Acta Biochim Biophys Sin, 2015, 47(7), 567–569
doi: 10.1093/abbs/gmv038
Advance Access Publication Date: 24 May 2015
New Phenomenon

New Phenomenon

d(GC)$_n$ repeats form Z-DNA within promoter region and repress the promoter activity in *Escherichia coli*

Shenghe Huang[1], Chuxin Wu[2], Dongming Li[1], Haizhou Wang[3], Zechang Rao[1], Qiling Shen[1], Chunxiang Chen[1], Yong Liu[3], Xun Xu[1], and Chengyu Hu[3,*]

[1]Fuzhou Medical College, Nanchang University, Fuzhou 344000, China, [2]Nanchang Teachers College, Nanchang 330103, China, and [3]Department of Bioscience, College of Life Science, Nanchang University, Nanchang 330031, China

*Correspondence address. Tel: +86-791-88317270; Fax: +86-791-83969530; E-mail: hucy2008@163.com

Z-DNA is a left-handed helical form of DNA in which sugar-phosphate backbone winds in a zig–zag pattern. DNA containing alternating purine and pyrimidine repeats has the potential to adopt the Z-DNA formation. *In vivo*, Z-DNA is formed and stabilized by negative supercoil or specific Z-DNA-binding proteins. Z-DNA-forming sequences are widespread in cells, especially in the promoter regions and centromere of chromatins, so it is thought to be involved in some biological processes such as gene expression, genetic instability, and DNA processing events [1]. Evidence about the effects of Z-DNA on gene transcription regulation is accumulating. Many studies showed that Z-DNA is closely related to gene transcription. On one hand, gene transcription can induce Z-DNA conformation because this process results in negative supercoiling behind a moving RNA polymerase, and Z-DNA form is facilitated at permissive regions. Three fragments of *c-MYC* promoter were found to bind to Z-DNA antibody only when the gene was actively transcribed [2]. On the other hand, Z-DNA in promoter can regulate gene transcription activity. Rat nucleolin (*Ncl*) promoter activity is increased by 36%–54% when Z1 [$(CA)_{10}(CG)_8$] (at −631 site) was deleted [3]. However, Z-DNA-forming sequence (at −815 site) increases the transcription level of *Escherichia coli gyrA* gene [4]. Therefore, the mechanism how Z-DNA sequence within promoter region modulates gene transcription is complicated, and the effect may be associated with the distance between Z-DNA sequence and certain *cis*-acting element. The extent of up-regulation is determined by its separation distance relative to TATA box in *CYC1* promoter in yeast [5]. It is possible that Z-DNA might regulate gene transcription by various approaches.

To test whether Z-DNA-forming sequences could play a regulatory role in gene transcription, a 125 bp fragment from *mreB* (*β-actin*) promoter P3 (constitutive promoter) was cloned and plasmid pET-Pm-luc was constructed (**Supplementary Fig. S1**). Overlapping PCR was performed for displacing relevant oligonucleotides with d$(GC)_5$ or d$(GC)_{10}$ at −70, −45, −20, and +15 sites from the transcription start site (TSS) (**Supplementary Fig. S2**) of *mreB* promoter P3. PCR product was subcloned into *Bgl*II/*Sal*I sites of pET-Pm-luc vector to replace *mreB* promoter P3. The primers used in this study are listed in **Supplementary Table S1**. Vectors were transformed into *E. coli* BL21 cells using heat shock method as described in *Molecular Cloning* (3rd edition, 2001). Cultures of the transformed *E. coli* cells were diluted from ~0.7 to 0.5 of OD_{600}. Luciferase activity was measured on a Hitachi fluorescence spectrophotometer. When the number of repeats in d$(GC)_n$ was increased, the inhibitory effect was more obvious (**Fig. 1A**); the inhibitory effect of d$(GC)_5$ replaced at −45 and +15 sites of *mreB* promoter was much weaker than that of d$(GC)_{10}$ (**Fig. 1A**). Furthermore, the effects of the identical d$(GC)_n$ substituted at different sites of promoters on gene transcription were different. For instance, other than that at −45 and +15 sites, d$(GC)_5$ or d$(GC)_{10}$ substituted at −70 and −20 sites was inefficient on the promoter activity of *mreB* (**Fig. 1A**). These results suggested that effects of d$(GC)_n$ on promoter activity were correlated with the length of d$(GC)_n$ and substitution sites.

