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Regulation of transcription by bent DNA through chromatin structure

Review Article

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Summary

A class of bent DNA is present in the genomic DNA of higher eukaryotes as a repeating unit (Kiyama, R., Gene Ther. Mol. Biol. 1, 641-647, 1998). This bent DNA appears once every 680 bp on average, and often shows periodicity, suggesting biological significance. By having a higher affinity for histone core particles, it has a role in designating a key nucleosome, which initiates nucleosome alignment. This modulates the enhancer activity of β-LCR and the silencer activity of the human β-globin locus and other loci. This suggests that this type of bent DNA plays important roles in various biological functions by affecting chromatin structure.

I. Periodic bent DNA

Recent advances in genome researches have made an extensive progress in the technology for identifying genes. A high attention, although less than that for identifying genes, has also been paid for the regulatory elements in the promoter regions of these genes. However, there are still a number of elements on the genomic DNA that control functions of the cell, and many exist as cryptic codes, i. e. elements that cannot be identified only from nucleotide sequence data. These include enhancers, silencers, insulators, matrix attachment regions and replication origins (Wolffe, 1995). Although the most advanced computer programs for gene identification have 80 ~ 90% accuracy in prediction or higher, the most reliable methods by which to identify these cryptic codes are experimental assays. One of the codes that are difficult to predict is the one for higher order chromatin structure. The difficulty in prediction of these codes is based mostly on the ambiguity of sequence-function relationships. There are many variations in the sequences of enhancers, for example, which confer specificity to species, genes and timing of expression.

It was a time of excitement when we first observed a very beautiful periodicity of DNA bend sites in the human ε-globin gene region (Wada-Kiyama & Kiyama, 1994; see **Fig. 1**). One of the reasons for this was that we were able to find an orderly entity within a not-well-ordered sequence, although it is not completely random. This was made possible only by assays based on biophysical properties of the structure within polyacrylamide gels, including the circular permutation assay (Wu & Crothers, 1984). This is still true now despite the availability of highly sophisticated computer programs. However, finding the relevance or significance of this entity, and especially proving it experimentally, which are not so simple, have not yet been completed. Here, we summarize the potential functions of these bend sites and the evidence to support them.

II. Signal for nucleosome alignment

DNA bend sites appear regularly and sometimes nearly periodically, as in the case of the ε-globin gene, at an average interval of 680 bp in the human β-globin locus as well as other loci (Kiyama, 1998; Wada-Kiyama *et al.*,

