Location of B- and Z-DNA in the Chromosomes of a Primitive Eukaryote Dinoflagellate

Marie-Odile Soyer-Gobillard,* Marie-Line Géraud,* Dominique Coulaud,‡ Martine Barray,‡ Bernard Théveny,‡ Bernard Révet,‡ and Etienne Delain‡

*Observatoire Océanologique de Banyuls, Département de Biologie Cellulaire et Moléculaire, Laboratoire Arago, Centre National de la Recherche Scientifique Unité de Recherche Associée 117, F-66650 Banyuls-sur-mer France; and ‡Laboratoire de Microscopie Cellulaire et Moléculaire, Centre National de la Recherche Scientifique Unité Associée 147, Institut Gustave Roussy, F-94805 Villejuif Cédex France

Abstract. The usual conformation of DNA is a right-handed double helix (B-DNA). DNA with stretches of alternating purine-pyrimidine (G-C or A-T) can form a left-handed helix (Z-DNA). The transition B→Z, facilitated by the presence of divalent cations, cytosine methylation, or constraints on DNA such as superhelicity may play a role in the regulation of gene expression and/or in DNA compaction (Zarling, D. A., D. J. Arndt-Jovin, M. Robert-Nicoud, L. P. McIntosh, R. Tomae, and T. M. Jovin. 1984. *J. Mol. Biol.* 176:369–415). Divalent cations are also important in the structure of the quasi-permanently condensed chromosomes of dinoflagellate protists (Herzog, M., and M.-O. Soyer. 1983. *Eur. J. Cell Biol.* 30:33–41) which also have superhelicity in their DNA. The absence of histones in dinoflagellate chromosomes suggest that the search for Z-DNA sequences might be fruitful and could provide one indication of the physiological role of this particular DNA conformation.

We report a complete immunofluorescent and immunogold analysis of the nuclei of the dinoflagellate *Prorocentrum micans E.* using monoclonal and polyclonal anti-B and anti-Z-DNA antibodies. Positive labeling was obtained with immunofluorescence using squash preparations and cryosections, both of which showed the intranuclear presence of the two DNA conformations. In ultrathin sections of aldehyde-prefixed, osmium-fixed, and epoxy-embedded cells, we have localized B-DNA and Z-DNA either with single or double immunolabeling using IgG labeled with 5- and 7-nm gold particles, respectively. Chromosomal nucleofilaments of dividing or nondividing chromosomes, as seen in ultrathin sections in their arch-shaped configuration, are abundantly labeled with anti-B-DNA antibody. Extrachromosomal anti-B-DNA labeling is also detected on the nucleoplasm that corresponds to DNA loops; we confirm the presence of these loops previously described external to the chromosomes (Soyer, M.-O., and O. K. Haapala. 1974. *Chromosoma (Berl.).* 47:179–192). B labeling is also visible in the nucleolus organizer region (NOR) and in the fibrillo-granular area (containing transcribing rDNA) of the nucleolus. Z-DNA was localized in limited areas inside the chromosomes, often at the periphery and near the segregation fork of dividing chromosomes. In the nucleolus, Z-DNA is observed only in the NOR area and never in the fibrillo-granular area. For both types of antibody experiments, controls using gold-labeled IgG without primary antibody were negative. A quantitative evaluation of the distribution of the gold-labeled IgG and a parametric test support the validity of these experiments. We also demonstrate the preservation of antibody activity on DNA molecules preincubated in OsO_4 solutions. The role of the Z-DNA conformation as a possible site for unwinding and DNA processing in chromosomes that lack nucleosomes and that are permanently condensed is discussed.

DINOFLAGELLATE protists are primitive eukaryotes, as demonstrated by ultrastructural, biochemical, and molecular biological studies (25, 27, 68), as well as by the study of their cell cycle and patterns of DNA synthesis (5). They are characterized by the presence of a permanent nuclear envelope, by chromosomes that are condensed throughout the cell cycle, and by the absence of histones and nucleosomes (6, 23). In ultrathin sections, chromosomes show an arrangement of the nucleofilaments in a series of arches (8, 15, 64). Chromosome spreadings revealed double-twisted helix organization (17, 18, 40, 52, 63), the architecture being maintained by the presence of divalent cations (24) and structural RNA (66). The bundles of nucleofilaments that form the double-twisted helix are supertwisted

with six hierarchical levels of organization (27). Other characteristics of these chromosomes are the total absence of banding either Q, G, or C (18), their high G and C content, and the presence of a rare base, hydroxymethyl uracile, in a high proportion (25). The presence of chromosomes in a permanently condensed state throughout the cell cycle raises the question of how such structures can be transcribed and replicated (2, 8, 40, 61, 68). As early as 1968, Bouligand et al. (8), then Babillot, 1970 (2), and Soyer and Haapala, 1974 (65) raised that question, the last authors describing extrachromosomal loops evidenced by pronase treatment. They proposed a model (65) in which a local untwisting of the supercoiling in these loops could allow the nonnucleolar or nucleolar transcription process to occur, a hypothesis later elaborated upon by other authors (40). More recently, Sigee (61) demonstrated the location of active chromatin in these extrachromosomal loops using tritiated adenine incorporation and autoradiography.

Various studies have demonstrated the stabilization of left-handed DNA structure (Z-DNA) by negative supercoiling, which implies that Z-DNA segments can reduce or suppress superhelicity in covalently closed circular DNA molecules. From these observations, several hypothesis have been proposed that link the existence of Z-DNA to potential roles in various physiological phenomena (for review, see references 32, 33, and 58). These mechanistic roles of Z-DNA still remain speculative (44, 76).

The absence of histones, the stabilization of DNA supercoiling by divalent cations, the presence of rare bases, and the high G and C content are factors that are known to facilitate local B→Z transitions of DNA. In the present work, we have detected and localized both right-handed double helix (B-DNA) and Z-DNA conformations in the nucleus of a dinoflagellate, *Prorocentrum micans E.*, by immunocytochemistry. Various antibodies and complementary techniques have been used: fluorescent optical microscopy on squashed unfixed, fixed, or frozen cells, as well as EM on osmium-fixed Epon-embedded cells, with single or double labelings. A critical study of these techniques is also presented, for example, the validity of immunocytochemical detection of DNA on osmium-fixed cells, which is demonstrated by a molecular approach. Quantitative measurements of grain densities in the nuclear compartments have been performed using an image processor. Preliminary results were presented at the 1987 meeting of the French Society for Electron Microscopy.

Materials and Methods

Cultures of Cells

Prorocentrum micans Ehrenberg (*P. micans*), from the Botany School of Cambridge (UK), was grown in Erdshreiber medium with continuous illumination of 2,000 lx. The cell cycle of this dinoflagellate lasts 5 d under these conditions with a discontinuous, eukaryotic interval of DNA synthesis (5).

Squashes for Optical Microscopy

Pellets of *P. micans* obtained at 900 t/min were fixed for 30 min in either a 3:1 mixture of ethanol/acetic acid, or in 2% paraformaldehyde in PBS (0.01 M PO_4HNa_2, 0.01 M PO_4H_2K, 0.15 M NaCl) pH 7.0, or not fixed at all. Controlled squashes were made between siliconed microscope slides and coverslips. Ethanol–acetic acid–fixed cells were washed with 45% ethanol. All cells were rinsed with PBS. All slides were frozen 1 h on a dry ice block, and stored at −20°C in a freezer. Slides can be processed for immunocytochemistry after a rapid wash with PBS.

Cryomicrotomy

P. micans pellets were fixed in a mixture of 1% glutaraldehyde and 2% formaldehyde in 0.2 M PIPES buffer, pH 7.0 (62). Pellets were washed 10 min in three changes of buffer then incubated 30 min in 2.3 M sucrose. Pellets were bound to blocks (LKB Instruments Inc., Bromma, Sweden), and rapidly frozen in liquid nitrogen. 1-μm thick cryosections obtained with the Cryonova ultramicrotome (LKB Instruments Inc.) were deposited on coverslips, where all immunological reactions were conducted after removal of sucrose by floating the coverslips on distilled water droplets.

EM Procedures

P. micans pellets were fixed 1 h with 0.2 M PIPES-buffered 2% paraformaldehyde and 1.25% glutaraldehyde, pH 6.4, according to Karnovsky's procedure, as modified by M.-O. Soyer (62). After washing in 0.2 M PIPES buffer, they were postfixed 1 h in similarly buffered 2% OsO_4 at room temperature, and embedded in Epon. Ultrathin sections were deposited on 300 mesh nickel grids; a slight etching was obtained by a 10-min treatment in 10% H_2O_2 at room temperature (3). Grids were then wash twice with double-distillated water. EM was on an Hitachi H-600 (Hitachi Ltd., Tokyo).

Antibodies

Anti-B-DNA. Two anti-B-DNA antibodies (Abs)[1] were used: a commercial polyclonal Ab (Sigma Chemical Co., St. Louis, MO), and the monoclonal IgG: E 77 isolated and characterized by Tron and co-workers (30, 73; see Table I). These two types of Ab have been used by several different laboratories (74; Delain, E., and B. Révet, unpublished results).

Anti-Z-DNA. Two anti-Z-DNA Abs produced in our Villejuif laboratory were used: a polyclonal (*n* 1,472) obtained by immunization of rabbits by brominated poly(dG.dC).poly(dG.dC) according to the technique described by Lafer et al. (36), and a mouse monoclonal IgG (3/1 C2) obtained by immunization with the same immunogen (Révet, B., G. Delage, and T. Huynh, unpublished results).

Screening and specificity testing of these Abs were done by the ELISA technique, with antigens immobilized on plastic wells (72). Antigens were either brominated poly(dG.dC).poly(dG.dC), or poly(dG.dC).poly(dG.dC) in 4 M NaCl, or poly(dG.m5dC).poly(dG.m5dC) in the presence of 10–20 mM $MgCl_2$. All polyclonal antibodies and mAbs reacted with the various Z-DNA species, except with the Z form of the methylated polynucleotide. None reacted with B-DNA.

Immunocytochemistry

In all situations, either squashes or sections, the nonspecific epitopes were saturated by a 15-min incubation at room temperature with a 10% dilution of normal goat serum in PBS buffer. All antibodies (first or second Ab) were diluted in PBS and 0.1% BSA. Incubations with the first anti-DNA Ab were performed for 1 h, at 37°C in a wet chamber. Then slides or sections were washed by alternating PBS in pipette and droplets application.

The second incubation Abs were either goat antirabbit (GAR), goat antimouse (GAM), or goat antihuman (GAHu) coupled with FITC, or 5- or 7-nm colloidal gold in PBS and 0.1% BSA. Preparations were washed as described for the first Ab. EM simultaneous detection of B- and Z-DNA were done according to Tapia et al. (69) by mixing identical volumes of 1/25 dilutions of the human anti-B-DNA and the polyclonal rabbit anti-Z-DNA. These ABs were revealed by a mixture of GAHu-5-nm (1/20 dilution) and GAR-7-nm (1/10 dilution).

Finally, squashes were mounted in glycerol containing 5% antifading compound *N*-propylgallate, when ultrathin sections were stained 15 min in saturated alcoholic uranyl acetate solution.

For squashes, controls consisted of a 1/100 dilution of rabbit antispasmine (a gift of Dr. Salisbury) as the primary Ab. The Ab does not react with any part of the dinoflagellate cell (45). For cryosections and EM, controls consisted used the second Ab alone or on sections that were not etched. For fluorescence a Reichert Polyvar optical microscope was used.

