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High sensitivity immunolocalization of double and single-stranded DNA by a monoclonal antibody

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A monoclonal antibody (AK 30-10) is described which specifically reacts with DNA both in double and single-stranded forms but not with other molecules and structures, including deoxyribonucleotides and RNAs. When used in immunocytochemical experiments on tissue sections and permeabilized cultured cells, this antibody detects DNA-containing structures, even when the DNA is present in very small amounts. Examples of high resolution detection include the DNA present in amplified extrachromosomal nucleoli, chromomeres of lampbrush chromosomes, mitochondria, chloroplasts and mycoplasmal particles. In immunoelectron microscopy using the immunogold technique, the DNA was localized in distinct substructures such as the "fibrillar centers" of nucleoli and certain stromal centers in chloroplasts. The antibody also reacts with DNA of chromatin of living cells, as shown by microinjection into cultured mitotic cells and into nuclei of amphibian oocytes. The potential value and the limitations of immunocytochemical DNA detection are discussed.

Introduction

Analysis of the distribution and topological organization of DNA in nuclei, chromosomes and cellular organelles requires cytochemical methods which allow the detection of DNA in situ with high specificity, as well as high resolution and sensitivity. Several cytochemical approaches are available for studying DNA distribution at the subcellular level (for reviews see [26, 48]). The most widely used techniques include the Feulgen-like reaction with osmium-ammine [17, 20, 21, 26] and tagging of DNA with DNase I-gold complexes [9, 10]. Another strategy for the selective labelling of DNA molecules involves the use of antibodies directed against DNA. Such antibodies occur frequently in sera from patients suffering from systemic lupus erythematosus (SLE; for reviews see [66, 69, 70, 72]) and have already been used to locate DNA at the ultrastructural level by means of postembedding cytochemistry [56]. However, the application of SLE sera is limited because of the limited supply of the individual antisera, thereby limiting the reproducibility, and because these sera usually contain a mixture of different antibodies with different specificities [5, 27]. These limitations are overcome by the use of monoclonal antibodies with a defined antigenic specificity.

Although native B-form DNA is a rather poor immunogen [70], several murine hybridoma clones secreting DNA antibodies have been established by using spleen cells of certain inbred strains of mice which spontaneously develop an autoimmune disorder similar to human SLE [2–4, 6, 7, 23, 36, 40–46, 49, 50, 54, 71, 74, 75]. However, none of the monoclonal DNA antibodies described in the literature has been used for systematic studies of DNA topology in situ.

In the present report we describe a novel murine monoclonal antibody reactive with both double and single-stranded DNA and demonstrate the utility of this immunological reagent for the localization of DNA at both the light and electron microscopic level in whole cell preparations as well as in sections.

Materials and methods

Biological materials

Mice and rats (Sprague Dawley) were obtained from local animal farms. Xenopus laevis were purchased from the South African Snake Farm (Fish Hoek/South Africa). Animals of the urodelan species Triturus cristatus carnifex and Pleurodeles waltlii were reared in our laboratory. The unicellular freshwater alga Cryptomonas ovata was cultured as described [29]. Dinoflagellates of the species Peridinium balticum (a gift from Dr. R. J. Blank, University of Erlangen/FRG) were grown in artificial seawater [55].

Glass slides with dried preparations of the hemoflagellate Crithidia luciliae were purchased from Kallestad Diagnostica (Freiburg/FRG).

Cell lines derived from rat vascular smooth muscle cells (line RVF-SM) and rat kangaroo kidney epithelium (line PtK_2) tissues were grown as described [24, 25].

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Monoclonal antibodies

BALB/c mice were given a course of two subcutaneous injections of 100 µg of a cytoskeleton preparation from murine liver as described by Hazan et al. [32]. Spleen cells were fused with cells of the mouse myeloma line Ag 8.653, and hybrid cells were propagated in hypoxanthine/aminopterin/thymidine/containing medium. Hybridoma supernatants were screened for antibodies by immunofluorescence microscopy, using frozen rat and mouse liver sections. Positive hybridoma colonies were subcloned twice by limited dilution.

Immunoglobulin subclasses were determined by double immunodiffusion [53], using subclass-specific antibodies (Miles-Yeda, Rehovot/Israel). To obtain large quantities of monoclonal antibodies, hybridoma cells were injected intraperitoneally into pristane-treated BALB/c mice. Ten to 14 days after inoculation ascites fluid was collected, centrifuged (200*g*, 10 min), and the supernatant used for antibody purification. The IgM-fraction was obtained by gel filtration on Sephacryl S-300 (Pharmacia, Freiburg/FRG) als described [35].

Chromatin, nucleic acids and histones

Chromatin fractions containing supranucleosomal particles were prepared from chick erythrocyte nuclei as described [77]. Calf thymus DNA was obtained from Boehringer (Mannheim/FRG), double and single-stranded DNA from replicating and non-replicating M13 phages was donated by T. Magin (Heidelberg/FRG). E. coli DNA was extracted and purified according to standard procedures. Protein-free mouse DNA was a gift of Dr. D. Werner (Heidelberg/FRG; cf. [52]). The plasmid HMT consisted of pBR 322 with an inserted rRNA gene of Xenopus laevis [58]. Ribosomal

