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DNA Methylation and Trinucleotide Repeat Expansion Diseases

Mark A. Pook
Division of Biosciences,
School of Health Sciences and Social Care,
Brunel University, Uxbridge
UK

1. Introduction

DNA methylation of CpG dinucleotides is essential for mammalian development, X inactivation, genomic imprinting, and may also be involved in immobilization of transposons and the control of tissue-specific gene expression (Bird & Wolffe, 1999). The common theme in each of these processes is gene silencing. Therefore, gene silencing is a major biological consequence of DNA methylation. As such, DNA methylation can play a very important role in human disease. For example, DNA methylation-induced silencing of tumour suppressor genes can result in cancer, while gain or loss of DNA methylation can produce loss of genomic imprinting in diseases such as Beckwith-Wiedermann syndrome (BWS), Prader-Willi syndrome (PWS) or Angelman syndrome (AS) (Robertson, 2005). Yet another group of diseases where DNA methylation has a prominent role to play in disease aetiology and pathology is that of the inherited trinucleotide repeat (TNR) expansion diseases.

TNR expansion diseases can be divided into two major subgroups: (i) those involving large non-coding repeats (typically 100-1000 repeats), and (ii) those involving short coding repeats (< 100 repeats, coding for polyglutamine or polyalanine). The majority of TNR expansion diseases that have disease-associated DNA hypermethylation are of the large non-coding repeat type. These include fragile X syndrome (FRAXA), which is caused by CGG repeat expansion in the 5′-untranslated region (UTR) of the *FMR1* gene (Verkerk et al., 1991), myotonic dystrophy type I (DM1), which is caused by CTG repeat expansion in the 3′-UTR of the *DMPK* gene (Brook et al., 1992), and Friedreich ataxia (FRDA), which is caused by GAA repeat expansion within intron 1 of the *FXN* gene (Campuzano et al., 1996). However, there is also evidence for possible involvement of DNA methylation in the short CAG repeat, polyglutamine-encoding, types of TNR expansion diseases, such as spinocerebellar ataxia type 1 (SCA1) (Dion et al., 2008) and spinocerebellar ataxia type 7 (SCA7) (Libby et al., 2008).

This review focuses on recent advances in our understanding of DNA methylation association with inherited TNR expansion diseases. It first describes the relevant TNR expansion diseases, the genes that are mutated and what is currently known about DNA methylation profiles in each case. This is followed by consideration of the potential causes of DNA methylation, the subsequent effects of DNA methylation on disease phenotype, and

how understanding the mechanisms of DNA methylation may benefit efforts towards therapy for TNR expansion diseases.

2. TNR expansion diseases with associated DNA methylation

2.1 Fragile site-related mental retardation syndromes

Seven folate-sensitive fragile sites have been identified within human chromosomes: FRAXA (Verkerk et al., 1991), FRAXE (Knight et al., 1993), FRAXF (Parrish et al., 1994), FRA10A (Sarafidou et al., 2004), FRA11B (Jones et al., 1995), FRA12A (Winnepenninckx et al., 2007) and FRA16A (Nancarrow et al., 1994) (Table 1). In each case, the fragile site is associated with a large non-coding CGG repeat expansion, together with methylation of the CpG sites within the repeat expansion as well as within an adjacent upstream CpG island (Lopez Castel et al., 2010a). In the majority of cases, the CGG repeat expansion occurs within the 5′-UTR of a specific gene and the CpG island resides within the promoter region of this gene (Table 1). The effect of DNA methylation is to induce silencing of the gene, and the outcome of this, for the majority of fragile site-expressing patients, is the development of mental retardation.

Disease	Fragile site	Chromosomal position	Associated Gene	CGG repeat size	
				Normal	Disease
FRAXA Fragile X syndrome	FRAXA	Xq27.3	*FMR1*	6-54	>200
FRAXE Fragile X syndrome	FRAXE	Xq28	*FMR2*	4-39	>200
None identified at present	FRAXF	Xq28	*FAM11A*	7-40	>300
None identified at present	FRA10A	10q23.3	*FRA10AC1*	8-14	>200
Jacobsen syndrome	FRA11B	11q23.3	*CBL2* (candidate)	11	>100
Mental retardation	FRA12A	12q13.1	*DIP2B*	6-23	>150
None identified at present	FRA16A	11q22	Unknown	16-49	>1000

Table 1. Fragile sites that are associated with aberrant methylated, expanded CGG repeats and methylated adjacent CpG sites in disease state.