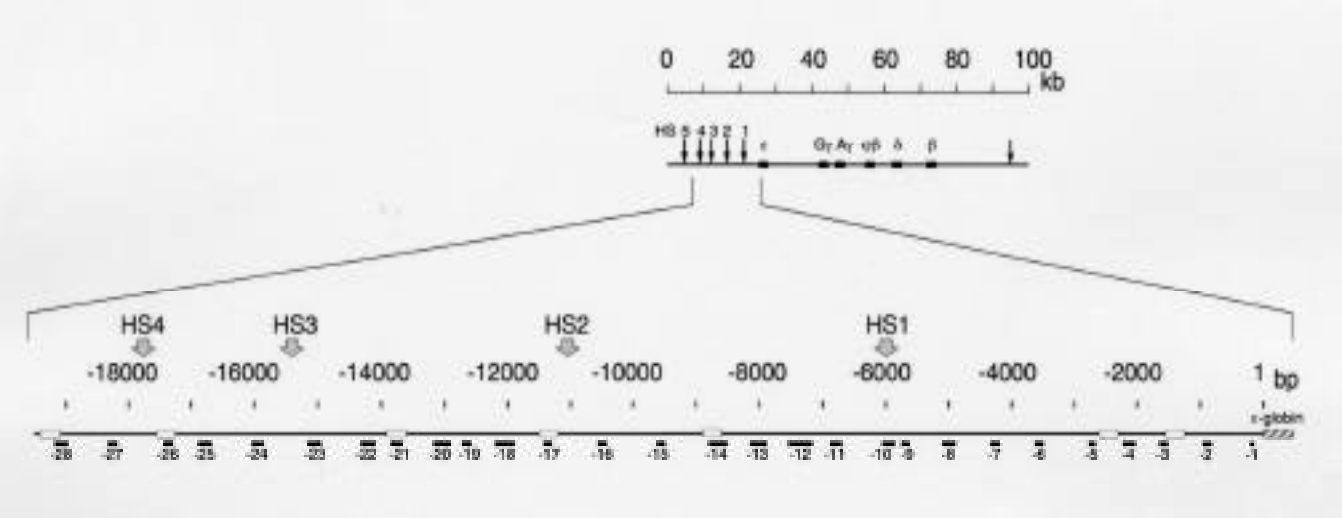

Fig. 1. The human β-globin locus and the positions of DNA bend sites in β-LCR. HS: DNase I hypersensitive site. The mapped bend sites (-1 to –28, solid boxes) are shown at the bottom. Note the periodicity between –1 and –9.

1999a). This interval length of 680 bp corresponds to that of four nucleosomes. Interestingly, this 680 bp interval actually exists in the human chromosomal DNA as alphoid satellite DNA occupying ~3% of the genomic DNA (Wu & Manuelidis, 1980), and therefore, is not unfamiliar as a unit of chromatin structure. In African green monkey genomic DNA, on the other hand, the equivalent of the human repeat family, α satellite DNA, is present at ~24% (Musich *et al.*, 1980) and this is the major structural component that determines the chromatin structure in this species. This 680 bp unit is further divided into four subunits ~170 bp in length, which are the nucleosome units in this repeat family. These DNA bend sites have a high affinity for histone core particles and their deletion results in disappearance of not only the phase at the bend site but also those in the immediate vicinity (Wada-Kiyama & Kiyama,1996; Onishi *et al.*, 1998). Furthermore, the positions of the sites have a good correlation with the positions of the dyad axis of 146 bp nucleosomal DNA (Wada-Kiyama *et al.*, 1999b). These lines of evidence support the idea that periodic bent DNA plays a key role in chromatin folding or nucleosome formation.

In addition to the rotational and translational positioning of nucleosomes, there is a third determinant for nucleosome position on the chromosomes: alignment of nucleosomes. Since chromosomal DNA in general is favorable in binding with core histones to form nucleosomes and each base pair has different affinities, there should be preference in binding driven by the differences in the nucleotide sequences of the DNA. Then, if one of the histone core particles occupies a space on DNA by having a higher affinity, there is no way of occupying the overlapping region by other particles. This indicates that overall alignment should be determined not only by rotational and translational positions of each nucleosome but also by a preference in binding among nucleosomes in the region. This led to the suggestion of the alignment *de facto* by key nucleosomes (Onishi *et al.*, 1998).

Determination of the best way to obtain perfect alignment with the least number of signals would facilitate our understanding of the role of key nucleosomes. It is not difficult to imagine that each nucleosome does not need its own positioning signal, because when every other nucleosome has a signal all positions are eventually determined. However, this will not be the case if every third or fourth are determined. To resolve this, it is necessary to know two parameters: the cooperativity of core histones and the force of random mutation. The former will result in preferential localization of nucleosomes next to the key nucleosomes, including preferential dinucleosome formation, and create more freedom for sequence variation, as the force becomes larger. The latter force, in contrast, decreases the chance of retaining the signal and increases the chances of sequence variation. A survey of more than 100 mapped bend sites indicated that four nucleosomes is the average unit. This suggests that both of the positions next to the key nucleosomes will be determined by the signal and one remaining position located just in the middle of the two neighboring key nucleosomes will be uniquely determined as a result.

This alignment of nucleosomes might have some effect on biological reactions and two such examples where the presence of these bend sites is closely associated with *cis*-acting elements in transcription are shown below.