Quantitative Analysis of Gold Labeling

A quantitative evaluation of the distribution of the gold-labeled IgG was done on enlargements of EM micrographs from different experiments per-

1. *Abbreviations used in this paper*: Ab, antibody; GAHu, goat antihuman; GAM, goat antimouse; GAR, goat antirabbit; NOR, nucleolar organizing region.

Table I. Antibodies and Complementary Techniques Used for the B- and Z-DNA Immunodetection

	1st antibody					2nd antibody	
Anti-DNA	Polyclonal antibody	Monoclonal antibody	From	For	Dilution	Type	Dilution
1 { Anti-B-DNA	X		Hu*	fluorescence	1/100	6 GAHu-FITC	1/25
1 { Anti-DNA	X		Hu	EM	1/25	7 GAHu-5 nm	1/20
2 Anti-B-DNA		X	M‡	fluorescence, cryosections	1/100	8 GAM-FITC	1/40
3 Anti-Z-DNA		X	M	fluorescence, cryosections	1/25	8 GAM-FITC	1/40
4 { Anti-Z-DNA	X		R§	fluorescence	1/200	9 GAR-FITC	1/25
4 { Anti-Z-DNA	X		R	EM	1/25	10 GAR-5 nm	1/20
4 { Anti-Z-DNA	X		R	EM	1/10	11 GAR-7 nm	1/10
5 Anti-spasmine	X		R	fluorescence	1/100	9 GAR-FITC	1/25

* Human.
‡ Mouse.
§ Rabbit.
1. From Sigma Chemical Co. ref 11F-62061.
2. From Dr. Tron's laboratory (30, 73).
3. From our Institut Gustave Roussy laboratory.
4. From our Institut Gustave Roussy laboratory.
5. A gift from Dr. Salisbury.
6–10. Janssen Pharmaceutica, Beerse, Belgium.
11. From Dr. P. Gounon, Institut Pasteur Paris.

formed on the following immunolabelings: anti-B-DNA revealed by 5-nm gold-labeled anti-IgG; anti-Z-DNA revealed by 5-nm gold-labeled anti-IgG; anti-Z-DNA revealed by 7-nm gold-labeled anti-IgG; anti-B and anti-Z revealed by 5- and 7-nm gold, respectively.

The surface areas of chromosomal and extrachromosomal regions were measured on 15 randomly chosen portions of nuclei in each series of experiments, using a TV camera and an Image Processing System (Kontron Analytical, Kontron, AG, Everett, MA), as seen in Fig. 1. The number of 5- or 7-nm gold particles in each compartment (chromosomal or extrachromosomal) was counted and the density of the labeling was expressed as the number of particles per μm^2. The nonparametric test of Mann and Whitney (43) was used to test the significance of the results (Table II).

Formation of Immune Complexes in the Presence of OsO_4

The Z-DNA-binding capacity and specificity of the mAb in the presence of OsO_4 were tested by using the already described property of these IgG to bind specifically to the Z sites of supercoiled ΦX174 RFI DNA (57): 20 μl samples contained ΦX174 RFI and IgG at final concentration of 20 μg/ml and 100 or 200 μg/ml, respectively. After reaction with the mAb, the supercoiled molecules were linearized by the restriction enzyme Ava II and the sites of binding were mapped and compared to the known Z-forming sites. The binding sites were similar to those detected by the T4 anti-Z antibody (57).

Most of the tests have been performed with the anti-Z mAb 3/1C2. Supercoiled ΦX174 DNA molecules were incubated 1 h at 4°C in the presence of OsO_4: 0.025, 0.05, 0.1, 0.25, 0.5, and 1% in 10 mM Tris-ethanolamine, NaCl 50 mM, pH 7.5. Without removing OsO_4, aliquots were incubated 15 min at 37°C with a concentration of anti-Z mAb 3/1C2 that previously gave a good reaction in normal conditions without OsO_4. After the reaction was complete, the DNA molecules were linearized by Ava II, always in the presence of OsO_4. To appreciate the possible effect of free OsO_4 on the IgG itself, a counter experiment was performed by incubating IgG for 1 h at 37°C in different concentrations of OsO_4 (0.005–0.05%), before incubation with the DNA molecules.

Results

Activity of Antibodies in the Presence of OsO_4

DNA Incubated in OsO_4. The results of four separate experiments showed that the binding of 3/1C2 IgG on DNA incubated in different concentrations of OsO_4 was not strongly affected by the presence of this fixative: 90% of the DNA molecules showed binding of some IgG in the control with 0.025% OsO_4; this fell to 78% in the presence of OsO_4 at concentrations of 0.25–1%. On the other hand, the activity of the restriction endonuclease Ava II was more affected by the fixative: 95% of the molecules were cleaved in the control, but only 50% were linearized in the presence of 0.05% OsO_4, and 2% with the highest possible concentration, 0.25%.

IgG Preincubated in OsO_4. IgG were incubated 1 h at 4°C in OsO_4 solution (0.005–0.05%) before testing their reactivity with DNA molecules. Their ability to bind DNA strongly decreased when OsO_4 concentration increased. Moreover, there was a strong tendency of the immune complexes to polymerize and precipitate, leading to large multimolecular aggregates. In this experiment, the maximal possible dose of OsO_4 was only 0.05%, due to precipitation at higher fixative concentration.

The two types of experiments above show that osmium tetroxide does not inhibit the rapid and efficient binding of 3/1C2 Ab to DNA in solution. Such a control, although not directly comparable to the status of osmium and DNA in plastic sections, demonstrates the accessibility of DNA epitopes in the presence of osmium. We considered it an acceptable control before our attempt to detect DNA in OsO_4-fixed cells in sections. We attempted to perform DNAse I digestion on thin sections of aldehyde-osmium fixed and epon-embedded cells, but osmium-treated DNA was insensitive to digestion whatever the enzyme concentration, which is in accord with the observations of Berger and Schweiger (4). So, another control before immunoassays (data not shown) consisted in floating thin sections of aldehyde-fixed and Lowicryl K4M-embedded cells on DNase I (Sigma Chemical Co.) 1 mg/ml solution in PBS for 3 h at room temperature, according to the method of Thiry et al. (70). In this case, no labeling occurred, neither after anti-B-DNA antibody nor after anti-Z-DNA antibody immunoassays. These negative results were not shown because of the rather poor preservation of chromosome structure.

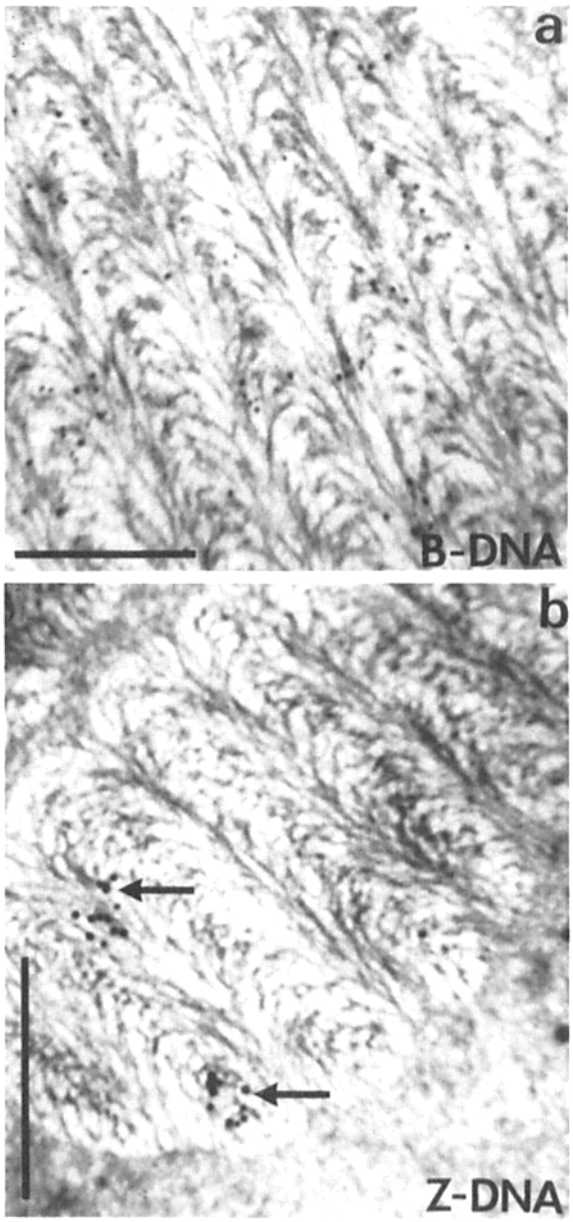


Figure 1. (*a*) Digitized image of a randomly chosen intrachromosomal region in which 5-nm gold particle labeling of B-DNA has been quantified. (*b*) Digitized image of an intra- and extra-chromosomal region in which 7-nm gold particle labeling of Z-DNA has been quantified. Bars: (*a*) 0.2 μm; (*b*) 0.5 μm.

Immunofluorescence Detection

The nucleus of *Prorocentrum micans* possesses ~100 chromosomes, each 1 μm in diameter and 10 μm in length. They remain permanently condensed throughout the cell cycle, as seen in Fig. 2 after squashing and 4'6 diamidino 2 phenyl inidole (DAPI) staining (*a*), or in epoxy semithin sections after acridine orange staining (*a'*).

B-DNA. B-DNA was detected either with polyclonal anti-B-DNA Ab (Table I, *1* and *2*) after squashing (data not shown) or with a monoclonal anti-B-DNA Ab for cryosections as seen in Fig. 2, *b* and *j*. In cells with telophase (*b*) or interphase (*j*) nuclei, sectioned chromosomes are brightly and homogeneously fluorescent (*j*), while in controls using the second Ab alone, the nucleus is totally black (Fig. *2*, *d* and *k*). In *b* the variations of the brightness in the nucleus are due to the fact that all the chromosomes are not in the same plan section.

Z-DNA. To prevent any modification or artifactual induction of Z form of the DNA due to chemical fixation (37) we detected Z-DNA using a monoclonal anti-Z-DNA Ab (Table I, *3* and *4*) without any preliminary fixation. In spite of a bad chromosomic preservation in squash technique due to lack of fixation, a positive fluorescent reaction was visible in the nuclear zone (Fig. 2 *f*), Fig. 2 *g* being the view of the same cell in phase contrast. Controls treated with an antispasmin as the first Ab (Table I, *5*) and a second GAR-FITC Ab are totally negative (Fig. 2 *h*), with the corresponding phase-contrast view (Fig. 2 *i*). No secondary staining of DNA with DAPI was done to avoid interference with the FITC fluorescence.

Immunoelectron Microscopy

Nondividing Chromosomes. Dinoflagellate chromosomes are composed of supertwisted nucleofilaments (17, 39, 52), which are visualized in thin sections as slack arches, whose structure depends on whether they are longitudinally, obliquely, or transversely sectioned. This visualization was interpreted by Bouligand et al. (8) by means of a model reproduced in Fig. 3. Referring to this drawing, Fig. 4 *a* represents interphasic chromosomes longitudinally sectioned, and Fig. 4 *b*, obliquely sectioned, *A* and *B* representing sections corresponding to the *A* and *B* planes of section in Fig. 3.

