

DNA methylation effects in Friedreich ataxia (FRDA)

by

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This is to certify that the work presented here is the result of my own investigation under the internal supervision of Professor M. Pook whom directly assisted when teaching me the techniques I used.

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LITERATURE REVIEW

Introduction

It has been more than 140 years since Nickolaus Friedreich described the “degenerative atrophy of the posterior columns of the spinal cord” (cited by Andermann, 1976). This pathology became the subject of intensive scientific discussion immediately after the first reports, but only during the last few decades determined the association of Friedreich ataxia severity with expansion of GAA triplet in the first intron of the gene coding specific mitochondrial protein.

The clinical signs of Friedreich ataxia (FRDA) include the slow progression of gait ataxia of cerebellar and sensory genesis, disarthria and other conditions like the absence of tendon reflexes in the legs, pes cavus, kyphoscoliosis, cardiomyopathy and other manifestations (Delatycki, Williamson, Forrest, 2000). This disease is the most common hereditary ataxia in the world today. Nevertheless only one child amongst 66,000 is affected (Pandolfo, 2003; Alper & Naraynan, 2003). The incidence of the disease in the United Kingdom is approximately 1 per 50,000 children (Leggo et al., 2003). The rate of carriers of this disease, inherited in an autosomal recessive pattern is significantly higher – about 1.1-1.5% (Mateo et al., 2004). The age of disease onset is before 25 years (Delatycki, Williamson & Forrest, 2000) and most of the patients become handicapped by the age of 45 (Lynch et al., 2005). This disease also significantly shortens the duration of life – by the assessment of the clinical specialists the average age of death in FRDA patients is approximately 37 years (Pandolfo, 2003; Alper & Naraynan, 2003). Thus the issues of prevention and screening are crucial for providing appropriate treatment and rehabilitation of those suffering from this disease.

FRDA is an autosomal recessive disease. Therefore, the determination of the gene responsible for neurodegenerative changes in the central nervous system, was the key development in the prognostication and treatment of the disease. Nevertheless the mechanisms of mutations leading to this inherited chronic pathology and the adequacy of laboratory models used for profound research of its genetics, are still the subjects of active debates.

Aims and objectives

The aim of the literature review is to describe and analyse data from recent studies dedicated to the effects of DNA methylation in Friedreich ataxia. This would entail:

- searching specialized databases with the use of appropriate exploration strategies,
- selecting sources of information for further analysis,
- discussing the modern state of the problem in the scientific and medical world,
- and providing a conclusion about the perspectives of further researches of DNA methylation effects in Friedreich ataxia.

This project will analyse the possible effects of DNA methylation at the FRDA gene promoter in FRDA in two sets of transgenic mouse tissues, one of which contains GAA repeat expansion mutations, whilst the other does not.

The role of frataxin in pathogenesis of Friedreich ataxia

There is evidence that Friedreich ataxia could be caused by an iron overload in the mitochondria (Pandolfo 2002, 2003). Figure 1 in the Appendix describes the modern concepts about iron metabolism in mitochondria. The data by Gakh et al. (2005) and Pandolfo (2002) demonstrated that frataxin, a 210-amino acid protein (see fig. 2 in the Appendix), is a mitochondrial iron-binding protein and its deficiency can interfere with some signalling pathways, in particular with the free radical-independent pathways. Many researchers urged that frataxin deficiency could result the abnormalities of the triplet GAA (guanidine-adenosine-adenosine) in the FRDA gene, For example repeating expansions of GAA triplets in the first intron of this gene is the frequent cause of the dysfunction of the sulphur-iron clusters and iron accumulation in the mitochondria. The sulphur-iron cluster serves as the universal coordinator of the delivery of both iron and sulphur. The most of authors (Ding B., Smith & Ding T., 2005) consider that sulphur in iron-sulphur clusters is derived from L-cysteine and can participate in the numerous biochemical processes, e.g. oxidation-reduction reactions, the tricarboxylic acid cycle (TCA), nitrogen fixation, amino acid biosynthesis, hem and biotin biosynthesis, DNA synthesis and repair, and regulation of gene expression, etc as one of the principal contributor of the processes of electron transferring. There is known (ibid) also that sulphur-iron clusters work as the modulators and can create complexes with proteins. They also can act as the catalytic centers and sensors of iron and oxygen. The control of iron level in the intracellular structure is the crucial for the normal functioning of cellular organelles because the intracellular 'free' iron concentration is tightly controlled, because the elevated levels of the plenty of 'free' iron may activate the peroxidative processes and, particularly, the production of hydroxyl free radicals and peroxydative oxidisation of lipids..

There was demonstrated that the clinical symptoms of Friedreich ataxia are associated with the progressive neurodegeneration in the posterior columns of medulla spinalis and a clear

correlation exists between the size of the repeating expansion of GAA triplet and severity of the phenotypic manifestations of Friedreich ataxia (Alper & Naraynan, 2003; McDaniel et al., 2001). However, some patients can have normal levels of frataxin (Kimura et al., 2002; van der Warrenburg, Knoers & Kremer, 2002), a phenomenon, which current studies cannot explain as yet.

It was also determined that at the promoter sequences DNA methylation can inhibit gene expression. Consequently, it is possible to suggest that DNA methylation of the FRDA gene promoter, can lead to the inhibition of frataxin synthesis and lead to the increased iron accumulation in mitochondria even if the individual has no expanded repeats of GAA triplets. Pook et al. (2001) suggested that the defect of mitochondrial iron metabolism is secondary, whereas the primary defects are presented by the disordered oxidative phosphorylation. On the other hand, French authors (Seznec et al., 2005) demonstrated that complete frataxin-deficiency does not induce oxidative stress in neuronal tissues and that Friedreich ataxia could be not associated with oxidative damage.

The results of recent laboratory research on the role of frataxin in pathogenesis of Friedreich ataxia are very promising developments for new therapeutic approaches in disease management (Lodi et al., 2002). These approaches are usually composed of antioxidant medications and iron chelators (ibid). Although the hypothesis surrounding the association of DNA methylation of the FRDA gene and the inhibition of frataxin synthesis still requires additional research to develop the required support. For example, the mechanisms of interaction between DNA methylation and GAA repeat expansion mutations are still unknown (Pook et al., 2001).

FRDA gene regulation – DNA methylation role.

Bhidayasiri et al. (2005), Napierala, Bacolla and Wells (2005) and Pandolfo (2002) assessed the frequency of homozygous and heterozygous variants of repeating GAA triplet expansion within intron 1 of the FRDA gene on chromosome 9q13, amongst Friedreich ataxia patients. They demonstrated that the ratio between these forms is equal to 24:1, i.e. the homozygous form is prevalent. The FRDA gene is located in a 150-kb region on chromosome 9q13 (see fig. 2 in Appendix), and was decrypted and cloned in the late 90s and the mouse model was successfully applied by Koutnikova et al. in 1997.

