveliev et al. [45] and clearly show that FRDA, like fragile X syndrome, is a chromatin disease (Fig. 2).

Towards the development of FRDA therapeutics that target the mutant gene

Given the parallels between FRDA and fragile X syndrome (both contain non-translated repeats that induce CpG hypermethylation and histone deacetylation), it would be logical to explore if therapies designed to treat fragile X syndrome also work in FRDA. Treatment of fragile X lymphoblasts with 5-azadeoxycytidine, a DNA-demethylating agent, has been shown to increase FMR1 message and protein [48]. Although 5-azadeoxycytidine is not clinically suitable due to toxicity effects, studies using this compound in FRDA lymphoblasts may clarify the role of CpG hypermethylation in Friedreich ataxia. To address hypoacetylated histones in fragile X syndrome, histone deacetylase (HDAC) inhibitors have been used and were found to increase message levels slightly in fragile X lymphoblasts [49]. Interestingly, combination treatment of fragile X lymphoblasts with 5-azadeoxycytidine and HDAC inhibitors demonstrated that transcript levels were increased over that obtained with 5-azadeoxycytidine alone [49].

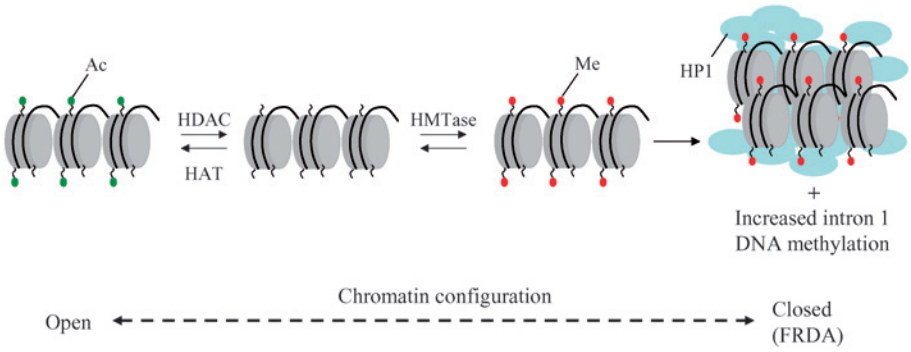

Figure 2. A model for heterochromatin formation in FRDA. Normal *FRDA* alleles contain open chromatin with acetylated histones (Ac, green). Non B-DNA structures, as a consequence of expanded GAA repeats, may induce histone deacetylase (HDAC) activity that, along with histone methyltransferases (HMTase), generate methylated histones (Me, red). The methylation of lysine 9 on histone H3 is a hallmark of heterochromatin and has been found in FRDA patient cells. Further chromatin compaction may be aided by the binding of HP1 and increased DNA methylation. Agents designed to increase histone acetyltransferase (HAT) activity or inhibit HDAC activity would be expected to increase frataxin levels. Indeed, some HDAC inhibitors have been found to increase frataxin expression [46]. Adapted from Festenstein [59].

Therefore, possible treatments for FRDA may likewise involve using a combination of drugs that facilitate chromatin opening.

To directly address if HDAC inhibitors could increase frataxin levels in FRDA, Herman and colleagues tested several different classes of HDAC inhibitors on an FRDA lymphoblastoid cell line [46]. Of the five different inhibitors tested, only one, BML-210 showed a significant increase (approximately two-fold) in frataxin message levels. Using BML-210 as a parent compound, additional compounds were generated and were also found to be effective at increasing frataxin message levels in both FRDA lymphoblastoid cells and primary lymphocytes. One compound in particular, 4b, was shown to increase the level of H3K14, H4K5 and H4K12 acetylation in FRDA lymphoblastoid cells. These are exciting results and demonstrate that therapeutics designed to reverse heterochromatin induced by expanded GAA repeats may benefit patients with FRDA.

