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Southern molecular hybridization experiments with parallel complementary DNA probes

Nickolai A. Tchurikov[a], Anna K. Shchyolkina[b], Olga F. Borissova[b] and Boris K. Chernov[c]

[a]*Department of Genome Molecular Organization,* [b]*Department of Biopolymers Physics* and [c]*Group of Genes Chemical Synthesis, V.A. Engelhardt Institute of Molecular Biology, Vavilov str. 32, Moscow B-334, 117984, Russia*

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We have detected the specific binding in Southern blot hybridization experiments of both complementary antiparallel and parallel 40 bp synthetic DNA probes, corresponding to a cloned *Drosophila* DNA fragment. The highly cooperative annealing and melting were observed in solution with these probes, which are complementary in the same direction and possess 17 GC pairs. The binding of ethidium bromide is indicative of formation of a perfect parallel DNA duplex. The specific binding was also detected in both genomic and in plaque hybridization experiments.

Parallel DNA; Molecular hybridization; Mirrored sequence

1. INTRODUCTION

Molecular hybridization techniques for nucleic acids have been known for many years [1–3]. Currently, the most widespread modifications are the Southern blot [4] and Northern blot hybridization methods [5]. These techniques use antiparallel complementary probes and provide a highly specific interaction between complementary polynucleotide strands simulating the natural pairing of nucleic acids.

Recently it was shown that artificial DNA sequences or DNA molecules corresponding to natural DNA are capable of forming a parallel double helix in vitro [6,7], and the main parameters of parallel DNA duplex were determined by scanning tunelling microscopy [8]. In different genomes there are many sequences which are complementary in the same orientation [9]. One of the direct approaches to study these 'mirrored' genomic sequences and their origin is molecular hybridization experiments with parallel complementary probes. The method described here provides a simple way of detecting of heterologous DNA sequences that are complementary in the same polarity on both Southern blots containing cloned DNA or genomic DNA and on replicas of genomic libraries.

2. MATERIALS AND METHODS

2.1. *Oligonucleotide probes*

Oligonucleotide probes were chemically synthesized by the phosphoramidite method using a DNA synthesizer. End-labelling was performed with T4 polynucleotide kinase. Specific activity of the probes was around 4×10^8 cpm/μg. Labelled oligonucleotides were purified on Sephadex G50 (superfine) columns.

Correspondence address: N.A. Tchurikov, Department of Genome Molecular Organization, V.A. Engelhardt Institute of Molecular Biology, Vavilov st. 32, Moscow B-334, 117984, Russia. Fax: (7) (095) 135 1405.

2.2. *Molecular hybridization*

Southern filters containing cloned DNA and nitrocellulose filters bearing plaques were prehybridized for 1 h at 65°C in solution containing 2×SSC (0.15 M NaCl and 0.015 M sodium citrate, pH 7.0), 0.5% sodium dodecyl sulphate (SDS), 10× Denhardt's solution, 0.05% sodium pyrophosphate, 100 μg/ml denatured salmon sperm DNA, 100 μg/ml yeast tRNA. The hybridization was performed in the same solution containing ^{32}P-labelled 40 bp oligonucleotide (2×10^6 cpm/ml, about 5 ng/ml) for 18 h. The filters were washed in 2× SSC, 0.5% SDS, 0.05% sodium pyrophosphate solution, 4× 1 h each, and autoradiographed. Hybridization and washing of the parallel probe was performed at 32°C and of the antiparallel one at 54°C.

2.3. *DNA hybridization with dried agarose gels*

About 20–30 μg of total *Drosophila* DNA digested with *Eco*RI endonuclease was electrophoresed on 0.8% agarose gel in Tris–acetate buffer (40 mM Tris base, 20 mM sodium acetate, 2 mM EDTA, pH 8.3). After photography of ethidium bromide (EtBr)-stained gel, it was prepared for hybridization as described by Tsao et al. [10]. The prehybridization was performed at 65°C for 1 h in the solution containing 0.3 M NaCl, 20 mM sodium phosphate, pH 7.5, 2 mM EDTA, 0.1% SDS, 10 μg/ml denatured salmon sperm DNA, 10 μg/ml yeast tRNA and 0.05% sodium pyrophosphate. The hybridization was performed for 48 h in the same solution with 2×10^6 cpm/ml of ^{32}P-labelled oligonucleotide. The washing was performed in 2× SSC, 0.1% SDS, 0.05% sodium pyrophosphate, 4× 1 h each. The parallel probe was hybridized and washed at 32°C and the antiparallel one at 54°C. To remove the probe for new hybridization, the gel was denatured/neutralized as described above and autoradiographed to ensure removal of the probe.