Bidichandani et al. (1998) demonstrated that FRDA patients have a noticeable deficiency of mature X25 mRNA. Also, it was evident that the GAA trinucleotide repeat expansion is associated with unusual DNA structure; a characteristic of the transcriptional block, which was confirmed in later studies.

A recent article by Clark et al. (2006) analysed Expansion of GAA trinucleotide repeats in eukaryotic genomes and demonstrated that the abundance of large expansions of GAA trinucleotide repeats is specific to mammals only. This fact is important for selecting appropriate laboratory models for inherited disorders, such as Friedreich ataxia. Another important result of this study is the significant mutation potential of GAA trinucleotide repeats expansion. This work was a continuation of the previous studies conducted by this team (Clark et al., 2004).

Sharma et al. (2004) reported the cases of heterozygous carriers of the abnormal alleles of the FRDA gene. Other researches conducted by this team (Pollard et al., 2004) produced evidence that the GAA triplet repeat mutation in Friedreich ataxia are related to the instability during DNA replication.

The OMIM database (2005) contains information about seven allelic variants of FRDA gene:

1. the FRDA0001 with (GAA)_n expansion,
2. FRDA0002 with T-to-G transversion in exon 3,
3. FRDA0003 with A-to-G transition at the end of the third intron,
4. FRDA0004 with conversion of isoleucine-154 to phenylalanine in exon 4,
5. FRDA0005 characterised by glycine to valine transversion (GLY130VAL mutation),
6. FRDA0006 with methionine to isoleucine mutation at the start codon,
7. and FRDA0007 (TGG-to-GGG change in exon 5a).

Interesting results were produced by Zuhlke et al. (2004). The authors found two mutations (Asn146Lys and p.Leu186Arg) which were not described in the OMIM database previously. Furthermore, in their study they reported a case that contained heterozygous 2776 bp deletion including exon 5a, which could have resulted in the removal 50 of the 210 residues of the frataxin, and consequently, its deactivation. This study also proved the possibility of the appearance of the Friedreich ataxia phenotype without any mutations within the FRDA gene. Indian authors (Chattopadhyay et al., 2004) found another novel haplotype, ACCT, and consider that it is a characteristic contained within the Indian population. The new alleles were also discovered by Italian researchers (Monticelli et al., 2004), who determined a new single-nucleotide polymorphism as the closest to the GAA trinucleotide repeats. The results of their study are useful for identifying the relationship between different GAA alleles and haplotypes. Monticelli et al. (2004) demonstrated that single-nucleotide variation T is strongly associated with GAA alleles. This research is also important for the understanding of principles and approaches of allele classification and origin.

In general, it was determined (see fig. 3 in the Appendix) that the individuals having less than 37 GAA triplets are under a lower risk of the development of clinical signs of Friedreich

ataxia while FRDA patients usually have allelic variants with 66 or more triplets. The intermediate number of GAA trinucleotide repeats may be associated with lower penetrance and somatic instability, which became an important factor of the disease occurrence. It is therefore expedient to include tests for somatic instability in the diagnostic programmes.

Ruggiero & Topal (2004) consider that human flap endonuclease 1 (h-FEN1) mutations can be important for GAA triplet-repeat expansion. Another study conducted by Kraslnikova & Mirkin (2004) showed that short (pre-mutation) and long (characteristic of disease) GAA trinucleotide repeats can delay the replication, whereas the normal sequence of the nucleotides in FRDA gene does not have an influence on the replication. Potaman et al. (2004) consider that the normal length of GAA repeats in the FRDA gene is less than 30, while the pre-mutation sequence length has an estimated 40 repeats. Mateo et al. (2004) found that the GAA1 size is the main determinant of Friedreich ataxia phenotype, whereas the GAA2 size is not relevant for clinical prognostication. The research conducted by Giotti et al. (2004) produced similar evidence. Furthermore, the authors extended their findings to the use of triplet repeat primed PCR (TP PCR) as a screening method in the management of Friedreich ataxia. Such molecular genetic screening could be useful for diagnosis of the milder forms of ataxia.

Italian authors (Colombo & Carobene, 2000) investigated the age of the intronic GAA trinucleotide repeat expansion mutation in Friedreich ataxia. According to their data, the average age of FRDA founding mutational events lies inside the interval of 564-801 generations. Colombo & Carobene (2000) considered that these data could be useful for historical assessment of the frequency of such pre-mutations.

Other investigations conducted with the use of the yeast frataxin homologue (Yfh1p) provided a significant contribution in terms of understanding the role of this protein for intracellular iron metabolism (Babcock et al., 1997; Branda et al., 1999; Cavadini et al., 2000, etc). The study of Mühlenhoff et al. (2002) assessed the role of Yfh1p depletion in assembling of mitochondrial proteins participating in the metabolism of iron and sulphur both in vitro (in detergent extracts of mitochondria) and in vivo. The authors demonstrated a strong reduction of mitochondrial Fe/S proteins under Yfh1p gene reduction. Theoretically, the yeast model could have some disadvantages due to the discrepancies between the prokaryotic and eukaryotic homologues of frataxin, although the compatibility of these homologues was proven in the numerous researches (Vazquez-Manrique et al., 2006).

DNA methylation in higher eukaryotes occurs at the 5-position of cytosine in the CpG dinucleotide (see fig. 4 in the Appendix). This biochemical process significantly changes the activity of specific genes and plays an important role in the ethiopathology of different inherited disorders (see Tab. 1 in the Appendix). The occurrence of GAA trinucleotide repeat expansion is strongly correlated with phenotypic expression of some neurodegenerative and neurological diseases (Pandolfo, 2003; Sharma et al., 2004). A recent publication of Pearson, Edamura & Cleary (2005), was dedicated to the problem of repeat instability as the factor of DNA mutations. Repeat instability is a distinctive dynamic mutation mechanism involving the construction of uncommon structures during DNA replication, repair and recombination. More usual static mutations could be reserved in the somatic cells and their transmission to the descendants is stable whereas the repeat mutations produce DNA structures which are able to continue transforming. The most frequent form of repeat instability is presented by trinucleotide repeats. Everett and Wood (2004) also considered that this instability could be both meiotic and mitotic. Not only GAA repeats exceeding 100 but also environmental impacts, gene modification and somatic mosaicism may play a role in FRDA aetiology

(pp.1395-2397). The original scheme of the mechanisms for regulation of frataxin expression in Friedreich ataxia is presented in fig. 5 in the Appendix.

Heidenfelder, Makhov & Topal (2003), show that GAA repeat expansion can result in DNA slippage during DNA replication. The authors developed in vitro models of large and growing (GAA)(n), and (TTC)(n) hairpins during DNA synthesis, and proved their role in the pathogenesis of Friedreich ataxia. Similar data is reported by Sinden et al. (2002).

