Volume 257, number 2, 415–418 FEB 07801 November 1989

Parallel DNA: generation of a duplex between two *Drosophila* sequences in vitro

Nickolai A. Tchurikov, Boris K. Chernov°, Yulia B. Golova° and Yurii D. Nechipurenko+

Department of Nucleic Acids Biosynthesis, °Department of Genetic Engineering and *+Department of Biopolymers Physics, Engelhardt Institute of Molecular Biology, the USSR Academy of Sciences, Vavilov str., 32, 117984 Moscow B-334, USSR*

Received 18 September 1989

We have observed the existence of a parallel complementary region between two *Drosophila* DNA sequences, fragments of the suffix [(1986) EMBO J, 5, 2341–2347] and a 5'-non-coding sequence of the alcohol dehydrogenase gene [(1983) Cell 33, 125–133]. The region includes ~40 bp, 76% of which are complementary in the same polarity. Synthetic complementary 16 bp oligonucleotides corresponding to this region which were bound by the 5'-ends through a 1.6-hexanediol bridge form a duplex which displays both melting and annealing as judged by UV absorbance. Anti parallel complementary 16 bp long oligonucleotides bound by the 5'-3' ends through the same bridge and a single-strand sequence were used as controls. The Hoechst 33 258 drug binds to this parallel duplex of DNA; however, the properties of such a complex testify against the B-form of the duplex.

DNA, parallel; Suffix element; Gene, Adh; Melting; Hoechst 33 258; CD; (Drosophila)

1. INTRODUCTION

Our fundamental knowledge of DNA structure is based on the Watson-Crick model of a double helix [4]. Although the model suggested an opposite polarity of the two strands, nevertheless the possibility of a parallel duplex has not been ruled out. Indeed, neither Chargraff's rules nor X-ray data have been inconsistent with the parallel duplex model. The first experimental evidence in favour of the opposite polarity was furnished by Josse et al. [5] who analysed the nearest neighbour frequencies of nucleotides. Nevertheless, the recent calculations and models are indicative of DNA capability to form a parallel duplex [6,7]. While this paper was in preparation, such a capability was confirmed by in vitro experiments with (AT)-oligonucleotides [8,9]. Although this structure may not be generally applicable to DNA, it is sufficiently attractive for us to consider the possibility that short parallel duplexes exist in vivo: in fact, we found a region of parallel complementarity between two DNA sequences in the *Drosophila* genome. Here, we present some evidence in favour of the fact that parallel duplexes of natural DNA do exist in vitro.

Correspondence address: N.A. Tchurikov, Department of Nucleic Acids Biosynthesis, the USSR Academy of Sciences, Vavilov str, 32, 117984 Moscow B-334, USSR

Abbreviations: Adh, alcohol dehydrogenase gene of *Drosophila melanogaster*; PC, parallel construction containing two parallel DNA strands; AC, antiparallel construction containing two antiparallel DNA strands; SS, single-stranded DNA; CD, circular dichroism

Preliminary report of a part of this work was published recently, Tchurikov et al. [3]

2. EXPERIMENTAL

The parallel and antiparallel DNA strands corresponding to these regions (16 bp) were synthesized by the phosphamidate method. The complementary strands were covalently bound by 5'-5' and 5'-3' ends through phosphodiester bonds to 1,6-hexanediol linkers [10]. The UV absorption spectra were recorded in 0.2 M Na-phosphate buffer, pH 7.2. Concentrations of PC, AC and SS were 1.6×10^{-6} M, 1.9×10^{-6} M and 3.1×10^{-6} M, respectively. CD spectra of PC and AC without and after addition of Hoechst 33 258 dye (a commercial preparation from Miles Laboratories, USA, also is manufactured under the name Bisbenzimid H 33 258) were recorded at 20°C in 0.2 M Na-phosphate buffer, pH 7.2, 10 mM $MgCl_2$. Concentration of dye was 1.1×10^{-6} M.