2.1.1 Fragile X syndrome (FRAXA)

The most prominent of the fragile site disorders is Fragile X syndrome (FRAXA), an X linked disorder that is recognized as the most common inherited form of mental retardation (Brouwer et al., 2009). FRAXA is caused by CGG repeat expansion within the 5′ UTR of the *FMR1* (fragile X mental retardation 1) gene, which is located at the FRAXA fragile site on chromosome Xq27.3 (Verkerk et al., 1991) (Fig. 1). Unaffected individuals have a range of allele sizes between 6-54 CGG repeats. However, allele sizes of 55-200 CGG repeats, known as 'premutations', are unstable and can expand upon transmission to FRAXA individuals,

who have alleles that exceed 200 CGG repeats, known as 'full mutations' (Fu et al., 1991). The expanded CGG repeats become methylated, as does the CpG island within the *FMR1* promoter, resulting in reduced expression of the *FMR1* gene product FMRP during development. Detailed analysis of the *FMR1* gene has revealed a distinct boundary of DNA methylation at a site between 650 and 800 nucleotides upstream of the CGG repeat in unaffected individuals that is lost in FRAXA patients (Naumann et al., 2009). This suggests that the *FMR1* promoter is normally protected from the spread of DNA methylation by a specific chromatin structure, which is somehow removed as a consequence of the expanded CGG repeat sequence.

Premutation CGG repeats ranging in size from 55-200 do not induce the typical DNA methylation and gene silencing that is seen with full mutations. Instead, unmethylated premutation CGG repeats produce overexpression of the *FMR1* gene, resulting in a toxic gain-of-function RNA that gives rise to the phenotypically distinct disorder called fragile X tremor/ataxia syndrome (FXTAS) (Jacquemont et al., 2003).

