# Triplex DNA in the nucleus: direct binding of triplex-specific antibodies and their effect on transcription, replication and cell growth

Yehenew M. AGAZIE*, Gary D. BURKHOLDER† and Jeremy S. LEE*‡

Departments of *Biochemistry and †Anatomy, 107 Wiggins Road, University of Saskatchewan, Saskatoon, Canada S7N 5E5

Jel 318 and Jel 466 are triplex-specific monoclonal antibodies which previously have been shown to bind to cell nuclei and chromosomes by immunofluorescence. Their interaction was further characterized by two methods. First, isolated intact nuclei were encapsulated in agarose. Both antibodies showed significant binding to the nuclei which could be inhibited by adding competing triplex DNA but not by adding *Escherichia coli* DNA to which the antibodies do not bind. Both triplex-specific antibodies inhibited replication and transcription in the nuclei by about 20 %. Secondly, the antibodies were introduced into synchronized myeloma cells by osmotic shock of pynocytic vesicles. Cell-cycle studies showed that the myeloma cells had an S phase of about 10 h and a doubling time of about 20 h. The cells were synchronized with thymidine and both cell growth and cell death were monitored. Introduction of the triplex-specific antibodies caused a marked decrease in cell growth without a significant increase in cell death. The effectiveness of the antibodies was improved by the addition of chloroquine diphosphate which inhibits degradation in the lysosomes. As a control, introduction of an antibody specific for a bacterial protein had little effect. In synchronized cells, inhibition of proliferation reached a maximum at 7 to 13 h after the release from the thymidine block. Thus, cells are most sensitive to the triplex-binding antibodies at the end of S phase and during G2. This result is consistent with the view that triplexes are involved in chromosome condensation/decondensation.

## INTRODUCTION

Pyrimidine·purine (pyr·pur) sequences are abundant in the eukaryotic genome and may represent up to 1 % of total DNA [1–3]. For example, the human genome contains more than 100000 elements of pyr·pur tracts which are approx. 200 to 300 bp in length [2]. A particularly abundant tract is $(TC)_n \cdot (GA)_n$ which accounts for 0.4 % and 0.75 % respectively of the primate and rodent genomes [4]. Interest in these sequences has been stimulated by the observation that pyr·pur tracts can form triplexes either in supercoiled plasmids or in the presence of physiological concentrations of spermine [5–10]. Thus, triple-helix formation could be exploited as a universal means of duplex DNA recognition to be used in chromosome mapping [11], gene regulation [12], mutagenesis experiments [13] and even in gene therapy [14,15].

An interesting aspect of pyr·pur sequences in the eukaryotic genome is their distribution; they have been mapped near genes, recombination hot spots and matrix attachment regions [16,17]. For example, pyr·pur tracts have been found in the 5′-flanking regions of the $\gamma$ and $\beta$ globin genes, the interleukin-2 receptor gene and the *c-myc* gene [12,18,19]. The involvement of triplexes in the control of $\gamma$ globin gene expression is most compelling; not only does the transcription factor BP-8 bind to a triplex in the promoter region but also mutations in the pyr·pur tract cause hereditary persistence of fetal haemoglobin [20]. Thus, the triplex structure might serve as a docking site for transcription factors or physically interfere with their binding to the promoter site [21].

Another possibility is that triplexes might play a role in chromosome organization. Pyr·pur tracts could form a loop of DNA that is held in place by triplex formation at its base; it is known that some pyr·pur tracts are found in the matrix-attached regions [17]. In support of this model was the finding that *in vitro* linear plasmids containing two separated pyr·pur tracts can form circles and Ω loop structures via triplex formation [22]. As well, the electrophoretic mobility of yeast and mouse chromosomes in pulsed-field gels is pH-dependent, and the mobility changes are consistent with triplex-mediated chromosome folding [23].

More direct evidence for the existence of triplex structures *in vivo* has come from immunofluorescent staining of fixed metaphase chromosomes with the triplex-specific monoclonal antibodies, Jel 318 and Jel 466 [24–26]. Staining patterns produced with Jel 318 correspond to G and Hoechst 33258 banding, whereas those produced with Jel 466 mainly correspond to R bands. Binding of Jel 318 was also observed to unfixed chromosomes [24].

In this report, further chromosome binding studies with the two triplex-specific antibodies are presented. First, the binding of Jel 318 and 466 to agarose-encapsulated and permeabilized nuclei was measured. These nuclear preparations contain intact chromatin that replicates and transcribes at about 85 % of the rate found *in vivo* [27,28]. It was found that the triplex-specific monoclonal antibodies bind to intact nuclear DNA preparations. Secondly, the effect of incorporating either Jel 318 or Jel 466 into cultured mammalian cells by osmotic lysis of pinocytic vesicles was investigated [29,30]. The triplex-specific antibodies suppress cell proliferation, particularly at the end of S phase and G2.