2.4. *Fluorescence measurements*

Steady-state fluorescence anisotropy was measured at 610 nm (excitation at 540 nm) on an Aminco SPF-1000 spectrofluorimeter. The concentration of adsorbed EtBr was calculated according to Le Pecq and Paoletti [11]. The fluorescence polarization of solutions containing EtBr and the oligonucleotide duplex were calculated according to Weber [12]. The relaxation time of the oligonucleotide parallel duplex was calculated as described earlier [13].

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                    10        20        30        40
probe A    5' ACTAACTAGCTAACAAACGTACGTGTGCAAAAACACTCGC 3'  ] parallel duplex
genomic   [5' TGATTGATCGATTGTTTGCATGCACACGTTTTTGTGAGCG 3'
  DNA     [3' ACTAACTAGCTAACAAACGTACGTGTGCAAAAACACTCGC 5'  ] antiparallel duplex
probe T    5' TGATTGATCGATTGTTTGCATGCACACGTTTTTGTGAGCG 3'  ]
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Fig. 1. Genomic double-stranded antiparallel sequence from the *cut* locus of *D. melanogaster* and sequences of parallel (A probe) and antiparallel (T-probe) probes.

3. RESULTS

3.1. *The genomic sequence and corresponding oligonucleotide probes*

In order to study the possibility of molecular hybridization with parallel complementary probes, we chose the unique genomic 8.3 kb *Eco*RI fragment from *D. melanogaster*, the *cut* locus. Its 40 bp region contains the hot spot specific for insertion of gypsy element [14]. Fig. 1 shows the 40 bp-long sequences of the genomic region and two probes possessing 17 GC bases. The probes are capable of forming parallel ('A'-probe) and antiparallel ('T'-probe) duplexes with different genomic strands. Thus, they are complementary in the same 5'–5' orientation. The latter circumstance permits the selection of the hybridization conditions by annealing and melting of the parallel complementary probes in preliminary experiments. The melting temperature was found to be equal to 53°C. Therefore the hybridization temperature for further experiments with parallel complementary probe A in 2× SSC solution was selected to equal 32°C.

3.2. *The study of parallel duplex by binding with ethidium bromide*

In order to estimate the quality of the parallel duplex formed by two probes in 2× SSC, we have evaluated by adsorption isotherm study the portion of double-stranded regions available for dye binding [13]. We concluded that at least 95% of bases in the parallel duplex