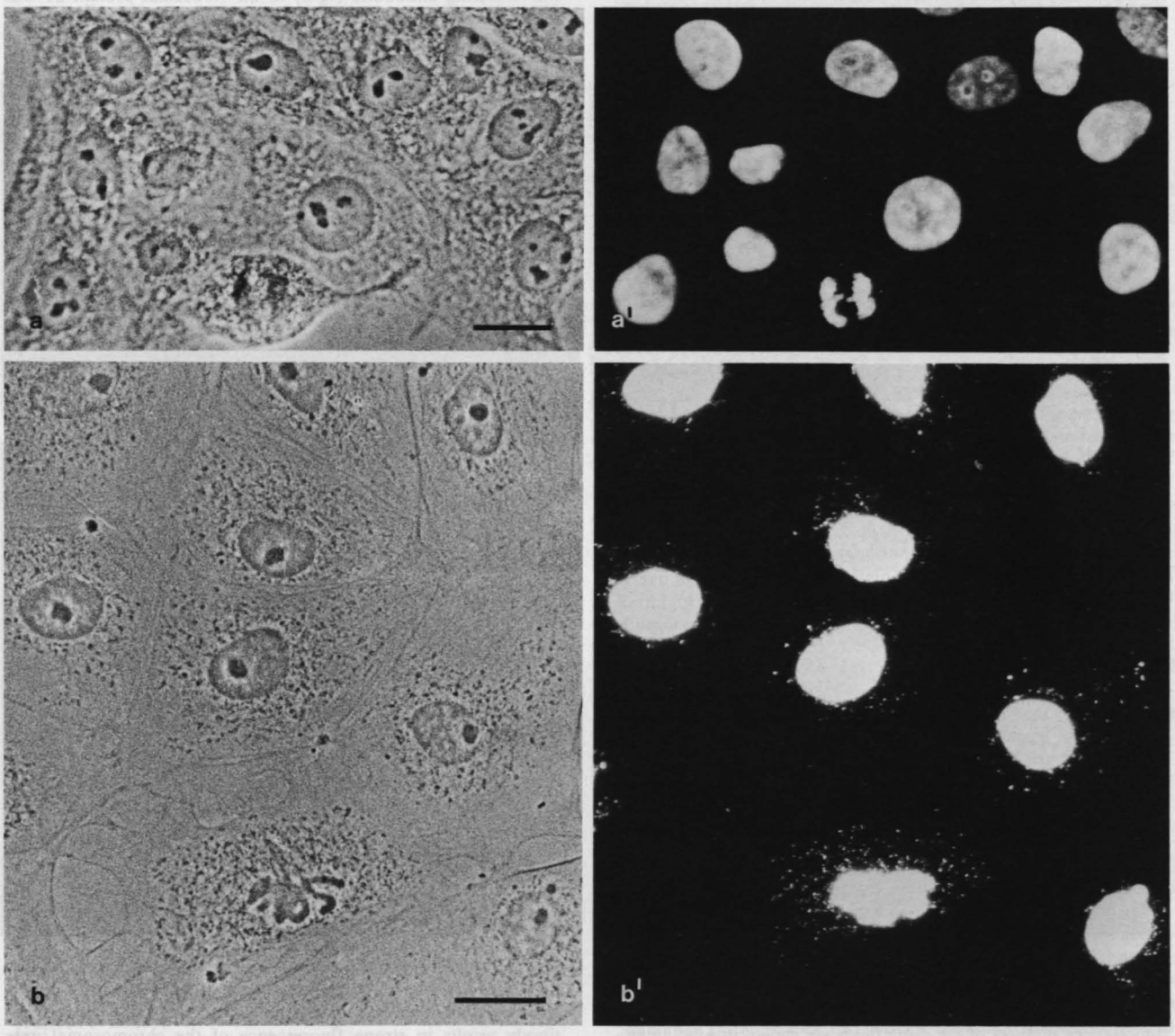


Fig. 1. Immunofluorescence microscopy of cultured cells after reaction with monoclonal DNA antibody AK 30-10. Rat (RV-SM, **a'**) and rat kangaroo (PtK_2, **b'**) cells show strong nuclear and chromosomal staining. The corresponding phase-contrast images are presented in (**a, b**). After treatment of PtK_2 cells with the detergent Triton X-100 prior to immunostaining, a finely punctate fluorescence is recognized in the cytoplasm (**b'**) which represents staining of mitochondrial DNA. — Bars 20 µm.

RNA was isolated from a crude ribosomal pellet obtained from a Xenopus laevis ovarian homogenate (see [35]). Yeast tRNA (Sigma Chemie, Deisenhofen/FRG) was treated with 50 µg/ml DNase I (RNase-free, Worthington, Freehold, NJ/USA) in 10 mM Tris-HCl, *p*H 7.2, 2 mM $MgCl_2$ in order to remove contaminating DNA. After 1 h incubation at 37 °C, sodium dodecyl sulfate (SDS) was added to 0.1% and tRNA purified by phenol extraction.

Nucleosides, nucleotides and synthetic polynucleotides were obtained from Boehringer (Mannheim/FRG) and Sigma. Double-stranded alternating copolymers included poly(dA-dC)·poly(dG-dT), polydG·polydC, polydA·polydT, poly(dA-dT) and poly(dG-dC). Calf thymus histones were purchased from Calbiochem (Frankfurt/FRG).

Enzyme-linked immunosorbent assay (ELISA)

Highly activated polyvinyl-chloride 96-well microtiter plates (Flow Laboratories, Meckenheim/FRG) were coated with E. coli DNA (10 µg/ml in phosphate-buffered saline, PBS; 50 µl per well) overnight at 4 °C. Using ^{32}P-labelled E. coli DNA, we estimated a binding efficiency of approximately 1.5% (see also [44]). Plates were then inverted, washed twice with PBS, followed by incubation in PBS containing 1% bovine serum albumin (BSA) to saturate any free binding sites. After another wash in PBS containing 0.05% (v/v) Tween-20, plates were incubated with undiluted supernatant from the hybridoma clones (50 µl/well) and incubated for 2 h at room temperature. The antibody-containing solutions were removed and, after several wash steps with PBS containing Tween, goat anti-mouse IgM coupled to alkaline phosphatase (Medac, Hamburg/FRG) was added at a 1:1000 dilution in PBS containing 1% BSA. At the end of the incubation period (2 h at room temperature) the plates were washed several times with PBS-Tween solution and bound enzymes were visualized by addition of substrate [30]. Absorbance was read at 405 nm using a Titertek Multiscan photometer (Flow Laboratories). The background binding was determined by using cell culture medium instead of hybridoma supernatant. For controls, the supernatant fluid of a hybridoma cell line secreting antibodies (IgM) to the ribosomal protein S1 was used [35].

