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# Association of Double-Stranded DNA Fragments into Multistranded DNA Structures

***Abstract:*** *We have previously observed that double-stranded DNA fragments containing a tract of the tandemly repeated sequence poly(CA) · poly(TG) can associate* in vitro *to form stable complexes of low electrophoretic mobility, which are recognized with high specificity by proteins HMG1 and HMG2. The formation of such complexes has since been observed to depend on interactions of DNA with polypropylene surfaces, with the suggestion that the formation of low mobility complexes might be the result of strand dissociation followed by misaligned reassociation of the repetitive sequences. The data presented here show that at high ionic strength the interactions of DNA with polypropylene are sufficiently strong for DNA to remain bound to the polypropylene surface, which suggests that DNA might also be involved in interactions with hydrophobic molecules* in vivo. *Under such conditions, low-mobility complexes are found only in the material adsorbed to the polypropylene surface, and all DNA fragments are able to form low-mobility structures, whether or not they contain repetitive sequences. Preventing the separation of strands by ligating hairpin loop oligonucleotides at both ends of the fragments does not prevent the formation of low-mobility complexes. Our results suggest two different pathways for the formation of complexes. In the first, dissociation is followed by misaligned reassociation of repetitive sequences, yielding duplexes with single-stranded end regions that associate to form multimeric complexes. In the second, repetitive as well as nonrepetitive DNA molecules bound to polypropylene adopt a conformation with locally unwound regions, which allows interactions between neighboring duplexes adsorbed on the surface, resulting in the formation of low-mobility complexes.* © 1999 John Wiley & Sons, Inc. Biopoly 50: 679–689, 1999

***Keywords:*** *low-mobility DNA complexes; DNA binding to hydrophobic surfaces; DNA strand exchange; poly(CA) · poly(TG) microsatellite*

## INTRODUCTION

The tandemly repeated sequence poly(CA) · poly(TG), also known as CA microsatellite, is found in tracts up to 60 base pairs (bp) long dispersed at thousands of sites in eucaryotic genomes. We have previously observed that double-stranded DNA fragments containing such sequences can associate with each other *in vitro*[1] to form complexes first observed on electrophoretic gels as a series of slow-migrating bands that formed a regular ladder. The complexes could be eluted from a preparative gel and visualized by electron microscopy, where they appeared as X-shaped structures, in which double-stranded DNA molecules seemed to be specifically associated at the level of their poly(CA) · poly(TG) tracts.[1] The complexes

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were also recognized with high specificity by the two nuclear nonhistone proteins HMG1 and HMG2.[1]

These early results, however, gave no precise indication as to the structure and the mechanism of formation of the complexes we observed. The first information on this subject came with the observation that the formation of low-mobility complexes was dependent on the interactions of DNA with polypropylene tube surfaces.[2,3] This observation was later documented by Belotserkovskii and Johnston,[4,5] who proposed a model based on the fact that interactions of DNA with polypropylene surfaces often induce some DNA denaturation. In the case of a repetitive DNA fragment, reassociation of the strands out of register in the repetitive region would produce duplexes with single-stranded regions. These could associate, in turn, to form multimeric low mobility complexes.[2,4,5]

In parallel to the work of Belotserkovskii and Johnston, we have obtained new results that are presented here on the interactions of DNA with polypropylene surfaces and on the formation of low-mobility complexes. We show that at high ionic strength the interactions of DNA with polypropylene surfaces are strong enough to maintain DNA adsorbed to the surface, held by interactions that have characteristics of hydrophobic interactions, suggesting that such interactions might also exist *in vivo*. Under such incubation conditions, low-mobility complexes are found only in the fraction bound to polypropylene. The formation of low-mobility complexes by nonrepetitive sequences had not been observed before. Here we show that any DNA fragment, repetitive or nonrepetitive, is able to form complexes of low mobility, even if the separation of its strands is prevented by ligation of hairpin oligonucleotides at its ends. Our results suggest two possibilities for the formation of complexes. The first possibility corresponds to the model of Belotserkovskii and Johnston, applies to repetitive sequences, and involves the misaligned reassociation of the single strands of repetitive fragments, yielding duplexes with single-stranded end-regions that associate to form multimeric complexes. The second possibility applies to any sequence, repetitive or nonrepetitive, and involves the partial unwinding of the double helix upon adsorption of DNA to polypropylene in high salt, followed by association of neighboring duplexes into low mobility complexes.

## MATERIALS AND METHODS

### DNA Fragments

All DNA fragments were derived from plasmids grown in *Escherichia coli*, and plasmid purification, restriction enzyme digestion, and gel electrophoresis were performed following standard procedures. For radioactive labeling, fragments were dephosphorylated with calf alkaline phosphatase, $^{32}$P-labeled at their 5′ ends with polynucleotide kinase and [$\gamma$-$^{32}$P]-ATP, and repurified by electrophoresis on polyacrylamide gels and electroelution.

Fragments containing the sequence poly(CA) · poly(TG) were obtained by digestion of plasmid pE10 and contain a 60 bp tract of poly(CA) · poly(TG). The partial nucleotide sequence of pE10 has been deposited at the EMBL database under accession number X96980. The fragment used for most experiments shown here is a 120 bp fragment obtained by digestion of pE10 by *Eco*RI + *Cla*I. A longer, 298 bp fragment, used for the experiment of Figure 3, was obtained by digestion by *Pvu*II + *Cla*I.

Two DNA fragments lacking poly(CA) · poly(TG) were derived from genomic sequences of SV40 virus that had been cloned in pBR322. Fragment HS, 197 bp, was obtained by digestion with *Hind*III + *Sfa*NI, map position −73 to 125. Fragment SB, 185 bp, was obtained by digestion with *Sty*I + *Ban*I, map position 37 to 294 with one of the 72 bp repeats in the enhancer deleted. The third nonrepetitive fragment was the 123 bp *Msp*I fragment from pBR322.