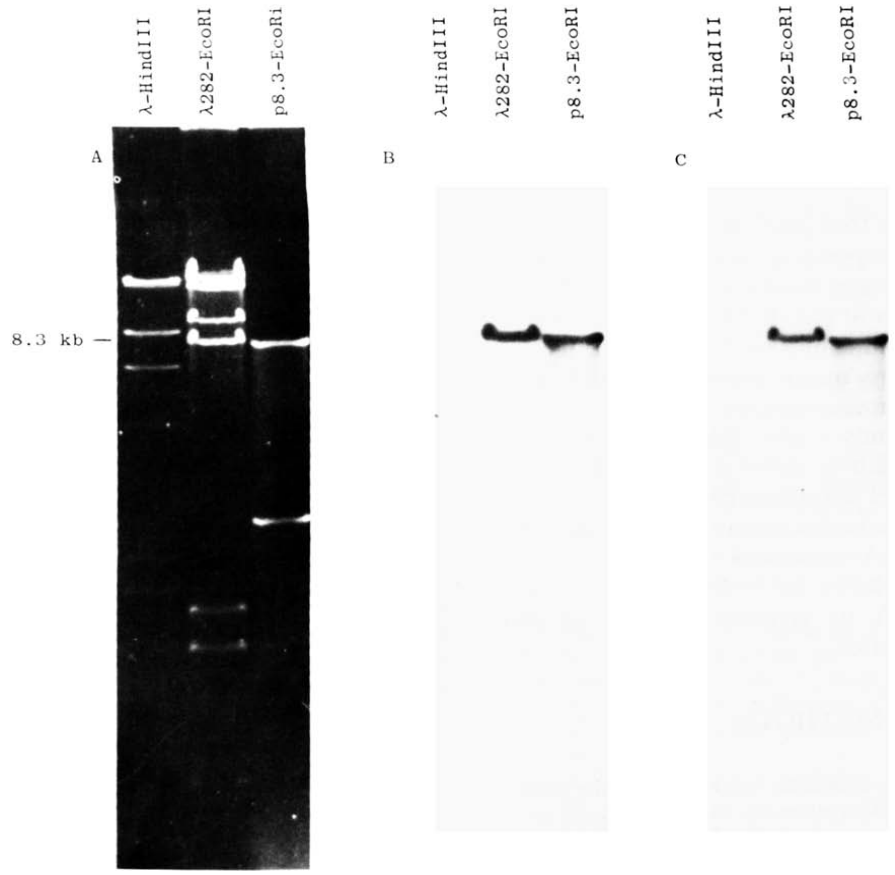

Fig. 2. Southern blot hybridization experiments with complementary parallel (A probe) and antiparallel (T probe) labelled oligonucleotides. (A) Ethidium bromide-stained 0.8% agarose gel containing *λ-Hind*III marker, *λ*282 and p8.3 digested with *Eco*RI endonuclease [11]. (B) The blot (Hybond-H) hybridized in 2× SSC (see Materials and Methods) at 32°C with parallel complementary probe. (C) The blot hybridized in 2× SSC (see Materials and Methods) at 54°C with antiparallel complementary probe.

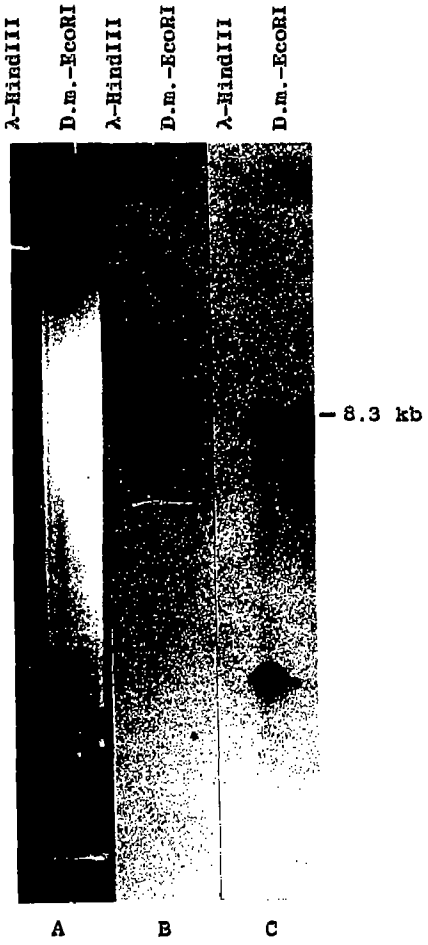

Fig. 3. Genomic DNA hybridization in dried agarose gel with parallel and antiparallel complementary probes. (A) Ethidium bromide-stained 0.8% gel containing λ-*Hin*dIII marker and *D. melanogaster* total DNA digested with *Eco*RI enzyme. (B) Autoradiograph after hybridization at 32°C in 2× SSPE solution (see Materials and Methods) with parallel 40 bp probe (A probe). (C) Autoradiograph after hybridization at 54°C in 2× SSPE solution (see Materials and Methods) with antiparallel 40 bp probe (T probe).

are involved in pairing, suggesting the high quality of the double helix. To study further the parallel duplex we have determined the relaxation time of the oligonucleotide duplex. The data indicated that this complex consists only of two strands of 40 bp in length and cannot be either a triplex or a hairpin. Thus, the high value of relaxation time (49 ± 3 ns), taken together with the high number of EtBr binding sites, independently confirm the high quality of the parallel duplex.

3.3. *Southern blot analysis of cloned DNA with parallel complementary oligonucleotide probes*

The previously cloned sequences of λ282 and p8.3, containing the same 8.3 kb *Eco*RI fragment from the *cut* locus of *D. melanogaster* [14] were used as the model for studying the hybridization of Southern filters with the parallel complementary probe (probe A). The same blot was hybridized in 2× SSC solution at 32°C with the parallel probe and then at 54°C with the antiparallel one. Fig. 2 shows the results. It is clear that both hybridizations take place only with the 8.3 kb *Eco*RI fragment and display the same efficiency specificity and low background. The same results were obtained with parallel 45 bp and 40 bp synthetic probes corresponding to the cloned sequences of the *D. melanogaster* suffix element [14] and *E. coli lon* gene [15], containing 26 and 17 GC pairs, respectively (not shown). Hybridization signals could also come from antiparallel hybridization of short stretches of parallel probe. To test this possibility, the longest 9 bp palindrome (from 21–29 bp, ACGTGTGCA) was hybridized in the conditions selected for parallel hybridization. No hybridization signal was detected (not shown). Therefore, we conclude that the hybridization band corresponding to the parallel probe (Fig. 2B) does indeed reflect the formation of parallel duplex. This is in agreement with our physical study, suggesting the high quality of the parallel duplex.

