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Noncanonical DNA Elements in the Lamin B2 Origin of DNA Replication*

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DNA replication origins of eukaryotes lack linear replicator elements but contain short $(dT)_n$ $(dA)_n$ sequences that could build mutually equivalent unorthodox structures. Here we report that the lamin B2 origin of DNA replication adopts an alternative form characterized by unpaired regions CTTTTTTTTTTCC/GGAAAAAAAAAAG (3900–3912) and CCTTTTTTTTC/GAAAAAAAAGG (4141–4151). Both unpaired regions are resistant to DNase and except in central parts of their homopyrimidine strands are sensitive to single strand-specific chemicals. Interactions that protect central pyrimidines probably stabilize the bubble-like areas. Because DNA fragments containing either one or both bubbles migrate in TBM (89 mM Tris base, 89 mM boric acid, and 2 mM $MgCl_2$) PAGE even faster than expected from their linear size, interacting regions are expected to belong to the same molecule. In an origin fragment containing a single bubble, free homopyrimidine strand can only interact with Hoogsteen hydrogen bonding surfaces from a complementary double stranded sequence. Indeed, this origin fragment reacts with triplex preferring antibody. In competition binding experiments control double stranded DNA or single stranded $(dT)_{40}$ do not affect origin-antibody interaction, whereas *T*AT and *G*GC triplexes exert competitive effect. Because the chosen fragment does not contain potential *G*GC forming sequences, these experiments confirm that the lamin B2 origin adopts a structure partly composed of intramolecular *T*AT triads.

Eukaryotic origins of DNA replication are sequences derived from fixed chromosomal regions that accommodate one or more DNA replication start sites and direct binding of origin recognition complexes (ORCs).[1] These regions are typically intergenic, adjacent to transcriptionally active genes, and vary in size from several hundred to several thousand base pairs, which is a consequence of natural divergence and/or of different mapping procedures (1–3). In some cases, origins colocalize with matrix attachment sites (4) or require two functional elements, only one of which serves for initiation, whereas both bind ORC (5–7). In addition, DNA replication origins do not share a common consensus sequence but still act as replicators when transferred to another chromosomal location (2, 8, 9). This important ability demonstrates that elements contained in naked DNA build structural basis for initiation of DNA synthesis.

Apart from AT richness, which seems to be the general feature of eukaryotic origins of DNA replication, elements that account for common function are very poorly defined (3, 10, 11). They could include $(dT)_n$ $(dA)_n$ sequences predominantly distributed in a mirror repeat manner, and as reported, involved in initiator protein binding (5, 12–18). The structural impact of these sequences was not investigated, but numerous other studies confirm that A and T tracts (depending on their distribution and size) could profoundly affect geometry of grooves, bending, and the ability to bend or formation of loops and multistranded structures (19). Having this in mind, we have probed the structure and shape of partly overlapping fragments lbo I-(3813–4052) and lbo II-(3871–4187) isolated from the human lamin B2 origin of DNA replication. This origin is very well characterized, accommodates the defined start site of DNA synthesis, binds ORC in living cells and *in vitro*, and supports the initiation of DNA synthesis at ectopic chromosomal locations (2, 18, 20–23). Origin regions implicated in the initiation of DNA synthesis and in protein binding are AT rich and have many T tracts distributed in the upper strand of DNA. To probe the structure and shape of these regions, we have used the agents that interact with different A or T rich elements in alternative forms of DNA. Our combined results show that at neutral pH, low ionic strength, and in the presence of Mg^{2+} ions, linear fragments of the lamin B2 contain nonhydrogen bonded $(dT)_n$ $(dA)_n$ tracts that most likely make part of the short triple stranded structures.

MATERIALS AND METHODS

Chemical Reactions Specific for Single Stranded DNA—For chemical reactions DNA fragments lbo I and -II were amplified from human genomic DNA using CAAAAACGGAGCTGGGCTGCAGCTG, GACATCCGCTTCATTAGGGCAGAGGCC, and TTTTTAAGAAGATGCATGCCTAGCGTGTTC, AGAGTCAGCTTGTGCAACAGCGTCG, respective primer pairs. In each reaction only one primer was 5′-end labeled. The lower or upper labeled strand containing fragments were electrophoretically purified and precipitated. The purity and integrity of isolated DNA fragments were checked by denaturing PAGE, and their sequence was checked by Maxam-Gilbert sequencing. Preparations containing ragged ends were discarded, and the good ones were subjected to further analysis. Chemical reactions were performed essentially as described (24, 25); 100–125 fmol of each labeled fragment were dissolved in buffer A (20 mM HEPES, pH 7.9, 30 mM NaCl, 0.1 mM EDTA, 6 mM $MgCl_2$, 0.2 mM $ZnCl_2$, 1 mM dithiothreitol, 10% glycerol), supplied with 10 μg of poly(dIdC)·poly(dIdC), and treated with either OsO_4 or diethylpyrocarbonate (DEPC). For lower strand modification, treatment was performed 15 min at room temperature in a total volume of 110 μl with 10 μl of DEPC added to the reactions. For upper strand modifications, binding mixtures (24 μl)

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[1] The abbreviations used are: ORC, origin recognition complex; DEPC, diethylpyrocarbonate.