Pollard et al. (2004) analysed the penetrance of FRDA gene in the patients inheriting alleles with the different GAA triplet expand. The authors suggested that FRDA mutations are reversible because in the laboratory experiments fully expanded GAA triplet repeat alleles at the *FRDA* locus have a marked tendency to reduce. Nevertheless the molecular mechanisms of the reversion of the *FRDA* mutation are still not elaborated properly. There were proposed two competitive models explaining the dynamical changes in the *FRDA* gene. The first is based on the supposition that small length changes of the repeat sequence may take place as a result of slippage and mispairing during DNA replication whereas the second mechanism is realised regarding to larger trinucleotide sequences. The authors suppose that this mechanism can include the processes of the hexanucleotide interruption (ibid). The contraction of the *FRDA* allelic variants with extended (GAA•TTC)_n repeat is associated with the significant mutational activity, nevertheless these mutations might be assessed as reversible.

It is well known that DNA methylation is of particular importance for gene regulation, and so alterations in DNA methylation may significantly affect the structure of DNA. This biochemical process leads to epigenetic but not genetic changes, as the genetic code is not affected (Mager & Bartolomei, 2005). Recently Chen & Riggs (2005) demonstrated that DNA methylation is an important mechanism for establishing stable heritable epigenetic

marks (p. 438). They showed that processes of DNA methylation are well regulated but the regulation mechanisms is still unclear and requires further research. DNA methylation is considered to be an important mechanism of gene regulation and epigenetic fidelity (Kapoor et al., 2001; Grabczyk, Kumari & Usdi, 2001). The frataxin gene is not an exception. The aberrant DNA methylation can have dramatic effects on DNA replication and repair and consequently on the gene expression.

Pearson, Edamura & Cleary (2005) wrote:

“A strong paternal contraction bias is evident for expanded repeats in patients with Friedreich ataxia (FRDA) and, in the third case, might be related to CpG methylation. Whether CpG methylation (or its absence) in the flanking regions of the SCA8, DM1 or FRDA repeats contributes to contractions in the male germ line is not known” (p. 732)

There was considered also that processes mentioned in the cited fragment could define both mutational bias and parent-of-origin effects. The last ones are an example of non-Mendelian inheritance but their role in FRDA pathogenesis was not confirmed. On the other hand there is no evidence to exclude the possibility of the association between the processes of DNA methylation and parent-of-origin effects.

DNA methylation was found to be a critical element in the imprinting process (Turek-Plewa & Jagodzinski, 2005), by marking and establishing a monoallelic expression pattern in the imprinted genes (ibid). There is also a causal link between DNA methylation and gene activity. In higher eukaryotes DNA methylation also affects genome stability and gene silencing (Baylin, 2005). Other methylation-dependent processes include X chromosome inactivation, histone modification and oncogene activation (Hatada et al., 2006). Therefore there is strong evidence that DNA methylation cannot influence the genome structure,

although it can be important for deactivation or activation of the genes and consequently, affect the penetrance of phenotype.

Saveliev et al. (2003) published interesting data, determining the role DNA trinucleotide repeats in the mediate heterochromatin variegated gene silencing. Even relatively short trinucleotide repeat expansions in Friedreich ataxia could lead to the clinical manifestations of disease due to the negative correlation between gene silencing and promoter accessibility. It was also shown that heterochromatin-mediated silencing might have a role in gene regulation and may modulate the gene expression in Friedreich ataxia and other trinucleotide-repeat diseases. The gene silencing could be explained by a mechanism resembling heterochromatin-mediated position effect variegation where CTG and GAA triplet repeats are crucial. Nevertheless, the role of DNA methylation, which may participate in the processes of repeat instability, was not discussed in this study.

DNA methylation mechanisms and effects

As previously discussed, DNA methylation is involved in numerous essential processes of gene expression regulation. The mechanisms of methylation and demethylation are still not fully understood but there are various chemical agents, which may induce these processes (Ehrlich, 2005). For example, DNA methylation might be modified by the nucleoside analogues (5-azacytidine etc), N-methyl-N-nitrosourea, etc. The clinical and experimental use of these agents is restricted due to the risk of inappropriate activation of the genes in normal cells. There is interestingly that demethylation though use of 5-azanucleosides could be associated with the possibility of toxic action and inappropriate gene activation (Estrell, 2004). Another barrier is presented by the phenomenon of resilencing after the removal of demethylating agent.

The majority of published articles are related to the relationship between oncogenesis and DNA methylation, however there are many articles discussing theoretical and methodological problems of genetic epigenesis and genetic silencing for example, interruption or suppression of the gene expression at the transcriptional or translational levels). Some of these publications are dedicated to the development of the issues of modifying of epigenetic mechanisms in the mouse models (Mager & Bartolomei, 2005; Reik & Dean, 2001, etc). The preferable studies in mice are presented by the loss-of-function researches, screening for mutagenesis, somatic cell nuclear transfer and others.

While appropriate targeting of methylation to specific loci of the genome is essential for normal growth and development, aberrant targeting of sequences for methylation can have adverse effects on health (Scarano et al., 2005). It is now well established that aberrant methylation plays a role in the deactivation of tumor suppressor genes and cancer development. In turn, the abnormal methylation of the expanded CGG triplet repeats at the

FMR1 locus leads to gene silencing and development of the fragile X syndrome (Grabczyk, Kumari & Usdin, 2001). Another possible mechanism of the involvement of DNA methylation in the etiopathogenesis of fragile X syndrome is the ability to prevent the binding of the transcription factor alpha-Pal/NRF-1, and affect the binding of other factors via the formation of transcriptionally silent chromatin. There is also possible that additional agents and processes are involved in the transcript deficiency in the patients with Friedreich ataxia. DNA methylation could be one of such processes.

The conceptual scheme of DNA methylation is presented in Fig. 6 in the Appendix. Primarily process of methylation of unmethylated nucleotide is termed as de novo promoter methylation (Zhu et al., 2006). Zhu and his co-authors found that epigenetic regulator, Lsh, is involved in the control of de novo methylation of DNA.

It is well known that methylation of DNA occurs at the 5-position of cytosine. In mammals the characteristic context of methylation is a CpG dinucleotide. In the fully methylated DNA duplex there are methylated cytosine (see fig. 6). However, during following DNA replication the parent strand of DNA became methylated while newly synthesized daughter strand is unmethylated. Such dinucleotides are termed as “hemimethylated” (Okano, 2002). Finally, the parent strand of DNA is copied to the daughter strand of DNA through a process termed maintenance methylation and fully methylated DNA duplex will occur. All described processes are controlled enzymatically. There are two diverse types of DNA methyltransferases dependently on the reaction they catalyse (Esteller, 2004). The reaction of de novo methylation of non-methylated DNA is catalysed by Dnmt3a, Dnmt3b and other methyltransferases while Dnmt1 methyltransferase catalyses mainly the methylation of hemimethylated DNA (see fig. 6). The intensity of DNA methylation can depend on the dietary pattern (consumption of the nutrients which are donors of methylgroup), presence of

methylation-modifying agents of chemical and physical origin (Watson & Goodman, 2002; Esteller, 2004).