## EXPERIMENTAL

### Preparation of agarose-encapsulated and permeabilized nuclei

The nuclei were prepared as first described by Jackson and Cook [27] with later improvements [28]. In short, mouse myeloma

Abbreviations used: pyr, pyrimidine; pur, purine; FCS, fetal calf serum.

‡ To whom correspondence should be addressed.

MOPC 315.43 cells [31] were washed twice each with 50 ml of PBS, collected by centrifugation and resuspended in 20 ml of the same buffer. Aliquots (5 ml) of 2.5 % agarose (low melting point, Sigma type IV), liquefied and cooled to 39 °C, and liquid paraffin (50 ml), warmed to 39 °C, were added to the cells in a 250 ml Erlenmeyer flask and emulsified by shaking at 400 rev./min for 30 s in a rotary shaker. After equilibration in an ice-water bath for 5 min, 100 ml of ice-cold PBS was added, mixed by manually rotating the flask, and the solution centrifuged at 3500 ***g*** at 0 °C for about 5 min. The microbead pellets (agarose-encapsulated cells) were washed three times with ice-cold PBS and resuspended in 3 vol. of ice-cold isotonic buffer (130 mM KCl, 10 mM $Na_2HPO_4$, 100 mM $KHPO_4$, 1 mM $MgCl_2$, 1 mM ATP, 1 mM dithiothreitol, pH 7.2) containing 0.5 % (v/v) Triton X-100 and kept in an ice-water bath for 20 min with gentle stirring. The permeabilized nuclei were collected by centrifugation at 4800 ***g***, washed three times with 5 vols. of isotonic buffer, and then resuspended in an equal volume of the same buffer before use in the binding experiments.

### Binding studies

Binding experiments were carried out as described previously [32,33]. An aliquot (500 $\mu$l) of the permeabilized nuclei microbeads was added to 500 $\mu$l of isotonic buffer in Eppendorf tubes. DNA-binding monoclonal antibodies Jel 275 (duplex-specific) [24] and Jel 318 and 466 (triplex-specific) [26] were added to the microbead suspension at various concentrations and incubated at room temperature for 2 h. Jel 42 (raised against HPr, a bacterial protein, [34]) was used as a control. After washing the microbeads three times with 2 vols. of isotonic buffer to remove the unbound antibodies, 50 $\mu$l of a secondary antibody [$^{125}$I-labelled sheep anti-(mouse IgG), $\sim$ 50000 c.p.m.] was added and the mixture incubated at room temperature for 2 h. The unbound anti-(mouse IgG) antibody was removed by washing three times with isotonic buffer, radioactivity was measured with a $\gamma$-counter model 1271 RiaGamma, and the results were expressed as c.p.m.

Competition experiments were also performed on the agarose-encapsulated nuclei. The competitors used were poly[d(Tm$^5$C)]·poly[d(GA)], which forms a stable triplex, and *E. coli* DNA which is not triplex-forming [21]. Poly[d(Tm$^5$C)]·poly[d(GA)] was treated at pH 5.0 in order to enhance triplex formation and was then brought back to pH 7.2 before use. Various concentrations of DNA were added to the nuclear preparations together with 3 $\mu$g of either Jel 318 or Jel 466. After incubation for 2 h, the preparations were washed three times with buffer. Binding of the secondary antibody and the remaining part of the experimental procedure were as described above.

### Transcription and replication

Transcription and replication assays were performed by measuring the incorporation of [$^{35}$S]uridine 5′-[$\alpha$-thio]triphosphate or [$^{35}$S]deoxythymidine 5′-[$\alpha$-thio]triphosphate respectively [27]. The maximum counts were about 40000 c.p.m. for transcription and 5000 c.p.m. for replication.

### Cell growth and synchronization

MOPC cells were synchronized by adding either colcemid [35] or thymidine [36] in total medium [RPMI 1640 with 10 % fetal calf serum (FCS), 50 $\mu$M 2-mercaptoethanol, 0.1 $\mu$M sodium selenate and 50 $\mu$g/ml gentamicin sulphate]. Synchronization at metaphase was achieved by adding 0.075 $\mu$g/ml of colcemid and incubating at 37 °C for 20 h. To release the cells from the block, they were washed three times and resuspended in total medium. Cells were also synchronized at the G1/S phase boundary by inhibiting DNA synthesis with thymidine in two stages. When thymidine is added to the medium at a concentration of 5 mM, nucleoside diphosphate reductase is inhibited, thus slowing down DNA synthesis. The first block was applied for 12 h. The cells were then washed three times and grown for 10 h to allow cells to finish the S phase. A second block was applied for a further 12 h to allow all cells to reach the G1/S boundary. Release from the second block by washing allows the cells to enter the S phase in synchrony. The cells were then resuspended in total medium and manipulated as desired.