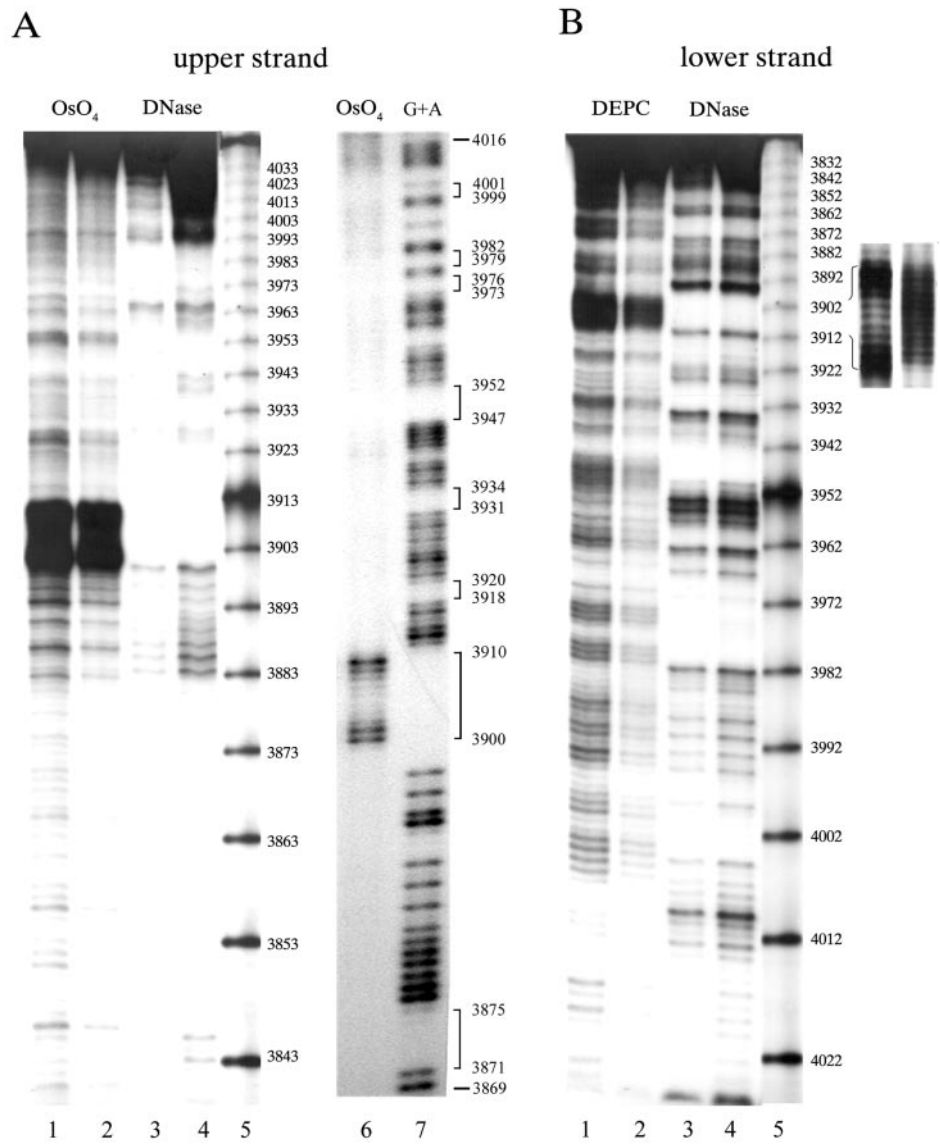

FIG. 1. **OsO_4, DEPC, and DNase I cutting profiles of lbo I.** *A*, *left panel*, the upper strand of DNA treated with OsO_4 (*lanes 1* and *2*) or DNase I (*lanes 3* and *4*) and a 10-bp ladder (*lane 5*). The *right panel* shows an extended picture of the 3869–4016 sequence, and *lane 6* shows the specificity of the OsO_4 reaction. T tracts are identified by a Maxam-Gilbert G+A reaction (*lane 7*) and marked by *square brackets*. Only the strongest signals are made visible by brief exposition on films. *B*, *left panel*, the lower strand of DNA treated with DEPC (*lanes 1* and *2*) or DNase I (*lanes 3* and *4*) and a 10-bp ladder (*lane 5*). *Right panel*, an extended picture of both strands of the bubble after treatment with single strand-specific chemicals.

were supplied with pyridine (2 μl per reaction) and treated with 30 μl of 2% solution of OsO_4 for 25 min on ice. After incubation reactions were precipitated and dry pellets were resuspended in pyperidine (150 μl) and incubated for 30 min at 90 °C. DNA fragments were extracted with *n*-butanol and reprecipitated. Reaction products were analyzed by 6% denaturing gels.

Electrophoretic Mobility Assays—DNA fragments prepared as described above were dissolved in buffer A without EDTA, supplied with 50 μM spermine or not, or were dissolved in 25 mM HEPES, pH 7.9, 2 mM $MgCl_2$, supplied with either 10% glycerol or 10% ethylene glycol, and were incubated overnight at 25 °C. Reaction mixtures were analyzed by 8% PAGE in 30-cm-long gels prepared and run in either TBE (89 mM Tris base, 89 mM boric acid, and 2 mM EDTA) or TBM (89 mM Tris base, 89 mM boric acid, and 2 mM $MgCl_2$) buffers.