To prepare fully closed linear molecules, hairpin oligonucleotides 26 bases long containing appropriate self-complementary sequences and cohesive ends were added to the ends of labelled linear fragments using T4 DNA ligase. After ligation, all the incompletely closed molecules were destroyed by digestion with a large excess of exonuclease III, and the fully closed, exonuclease III-resistant remaining molecules were purified on a polyacrylamide gel. The resulting molecules were resistant to heat denaturation and to phosphatase treatment, as expected.

### Formation of Low-Mobility Complexes

In a polypropylene tube (1.5 mL SafeLock tubes from Eppendorf, Hamburg), ~ 50,000 cpm of labeled DNA fragment, in 3 $\mu$L of 50 m*M* Tris HCl pH 7.5, 1 m*M* EDTA, are gently mixed with 3 $\mu$L of 5*M* NaCl with the tip of a micropipette. The samples are incubated overnight at 37°C in an oven to prevent water condensation in the tube caps. As described in Results, a large fraction of DNA binds to the tube walls during this incubation period and contains the low-mobility complexes. After incubation, the liquid is transferred to a new tube, and the DNA bound to the wall of the first tube is redissolved in 20 $\mu$L of 10 m*M* Tris HCl pH 7.5, 1 m*M* EDTA, 0.1% Triton X100. Cerenkov counting of both tubes yields an estimate of the fraction of DNA bound, usually 75–95% of the total added.

The above procedure is the most reliable that we have found, and in the course of developing this procedure we have made the following additional observations. First, some batches of polypropylene tubes are more efficient than others for obtaining complexes, and washing tubes with water before incubation was sometimes necessary, suggesting the presence of binding inhibitors in some tubes. Second, Triton X100 completely inhibits DNA binding to tube

walls as well as formation of low-mobility complexes. However, once the complexes are formed they are perfectly stable in the presence of this detergent, and to improve recovery we add 0.1% Triton to the buffer used to redissolve the complexes. Third, siliconizing the tubes also prevents the DNA from binding to polypropylene. However, with some DNA fragments containing poly(CA) · poly(TG) we observe the formation of complexes in siliconized tubes at low ionic strength as in our initial observations.[1] Fourth, the nature of the salt seems unimportant. We routinely use sodium chloride, but substituting potassium makes no difference, and using cesium chloride or ammonium sulfate gives similar or even slightly better results. Fifth, the overnight incubation can be reduced to a few hours with little change in the results. And finally, the air–water interphase plays no role in the phenomenon: when incubated in a large volume, DNA binds all over the immersed polypropylene surface, and the same binding of DNA to polypropylene also occurs under an oil layer.

Low-mobility complexes are usually analyzed by electrophoresis in 4% polyacrylamide gels (acrylamide : bisacrylamide ratio 29:1) in 6.7 m*M* Tris acetate, 3.3 m*M* Na acetate, 1 m*M* EDTA, pH 7.8, at 4°C, with buffer recirculation. However, electrophoresis at room temperature in 40 m*M* Tris acetate, 20 m*M* Na acetate, 1 m*M* EDTA, pH 7.8, usually gives very similar results.

## Chemical and Enzymatic Probing of DNA Complexes

For in-gel chemical modification of DNA complexes, bands were cut from a preparative polyacrylamide gel and electrophoresis buffer added to bring the volume to a total of 200 $\mu$L. Following addition of 3 $\mu$L of diethylpyrocarbonate (DEPC), the samples were incubated at room temperature for 20 min with occasional vortexing. DNA was immediately electroeluted, precipitated twice with ethanol, cleaved at DEPC-modified sites by incubation at 90°C for 20 min in the presence of 1*M* piperidine, and lyophylized.

DNAseI digestion of DNA complexes was carried out in gel slices incubated in a total volume of 200 $\mu$L in 5 m*M* $MgCl_2$, 2 m*M* $CaCl_2$, and 0.01 $\mu$g/mL DNAseI in electrophoresis buffer. After a 30 min incubation at 25°C, the digestion was stopped by addition of EDTA to 10 m*M*, and DNA was electroeluted and precipitated with ethanol.

Electrophoretic analysis of the products was performed on an 8% denaturing polyacrylamide gel in 7*M* urea, using a Maxam-Gilbert G+A sequencing reaction as size marker.

# RESULTS

## Formation of Low Mobility Complexes by DNA Fragments Containing Poly(CA) · Poly(TG)

We have previously shown that DNA fragments containing the repetitive sequence poly(CA) · poly(TG) can form low mobility complexes.[1] This phenomenon was initially observed after storage of DNA fragments for several days at 4°C in 10 m*M* Tris HCl, 1 m*M* EDTA, pH 7.5. During our further work aimed at studying these complexes more precisely, we found that their formation was not perfectly reproducible and that their yield was very variable, which led us to search for conditions in which the phenomenon would appear in a more reproducible way. Attempts to identify a chemical that might be present in trace amounts and stimulate the phenomenon were unsuccessful. Instead, we observed that the formation of complexes was much more reproducible and strongly stimulated by incubating the fragments at 37°C and high ionic strength. More recently, we observed that this incubation at 37°C and high ionic strength induced the binding of DNA fragments to the walls of the polypropylene reaction tubes, and that the low mobility complexes were found specifically in the bound fraction. This result is illustrated in Figure 1, where a 120 bp fragment containing 60 bp of poly(CA) · poly(TG) was incubated overnight at 37°C in 2.5*M*