### Exploration of the MOPC cell cycle

Analysis of the cell cycle was carried out by measuring the activity of [$^3$H]thymidine incorporated into DNA [37]. The cells were continuously exposed to [$^3$H]thymidine at a radioactivity of 2.5 $\mu$Ci/ml for a total of 25 h in the case of thymidine-synchronized cells or 17 h in the case of colcemid-synchronized cells. Samples that contained approx. $10^5$ cells were taken at zero time and every hour thereafter and processed as described previously [27].

### Incorporation of monoclonal antibodies into cells

Antibodies were incorporated into MOPC cells by osmotic permeabilization as described previously [29,30], with some changes. Briefly, MOPC cells growing in total medium after synchronization were washed three times with RPMI 1640. The cells were exposed to 1 ml of hypertonic medium [1 M sucrose, 10 % poly(ethylene glycol)$_{1000}$] with 300 $\mu$g/ml of either Jel 318 or Jel 466 for 15 min. Controls were exposed to a hypertonic medium alone or a hypertonic medium containing Jel 42 at 300 $\mu$g/ml. (It should be noted that the duplex-specific antibody, Jel 275, was not available in sufficient quantities for these experiments since ascites production with Jel 275 is very poor.) The four experimental groups were then exposed to a hypotonic medium (6 vol. RPMI and 4 vol. water) for 2 min. Finally, the cells were washed three times with RPMI containing 10 % (v/v) FCS and resuspended in total medium containing 1 $\mu$g/ml of chloroquine diphosphate and incubated for 3 h. They were then washed and grown in total medium at an initial cell density of about $10^4$ cells/ml. Cell proliferation was evaluated by determining the total cell population with a haemocytometer at zero time and every 24 h thereafter for six days. At the same time, the percentage of dead cells was determined by Trypan Blue (0.025 %) exclusion staining and counting cells in three random microscopic fields under the $\times$40 objective; an average of three counts was taken each time.

## RESULTS

It was demonstrated previously that the triplex-specific monoclonal antibodies, Jel 318 and Jel 466, stained fixed metaphase chromosomes and unfixed chromosomes, as assessed by indirect immunofluorescence microscopy [24–26]. In order to obtain more direct evidence for the existence of triplexes, nuclei were encapsulated in agarose and the binding of the antibodies was measured. Individual binding experiments were performed in a volume of 1 ml containing 500 $\mu$l of packed agarose beads. The total DNA concentration was estimated to be 2.5 $\mu$g/ml [38]. As expected, the duplex-specific monoclonal antibody, Jel 275, binds well and a maximum of about 11000 c.p.m. was reached at 60 $\mu$g of antibody (Figure 1). Jel 42, which binds a bacterial protein,

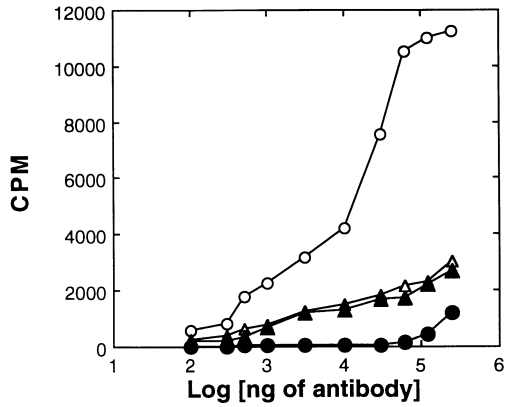


**Figure 1 Direct binding of the antibodies to agarose-encapsulated nuclei**

○, Jel 275; ●, Jel 42; △, Jel 318; ▲, Jel 466.

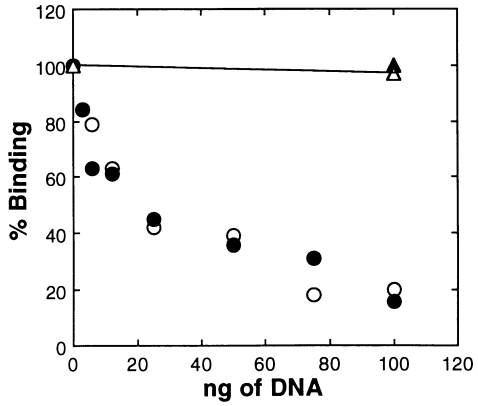


**Figure 2 Competitive binding of the triplex-specific antibodies to agarose-encapsulated nuclei**

The percentage binding was measured as a function of added competing DNA. ●, Jel 466 with triplex DNA; ○, Jel 318 with triplex DNA; ▲, Jel 466 with *E. coli* DNA; △, Jel 318 with *E. coli* DNA.

was used as a negative control and the background level of binding was insignificant below 100 $\mu$g of antibody. Above this level, some binding is apparent and the antibody may not be completely eliminated by the washing procedure. Both triplex-specific monoclonal antibodies, Jel 318 and Jel 466, showed a steady increase in binding from 0.1 to 100 $\mu$g of antibody but the maximum c.p.m. was about 5-fold lower than for Jel 275.

