Collapse of the waves on “soft” DNA sites can be a physical basis of the regulation of genes expression

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NMR data demonstrate the existence of Hoogsteen base pairs in double-stranded DNA. In this work, a possibility of implication of these pairs in regulation of genes expression is discussed. The author suggests a physical mechanism for switch between different states of regulatory DNA sites as the result of collapse of rotational waves at these sites.

1. Introduction. Waves that spread along DNA chain and leading to a co-operative rearrangement of its specific sites can play an important role in regulation of genes expression, which might be revealed by modern NMR methodologies. It is worth to begin with a brief discussion of works where Hoogsteen base pairs were detected by NMR, which directly relate to the co-operative rearrangements under discussion. Then the attention will be paid to peculiarities of sequences of DNA sites implicated in the regulation of genes expression with the subsequent consideration of spread of waves along DNA chain, mechanism of collapse of the waves and possible role of this collapse in the regulation of genes expression.

2. Al Hashimi et al. [1-5] have experimentally demonstrated that in double-stranded DNA constantly some base pairs turn from Watson-Crick to Hoogsteen ones. Methodology elaborated in these works is based on the evaluation of relaxation dispersion of ^{13}C signals in NMR [1]. An unexpected result was that rather often turn of base pairs in the excited state, which requires the break of 1-2 hydrogen bonds and release of nucleotides from stacking with neighboring base pairs. It has been found the dependence of the Hoogsteen pair appearance on their close environment [5], which may be due to the need to overcome stacking and a possibility to form hydrogen bonds in the intermediate state [1].

Hoogsteen base pairs are widely spread, which leads to an assumption on possible role of their formation in biological processes involving DNA, such as replication, mutagenesis, repair and transcription. Al-Hashimi et al. have made attempts to study the dependence of Hoogsteen base pairs formation in DNA on the close environment of the particular base pair as well as on cytidine methylated at the C5 [5] in this pair. The latter suggest that that

authors assumed the existence of a link between formation of Hoogsteen pairs and mechanisms of epigenetic regulation of DNA transcription, in which 5-methyl-C plays essential role. It is believed that methylation of C in DNA is one of the modes of the regulation of transcription, and methylated C often resides in CpG-islands (CG repeats). It is also adopted to link the presence of CG repeats with a possibility of DNA transformation into Z-form [6]. In the thesis by Alvey (a co-worker of Al-Hashimi), the process of DNA transformation from B to Z form is considered separately [7], and further it will be discussed in this paper to combine all elements of a puzzle discovered by Al-Hashimi et al. into the known things related to regulation of genes expression. Besides, the picture will be added with a brief review on the currently existing ideas on oscillations in DNA, and these oscillations will be linked both to the transcription regulation and to the formation of Hoogsteen base pairs.

Z form of DNA (Z-DNA) has been discovered in the end of 70s of the last century [8, 9]. DNA bases in this form are complementary paired according to Watson-Crick regularities as in the canonical B-DNA but Z-DNA is remarkable by its reverse left-handed twist of the double helix and by the fact that only definite DNA sequences can adopt this conformation, namely, the sequences should have purines are alternated with pyrimidines. The algorithm search for DNA sites possessing features necessary for Z-DNA formation has been found long ago [10]. This algorithm recognizes any site where purines are alternated with pyrimidines as Z-DNA-like. The use of this algorithm has revealed that such sites are clustered in regulatory parts of genes.

3. It is worth to note that TATA-box, CpG islands and many promoters prone to form Z-DNA according to evaluation by Z-hunt algorithm. It is believed that regulatory proteins recognize the respective DNA sites where it exists in Z form and bind to these sites [11]. It has been suggested that under physiological conditions, negative supercoiling of DNA facilitates to overcome the activation barrier preventing DNA transformation to Z form

   [11]. To my opinion, supercoiling has developed in evolution later than capability of several DNA sites of the formation of Z-DNA because formation of Z-DNA is predetermined by the DNA sequence whereas to supercoiling requires a complex of molecular machineries to perform DNA

cleavage, its e subsequent energy-consuming twisting providing additional tension, and final sew of the DNA chains.