Similar experiments were carried out for a 361 bp fragment from *dnaK* (*hsp70*) promoter P1 (inducible promoter) at −290, −180, −55, −20 and +20 sites from TSS (**Supplementary Figs. S1** and **S2**). But *E. coli* cells with vectors were divided into two parts after being cultured at 30°C to $OD_{600} \approx 0.55$. One part was cultured at 30°C until $OD_{600} \approx 0.7$ and another part was cultured at 42°C for 20 min. All cultures were diluted to $OD_{600} = 0.5$. The results showed that d$(GC)_{10}$ tremendously suppressed *dnaK* promoter activity at each site substitution. The repression effect was most remarkable at −20 site substitution, followed by −55 site substitution (**Fig. 1B**). Furthermore, the expression of luciferase gene was increased after heat shock at 42°C

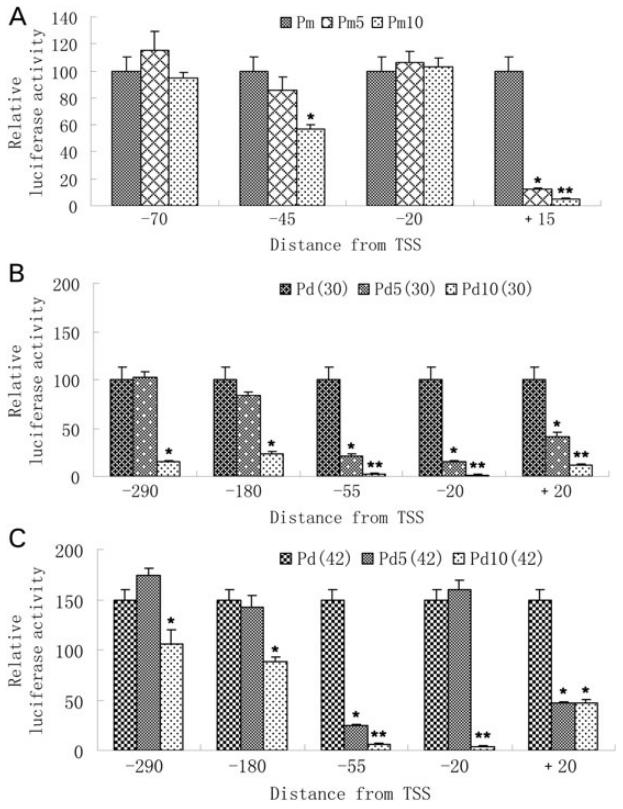

Figure 1. The down-regulation of the promoter activity of *mreB* and *dnaK* by d(GC)$_5$ and d(GC)$_{10}$ (A) The variation of *mreB* promoter activity. (B) The variation of *dnaK* promoter activity; *E. coli* were cultured at 30°C. (C) The variation of *dnaK* promoter activity; *E. coli* were cultured at 42°C for 20 min after growth at 30°C. The results represent an average of three independent experiments. Pm, *mreB* promoter P3; Pm5, *mreB* promoter P3 with d(GC)$_5$; Pm10, *mreB* promoter P3 with d(GC)$_{10}$; Pd, *dnaK* promoter P1; Pd5, *dnaK* promoter P1 with d(GC)$_5$; Pd10, *dnaK* promoter P1 with d(GC)$_{10}$. *P < 0.05; **P < 0.01.

at each site substitution (**Fig. 1C**). Meanwhile, the inhibitory effects caused by d(GC)$_{10}$ were clearly alleviated at −290, −180, and +20 site substitution after heat shock (**Fig. 1C**). When *E. coli* cells were cultured at 30°C, d(GC)$_5$ reduced *dnaK* promoter activity at −55, −20, and +20 site substitution (**Fig. 1B**). Interestingly, the inhibitory effect caused by d(GC)$_5$ occurred only at −55 and +20 site substitution rather than at −20 site substitution after heat shock (**Fig. 1C**).