Formation of Intermolecular Triplexes—Short intermolecular triplexes were formed from either $(dA)_n$ and $(dT)_n$ ($n = 34$ or $n = 40$) or $(dC)_{34}$ and $(dG)_{34}$ single stranded constituents. To determine the amount of complementary strand necessary for triplex formation, single stranded oligomers were incubated in buffer A without EDTA by mixing 5′-end labeled A or C with increasing amounts of unlabeled complementary strand. Reaction mixtures were incubated for 30 min at 37 °C and the products were analyzed by 10% TBM PAGE as essentially described in Ref. 26. For competition binding experiments triplexes were prepared in the same manner but from unlabeled constituents. Typically, 1 pmol of either single stranded A or C oligomer was incubated with corresponding complementary strand in a total volume of 3 μl. To prepare *T*AT triplex it was necessary to use either 50- or 100-fold molar excess of single stranded T oligomer. To prepare *G*GC triplex it was enough to use 10-fold molar excess of single stranded G oligomer. Under these conditions, *T*AT triplex was contaminated with small amount of double stranded oligomer, whereas *G*GC seemed fully converted into a triplex.

Competition Binding Experiments—To determine the amount of antibody necessary for quantitative interaction with origin DNA, 1–5 fmol of 5′-end labeled lbo I dissolved in buffer A without EDTA was incubated with 1–5 μl of 20-fold diluted mouse polyclonal serum (the preparation and detailed affinity of the serum will be described elsewhere) in a total volume of 8–10 μl. Competition binding experiments were typically performed in buffer A without EDTA using 5 fmol of 5′-end labeled lbo I, 1 μl of serum, and different cold competitors. Respective molar excesses of each competitor over labeled probe were as follows: 100–400-fold of double or triple stranded DNA and 10,000–20,000-fold of single stranded dT oligomer. After the addition of labeled probe reactions were incubated for 30 min at room temper-

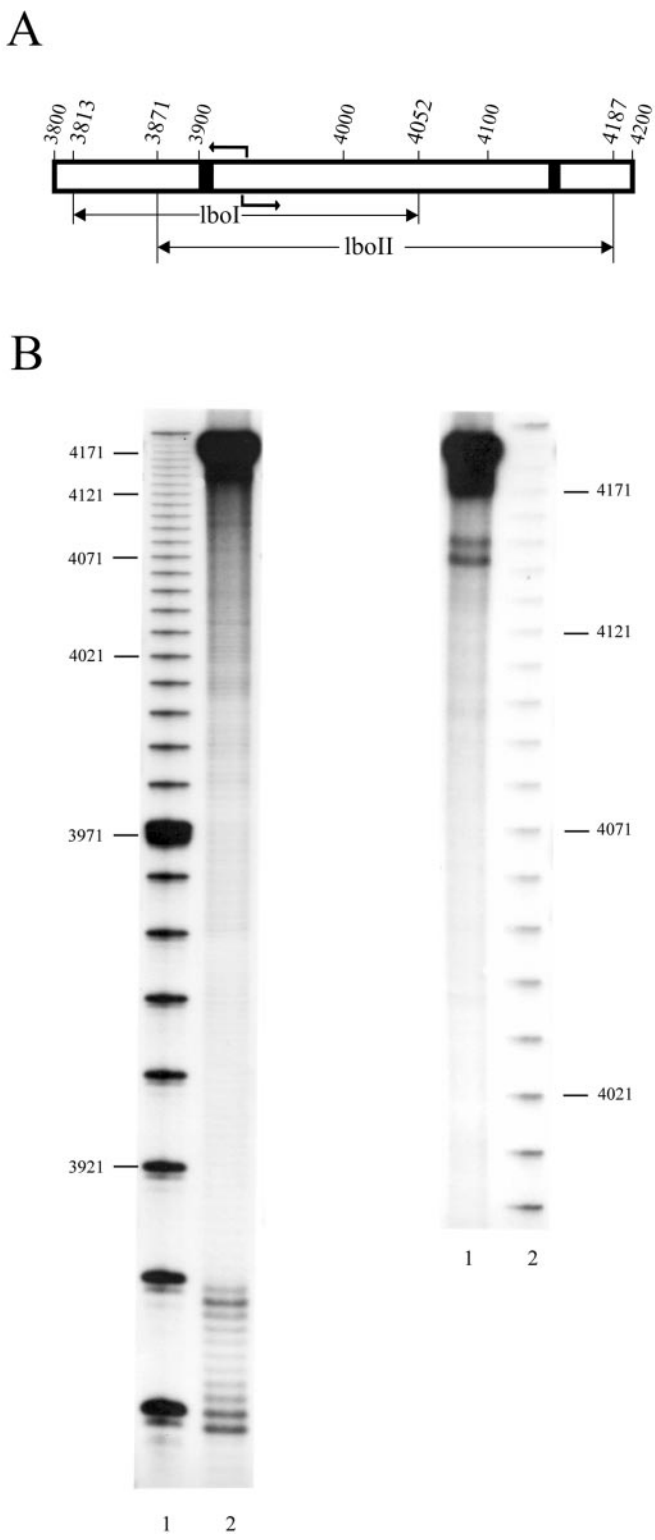

FIG. 2. **OsO_4 cutting profile of lbo II.** *A*, schematic representations of lbo I and -II origin fragments. *Black boxes* mark respective unpaired regions, and *arrows* mark the previously determined start site of DNA synthesis. *B*, *left panel*, a 10-bp ladder (*lane 1*) and the position of unpaired regions in upper strand of the entire lbo II fragment (*lane 2*). *Right panel*, an extended picture of the 4141–4151 sequence (*lane 1*) and 10-bp ladder (*lane 2*).