Models of transition. It is often suggested that transition to Z-DNA occurs via disruption of several base pairs that further are formed again in the reformed DNA (e.g. see [12]). Evidently, Z-DNA is energetically unfavorable as compare to canonical B-DNA since base pairs are taken out of the stacking (Fig. 1).

Alternatively, there are models of DNA transition from B to Z-form that consider sequential turn of the bases. In this line, in the Alvey's thesis at least three such type models are presented [13-15]. The author of this paper agrees with Alvey [7] and suggest that transition to Z-form is precede by the turn of base pairs to Hoogsteen structure. An important distinction of the author's suggestion is the turn not one but a cluster of pairs co-operatively at the whole site that undergoes restructuring.

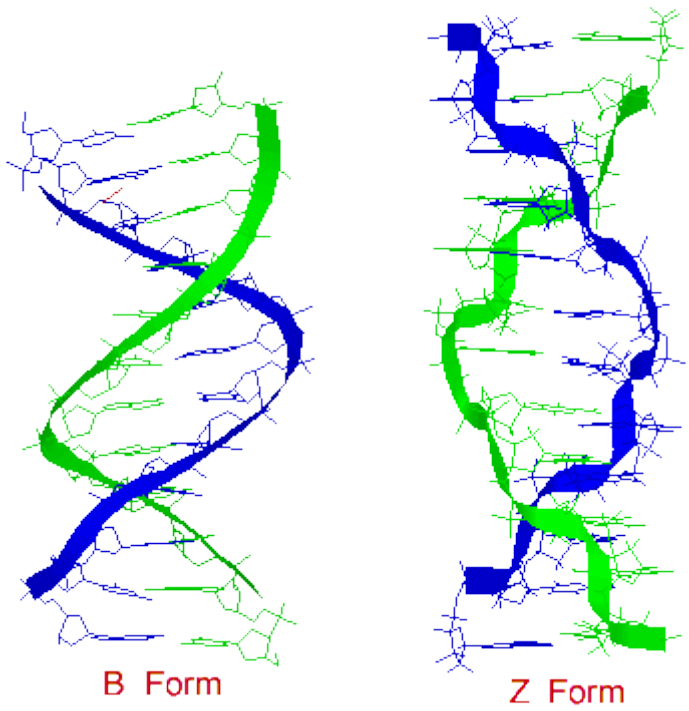

Figure 1. B and Z forms of DNA (http://www.web-books.com/MoBio/Free/Ch3B3.htm).

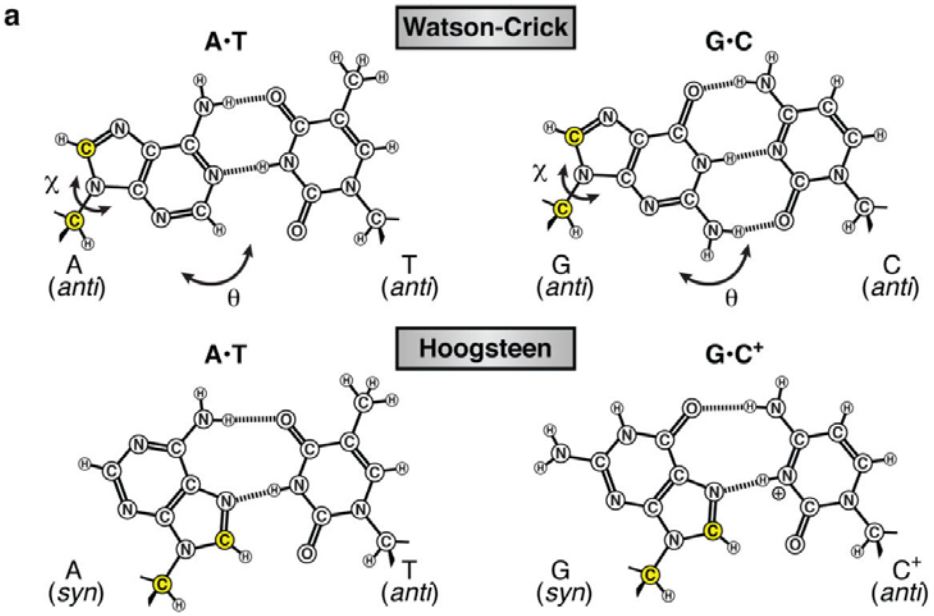

Figure 2. Turn of the Watson-Crick base pair to the Hoogsteen one [1].