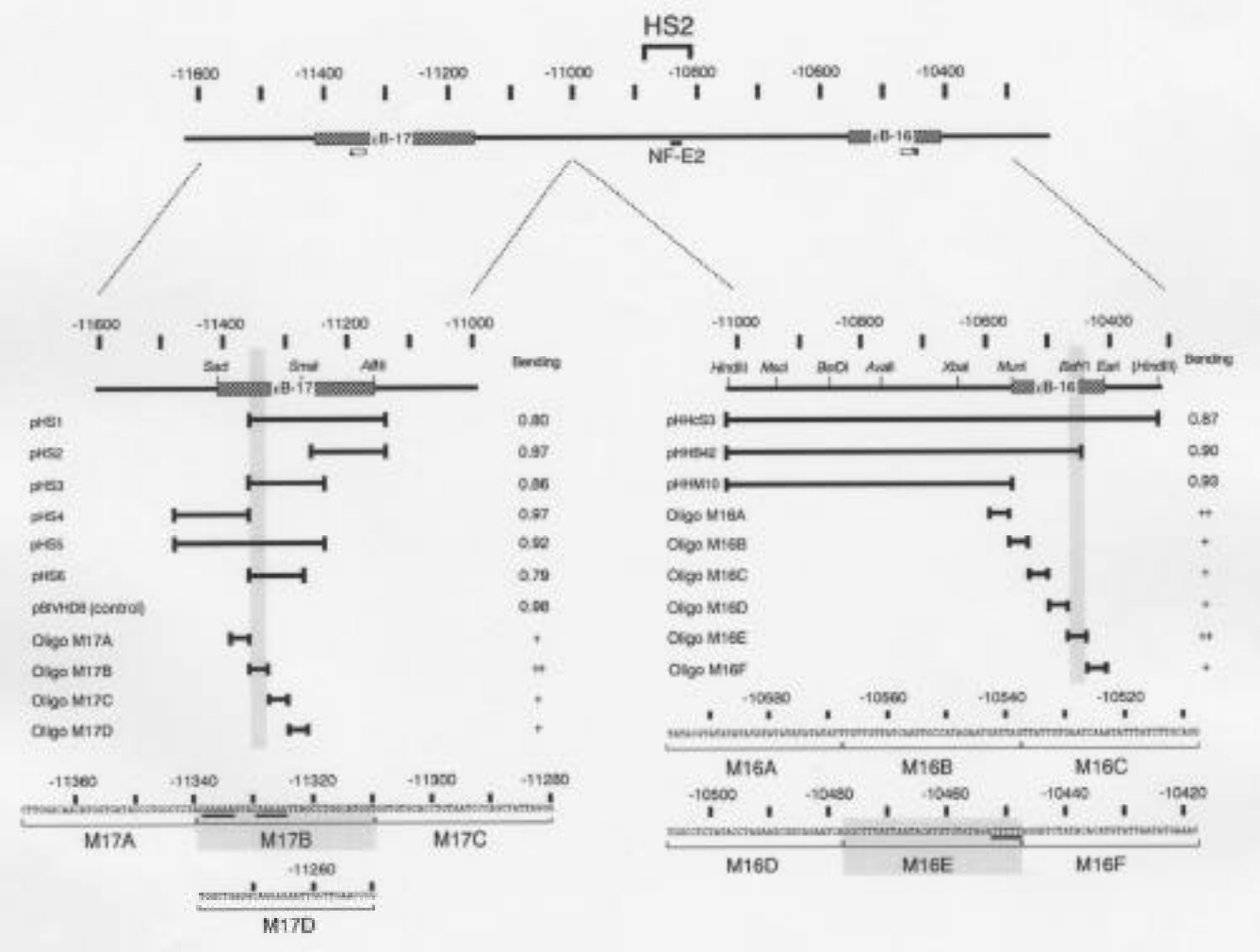

Fig. 2. DNA bend sites at HS2 of β-LCR. Two DNA bend sites of the region, εB-16 and εB-17, were determined by circular permutation assay. DNA bend centers of these sites were determined by assays first with plasmids containing deletions and then with oligonucleotides. The highest degrees of bending were shown on the right for plasmid assays and the locations are shaded. The regions used for the bending assay with the concatenated oligonucleotides M16A to M16F and M17A to M17D, and their nucleotide sequences are shown below. A T5 tract in εB-16 and two A5 tracts in εB-17, which are most likely the bend centers, are shown by horizontal lines. For more details, see Onishi *et al.,* 1998.