3. RESULTS

Recently we have described a suffix element in the *Drosophila* genome [1]. The element is a presumptive retroposon which was found to constitute the 3'-end exons in many different *Drosophila* genes and to be involved in processing the 3'-ends of mRNAs. A fragment of the suffix non-coding exon sequence (from 48 to 88 bp) was found to be complementary in a parallel orientation to the fragment of the 5'-non-coding sequence in adult mRNA of the alcohol dehydrogenase gene (from +47 to +88 bp) [1,2]. There are three 'insertions' in each sequence as well as four non-complementary pairs (fig.1A). Thus, the parallel DNA strands of the suffix (41 bp) and Adh (42 bp) have 32 complementary nucleotides.

The probability of occasional occurrence of such regions in the genome seems to be rather low. Therefore it was suggested that the fact may reflect a relationship between sequences which occurred during

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

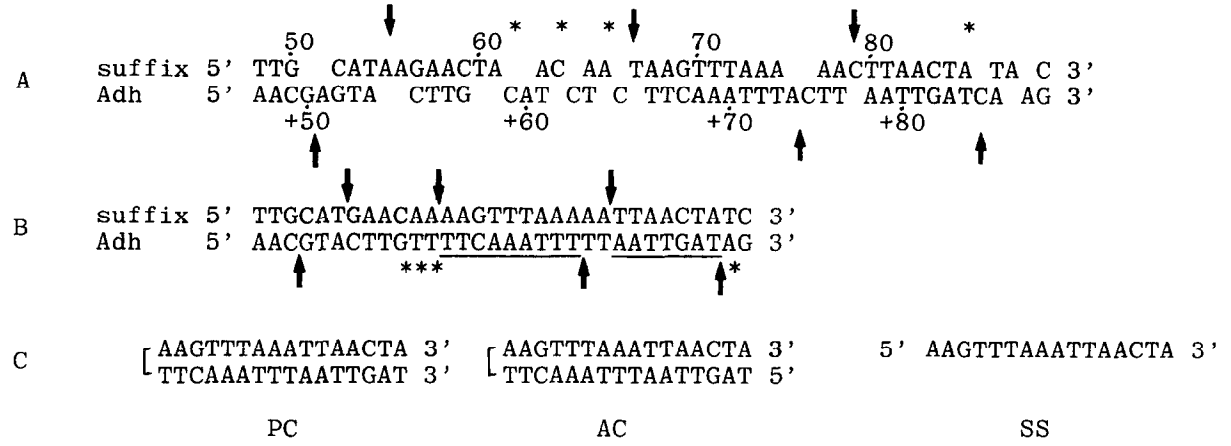

Fig.1. Complementary regions of the suffix and Adh. (A) Fragments as a whole containing non-complementary 1 or 2 bases (*) and insertions of 1 or 2 nucleotides (↓). (B) Mismatched regions are removed and two entirely complementary regions are underlined. (C) Oligonucleotide constructions: a parallel complex (PC) and an antiparallel complex (AC) bound with 1,6-hexanediol linkers and a single strand (SS) which were used in biophysical experiments. The brackets indicate 1,6-hexanediol bridges.

evolution. We wondered whether parallel DNA can interact to form a duplex. To check this possibility, we decided to use these sequences for in vitro experiments. Fig.1B presents strands without insertions and non-complementary bases. Such a 'duplex' is AT-rich (78%) and contains two entirely complementary regions (9 and 7 bp long).

Fig.1C shows such a parallel construction (PC), an antiparallel construction (AC), and a single strand (SS) which were used in thermal-denaturation experiments.

To characterize the structure of PC, UV absorption spectra were determined at 3 and 90°C (fig.2A). PC displays a 20% hyperchromicity and a small apparent shift of the absorbance maximum to long wavelengths. By contrast, the maximum PC absorbance at 3°C is shifted slightly to shorter wavelengths as compared