What leads the author to suggest a possibility of the formation of Hoogsteen base pairs at relatively long DNA site?

First, experimental data indicating that these pairs are quite common in DNA [1], i.e., are formed often and exist relatively long time.

Second, the turn of purine leading to the Hoogsteen base pair formation does not require disruption of all hydrogen bonds in the pair since the purine can rotate around one remained bond and the glycoside bond. Such turn might provide stepwise transformation of the B helix without its disruption prior to the formation of another structure type.

Third, the turn of purine transforms its conformation relative to the deoxy-ribose to syn-type, which is common in Z-DNA (Fig. 2). The subsequent steps of transformation of B-DNA to Z form might be conformational change of deoxy ribose itself and the turn of purine together with the sugar relative the remaining hydrogen bond back to Watson-Crick pair but already in Z-DNA. Pyrimidine at this transition has no necessity to turn since in the final state its arrangement does not change, namely, its sugar remain in *C2'-endo conformation*, and the base in *anti-conformation* as before. In other words, transition to Z-DNA requires turn of every second sugar (Fig. 3), namely, of each sugar bound to purine. Since purines in DNA are alternated with pyrimidines, an alternation of sugar orientations arises (Fig. 3).

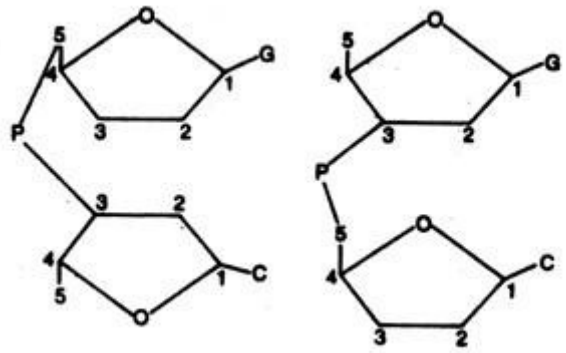

Figure 3. Mutual positioning of sugars in the ribose-phosphate backbone of Z-DNA (left) and B-DNA (right).

Fourth. It is worth mentioning that the formation of Watson-Crick base pair in Z-DNA is not necessary to turn back and one can imagine a complete 360^{o} turn of purine without disruption of its hydrogen bond. This turn should lead to accumulation of a noticeable tension in the sugar-phosphate backbone of the molecule and to provoke reverse helix coiling. The turned purine might bind to the backbone by a hydrogen bond via its N3, or might affect the backbone conformation due to steric hindrance at the turn. According to the author's hypothesis, the full turn should occur not too fast, a time is required to accumulate all conformational changes in the sugar-phosphate backbone. Hoogsteen base pair is considered as a relatively stable intermediate, which is formed fast. Then relaxation of the backbone occurs, and during this process part of the energy spent for the turn dissipates at various degrees of freedom, which prevents reverse turn of the purine back. The next stage of the double helix relaxation is expected to occur slower, and in the course of this stage purines would stepwise transform to Z-DNA by thermal fluctuations since they cannot co-operatively reverse to the B-DNA. Thus, the existence of a Hoogsteen base pair as relatively stable intermediate is desirable for further DNA transformation from B to Z form. In this respect, it is worth to mention experimentally found biphase nature of this transformation considered in the Alvey's thesis [7].