B-DNA Detection. EM detection utilized a polyclonal anti-B-DNA Ab labeled with colloidal gold (Table I, *1*). On low magnification micrographs of longitudinally (Fig. 4 *c*) or obliquely sectioned chromosomes (Fig. 4, *d* and *e*), 5-nm gold particles coupled to second Ab (*GAHu*, Table I, *1*) were visible over the entire chromosome as seen in Fig. 4, *c* and *d*. The labeling was visible on chromosomal as well as on extrachromosomal nucleofilaments. On higher magnification micrographs (Fig. 5, *a*, *b*, and *d*), the distribution of 5-nm gold particles was visible on arch-shaped nucleofilaments (Fig. 5 *d*, *arrow*) as well as on interarch nucleofilaments (Fig. 5, *a* and *b*). The labeling was equally intense in *A* and *B* thin sections (Fig. 5 *b*) and as well in transversally as longitudinally sectioned nucleofilaments.

Z-DNA Detection

Detection was with a polyclonal anti-Z-DNA as primary Ab (Table I, *4*) visualized with either 5- or 7-nm gold particles (GAR 5 or GAR 7) (Table I, *4*). The localization of Z-DNA was at the level of chromosome nucleofilaments, although a few particles were seen in the nucleoplasm. Gold particles were often grouped in clusters of 30–40 (Fig. 6, *b–f*) located in the chromosomes (Fig. 6, *a*, *b*, and *c*) and especially in the peripheral regions (Fig. 6, *c–f*). Controls using only the secondary Ab (GAR 5 or GAR 7) showed no labeling (Fig. 6 *g*). (GAR 7-nm control).

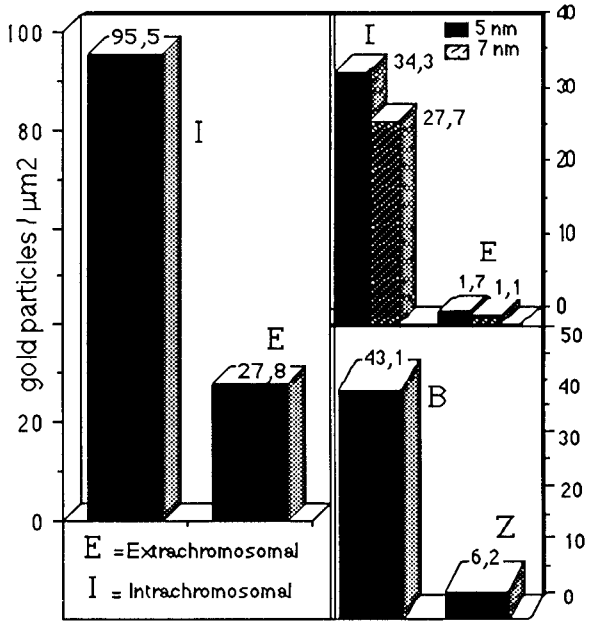
Simultaneous Detection of B- and Z-DNA

Using a mixture of polyclonal anti-B-DNA and polyclonal anti-Z-DNA as primary Ab and GAHu-5-nm and GAR-7-nm

Table II. Quantitative Evaluation of EM Immunolabeling

Average density			Difference at 5%
		(particles/μm²)	
B-DNA	Intrachromosomal	95,5	Significant
	Extrachromosomal	27,8	
Z-DNA (5 nm)	Intrachromosomal	34,3	Significant
	Extrachromosomal	1,7	
Z-DNA (7 nm)	Intrachromosomal	27,7	Significant
	Extrachromosomal	1,1	
Doublelabeling	B-DNA (5 nm)	43,1	Significant
	Z-DNA (7 nm)	6,2	

a



b

(*a*) Average density of gold particles on intra and extrachromosomal areas and significance of the comparison between these two areas determined by the Mann and Whitney statistical test. (*b*) Corresponding histogram of B-DNA (*1*) in intra (*I*) and extra (*E*) chromosomal areas, Z-DNA (*2*) labeled by 5- or 7-nm gold particles in intra and extrachomosomal areas, and double labeling of B(5-nm)- and Z(7-nm)-DNA (*3*).

as second Ab, the 5-nm gold particles were localized either in the chromosomes at the level of nucleofilaments or in the nucleoplasm (Fig. 7 *a*). On this micrograph, an extrachromosomal loop is visible (*arrow*) that is only labeled with anti-B-DNA. The presence of Z-DNA was indicated by clumps of 7-nm gold particles (Fig. 7, *black and white arrows*), very few of which are visible in the nucleoplasm (Fig. 7 *b*). Controls using only secondary antibodies showed no labeling (Fig. 7 *c*).

Quantitative Evaluation of the Distribution of Gold-labeled IgG

We have compared the distribution and number of gold particles per μm^2 on intra(*I*)- and extra(*E*)-chromosomal compartments for the two forms of DNA after single or double labeling of interphase nuclei (Table II, *a*).

In Table II, *b*, the histogram (*1*) shows that the density of B-DNA–bound gold particles is three times higher on chromosome sections (*I*) than in the nucleoplasm (*E*). The parametric test of Mann and Whitney (43) indicates that the difference is significant at the 5% level.

The distribution of Z-DNA was evaluated by counting both 5- and 7-nm gold particles (Table II, *b*, histogram (*2*)). In both cases, Z-DNA was ~20 times more abundant on chromosome sections (*I*) than in the nucleoplasm (*E*). The Mann and Whitney test confirms the significance of this difference ($P < 0.05$). In both nuclear compartments, the density of 5-nm gold particles is a little higher than those of 7-nm gold particles, but this difference was not significant ($P > 0.05$).

The histogram (3) of Table II, *b* shows the significant difference between the density of B and Z forms of DNA detected simultaneously, when B- and Z-DNA were labeled by 5- and 7-nm gold particles, respectively.

Dividing Chromosomes

5-nm gold particles indicative of the presence of B-DNA were localized on numerous nucleofilaments of the dividing chromosomes (Fig. 8 *a*). Several clumps of ~30–40 Z-DNA gold particles were visible on the dividing chromosomes. Fig. 8 *b* is especially interesting, as the cluster occurs at the chromosome segregation fork. As seen in Fig. 8 *c*, 5- and 7-nm gold particles are visible over the entire chromosome. A clump of the two types of particles is visible in the chromosome and in the segregation fork. Controls using only a mixture of GAR7 and GAHu5 were negative as seen in Fig. 8 *d*.

Nucleolus Area

In dinoflagellates, the active nucleolus is composed of an unwound part of the otherwise always condensed chromosome, corresponding to the nucleolar organizing region of the higher eucaryotes (NOR), a fibrillo-granular area containing the transcription complexes, and a granular area composed of preribosomes (64). As seen in Fig. 9 *a* and *c*, the unwound part of the chromosome was labeled by anti-B-DNA, as was the fibrillo-granular area. The granular region was never labeled. 7-nm gold particles (labeling Z-DNA) organized in clumps were visible only in the NOR (Fig. 9 *b*). Neither the fibrillo-granular area nor the granular area were labeled. Controls using GAHu 5 or GAR 7 alone were negative (Fig. 9 *d* [GAR 7]).

Discussion

DNA Immunolocalization

The immunodetection of DNA inside the nucleus of dinoflagellates has already been described by different work-

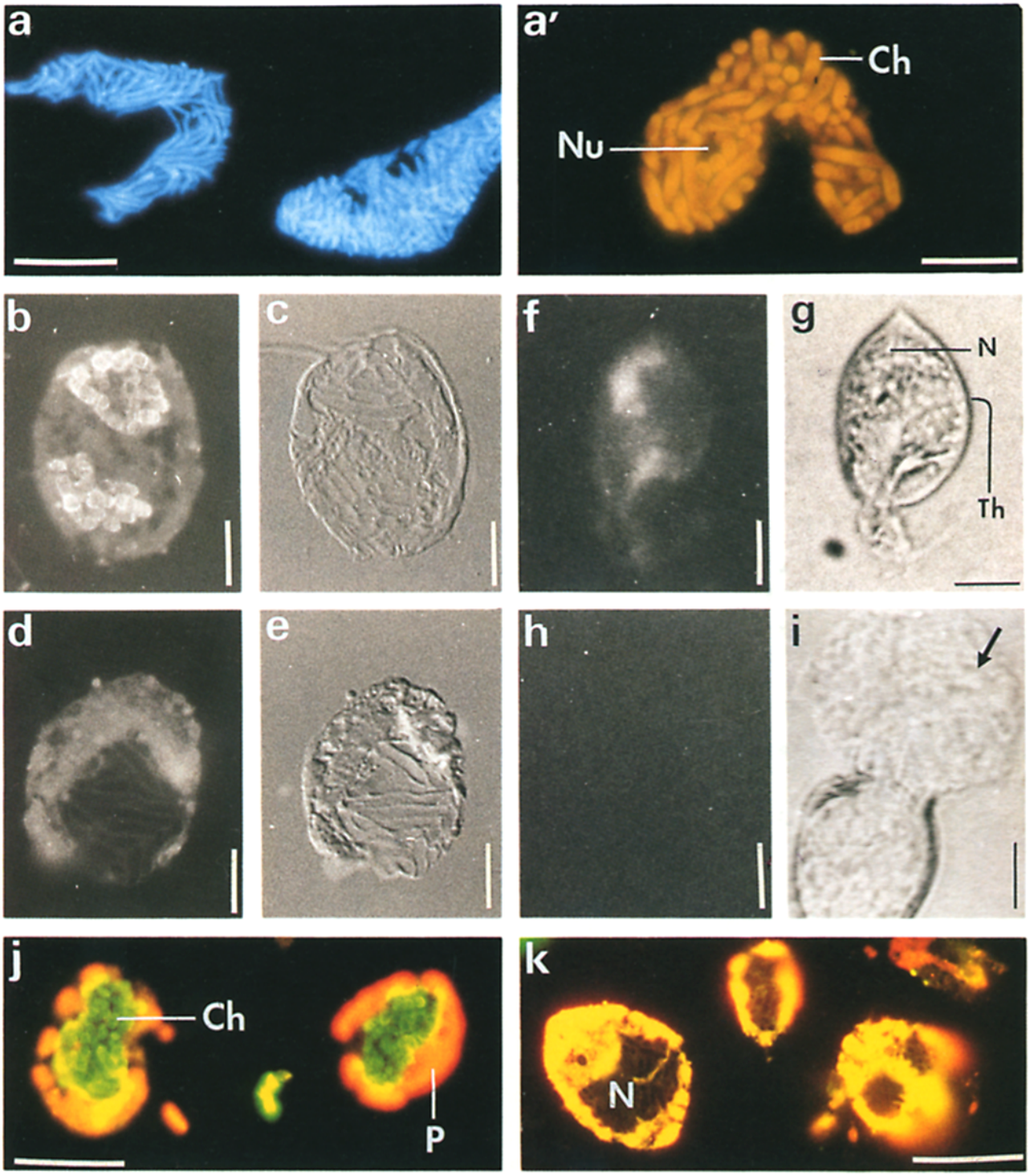


Figure 2. (*a* and *a'*) Nuclei of *Prorocentrum micans* as seen after squashing and DAPI chromosome staining (*a*), or in a thick section of epoxy-embedded cells and acridine orange staining (*a'*). Chromosomes (*Ch*), nucleolus (*N*). (*b* and *j*) B-DNA in chromosomes of telophase (*b*) or interphase (*j*) nuclei of *P. micans* localized on 1-μm cryosections treated with 1/100 dilution monoclonal anti-B-DNA Ab. Observe the bright white (*b*) or green (*j*) fluorescence localized on the chromosomes. Orange color is due to the autofluorescence of plastids (*P*) and yellow color to that of the cytoplasm. *d* and *k* are the cryosectioned control cells treated with 1/40 GAM-FITC only, in which nuclei are negative. *c* and *e* are the interference contrast views of *b* and *d*, respectively. (*f*) Z-DNA in an unfixed cell of *P. micans* revealed by monoclonal anti-Z-DNA Ab (1/25) and GAM-FITC (1/40) as the second Ab. A part of the nucleus (*N*), as seen in the phase-contrast view in *g*, is fluorescent. *Th*, theca. Control cells (*h*), treated with 1/100 antispasmine as first Ab is totally negative; (*i*) phase-contrast view of the same cell. Bars: (*a*) 5 μm; (*a'*) 2 μm; (*b–i*) 5 μm; (*j* and *k*) 10 μm.