Methylation mark directly affects the gene expression, a numerous proteins could participate in the regulatory processes through the interaction either with methylated or unmethylated DNA sequences (Majumder et al., 2005). This attribute allows the presence of DNA methylation in the gene promoter to affect its expression. The proteins participating in methylation processes may bind specifically to methylated sequences or specifically to DNA chains containing unmethylated CpGs dinucleotides. In some cases, these proteins also interact with the DNA methyltransferases and, therefore, influence on the transcriptional activation or repression (Baylin, 2005). A recent article produced by Morgan et al. (2005) has been dedicated to the problem of epigenetic reprogramming in mammals. Authors considered that DNA methylation could be an important factor of reprogramming in the somatic cells with epigenetic marks. Santos & Dean (2004) also obtained similar data.

In summary, the normal functions of DNA methylation could be described as (Scarano et al., 2005): providing chromosomal stability, silencing of parasitic and viral sequences, imprinting, X-chromosome inactivation, tissue specific expression and compartimentisation of chromatine. At the same time, aberrant DNA methylation leads to the risk of carcinogenesis due to methylation-associated silencing of tumour suppressors and hypomethylation of oncogenes (ibid). Similar changes can occur in cardiovascular and immune diseases and, theoretically, can be related to the risk of neurodegenerative diseases, such as Friedreich ataxia.

The most widely used methods of studying DNA methylation are presented by quantitative determination of 5-methylcytosine in DNA content (HPCE and HPLC), global genomic

screening, the use of DNA demethylating drugs (e.g. 5-azacytidine and decitabine) in vitro and in the animal models (Yoder & Bestor, 1996; Esteller, 2004).

Grabczyk, Kumari & Usdin (2001) described the relationship between the processes of methylation and phenotypical characteristics of the patients suffering fragile X syndrome. The authors argued that the expansion of CGG and CCG sequences can lead to hypermethylation of the promoter. This phenomenon can occur due to the presence of mispaired cytosine. Grabczyk, Kumari & Usdin (2001) wrote about this as:

“Methylation may ... affect promoter activity not only by establishing transcriptionally silent chromatin but also by directly inhibiting transcription factor binding... The direct effect of methylation on transcription factor binding suggests that significantly increasing the activity of the FMR1 [gene of fragile X syndrome] promoter in individuals with FXS [fragile X syndrome] may require not only restoring transcriptionally active chromatin but also demethylating key CpG residues” (p. 369).

But some patients with FXS can have normal levels of FMR1 mRNA even though promoter hypermethylation takes place. However when more exact methods are applied there is determined the levels of FMR1 mRNA exceed the normal values and there could be evidence of certain transcriptional silencing. Nevertheless there are no references that patients with extend of the polynucleotides, which are not include cytosine (e.g. GAA triplet expansion) could have similar patterns of methylation.

Mouse models in Friedreich ataxia studies

The use of a representative mouse model for Friedreich ataxia can be helpful for developing knowledge of this inherited disease. Mouse models for human diseases could be the best choice, because they offer the opportunity to examine the pathogenesis of inherited disorders in higher eukaryotes and mammals. Successful experiments by Pook et al. (2001) have allowed the use of transgenic mouse models in testing new drugs and developing gene therapy in the cases of Friedreich ataxia. Alternative models can be represented by FRDA conditional knockout (Seznec et al., 2004; Simon D., 2004; Sandos et al., 2003, etc) and GAA knock-in mouse (Miranda et al., 2002) models.

British scientists (Al-Mahdawi et al., 2004) proposed the mouse model of FRDA GAA repeat instability, which could be useful for developing new methods of Friedreich ataxia prevention and treatment. Many authors (Sarsero et al., 2005, 2004, 2003) use transgenic mice for examination of spatial and temporal features of FRDA gene expression. The animal model is commonly used for pre-clinical tests of new medications. Also, the possibility of using the transgenic mice for experimental therapy with stem cells has been discussed.

Puccio et al. (2001) performed the successful application of a mouse model for assessing intramitochondrial disorders of iron accumulation. They generated several frataxin-deficient lines of mice and demonstrated time-dependent iron accumulation in the mitochondria of animals. The generated transgenic lines of mice were recommended for wide use in the pre-clinical studies.

Yet, these did not indicate the research, which the authors had studied on the impact of DNA methylation on FRDA gene expression. Also, there are no studies that have analysed DNA

methylation at the FRDA promoter in FRDA transgenic mouse tissues that either do or do not contain GAA repeat expansion mutations.

Discussion

Methylation of cytosines in the 5th position of the pyrimidic ring is the most important epigenetic modification in eukaryotes. This process is associated with gene silencing and, theoretically, could be one of the causes of the suppression of FRDA gene. In contrast, artificial stimulating of demethylation could increase risk of oncogenesis thus this approach should not be used for Friedreich ataxia treatment. The reviewed literature does not contain clear evidence of the DNA methylation role in FRDA pathogenesis; however some authors mentioned the possibility of DNA methylation effects in the patients with heterozygous genotype and/or non-typical allelic variants of the FRDA gene (Grabczyk, Kumari & Usdin, 2001; LeProust et al., 2000). Thus, contrarily to Jacobsen syndrome and Fragile X syndrome the role of methylation in the pathogenesis of Friedreich ataxia is not proved and the DNA methylation status of the FRDA promoter, and how this may interact with GAA repeat expansion mutations, is unknown.

In spite of presence of the FRDA gene homologue in the yeast, the use of prokaryotic model of Friedreich ataxia is not expedient for testing new pharmacological agents. The mouse model has more advantages and can be used for analysing gain or loss of function mutants, genetic interactions and modifiers etc. In the case of FRDA modelling, there is the possibility to generate a transgenic line of mice where functions of frataxin and intramitochondrial metabolism of iron will be analysed through introducing a mutated human gene into a null mouse background. It is important that human frataxin is able to substitute for mouse frataxin.

The contradictory data of some research is related to the role of oxidative phosphorylation in the pathogenesis of Friedreich ataxia. The possible interaction between the processes of oxidative phosphorylation and DNA methylation were not discussed in the reviewed sources of information. Also, a key factor is how frataxin activity is correlated with the oncogenesis induced by hypermethylation and what remote effects DNA methylation could have on the use of antioxidant agents recommended for FRDA treatment.