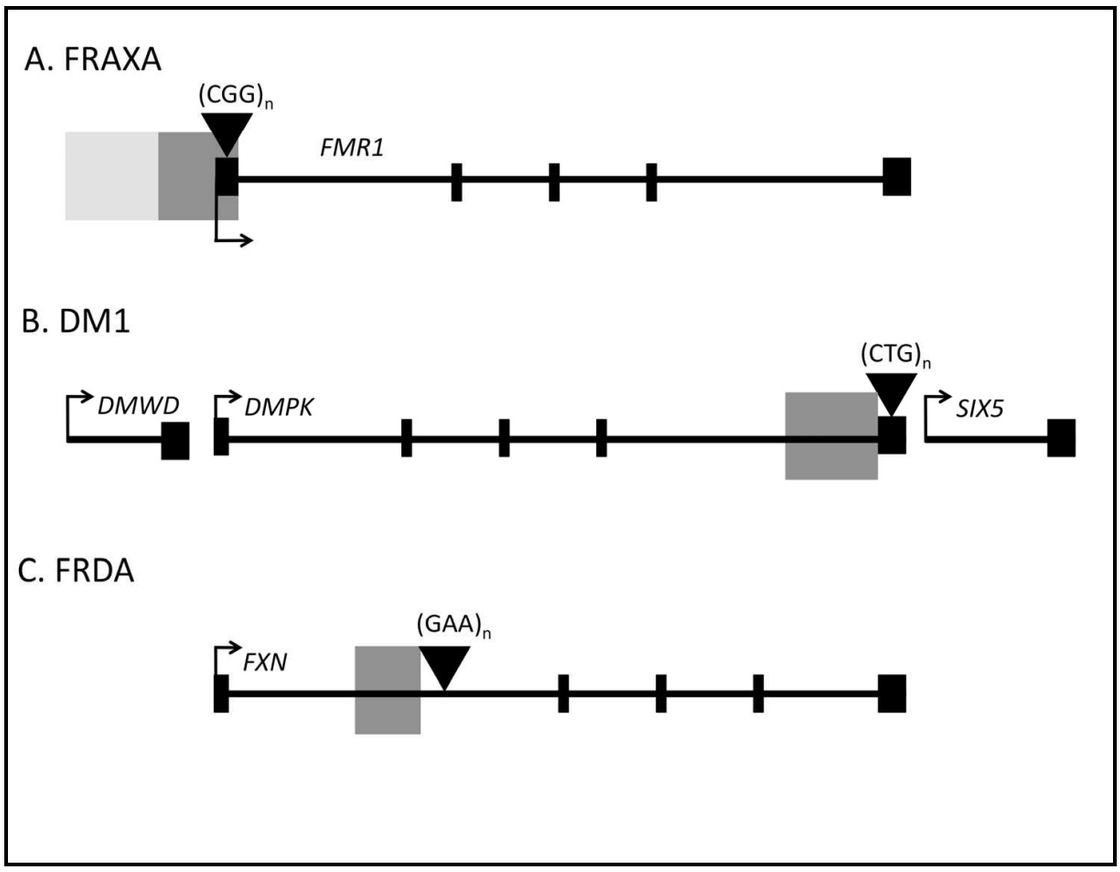


Fig. 1. Location of DNA methylation within expanded TNR loci. (A) FRAXA: The expanded CGG repeat is located in the 5′-UTR of the *FMR1* gene. A boundary of DNA methylation associated with normal CGG alleles (light grey box) shifts upon CGG expansion to enclose the CGG repeat (dark grey box). (B) DM1: The expanded CTG repeat in the 3′-UTR of the *DMPK* gene, and (C) FRDA: The expanded GAA repeat in intron 1 of the *FXN* gene are each associated with regions of DNA methylation just upstream of the expanded repeat.

2.2 Myotonic Dystrophy type 1 (DM1)

Myotonic dystrophy type 1 (DM1) is an autosomal dominant inherited multisystem disorder characterized by clinical features such as muscle weakness, myotonia and heart conduction defects (Schara & Schoser, 2006). The molecular basis for DM1 is expansion of a CTG repeat sequence within the 3′-UTR of the *DMPK* gene (Brook et al., 1992). Unaffected individuals have CTG repeat sizes of 5-37, and there is a premutation range of 34-90 CTG repeats, whereas affected individuals have expanded CTG repeat sizes that can range from 90 to thousands of units (Lopez Castel et al., 2010a). Both intergenerational and somatic instability of the CTG repeat are evident, providing a molecular basis for the anticipation phenomenon observed in DM1 families, together with tissue specific differences in disease pathology (Lavedan et al., 1993; Monckton et al., 1995). The effects of expanded CTG repeats are two-fold. Firstly, expression of an expanded CUG RNA sequence causes a toxic gain-of-function effect by altering the activity of RNA splicing factors (Ranum & Cooper, 2006). Secondly, the expanded CTG repeat induces epigenetic changes, including DNA methylation, at the DM1 locus that result in reduced expression of the *DMPK* gene and upstream and downstream genes *SIX5* and *DMWD* (Klesert et al., 1997; Alwazzan et al., 1999; Eriksson et al., 2001). Disease-associated DNA methylation was first reported to occur within a region approximately 1kb upstream of the *DMPK* gene (Steinbach et al., 1998). However, this was a rather restricted study based on the use of methylation-sensitive restriction enzymes that do not identify all CpG sites. A more recent study, which used bisulphite sequencing to characterize the DM1 locus at higher resolution, confirmed the disease-associated DNA methylation upstream of the expanded CTG repeat and further identified a distinct boundary at the expanded CTG repeat beyond which there is no DNA methylation (Lopez Castel et al., 2010b) (Fig.1).