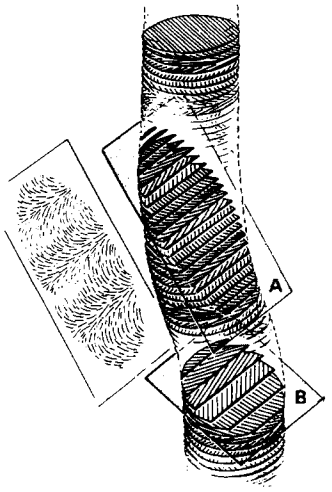


Figure 3. Schematic interpretation (Bouligand's plywood model) of dinoflagellate chromosome ultrastructure as seen in thin sections. Reproduced from Bouligand et al. (8; see Fig. 8 reproduced from *Chromosoma (Berl.)*, 1968, 24:251–287, by copyright permission of *Chromosoma (Berl.)*.) Oblique (*A*) and quite transverse (*B*) plan section were represented on the draw.

ers (22, 60, 68). Spector (68) used immunofluorescence to demonstrate the localization of an anti-DNA antibody at the periphery of *Crypthecodinium cohnii* chromosomes, with no labeling in the nucleolus (68). Our present observations in immunofluorescence show a homogenous distribution of anti-B-DNA antibody across entire chromosomes. However, in spread preparations, the chromosomes of *Crypthecodinium* and *Prorocentrum* seem to have a quite different fibrillar organizations (19, 63), so the localization of DNA in these different studies is difficult to compare. Scheer et al. (60) using a monoclonal anti-single or double-stranded DNA antibody, described the electro-immunolocalization of DNA in *Peridinium balticum* as being essentially limited to the fibrillar chromosomes, with a weak labeling of the nucleolus, perhaps in the NOR. Our data complement these results by showing detailed localization of B-DNA and provide the first description of the existence of Z-DNA sequences in a dinoflagellate.

Z-DNA in Various Cells: A Controversy

The location of Z-DNA in various cell types has been controversial in part due to concerns about the fixation procedures (1, 28, 37, 41, 42, 48, 50, 51, 59, 75). For this reason, we tried to avoid systematic artifacts by using various preparation methods including freezing, no fixation, and fixation with aldehydes or OsO_4, with visualization under optical and EM. All these methods led to similar results. Preliminary efforts using squashed cells, either fixed or unfixed in different conditions, revealed that B- and Z-DNA could be detected in the chromosomes of *Prorocentrum micans*. Subsequent EM work allowed us to define the location of both DNA conformations as detected by our Abs (polyclonal and monoclonal), or others that were purchased or obtained from our laboratory or from colleagues.

Detection of B or Z Conformations

Various discussions can be found in the literature concerning the feasibility of detecting the B or Z conformations of DNA in cells or isolated chromosomes. The most important issue relates to whether the fixation procedure with alcohol or acetic acid reveals sequences actually in Z conformation or simply generates this conformation (32). Other concerns have been raised as well anti-B DNA mAb (from autoimmune diseases) have been found to cross react with substances very different from DNA (14, 31). In other words, the specificity of the recognition of DNA by antibodies is questionable (56). Anti-Z-DNA Abs have been shown to present various specificities, possibly distinguishing different Z forms of DNA: in particular, the Z form of poly(dG-m5dC).poly(dG-M5dC) is often not recognized by Ab able to bind brominated-poly(dG-dC).poly(dG-dC) or poly(dG-dC).poly(dG-dC) in high salt concentrations (36, 47, 77). The argument that anti-Z-DNA Ab can stabilize the Z structure of DNA and even modify the B ↔ Z equilibrium (76) is not a concern for studies such as ours that used embedded specimens, even if one cannot rule out equilibrium changes by the Ab of the DNA conformation in the plastic section.

Our anti-Z-DNA antibodies are able in vitro to bind specifically to Z sequences in naturally supercoiled DNAs such as ΦX174 RFI (57). It is known that OsO_4 alone does not react readily with DNA (7, 11, 16, 53, 54), but nothing is known on the effect of residual osmium on the reactivity of anti-DNA antibodies itself in fixed material. To test the effectiveness of these Abs in the presence of osmium, we have performed DNA and anti-DNA in vitro experiments in the presence of free OsO_4 in excess. We have shown that even if Abs alone are sensitive to the presence of free osmium, the preincubation of DNA in OsO_4 up to 1% did not inhibit its recognition by our anti-DNA Ab. This strongly confirmed the observation of Palecek and co-workers that the alterations of the DNA secondary structure by osmium are minimal. These experiments, although not really comparable to the detection on plastic sections, give another example of the detectability of some epitopes in OsO_4-fixed cells (3, 49).

In Situ Detection

In general, our experiments demonstrated a very good correspondence between the localizations obtained on unfixed, aldehyde-fixed, alcohol- or OsO_4-fixed cells, giving a strong indication that valid conclusions can be drawn from these experiments. However, the actual effects of various fixatives, dehydratants, and embedding materials on the DNA conformations are not known. Most papers reporting the location of B- or Z-DNA in chromosomes used eukaryotic *Drosophila* polytenic material. There is a strong difference between these nucleosome-containing structures and the nucleosome-free fibrillar ones of dinoflagellates (23). It is tempting to compare our results with those obtained by Kellenberger and co-workers who have shown bacterial nucleoids to be comparable to dinoflagellate chromosomes (29, 34). Some basic proteins like HU in the bacterial nucleoid (12, 13) or mitochondria-DNA binding protein-C (mt-DBP-C) in *Xenopus* mitochondria (46) are comparable to the 13–14-kD basic proteins found in dinoflagellate chromosomes (23). In the context of our study, this raised the problem of the presence of proteins specifically bound to some sequences of DNA, either for structural or functional purposes, that might protect or hide epitopes.

Fixations are always imperfect with regard to ultrastructure when freezing and limited chemical fixation are considered optimal for conserving epitopes and molecular organi-

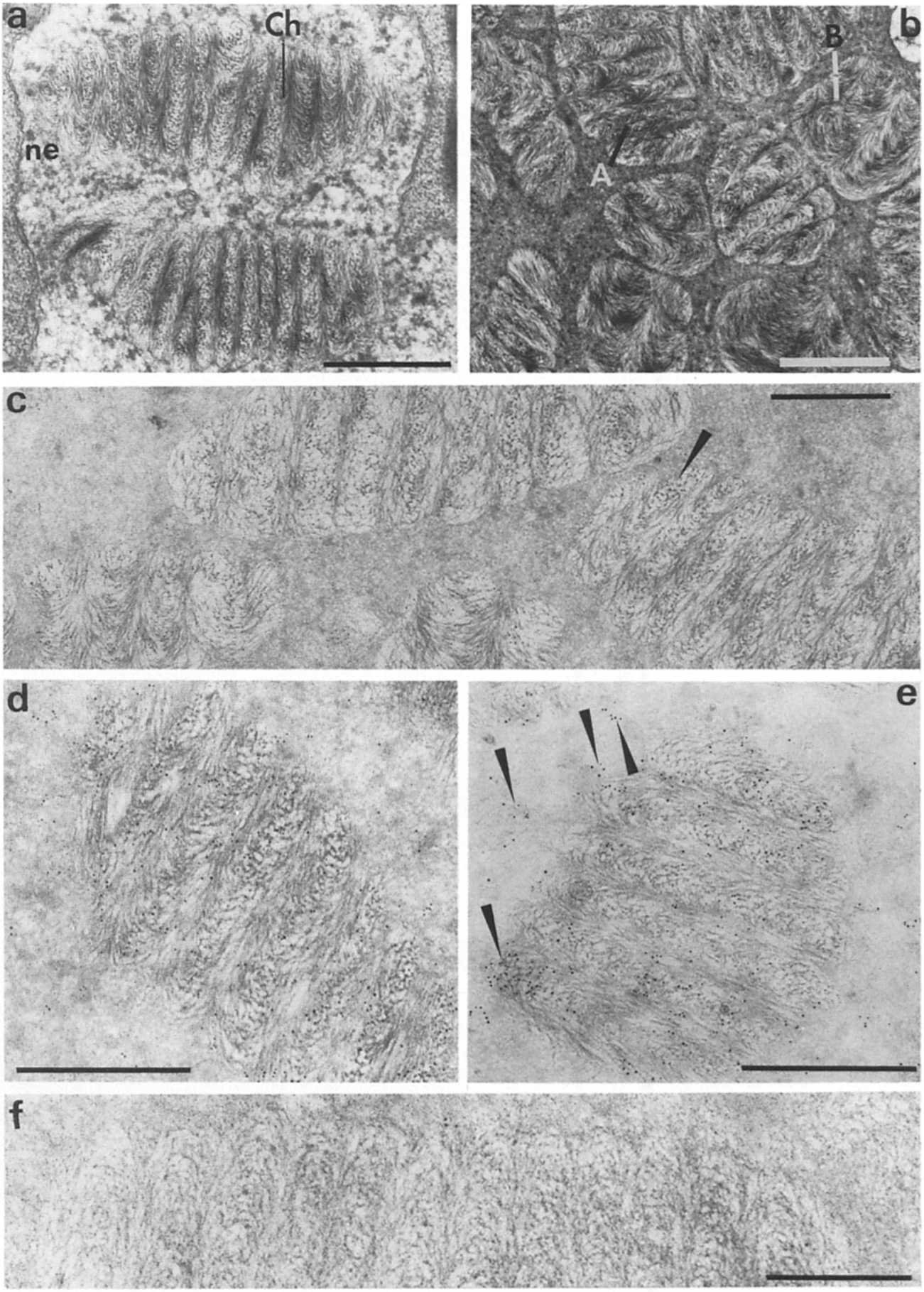


Figure 4. (*a* and *b*) EM of near longitudinal and oblique ultrathin sections of *P. micans* chromosomes (*Ch*) in interphase nuclei. Arch-shaped aspect of nucleofilaments is visible, A section corresponding to A-plan-section and B section to B plan section of the Bouligand model (Fig. 3). *ne*, Nuclear envelope. (*c–e*) B-DNA in interphase chromosomes after 1/25 polyclonal anti-B-DNA immunolabeling. In overview (*c*) or in higher magnification micrographs (*d* and *e*) of oblique section, 5-nm gold particles revealed the distribution of B-DNA in the chromosomes and the adjacent nucleoplasm (*e*, *black arrows*). Control using only the second GAHu (1/20) Ab is negative (*f*). Bars: (*a* and *b*) 1 μm; (*c*) 1 μm; (*d* and *e*) 0.5 μm; (*f*) 0.3 μm.