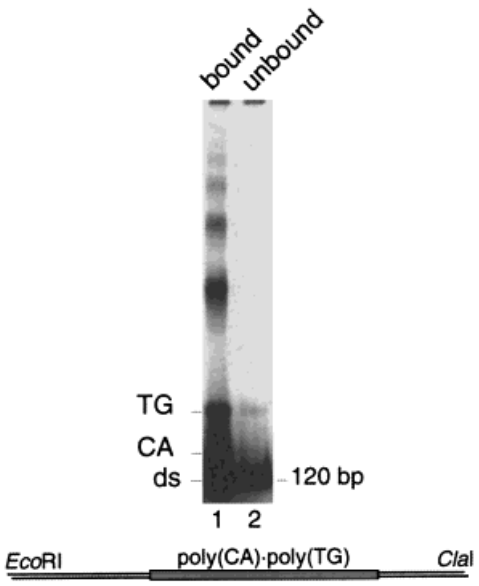


**FIGURE 1** Formation of low-mobility complexes and interaction of DNA with polypropylene. A 120 bp DNA fragment (map at the bottom) containing a 60 bp tract of poly(CA) · poly(TG) was incubated overnight at high ionic strength at 37°C. The material bound to the polypropylene tube walls was redissolved in TE buffer containing 0.1% Triton X-100 (see Materials and Methods) and analyzed on a 4% polyacrylamide gel (lane 1) along with the material remaining in solution (lane 2). TG and CA indicate the positions of the poly(TG)- and poly(CA)-containing strands, respectively. ds: double-stranded fragment. Note that the low-mobility complexes are all in the bound fraction.

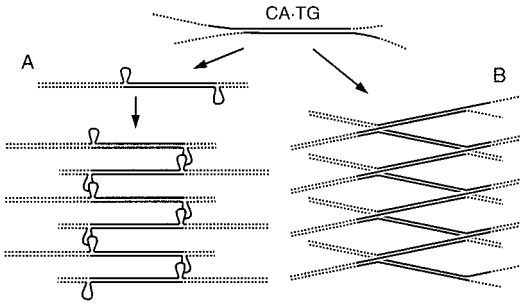


**FIGURE 2** Models of low-mobility structures that can be formed by a DNA fragment containing the sequence poly(CA) · poly(TG). After strand dissociation followed by misaligned reassociation in the central repetitive region, two models are possible: formation of single-stranded loops (A) or formation of four-way junctions (B), both of which lead to the formation of low-mobility complexes.

NaCl in a polypropylene tube. The fraction bound to the tube wall was then redissolved in a buffer of low ionic strength containing 0.1% Triton and analyzed on a polyacrylamide gel along with the unbound fraction. The low-mobility complexes, which form a ladder of bands extending all along the gel, are found in the fraction bound to the tube (lane 1) and are not visible in the unbound fraction (lane 2), even after long exposure (not shown). The percentage of this DNA bound can be as high as 95%, but the fraction of DNA that we observe as retarded material on polyacrylamide gels remains always less than 5% of the total.

At the same time, the formation of complexes was observed to be linked to the presence of single strands in the samples, resulting from the interactions of DNA fragments with polypropylene surfaces of the tubes.[2–5] This observation led Belotserkovskii and Johnston to propose a model for the low-mobility structures,[2,4,5] which is shown in Figure 2. In this model the dissociation of the strands of a duplex containing a poly(CA) · poly(TG) tract, followed by their reassociation out of register in the central repetitive region, leads to the formation of duplexes with single-stranded loops (Figure 2A) or to the formation of four-way junctions (Figure 2B), which can, in turn, form oligomers.

Indeed, the results recently published by Belotserkovskii and Johnston[5] are in good agreement with our unpublished data, and suggest that the complexes observed, for example in Figure 1, with short fragments containing the poly(CA) · poly(TG) sequence correspond to four-way junctions and their oligomers, as shown in Figure 2B. An additional result supporting this model is shown in Figure 3. By incubating at high ionic strength a mixture of two fragments of different lengths with the smaller corresponding to the right-hand part of the larger (see map Fig. 3D), a new band of low mobility is observed (band Y, Figs. 3A and 3B). This band does not appear when either fragment is incubated alone (lanes 0, Figure 3A). It contains all four different strands, as shown by individual labeling of all four ends (data not shown). As shown in Figure 3B, the structure in band Y can be cut by restriction enzymes with recognition sites in the fragments, with the remarkable exception of the *Eco*RI site, which is resistant (other restriction enzymes not shown on the figure gave similar results). These results do not suggest an association of duplexes containing single-stranded loops, as in such a case the *Eco*RI site would remain sensitive to digestion. Rather, a model of Y-shaped structure such as shown in Figure 3D is in good agreement with the data, since in such a model the *Eco*RI site is located precisely at the junction and is therefore expected to be resistant to digestion. This model was confirmed by enzymatic and chemical probing of band Y directly in the polyacrylamide gel slice. Chemical probing was performed with DEPC, which detects unpaired purines. Figure 3C shows that a single site is sensitive to DEPC in form Y as compared to the double-stranded fragment, and that this sensitive site is precisely at the *Eco*RI site. No other structure modification can be detected with DEPC elsewhere along the fragment. DNAseI, which cuts single-stranded DNA less efficiently than double-stranded DNA, was also used to probe the structure of band Y, and failed to detect any major change of DNA conformation between form Y and the regular double-stranded fragment (Fig. 3C).

The most likely model for the structure of the low-mobility complexes formed by poly(CA) · poly(TG)-containing fragments is therefore the model of Figure 2B, in which associations are mediated by strand exchanges between different duplexes. In addition, this model is in good agreement with the observation that proteins HMG1 and HMG2 interact specifically with these complexes,[1] since these two proteins are known to interact specifically with four-way junctions.[6]

The following results, however, show that not all low mobility structures fit this model and that the mechanism of their formation may be more complex than a simple dissociation–reassociation.