2.3 Friedreich Ataxia (FRDA)

Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative disorder caused by homozygous GAA repeat expansion within intron 1 of the *FXN* gene (Campuzano et al., 1996). The effect of the expanded GAA repeat is to reduce expression of the essential mitochondrial protein frataxin (Campuzano et al., 1997), which results in progressive spinocerebellar neurodegeneration and cardiomyopathy (Pandolfo, 2009). Unaffected individuals have *FXN* alleles containing 5-32 GAA repeats, there is a premutation range of 33-65 GAA repeats, and affected individuals have alleles of 66-1700 GAA repeats. Both intergenerational and somatic instability of the GAA repeat are evident in FRDA, with expanded GAA repeats occurring prominently in disease-related CNS tissue (De Michele et al., 1998; De Biase et al., 2007a, 2007b). There is no disease-associated change in the DNA methylation status of the CpG island that spans the *FXN* 5′-UTR and exon 1 regions. However, disease-associated DNA hypermethylation has been identified within a region of *FXN* intron 1 immediately upstream of the expanded GAA repeat in FRDA cell culture, FRDA patient tissues and FRDA mouse models (Greene et al., 2007; Al-Mahdawi et al., 2008). Furthermore, the level of DNA methylation in this region correlates with expanded GAA repeat size and inversely correlates with age of FRDA disease onset (Castaldo et al., 2008). Interestingly, DNA hypomethylation has been identified in the *FXN* intron 1 *Alu* repeat sequence (which is normally fully methylated) immediately downstream of the expanded GAA repeat (Al-Mahdawi et al., 2008) (Fig.1). This effect of demethylation may have some, as yet unknown, relevance for GAA repeat instability and frataxin expression.

2.4 Polyglutamine-encoding TNR expansion disorders

To date, eleven inherited disorders are known to be caused by expansion of CAG repeats within the coding region of genes, resulting in the production of abnormal proteins that have long stretches of polyglutamine repeats (Lopez Castel et al., 2010a). Included within this group of polyglutamine disorders are Huntington disease (HD) and the spinocerebellar ataxias (SCAs). In each case, the severity of disease correlates with the size of the expanded CAG repeat, which is subject to both intergenerational and somatic instability (Koefoed et al., 1998; Wheeler et al., 2007). There is currently no evidence to support a disease-associated role for DNA methylation in HD (Reik et al., 1993) or SCA3 (Emmel et al., 2011). However, DNA methylation has been implicated in stability of CAG repeats in SCA1 (Dion et al., 2008) and in increased instability of CAG repeats in SCA7 (Libby et al., 2008).

3. Causes of DNA methylation

DNA methylation is involved in human diseases such as cancer, imprinting disorders and inherited TNR disorders, but at present it is not known why certain CpG sequences succumb to disease-associated aberrant DNA methylation. When considering the causes of DNA methylation in the TNR disorders, distinction must be made between DNA methylation of the expanded CGG repeat itself in the fragile site disorders, such as FRAXA and FRAXE, and DNA methylation of the flanking CpG sites in both fragile site disorders and other TNR disorders, including DM1 and FRDA. In the case of expanded CGG repeat disorders, the CGG repeat contains CpG residues that may be subject to methylation by direct effects. Thus, expanded CGG repeats have been shown to form single-stranded hairpins that lead to slippage structures during replication. Unrepaired slippage structures that contain extrahelical and mispaired cytosines may then act as substrates for direct *de novo* methylation by DNA methyltransferase enzymes (Chen et al., 1995; Laayoun & Smith, 1995; Chen et al., 1998). On the other hand, a common theme for all of the large non-coding TNR expansion diseases is DNA hypermethylation of CpG dinucleotides in the local vicinity of the TNR expansion. This suggests the action of a unified, but as yet unknown, secondary molecular mechanism. Evidence in favour of both *cis*- and *trans*-acting secondary effects has been put forward. Thus, aberrant DNA methylation of expanded CGG repeats or CpG sequences flanking expanded TNR sequences may be based upon underlying *cis*-acting DNA sequence context. For example, there is evidence to suggest that methylation can spread from core repetitive DNA sequences (Yates et al., 1999), and particular motifs have been identified as candidates for methylation-targeting DNA sequences (Feltus et al., 2006).