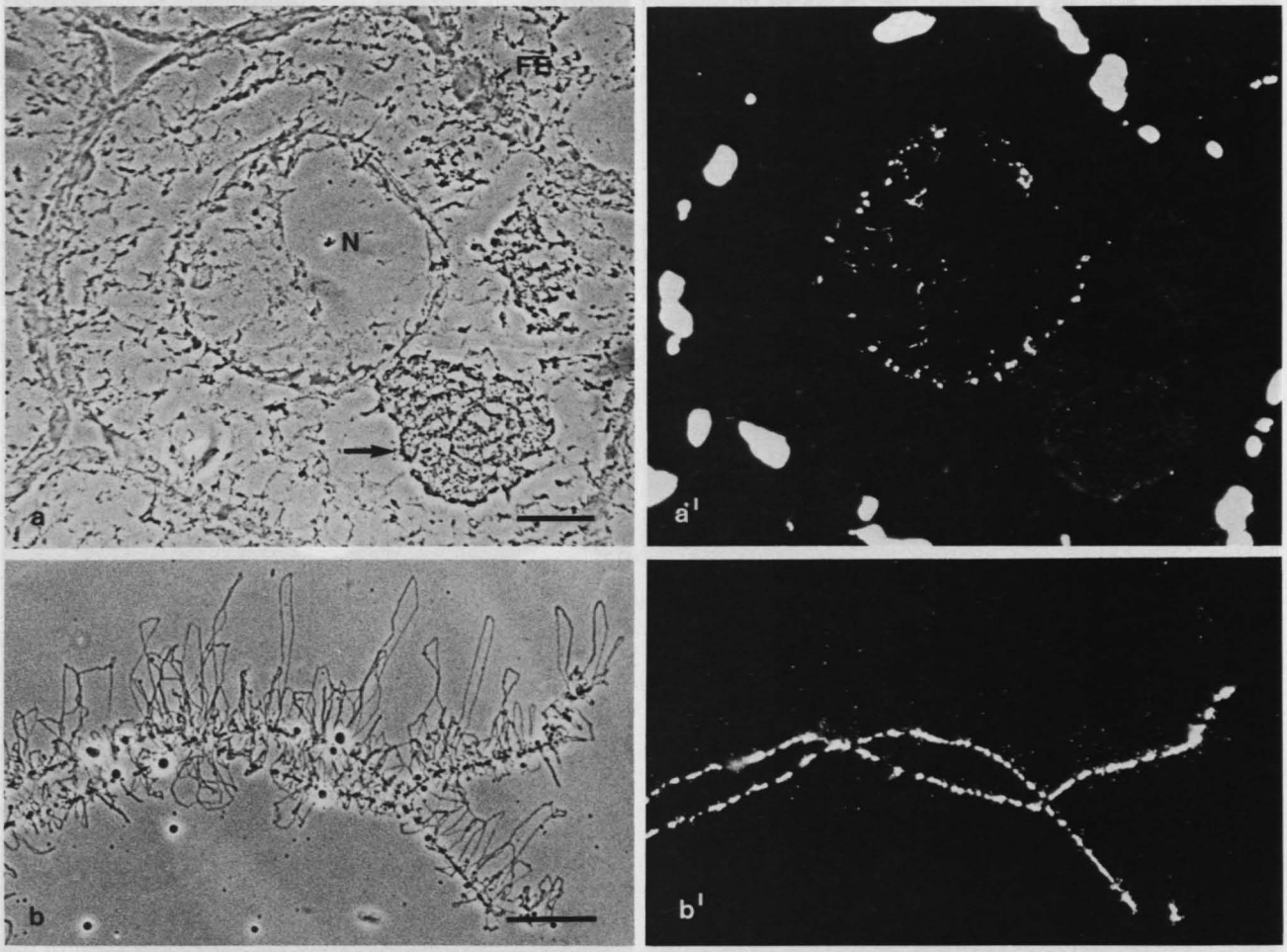


Fig. 2. Detection of small amounts of DNA by immunofluorescence microscopy of frozen sections of a previtellogenic Xenopus laevis ovary, using antibody AK 30-10. Phase-contrast and epifluorescence pictures of the same fields are shown in **a, a'**. Immunoreactivity is observed in the nuclei of the cells of the follicle epithelium surrounding the oocytes (FE), in the numerous amplified nucleoli in the periphery of the oocyte nucleus (N) and in the mitochondria of the "mitochondrial cloud" (**a'**). The mitochondrial cloud is visible in the corresponding phase-contrast micrograph as a distinct cytoplasmic aggregate (*arrow* in **a**). Incubation of isolated lampbrush chromosomes from Triturus cristatus with the antibody results in strong fluorescence of the chromosomal axes whereas the lateral loops are barely stained (**b'**). The corresponding phase-contrast image is shown in (**b**). The numerous fluorescent dots surrounding the chromosome axes may reflect sites of higher compaction states of the loop DNA. — Bars 20 µm.

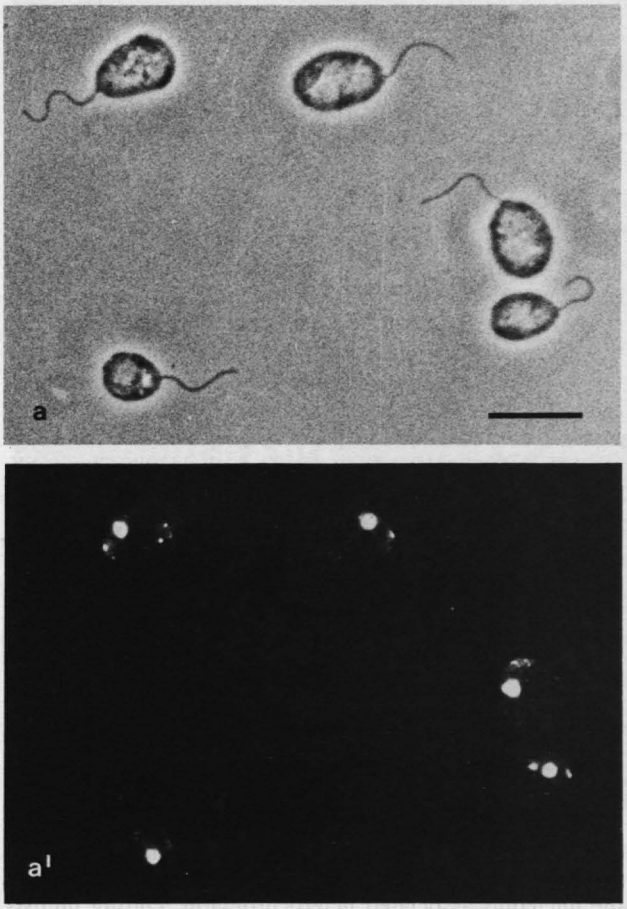


Fig. 3. Immunofluorescent staining of kinetoplast DNA of the flagellated protozoon Crithidia luciliae by monoclonal antibody AK 30-10 (**a'**). Nuclei are only weakly fluorescent. The corresponding phase-contrast image is shown in (**a**). — Bar 10 μm.

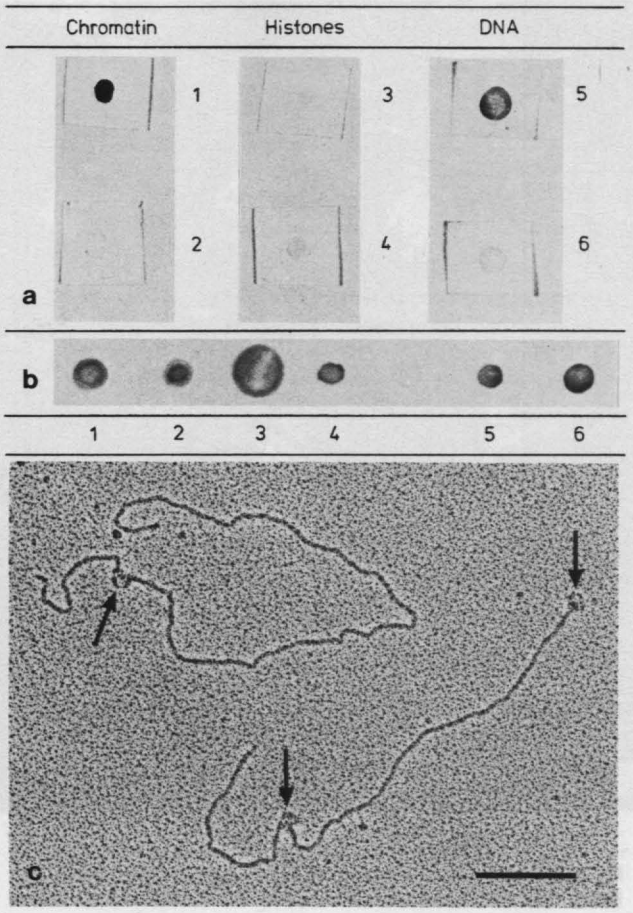


Fig. 4. Binding of antibody AK 30-10 to DNA as demonstrated by a dot-immunobinding assay (**a, b**) and electron microscopy of spread DNA molecules (**c**). — **a.** The antibody reacts with chromatin from chick erythrocytes (dot 1) and deproteinized calf thymus DNA (dot 5) but not with calf thymus core histones (dot 3). Controls were incubated with a murine monoclonal antibody (IgM) directed against ribosomal protein S1 (dots 2, 4, 6). — **b.** Binding of antibody AK 30-10 to double and single-stranded DNA from a diversity of species: Dot 1, native calf thymus DNA; dot 2, heat-denatured calf thymus DNA; dot 3, double-stranded M13 DNA; dot 4, single-stranded M13 DNA; dot 5, recombinant plasmid HMT; dot 6, double-stranded mouse DNA. — **c.** Electron microscopic visualization of antibody-DNA complexes. Several large IgM molecules are bound to mouse DNA *(arrows)*. — Bar 0.2 μm.

Competition studies

Hybridoma supernatant or purified DNA antibodies were preincubated for 30 min at room temperature with various quantities of defined nucleotides and natural and synthetic nucleic acids (see above) as competing substrates. Remaining binding capacities of the antibodies were assessed by means of immunofluorescence microscopy or ELISA. For the competitive ELISA purified DNA antibodies were used at a concentration of 50 μg/ml in PBS. Under these conditions the competing rRNA and tRNA remained largely intact as shown by gel electrophoresis of the assay mixture after the incubation period.