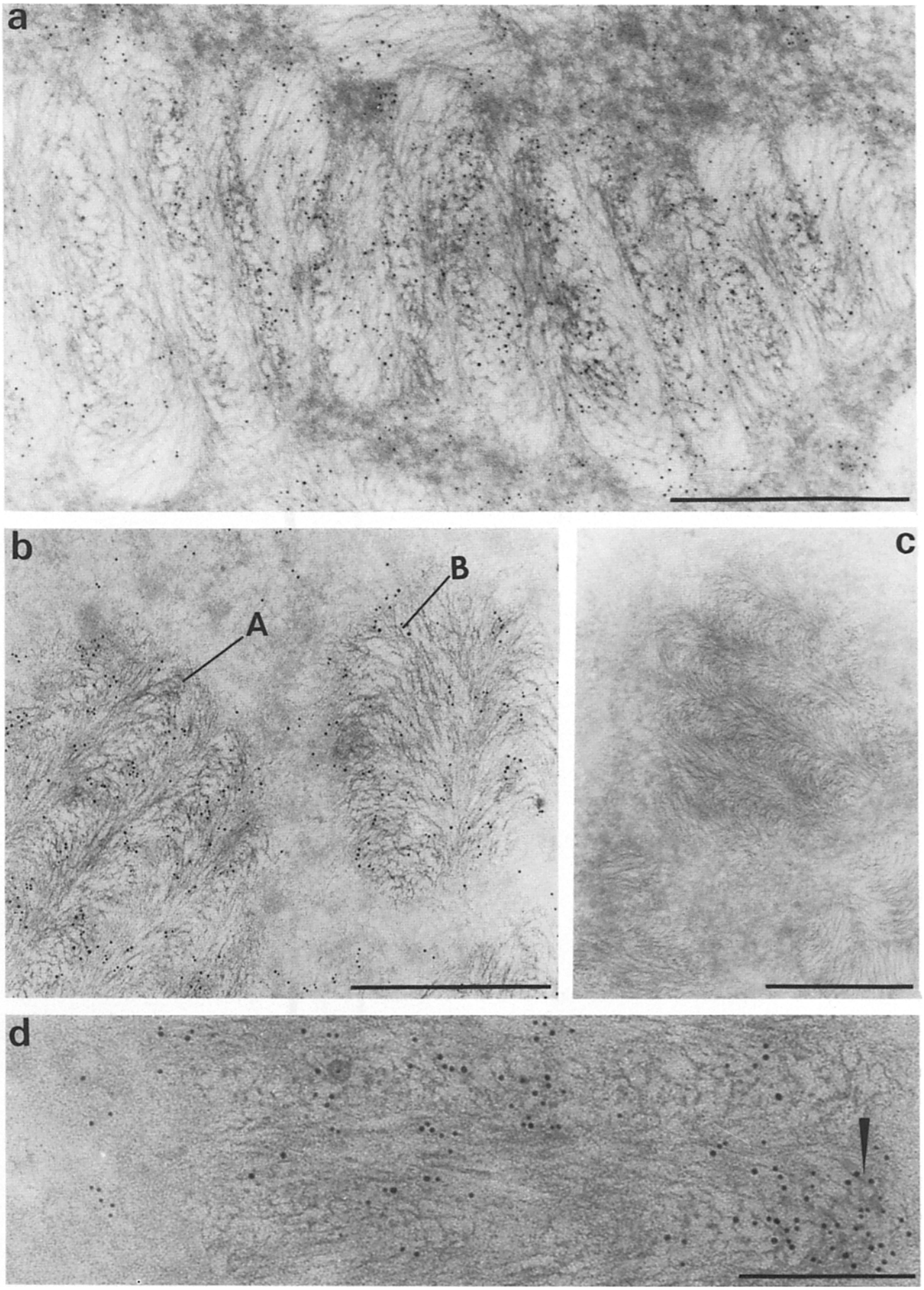


Figure 5. High magnification of longitudinal (*a* and *d*) and oblique (*b*) sections of *P. micans* chromosomes after polyclonal anti-B-DNA immunolabeling. 5-nm gold particles are localized on the nucleofilaments as well on the arch-shaped configuration (*a*, *b*(*A*), and *d*), as on the longitudinally sectioned one (*a*, *b*(*B*), and *d*). A nonnegligible quantity of gold particles are visible in the nucleoplasm, on peripheral nucleofilaments (*a*, and *b*(*B*)). (*c*) Control treated with only 1/20 GAHu-5-nm was negative. Bars: (*a–c*) 0.5 μm; (*d*) 0.2 μm.

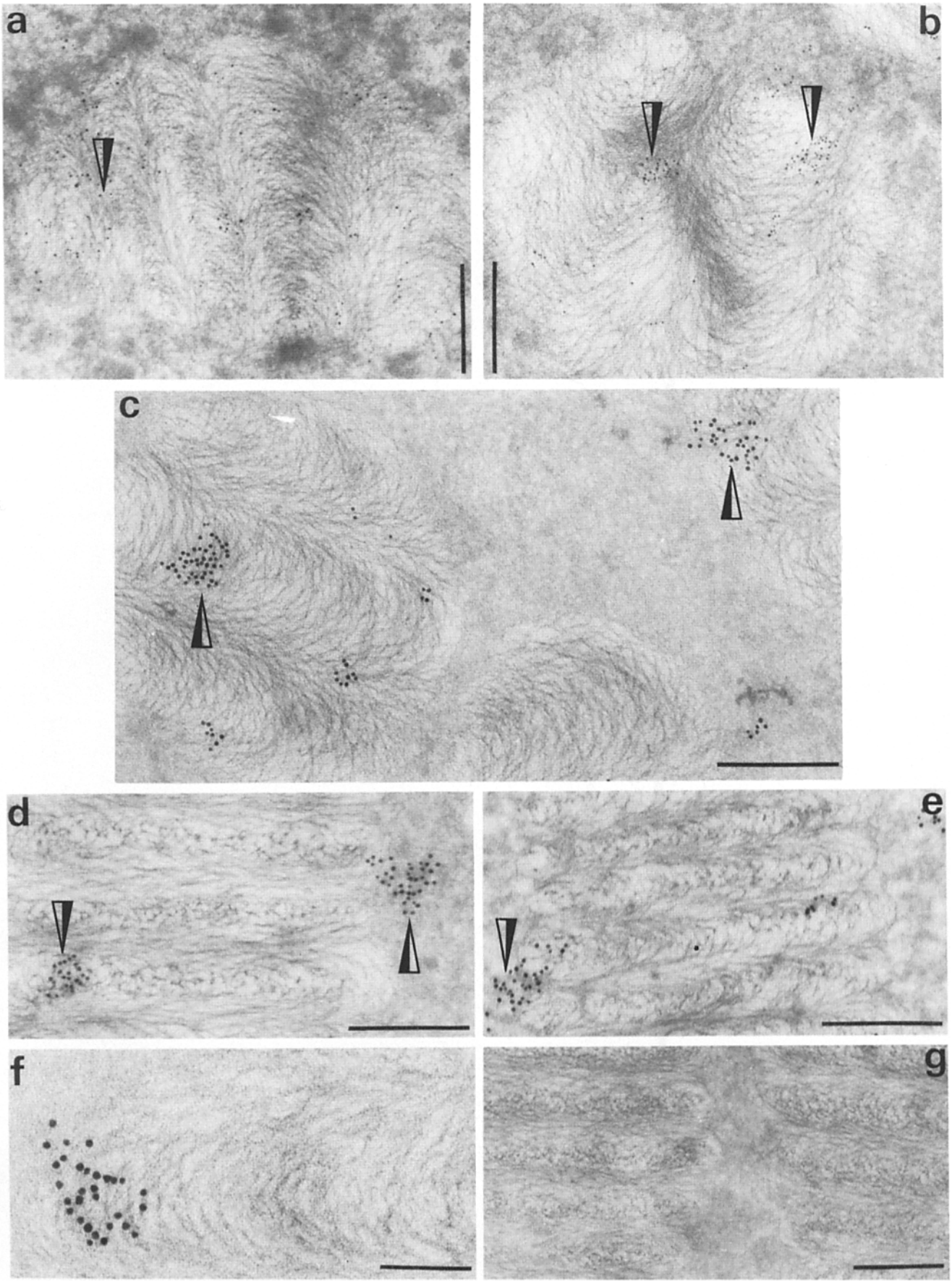


Figure 6. Z-DNA in *P. micans* chromosomes after 1/25 polyclonal anti-Z-DNA as first Ab and 1/20 GAR-5-nm (*a* and *b*), or 1/10 GAR-7-nm (*c–f*) as second Ab. (*a* and *b*) 5-nm gold particles are visible on the nucleofilaments, often grouped in clumps (*b, black and white arrows*). (*c–f*) 7-nm gold particles are visible on nucleofilaments (*f*), as seen in oblique (*c*) or longitudinal (*d* and *e*) sections, located within the chromosome (*c*) and on its periphery (*d–f*). (*g*) Control treated with only 1/10 GAR-7-nm Ab is negative. Bars: (*a–e, g*) 0.25 μm; (*f*) 0.1 μm.