Fifth. Co-operative phase transition has an advantage as compared to uncoordinated turn of base pairs. It seems that an event implicating simultaneously several base pairs is unlikely, but it is not the case. The co-operative turn requires much less energy than the sum of energies of the single turns of each pair studied by Al-Hashimi [1-5]. The thing is that in the course of co-operative turn, the symmetry of the reforming DNA site

changes sharply, namely, that of the ribose-phosphate backbone. One can see from Figure 3 that in Z-DNA positioning of sugars repeats with periodicity of two base pairs, while in B-DNA sugars are oriented identically in each pair. That means decrease of the symmetry extent upon transition from B-DNA to Z-DNA. The effect of symmetry decrease is equivalent to the entropy increase; for example, this effect is the basis for the theory of second-order phase transitions. A possibility of symmetry decrease without transition to a completely disordered state distinguishes the co-operative DNA rearrangement as compared to that occurred as a random transition. The co-operative transition increases entropy but increases it in minimal way. A scenario implicating minimal entropy increase requires less energy for rearrangements to occur. Another point of view is that the sites of alternation of purines and pyrimidines contain in their structures a possibility of a co-operative turn since at these sites upon the turn entropy increases minimally. Thus, a co-operative transition has an advantage in energy requirements as compared to other scenarios. It should be mentioned here that to decrease symmetry of the ribose-phosphate backbone, final transition to Z-DNA is not necessary. Co-operative turn of purines to Hoogsteen base pair would be enough, and in this case, mutual positioning of sugars and bases would fit that in Z-DNA (Figure 3).

Finally, the author can suggest a mechanism of accumulation of energy required for transition to Z-DNA precisely in sites prone to such kind transition. The four above-discussed points are close to what could be found in the Alvey's thesis [7]; if these considerations are not formulated precisely, the author readily admits the priority of her research. The two final points requires to go out from considerations of structural chemistry and to refer to dynamics of waves propagation in the DNA.

Currently, transition to Z form in model DNAs in vitro is achieved either by using high salt concentrations or by supercoiling. Both ways are not feasible under physiological conditions; therefore, it is interesting to find mechanism that governs the transition under the mildest conditions. Waves in DNA, including those generated by thermal fluctuations, might be a natural reason for transition of DNA from b to Z form.

4. Further, the author suggests that it there is a mechanism enabling simultaneous involvement of several base pairs in Hoogsteen base pairs. This suggestion contradicts to an idea that cytidine in Hoogsteen base pair should be protonated [1, 4]. The protonated C is carries a positive charge, and it is

impossible to place several positively charged cytidines close to each other in DNA, therefore, co-operative turn of CpG island is possible only if GC-Hoogsteen pair is not charged.

This contradiction could be overcome if consider a possibility of the formation of Hoogsteen base pair by cytidine in imino-form, i.e., taking into account a possibility of the tautomerism. This possibility has been observed by the author earlier [16], which allowed revelation of a relationship between epigenetic and tautomeric properties of cytidine derivatives. In particular, methylation of cytidine somewhat destabilizes the amino-form and stabilizes the imino-form of cytidine, which should facilitate the transition to Hoogsteen base pair. It is worth to note that it is typical for CpG-islands to participate in regulation of expression after methylation of their cytidines, that is, after the modification facilitating the transition to imino-form.

The above suggestions do not imply that protonated form of cytidine is completely out of consideration when transition of GC pairs to Hoogsteen base pair is discussed. At the respective pH protonated cytidine can arise and facilitate the transition to Hoogsteen base pair, but protonation could not be ubiquitous. Here the author's suggestion does not contradict experimental data that decrease of pH facilitates the formation of GC Hoogsteen base pair [1, 4]. Methylation of cytidine is a way to manage tautomeric balance, which indicates to a possibility of the formation both Hoogsteen base pair and Z-DNA at neutral pH.

5. The basis of the author's mechanism of energy accumulation for DNA transition from B to Z-form is the spread of oscillation along the DNA molecule. Analogous process along a hypothetic polypeptide consisting completely of an alpha-helix has been observed by Davydov [17]. The choice of the model molecule was not very good, but the main message of the work had a biological significance by demonstrating a possibility of loss-free transportation of energy comparable to the energy of ATP hydrolysis. This approach has been applied in theoretical works on spread of wave along DNA. One of the well-known models described in [18] showed a theoretical possibility of spontaneous generation of wave having relatively high energy, and possible implication of this energy for melting of transcription start sites (promoters) have been shown. Both authors of the mentioned paper later have contributed to the experimental verification of their model [19, 20]. It is worth mentioning here that the experimental approaches applied in [19,20]

have used an external energy supply to the DNA. An important thing for the further understanding is the fact that in the models rotational wave have been observed [18]. The authors of [18-20] suggested that these degrees of freedom, which associate with rotations in the plane of bases might lead to break of hydrogen bonds in the DNA double helix.