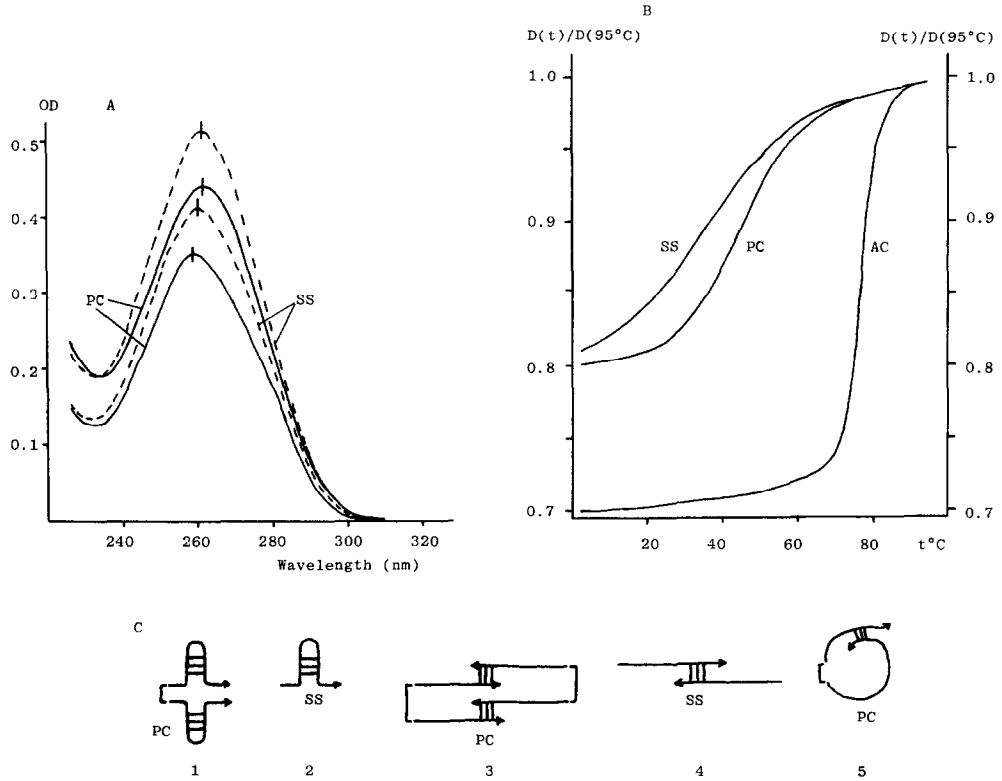

Fig.2. UV absorption spectra of PC and SS (A) and thermal-denaturation profiles (B). The spectra were recorded at 3 and 90°C in 0.2 M Na-phosphate buffer, pH 7.2. The profiles were determined in the same solution, $D(t)/D(96°C)$ versus t. (C) Possible structures of an opposite polarity in PC and SS: hairpins (1,2), short duplexes between two molecules (3,4) and a sigma structure (5). The brackets indicate 1,6-hexanediol linkers in PC.

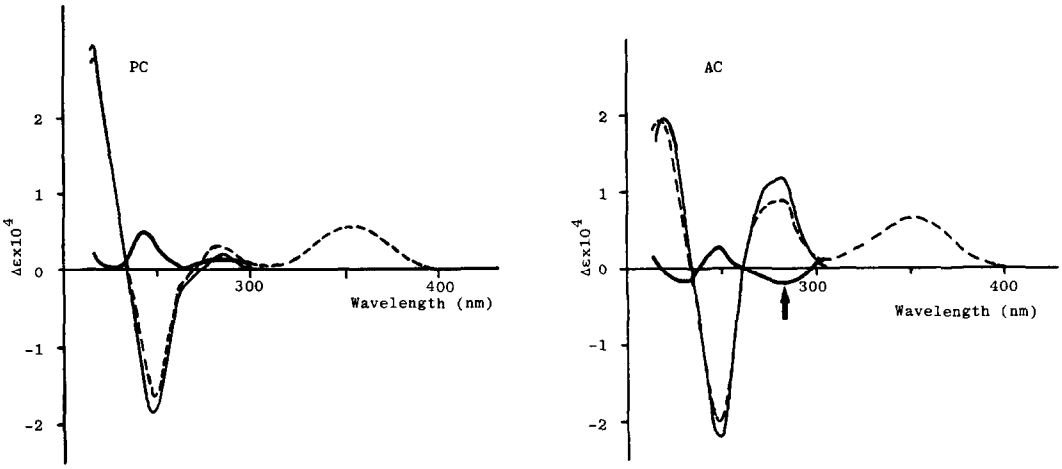

Fig.3. Circular dichroism spectra of PC and AC complexes with Hoechst 33 258 drug. The dye is optically inactive in the free state. CD spectra of free PC and AC (unbroken thin lines) and after addition of Hoechst (broken lines) were recorded from 215 to 420 nm. Thick unbroken lines present the result of subtraction of CD spectrum of free DNA from CD spectrum of the DNA-Hoechst complex. The arrow indicates the position of a negative band in AC.