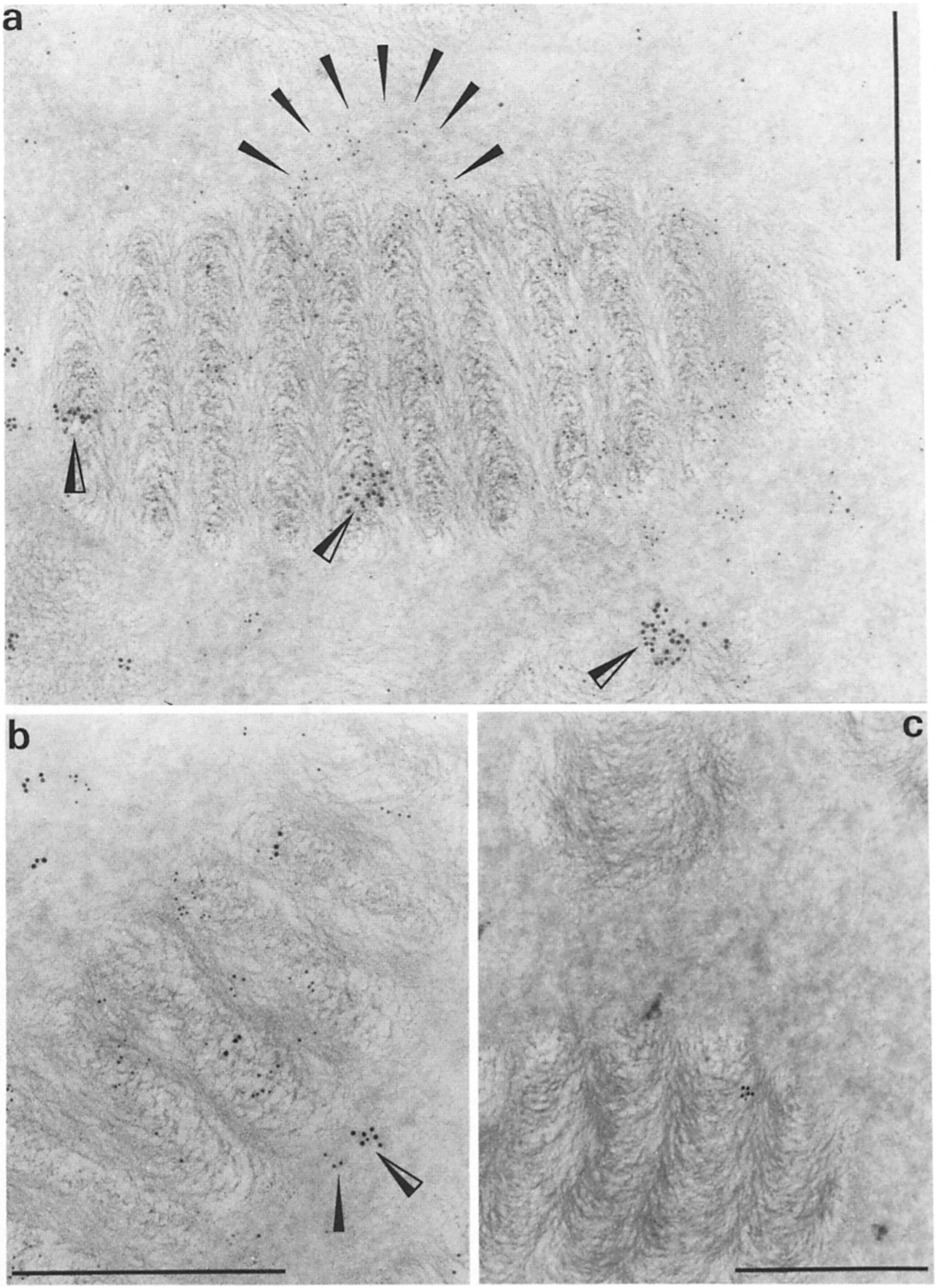
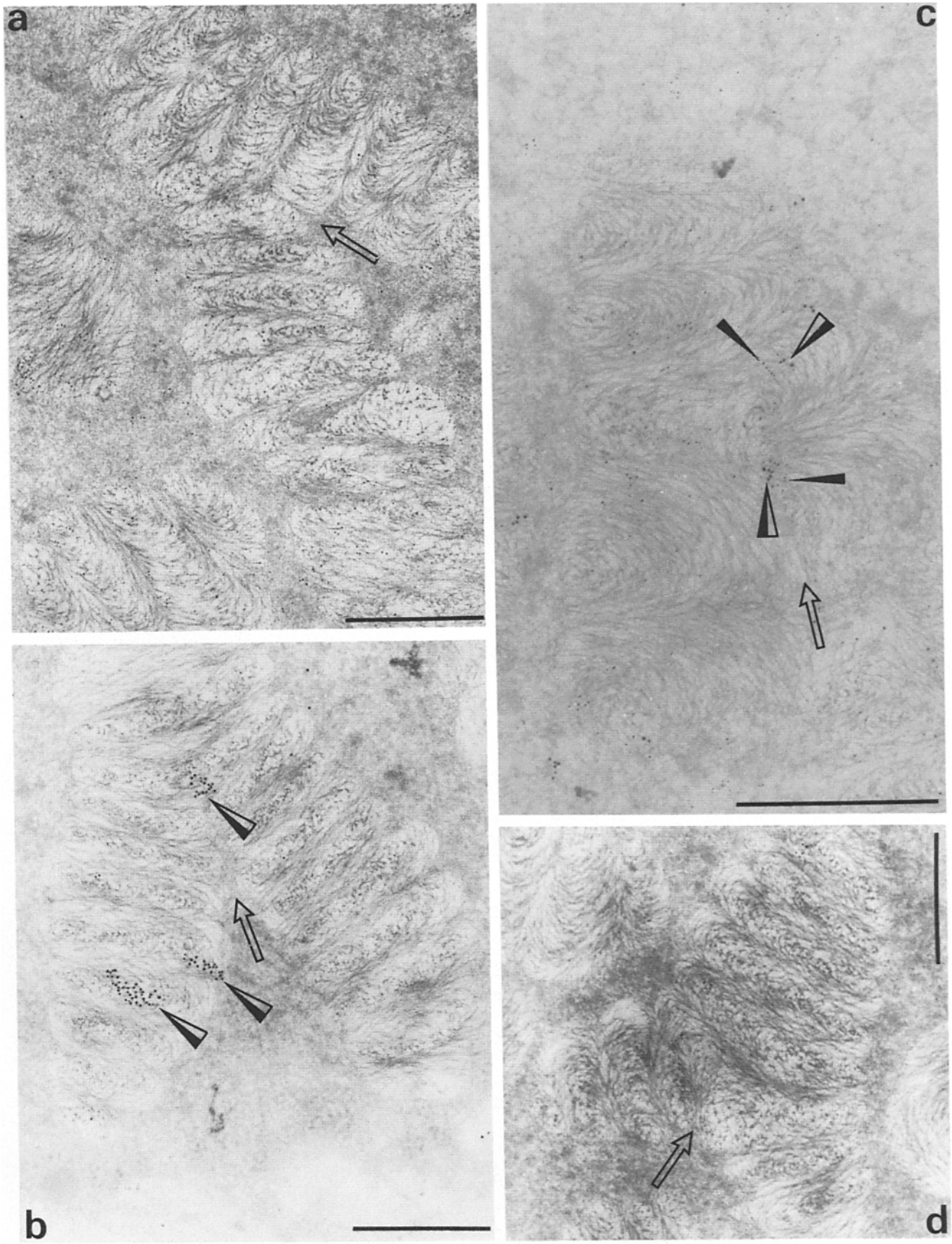


Figure 7. (*a* and *b*) Double labeling of B- and Z-DNA in nuclei treated by a 1/25 dilution of polyclonal anti-B-DNA Ab and a 1/10 dilution of polyclonal anti-Z-DNA followed by 1/20 GAHu-5-nm and 1/10 GAR-7-nm, respectively. 5-nm gold particles (indicative of B-DNA) are visible in the chromosome and in the nucleoplasm in which an extrachromosomal loop is visible (*black arrow*). 7-nm gold particles (indicative of Z-DNA) are visible in clumps in the chromosome typically located in peripheral regions (*black and white arrows*). (*c*) Control treated simultaneously with GAHu-5-nm and GAR-7-nm is negative. Bars: (*a* and *c*) 0.5 μm; (*b*) 0.1 μm.


a
b
c
d

zation (13, 29). Our results with various experimental conditions are comparable to those using frozen cells, and thus should be considered as reliable. Our preliminary controls after DNase I digestion on Lowicryl K4M thin sections showed that our antibodies reacted specifically with DNA since the labeling with anti-B- or anti-Z-DNA antibodies was negative. Our immunodetection of the presence of a B ↔ Z transition at a location where DNA could be considered as "active" has already been proposed (38, 55), but thus far only at a theoretical level based on its presumed role in untwisting supercoiled zones (21).

Specific Sites of the B ↔ Z Transitions

We observed abundant B-DNA immunolabeling on the interphase and dividing chromosomes as well as in longitudinally or transversally sectioned nucleofilaments; this can be explained by the good accessibility of most of the epitopes after light etching of the thin sections with no adverse effects from the OsO_4 fixation. With these methods, specific localizations were more easily detected than with only aldehyde fixation followed by low temperature–embedding medium (Lowicryl) (data not shown).

Our observations of the localization of Z-DNA at the periphery of the chromosomes are in accord with the already described loops protruding from the chromosome masses (65) and visualized by immunolabeling with anti-B-DNA Ab. These loops which can be experimentally induced by alcali-urea treatment (20), could be interpreted as possible sites of untwisting at the periphery of the chromosome, left-handed Z-DNA sequences being likely to induce right-handed DNA helicity by relieving negative superhelical stress. This, plus the fact that the presence of Z-DNA sequences is transitory, may explain the absence of 5- or 7-nm gold particles labeling the Z-DNA Ab in the extrachromosomal loops (Fig. 7 *a*). These observations complement those of Briane et al. who found a progressive decrease of Z-DNA configuration when myoblasts were in the process of terminal differentiation (9).

Nucleolar DNA

In the interphase nucleolus, our immunolabeling shows that B-DNA is present in the unwound NOR and in the fibrillogranular area which contains transcribing rDNA. The granular area containing preribosomes was not labeled. On the other hand, in Ehrlich tumor cell nucleoli (70, 71) perinucleolar chromatin was B labeled, but dense fibrillar component and granular component were not. Nevertheless, the two nucleolar systems are too different to be compared. The only possible relation between our nucleosome-deprived material and higher eukaryotes is that nucleosomes are also absent in transcribing eukaryotic rDNA (35). As for B-DNA, the Z immunolabeling was only observed in the unwound NOR; neither fibrillogranular area nor granular area were labeled. This could be explained by the fact that transcribing rDNA has recovered its right-handed form after unwinding of the concerned part of the chromosome with the ribosomal genes.

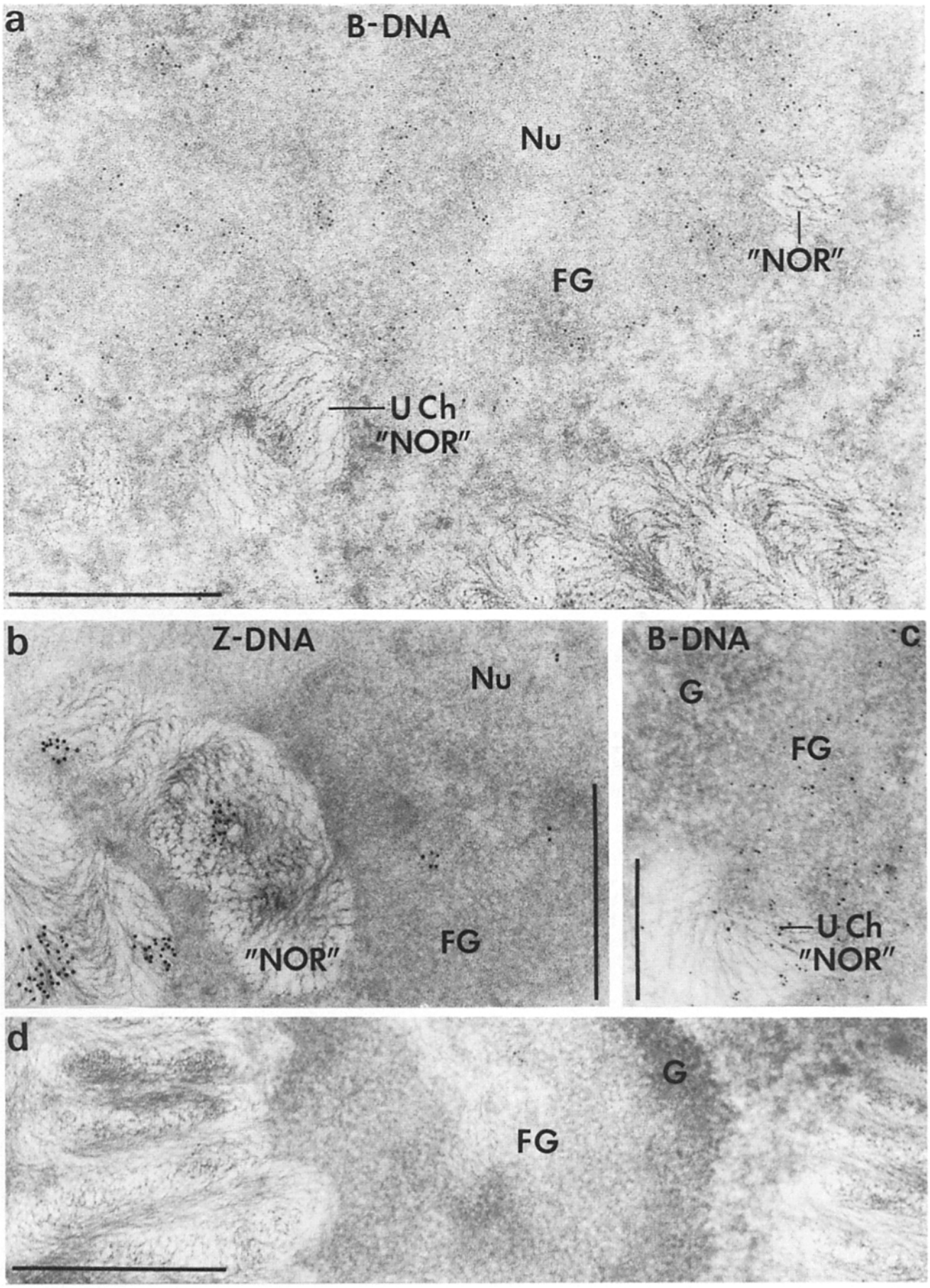
Quantitative Evaluation of the Gold Particle Distributions