Dot-immunobinding assay

Antigen solutions (nucleic acids, histones, chromatin) were applied directly to nitrocellulose paper (between 0.1 and 1 μg) and dried. Then the nitrocellulose strip was baked for 1 h at 70°C and incubated with hybridoma supernatant followed by peroxidase-conjugated secondary antibodies essentially as described by Hawkes et al. [31].

Immunofluorescence microscopy

Cryostat sections (~5 μm thick) and cultured cells grown on coverslips were processed for indirect immunofluorescence microscopy as described [35, 62]. Hybridoma supernatant was used undiluted. In some experiments PtK_2 cells were treated with 1% (w/v) Triton X-100 for 1 min to ensure accessibility of the mitochondrial DNA to the antibodies. To define the nature of the antigen, cryostat sections were treated with DNase I (Worthington Biochemicals; 0.1 mg/ml in 10 mM Tris-HC1, *p*H 7.2, 2 mM $MgC1_2$) or pancreatic RNase (Boehringer, Mannheim; 0.1 mg/ml in 50 mM NaC1, 10 mM Tris-HC1, *p*H 7.2) for 30 min at 37°C. The slides were then rinsed in PBS and used for immunofluorescence microscopy.

Lampbrush chromosomes prepared from mid-sized oocytes of Triturus oocytes were processed for immunofluorescence microscopy as outlined elsewhere [63].

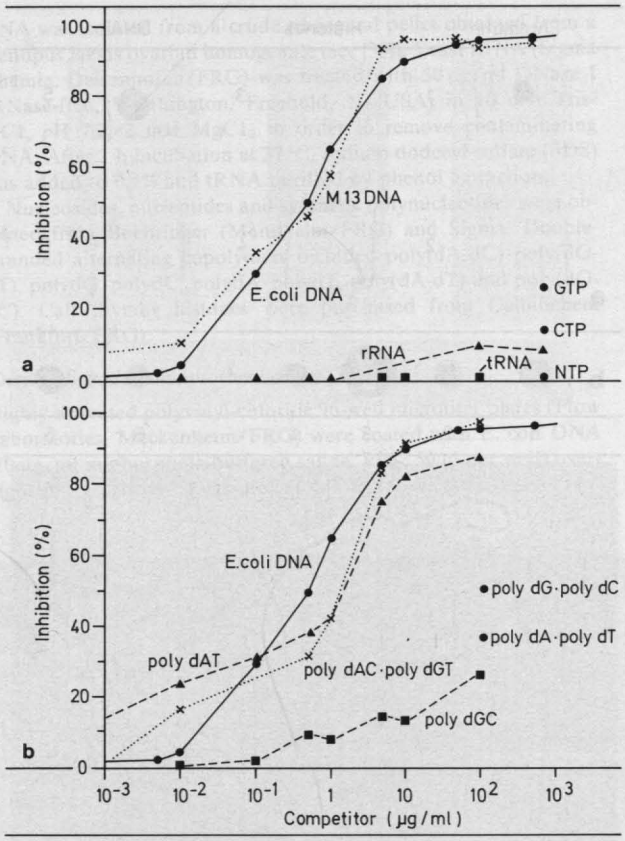


Fig. 5. Inhibition of binding of antibody AK 30-10 to E. coli DNA that had been immobilized on the surface of microtiter plates by various nucleic acids and nucleotides (**a**) as well as synthetic polynucleotides (**b**). — **a.** Competitive ELISA using E. coli DNA, double-stranded; M13 DNA, single-stranded; rRNA from Xenopus laevis ovary ribosomes; tRNA from yeast; NTP: dATP, dGTP, dCTP, dTTP, ATP, UTP. — **b.** Competitive ELISA with various synthetic polynucleotides. PolydG·polydC and polydA·polydT were tested at a concentration of 100 µg/ml only.

Electron microscopic immunolocalization

Fixation of the algae, embedding in Lowicryl K4M and antibody labelling of the ultrathin sections have been described in detail [29]. Small pieces of rat liver were fixed in 4% formaldehyde, freshly made from paraformaldehyde, and 0.1% glutaraldehyde in 0.2 M Pipes buffer, *p*H 7.0 for 60 min at room temperature and processed for postembedding electron microscopic immunocytochemistry using the same protocol. Preembedding antibody labelling on cryostat sections of rat liver was performed as outlined recently [62]. Secondary antibodies coupled to 5 or 10 nm gold particles (Janssen Life Sciences, Beerse/Belgium) were used at dilutions ranging from 1:75 to 1:20. Electron microscopic visualization of antibody complexes on spread DNA molecules was performed by the mica technique essentially as described [51, 67].

Microinjection of antibodies into cells

Purified DNA antibodies were microinjected with glass capillaries into mitotic cells. Two hours later the cells were fixed in cold methanol, air-dried and incubated with fluorescein isothiocyanate(FITC)-conjugated secondary antibodies. Injection of purified DNA antibodies into nuclei of Pleurodeles oocytes was done as described [12, 60]. Each nucleus received 5 to 10 nl of a 1 mg/ml antibody solution in PBS. After varying times lampbrush chromosomes were isolated and examined by phase-contrast microscopy [12, 60].

Results

Antibody AK 30–10

The hybridoma clone AK 30–10 was selected during a routine screening by immunofluorescence microscopy of hybridomas derived from a BALB/c mouse immunized with cytoskeletal preparations. Although the material used for the immunization also contained high salt-dehistoned DNA we do not know whether the clone AK 30–10 was induced by the immunization procedure or reflects the fusion of an autoantibody producing spleen cell with the myeloma partner (for discussion see also [70]). Clone AK 30–10 was selected because the secreted antibodies gave a very strong nuclear fluorescence when frozen sections of rat liver were examined. The antibody subclass was determined to be IgM.

Immunofluorescence microscopy

Incubation of frozen sections of tissues of human, animal (ranging from mammals to insects) and plants with antibody AK 30–10 resulted in a specific and bright fluorescence of all cell nuclei (see below). Cultured cells from a variety of species also revealed a strong nuclear fluorescence, with exception of the nucleoli (Figs. 1a′, b′). The periphery of the nucleoli was often accentuated by a fluorescent ring, probably due to the densely packed DNA in the nucleolus-associated heterochromatin. During mitosis chromosomes stood out as clearly demarcated, uniformly fluorescent structures (Figs. 1a′, b′). In addition to the striking nuclear and chromosomal staining, a finely punctate fluorescence was frequently noted in the cytoplasm of cultured cells (Fig. 1b′). This cytoplasmic fluorescence was seen especially clearly after treatment of cells with detergents such as Triton X-100 and saponin which disrupt membrane barriers and facilitate accessibility of cellular organelles to antibodies. The dot-like cytoplasmic staining was precisely superimposed on the distribution of mitochondria as seen in the corresponding phase-contrast image (Figs. 1b, b′). Recently, it has been demonstrated that human autoimmune antibodies to native DNA bind to nuclear as well as mitochondrial DNA [57]. In agreement with these authors we interpret the finely punctate pattern of cytoplasmic fluorescence as the result of the binding of the antibodies to mitochondrial DNA.