Another potential mechanism, which could be either *cis*- or *trans*-acting, is the induction of DNA methylation by short interfering RNAs (siRNAs). Studies of human cells have shown that long CNG repeat hairpins can be cleaved by the ribonuclease Dicer to form short double-stranded siRNAs (Krol et al., 2007), which may then induce DNA methylation as a process of transcriptional gene silencing (Kawasaki & Taira, 2004; Morris et al., 2004). Bidirectional transcription across TNRs may also produce siRNAs, which then recruit histone methyltransferases, HP1 and DNA methyltransferases to result in DNA methylation, as proposed for a general model of heterochromatin formation at repetitive elements (Grewal & Jia, 2007). Alternatively, siRNAs targeted to gene promoter CpG islands may be produced by bidirectional transcription at these regions (Morris et al., 2008), and such bidirectional transcripts have indeed been identified at several TNR loci (Cho et al.,

2005; Moseley et al., 2006; Ladd et al., 2007; De Biase et al., 2009; Chung et al., 2011). Furthermore, DNA methylation at the TNR locus may be induced by *trans*-acting siRNAs that are generated from a different locus (Watanabe et al., 2011). In each case, DNA methylation is likely to be a later long-term gene silencing effect, following on from earlier increases in histone methylation (Hawkins et al., 2009). Interestingly, siRNA targeting of the huntingtin gene promoter has failed to induce DNA methylation (Park et al., 2004), agreeing with a lack of any evidence for DNA methylation induction by CAG repeat expansion (Reik et al., 1993).

Another general mechanism that may be involved in the formation of DNA methylation at TNR loci is the loss of a methylation-sensitive chromatin insulator and subsequent spreading of DNA methylation. Of particular note is the chromatin insulator protein CTCF (CCCTC-binding protein), since CTCF binding sites have been identified in the flanking regions of *FRAXA* CGG repeats (Ladd et al., 2007), *DM1* CTG repeats (Filippova et al., 2001) and *SCA7* CAG repeats (Libby et al., 2008), and also in the upstream region of *FXN* GAA repeats (De Biase et al., 2009) (Fig.2).