III. Modulator of enhancer activity

The first example is the enhancer of the human β-globin locus, which is located 11 kb upstream of the most upstream gene, the ε-globin gene, among the five (ε, ^{G}γ, ^{A}γ, δ, and β) active genes in the locus (Stamatoyannopoulos *et al.,* 1994). The enhancer is located within the region HS2, which shows tissue-specific DNase I-hypersensitivity, and contains the binding site for an erythroid-specific transcription factor, NF-E2 (reviewed in Hardison *et al.,* 1997). The enhancer was mapped between two DNA bend sites the distance between which was longer than the average and can accommodate five nucleosomes (**Fig. 2**). The nucleosomes in this region were regularly phased except that located in the middle which corresponded to the precise location of HS2 and included the binding site for NF-E2 (Region II, **Fig. 3**). Several phases were adopted in this region in the reconstituted chromatin and in erythroid K562 cells where the globin genes are expressed, whereas only one phase was adopted in non-erythroid HeLa cells. Meanwhile, almost unique phases were adopted at the flanking bend sites *in vitro* as well as *in vivo* (Regions I and III). These observations suggest that HS2 is placed at a region of weak nucleosome phasing activity along with factor binding sites and could be influenced by the nucleosomal phases determined by those located at the bend sites (**Fig. 4**).

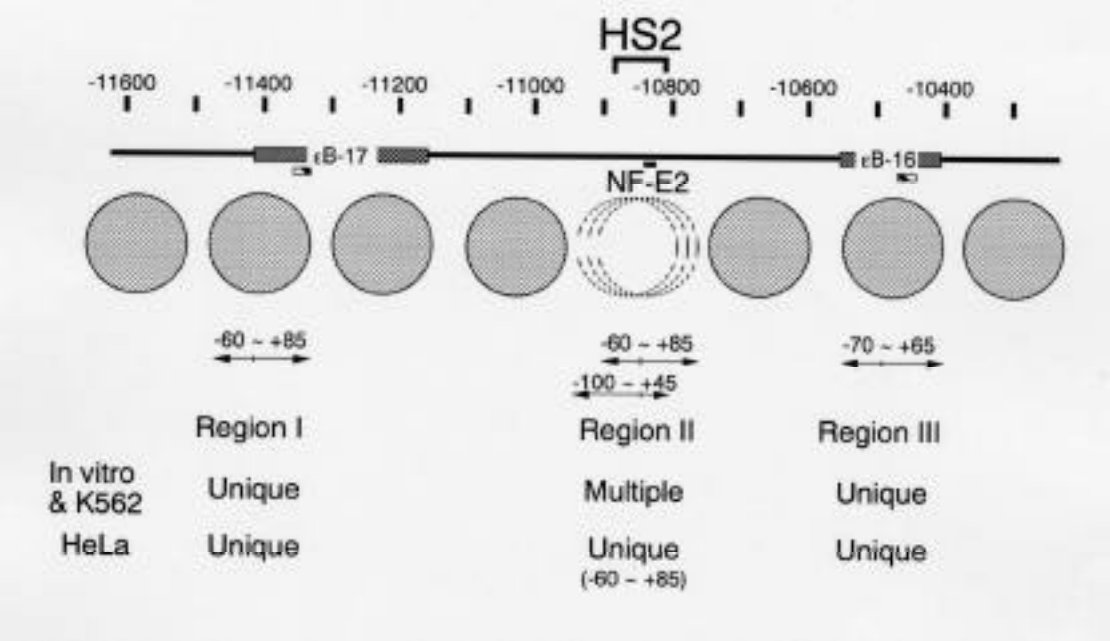

Fig. 3. *In vitro* and *in vivo* nucleosomal phases at HS2. Nucleosomal phases were determined by restriction analysis of the ~146-bp core DNA fragments for all nucleosomes or by inverse PCR for Regions I to III with reconstituted chromatin (*in vitro*) and with nuclei isolated from K562 and HeLa cells (*in vivo*). The relative positions of nucleosome boundaries from nucleotides –11,417 (Region I), -10,848 (Region II) and –10,496 (Region III) are shown in the figure. For more details, see Onishi *et al.*, 1998.

