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Zintrons in rat α-lactalbumin gene

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The eukaryotic genome is characterised by the occurrence of a large amount of repetitive DNA which exists within and around coding sequences. Random repeats of $(TG)_n$ sequences have been observed in several introns. In this paper we show that $(TG)_{14}$ and $(TG)_{24}$ sequences present in the third intron of rat α-lactalbumin gene adopt left-handed Z-helices under varying superhelical densities. The overall sequence of the parent plasmid further influenced the level of supercoiling at which the B to Z transition could be induced in $(TG)_n$ sequences. Such Z-potential intervening sequences (zintrons) could act as buffers to maintain desired levels of supercoiling near the transcribed sequences.

Lactalbumin, α-; Intron sequence; DNA, Z-; DNA supercoiling; (Rat)

1. INTRODUCTION

Rapid developments in knowledge concerning unusual DNA structures, such as left-handed Z-DNA, bent DNA, cruciform, and heteronomous DNA, have occurred in recent years [1–8]. This has been achieved through rapid progress in DNA synthesis, sequencing, cloning and development of diverse methods for the detection and analysis of unusual DNA structures. The possible roles of such structures in key cellular processes like replication [9], recombination [10] and transcription [11,12] have emerged from in vitro experiments using synthetic and cloned DNA segments. However, very little effort has been made to study unusual DNA structures at the chromosomal level and the role of such structures in vivo. The eukaryotic genome is characterised by the occurrence of a large amount of repetitive DNA which exists within and around the coding sequences [13]. In addition to longer repeats, short repeats of TG sequences have been identified in the introns of several genes including mouse immunoglobulin genes [14], rat α-lactalbumin gene [15], human actin gene [16] and human γ-globin genes [17]. The functional role of such intervening repetitive alternating purine-pyrimidine sequences, if any, is yet to be understood. Synthetic $(TG)_n$ sequences have been shown to adopt the Z-conformation when cloned in supercoiled plasmids [18]. Such Z-potential $(TG)_n$ sequences observed in the introns of several genes have been postulated to adopt Z-helices (zintrons) [3].

We examined the plasmid pBαLA 0.9 which contains a 0.9 kb insert from the third intron of the rat α-lactalbumin gene having two TG repeats $(TG)_{14}$ and $(TG)_{24}$ separated by 318 bp. By two-dimensional chloroquine gel electrophoresis we could show that the two stretches of $(TG)_n$ sequences adopt Z-conformation at different levels of supercoiling. The presence of Z-conformation in this molecule was further confirmed by monoclonal anti-Z-DNA antibody binding studies.

2. MATERIALS AND METHODS

pBαLA 0.9 is a pBR322 derivative containing a 957 bp *Eco*RI fragment from the third intron of rat α-lactalbumin gene in the *Eco*RI site [15]. pUαLA 0.9 was constructed with the same insert in the *Eco*RI site of pUC19. The insert contains a perfect $(TG)_{14}$ separated from another $(TG)_{24}$ sequence by 318 nucleotides.

Plasmid DNA was isolated according to a modification of the

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alkaline lysis method and purified by cesium chloride-ethidium bromide density gradient centrifugation [19]. Topoisomerase I was isolated as reported [20].

2.1. *Two-dimensional gel electrophoresis*

Topoisomers of pBαLA 0.9 were prepared as in [21]. The mixture of topoisomers was electrophoresed in a 20 × 20 cm gel of 1% agarose in Tris-borate buffer (TBE) in the first dimension at 2 V/cm for 20 h. The gel was soaked in TBE containing 750 μg/l chloroquine for 12 h following which the gel was rotated through 90° and electrophoresis was performed in the second dimension at 2 V/cm for 16 h in the above buffer. The gel was soaked in TBE for 5 h with shaking to remove chloroquine. It was then stained with ethidium bromide (1 μg/ml) and the DNA visualised on a UV transilluminator.

2.2. *Gel mobility retardation assay with anti-Z-DNA antibody*

Monoclonal Z-22 antibody prepared against the Z-form of brominated poly(dG-dC) (a gift from Dr B.D. Stollar) was used for gel mobility retardation studies. Binding assay was performed in a buffer containing 60 mM sodium phosphate (pH 7.9), 200 mM NaCl and 30 mM EDTA for 30 min at room temperature with different concentrations of the antibody. The mixtures were analysed on a 1% agarose gel in Tris-acetate buffer.