In frozen sections through ovaries of Xenopus laevis, the chromatin of the somatic cell nuclei of the follicle epithelial cells was intensely stained (Fig. 2a′), as in other tissues from a broad range of species (not shown). In the previtellogenic oocytes the numerous amplified nucleoli in the nuclear periphery showed a remarkably bright staining, in addition to some intranuclear fluorescence due to portions of the lampbrush chromosomes included in the tissue section (Fig. 2a′). This demonstrates that the low concentrations of rDNA present in individual amplified nucleoli (0.007 – 0.15 pg; [73]) is detected by this reaction. In addi-

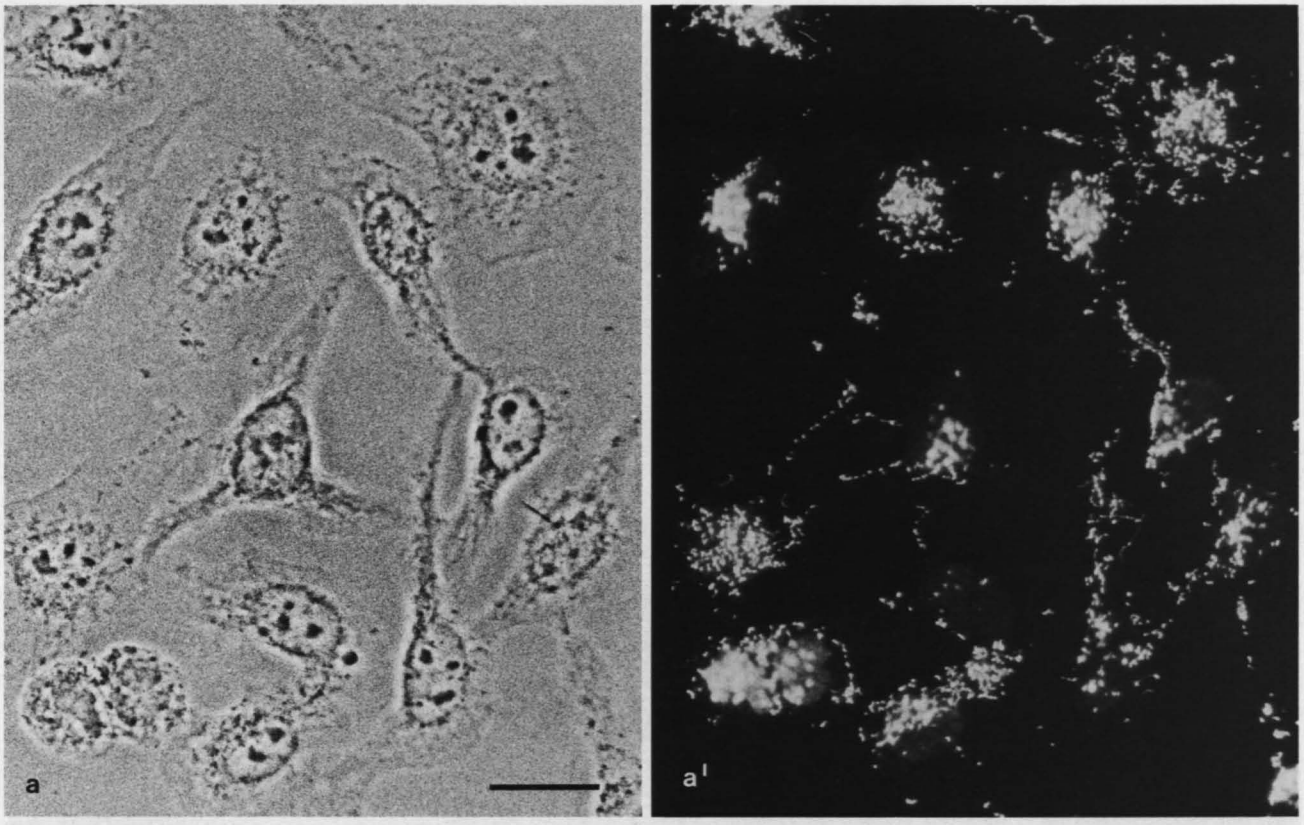


Fig. 6. Immunofluorescence microscopic detection by monoclonal antibody AK 30-10 of mycoplasma contamination in a culture of a human bladder carcinoma cell line (RT 112; **a'**). The corresponding phase-contrast image is shown in (**a**). — Bar 20 μm.

tion, the cytoplasmic "mitochondrial cloud" of previtellogenic Xenopus oocytes, which represents a morphologically distinct aggregate of mitochondria [33], revealed a very delicate punctate fluorescence, indicating again that mitochondrial DNA was visualized by antibody AK 30–10 (Fig. 2a'). In both sections and whole cell preparations, antibody AK 30–10 did not decorate the surface membranes, indicating that it did not react with an epitope also present in plasma membrane proteins, as is the case for some other monoclonal DNA antibodies [37, 76].

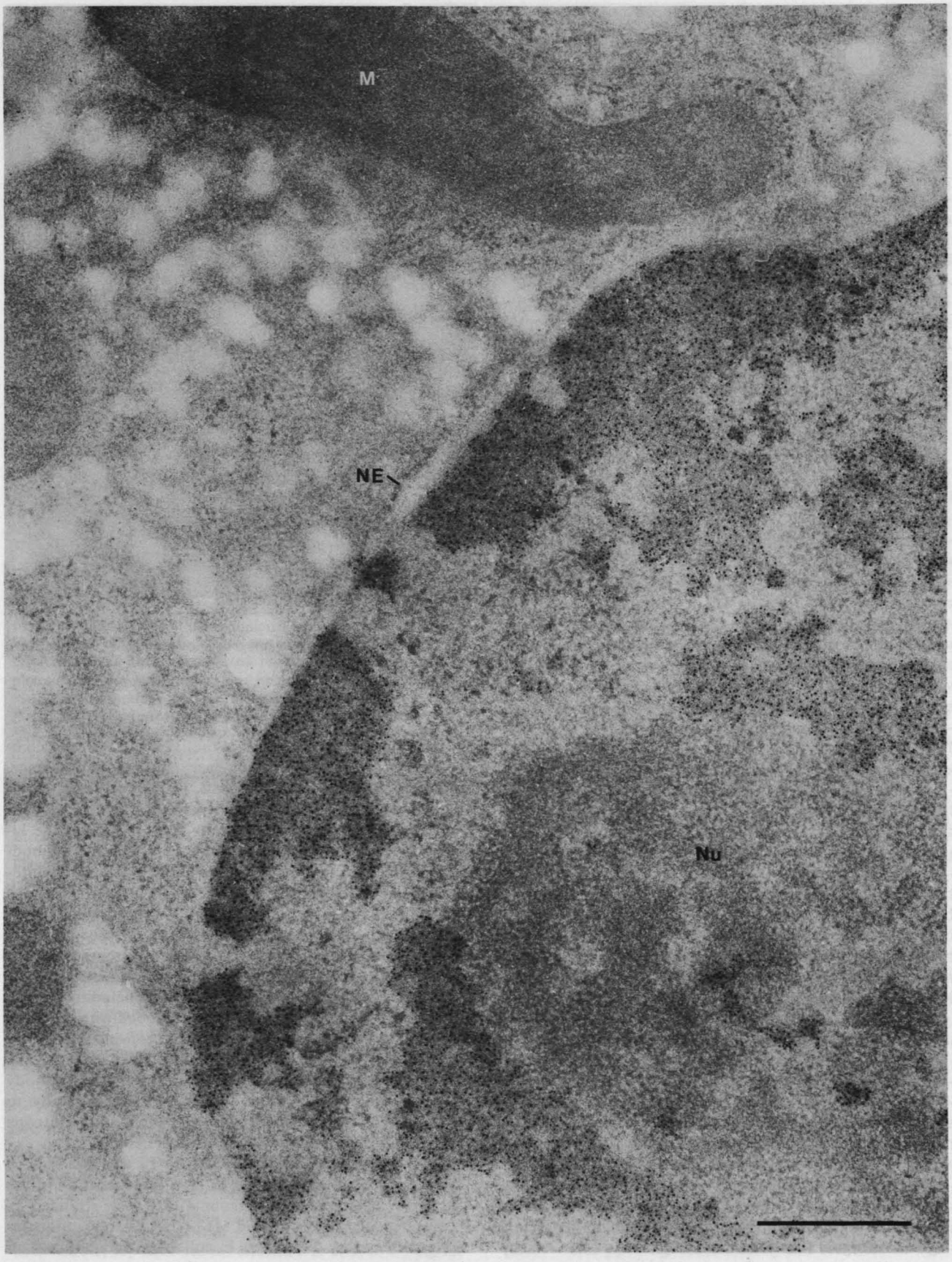
When lampbrush chromosomes isolated from Triturus oocytes were incubated with antibody AK 30–10, the chromosomal axes were heavily stained, in contrast to the lateral loops which appeared negative (Fig. 2b'). This result is consistent with the general view of lampbrush chromosome organization [13] which implies that the majority of lampbrush chromatin is transcriptionally inactive and highly compacted in form of the linear arrays of chromomeres constituting the chromosome axis. Only a small proportion of the total chromosomal DNA (about 5%) is transcriptionally active and present as highly extended chromatin in the numerous loops. The absence of detectable fluorescence along the lateral loops indicated that in a single double-stranded molecule in the extended state the local concentration of DNA is not sufficient to generate a significant signal (for details see [63]).