## Reassociation of DNA Fragments at High Concentration

First, as we mentioned briefly earlier,[3] this formation of low-mobility complexes is not simply the result of strand dissociation followed by reassociation, as the reassociation of single strands containing the se-

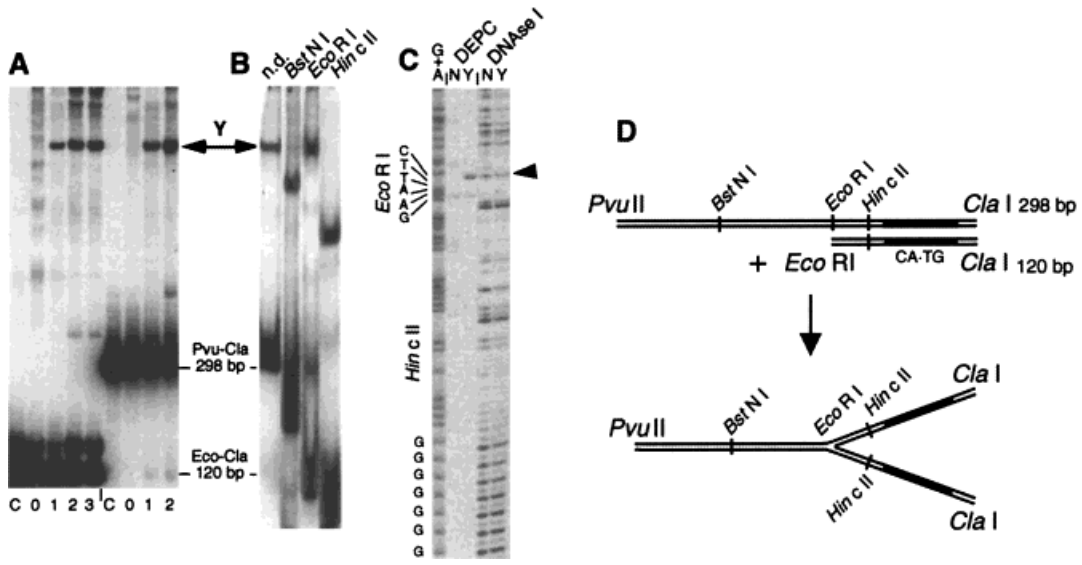


**FIGURE 3** Formation of complexes by two fragments of different lengths. In these experiments, two fragments of different lengths were used, the smaller 120 bp fragment consisting of the right-hand part of the larger 298 bp fragment. The map shows the positions of the restriction sites and of the 60 bp poly(CA) · poly(TG) tract on the fragments. (A) Appearance of a new band of low mobility, Y, when both fragments are incubated together. Two series of samples are shown, the first one with the smaller fragment labeled, the second one with the larger fragment labeled. Lanes C: nonincubated controls. Lanes 0: labeled fragment incubated alone. Lanes 1–3: labeled fragment incubated with 3, 6, and 12 ng of the other fragment unlabeled. After incubation, the complexes were analyzed by electrophoresis on a 4% polyacrylamide gel. (B) Digestion of band Y with restriction enzymes. The complexes were incubated in restriction buffer in the absence (nd) or in the presence of the restriction enzymes *Bst*NI, *Eco*RI, and *Hinc*II, as indicated. Note that the material in the upper band Y is not cut by *Eco*RI. (C) Enzymatic probing with DNAseI and chemical probing with DEPC. With the larger fragment 5′ end labeled at its ClaI site, low-mobility complexes were formed and fractionated on a preparative polyacrylamide gel. Band Y and free DNA were cut, and both gel slices were incubated with DNAseI or DEPC as described in Materials and Methods. DNA was electroeluted, the DEPC-treated samples were cleaved with piperidine at modified sites, and all samples were analyzed on a sequencing gel along with a G + A size marker. Note in band Y the DEPC-sensitive site at the position of the EcoRI site (arrowhead). (D) Maps of the fragments and model of the structure in band Y.

quence poly(CA) · poly(TG) does not yield low-mobility complexes when care is taken to suppress all interactions between DNA and polypropylene surfaces. To do so we have used the nonionic detergent Triton-X100, which does not interfere with DNA renaturation but completely suppresses both the binding of DNA to polypropylene at high ionic strength and the formation of multistranded complexes (data not shown). Under such conditions, the 120 bp DNA fragment containing the poly(CA) · poly(TG) sequence was heat denatured and allowed to renature, at DNA concentrations ranging between 0.1 and 100 ng/$\mu$L (1.3 $\mu M$ and 1.3 m$M$, respectively). The result is shown on Figure 4B: the band ladder seen before is not detectable in this experiment, even at the highest concentration (lane 6), and even upon overexposure of the autoradiogram (Figure 4C). The only band of low mobility (bracket Figure 4C) was found to contain only the CA strand (data not shown), which seems to be able to associate with itself. Renaturation in glass capillary tubes gave identical results (not shown). These experiments confirm and extend to much higher fragment concentrations the last results of Belotserkovskii and Johnston,[5] who also showed that no ladder formation occurs upon reassociation at a fragment concentration of $\sim$ 10 n$M$ in borosilicate glass, unless they modified the conditions by using polyethylene glycol or a high magnesium concentration.