In addition to the HDAC inhibitors identified by the Gottesfeld group, other agents have been shown to increase frataxin expression or transcription through GAA repeats (Table 2). Butyric acid, an HDAC inhibitor, was shown to increase a frataxin reporter gene expression in a stable cell line [24]. The reporter consisted of the normal frataxin gene (lacking expanded GAA repeats) with a fusion of exon 5a to GFP. Consequently, this line is beneficial for the identification of compounds that induce frataxin expression, but do not necessarily target GAA repeats. Hemin was another compound identified in this study that increased reporter expression. Hemin, along with cisplatin [50], 3-nitropropionic acid [51] and erythropoietin [23], most likely increase frataxin levels due to the fact that these compounds impact normal frataxin function (e.g. oxidative stress and iron management). Thus it is possible that frataxin induction in the presence of these compounds is the result of specific transcription factor activation, and not a direct consequence of increasing transcription through GAA repeats or inhibiting HDAC activity.

In contrast, direct targeting of the GAA repeat by polyamides was shown to increase frataxin message and protein levels in FRDA lymphoblastoid cells [43]. These compounds were also shown to disrupt GAA-repeat-induced sticky DNA. Using stable cell lines containing 15 GAA or 148 GAA repeats fused to green fluorescent protein (GFP), Grant et al. [52] identified additional DNA-binding compounds that increase GFP expression. One of these compounds, pentamidine, was also shown to increase frataxin expression in primary FRDA lymphocytes. It is suspected that certain DNA-binding compounds are effective at increasing transcription through GAA repeats because they strongly prefer duplex B-form DNA over alternate structural forms, particularly triplex forms. Another possibility is that specific DNA-binding compounds may displace proteins that promote heterochromatin formation, such as HP1, thereby resulting in a more open, transcriptionally conducive form of the *FRDA* gene [43]. Regardless of mechanism, these two studies demonstrate that strategies designed to target the alternative DNA structures found in FRDA are, like HDAC inhibitors, viable therapeutic options.

Currently, the mechanism by which expanded GAA repeats lead to the formation of heterochomatin in FRDA is unknown (Fig. 3). One possibility is that the unusual structure formed by GAA repeats is recognized as a signal for the recruitment of proteins that form heterochromatin such as high-mobility-group

Table 2. Studies that identify compounds that increase frataxin expression or promote transcription through GAA repeats (viral gene therapeutic studies are omitted).

Paper	Compound(s)	Screening method(s)
Grabczyk and Usdin [36]	oligodeoxyribonucleotides	*in vitro* transcription of GAA repeats
Sarsero et al. [24]	hemin and butyric acid (an HDAC inhibitor)	stable cells containing GFP fusion in exon5a of *FRDA*
Ghazizadeh [50]	cisplatin	Cisplatin-resistant ovarian carcinoma cells
Turano et al. [51]	3-nitropropionic acid	FRDA patient lymphoblastoid cells, frataxin transgenic rat PC12 cells
Sturm et al. [23]	erythropoietin	FRDA patient lymphocytes, cardiac cells from non-FRDA individuals, neuronally differentiated P19 mouse cells
Burnett et al. [43]	GAA-repeat-binding polyamides	FRDA patient lymphoblastoid cells, *in vitro* sticky DNA studies
Herman et al. [46]	BML-210 and derivatives (HDAC inhibitors)	FRDA patient lymphocytes and lymphoblastoid cells
Grant et al. [52]	DNA minor-groove binders, including pentamidine	FRDA patient lymphocytes, $GAA_{(148)}$:GFP stable cells