ature in a total volume of 8 μl. Reaction products were analyzed by electromobility shift assay using 4% TBE PAGE. For competition binding experiments cold lbo I was prepared as described. Nonorigin control was amplified from human genomic DNA using a CCTCAGAACCCAGCTGTGGA and GCCAGCTGGGTGGTGATAGA primer pair and purified by PAGE. Cold double stranded DNA was either preincubated with the antibody for 30 min at room temperature or added together with the labeled probe. Both procedures gave similar results. For competition binding experiments *T*AT and *G*GC triplexes were prepared as described. Where indicated, parallel reactions containing corresponding amounts of single and double stranded constituents of *T*AT triplex were processed in the same manner. For comparison of competitive efficiencies of *T*AT and *G*GC triplexes the amount of single stranded DNA in each reaction was made approximately equal by adding 10 pmol of single stranded G oligomer to the *T*AT mixture and 50 or 100 pmol of single stranded T oligomer to the *G*GC mixture. In all competition binding experiments with triplex DNA the antibody was first challenged with competitors and then incubated with labeled probe.

RESULTS

To check whether the lamin B2 origin adopts a structure that in some respect deviates from canonical form, we have first performed experiments with chemicals that poorly react with B-DNA but recognize unpaired regions usually present in junctions between canonical and noncanonical intramolecular elements or in some multistranded structures. We have chosen two assays, one with OsO_4–pyridine and the other with DEPC. DEPC reacts with unpaired purines in single stranded DNA or with purines in *syn* conformation, as in Z-DNA, and for that reason we have used it for the lower, purine rich strand of the origin. OsO_4–pyridine, predominantly reacting with unpaired thymines and partly with unpaired cytosines, was chosen for the upper strand of the origin. Experiments were performed with origin fragment lbo I-(3813–4052), previously shown to encompass the initiation site and regions interacting with ORC *in vivo* and *in vitro*. In addition to chemical assays, origin fragment was also subjected to limited hydrolysis using DNase I (Fig. 1); DNase was expected to strongly react with double stranded DNA and to be significantly less reactive with single or multistranded regions if present in the origin (27, 28).

The chemical reaction with OsO_4–pyridine was specific for a small fraction of pyrimidines present in the upper strand of lbo I (Fig. 1*A*); the most reactive was the stretch CTTTTTTTTTTCC, spanning nucleotide positions 3900–3912. The sequence TGCATGCCTAGCGTG (3883–3897) adjacent to the affected pyrimidine tract was also reactive. Weak cleavages of pyrimidines not belonging to T tracts were also detected at positions 3925, 3927, 3937, 3938, 3954, 3956, 3959, and 3961–3964, but none of them matched the intensity of the sequence CTTTTTTTTTTCC, and none of the shorter T tracts was significantly affected (Fig. 1*A*, *lanes 6* and *7*). In the lower strand, the region 3900–3912 was also the most reactive one, although adenines complementary to poorly reactive T tracts and purines in the 3883–3897 sequence exhibited some reactivity, too (Fig. 1*B*). In addition, all T tracts were resistant to DNase, whereas the region 3883–3897 exhibited significant sensitivity.

According to the results from Fig. 1, two clearly noncanonical regions at positions 3900–3912 and 3883–3897 were present in lbo I; the latter, composed of alternating purines and pyrimidines, highly sensitive to DNase and only moderately to single strand specific agents, was consistent with noncanonical double stranded structure such as left-handed, Z-DNA (29–32 and Z-Hunt algorithm therein). The former region, composed of pyrimidines in the upper and purines in the lower strand and containing the stretch of 10 consecutive T residues, was sensitive to OsO_4–pyridine and DEPC but resistant to DNase I, which demonstrated its nonhydrogen bonded, bubble-like nature.

In the lamin B2 origin region there was another similar motif, CCTTTTTTTTC, spanning nucleotide positions 4141–4151 (Fig. 2*A*). Because the motifs CTTTTTTTTTTCC and CCTTTTTTTTC contained two longest T tracts present in the