Characterization of the epitope

Digestion of frozen sections of rat liver with DNase I completely abolished the nuclear immunofluorescence, whereas RNase treatment had no effect (results not shown; cf. [47]). When smears of Crithidia luciliae were incubated with AK 30–10, a strong fluorescence of the kinetoplast was noted (Fig. 3a') indicating that the antibodies reacted with this form of double-stranded mitochondrial DNA [1].

In "dot-immunobinding" assays [31], calf thymus DNA and chromatin prepared from chicken erythrocytes were clearly positive, in contrast to purified histones (Fig. 4a) and many other proteins tested (data not shown). With the same approach various natural DNAs, including double and single-stranded molecules, were probed with antibody AK 30–10. All scored positive, without conspicuous differences in staining intensities (Fig. 4b). In contrast, rRNA from Xenopus ovary was not recognized by the antibody (not shown). Furthermore, binding of the antibody to DNA could be directly visualized by electron microscopy of spread DNA antibody complexes (Fig. 4c).

The specificity of antibody AK 30–10 was analyzed in greater detail by competitive ELISA. Preincubation of the antibody with increasing amounts of double-stranded E. coli DNA or single-stranded M13 DNA as competitors reduced its ability to bind to E. coli DNA immobilized on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the plastic surface of the microtiter plates (Fig. 5a). In contrast, rRNA and tRNA were practically not inhibitory, nor were the four deoxyribonucleotides and the ribonucleotides ATP and UTP. When tested at relatively high concentrations, CTP and GTP displayed a modest but significant inhibitory effect (Fig. 5a).

From these data we conclude that the monoclonal antibody AK 30–10 recognizes an epitope present on both single and double-stranded DNA but absent from RNA. However, when various synthetic double-stranded polynucleotides were tested by competitive ELISA, it became apparent that the antibody recognition was not exclusively based on a feature of the deoxyribose-phosphate backbone of the DNA chain but was also influenced to some extent by the base composition. For instance, the double-helical copolymer poly(dG–dC) was a much poorer competitor than poly(dA–dT) (Fig. 5b). A potent inhibitor was also poly(dA–dC)·poly(dG–dT) whereas the polynucleotides polydG·polydC and polydA·polydT inhibited only poorly (Fig. 5b).

Detection of mycoplasma cells

Mycoplasmas were readily detectable by immunofluorescence microscopy after incubation of cell cultures grown on coverslips with DNA antibody AK 30–10. Contaminated cells were identified by the presence of numerous strongly fluorescent bodies usually located at cell peripheries and often lined up along cellular extensions (Fig. 6a′). The pattern of fluorescence was essentially identical to that obtained with the DNA fluorochromes Hoechst 33258 or DAPI which are routinely used for screening of cell cultures to detect mycoplasma contaminations [15, 59]. The sensitivity of the immunofluorescence assay appeared comparable, if not superior, to the cytochemical staining with the DNA-specific fluorescent stains.

DNA localization by immunogold electron microscopy

Ultrathin sections of Lowicryl-embedded biological material were floated on droplets of antibody solution, followed by incubation with secondary, gold-coupled antibodies. The colloidal gold particles seen in Figure 7 illustrate the specific binding of antibody AK 30–10 to DNA-containing structures, notably chromatin, of a rat hepatocyte. The gold particles were particularly enriched in the condensed chromatin of the nuclear periphery and in the perinucleolar region. In contrast, the nucleoplasmic spaces between the heterochromatin blocks were devoid of gold particles (Fig. 7). Whether the absence of labelling of the spaces between these chromatin blocks reflects a very low local DNA concentration in these regions, perhaps due to an extended conformation as in the lampbrush chromosome loops (see above), or whether DNA is actually absent cannot be decided at present.

The nucleolus usually appeared as a zone of exclusion of gold particles, except for small "islands" of gold grain clusters within the nucleolar body, which either represented invaginations of the surrounding nucleolus-associated heterochromatin or true nucleolar, i.e. rDNA-containing, chromatin as present in the "fibrillar centers" (see below).

Essentially the same results were obtained by postembedding immunolabelling of the alga Cryptomonas ovata as a representative of the plant kingdom (Fig. 8a). Immunogold particles were confined to the numerous patches of condensed chromatin and to small intranucleolar regions. In addition, gold clusters were also present at several distinct sites in the stroma of the plastids surrounding the nucleus (Fig. 8a; for details see [29]).