## Formation of Low-Mobility Structures by Nonrepetitive DNA Fragments

Given the efficiency with which fragments containing poly(CA) · poly(TG) form low-mobility complexes

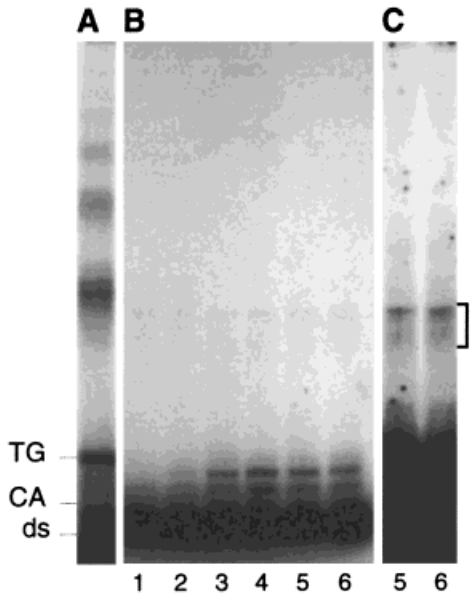


**FIGURE 4** Reassociation of DNA strands under conditions that suppress interactions of DNA with polypropylene. The 120 bp fragment containing a 60 bp tract of poly(CA) · poly(TG) was heat denatured at different concentrations, and allowed to renature in the presence of a nonionic detergent, which suppresses all interactions between DNA and polypropylene tube walls. (A) Control: ladder of low mobility complexes formed by adsorption of the fragment to polypropylene surface. TG and CA indicate the positions of the TG- and CA-containing single strands, respectively. ds: double-stranded 120 bp fragment. (B) Reassociation of the fragment in 0.1% Triton-X100. The total fragment concentration was varied by addition of increasing amounts of the same fragment unlabeled. Total fragment concentration: 0.1, 1, 3, 10, 30, and 100 ng/$\mu$L in lanes 1–6, respectively. After reassociation, the material was analyzed by electrophoresis on a 4% polyacrylamide gel and autoradiography. (C) Overexposure of lanes 5 and 6. The bracket indicates a low-mobility form that contains only the CA strand.

under high ionic strength conditions, we used the same conditions with DNA fragments containing no repetitive sequences. Two 190 bp fragments, HS and SB from the genome of SV40, were used. Figure 5 shows that they both yield retarded material appearing as a smear all along the gel with some discrete bands superimposed. No regular ladder is observed with these fragments. As before, retarded material represents only a few percent of total DNA. As was previously performed with poly(CA) · poly(TG) fragments, slices were cut from a preparative gel as indicated on Figure 5A, the material was electroeluted, precipitated with ethanol, redissolved, and analyzed on a second gel (Figure 5B). While more than 50% of the material becomes dissociated during the elution process and migrates as the initial double-stranded fragment, a significant fraction of the eluted material migrates at the position where it was cut from the preparative gel. This result is very similar to that obtained with poly(CA) · poly(TG); however, some complexes formed with fragments containing no repetitive sequence appear to be somewhat less stable. This conclusion might be drawn from the fact that slices HS3 and SB5 that were apparently uniform before elution each show two sharp bands on the second gel, indicating a selective dissociation of some of the complexes.

A study of the stability of the complexes as a function of temperature is shown in Figure 5C. It is observed that the stability of the eluted structures decreases when the temperature increases, up to the melting temperature of the fragment when all structures dissociate into the single strands of the starting fragment. This proves incidentally that the retarded material is not due to a contamination by long DNA fragments.

## Formation of Low-Mobility Structures by Nonrepetitive Closed Linear Molecules

To test the hypothesis that the formation of complexes involves a denaturation of the fragments, we studied whether closing the extremities of DNA fragments by ligation of hairpin oligonucleotides at both ends would have an effect on the formation of complexes. Figure 6 shows that this is not the case. High ionic strength incubation of a 123 bp fragment from pBR322, either linear, or after ligation of hairpins at both ends (see Materials and Methods), yields basically similar results (Figure 6A), with some differences in the pattern of faint bands superimposed over the continuous background. In addition, the elution of the retarded material and its analysis on a second gel gives similar results whether or not the DNA fragment is closed with ligated hairpins (Figure 6B).

A similar experiment was performed with the initial 120 bp fragment that contains the poly(CA) · poly(TG) sequence. The ligation of hairpins at both ends of this fragment should prevent the formation of the structures shown in the models of Figure 2. In the case of single-stranded loops, their formation should be prevented by an ultrafast reassociation starting at the palindromic ends to extend in register all along the fragments. In the case of four-way junctions, their formation is simply impossible since strand exchange between duplexes can no longer occur. The result of the experiment is striking: in contrast to the ladder observed with the starting 120 bp fragment (Figure 7A, lane 1), no band ladder is observed with closed linear molecules (Figure 7A, lane 2), a result in good

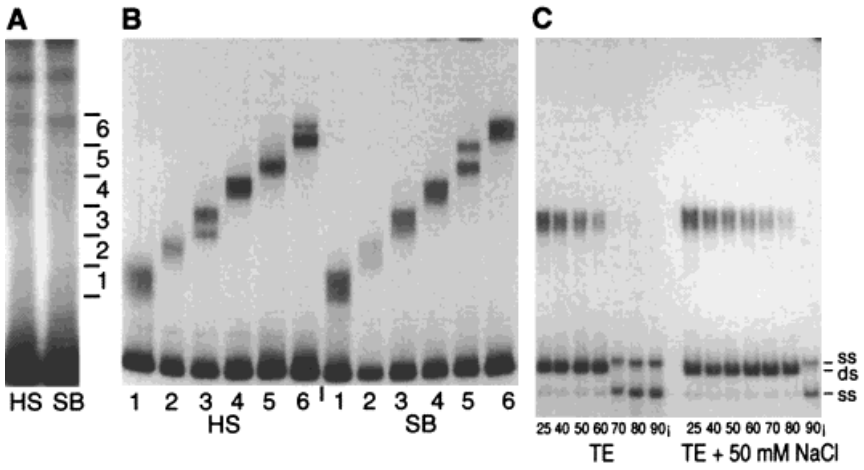


**FIGURE 5** Complexes formed by DNA fragments containing no repetitive sequence. (A) Two 190 bp fragments from the SV40 genome, HS and SB, were incubated at high ionic strength and analyzed on a 4% polyacrylamide gel. The retarded material forms a smear with some discrete bands superimposed. (B) Stability of the complexes. For each of the fragments, bands labeled 1–6 in (A) were cut from a preparative gel, DNA was electroeluted, ethanol precipitated, and analyzed on a second gel. (C) Thermal stability of the complexes. Material from band SB3, dissolved either in TE or in TE + 50 m*M* NaCl, was incubated 10 min at the indicated temperatures and loaded on a polyacrylamide gel. The positions of the single strands (ss) and of the double strand (ds) are indicated.