One advantage of encapsulated nuclei is that competition experiments can be performed. Thus, it was anticipated that addition of synthetic triplex DNA {prepared from poly[d(Tm$^5$C)]·poly[d(GA)]} would compete with the antibodies binding to the nuclei (Figure 2). The antibody was chosen at 3 $\mu$g which corresponds to the middle of the binding curve for Jel 318 and 466; at this level the antibodies are not in excess yet still give over 1000 c.p.m. Higher concentrations of antibody (50–100 $\mu$g) were problematic because the antibodies precipitated with the competing triplex DNA (results not shown). Addition of 100 ng of triplex DNA reduces the binding of both Jel 318 and Jel 466 to the nuclei by about 80 % whereas addition of *E. coli* DNA did not result in any competition.

Another advantage of the encapsulated nuclei is that they are

**Table 1 The effect of anti-triplex antibodies, Jel 318 and Jel 466, and the duplex-DNA-specific antibody, Jel 275, on (a) transcription and (b) replication in agarose-encapsulated nuclei**

The maximum c.p.m. was calculated as a percentage of the control with BSA and is the average of at least three determinations (±S.D.).

| Experimental group | Percentage of maximum c.p.m. |
|---|---|
| (a) | |
| Control, actinomycin D | 28±3 |
| Control, no ribonucleotides + [$^{35}$S]UTP | 12±3 |
| Control, BSA | 100 |
| Control, Jel 42 | 99±1 |
| Jel 275 | 80±2 |
| Jel 318 | 80±3 |
| Jel 466 | 80±3 |
| (b) | |
| Control, no nucleotides + [$^{35}$S]dTTP | 13±2 |
| Control, BSA | 100 |
| Control, Jel 42 | 100±1 |
| Jel 275 | 81±3 |
| Jel 318 | 82±3 |
| Jel 466 | 82±4 |

active in transcription and replication [27,28]; thus, the effect of the antibodies on these processes could also be monitored. As shown in Table 1, 15 $\mu$g of actinomycin reduced transcription to about 35 % of the control value. Addition of 60 $\mu$g of Jel 42 had no effect, whereas the duplex-specific and both triplex-specific antibodies reduced incorporation by about 20 %. Similarly, Jel 275, 318 and 446 caused a small reduction in replication to about 80 % of the control and again Jel 42 was ineffective. Thus, the antibodies produce a small but reproducible reduction in these essential cellular processes.

Antibodies can also be incorporated into living cells by osmotic shock [29,30]. The myeloma cell line MOPC was chosen for this work because it is robust and grows rapidly. Studies of the MOPC cell cycle revealed a doubling time of 20 h. Synchronization of the cells was achieved with colcemid, which blocks in metaphase, or a double thymidine treatment which blocks at the beginning of S phase; DNA synthesis was followed by measuring the incorporation of [$^3$H]thymidine. After treating with colcemid and then removing the block by washing, there was a slow rate of incorporation for 5 h followed by a burst of synthesis (results not shown). From this, it was deduced that the G1 phase lasts about 5 h but also that synchrony with colcemid was only about 70 % to account for the initial incorporation. After releasing from the double thymidine block, rapid synthesis occurred immediately and then ceased abruptly after 10 h (results not shown). Thus, S phase lasts about 10 h and the abrupt cessation of synthesis suggests that the initial synchrony was excellent (> 90 %). Finally, direct observation of the cells after release from the double thymidine block showed that cell division occurred from 14 to 15 h. Typically, mitosis lasts for 1 h so that G2 is calculated to be 3 to 4 h and the complete cell cycle of 20 h can be constructed, i.e. G1, 5–6 h; S, 10 h; G2, 3–4 h; and M, 1 h.