The alternating purine–pyrimidine tracts have been shown to inhibit the expression of the tRNAPro gene from *Caenorhabditis elegans* when placed in the flanking regions of the gene or in the promoter, because Z-DNA formation can affect transcriptional activity by interfering with the binding of required transcription factors [1]. In other words, Z-DNA may regulate gene expression, which perhaps is dependent on its location. Thus, the original sequences were substituted with d(GC)$_n$ at some specific sites of *mreB* and *dnaK* promoters, such as −290 site at the distant end, −180 site next to heat shock response element, −55 site beside sexfama box, and −20 site between sexfama box and pribnow box (**Supplementary Fig. S2**).

Gene transcription requires transient melting of DNA duplex in the promoter, and this process can be facilitated by local negative supercoiling stress [6]. However, a left-handed helical turn of Z-DNA can relieve 1.8 helical turns of right-handed negative superhelical twisting [1] and Z-DNA structure can absorb the supercoiling stress that is required for promoter activation, thereby repressing *ilv*P$_G$ promoter activity (Z-DNA sequence at −449 site) [4]. Likewise, d(GC)$_{10}$ sequence at −45 site from TSS reduced *mreB* promoter activity (**Fig. 1A**) and inhibited *dnaK* promoter activity at −290, −180, −55, and −20 sites from TSS (**Fig. 1B,C**). Moreover, partial transcription blockage with T7 RNA polymerase is detected within d(CG)$_{14}$ sequence at +252 site from TSS *in vitro* because Z-DNA conformation pauses or terminates upon RNA polymerase movement into this sequence when transcription elongation is in progress [7]. Similarly, d(GC)$_n$ at +15 site of *mreB* promoter and +20 site of *dnaK* promoter interfered with the expression of luciferase gene *in vivo* (**Fig. 1**). However, it is not efficient when d(GC)$_{10}$ is at −70 and −20 sites of *mreB* promoter (**Fig. 1A**). This variable response is probably resulted from the intrinsic affinity of each promoter DNA sequence for transcription apparatus and may be associated with the location and/or intensity when transcription factors recognize and bind to promoter [1].

In *E. coli*, *rpoH* translation is rapidly derepressed and σ^{32} becomes transiently stabilized upon temperature up-shift from 30°C to 42°C. Then, σ^{32} recognizes the correlative promoter, and the synthesis of heat shock proteins is rapidly induced. Z-DNA form may alter the recognizing and binding activity of transcriptional regulators to the promoter [8]. Our results indicated that temperature up-shift contributes to overcome the barriers of Z-DNA for temperature-induced promoter and Z-DNA down-regulates *dnaK* promoter activity in a temperature-dependent manner (**Fig. 1B,C**).