Other weaknesses of the available research are related to their particularistic approach in the assessment of FRDA mutations effects. Many of the authors concentrated their attention on seeking the evidence of the importance of expansion of GAA trinucleotide repeats in Friedreich ataxia pathogenesis, whereas other possible mechanisms were not investigated deeply. For example, the role of epigenetic modifiers as potential disease modifying factors in Friedreich's ataxia was not assessed in the available bibliographic sources.

It is important that for some further research to be based on the analysis of DNA methylation at the FRDA promoter in FRDA transgenic mouse tissues knocking-in and knocking-out with GAA repeat expanded mutations.

REFERENCES:

1. Al-Mahdawi et al. (2004) GAA repeat instability in Friedreich ataxia YAC transgenic mice. *Genomics*. 84(2) pp. 301-310.
2. Alper & Narayanan. Friedreich's ataxia. *Pediatr Neurol*. 2003 May;28(5):335-41.
3. Andermann (1976) Nicolaus Friedreich and degenerative atrophy of the posterior columns of the spinal cord. *Can J Neurol Sci*. 3(4) pp. 275-277
4. Babcock et al. (1997) Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of frataxin. *Science* 276: pp. 1709-1712.
5. Baylin (2005) DNA methylation and gene silencing in cancer. *Nat Clin Pract Oncol*. 2 Suppl 1(S1) pp. S4-S11.
6. Bhidayasiri et al. (2005) Late-onset Friedreich ataxia: phenotypic analysis, magnetic resonance imaging findings, and review of the literature. *Arch Neurol*. 62(12) pp. 1865-1869.
7. Bidichandani et al.(1998) 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(1) pp. 111-121.
8. Branda et al. (1999). Mitochondrial intermediate peptidase and the yeast frataxin homolog together maintain mitochondrial iron homeostasis in *Saccharomyces cerevisiae*. *Hum Mol Genet*. 8(6) pp. 1099-110
9. Cavadini et al. (2000) Human frataxin maintains mitochondrial iron homeostasis in *Saccharomyces cerevisiae*. *Hum Mol Genet*. 9(17) pp. 2523-2530
10. Chattopadhyay et al. (2004) Molecular analysis of GAA repeats and four linked bi-allelic markers in and around the frataxin gene in patients and normal populations from India. *Ann Hum Genet*. 68(Pt 3) pp. 189-195.
11. Chen & Riggs (2005) Maintenance and regulation of DNA methylation patterns in mammals. *Biochem Cell Biol*. 83(4) pp. 438-448.

12. Ciotti et al. (2004) Triplet repeat primed PCR (TP PCR) in molecular diagnostic testing for Friedreich ataxia. *J Mol Diagn.* 6(4) pp. 285-289.
13. Clark et al. (2004) Expansion of GAA triplet repeats in the human genome: unique origin of the FRDA mutation at the center of an Alu. *Genomics.* 83(3) pp. 373-383.
14. Clark et al. (2006) Expansion of GAA trinucleotide repeats in mammals. *Genomics.* Vol. 87(1) pp. 57-67.
15. Colombo & Carobene (2000) Age of the intronic GAA triplet repeat expansion mutation in Friedreich ataxia. *Hum Genet.* 106(4) pp. 455-458.
16. Delatycki, Williamson & Forrest (2000) Friedreich ataxia: an overview. *J Med Genet* 37(1): pp. 1-8
17. Ding, Smith & Ding (2005) Mobilization of the iron centers in IscA for the iron-sulfur cluster assembly in IscU. *Biochem.* 389 pp. 797-802
18. Ehrlich M. (2005) The controversial denouement of vertebrate DNA methylation research. *Biochemistry (Mosc).* 70(5) pp. 568-575
19. Esteller (2004) *DNA methylation: Approach and Applications.* CRC, 240 p.
20. Everett & Wood (2004) Trinucleotide repeats and neurodegenerative disease. *Brain.* 127(Pt 11) pp. 2385-2405.
21. Gakh et al. (2005) Mitochondrial iron detoxification is a primary function of frataxin that limits oxidative damage and preserves cell longevity. *Hum Mol Genet.* [Epub ahead of print]
22. Grabczyk, Kumari & Usdin (2001) Fragile X syndrome and Friedreich's ataxia: two different paradigms for repeat induced transcript insufficiency. *Brain Res Bull.* 56(3-4) pp. 367-373
23. Hatada et al. (2006) Genome-wide profiling of promoter methylation in human. *Oncogene.* [Epub ahead of print]

24. Heidenfelder, Makhov & Topal. (2003) Hairpin formation in Friedreich's ataxia triplet repeat expansion. *J Biol Chem.* 278(4) pp. 2425-2431.
25. Isaya et al. (2004) Functional studies of frataxin. *Acta Paediatr Suppl.* 93(445) pp. 68-71
26. Kapoor et al. (2005) Mutations in a conserved replication protein suppress transcriptional gene silencing in a DNA-methylation-independent manner in *Arabidopsis*. *Curr Biol.* 15(21) pp. 1912-1918
27. Kimura et al. (2002) [A case of Friedreich's ataxia having no abnormal gene] *No To Hattatsu.* 34(4) pp. 343-346.
28. Koutnikova et al. (1997) Studies of human, mouse and yeast homologues indicate a mitochondrial function for frataxin. *Nature Genet.* 16: pp. 345-351
29. Krasilnikova & Mirkin (2004) Replication stalling at Friedreich's ataxia (GAA)_n repeats in vivo. *Mol Cell Biol.* 24(6) pp. 2286-2295.
30. Leggo et al. (1997) Analysis of spinocerebellar ataxia types 1, 2, 3, and 6, dentatorubral-pallidoluysian atrophy, and Friedreich's ataxia genes in spinocerebellar ataxia patients in the UK. *J Med Genet.* 34(12) pp. 982-985.
31. LeProust et al. (2000) Unexpected formation of parallel duplex in GAA and TTC trinucleotide repeats of Friedreich's ataxia. *J Mol Biol.* 302(5) pp. 1063-1080
32. Lodi et al. (2002) Mitochondrial dysfunction in Friedreich's ataxia: from pathogenesis to treatment perspectives. *Free Radic Res.* 36(4) pp. 461-466.
33. Lynch et al. (2005) Performance measures in Friedreich ataxia: potential utility as clinical outcome tools. *Mov Disord.* 20(7) pp. 777-782
34. Mager & Bartolomei (2005) Strategies for dissecting epigenetic mechanisms in the mouse. *Nat Genet.* 37(11) pp. 1194-200
35. Majumder et al. (2005) Epigenetic regulation of metallothionein-i gene expression: Differential regulation of methylated and unmethylated promoters by DNA