Monoclonal antibodies were introduced at various times into MOPC cells by osmotic lysis of pinocytic vesicles. The effect of incorporation was evaluated by determining total cell population and percentage of dead cells at 0 time and every 24 h for a total of 120 h (see the Experimental section). In unsynchronized cells, incorporation of either Jel 318 or Jel 466 suppressed cell growth by about 15 % after 120 h compared with Jel 42 and another

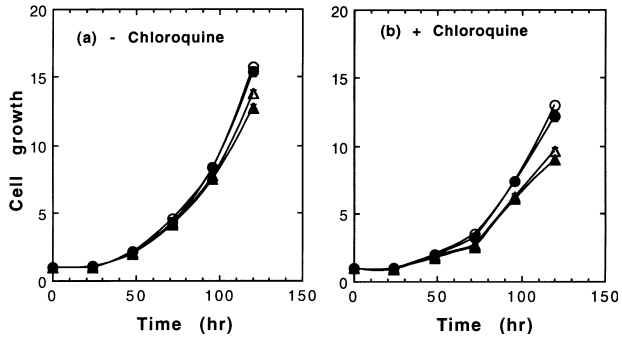


**Figure 3 Effect of incorporation of the antibodies on the growth of unsynchronized cells in (a) the absence or (b) the presence of chloroquine**

The growth of approx. $10^5$ cells was followed for 120 h. The ordinate is net fold growth from an initial value of 1. Each point is the average of at least three determinations. The standard deviation is also shown except in cases where it was smaller than the symbol itself. ○, No antibody; ●, Jel 42; ▲, Jel 318; △, Jel 466.

control with no antibody (Figure 3a). However, if this experiment was repeated with chloroquine treatment, cell growth was suppressed by 30% with the triplex-specific antibodies (Figure 3b). Chloroquine is known to inhibit degradation by lysosomes and may prolong the half-life of the antibodies [39]. Thus, the following experiments were all performed with chloroquine

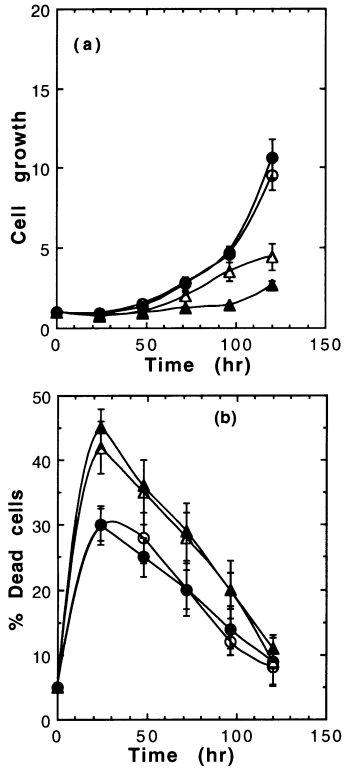


**Figure 4 Effect of incorporation of the antibodies on (a) cell growth and (b) percentage of dead cells**

The cells were synchronized with colcemid 12 h before incorporation. Each point is the average of at least three determinations. The standard deviation is also shown except in cases where it was smaller than the symbol itself. ○, No antibody; ●, Jel 42; △, Jel 318; ▲, Jel 466.

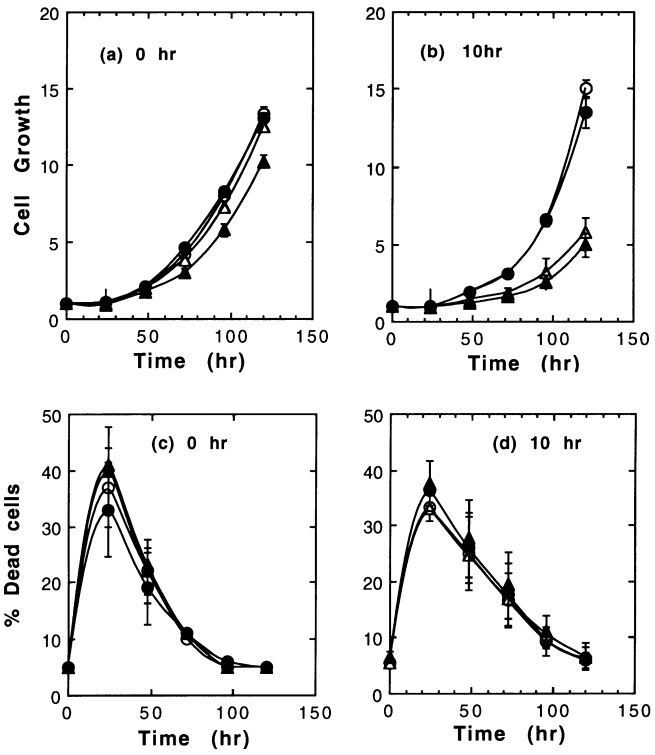


**Figure 5 Effect of incorporation of the antibodies on cell growth (a) and (b) and percentage of dead cells (c) and (d)**

The cells were synchronized with thymidine (**a**) and (**c**) 0 h before incorporation or (**b**) and (**d**) 10 h before incorporation. Each point is the average of at least three determinations. The standard deviation is also shown except in cases where it was smaller than the symbol itself. ○, No antibody; ●, Jel 42; △, Jel 318; ▲, Jel 466.