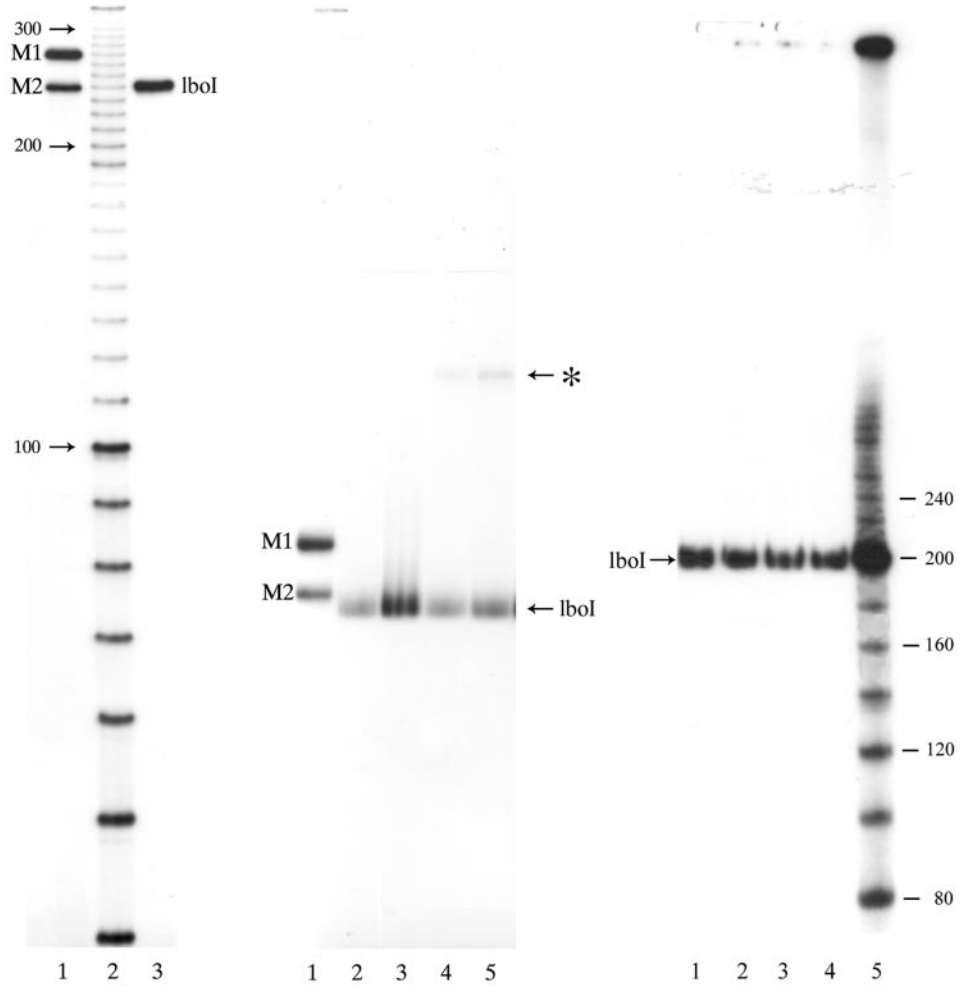

FIG. 3. **Anomalous electrophoretic migration of lbo I.** *Left panel* is denaturing PAGE; *M1* and *M2* are respective 267-bp and 237-bp markers (*lane 1*), a 10-bp ladder (*lane 2*), and lbo I (*lane 3*). *Middle panel* is nondenaturing TBE PAGE; M1 and M2 (*lane 1*), lbo I in buffer A without (*lane 2*) or with 50 μM spermine (*lane 3*) or in 25 mM HEPES, pH 7.9, 2 mM $MgCl_2$, with either 10% glycerol (*lane 4*) or 10% ethylene glycol (*lane 5*). *Right panel* is nondenaturing TBM PAGE; *lanes 1–4* are the same as *lanes 2–5* in the *middle panel*, and *lane 5* is a 20-bp ladder.

lamin B2 origin region, they were both analyzed with OsO_4–pyridine using DNA fragment lbo II-(3871–4187). The analysis confirmed sensitivity of both pyrimidine motifs to single strand specific agent (Fig. 2*B*); despite frequent occurrence of shorter T tracts in lbo II DNA, only these motifs were significantly affected.

The next step was to check whether the occurrence of partly single stranded regions affected migration of lbo I and -II in nondenaturing PAGE. For that purpose DNA was incubated in different buffers, and its purity and integrity after incubation were checked and confirmed by denaturing gels and by chemical sequencing. As expected, lbo I migrated as a single band of 240 bp (Fig. 3). The same fragment was then analyzed by either TBE or TBM nondenaturing PAGE. Both types of gels gave essentially the same results; lbo I migrated faster than expected from its linear size. The effect was more prominent in TBM PAGE, where lbo I, composed of 240 bp, migrated as linear DNA of 200 bp. Similar to that, lbo II, composed of 317 bp, migrated as a linear DNA slightly shorter than 240 bp (Fig. 4).

Faster migration of both DNA fragments could be a consequence of the increased flexibility of their unpaired regions. However, central pyrimidines of the lbo I bubble were partly protected from single strand-specific chemical, whereas their complementary purines were not (Fig. 1*B*, *right panel*). This suggested an intramolecular interaction involving single stranded T and probably compensating for the loss of stacking and hydrogen bonding in the bubble. The results of chemical assays identified a single nonhydrogen bonded region within lbo I, which pointed to a complementary double stranded sequence as other interacting element and to a sort of triple stranded DNA as an outcome of this interaction. Because Mg^{2+} stabilizes triple stranded *T*AT elements (26), its effect was also consistent with this idea.

lbo I elements that could interact with the bubble are other $(dT)_n$ $(dA)_n$ sequences. Indeed, intramolecular triplexes were previously shown to form between nonadjacent sequences contained in the same linear plasmid and relating to each other as either mirror or glide reflection images (33, 34). Similar to that, the lbo I bubble could donate its pyrimidine strand for interaction with the purine strand of one of the shorter symmetrical sequences. Because the origin contains more than one potential acceptor, alternative structures containing short double stranded loops could be possible (Fig. 5).

To further test the structure of origin DNA we have used mouse polyclonal antibody reacting with triple stranded elements, and this antibody recognized lbo I (Fig. 6*A*). Origin-antibody reaction was also performed in the presence of control

double stranded DNA derived from chromosomal region not involved in initiation of DNA synthesis (35). As presented in Fig. 6*B*, binding of the antibody to lbo I was not affected by a 100–400-fold molar excess of control DNA, thus confirming the presence of origin element nonexistent in the double stranded control. The antibody was then challenged with triple stranded 40-mer *T*AT prepared as described under "Materials and Methods" and in Fig. 7A, and incubated with lbo I. As demonstrated in Fig. 7*B*, preincubation with triple stranded 40-mer significantly impaired the antibody-origin interaction, whereas preincubation with its double or single stranded constituents exhibited less significant effect (Fig. 7*B*). In addition to *T*AT triplexes, *G*GC triplexes were efficient competitors (Fig. 7, *C* and *D*), and the antibody could not discriminate between these two structures.