- methyltransferases and methyl CpG binding proteins. *J Cell Biochem.* [Epub ahead of print]
36. Mateo et al. (2004) Expanded GAA repeats and clinical variation in Friedreich's ataxia. *Acta Neurol Scand.* 109(1) pp. 75-78.
 37. McDaniel et al. (2001) Sequence variation in GAA repeat expansions may cause differential phenotype display in Friedreich's ataxia. *Mov Disord.* 16(6) pp. 1153-1158.
 38. Miranda et al. (2002) Frataxin knockin mouse. *FEBS Lett.* 512(1-3) pp. 291-297.
 39. Monticelli et al. (2004) New clues on the origin of the Friedreich ataxia expanded alleles from the analysis of new polymorphisms closely linked to the mutation. *Hum Genet.* 114(5) pp. 458-463
 40. Morgan et al. (2005) Epigenetic reprogramming in mammals. *Hum Mol Genet.* 14 Spec No 1 R47-58.
 41. Muhlenhoff et al. (2002) The yeast frataxin homolog Yfh1p plays a specific role in the maturation of cellular Fe/S proteins. *Hum Mol Genet.* 11(17) pp. 2025-2036.
 42. Napierala, Bacolla & Wells (2005) Increased negative superhelical density in vivo enhances the genetic instability of triplet repeat sequences. *J Biol Chem.* 280(45):37366-37376.
 43. Okano & Li (2002) Genetic analyses of DNA methyltransferase genes in mouse model system. *J Nutr.* 132(8 Suppl) pp. 2462S-2465S
 44. OMIM (2005) FRDA GENE. Available at the web-site <<http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=606829>>. Retrieved on 12.01.2005
 45. Palau (2001) Friedreich's ataxia and frataxin: molecular genetics, evolution and pathogenesis). *Int J Mol Med.* 7(6) pp. 581-589.

46. Pandolfo (2002) Frataxin deficiency and mitochondrial dysfunction. *Mitochondrion*. 2(1-2) pp. 87-93.
47. Pandolfo (2003) Friedreich ataxia. *Semin Pediatr Neurol*. 10(3) pp. 163-72.
48. Pearson et al. (2005) Repeat instability: mechanisms of dynamic mutations. *Nat Rev Genet*. 6(10) pp. 729-742.
49. Pollard et al. (2004) Replication-mediated instability of the GAA triplet repeat mutation in Friedreich ataxia. *Nucleic Acids Res*. 32(19) pp. 5962-5971
50. Pook et al. (2001) Rescue of the Friedreich's ataxia knockout mouse by human YAC transgenesis. *Neurogenetics*. 3(4) pp. 185-193
51. Potaman et al. (2004) Length-dependent structure formation in Friedreich ataxia (GAA)_n*(TTC)_n repeats at neutral pH. *Nucleic Acids Res*. 32(3) pp. 1224-1231.
52. Puccio et al. (2001) Mouse models for Friedreich ataxia exhibit cardiomyopathy, sensory nerve defect and Fe-S enzyme deficiency followed by intramitochondrial iron deposits. *Nat Genet*. 27(2) pp. 181-186.
53. Ruggiero & Topal (2004) Triplet repeat expansion generated by DNA slippage is suppressed by human flap endonuclease 1. *J Biol Chem*. 279(22) pp. 23088-23097.
54. Santos & Dean (2004) Epigenetic reprogramming during early development in mammals. *Reproduction*. 127(6) pp. 643-651.
55. Santos et al. (2003) Iron metabolism in mice with partial frataxin deficiency. *Cerebellum*. 2(2) pp. 146-153.
56. Sarsero et al. (2003) Upregulation of expression from the FRDA genomic locus for the therapy of Friedreich ataxia. *J Gene Med*. (1) pp. 72-81.
57. Sarsero et al. (2004) Human BAC-mediated rescue of the Friedreich ataxia knockout mutation in transgenic mice. *Mamm Genome*. 15(5) pp. 370-382.
58. Sarsero et al. (2005) Evaluation of an FRDA-EGFP genomic reporter assay in transgenic mice. *Mamm Genome*. 16(4) pp. 228-241.

59. Saveliev et al. (2003) DNA triplet repeats mediate heterochromatin-protein-1-sensitive variegated gene silencing. *Nature*. 422(6934) pp. 909-913
60. Scarano et al. (2005) DNA methylation 40 years later: Its role in human health and disease. *J Cell Physiol*. 204(1) pp. 21-35
61. Seznec et al. (2004) Idebenone delays the onset of cardiac functional alteration without correction of Fe-S enzymes deficit in a mouse model for Friedreich ataxia. *Hum Mol Genet*. 13(10) pp. 1017-24.
62. Seznec et al. (2005) Friedreich ataxia: the oxidative stress paradox. *Hum Mol Genet*. 14(4) pp. 463-474.
63. Sharma et al. (2004) Friedreich ataxia in carriers of unstable borderline GAA triplet-repeat alleles. *Ann Neurol*. 56(6) pp. 898-901.
64. Simon et al. (2004) Friedreich ataxia mouse models with progressive cerebellar and sensory ataxia reveal autophagic neurodegeneration in dorsal root ganglia. *J Neurosci*. 24(8) pp. 1987-1995.
65. Sinden et al. (2002) Triplet repeat DNA structures and human genetic disease: dynamic mutations from dynamic DNA. *J Biosci*. 27(1 Suppl 1) pp. 53-65
66. Strachan & Read (1999) *Human Molecular Genetics*. Willey-Liss 2nd edition. pp. 386-388
67. Turek-Plewa & Jagodzinski (2005) The role of mammalian DNA methyltransferases in the regulation of gene expression. *Cell Mol Biol Lett*. 10(4) pp. 631-47
68. van de Warrenburg, Knoers & Kremer (2002) [Friedrich's ataxia: clinical difficulties and genetic possibilities] *Ned Tijdschr Geneesk*. 146(36) pp. 1669-1672
69. Vazquez-Manrique et al. (2006) Reduction of *Caenorhabditis elegans* frataxin increases sensitivity to oxidative stress, reduces lifespan, and causes lethality in a mitochondrial complex II mutant. *FASEB J*. 20(1) pp. 172-174.

70. Watson & Goodman (2002) Epigenetics and DNA methylation come of age in toxicology. *Toxicol Sci.* 67(1) pp. 11-16
71. Yoder & Bestor (1996) Genetic analysis of genomic methylation patterns in plants and mammals. *Biol Chem.* 377(10) pp. 605-610.
72. Zhu et al. (2006) Lsh is involved in de novo methylation of DNA. *EMBO J.* [Epub ahead of print]
73. Zuhlke et al. (2004) Extension of the mutation spectrum in Friedreich's ataxia: detection of an exon deletion and novel missense mutations. *Eur J Hum Genet.* 12(11) pp. 979-982.