treatment. First, the antibodies were introduced 12 h post-colcemid synchronization, at which time the cells would be in late S phase. As shown in Figure 4(a), Jel 318 inhibits cell growth significantly and Jel 466 almost completely prevents cell growth at least for the first 96 h. The percentage of dead cells is shown in Figure 4(b). It is clear that this treatment is very harsh since about 30% of the cells in the control groups are dead after 24 h and incorporation of the triplex-specific antibodies increases the killing to over 40%. Thus, Jel 318 and 466 cause cell death as well as slowing the rate of growth. When the osmotic shock was performed at earlier post-colcemid synchronization times the cells became very fragile and cell death was as high as 80% even in the absence of antibodies. As well, the synchronization with colcemid was relatively poor (see above) and was expected to become worse as the length of time after release from the block was increased. Consequently, the remaining incorporation studies were performed on cells which were synchronized with the double thymidine block.

When the antibodies were incorporated immediately after release from the thymidine block, only Jel 466 showed slight suppression of cell growth (Figure 5a). At 10 h (Figure 5b), however, both triplex-specific antibodies reduced cell growth to only 5-fold compared with about 15-fold for the controls. The percentage of dead cells was also determined (Figures 5c and 5d). For both the 0 and 10 h experiments the amount of cell death is about 35% 24 h after osmotic shock and is unaffected by the incorporation of the antibodies. Thus, the triplex-binding antibodies are not killing the cells but are very effective at slowing

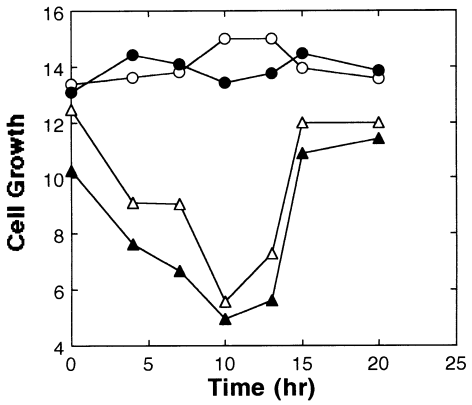


**Figure 6 Summary of the incorporation experiments after synchronization with thymidine**

The cell population (net fold growth) at 96 h (see Figures 4 and 5) is shown as a function of the time of incorporation of the antibodies after release from the thymidine block. ○, No antibody; ●, Jel 42; △, Jel 318; ▲, Jel 466.

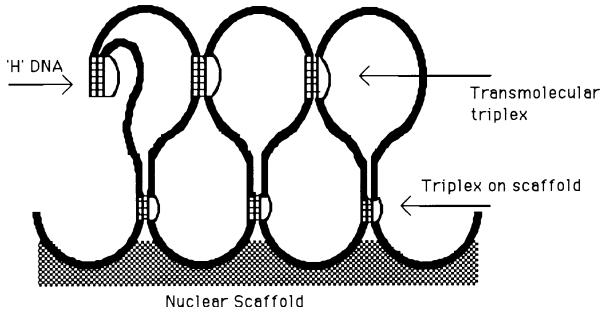


**Figure 7 Possible role of triplexes in eukaryotic chromosomes**

See text for details.

cell growth when incorporated at the end of S phase. Experiments were also performed at 4, 7, 13, 15 and 20 h after release from the thymidine block. These results are summarized in Figure 6. It is clear that both triplex-specific antibodies are most effective at inhibiting cell growth at 7 to 13 h, which corresponds to the end of DNA synthesis and the beginning of chromosome condensation during G2. At 15 h, after metaphase, the suppression is relaxed and by 20 h, at the beginning of another cell cycle, only slight suppression is apparent.

## DISCUSSION

Nuclei which have been encapsulated in agarose provide a very useful model for studying nuclear function because they are freely permeable and yet are still metabolically active [27,28]. Such nuclei have been used previously to study the presence of 'Z' DNA with the aid of structure-specific monoclonal antibodies [32,33]. It was found that the level of 'Z' DNA was dependent on the degree of supercoiling and the transcriptional activity of the nuclei. In this paper, Jel 318 and Jel 466 have been used to probe for triplexes. From the direct binding experiments (Figure 1) it is clear that the amount of triplex in the nuclei is smaller than the amount of duplex. Quantitative comparisons, however, are difficult because the proportion of either type of DNA structure which is accessible to the antibodies is not known and the binding constants of the various antibodies may be very different. Another difficulty is that the antibodies themselves may promote triplex formation. However, for 'Z' DNA-specific antibodies it was found that at least 40 $\mu$g of antibody was required to promote the formation of 'Z' DNA [32]. In the present case, significant binding of the triplex-specific antibodies was observed at less than 10 $\mu$g of antibody (Figure 1). Therefore, it seems likely that a large proportion of the observed antibody binding is due to the presence of existing triplexes. The competition experiments (Figure 2) provide further evidence for the presence of triplex DNA under physiological conditions since only a triplex-forming DNA was an effective competitor.