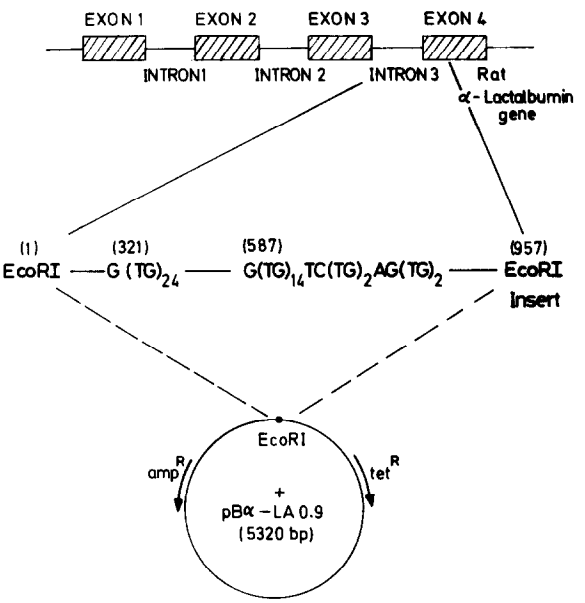

Fig.1. Map of pBαLA 0.9 plasmid.

3. RESULTS AND DISCUSSION

Fig.1. shows the construct of plasmid pBαLA 0.9 (5320 bp), a pBR322 derivative containing a 0.9 kb insert from the third intron of rat α-lactalbumin gene with two $(TG)_n$ stretches. Topoisomers of pBαLA 0.9 were generated by relaxation with topoisomerase I in the presence of 0, 0.5, 1 to 10 μM ethidium bromide. Topoisomers generated in this way were mixed to ensure a complete topoisomer distribution. On analysis of the population of topoisomers on a two-dimensional agarose gel two breaks appeared, one at 22 and another at 31 supercoils (fig.2). Such breaks, which are the consequence of structural alteration within the plasmid, indicate that the two (TG) stretches undergo structural transition at different superhelical densities $\sigma = -0.041$ and -0.058. Such a transition was not observed for the parent plasmid without the insert. Since TG stretches cannot adopt a cruciform structure, the change in supercoiling at the point of the break is indicative of a structural transition to the left-handed Z-form. The two transitions observed are associated with a change in 5 and 3 supercoils. The transitions of $(TG)_{24}$ and $(TG)_{14}$ sequences from Z to B should cause a respective gain of 4.5 and 2.5 supercoils, in agreement with the observed first and second transitions.

The presence of the Z-conformation in the plasmid containing the third intron of the α-lactalbumin gene was confirmed by binding experiments with a Z-specific monoclonal antibody (Z-22).

Z-22 has been shown to bind both TG and CG sequences in the Z-conformation [22]. Fig.3 shows results from a gel mobility retardation assay using increasing concentrations of antibody. With increasing antibody concentrations, more of the supercoiled band was found to be retarded for pUαLA 0.9. Similar results were obtained for pBαLA 0.9 (not shown). Supercoiled pLP332, which has a $(CG)_{16}$ stretch in the Z-DNA conformation, displayed binding to antibody while the parent plasmid without the insert did not. Since there exists a Gaussian distribution of the number of supercoils in the isolated plasmid DNA, the retarded population could represent that fraction wherein the superhelical density is sufficient to cause a B to Z transition in one or both of the TG stretches. Also, binding of Z-specific antibody could shift the equilibrium of B to Z transition [23] in (TG) stretches to a lower superhelical density.

In table 1, the superhelical density at which the (TG) repeats, present in the rat α-lactalbumin

gene, undergo structural transition is compared with that required for B to Z transition in (TG) repeats cloned in other plasmids. Superhelical densities ranging from -0.03 to -0.06 seem to favour B to Z transition for inserts containing 14–32 (TG) repeats [24–27]. Although it is known that the B to Z transition is chain-length-dependent, an apparent correlation is not evident in table 1 for cases where variation occurs in the plasmid type containing the $(TG)_n$ inserts. $(TG)_{15}$ and $(TG)_{25}$ inserts undergo the B to Z transition at identical superhelical density, however $(TG)_{30}$ and $(TG)_{32}$ undergo transition at a level higher than that expected. Thus, the junction sequences as well as the global sequence of the plasmid could influence B to Z transitions in TG stretches for a given superhelical stress. It is interesting to note that although the $(TG)_{14}$ repeat in pBαLA is long enough to adopt a stable Z-conformation it flips over to the Z-form at a much higher effective superhelical density.