agreement with the models of Figure 2. But structures of low mobility are still present, albeit in lower amount, and are still stable enough to withstand purification and migrate at the same position on a second gel after electroelution and ethanol precipitation (Figure 7B). Repetitive fragments therefore behave like nonrepetitive fragments when their ends are closed.

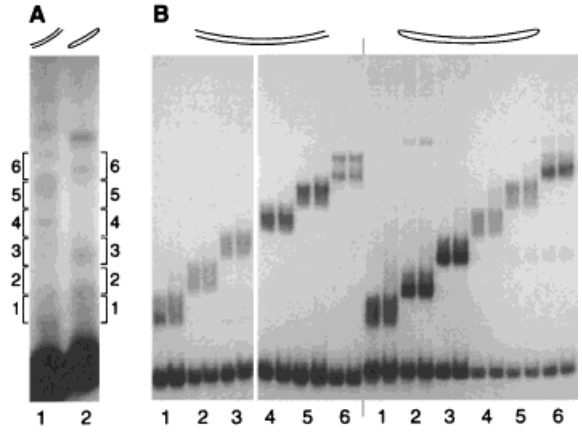


**FIGURE 6** Complexes formed by a nonrepetitive fragment with hairpin loops ligated at both ends. A 123 bp nonrepetitive fragment from plasmid pBR322 was ligated with hairpin loops formed by palindromic synthetic oligonucleotides (see Materials and Methods) to form a linear molecule with no free ends, as represented at the top of the figure. (A) This substrate was adsorbed to polypropylene and analyzed on a polyacrylamide gel (lane 2A), in parallel with the starting linear fragment incubated in the same way (lane 1A), showing the presence of retarded material. (B) Material from slices 1–6 cut from the first gel was electroeluted and analyzed on a second polyacrylamide gel.

## Electron Microscopy of the Complexes

We have previously shown that a linear plasmid, 2390 bp long containing a $(CA)_{30}$ insert, forms low-mobility complexes when incubated at high ionic strength. After their purification on agarose gels (Figure 8A), analysis of these complexes by electron microscopy showed X-shaped structures, with the location of the junction relative to the ends being compatible with a specific association of the poly(CA) · poly(TG) sequences, although electron microscopy was not accurate enough to determine precisely the location of the junctions along the DNA molecule. Some examples of such X-shaped structures are shown again in Figure 8B. A further analysis of this material has also shown other structures present in very variable yield from one preparation to another. Approximately as frequent as X-shaped structures are T-shaped complexes (Figure 8C), with one of the ends of the first duplex bound to an internal point of the other duplex. The binding point seems located at random along the second molecule, and shows no correlation with the position of the poly(CA) · poly(TG) sequence. Rather infrequently, X-shaped structures in which the junction is

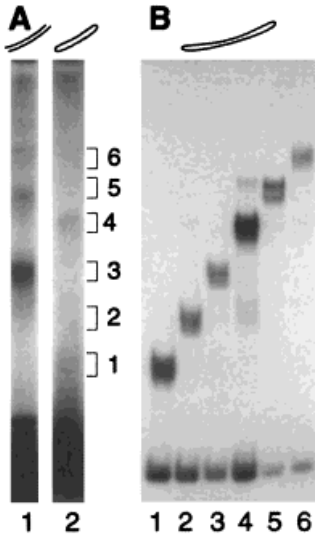


**FIGURE 7** Complexes formed by a fragment containing poly(CA) · poly(TG) with hairpin loops ligated at both ends. The experiment is similar to the experiment shown in Figure 6. The 120 bp fragment containing poly(CA) · poly(TG) was ligated with hairpin loops formed by palindromic synthetic oligonucleotides (see Materials and Methods) to form a linear molecule with no free ends, as represented at the top of the figure. (A) This substrate was adsorbed to polypropylene and analyzed on a polyacrylamide gel (lane 2A), in parallel with the starting linear fragment (lane 1A), showing the presence of retarded material. (B) Material from slices cut from lane 2 of the first gel was electroeluted and analyzed on a second polyacrylamide gel.

not at the level of the poly(CA) · poly(TG) insert are also observed (Figure 8D). Work is in progress to try to directly visualize these structures by atomic force microscopy of DNA complexes bound to polypropylene surfaces.

These two kinds of structures do not seem to be linked to the presence of poly(CA) · poly(TG). This result is confirmed by the fact that upon incubation of linear pBR322 at high ionic strength, electrophoresis on agarose gels also shows the presence of slowly migrating complexes (Figure 8E). These results show by an entirely different technique the possibility of forming complexes with any DNA sequence. We should mention, however, that our experiments show the difficulty of forming such structures with circular DNA molecules, even nicked (data not shown). Whether this is simply due to steric problems or to restrictions on the partial opening of the duplexes is still unclear.

### Mechanism of Complex Formation

Finally, two results obtained with DNA adsorbed to the polypropylene surface give indications as to the possible mechanism of formation of low-mobility complexes. First, different amounts of the 120 bp fragment containing the poly(CA) · poly(TG) sequence were incubated at high ionic strength, with all other conditions being identical. The adsorbed material was then analyzed on a gel (Figure 9), showing that the percentage of denaturation remains constant when the amount of DNA decreases, a result confirmed by quantitation of bands with a Phosphorimager. This strongly suggests that DNA binding to polypropylene leads to complete strand separation of only a small fraction of all adsorbed molecules, while the two strands of the majority of the bound fragments remain in contact with each other.