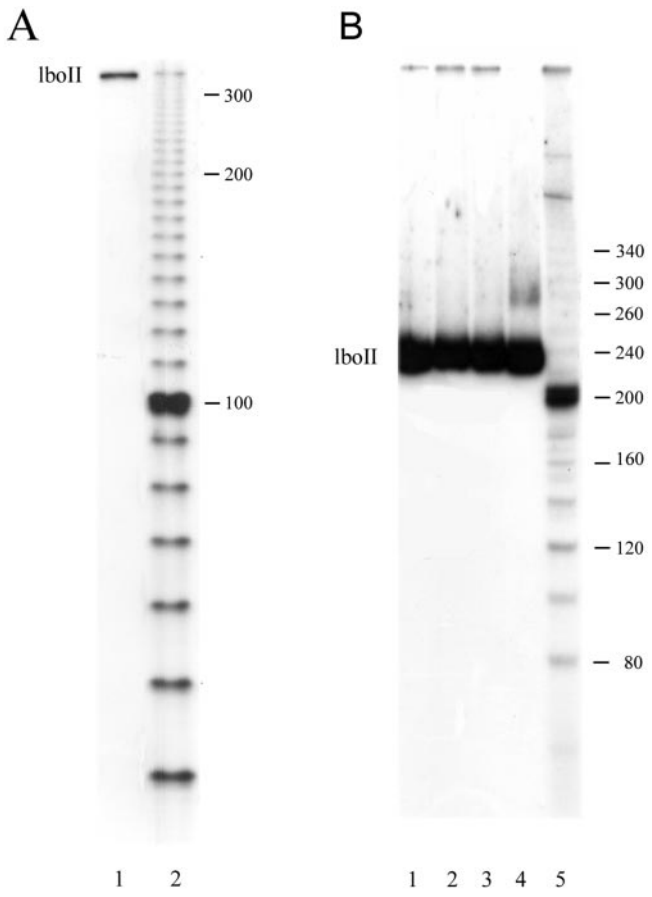

FIG. 4. **Anomalous electrophoretic migration of lbo II.** *A*, denaturing PAGE; lbo II (*lane 1*), a 10-bp ladder (*lane 2*). *B*, nondenaturing TBM PAGE; lbo II (*lanes 1–4*) in different buffers and the same as for lbo I in nondenaturing PAGE and a 20-bp ladder (*lane 5*).

In conclusion, we want to emphasize that in lbo I we detected a single OsO_4–pyridine sensitive and DNase resistant stretch of T residues and within that stretch a partly protected portion. Protecting interaction is unlikely to be intermolecular because native PAGE does not reveal significant multimerization, and therefore cannot involve Watson-Crick bonding, because out of the bubble lbo I contains no free Watson-Crick hydrogen bonding surfaces. The antibody reacts well with lbo I but not with its double stranded portion as revealed by competitive inefficiency of double stranded nonorigin DNA and would not react with single stranded T even if fully available, as revealed by competitive inefficiency of excessive amounts of single stranded $(dT)_{40}$. Consequently, our combined results point to intramolecular triplex as the most likely unorthodox element of origin DNA. Because lbo I contains no potential *G*GC forming sequences, the triplex ought to be of the *T*AT type.

DISCUSSION

In this study we have probed the structure of the human lamin B2 origin, focusing on sequence elements common for eukaryotic DNA replication origins in general and using chemical and electrophoretic assays that we considered suitable for analysis of noncircular molecules that are several hundred base pairs long. Importantly, all assays were performed at neutral pH, low or moderate ionic strength, and in the presence of Mg^{2+} ions, which indicated that observed noncanonical elements could form under physiological conditions.