What could be a source for the energy of rearrangements in DNA site comprising several nucleotide base pairs? To address this question, let us discuss which features should be peculiar to sites where energy of rotational waves should be liberated. These sites should have features necessary for realization of so-called “collapse of the waves”. The wave front should slow down at this site, and as the result, the wave catches up with its front and concentrated on this site, which leads to an abrupt energy release. One can observe such effect on a sea shore where the oncoming wave slows down in shallow water, grows high and the rear part of the wave catches up and hangs over the front of the wave; finally, the wave collapses with a character noise. Where is “shallow water” in DNA? One can assume that these should be sites where DNA is somewhat “softer”. This softness would not relate to hight AT-base pairs content since the amount of hydrogen bonds is not important for movements of rotational oscillations. The important is another thing, namely, the strength of stacking between the neighbor nucleotides. Rotational oscillations require removing the bases from the stacking. The stacking defines rigidity of the rotational waves. The stacking strength decreases in the row (Pu-Pu)>(Pu-Py)>(Py-Py), i.e., stacking between two purines is stronger than between purine and pyrimidine, and the stacking between two pyrimidines is the weakest. How this would affect the spread of waves along the DNA chain? Two sequential purines provide the strongest stacking (which significantly reduces the amplitude of rotational fluctuations) and therefore the most rigid site. Since DNA is a double helix, two sequential pyrimidines in one chain mean two purines in the complementary one, which means again a rigid site. The complementary pyrimidines do not stack so strong but they would participate in vibrations with the same amplitude as the purines. Thus, one could expect that purine-pyrimidine neighborhood in the DNA chain would allow maximum amplitude of the bases displacement.

To make a site soft, purines and pyrimidines should be alternated. On this basis, TATA-box occurs close to CpG islands that are implicated in regulation of eukaryotic genes expression. Almost strict alternation of purines and pyrimidines takes place in both cases. Already mentioned above

algorithm [10] search for DNA sites possessing features necessary for Z-DNA formation finds in DNA alternation of purines and pyrimidines finds alternation of purines and pyrimidines and it turns out that these alternations are clustered in regulatory gene regions [10]. This indicates that these regions typically have increased frequency of purine-pyrimidine neighborhood, and it is remarkable that the same regions have a useful feature since they are capable of accumulation of rotational waves energy.

Z-DNA has been predicted only for the sequence GCGCGCGC. Violation in the alternation of purines and pyrimidines as well as replacement of GC pair to AU pair should destabilize Z-DNA structure. However, the above-mentioned Z-hunt algorithm [10] finds many sites resembling GCGCGCGC in regulatory gene sequences. The similarity might be limited to purine-pyrimidine alternation, or this alternation might be somewhat violated as in TATA-box: TATAAA. Probably, regulatory sites similar to Z-DNA according to their sequence does not adopt a form of true Z-DNA, and functional significance of these sites might relate to their capability of energy accumulation via the above-mentioned mechanism of wave collapse and rearrangements via a step of Hoogteen pair formation. Thus, one can suggest that physical basis for the functioning of regulatory gene sequences is their softness regarding rotational oscillations caused by weakened stacking between DNA nucleotides in such sites. Now it is possible to formulate the same assertion with regard to the conditions necessary for Hoogsteen base pair formation and the subsequent transition to Z-DNA. To form a Hoogsteen base pair, it is important to bring a purine out from the stacking, because it is purine that further has to turn. Releasing of purine from stacking occurs most easily when its neighbors from both sides are pyrimidines. A necessity to turn all purines at an intermediate step in the course of transition to Z-DNA results in a need of alternation of purines and pyrimidines in the respective DNA site.