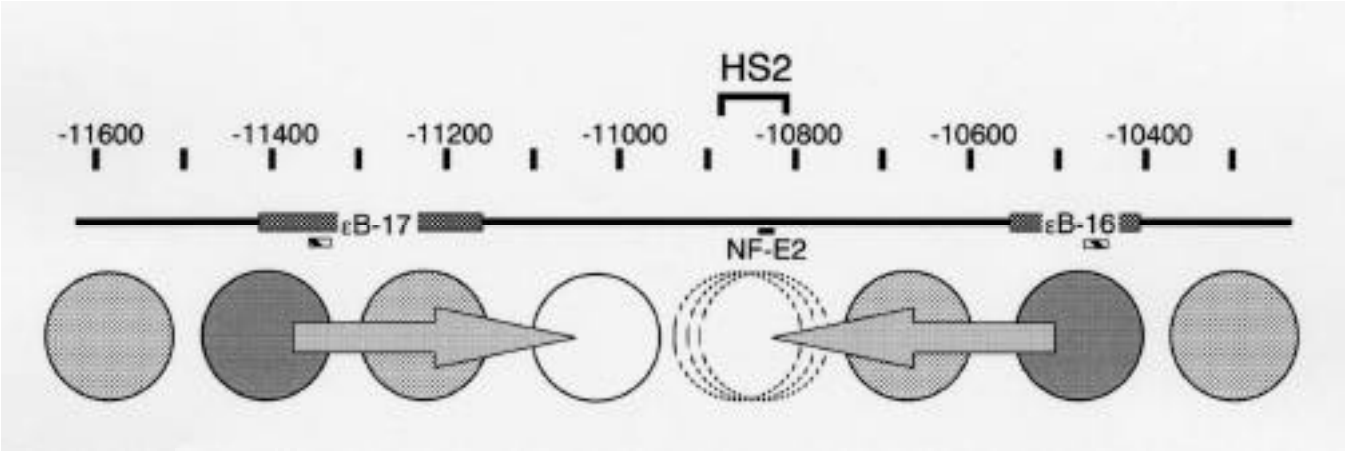

Fig. 4. Effects of the key nucleosomes at εB-16 and εB-17.

Interestingly, removing εB-16 or changing the distance between εB-16 and NF-E2 site affected transcription efficiency, while removing εB-17 did not (Onishi *et al.*, unpublished results). This can be explained if the effect of the bend sites reaches the next and the second closest nucleosomes but not the third closest, and supports the idea of four nucleosomes as a unit.

IV. Modulator of silencer activity

The other example is the silencers, which are present in the promoter regions of the β-like globin genes. The best characterized silencer in this locus is located 200 to 300 bp upstream of the ε-globin gene (Wada-Kiyama *et al.*, 1992) and overlaps the first DNA bend site from the cap

site (**Fig. 5A**). This co-localization of the silencer or repressor activity was observed in the β-globin gene (**Fig. 5B**), and also in other genes including the erythropoietin receptor (**Fig. 5C**), c-*myc* and estrogen receptor genes (see Lang *et al.*, 1991, DeConinck *et al.*, 1995, Ohki *et al.*, 1998, Wada-Kiyama *et al.*, 1999b). Furthermore, co-localization was observed among all of the human β-like globin genes and the globin genes of the other species where the locations of the first bend site upstream from the cap site are conserved (Wanapirak *et al.*, unpublished results). Although these sites contain transcription factor binding sites and binding sites for less well characterized proteins, the mechanism of silencing gene expression is not clear. Therefore, co-localization of DNA bend sites with silencers could yield clues for understanding the mechanism of regulation.