In the lamin B2 origin of DNA replication, noncanonical

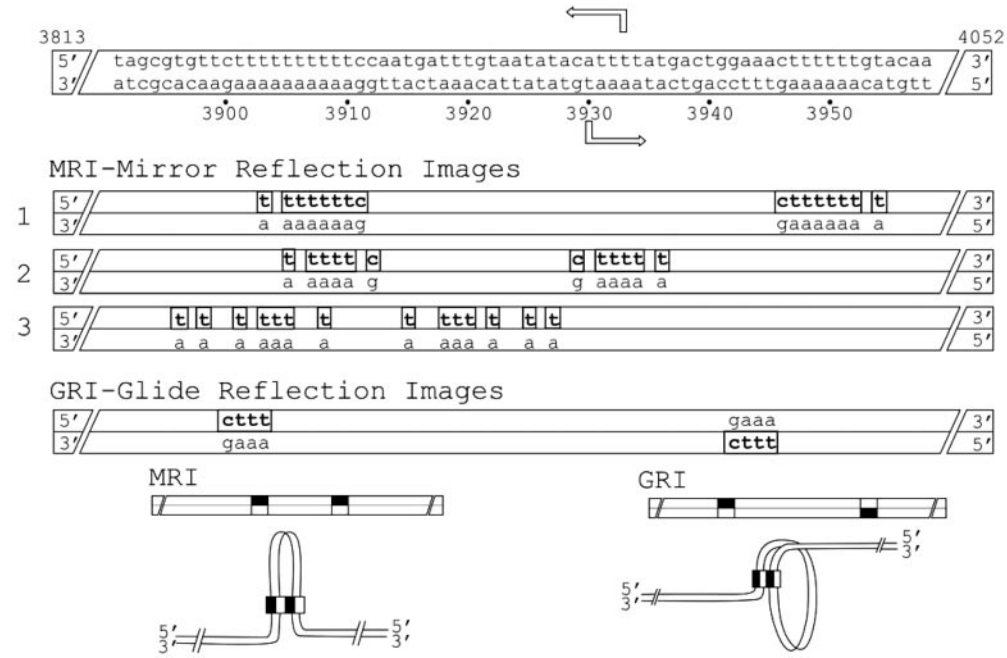

FIG. 5. **Mirror and glide reflection images of lbo I.** Pairs of mirror (*MRI*) and glide reflection elements (*GRI*) from lbo I and respective loops based on possible intramolecular triplexes. *Black* and *white boxes* represent respective pyrimidines and purines. In the entire lbo I there are two GRI and nine MRI pairs in which one symmetrical sequence of each pair partly overlaps the bubble.

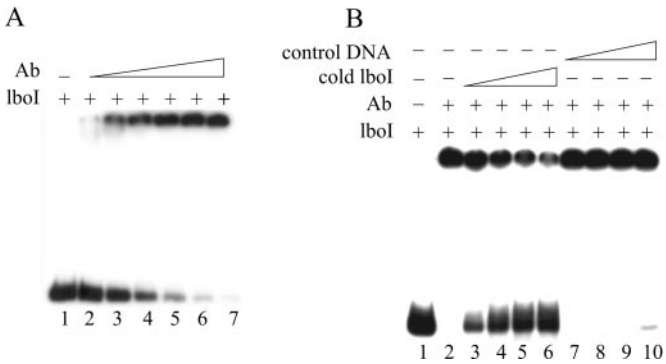

FIG. 6. **Reaction of lbo I with triplex preferring antibody.** *A*, labeled lbo I (*lanes 1–7*) incubated with increasing amounts of the triplex preferring antibody (*lanes 2–7*). *B*, labeled lbo I incubated with the antibody in the presence of 100-, 200-, 300-, and 400-fold molar excess of either cold lbo I (*lanes 3–6*) or control DNA (*lanes 7–10*).

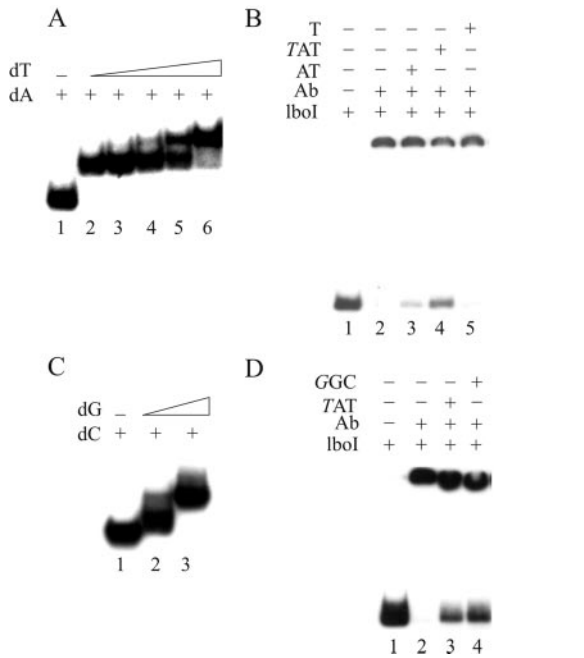

FIG. 7. **Competitive effect of *T*AT and *G*GC triplexes.** *A*, formation of *T*AT triplex using labeled $(dA)_{40}$ (*lanes 1–6*) and increasing amounts of unlabeled $(dT)_{40}$ (*lanes 2–6*). *B*, labeled lbo I (*lanes 1–5*) incubated with the antibody (*lanes 2–5*) in the presence of *T*AT triplex (*lane 4*) or corresponding amounts of its double (*lane 3*) and single stranded (*lane 5*) constituents. *T*AT triplex was prepared as in *A* (*lane 6*) but with unlabeled $(dA)_{40}$ using a 50-fold molar excess of single stranded T over single stranded A and 200-fold molar excess of single stranded A over lbo I. *C*, formation of *G*GC triplex using labeled $(dC)_{34}$ (*lanes 1–3*) and increasing amounts of unlabeled $(dG)_{34}$ (*lanes 2* and *3*). *D*, lbo I (*lanes 1–4*) incubated with the antibody (*lanes 2–4*) in the presence of *T*AT (*lane 3*) and *G*GC (*lane 4*) triplexes. The *G*GC triplex was prepared as in *C* (*lane 3*) but from unlabeled constituents using a 10-fold molar excess of single stranded G over single stranded C and a 200-fold molar excess of single stranded C over labeled lbo I. The *T*AT triplex was essentially prepared as in *B* (*lane 4*) but using single stranded 34-mers and a 100-fold excess of single stranded T. The amount of unincorporated single stranded oligomer present in each reaction is made equal by adding single stranded G to the *T*AT reaction or single stranded T to the *G*GC reaction.

elements included the bubbles within homopyrimidine/homopurine tracts CTTTTTTTTTTCC/GGAAAAAAAAAAG and CCTTTTTTTTC/GAAAAAAAAGG and one sequence composed of alternating purines and pyrimidines, consistent with Z-DNA. To understand the form that keeps the bubbles open despite the loss of stacking and hydrogen bonding in corresponding DNA fragments, we have performed more detailed analysis using lbo I.