with SS spectrum. The absorbance melting profiles of AC, PC and SS are given in fig.2B. SS demonstrates a 19% hyperchromicity and a melting profile which distinctly differs from that of PC. PC displays a more cooperative effect and a higher melting temperature. Short antiparallel hairpins may occur inside SS or PC molecules. The latter may also possess 'sigma' structures (see fig.2C). However, the difference in their melting profiles suggests that the melting of PC cannot be attributed only to the generation of hairpin or sigma structures. In addition, short duplexes of an opposite polarity may also be formed between different PC or SS molecules (see fig.2C). However, such interactions cannot be a principal cause of the hyperchromicity and melting profiles because even dilutions of PC and SS samples greater than tenfold do not interfere with the results (not shown). Therefore, the behaviour of a PC melting curve cannot be explained only by the presence of short antiparallel duplexes.

We believe that our experimental results suggest the existence of both parallel and short antiparallel duplexes in PC. We propose that the presence of a parallel duplex explains the differences in absorption spectra and thermal-denaturation profiles.

The melting of AC is highly cooperative; it gives a 28% hyperchromicity and a normal absorption spectrum (not shown). Hence, a 1,6-hexanediol linker is sufficiently flexible to determine the formation of a DNA duplex with no noticeable effect exerted by the hydrophobic bridge which follows from the absorption spectrum data and thermal-denaturation studies. The comparison of AC and PC melting profiles suggests that PC has apparently a less compact structure.

To characterize further the structure of PC, the binding of Hoechst 33 258 drug to PC and AC was determined. It has been shown that the drug binds to DNA providing strong interactions specifically with the B-form of DNA and such a complex has a typical CD

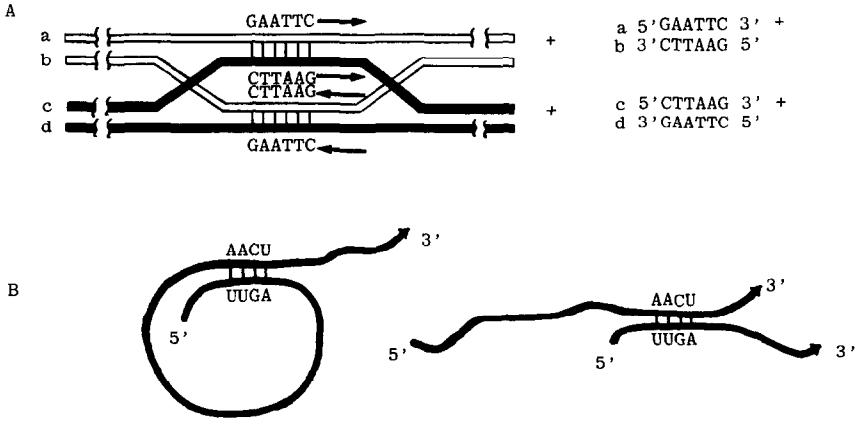

Fig.4. Hypothetic DNA and RNA parallel duplexes. (A) Parallel interactions between two Watson-Crick double helices (*a–d*, a family of symmetric DNA sequences). (B) Parallel RNA duplexes inside one molecule or between two molecules.

spectrum with a negative band in the area near 270 nm [11]. We have found that Hoechst binds to PC and that the CD spectrum of Hoechst-PC presents a contrast (fig.3). The result is indicative of the fact that PC does not belong to the B-DNA family. However, the final conclusion about the structure of a parallel DNA duplex should be drawn on the basis of X-ray diffraction studies.