Appendix:

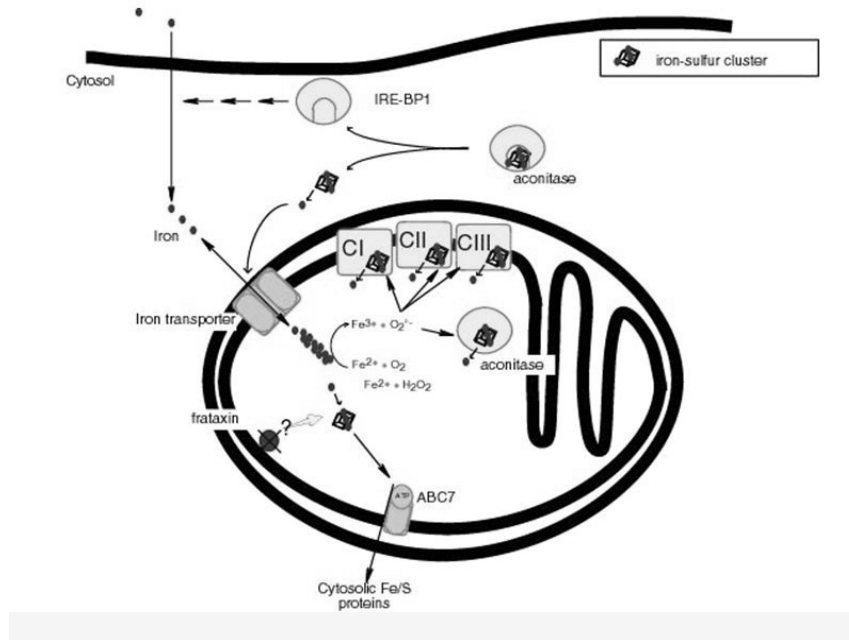


Figure 1. Intramitochondrial iron metabolism: the role of frataxin.

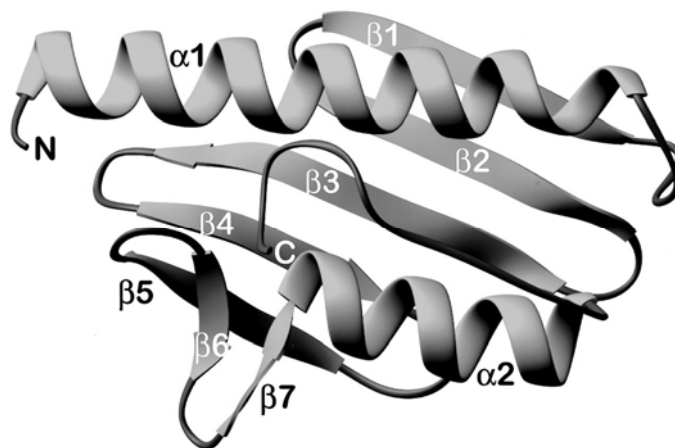


Figure 2. Frataxin structure.

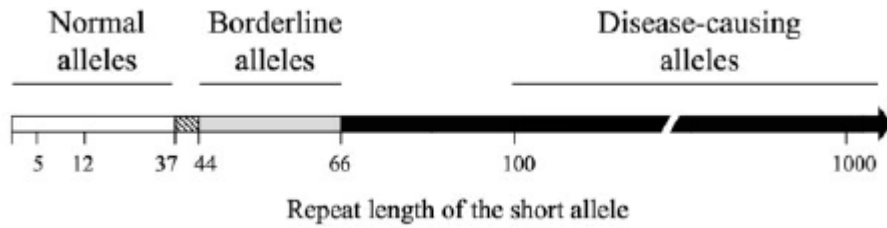


Figure 3. Genotype–phenotype correlation of different FRDA allelic variants.