As judged by the intensity of cleavage reactions, the bubble element existed in majority of tested molecules, whereas other affected spots indicated a sort of structural polymorphism. Because in TBE and TBM PAGE lbo I generally migrated as single band, polymorphism occurred within one dominant form of the molecule, or less likely, in minor alternative forms undetectable by PAGE. As judged by partial protection of the bubble pyrimidines and by the apparent size of lbo I in TBM PAGE, the dominant form was created by intramolecular interaction of free T residues with nonadjacent $(dT)_n$ $(dA)_n$ sequences. By studying the results of chemical assays, it was not possible to identify which of 11 short $(dT)_n$ $(dA)_n$ sequences present in lbo I actually interacted with the bubble. Because of their narrow minor groove, double stranded T tracts were poorly sensitive to DNase I (36), and because of their double stranded nature, poorly sensitive to OsO_4-pyridine, as they would be in triplex or duplex structures alike. However, the experiments with triplex preferring antibody strongly suggested that triple stranded *T*AT regions did form within lbo I.

Formation of noncanonical elements within origin DNA is not exceptional. Potential triplex forming sequences were previously detected in viral and cellular DNA replication origins (37, 38). Furthermore, dihydrofolate reductase origin was shown to contain both triplex and Z-DNA (39). Described structures did not include T tracts and were formed in circular molecules at acidic pH. Triplexes independent of superspiralization were shown previously to form between distant pyrimidine tracts, contained in the same linear plasmid and relating to each other as either mirror or glide reflection images (33, 34). In this structure, the purine strand of accepting tract provided both Watson-Crick and Hoogsteen hydrogen bonding surfaces, whereas the bubble provided the complementary third strand. To initiate triplex formation, one end of the linear molecule had to pass through a bubble within a donor pyrimidine tract, and its acceptor duplex had to wind around free third strand. The resulting structure was a hydrogen bonded (braided) knot, which did not fall apart even in the absence of Hoogsteen bonding because the reforming duplex prevented the separation of interwound strands (34). Triplexes of the same type were also formed between two or more molecules (34), which we believe occasionally occurred even with lbo I (a very slowly migrating species is indicated by an *asterisk* in Fig. 3). However, under conditions of our experiments, the predominant mode of interaction was intramolecular. Because in general, *pyr*purpyr triplexes have antiparallel pyrimidine strands, the resulting structure was expected to involve Hoogsteen hydrogen bonding between third and central strand (Figs. 5 and 8).

Having such a structure in mind we were surprised by the competitive efficiency of *G*GC. The same as with *T*AT, this effect was dependent on a triple helical form and neither alternating nor monotonous GC duplexes exhibited significant competition (data not shown). Because lbo I contains no potential *G*GC forming sequences, the possibility that it adopted a form of this particular triplex was excluded. However, we cannot exclude the possibility that origin *T*AT elements have parallel pyrimidine strands (Fig. 8) and pairing schemes that include reverse Hoogsteen bonding (40, 41). In parallel stranded *T*AT triplexes third strand phosphates would be placed in a major groove (and not out of it as in triplexes with antiparallel pyrimidine strands), and this would make them more similar to *G*GC structures. The detailed structure of the origin-based *T*AT triplex could be biologically relevant, especially if it builds binding sites for the origin recognition complex. In lbo I, the whole region encompassing the bubble participates in interaction with

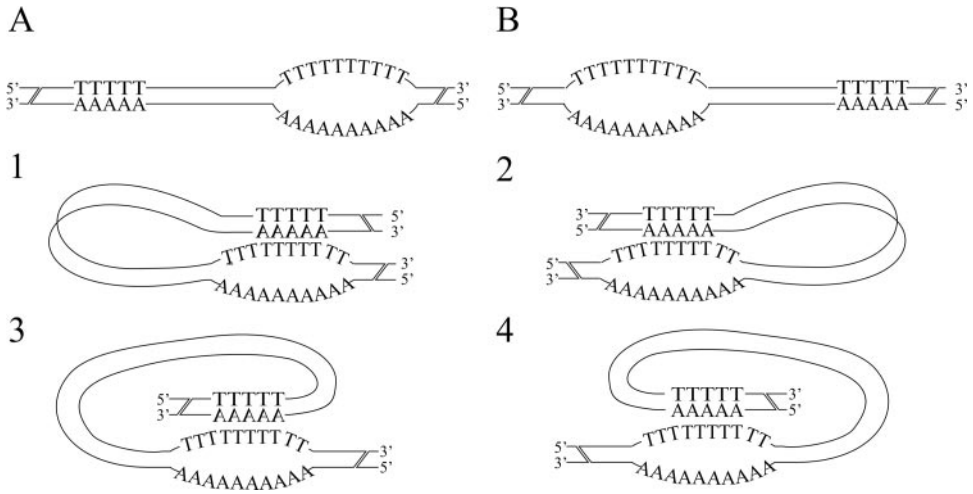

FIG. 8. **Hypothetical acceptors of lbo I interacting with the bubble T strand.** Double stranded mirror-like sequences occurring upstream (*A*) or downstream (*B*) of the donor sequence could form triple stranded elements with either antiparallel (*1* and *2*) or parallel (*3* and *4*) pyrimidine strands.

ORC (18), and it would be interesting to check whether this protein could recognize any specific structural element.

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