Meanwhile, the inhibition of methylation at *Hha*I sites in vectors was used to determine the Z-DNA conformation [9]. Methylase *Hha*I is able to methylate C-5 position of cytosine in d(GCGC) sequences, resulting in the inhibition of cleavage at *Hha*I sites. If the sequence is in Z-DNA conformation, methylation will be restrained, and it will be cleaved at *Hha*I sites; while if it is in B-DNA form when incubated with *Hha*I methylase, it will be methylated and stay intact [9]. In this study, there are 52 *Hha*I sites in pET-Pm-luc or pET-Pd-luc in addition to d(GC)$_n$ sequence. Many fragments were cleaved in plasmids by *Hha*I in the absence of *Hha*I methylase (**Supplementary Fig. S3**, lanes 3, 6, 9, 12, and 15). After pre-incubation with methylase *Hha*I, recombinant plasmids were mostly cleaved in linear by *Hha*I (**Supplementary Fig. S3**, lanes 5, 8, 11, and 14). Served as control, plasmids containing completely methylated GCGC sequences remained intact (**Supplementary Fig. S3**, lane 2). In lanes 5, 8, 11, and 14, plasmids with d(GC)$_{10}$ or d(GC)$_5$ had a band of supercoiling plasmid below linear DNA because of incomplete digestion of Z-DNA sequences or instability of Z-DNA conformation and methylation of these sites. These results indicated that the d(GC)$_5$ and d(GC)$_{10}$ in the plasmids could form a potential Z-DNA conformation. Moreover, the vectors containing d(GC)$_{10}$ could be liable to adopt Z-DNA form than those containing d(GC)$_5$ (**Supplementary Fig. S3**), and d(GC)$_{10}$ might repress the transcription activity of *mreB* and *dnaK* promoter more significantly than d(GC)$_5$ (**Fig. 1**). So, the repression effect of Z-DNA on gene transcription may be more obvious with the increasing tendency of B-DNA to Z-DNA conformational transition.

In conclusion, d(GC)$_n$ within promoter regions may form Z-DNA conformation and repress *mreB* and *dnaK* promoter activity in *E. coli* cells, and the inhibitory effects are dependent on Z-DNA-forming location and Z-DNA conformational stability. Future studies are needed to detect the Z-DNA conformation of vectors *in vivo* and to explore the molecular mechanism of transcriptional regulation by Z-DNA.

Supplementary Data

Supplementary data is available at *ABBS* online.

Funding

This work was supported by the grants from the National Natural Science Foundation of China (Nos. 31360515 and 31472304) and the Science and Technology Project of Fuzhou Medical College (No. CDFY-KJ1402).

References

1. Wang G, Vasquez KM. Z-DNA, an active element in the genome. *Front Biosci* 2007, 12: 4424–4438.
2. Wittig B, Wölfl S, Dorbic T, Vahrson W, Rich A. Transcription of human c-myc in permeabilized nuclei is associated with formation of Z-DNA in three discreteregions of the gene. *EMBO J* 1992, 11: 4653–4663.
3. Rothenburg S, Koch-Nolte F, Rich A, Haag F. A polymorphic dinucleotide repeat in the rat nucleolin gene forms Z-DNA and inhibits promoter activity. *Proc Natl Acad Sci USA* 2001, 98: 8985–8990.
4. Sheridan SD, Opel ML, Hatfield GW. Activation and repression of transcription initiation by a distant DNA structural transition. *Mol Microbiol* 2001, 40: 684–690.
5. Wong B, Chen S, Kwon JA, Rich A. Characterization of Z-DNA as a nucleosome-boundary element in yeast *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* 2007, 104: 2229–2234.
6. Wang JC, Lynch AS. Transcription and DNA supercoiling. *Curr Opin Genet Dev* 1993, 3: 764–768.
7. Ditlevson JV, Tornaletti S, Belotserkovskii BP, Teijeiro V, Wang G, Vasquez KM, Hanawalt PC. Inhibitory effect of a short Z-DNA forming sequence on transcription elongation by T7 RNA polymerase. *Nucleic Acids Res* 2008, 36: 3163–3170.
8. Thomas T, Kiang DT. A twenty-two-fold increase in the relative affinity of estrogen receptor to poly(dA-dC).poly(dG-dT) in the presence of polyamines. *Nucleic Acids Res* 1988, 16: 4705–4720.
9. Zacharias W, Larson JE, Kilpatrick MW, Wells RD. *Hha*I methylase and restriction endonuclease as probes for B to Z DNA conformational changes in d(GCGC) sequences. *Nucleic Acid Res* 1984, 12: 7677–7692.