In this work, we have shown that B-DNA is located either on the chromosome nucleofilaments or on nucleoplasm fibers corresponding to nucleofilaments protruding from chromosomes. Our quantitative analysis demonstrated that there is more B-DNA than Z-DNA in the chromosomes and especially in the nucleoplasm where Z-DNA is not very abundant. When Z-DNA configuration is labeled with 7-nm gold particles, the density of the particles is less abundant than after the 5-nm labeling; this is due to the size of the gold particles and to their steric overcrowding. This is also the case for the double labeling with B(5-nm)-Z(7-nm)-DNA Ab. Nevertheless, the quantitative parametric test has shown that this relatively low density of 7-nm gold particles cannot be considered significant. So, double labelings were valuable, B-DNA labeling being about seven times more abundant than Z-DNA one (Table II *b*). However, with immunocytochemistry, it is hazardous to strictly correlate the gold particles number with the real B- or Z-DNA amount. So, we have not concluded that 20–30% of the total DNA is on a Z form. Moreover, it should be noted that all the DNA visible on the micrographs is not revealed with the B or Z antibodies because of its orientation; the relative accessibility of B- and Z-DNA is still unknown, especially on sections. Our results showing the presence of a relatively important quantity of clustered gold particles labeling Z-DNA are probably a good indication of a preferential localization of Z-DNA sequences.

Conclusion

It remains, however, impossible to correlate all the abundant data from the literature on eukaryotic chromosomes with those presented here. The dinoflagellates have the advantage of showing a permanently well-organized DNA structure, less compact than that of higher eukaryotic chromatin; thus, it was possible to analyze the position of accessible DNA sequences in dinoflagellates by various optical and EM techniques. Finally, it must be kept in mind that the B ↔ Z transition in DNA is a dynamic phenomenon, probably connected to various biological processes where DNA supercoiling must be shortly and locally inversed, reduced, or suppressed. This makes the dinoflagellate chromosome a very suitable model for studying this B ↔ Z transition as it does not contain the nucleosomal system that would modulate local supercoiling. The presence of an unusual base, divalent cations, and specific basic proteins in the nucleus of these

Figure 8. B- and Z-DNA in dividing chromosomes. (*a*) 5-nm gold particles (B-DNA) are localized on most of the nucleofilaments of a Y-shaped dividing chromosome. Empty arrow indicates the chromosome segregation fork. (*b*) 7-nm gold particles (Z-DNA) are localized in clumps within and at the periphery of the dividing chromosome and at the origin of the segregation fork (*black and white arrows*). (*c*) Simultaneous detection of B- and Z-DNA in a dividing chromosome. 5- and 7-nm gold particles are visible at the level of the segregation fork (*black*, and *black and white arrows*). Empty arrow indicates the direction of the chromosome segregation. (*d*) Control simultaneously treated with GAHu-5-nm and GAR-7-nm was negative. Bars: (*a–d*) 0.5 μm.


a
B-DNA
Nu
"NOR"
FG
U Ch
"NOR"
b
Z-DNA
Nu
"NOR"
FG
B-DNA
c
G
FG
U Ch
"NOR"
d
G
FG

flagellates are also arguments in favor of their use as models for fundamental biological and ultrastructural study of DNA processing in primitive eukaryotes.

We gratefully acknowledge the assistance of B. Hervé, D. Delain, M. Albert, M. J. Bodiou, J. Lecomte, P. Prevot, and of S. von Boletzky, R. Fester, and especially Don Anderson for critical reading of the manuscript. Dr. F. Tron (Paris Hopital Necker) kindly provided the PME 77 and Dr. P. Gounon (Paris Institut Pasteur) the GAR-7-nm antibody.

This work was supported by Centre National de la Recherche Scientifique.

Received for publication 31 August 1989 and in revised form 2 April 1990.

References

1. Arndt-Jovin, D. J., M. Robert-Nicoud, D. A. Zarling, C. Greider, E. Weimer, and T. M. Jovin. 1983. Left-handed Z-DNA in bands of acid-fixed polytene chromosomes. *Proc. Natl. Acad. Sci. USA.* 80:4344–4348.
2. Babillot, C. 1970. Etude de l'incorporation d'uridine-H3 dans le noyau chez *Amphidinium carterae*, Dinoflagellé. *C. R. Hebd. Seances Acad. Sci.* D271:828–831.
3. Bendayan, M., and M. Zollinger. 1983. Ultrastructural localization of antigenic sites on osmium-fixed tissues: applying the protein A gold technique. *J. Histochem. Cytochem.* 31:101–109.
4. Berger, S., and H. G. Schweiger. 1986. Perinuclear dense bodies: characterization as DNA- containing structures using enzyme-linked gold granules. *J. Cell Sci.* 80:1–11.
5. Bhaud, Y., and M.-O. Soyer-Gobillard. 1986. DNA synthesis and cell cycle of a primitive Dinoflagellate, *Prorocentrum micans* E. *Prothistologica.* 22:23-30.
6. Bodansky, S., L. B. Mintz, and D. S. Holmes. 1979. The mesocaryote *Gyrodinium cohnii* lacks nucleosomes. *Biochem. Biophys. Res. Commun.* 88:1329–1336.
7. Boublikova, P., and E. Palecek. 1989. Probing of B-Z junctions in recombinant plasmids *in vitro* and in the cell with different osmium tetroxide complexes. *Gen. Physiol. Biophys.* 8:475–490.
8. Bouligand, Y., M.-O. Soyer, and S. Puiseux-Dao. 1968. La structure fibrillaire et l'orientation des chromosomes chez les Dinoflagellés. *Chromosoma (Berl.).* 24:251–287.
9. Briane, D., D. Delain, H. Sénéchal, E. Taillandier, J. P. Wahrmann, and B. Hervé. 1987. Differential immunoreactivity for Z-DNA in rat myoblast nuclei during their terminal differentiation. *Exp. Cell Res.* 170: 453–468.
10. Deleted in proof.
11. Dizdaroglu, M., E. Holwitt, M. P. Hagan, and W. F. Blakely. 1986. Formation of cytosine glycol and 5,6-dihydroxycytosine in deoxyribonucleic acid on treatment with osmium tetroxide. *Biochem. J.* 235:531–536.
12. Drlica, K., and J. Rouvière-Yaniv. 1987. Histonelike proteins in bacteria. *Microbiol. Rev.* 51:301–309.
13. Dürrenberger, M., M.-A. Bjornsti, T. Uetz, J. A. Hobot, and E. Kellenberger. 1988. Intracellular location of the histonelike protein HU in *Escherichia coli. J. Bacteriol.* 170:4757–4768.
14. Faaber, P., T. P. M. Rijke, L. B. A. van de Putte, P. J. A. Capel, and J. H. M. Berden. 1986. Cross reactivity of human and murine anti-DNA antibodies with heparan sulfate. *J. Clin. Invest.* 77:1824–1830.
15. Gautier, A., L. M. Salamin, E. Couture-Tosi, A. W. McDowal, and J. Dubochet. 1986. Electron microscopy of the chromosomes of dinoflagellates *in situ*: confirmation of Bouligand's liquid crystal hypothesis. *J. Ultrastruct. Res.* 97:10–30.
16. Glikin, G. C., M. Vojtiskova, L. Rena-Descalzi, and E. Palecek. 1984. Osmium tetroxide: a new probe for site-specific distortions in supercoiled DNA. *Nucleic Acids Res.* 12:1725–1735.
17. Haapala, O. K., and M.-O. Soyer. 1973. Structure of dinoflagellate chromosomes. *Nature (Lond.).* 244:195–197.
18. Haapala, O. K., and M.-O. Soyer. 1974. Absence of longitudinal differenciation of dinoflagellate (*Prorocentrum micans*) chromosomes. *Hereditas.* 78:141–145.
19. Haapala, O. K., and M.-O. Soyer. 1974. Electron microscopy of whole mounted chromosomes of the Dinoflagellate *Gyrodinium cohnii. Hereditas.* 78:146–150.
20. Haapala, O. K., and M.-O. Soyer. 1974. Effect of alkali-urea treatment on dinoflagellate chromosomes. *Hereditas.* 78:295–298.
21. Haniford, D. B., and D. E. Pulleyblank. 1983. The *in-vivo* occurrence of Z-DNA. *J. Biomol. Struct. & Dyn.* 1:593–609.
22. Hansmann, P., H. Falk, U. Scheer, and P. Sitte. 1986. Ultrastructural localization of DNA in two *Cryptomonas* species by use of a monoclonal DNA antibody. *Eur. J. Cell Biol.* 42:152–160.
23. Herzog, M., and M.-O. Soyer. 1981. Distinctive features of Dinoflagellate chromatin: absence of nucleosomes in a primitive species *Prorocentrum micans.* E. *Eur. J. Cell Biol.* 23:295–302.
24. Herzog, M., and M.-O. Soyer. 1983. The native structure of Dinoflagellates chromosomes and their stabilization by Ca2+ and Mg2+ cations. *Eur. J. Cell Biol.* 30:33–41.
25. Herzog, M., and L. Maroteaux. 1986. Dinoflagellate 17S ribosomal RNA sequence inferred from the gene sequence. Evolutionary implications. *Proc. Natl. Acad. Sci. USA.* 83:8644–8648.
26. Herzog, M., G. D. de Marcillac, and M.-O. Soyer. 1982. A high level of thymine replacement by 5-hydroxymethyluracil in nuclear DNA of the primitive dinoflagellate *Prorocentrum micans* E. *Eur. J. Cell Biol.* 27:151–155.
27. Herzog, M., S. von Boletzky, and M.-O. Soyer. 1984. Ultrastructural and biochemical nuclear aspects of Eukaryote classification: independent evolution of the Dinoflagellates as a sister group of the actual eukaryotes? *Origins Life.* 13:205–215.
28. Hill, R. J., and D. Stollar. 1983. Dependance of Z-DNA antibody binding to polytene chromosomes on acid fixation and DNA contorsional strain. *Nature (Lond.).* 305:338–340.
29. Hobot, J. A., M.-A. Bjornsti, and E. Kellenberger. 1987. The distribution of bacterial DNA investigated by on-section immunolabeling and cryosubstitution. *J. Bacteriol.* 169:2055–2062.
30. Jacob, L., and F. Tron. 1982. Monoclonal anti-deoxyribonucleic antibodies. *J. Immunol.* 128:895–898.
31. Jacob, L., F. Tron, J. F. Bach, and D. A. Louvard. 1984. A monoclonal anti-DNA antibody also bind to cell-surface protein(s). *Proc. Natl. Acad. Sci. USA.* 81:3843–3845.
32. Jovin, T. M., L. P. McIntosh, D. J. Arndt-Jovin, D. A. Zarling, M. Robert-Nicoud, J. H. van de Sande, K. F. Jorgenson, and F. Eckstein. 1983. Left handed DNA: from synthetic polymers to chromosomes. *J. Biomol. Struct. & Dynam.* 1:21–57.
33. Jovin, T. M., D. M. Soumpasis, and L. P. McIntosh. 1987. The transition between B-DNA and Z-DNA. *Annu. Rev. Phys. Chem.* 38:521–557.
34. Kellenberger, E. 1988. About the organization of condensed and decondensed non-eukaryotic DNA and the concept of vegetative DNA (a critical review). *Biophys. Chem.* 29:51–62.
35. Labart, P., and T. Koller. 1982. Structure of the active nucleolar chromatin of *Xenopus laevis* oocytes. *Cell.* 28:279–292.
36. Lafer, E. M., A. Möller, R. P. C. Valle, A. Nordheim, A. Rich, and B. D. Stollar. 1983. Antibody recognition of Z-DNA. *Cold Spring Harbor Symp. Quant. Biol.* 47:155–162.
37. Lancillotti, F., M. C. Lopez, C. Alonso, and B. D. Stollar. 1985. Locations of Z-DNA in polytene chromosomes. *J. Cell Biol.* 100:1759–1766.
38. Lancillotti, F., M. C. Lopez, P. Arias, and C. Alonso. 1987. Z-DNA in transcriptionally active chromosomes. *Proc. Natl. Acad. Sci. USA.* 84: 1560–1564.
39. Livolant, F., and Y. Bouligand. 1978. New observations on the twisted arrangement of Dinoflagellate chromosomes. *Chromosoma (Berl.).* 68: 21–44.
40. Livolant, F., and Y. Bouligand. 1980. Double helical arrangement of spread Dinoflagellate chromosomes. *Chromosoma (Berl.).* 80:97–118.
41. Magaud, J. P., J. Brochier, R. Rimokh, and D. Germain. 1984. Marquage chromosomique en bandes R par un anticorps monoclonal anti-ADN natif (specificité dG-dC). *C. R. Hebd. Seances Acad. Sci.* 299:135–138.
42. Malmqvist, M., J. Carlsson, B.-A. Fredriksson, L. Johnsson, P. Matsson, B. Nilsson, and K. Ruuth. 1987. Characterization of monoclonal anti-DNA antibodies and visualization of binding to chromosomes and cell nuclei. *J. Immunol. Methods.* 103:109–119.
43. Mann, H. B., and D. R. Whitney. 1947. On a test of whether one or two random variables is stochastically larger than the other. *Ann. Math. Stat.* 18:50–60.
44. Marx, J. L. 1985. Z-DNA: still searching for a function. *Science (Wash.*