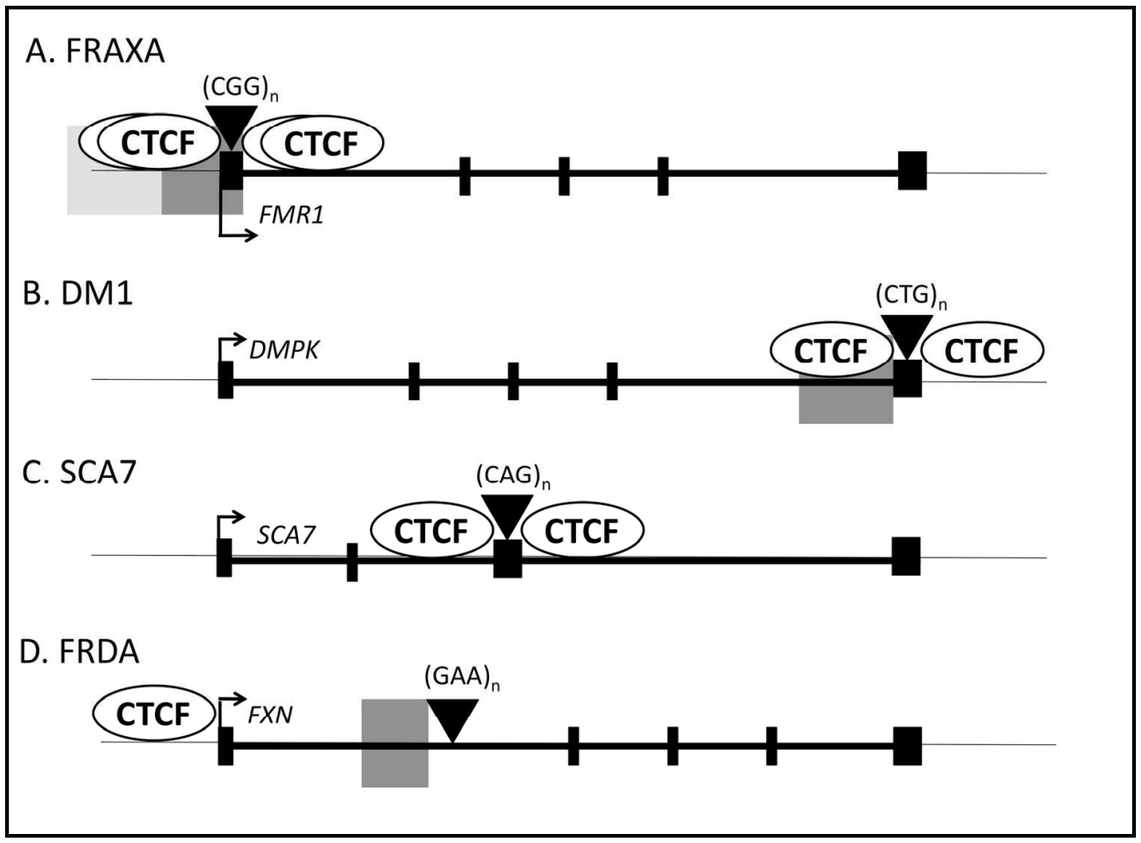


Fig. 2. The position of CTCF binding sites within TNR expansion loci (A) FRAXA, (B) DM1, (C) SCA7 and (D) FRDA. Grey boxes represent regions of DNA methylation.

A model has been proposed for the *DM1* locus whereby the normal CTG repeat allele is associated with bidirectional transcription, siRNAs, H3K9 dimethylation and HP1 recruitment in the region of the CTG repeats, but without any associated DNA methylation.

This local heterochromatin formation is limited to a small region by flanking CTCF sites and it is likely that CTCF binding protects the *DM1* CTG region from DNA methylation. However, expanded *DM1* CTG repeats are associated with loss of CTCF binding, spread of heterochromatin and regional CpG methylation (Cho et al., 2005). In FRDA, expanded GAA repeats are similarly associated with depletion of CTCF binding in the 5′-UTR of the *FXN* gene and associated hypermethylation of CpG sites just upstream of the GAA repeat. (Greene et al., 2007; Al-Mahdawi et al., 2008; De Biase et al., 2009). However, it is not currently known if depletion of CTCF actually precedes DNA methylation, or vice versa, in the context of TNR expansion diseases. For example, DNA methylation is known to inhibit CTCF binding at the *DM1* locus and other genetic loci (Filippova, 2008), whereas it does not appear to inhibit CTCF binding at the *FXN* locus (De Biase et al., 2009). Therefore, further studies will be required to determine the precise order of events that connect TNR expansions, CTCF binding and DNA methylation.

4. Effects of DNA methylation

There are two principal effects of DNA methylation on TNR expansion disorders. Firstly, silencing of gene transcription may take place, exerting profound effects on the subsequent disease phenotype. Secondly, modulation of TNR instability may occur throughout development and within different specific tissues, which will also impact upon progression of the disease phenotype.

4.1 Silencing of gene expression

The main effect of DNA methylation in TNR expansion disorders, as with other diseases such as cancer, is silencing of gene transcription. DNA methylation inhibits transcription by two general mechanisms: (i) preventing binding of basal transcription proteins or other regulatory DNA binding proteins (e.g. CTCF), and (ii) influencing nucleosome positioning or stability and reinforcing heterochromatin formation through the actions of methyl-CpG-binding proteins (MBPs), histone modifications and chromatin remodeling (Klose & Bird, 2006). There is evidence for both mechanisms at play in silencing of gene transcription in TNR expansion disorders. For FRAXA, expansion of CGG repeats in the 5′-UTR of the *FMR1* gene to greater than 200 units induces CpG methylation within the repeat tract and also the adjacent promoter region, leading to transcriptional silencing (Pieretti et al., 1991; Sutcliffe et al., 1992). Hypermethylation of the *FMR1* region is associated with histone deacetylation, H3K9 methylation and chromatin remodeling, which may impact upon *FMR1* transcription (Coffee et al., 1999; Coffee et al., 2002). However, it is suggested that DNA methylation, rather than histone modifications, is the key event for silencing of *FMR1* transcription (Pietrobono et al., 2002; Pietrobono et al., 2005). In contrast to FRAXA, the unmethylated 55-200 CGG repeats of the *FMR1* gene that characterize FXTAS produce a 2-10 fold increase of *FMR1* transcription, leading to an RNA toxic gain-of-function disease effect (Tassone et al., 2000). For DM1, the expanded CTG repeat, which is situated in the 3′-UTR of the *DMPK* gene, induces CpG methylation and H3K9 methylation flanking the repeat. This then silences transcription of *DMPK* and the neighbouring *SIX5* and *DMWD* genes (Klesert et al., 1997; Thornton et al., 1997; Alwazzan et al., 1999; Eriksson et al., 2001), likely by mechanisms that involve bidirectional transcription and siRNA formation (Cho et al., 2005). For FRDA,