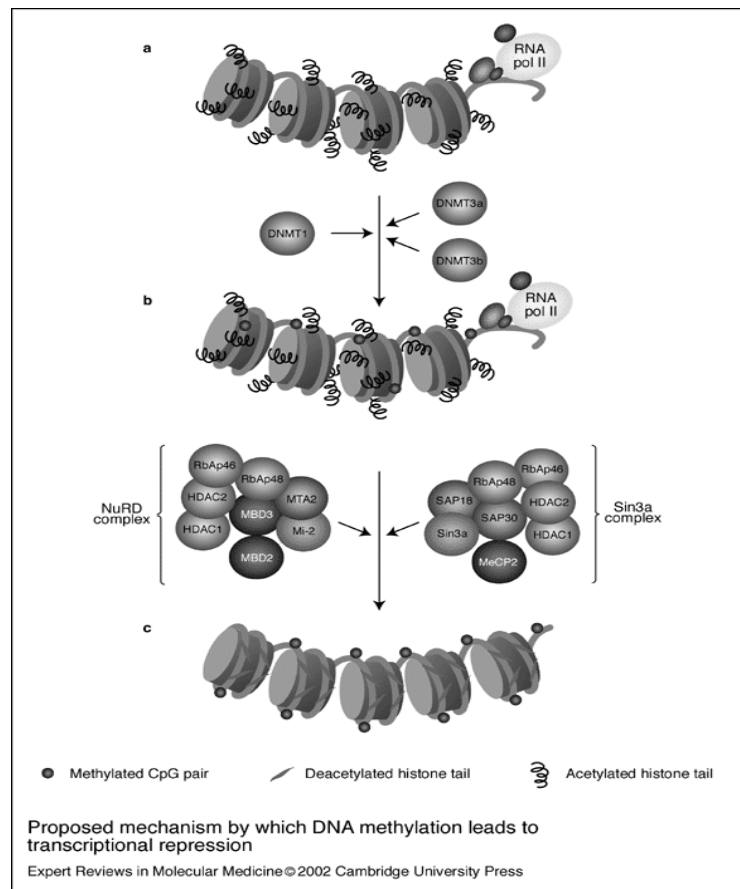


Figure 4. Mechanism of transcriptional repression due to DNA methylation

Disease	Gene	Locus	Repeat ^a	Repeat length			Protein/possible biological effect of expansion
				Normal	Pre-mutation	Disease	
Fragile X syndrome	FMR1 (FRAXA)	Xq37-3	(CGG) _n	6–52	59–230	230–2000 (pure)	FMR1 protein (FMRP)/promoter methylation, gene silencing, loss of FMRP function
Fragile XE syndrome	FMR2 (FRAXE)	Xq28	(CCG) _n	4–39	? (31–61)	200–900	FMR2 protein/methylation
None, fragile site	Fragile XF (FRAXF)		(CGG) _n	7–40	?	306–1008	Methylation
None, fragile site	FRA16A		(CCG) _n	16–49	?	1000–1900	Methylation
None, Jacobsen syndrome	FRA11B		(CGG) _n	11	80	100–1000	Methylation
Spinobulbar muscular atrophy (SBMA) (Kennedy's disease)	AR	Xq13–21	(CAG) _n	14–32	?	40–55	Androgen receptor (AR)/polyglutamine tract expansion
Myotonic dystrophy type 1 (DM1)	DMPK	19q13	(CTG) _n	5–37	50–80	80–1000	Myotonic dystrophy protein kinase/altered mRNA processing and transport, altered gene expression due to chromatin changes
Huntington disease	HD	4p16-3	(CAG) _n	10–34	36–39	40–121	Huntingtin/polyglutamine expansion
Spinocerebellar ataxia 1	SCA1	6p23	(CAG) _n	6–44	–	39–82 (pure)	Ataxin-1/polyglutamine expansion
Spinocerebellar ataxia 2	SCA2	12q24-1	(CAG) _n	14–31	–	34–59 (pure)	Ataxin-2/polyglutamine expansion
Spinocerebellar ataxia 3 (Machado Joseph disease)	SCA3	14q32-1	(CAG) _n	13–44	NA	55–84	Ataxin-3/polyglutamine expansion
Spinocerebellar ataxia 6	SCA6	19p13	(CAG) _n	4–18	NA	21–33	α _{1A} -voltage-dependent calcium channel subunit/polyglutamine expansion
Spinocerebellar ataxia 7	SCA7	13p12–13	(CAG) _n	4–34	NA	37–306	Ataxin-7/polyglutamine expansion
Spinocerebellar ataxia 8	SCA8	13q21	(CTG) _n	15–27?	110–200	–	3' to SCA8 gene/alters gene expression of adjacent genes?
Spinocerebellar ataxia 12	SCA12	5q31–33	(CAG) _n	7–28	?	66–78	5' to SCA12 gene/alters gene expression?
Dentatorubral-pallidolysian atrophy (Haw River syndrome)	DRPLA	12p13-31	(CAG) _n	7–25	?	49–75	Polyglutamine expansion
Friedreich ataxia (FRDA)	X25	9q13–21-1	(GAA) _n	6–29	? (> 34–40)	200–900	Frataxin/expand tract in an intron; altered mRNA production, altered replication

^aTypically repeat tracts contain sequence interruptions. See Pearson and Sinden (1998a) for a discussion of the sequence interruptions. ?, Potential mutagenic intermediate length. Not all diseases are associated with a premutation length repeat tract or premutation disease condition. –, None; NA, Not applicable.

Table 1. Aetiology of inherited health conditions.

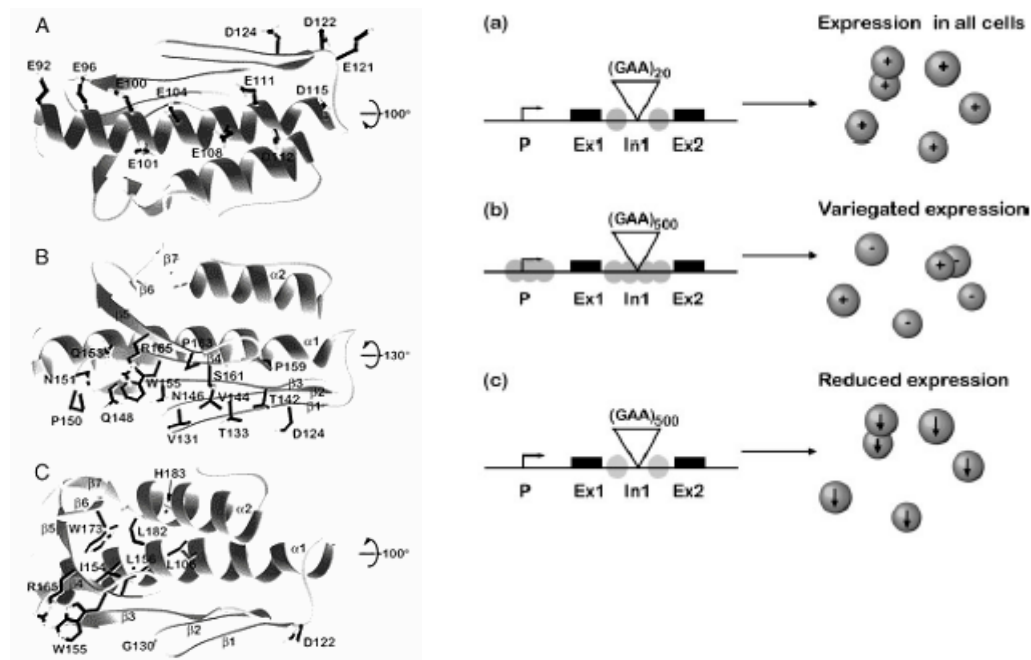


Figure 5. Mechanisms for regulation of frataxin expression in FRDA.

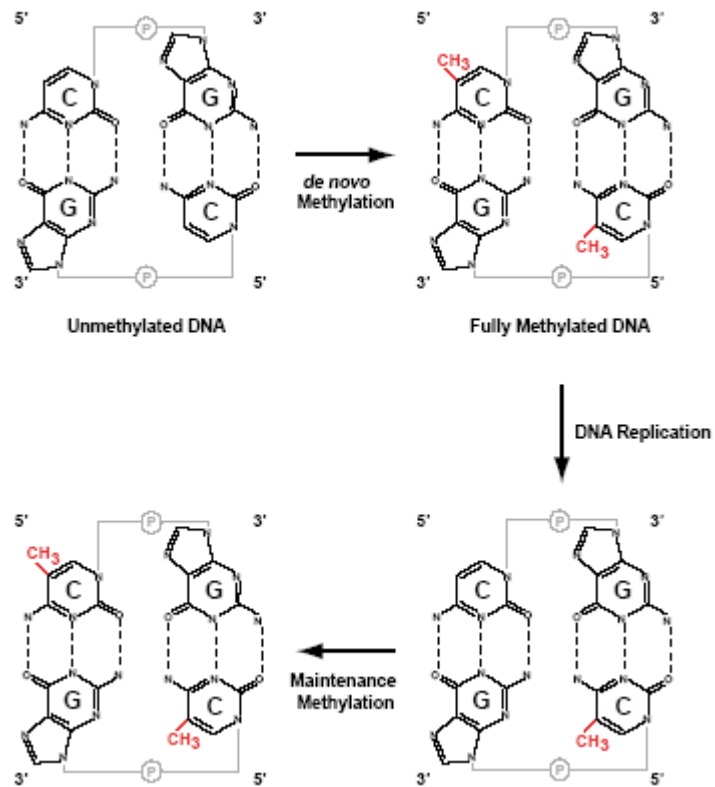


Figure 6. DNA methylation