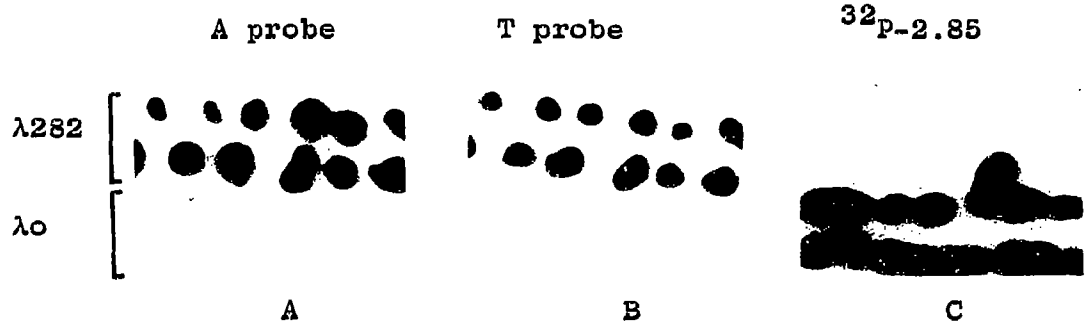

Fig. 4. Plaque hybridization experiments. (A) Autoradiograph of a replica (Schleicher & Schuell, 0.45 μm nitrocellulose filter) bearing λ282 (positive) and λo (negative) plaques [11] after hybridization at 32°C in 2× SSC solution (see Materials and Methods) with parallel probe (A probe). (B) Autoradiograph of a duplicate replica after hybridization at 54°C in 2× SSC solution (see Materials and Methods) with antiparallel probe (T probe). (C) Autoradiograph of a duplicate replica after hybridization at 65°C in 2× SSC solution with nick-translated 2.85 kb *Eco*RI fragment from plasmid p2.85, containing the fragment from λo [14].

3.4. *The hybridization analysis of genomic DNA with parallel complementary probes*

Encouraged by efficient hybridization of Southern blots containing cloned sequences with parallel complementary probes, we attempted to hybridize the probes with genomic Southern blots in 2× SSC solution, as well as in solutions containing 3 M tetramethylammonium chloride [16] or in 3 M tetramethylammonium bromide, but we observed a high background. Therefore we have used molecular hybridization in the agarose dried gels [10]. Fig. 3 presents the results of the hybridization of the previously used 40 bp probes with *Eco*RI digest of *D. melanogaster* DNA. The same 8.3 kb band hybridized with both probes. The efficiency and specificity of hybridization with the parallel complementary probe is very close to that of the antiparallel one. Low background in genomic hybridization suggests a rather small effect coming from antiparallel hybridization of short palindromes in parallel probe. Therefore, we conclude that parallel complementary probes could be used for analysis of genomic DNA digests.

3.5. *Plaque hybridization with parallel complementary probe*

For isolation of 'mirrored' antiparallel duplexes from different genomes, one needs a technique which allows the screening of recombinant DNA clones with parallel complementary probes. Fig. 4 demonstrates the autoradiogram after hybridization of duplicate nitrocellulose filters. The quality of signals obtained with both probes is very close and permits one to use parallel probes for isolation of clones from genomic libraries. We have isolated the corresponding region from *Drosophila* genomic library with the parallel probe. Only one false clone amongst the five selected was found: four clones possess the same 8.3 kb *Eco*RI fragment.

4. DISCUSSION

A primary purpose of our experiments was to elaborate the method allowing the detection of parallel complementary sequences in different genomes. Although several previous studies showed that, physically, DNA may form a parallel duplex [6–8,13], the question of whether a cell is using this possibility is still open. It has been speculated that families of 'mirrored' antiparallel duplexes may appear as a result of parallel biosynthesis [9,17]. One possible direct way to study these different non-homologous, although symmetric, sequences is to detect and isolate them from genomes by molecular hybridization techniques with synthetic parallel complementary probes.

This runs counter to the current view that molecular hybridization experiments may detect only homologous antiparallel complementary sequences. Moreover, prior to these experiments it was not at all obvious that rather long DNA molecules possessing an average GC content may form perfect and stable parallel duplexes. Nonetheless, the results reported here suggests that parallel complementary oligonucleotides can be used successfully as probes in molecular hybridization experiments with cloned and genomic sequences, as well as for effective screening of genomic libraries. Here we show that the methods for molecular hybridization with parallel probes on nitrocellulose filters and in dried agarose gels have specificity and background satisfying all classical criteria for molecular hybridization techniques with antiparallel probes, and can be used for the study of genomic 'mirrored' duplexes.

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