Figure 9. (*a–c*) Localization of B- and Z-DNA in the nucleolus (*Nu*) of *P. micans*. (*a*) B-DNA is evidenced by 5-nm gold particles after 1/25 polyclonal anti-B-DNA Ab immunolabeling. Particles are visible on the nucleofilaments of the unwound part (*U Ch*) of the chromosome corresponding to the *NOR* (*a* and *c*) and on the fibrillo-granular area (*FG*) while the granular area (*G*) is not labeled (*c*). Z-DNA is evidenced by clumps of 7-nm gold particles after 1/10 polyclonal anti-Z-DNA labeling. Only the nucleofilaments of the unwound part of the NOR are labeled (*b*). A few particles are visible in the fibrillo-granular area otherwise not labeled. (*d*) Control using only second Ab (GAR-7-nm) is not labeled. Bars: (*a*, *b*, and *d*) 0.5 μm; (*c*) 0.3 μm.

DC). 230:794–796.
45. Métivier, C., and M.-O. Soyer-Gobillard. 1987. Biochemical characterization and immunological localization of 45 Kda cytoskeletal protein in the Dinoflagellate: *Noctiluca scintillans. Eur. J. Cell Biol.* 44:42–49.
46. Mignotte, B., E. Delain, D. Rickwood, and M. Barat-Gueride. 1988. The *Xenopus laevis* mitochondrial protein mt-DBP-C cooperatively folds the DNA in vitro. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:3873–3879.
47. Möller, A., J. E. Gabriels, E. M. Lafer, A. Nordheim, A. Rich, and B. D. Stollar. 1982. Monoclonal antibodies recognize different part of Z-DNA. *J. Biol. Chem.* 257:12081–12085.
48. Morgenegg, G., M. R. Celio, B. Malfoy, M. Leng, and C. C,. Kuenzle. 1983. Z-DNA immunoreactivity in rat tissues. *Nature (Lond.).* 303: 540–543.
49. Nicolas, M. T., G. Nicolas, C. H. Johnson, J. M. Bassot, and J. W. Hastings. 1987. Characterization of the bioluminescent organelles in *Gonyaulax polyedra* (Dinoflagellates). *J. Cell Biol.* 105:723–735.
50. Nordheim, A., M. L. Pardue, E. M. Lafer, A. Möller, B. D. Stollar, and A. Rich. 1981. Antibodies to left-handed Z-DNA bind to interband region of *Drosophila* polytene chromosomes. *Nature (Lond.).* 294:417–422.
51. Nordheim, A., M. L. Pardue, L. M. Weiner, K. Lowenhaupt, P. Scholten, A. Möller, A. Rich, and B. D. Stollar. 1986. Analysis of Z-DNA in fixed polytene chromosomes with monoclonal antibodies that show base sequence-dependent selectivity in reactions with supercoiled plasmids and polynucleotides. *J. Biol. Chem.* 261:468–476.
52. Oakley, B. R., and J. D. Dodge. 1979. Evidence for a double helically coiled toroidal chromonema in the dinoflagellate chromosome. *Chromosoma (Berl.).* 70:277–291.
53. Palecek, E., E. Rasovska, and P. Boublikova. 1988. Probing of DNA polymeric structure in the cell with osmium tetroxide. *Biochem. Biophys. Res. Commun.* 150:731–738.
54. Palecek, E., P. Boublikova, and K. Nejedly. 1989. Probing of DNA structure with osmium tetroxide: effect of ligands. *Biophys. Chem.* 34:63–68.
55. Rahmouni, R., and R. Wells. 1989. Stabilization of Z-DNA *in vivo* by localized supercoiling. *Science (Wash. DC).* 246:358–363.
56. Rauch, J., H. Tannenbaum, B. D. Stollar, and R. S. Schwartz. 1984. Monoclonal anti-cardiolipin antibodies bind to DNA. *Eur. J. Immunol.* 14:529–534.
57. Révet, B., D. A. Zarling, T. M. Jovin, and E. Delain. 1984. Different Z-DNA forming sequences are revealed in *fX*174 RFI by high resolution darkfield immunoelectron microscopy. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:3353–3358.
58. Rich, A., A. Nordheim, and A. H.-J. Wang. 1984. The chemistry and biology of left-handed Z-DNA. *Annu. Rev. Biochem.* 53:791–846.
59. Robert-Nicoud, M., D. J. Arndt-Jovin, D. A. Zarling, and T. M. Jovin. 1984. Immunological detection of left-handed Z-DNA in isolated polytene chromosomes: effects of ionic strength, pH, temperature and topological stress. *EMBO (Eur. Mol. Biol. Organ). J.* 3:721–731.
60. Scheer, U., K. Messner, R. Hazan, I. Raska, P. Hansmann, H. Folk, E. Spiess, and W. W. Franke. 1987. High sensitivity immunolocalization of double and single-stranded DNA by a monoclonal antibody. *Eur. J. Cell Biol.* 43:358–371.
61. Sigee, D. C., 1983. Structural DNA in genetically active DNA in dinoflagellate chromosomes. *Biosystems.* 16:203–210.
62. Soyer, M.-O. 1977. Une modification de la technique de Karnovsky pour la préservation optimale des structures nucléaires chez les Dinoflagellés. *Biol. Cell.* 30:297–300.
63. Soyer, M.-O., and O. K. Haapala. 1973. Filaments extrachromosomiens: variations et relations avec l'enveloppe nucléaire pendant la division chez les Dinoflagellés. *J. Microsc. (Paris).* 18:267–270.
64. Soyer, M.-O., and O. K. Haapala. 1974. Division and function of dinoflagellate chromosomes. *J. Microsc. (Paris).* 19:137–146.
65. Soyer, M.-O., and O. K. Haapala. 1974. Structural changes of dinoflagellate chromosomes by pronase and ribonuclease. *Chromosoma (Berl.).* 50:179–192.
66. Soyer, M.-O., and M. Herzog. 1985. The native structure of dinoflagellate chromosomes. Involvement of structural RNA. *Eur. J. Cell Biol.* 36: 334–342.
67. Deleted in proof.
68. Spector, D. L. 1984. Dinoflagellate nuclei. *In* Dinoflagellates. D. L. Cantor, editor. Academic Press, Inc., Orlando, FL. 107–147.
69. Tapia, F. J., I. M. Varnell, L. Probert, J. de Mey, and J. M. Polak. 1983. Double immunogold staining method for the simultaneous ultrastructural localization of regulatory peptides. *J. Histochem. Cytochem.* 31:977–981.
70. Thiry, M., U. Scheer, and G. Goessens. 1988. Localization of DNA within Ehrlich tumour cell nucleoli by immunoelectron microscopy. *Biol. Cell.* 63:27–34.
71. Thiry, M., U. Scheer, and G. Goessens. 1988. Immunoelectron microscopic study of nucleolar DNA during mitosis in Ehrlich tumor cells. *Eur. J. Cell Biol.* 47:349–357.
72. Thomas, T. J., M. J. Baarsch, and R. P. Messner. 1988. Immunological detection of B-DNA to Z-DNA transition of polynucleotides by immobilization of the DNA conformation on a solid support. *Anal. Biochem.* 168:358–366.
73. Tron, F., D. Charron, J. F. Bach, and N. Talal. 1980. Establishment and characterization of a murine hybridoma secreting monoclonal anti-DNA autoantibody. *J. Immunol.* 125:2805–2809.
74. Tron, F., L. Jacob, and J. F. Bach. 1973. Murine monoclonal anti-DNA antibodies with an absolute specificity for DNA have a large amount of idiotypic diversity. *Proc. Natl. Acad. Sci. USA.* 80:6024–6027.
75. Viegas-Péquignot, E., C. Derbin, B., Malfoy, M. Leng, and B. Dutrillaux. 1983. Z-DNA immunoreactivity in fixed metaphase chromosomes of primates. *Proc. Natl. Acad. Sci. USA.* 80:5890–5894.
76. Wittig, B., T. Dorbic, and A. Rich. 1989. The level of Z-DNA in metabolically active, permeabilized mammalian cell nuclei is regulated by torsional strain. *J. Cell Biol.* 108:755–764.
77. Zarling, D. A., D. J. Arndt-Jovin, M. Robert-Nicoud, L. P. McIntosh, R. Tomae, and T. M. Jovin. 1984. Immunoglobulin recognition of synthetic and natural left-handed Z-DNA conformations and sequences. *J. Mol. Biol.* 176:369–415.