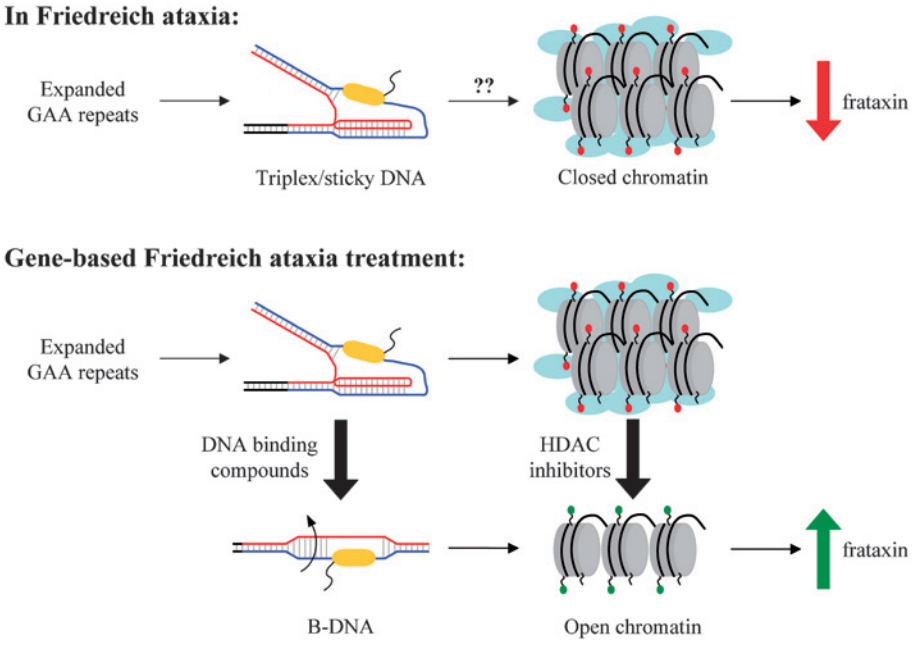

Figure 3. Gene-based strategy to obtain FRDA therapeutics. In FRDA, non B-form DNA structures and heterochromatin both contribute to reduce *FRDA* transcription and result in pathogenically low levels of frataxin. The mechanism by which this takes place is unknown (??). An ideal gene-based treatment would overcome both obstacles (non B-form DNA and heterochromatin) to increase frataxin production. Such a therapy might include specific DNA-binding compounds that stabilize B-form DNA and HDAC inhibitors that facilitate chromatin decondensation.

(HMG) proteins, HDACs and histone methyltransferases [46]. Alternatively, heterochromatin formation at GAA-expanded alleles may be mediated by the formation of small double-stranded or antisense RNAs that induce heterochromatin [47]. Nevertheless, it is clear from the studies described here that treatments designed to increase B-form DNA and/or open chromatin should be effective in treating FRDA (Fig. 3). Future studies should address if these compounds can work synergistically to increase frataxin levels greater than that found for the individual agent.

An essential resource: animal models of FRDA with GAA repeats

To further validate the potential of FRDA therapeutics designed to reverse heterochromatin or triplex/sticky DNA formation, animal studies are needed. Fortunately, several groups have, or are working on the generation of, mouse models of FRDA that contain expanded GAA repeats [53, 54], J. Sarsero, personal communication]. In a collaborative effort between J. Gottesfeld and M. Pandolfo, certain HDAC inhibitors (4b and its derivatives) have been shown to increase frataxin transcription in the brain and cerebellum of a frataxin KIKI mouse model [personal communication]. We have recently identi-

fied several aromatic amidines that increase transcription through GAA repeats [M. Hebert and A. Whittom, unpublished observations] and are currently in collaboration with M. Pook to test some of these compounds in his FRDA mouse model (described in Al-Mahdawi et al.) [54]. Finally, the Sarsero group is poised to identify additional compounds that increase frataxin expression by screening approximately 2000 primarily FDA-approved compounds in a GAA-containing reporter line. Promising compounds will then be tested in a GAA-repeat mouse model that this group is developing to see if any can increase frataxin levels in an animal model of FRDA [J. Sarsero, personal communication].

Summary

The hard work of numerous individuals on the development of a treatment for Friedreich ataxia has yielded many therapeutic targets. Strategies designed to treat the symptoms resulting from frataxin reduction (antioxidants, for example) are promising and are in phase I and phase II trials. In contrast, gene-based approaches, which address the fundamental cause of FRDA and seek to increase frataxin levels, have lagged behind. Recent studies demonstrating that HDAC inhibitors and DNA-binding compounds are effective in enhancing frataxin levels in cells from FRDA patients represent a turning point in the development of *FRDA*-gene-based therapeutics. Animal studies, currently underway, will be crucial in the assessment of whether any of these compounds translates into an effective FRDA therapy. Taking a cautiously optimistic stance, one can envisage a therapy for FRDA that includes not only antioxidant agents, but compounds that target the GAA-expanded allele and upregulate frataxin levels.

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