When two B to Z transitions occur within a single plasmid, the effective superhelical density (σ_E) experienced by the plasmid at the point of the second transition is lower than the value obtained by band counting. This is a consequence of the absorption of supercoil following the first transition. In an earlier study, two $(TG)_{20}$ blocks were shown to undergo the B to Z transition at different superhelical densities when present together in the same plasmid [27]. On applying the correction for the change in level of supercoiling following the first transition, it can be demonstrated that the effective superhelical density (σ_E) for the second transition is indeed identical to that of the first. The apparent superhelical density required to bring about the second transition is higher by the number of supercoils absorbed due to the first transition. Further, the possibility of the effect of the junction regions, as proposed earlier [27], could be ruled out as both segments are flanked by *Bam*HI sites. Our results showed that within a plasmid two $(TG)_n$ sequences could undergo the B to Z transition at different effective superhelical density modulated by the length of the TG stretch and near-neighbour sequences (table 1).

In addition to the above observation, the effective superhelical densities at which the transitions occur for $(TG)_{24}$ and $(TG)_{14}$ sequences were found to differ between the two plasmids (table 1).

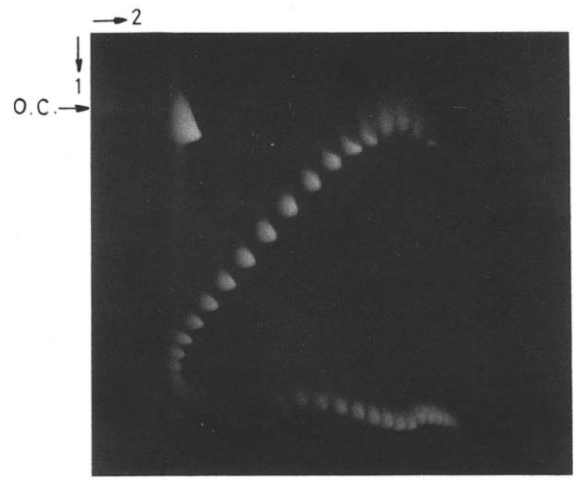

Fig.2. Two-dimensional gel electrophoresis of the topoisomers of pBαLA 0.9. O.C., position of open circle form. 1 and 2 indicate the first and second directions of electrophoresis, respectively.

However, the difference between the transition thresholds for $(TG)_{14}$ and $(TG)_{24}$ remains the same (0.008), irrespective of the plasmid type. In the case of pBαLA 0.9 and pUαLA 0.9, the (TG) stretches are surrounded by identical sequences for more than 100 bp. Hence, the ability of the remaining sequences to absorb the changes in twist may be a major factor responsible for the higher effective superhelical density requirement for the transitions in pUαLA 0.9 compared to pBαLA 0.9 (table 1). In view of these observations, it seems necessary to exercise caution while deciding the level of supercoiling at which a particular sequence would undergo structural transition without considering the effect of the bulk of the sequences surrounding the sequence of interest.

A circular DNA molecule may be considered to mimic a domain of chromosomal DNA. The ability of a given segment to undergo structural transition would depend not only upon its length and sequence but also on the location within the domain, the size of the domain, and the twist-absorbing capacity of the domain. The above results fit well with our earlier proposition that repetitive DNA can buffer sequence-dependent structural deviations from an ideal double helix [28]. Buffering can serve a mechanistic function by reducing extraneous conformational effects which could interfere with the process of transcription.