the expanded GAA repeat within intron 1 of the *FXN* gene induces CpG methylation, histone deacetylation and H3K9 methylation in the region immediately upstream of the GAA repeat, but the *FXN* promoter appears to be unaffected (Greene et al., 2003; Herman et al., 2006; Al-Mahdawi et al., 2008). Since DNA methylation within the body of a gene has not been linked to transcriptional silencing (Brenet et al., 2011), it is unlikely that CpG methylation within intron 1 is the primary cause of *FXN* gene silencing. The increased DNA methylation within intron 1 of the *FXN* gene is more likely to be secondary to gene silencing caused by chromatin changes (Greene et al., 2007) or changes in bidirectional transcription and CTCF binding (De Biase et al., 2009).

4.2 Modulation of TNR instability

Another major effect of DNA methylation, specifically related to TNR expansion disorders, is the ability to influence the dynamics of the trinucleotide repeat stability. Both germline and somatic instability of TNR sequences are known to play major roles in the aetiology and progression of all TNR expansion disorders (Lopez Castel et al., 2010a). For FRAXA, germline instability of the CGG repeat involves maternally derived expansions, but deletion in the gametes of full-mutation males. The CGG deletions occur during replication and are dependent on replication fork dynamics, size of repeat and CpG methylation status (Nichol Edamura et al., 2005). The period of somatic CGG instability is restricted to early stages of embryonic and foetal growth and ends when expanded CGG sequences become abnormally methylated (Devys et al., 1992; Taylor et al., 1999). Subsequent CpG methylation of FRAXA 'full mutation' expanded CGG repeats causes somatic stability (Wohrle et al., 1995). Several studies, which have examined the effect of DNA methylation on germline TNR instability using mouse models of DM1, HD and SCA1, find that instability is particularly associated with periods of demethylation in the developing germline (Kaytor et al., 1997; Kovtun & McMurray, 2001; Savouret et al., 2004). Furthermore, treatment of cultured cells from DM1 patients with DNA demethylating compounds produced destabilization of CTG repeats, with a bias towards expansion (Gorbunova et al., 2004). Thus, it appears that changes in DNA methylation patterns during germline epigenetic reprogramming may trigger the intergenerational TNR expansions that lead to disease. It is currently not known how DNA methylation stabilizes germline TNR sequences. However, several hypotheses have been proposed. For example, a recent study of heterozygous DNA methyltransferase 1 (*Dnmt1*$^{+/-}$) knockout SCA1 mice revealed Dnmt1-deficient promotion of CAG intergenerational instability, together with aberrant DNA methylation and histone methylation within the CpG island adjacent to the CAG repeat, suggesting a role for local chromatin structure in germline TNR instability. However, no effect of Dnmt1 deficit was seen on somatic instability (Dion et al., 2008). Another study, which investigated somatic instability of the *DM1* CTG repeat in relation to replication and CTCF binding, has led to the suggestion that CpG methylation may regulate, in a tissue-specific manner, the role of CTCF in DNA replication and thereby CTG repeat instability (Cleary et al., 2010). Yet another connection between DNA methylation, CTCF and TNR expansion has been identified in studies of *SCA7* transgenic mice, which revealed further destabilization of unstable expanded CAG repeats by CpG methylation of CTCF binding sites (Libby et al., 2008). Furthermore, other studies suggest a potential connection between DNA methylation, transcription and TNR instability. For example, cyclical changes in promoter CpG methylation have been identified