In a second experiment, the end-labeled DNA fragment containing poly(CA) · poly(TG) was incubated at high ionic strength in the presence of increasing amounts of one of its strands, unlabeled. In parallel, identical samples were heat denatured and allowed to renature. The result of the denaturation–renaturation is, as expected, a complete redistribution of the strands, revealed by the presence of an intense band corresponding to the labeled TG strand displaced by the excess of unlabeled TG strand in lanes 5 and 6. Strikingly, a similar result is seen in lanes 2 and 3, whereas DNA was not heat denatured but was simply adsorbed to polypropylene, indicating that all labeled double-stranded DNA fragments have exchanged their TG strand with the free unlabeled TG single strands. The implications of these results with respect to the structure of DNA adsorbed to polypropylene surfaces will be discussed later.

## DISCUSSION

It was previously shown that DNA can interact with polypropylene surfaces and that these interactions can induce the denaturation of DNA fragments.[2–5] We have now shown that, at high ionic strength, these interactions are strong enough to allow stable binding of DNA to polypropylene. Keeping the ionic strength high, we observe similar interactions with polyethylene and paraffin, suggesting that we are dealing with interactions of DNA with hydrophobic polymers, not with a chemical added during the tube manufacturing process. Similarly, the fact that such interactions are strongly stimulated by high ionic strength and completely inhibited by nonionic detergents suggests that these are interactions of a hydrophobic nature. While the stickiness of DNA to plastic tubes is well known to experimenters, very little is known about the interactions of DNA with purely hydrophobic macromol-

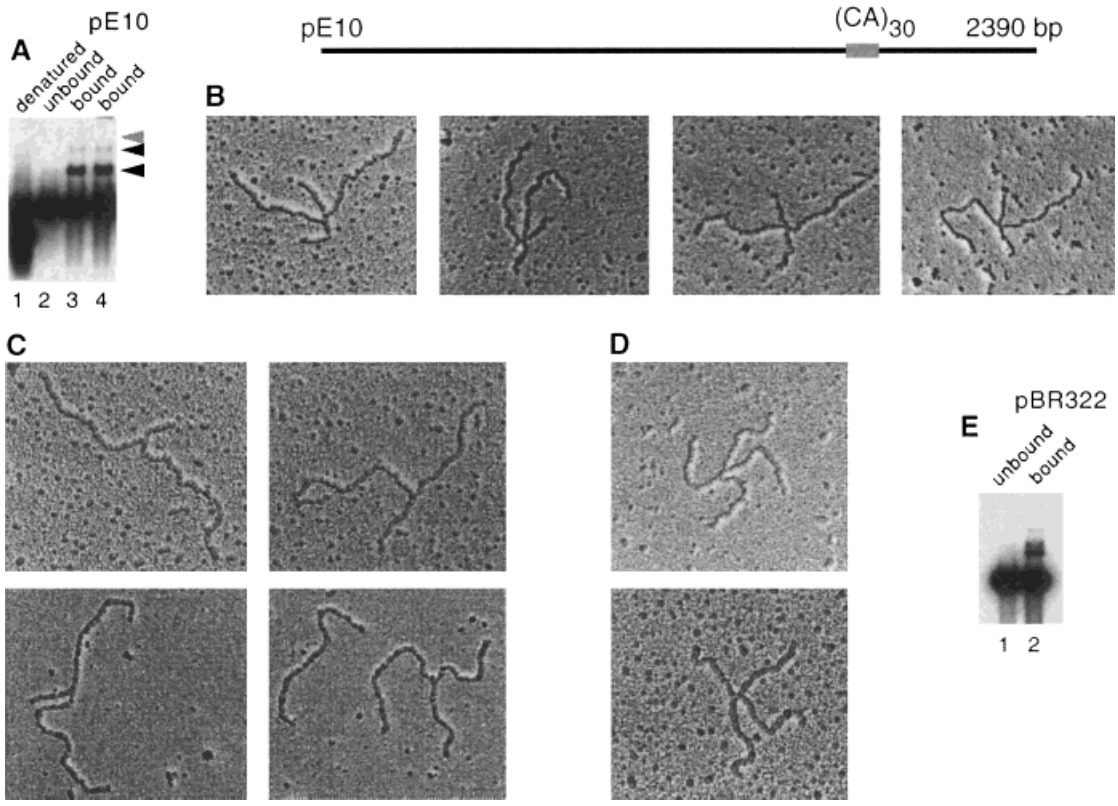


**FIGURE 8** Formation of complexes with long DNA fragments. (A) Linear plasmid pE10, which contains a 60 bp tract of poly(CA) · poly(TG) (map at the top of the figure), was incubated at high ionic strength and analyzed on an agarose gel. The material in the first retarded band was eluted and analyzed by electron microscopy. Several different structures were observed: (B) X-shaped structures with association of two duplexes at the level of the poly(CA) · poly(TG) tract.[1] (C) T-shaped structures, with the end of one of the fragments bound to a random point inside the other fragment. (D) X-shaped structures with the junction elsewhere than at the poly(CA) · poly(TG) sequence. (E) Same experiment as in (A) with linear pBR322, which contains no poly(CA) · poly(TG), analyzed on an agarose gel after incubation at high ionic strength.

ecules and our results suggest that DNA might also interact with hydrophobic molecules *in vivo.*

At 37°C and high ionic strength, fragments containing the sequence poly(CA) · poly(TG) are not the only ones able to form low-mobility complexes. Actually any fragment, repetitive or not, can form structures of low mobility. With nonrepetitive fragments, the yield and stability of the complexes are somewhat lower, but the low-mobility structures are still stable enough to partially withstand purification by electrophoresis, electroelution, and ethanol precipitation.