A possible involvement of triplexes in transcription and replication is suggested by the suppression of these processes by the triplex-specific antibodies (Table 1). Although the inhibition was small (20%), the triplex-binding antibodies were as effective as Jel 275 which is duplex-specific. In the case of transcription, pyr·pur tracts are generally found in the 5′ flanking regions of genes rather than within the genes. Therefore, the inhibition probably occurs at the level of initiation rather than elongation. A role for triplexes in the control of gene expression has been demonstrated for the $\gamma$-globin genes for example, whereas in other cases such as c-K-*ras*, triplexes may not be involved [20,40]. It is also possible that triplex formation might stimulate rather than inhibit transcription. Therefore, it will be important to study individual genes rather than total transcription as reported here. The inhibition of replication by Jel 318 and Jel 466 is perhaps more suprising because the majority of the DNA will be duplex and will not bind the antibodies. One explanation is that a replication fork may not be able to proceed through a triplex region when it is stabilized by a bound antibody.

Finally, the effect of the antibodies on cell growth was determined by incorporating the antibodies into living cells (Figures 4, 5 and 6). Chloroquine was included because it enhanced the growth retardation of unsynchronized cells in the presence of the antibodies (Figure 3). As well as inhibiting antibody degradation, chloroquine binds to DNA and may possibly interfere with triplex formation or other cell functions [39]. However, this cannot explain the cell-cycle dependence of the antibodies on cell growth (Figure 6). Synchronization of the cells was achieved with colcemid or thymidine. The thymidine block appeared to be more suitable since the synchrony was more complete and the cells could be immediately subjected to the osmotic shock treatment. After the colcemid block, the cells became very fragile and required at least 6 h before further manipulation. A similar effect was obtained following a vinblastine block (results not shown). By 12 h after release from the colcemid block, most of the cells would be in S phase. At this time their growth was extremely sensitive to the triplex-specific antibodies. This result was confirmed with the thymidine block because the maximum effect occurred at the end of S phase. Therefore, the antibodies may be inhibiting some aspect of the termination of replication, although it is not clear how triplexes might be involved in this process. Suppression of cell growth also occurred when the antibodies were incorporated in the middle of S phase and in G2; but during G1, incorporation of the antibodies had little effect. During late S phase the condensed heterochromatin is being replicated and in G2 the chromosomes are beginning to condense towards metaphase. At the end of M phase the chromosomes once again decondense and the interphase nucleus reforms. If triplexes are involved in some aspect of chromosome structure, particularly chromatin condensation, then the presence of the triplex-binding antibodies may be most detrimental during the condensation/

decondensation cycle of M phase. As well, the breakdown of the nuclear envelope at M phase may facilitate access of the antibodies to the triplex DNA.

Several models of chromosome structure have been proposed which consist of loops of DNA radiating from a core or scaffold [41,42]. Three potential roles for triplexes are shown in Figure 7. First, intramolecular triplexes (or 'H' DNA) may be present in the loops of DNA and be involved in the control of gene expression. Secondly, transmolecular triplexes could form at the base of the loop and be involved in assembling the loops of DNA on to the nuclear scaffold. The loops would then be topologically constrained, as has been demonstrated [43,44]. Finally, transmolecular triplexes may form between loops and organize them into an ordered array. As well, this type of triplex could be involved in the control of gene expression by interaction between homologous sequences which are distantly related on the chromosome. Thus, this last type of triplex may be very difficult to find experimentally.

This research was supported by MRC Canada by grants to J.S.L.