during transcription (Kangaspeska et al., 2008; Metivier et al., 2008), and at the same time, transcription through the repeat tract has been identified as a major contributor to expansion of GAA repeats (Ditch et al., 2009). Therefore, periods of active demethylation during transcription may present a window of opportunity for TNR expansion. Finally, several studies have highlighted an important role of DNA repair mechanisms, and in particular the mismatch repair (MMR) system, in TNR instability (Savouret et al., 2003; Wheeler et al., 2003; Dragileva et al., 2009), and DNMT1 deficiency has been shown to result in MMR defects that increase the rate of CAG repeat contraction (Lin & Wilson, 2009). Therefore, it may be interesting to further pursue connections between DNA methylation, DNA repair and TNR instability.

5. Demethylation therapy for TNR expansion diseases

The finding that DNA methylation of the CGG repeat and flanking CpG sequences of the *FMR1* promoter cause transcriptional silencing of the *FMR1* gene in FRAXA, has lead to consideration of DNA demethylation as a potential therapy. To date, investigations have focused on the use of the cytidine analogue DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine (5-aza-CdR), which is an FDA approved drug (decitabine) used for the treatment of myelodysplastic anaemia (Oki et al., 2007). Treatment of fully methylated FRAXA patient cell lines with 5-aza-CdR leads to a decrease in promoter CpG methylation, together with increased histone acetylation, decreased H3K9 methylation and increased H3K4 methylation, that result in an increase in *FMR1* transcription (Chiurazzi et al., 1998; Pietrobono et al., 2002; Tabolacci et al., 2005). Combined treatment with 5-aza-CdR and histone deacetylase (HDAC) inhibitors have also been shown to produce a synergistic increase in *FMR1* transcription (Chiurazzi et al., 1999). However, 5-aza-CdR is a drug that induces substantial cytotoxicity, and therefore the development and testing of other less toxic DNA methylation inhibitors, such as zebularine (Cheng et al., 2003) or hydralazine (Cornacchia et al., 1988) may be necessary before treatment for FRAXA can be considered further. Another potential adverse effect of DNA methylation inhibitor treatment that will have to be considered is the finding that DNA demethylation can induce TNR instability, with a bias towards expansions (Gorbunova et al., 2004), which may then negatively impact upon gene expression.

6. Conclusions

DNA methylation is a molecular process that is clearly associated with TNR expansion disorders, particularly those of the long non-coding repeat type. Recent studies have revealed common themes for TNR gene silencing, including bidirectional transcription, siRNA formation, CTCF binding, histone modifications and chromatin remodeling. However, the exact role that DNA methylation plays within TNR expansion disease pathogenesis remains uncertain and further investigations are still needed. At the same time, DNA methylation also appears to impact upon TNR instability, which is an important part of TNR expansion disease progression. Therefore, the interplay between DNA methylation, DNA replication, DNA repair and transcription will need particular investigation if future consideration can realistically be given to DNA demethylation therapies for TNR expansion disorders.

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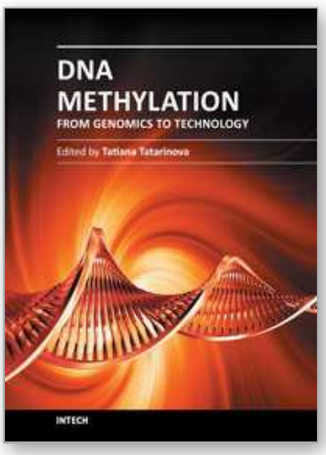
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Epigenetics is one of the most exciting and rapidly developing areas of modern genetics with applications in many disciplines from medicine to agriculture. The most common form of epigenetic modification is DNA methylation, which plays a key role in fundamental developmental processes such as embryogenesis and also in the response of organisms to a wide range of environmental stimuli. Indeed, epigenetics is increasing regarded as one of the major mechanisms used by animals and plants to modulate their genome and its expression to adapt to a wide range of environmental factors. This book brings together a group of experts at the cutting edge of research into DNA methylation and highlights recent advances in methodology and knowledge of underlying mechanisms of this most important of genetic processes. The reader will gain an understanding of the impact, significance and recent advances within the field of epigenetics with a focus on DNA methylation.

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University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
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InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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