An increased flexibility of double helix DNA sites prone to form Z-DNA has been registered experimentally by NMR, which is one more valuable finding in the works of Al-Hashimi et al. [21, 22].

It is worth to mention that rotational waves can be both left- and right-rotating. The former should be useful for transformation of B-DNA to Z-DNA since they make an effect of reverse supercoiling twisting the double helix in a direction character for Z-DNA. The site of purine-pyrimidine alternation concentrates the twisting effect and the resulting Z-helix

fragment is a “stopped wave” where the kinetic energy of the fluctuation is transformed to the potential energy of Z-DNA.

Here it is worthwhile to present two “predictions” related to epigenetic role of rotational waves in DNA. First, if the evolution has created “devices” capable of the directed induction of fluctuations in DNA, the direction of the double helix rotation of should not be chosen randomly. Activation of regulatory DNA sites requires left-hand rotation. Probably, right-handed rotation might reverse formation of B-DNA from the Z-dNA. The author suggests that the evolution could not pass by a possibility of such kind control of the state of regulatory DNA sites. If so, one has to expect a discovery of biomolecules capable of the induction of DNA rotational waves. One can suggest an idea of experiment for studying effects of wave collapse in DNA to find molecules that transform the energy of ATP into the energy of rotational DNA vibrations. In [20] it has been demonstrated that vibrations in DNA can be shacked by terahertz radiation; this probably can be done separately with left-hand and right-hand vibrations by the application of the circularly polarized radiation. Hoogsteen base pair formation and the subsequent transition of the “soft” DNA site into Z-form might be detected by NMR or by optical methods.

The second prediction relates to the methylation of CpG islands. What could be the effect of cytosine methylation on the wave collapse at such sites? Taking into account the data of [16], methylation of cytosine stabilizes imino-form of C and somewhat destabilizes GC Watson-Crick base pair. Thereby methylation of cytidine facilitates the formation of GC Hoogsteen base pair with cytidine in imino-form. That is, wave collapse at this site requires less energy. What can happen after methylation and turn of the bases? One possibility is recognition of the site by a specific protein. But even without such a protein, a link between methylation and regulation of genes expression in frames of this hypothesis can be traced. In particular, before methylation the wave is not capable of collapse at the CpG island, whereas after the methylation the collapse at this site becomes likely, which excludes a possibility of the wave to get to the promoter and collapse there. Currently, it is believed that methylation of C switches off genes expression [23], which is in agreement with the author’s prediction. It has been also shown that after methylation of the respective sites transcription of viral elements integrated in DNA is repressed [24]. Such mechanism of control a regulatory site by cytidine methylation could exist before the appearance of specific proteins binding to methylated CpG islands [25], which might be of

interest to clarify possibility of appearance of cytidine methylation in evolution.

Finally, it is worth to note that registration of purine turn in Watson-Crick base pairs leading to their transition to Hoogsteen pairs shows that even without active energy supply in DNA there exist rather active thermal fluctuations. It seems likely that general thermal noise might be the origin for wave collapse and transition of B-DNA to Z-form, which could be potentially registered. This could enable creation of a good model for the emergence of the mechanism of transcription regulation in evolution.

6. Conclusion remarks concerning the novelty of the suggested hypothesis.

1) Transition of DNA to Z-form is precede by co-operative turn of Watson-Crick base pairs to Hoogsteen pairs involving rather long DNA sequence.

2) The turn of several neighboring GC pairs to Hoogsteen base pairs is possible only at transition of cytosine to the imino-form. The postulation of the existence of only protonated form GC+ Hoogsteen base pair does not enable understanding mechanism of DNA transition from B to Z form.

3) The total (360 degree) turn of purine relative to pyrimidine leaving one hydrogen bond unbroken leads to the accumulation of substantial tension in the sugar-phosphate backbone and induces reverse change of coiling, which underlies the mechanism of transformation of DNA from B to Z-form.

4) Energy necessary for DNA transition from B to Z-form might be accumulated at sites where purines and pyrimidines are alternated in the DNA sequence. The mechanism of the energy accumulation is based on the wave collapse at the most “soft” DNA sites.

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