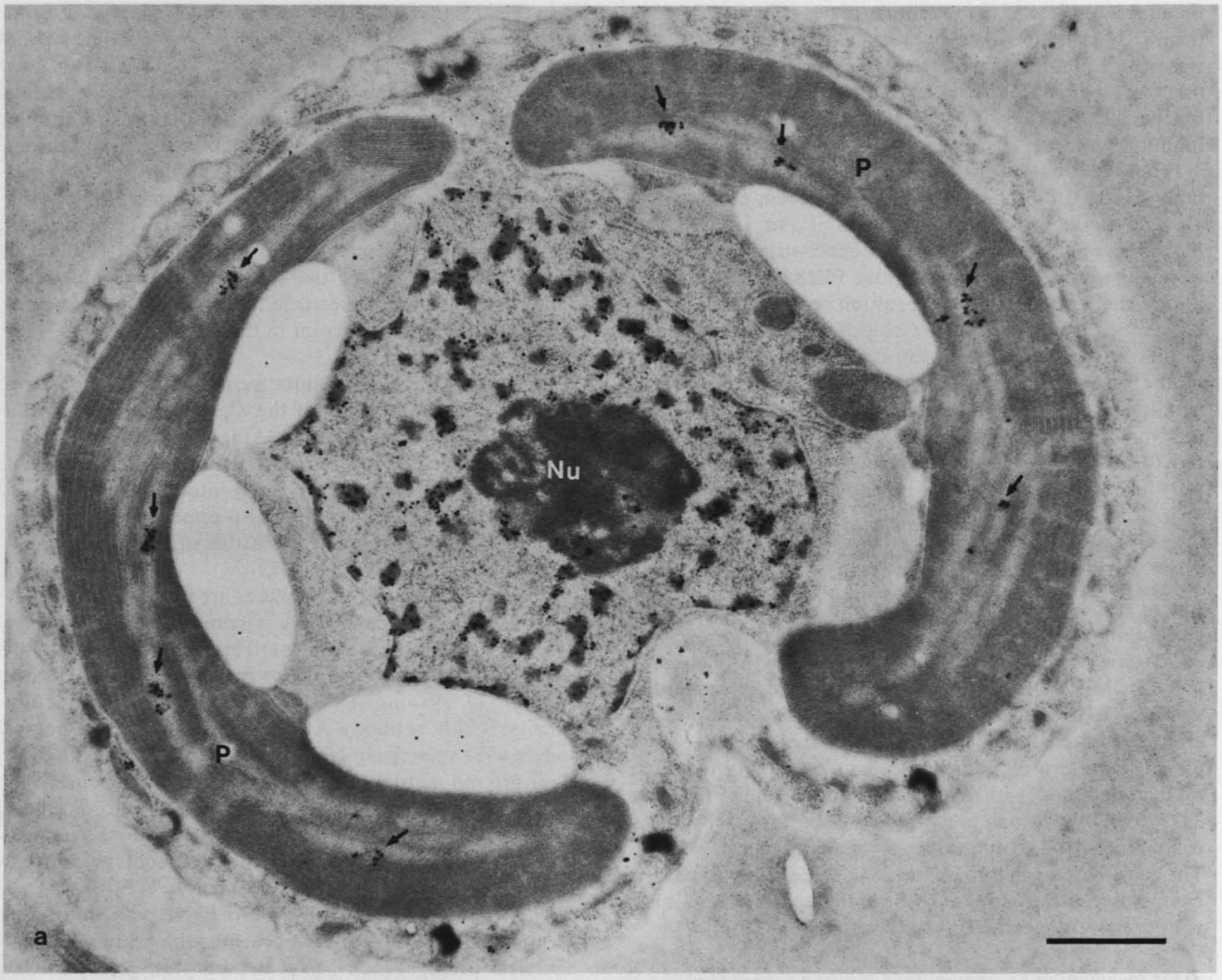
Chromosomes of dinoflagellates are unique in that they lack histones and their DNA is compacted in a manner which is structurally different from the organization of "true"eukaryotic chromatin (for review see [22]). After incubation of sections of Peridinium balticum embedded in Lowicryl with antibody AK 30–10, the fibrillar chromosomes were selectively labelled (Fig. 8b), whereas the nuclear ground substance in between the chromosomes was essentially unlabelled. Gold-associated chromosomal structures at the nucleolar periphery, or inside the nucleolar body, probably represented the "nucleolus organizer" regions (Fig. 8b).

The intranucleolar localization of DNA was studied in greater detail by following a preembedding immunocytochemical protocol [62]. In rat hepatocytes, gold particles were heavily concentrated in the perinucleolar chromatin but were absent from the nucleolar body, except for the fibrillar centers (Fig. 9; for nomenclature see [28, 62]). Inside the fibrillar centers a network of fine filaments, most likely the rDNA-containing chromatin, was selectively decorated unlike the surrounding dense fibrillar component which appeared totally negative. This finding supports our earlier conclusion, based on the use of antibodies to RNA polymerase I, that transcriptionally active rRNA genes are located within the fibrillar centers of nucleoli [62, 64].

Fig. 7. Immunoelectron microscopy of DNA on a section of Lowicryl-embedded rat liver tissue. The ultrathin section was incubated with antibody AK 30-10, followed by secondary antibodies coupled to 5 nm gold particles. The hepatocyte shows strong and selective labelling of the condensed chromatin regions which are preferentially located in the nuclear periphery and around the nucleolus (Nu). The nucleolar body is essentially free of gold particles, with the exception of small intranucleolar components which represent either rDNA chromatin or invaginations of the surrounding perinucleolar heterochromatin. The low contrast regions labelled with immunogold (in the *lower right*) probably represent the "fibrillar centers". — NE Nuclear envelope. — M Mitochondrion. — Bar 0.5 μm.

Binding of antibody AK 30–10 to chromatin DNA in vivo

Mitotic PtK_2 cells were microinjected with purified AK 30–10 antibodies and the distribution of the injected immunoglobulins was visualized 2 h later by gently permeabilizing the cells and labelling them with FITC-conjugated anti-mouse IgM. As shown in Figure 10a, immunofluorescence was only seen in the two daughter nuclei formed from the injected mitotic cell, whereas the adjacent non-injected cells were not stained. This observation clearly indicates *(i)* that in living cells DNA is available to the relatively large IgM molecules, even though it is packaged into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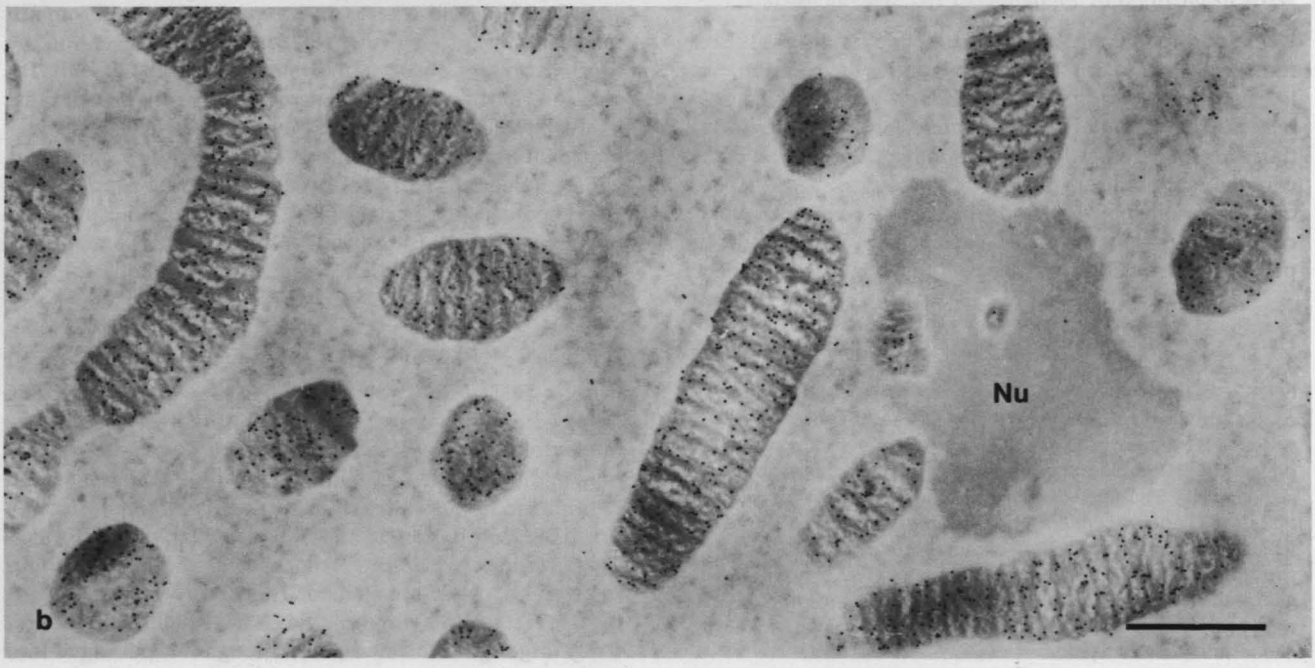
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interphase chromatin or mitotic chromosomes, and *(ii)* loading of the chromatin with such antibodies does not interfere with chromatin organization.