The precise structure of the low-mobility material remains to be determined. For short DNA fragments containing the sequence poly(CA) · poly(TG), our results are in good agreement with the results and the model of Belotserkovskii and Johnston. They suggest a structure as shown in Figure 2B, formed of four-way junctions and of their oligomers, resulting from shifted associations of the DNA strands in the repetitive sequence.

This model is satisfactory for short, partially repetitive fragments, but must be modified if it is to fit other examples observed. Nonrepetitive sequences can form stable structures of low mobility even when the fragments are closed by ligation of hairpin oligonucleotides at their ends. Similarly, repetitive fragments with closed ends behave exactly as nonrepetitive fragments. While such low-mobility structures cannot be the result of complete strand separation, it seems very likely that their formation also involves some unwinding of the double helix mediated by interactions with polypropylene. Theoretical models have been built of four-stranded structures formed by the association of two double-stranded DNA molecules.[7–9] One of these can apply to DNA with no free ends and requires an unwinding step,[9] and it will be

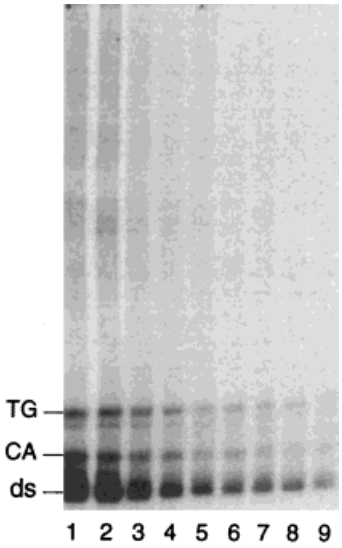


**FIGURE 9** Adsorption of decreasing amounts of DNA to polypropylene. The 120 bp fragment with a 60 bp tract of poly(CA) · poly(TG) was adsorbed to polypropylene in decreasing amounts to study the influence of the concentration on the percentage of denaturation. After electrophoresis, the percentages of denaturation in each sample were measured by quantitation of the bands with a Phosphorimager. Fragment amounts: 400, 200, 100, 50, 25, 6, 3, 1.5, and 0.8 pg in lanes 1–9, respectively.

very interesting to determine whether it is related to our experimental observations. Finally, the basic question is whether the low-mobility structures that we observe can all be explained by Watson–Crick pairing.

The possible conformation of DNA bound to polypropylene surfaces is an interesting question. In solution, high ionic strength stabilizes the DNA duplex, and poly(CA) · poly(TG) is as stable as nonrepetitive DNA to heat denaturation.[10] When adsorbed to polypropylene, DNA behaves differently: high ionic strength does not stabilize the adsorbed duplexes and all bound fragments are able to exchange one of their strands with a free single strand, so that one might consider that the adsorbed DNA is completely denatured and that it renatures upon redissolution. However, in contrast to renaturation in solution, a large amount (>90%) of the double stranded fragment is always present immediately after redissolution, and the percentage of single strands is not concentration dependent. Therefore adsorbed DNA has quite different properties from DNA in solution, and it will be interesting to study to what extent the bases remain in interaction in the adsorbed DNA.

The question can be raised as to whether our results, which depend strongly on the interactions of DNA with polypropylene at high ionic strength, have a relation with DNA structure *in vivo.* While polypropylene is required for complex formation, high ionic strength is not an absolute requirement. In our initial work with fragments containing poly(CA) · poly(TG) we observed the formation of complexes at low or physiological ionic strength, and it is possible to suggest that molecules might exist inside a cell with a similar effect to polypropylene. In any case, our results constitute still another example of the structural variability of DNA (for a recent review, see Ref. 11), and of its ability to remain in stable conformations that are very different from that of the B-form double helix. As B-DNA has a fairly uniform structure, any different conformation is interesting as a potential signal or intermediate for a particular process. For example, the strand exchange and the association be-

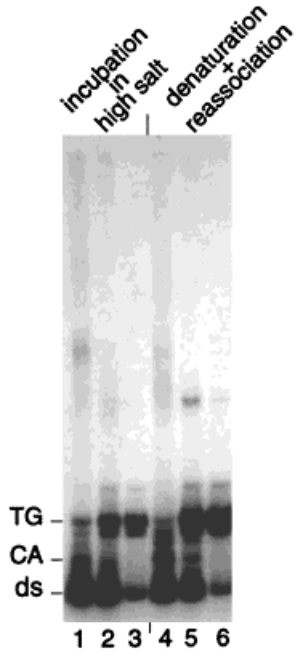


**FIGURE 10** Strand exchange between a labeled duplex and an unlabeled single strand. The same 120 bp fragment as above, labeled at both ends, was adsorbed to polypropylene at high ionic strength either alone (lane 1), or in the presence of increasing amounts of one of its unlabeled strands (TG strand, lanes 2–3). After overnight incubation, the adsorbed material was redissolved and analyzed on a polyacrylamide gel. In parallel, identical samples were heat denatured and allowed to reassociate in the presence of Triton-X100 to inhibit interactions of DNA with polypropylene (lanes 4–6). Labeled fragment amount: ~ 0.2 ng in all lanes. Amount of unlabeled single strand: 5 ng in lanes 2 and 5, 25 ng in lanes 3 and 6. Note in lanes 2 and 3 the large amount of free labeled TG strand, which has been displaced from the duplex upon high salt incubation of the double-stranded fragment in the presence of its homologous TG strand unlabeled.

tween DNA duplexes that we observe are very reminiscent of recombination processes.

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