## REFERENCES


1 Birnboim, H. C., Sederoff, R. R. and Paterson, M. C. (1979) Eur. J. Biochem. **98**, 301–307
2 Hoffman-Liebermann, B., Liebermann, D., Troutt, A., Kedes, L. H. and Cohen, S. N. (1986) Mol. Cell Biol. **6**, 3632–3642
3 Wong, A. K. C., Yee, H. A., van de Sande, J. H. and Rattner, J. B. (1990) Chromosoma **99**, 344–351
4 Manor, H., Rao, B. S. and Martin, R. G. (1988) J. Mol. Evolution **27**, 96–101
5 Mirkin, S. M., Lamichev, V. I., Drushlyak, K. N., Dobrynin, V. N., Filippov, S. A. and Frank-Kamenetskii, M. D. (1987) Nature (London) **330**, 495–497
6 Kohwi, Y. and Kohwi-Shigematsu, T. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 3781–3785
7 Htun, H. and Dahlberg, J. E. (1988) Science **241**, 1791–1796
8 Htun, H. and Dahlberg, J. E. (1989) Science **243**, 1571–1576
9 Collier, D. A. and Wells, R. D. (1990) J. Biol. Chem. **265**, 10652–10658
10 Hampel, K. J., Crosson, P. and Lee, J. S. (1991) Biochemistry **30**, 4455–4459
11 Strobel, S. A. and Dervan, P. B. (1991) Nature (London) **350**, 172–174
12 Orson, F. M., Thomas, D. W., McShan, W. M., Kessler, D. J. and Hogan, M. E. (1991) Nucleic Acids Res. **19**, 3435–3440
13 Havré, P. A., Gunther, E. J., Gasparro, F. P. and Glazer, P. M. (1993) Proc. Natl. Acad. Sci. U.S.A. **90**, 7879–7883
14 Wilson, W. D., Tanoius, F. A., Mizan, S., Yao, S., Kiselyov, A. S., Zon, G. and Strekowski, L. (1993) Biochemistry **32**, 10614–10621
15 Maher, L. J. (1992) BioEssays **14**, 807–815
16 Wells, R. D., Collier, D. A., Hanvey, J. C., Shimizu, M. and Wohlrab, F. (1988) FASEB J. **2**, 2939–2949
17 Boulikas, T. (1993) J. Cell Biochem. **52**, 14–22
18 Ulrich, M. J., Gray, W. J. and Ley, T. J. (1992) J. Biol. Chem. **267**, 18649–18658
19 Kolluri, R., Torreyu, T. A. and Kinniburg, A. J. (1992) Nucleic Acids Res. **20**, 111–116
20 Horwitz, E. M., Maloney, K. A. and Ley, T. J. (1994) J. Biol. Chem. **269**, 14130–14139
21 Lee, J. S., Woodsworth, M. L., Latimer, L. J. P. and Morgan, A. R. (1984) Nucleic Acids Res. **12**, 6603–6613
22 Hampel, K. J., Ashley, C. and Lee, J. S. (1994) Biochemistry **33**, 5674–5681
23 Hampel, K. J. and Lee, J. S. (1993) Biochem. Cell Biol. **71**, 190–196
24 Burkholder, G. D., Latimer, J. P. and Lee, J. S. (1988) Chromosoma **97**, 185–192
25 Burkholder, G. D., Latimer, J. P. and Lee, J. S. (1991) Chromosoma **101**, 11–18
26 Agazie, Y. M., Lee, J. S. and Burkholder, G. D. (1994) J. Biol. Chem. **269**, 7019–7023
27 Jackson, D. A. and Cook, P. R. (1985) EMBO J. **4**, 913–918
28 Jackson, D. A., Yuan, J. and Cook, P. R. (1988) J. Cell Sci. **90**, 365–378
29 Okada, C. Y. and Rechsteiner, M. (1982) Cell **29**, 33–41
30 Chakrabarti, R., Pfeiffer, N. E., Wylie, D. E. and Schuster, S. M. (1989) J. Biol. Chem. **264**, 8214–8221
31 Mosmann, T. R., Baumal, R. and William, A. R. (1979) Eur. J. Immunol. **9**, 511–516
32 Wittig, B., Dorbic, T. and Rich, A. (1989) J. Cell Biol. **108**, 755–764
33 Wittig, B., Dorbic, T. and Rich, A. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 2259–2263
34 Vandonselaar, M., Lee, J. S. and Delbare, L. T. J. (1984) J. Mol. Biol. **177**, 369–371
35 Kasten, F. H., Strasser, F. F. and Turner, M. (1965) Nature (London) **207**, 161–164
36 Prescott, D. M. (1976) in Reproduction of Eukaryotic Cells, pp. 19–35, Academic Press, NY
37 Adams, R. L. P. (1980) Laboratory Techniques in Biochemistry and Molecular Biology: Cell Culture for Biochemists (Work, T. S. and Burdon, R. H., eds.), Elsevier, Amsterdam
38 Morgan, A. R., Lee, J. S., Pulleyblank, D. E., Murray, N. L. and Evans, D. H. (1979) Nucleic Acids Res. **7**, 547–569
39 Luthman, H. and Magnusson, G. (1983) Nucleic Acids Res. **11**, 1295–1301
40 Raghu, G., Tevosian, S., Anant, S., Subramanian, K. N., George, D. L. and Mirkin, S. M. (1994) Nucleic Acids Res. **22**, 3271–3279
41 Rattner, J. B. and Lin, C. C. (1985) Cell **42**, 291–296
42 Saitoh, Y. and Laemmli, U.K. (1994) Cell **76**, 609–622
43 Adachi, Y., Luke, M. and Laemmli, U.K. (1991) Cell **64**, 137–148
44 Gasser, S. M. and Laemmli, U.K. (1987) Trends Genet. **3**, 16–22


Received 20 December 1995/26 January 1996; accepted 31 January 1996