4. DISCUSSION

The observation of parallel DNA duplex between two short DNA sequences from the same genome and the generation of such a duplex in vitro raises the question about the possible biological significance of parallel DNA. The formation of a parallel DNA duplex was recently demonstrated for short AT-containing oligonucleotides [8,9]. Later it was also shown by Shchelkina et al. [12]. Our 16-bp sequence contains two GC pairs. We believe that the fact is responsible for a less cooperative melting of PC as compared with AT-containing oligonucleotides. Short (15 bp) DNA sequences from sea urchin genome give one more example of natural DNA sequences which are composed of complementary bases with a similar polarity. They also contain two GC pairs [13]. DNA sequence banks provide a great number of such examples in different genomes (Tchurikov, unpublished results). This is not indicative but at least predictable because genomic DNA contains AT-rich tracks. Although such evidence is clearly not sufficient to consider these peculiar (*Drosophila* or sea urchin) sequences as natural parallel DNA duplexes, the examples may provide a paradigm in favour of existence of short segments of a parallel DNA helix in cells. Indeed, this fact taken together with the in vitro generation of such unusual structures is worthy of both theoretical and experimental consideration.

It is clear that two fragments of the Watson-Crick DNA helix which possess sequences composed of complementary nucleotides in the same orientation are different although symmetric. The family includes four DNA sequences which can generate both types of duplexes having either an opposite or a similar polarity (fig.4). It is obvious that one of the duplex cannot arise from another by duplication. Alternatively, such DNA fragments may originate as a result of molecular convergence or unusual template synthesis. The latter should require a special enzyme, at least in earlier evolution. Fig.4 demonstrates some hypothetic interactions of parallel DNA and RNA sequences. From the general point of view, parallel duplexes may play a role in recombination and genomic rearrangements (in sites of a parallel DNA helix), RNA processing, regulation of expression (by specific parallel DNA-protein binding), evolution, etc. We believe that only further experimental studies will allow us to answer some of the questions raised by the generation of parallel DNA duplexes in vitro.

Acknowledgements: We are grateful to A.K. Naumova for her help in search of suffix sequence homologies. We also thank Dr G.P. Georgiev, Dr A.S. Zasedatelev, Dr V.Yu. Shumilov and Dr C.R. Cantor for their stimulating discussions, criticism and advice.

REFERENCES

[1] Tchurikov, N.A., Ebralidze, A.K. and Georgiev, G.P. (1986) EMBO J. 5, 2341–2347.
[2] Benyajati, C., Spoerel, N., Haymerle, H. and Ashburner, M. (1983) Cell 33, 125–133.
[3] Tchurikov, N.A., Chernov, B.K., Golova, Yu.B. and Nechipurenko, Yu.D. (1988) Dokl. Akad. Nauk SSSR (USSR) 303, 1254–1258.
[4] Watson, J.D. and Crick, F.H.C. (1953) Nature 171, 737–738.
[5] Josse, J., Kaiser, A.D. and Kornberg, A. (1961) J. Biol. Chem. 236, 864–875.
[6] Pattahiraman, N. (1986) Biopolymers 25, 1603–1606.
[7] Kurjav'y, V.V. (1987) Mol. Biol. (USSR) 21, 1486–1496.
[8] Ramsing, N.B. and Jovin, T.M. (1988) Nucleic Acids Res. 16, 6659–6676.
[9] Van de Sande, J.H., Ramsing, N.B., Germann, M.W., Elhorst, W.E., Kalisch, B.W., Bitzing, E.V., Pon, R.T., Clegg, R.C. and Jovin, T.M. (1988) Science 241, 551–557.
[10] Chernov, B.K. and Golova, Yu.B. (1989) Dokl. Akad. Nauk SSSR (USSR), in press.
[11] Mihailov, M.V., Zasedatelev, A.S., Krylov, A.S. and Gursky, G.V. (1981) Mol. Biol. (USSR) 15, 690–705.
[12] Shchelkina, A.K., Lysov, Y.P., Ilyicheva, I.A., Cherny, A.A., Golova, Yu.B., Chernov, B.K., Gottikh, B.P. and Florentiev, V.L. (1989) Mol. Biol. (USSR) 23, 295–305.
[13] Johnson, S.A., Davidson, E.H. and Britten, R. (1984) J. Mol. Evol. 20, 195–201.