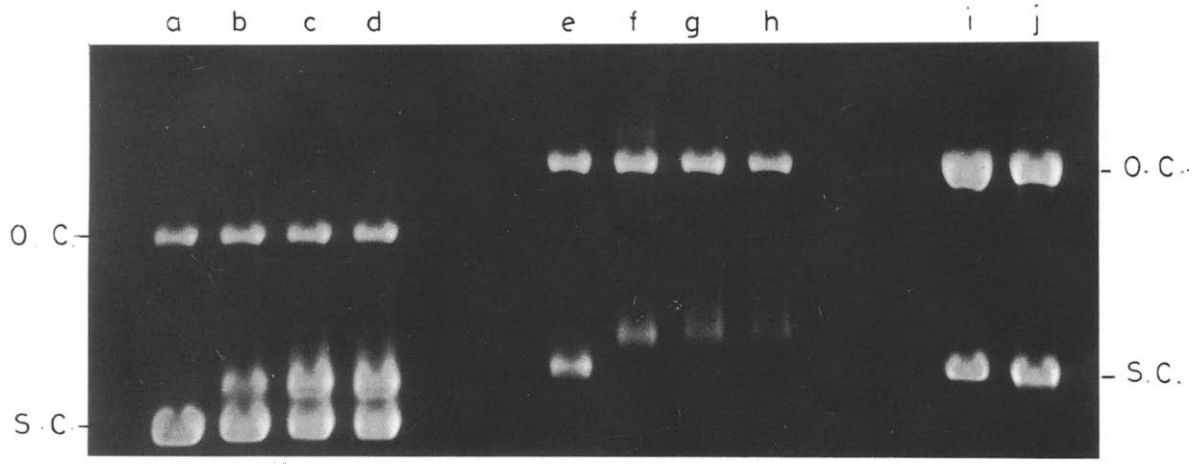

Fig.3. Gel mobility retardation assay of plasmids in the presence of monoclonal anti Z-DNA antibody (Z-22). Lanes: (a–d) pUαLA 0.9 with 0, 0.3, 0.6 and 0.9 μg antibody; (e–h) pLP332 with 0, 0.15, 0.3 and 0.45 μg antibody; (i,j) pBR322 with 0 and 0.9 μg antibody, respectively. O.C., open circle; S.C., supercoiled.

Recently, the generation of supercoil waves during the process of transcription has been proposed [29,30]. Accumulation of positive supercoils in front of the transcribing RNA polymerase demands topoisomerase action to release the torsional stress generated for efficient transcription. The presence of zintron sequences could help in absorbing conformational stress generated due to positive supercoiling by flipping over to the B-form, and thus maintaining a desired level of supercoiling near the transcribed sequences for efficient read-through.

Table 1

Comparison of superhelical density for structural transition

Plasmid	Length of insert	Superhelical density for B to Z transition		Reference
		Apparent	Effective	
1. pDHfl4	$(TG)_{30}$	−0.048	−0.048	24
2. pAN064	$(TG)_{32}$	−0.068[a] −0.057[b]	−0.068[a] −0.057[b]	25
3. pRSBP3	$(TG)_{32}$	−0.03	−0.03	26
4. pRSλHE5	$(TG)_{25}$	−0.03	−0.03	26
5. pRW155	$(TG)_{20}$—95 bp—$(CA)_{20}$	−0.042[c] −0.055[d]	−0.042[c] −0.042[d]	27
6. pBαLA 0.9	$(TG)_{24}$—318 bp—$(TG)_{14}$	−0.041[e] −0.058[f]	−0.041[e] −0.049[f]	this work
7. pUαLA 0.9	$(TG)_{24}$—318 bp—$(TG)_{14}$	−0.046[e] −0.063[f]	−0.046[e] −0.054[f]	this work

All transitions were monitored by two-dimensional gel electrophoresis except in pAN064 where filter binding with anti-Z-DNA antibody was performed in the presence of NaCl. [a] 300 mM; [b] 150 mM; [c,d] 1st and 2nd transitions, respectively; [e,f] transitions in $(TG)_n$ where n = 24 and 14, respectively

4. CONCLUDING REMARKS

We have shown here that tandem repeats of (TG) sequences in the third intron of the rat α-lactalbumin gene can adopt left-handed Z-helices at different superhelical densities. The length of the TG stretch as well as the junction sequences influence such transitions. The superhelical density at which the $(TG)_n$ segments undergo structural transition is strongly influenced by the overall sequence characteristics of the plasmid bearing the insert. The presence of $(TG)_n$ sequences, within the intron of the rat α-lactalbumin gene, which are capable of undergoing structural transition at different levels of superhelical density suggests a possible functional role for such zintron sequences in efficient transcription. It would be interesting to measure the change in histone-DNA contacts with such intervening sequences in vivo through cross-linking experiments [31]. The inability of Z-DNA structure to be accommodated on nucleosomes shown recently [32] could provide another level of regulation where such zintron sequences could exclude nucleosome organization when in the Z-form, thereby causing phasing of the nucleosomes found to be associated with activation of the concerned gene.

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