V. Other functions

Although experimental evidence is lacking, these bend sites could have functional relationships with other functions through regulation of nucleosome positioning. One such example is recombination. As discussed previously (Kiyama, 1998; Ohki *et al.*, 1998), the continuity of periodic bent DNA was conserved after rearrangement between the c-*myc* and immunoglobulin genes. Recombination is likely to be mediated by specific proteins that interact with DNA through chromatin, and thus chromatin structure is an important factor that determines the recombination efficiency in that the structure at least affects accessibility of the proteins to the binding sites. Transcription factors and the integrases require specific nucleosome positions when they recognize the motifs located within the nucleosome structure (Pina *et al.*, 1990; Pruss *et al.*, 1994). Determination of the precise nucleosome structure based on X-ray diffraction analysis and establishing databases of nucleosome positions will provide more insight into the biological relevance of periodic bent DNA.

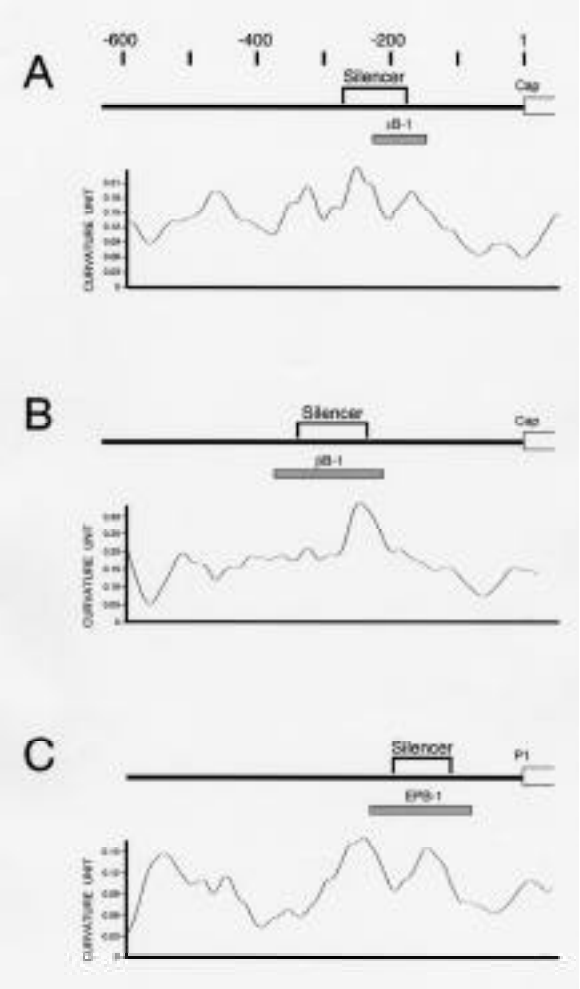

Fig. 5. Co-localization of DNA bend sites with the silencers of the ε-globin (**A**), β-globin (**B**) and erythropoietin receptor (**C**) genes. Silencer activities were found between -274 and -177 bp (ε-globin; Wada-Kiyama *et al.*, 1992), between -338 and -233 bp (β-globin; Berg *et al.*, 1989) or between -194 and -116 bp (erythropoietin receptor; Chin *et al.*, 1995) from the cap sites and overlapped the bend sites located between -221 and -142 bp (εB-1; Wada-Kiyama and Kiyama, 1994), between –373 and -210 bp (βB-1; Wada-Kiyama & Kiyama, 1995) or between -517 and -289 bp (EPB-1; Rodley *et al.*, 1998). These were included in the regions with several peaks in the curvature plots by TRIF analysis (Shpigelman *et al.*, 1993; Wada-Kiyama *et al.*, 1999a).

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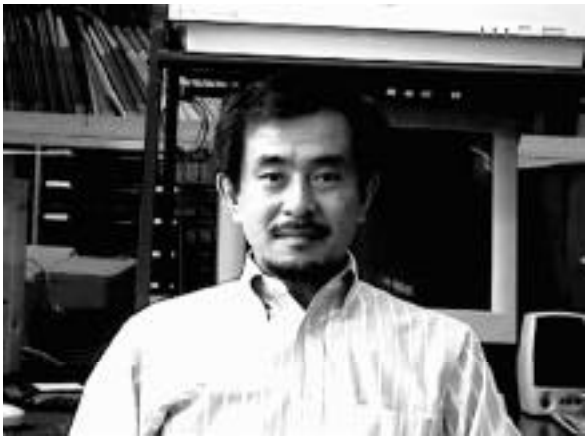

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