To examine specifically whether binding of the antibodies to DNA would interfere with transcriptional events in the living cell, we injected antibody AK 30–10 into nuclei of Pleurodeles oocytes. Within 1 h after injection, the lateral loops of the lampbrush chromosomes were considerably foreshortened, indicating that the antibodies, by binding to the loop DNA, interfered with transcription (Fig. 10d; for relation between loop size and transcriptional activity see [63]). In addition, a network of actin filaments was induced by the microinjection of DNA antibodies which is also indicative of inactivation of loop transcription [61]. Three hours after microinjection, the majority of the lateral loops was completely retracted into the chromosome axis, indicating total arrest of loop transcription (Fig. 10e).

Discussion

The monoclonal antibody described in the present study recognizes both double and single-stranded DNA with no apparent preference for the one or the other form. Similar binding to both forms of DNA has been described for a variety of murine monoclonal antibodies derived from mouse strains developing autoimmune disorders similar to systemic lupus erythematosus (SLE; [7, 43, 46, 74]; for further refs. see [23, 70]). Our ELISA results of competitive binding of a series of synthetic helical polynucleotides suggest that not only is the phosphodiester-deoxyribose backbone involved in the binding reaction but also, to some extent, is the nucleotide base. In fact, it has been demonstrated for several monoclonal DNA antibodies that certain bases are preferred over others ([44, 71]; similar phenomena have also been described for DNA autoimmune antibodies from SLE patients; see [14]). Of course, we cannot decide whether the base moieties are part of the epitope or whether they are indirectly involved, as has been suggested for several monoclonal antibodies to oligonucleotides [39].

The strong binding of antibody AK 30–10 to DNA and the absence of binding to RNA makes it a useful immunological tool for the identification of DNA-containing structures in cytological preparations at the light and electron microscopic level. Immunofluorescence microscopy permits the detection of very low levels of DNA, as illustrated by our observation that mitochondrial DNA can be visualized in cultured mammalian cells and in frozen sections of previtellogenic Xenopus oocytes. Vertebrate mitochondria contain multiple (2–14) copies of circular DNA molecules with molecular weights of approximately 11×10^6 [11, 65]. Sera of SLE patients with high titers of antibodies reactive with native DNA have also been reported to stain mitochondria [57]. Other cytologically well-defined structures with known DNA content are the amplified nucleoli of Xenopus laevis oocytes. They contain rDNA quantities ranging from 0.007 to 0.15 pg [73] which is sufficient to generate a strong immunofluorescence signal with antibody AK 30–10. Therefore, we propose the use of antibody AK 30–10 or other antibodies with similar specificity and affinity to identify DNA in structures of unknown composition.

Due to its high sensitivity antibody AK 30–10 is also of potential value for monitoring mycoplasma contamination of cell cultures. As we have shown in this study immunofluorescence microscopy is at least as sensitive as the routine techniques based on the use of DNA-specific fluorochromes such as Hoechst 33258 or DAPI [15, 59]. The sensitivity can certainly be increased by optimizing the secondary antibody combinations.

Unlike DNA-intercalating fluorochromes, which are restricted in their use to the light microscopic level, the monoclonal DNA antibody described in this study opens sensitive approaches for the identification of DNA at the ultrastructural level. We have presented several examples which illustrate that immunogold electron microscopy is a powerful method for achieving both high resolution of DNA detection and optimal structural preservation, since fixation with formaldehyde or glutaraldehyde did not interfere with the binding of antibody AK 30–10. For example, the present approach has enabled us to extend earlier studies of the topological distribution of the plastidal genome of plant cells, which were based on the use of fluorochromes [16, 18, 19, 38], to the electron microscopic level (see also [29]).

It is to be hoped that immunogold electron microscopy used in conjunction with antibodies directed against DNA and those directed against components of the transcriptional and translational apparatus will help in elucidating the functional organization of DNA, i.e. "nucleoids", in plastids and mitochondria in greater detail.

Another problem that has received much attention recently is that of detection of low local concentrations of DNA such as in transcriptionally active rRNA genes in nucleoli [28, 34, 62, 64]. With our present immunocytochemical approach we detected intranucleolar DNA exclusively in the fibrillar centers and not in the surrounding dense fibrillar component.This observation confirms and extends our earlier conclusion, based on the immunolocalization of RNA polymerase I-complexes, that transcription of rDNA takes place in the fibrillar centers of nucleoli ([62, 64]; for contrasting views see, e.g., [28, 34]).

On the other hand, we have also shown the limitation of identification of extremely low concentrations of DNA, even with this sensitive immunocytochemical technique,

Fig. 8. Immunocytochemical detection of DNA on ultrathin sections of Lowicryl-embedded algae of the species Cryptomonas ovata (**a**) and Peridinium balticum (**b**). Same procedure as described in the legend to Figure 7, with the exception that 10 nm gold particles were used. In Cryptomonas, regions of condensed chromatin which are scattered throughout the nucleus are strongly labelled as are certain intranucleolar blocks of relatively low contrast (**a**). In addition, distinct gold clusters are seen in thylakoid-free spaces of the stroma of two large plastids (P, *arrows*). The dinoflagellate-type chromosomes of Peridinium are also intensely labelled by the DNA antibody (**b**). The spaces between the chromosomes and the nucleolus (Nu) are essentially free of gold particles (**b**). — Bars 1 μm.

by the observation that the extended B-form double-stranded DNA present in the transcriptionally active loops of lampbrush chromosomes is not detected by this or any other DNA antibody so far tested. Inhibition of transcription of these strands, which results in progressive detachment of ribonucleoprotein fibrils from the loop chromatin concomitant with an increased nucleosomal packing to higher order structures and loop retraction, is correlated with the appearance of immunofluorescent DNA in the retracting loops [63]. At present, we cannot decide whether the failure to detect the single DNA molecule of actively transcribed lampbrush chromosome loops is due to the inability of the antibody to bind to the DNA because of the steric hindrance provided by the masses of associated ribonucleoprotein material or merely to a local DNA concentration along the loop that is below the limit of detection of this method.

Our finding that DNA, although packaged with histones into chromatin, is available in the living cell for reaction with microinjected DNA antibodies is of special importance for experiments aimed at the elucidation of DNA and chromatin interactions in the living cell. Clearly, the presence of antibodies bound to the highly condensed mitotic chromosomes after microinjection into dividing metaphase cells does not inhibit chromatin decondensation and dispersion and the appearance of "normal" interphase chromatin morphology. It has been generally estimated that 1 to 5% of the DNA in nucleoprotein complexes is accessible to DNA antibodies from SLE patients [68]. On the other hand, our microinjection experiments with amphibian oocytes indicate that binding of the antibodies to the intensely transcribed chromatin of amphibian lampbrush chromosomes interferes with and eventually inhibits RNA polymerase II-dependent transcription. We do not know whether the antibody loading of interphase chromatin after microinjection of DNA antibodies into mitotic cells is comparable to that attained in the oocyte microinjection experiments. Future experiments will have to show whether the introduction of DNA antibodies into the interphase nucleus, be it directly by nuclear microinjection or by injection into mitotic cells, also has inhibitory effects on transcription.

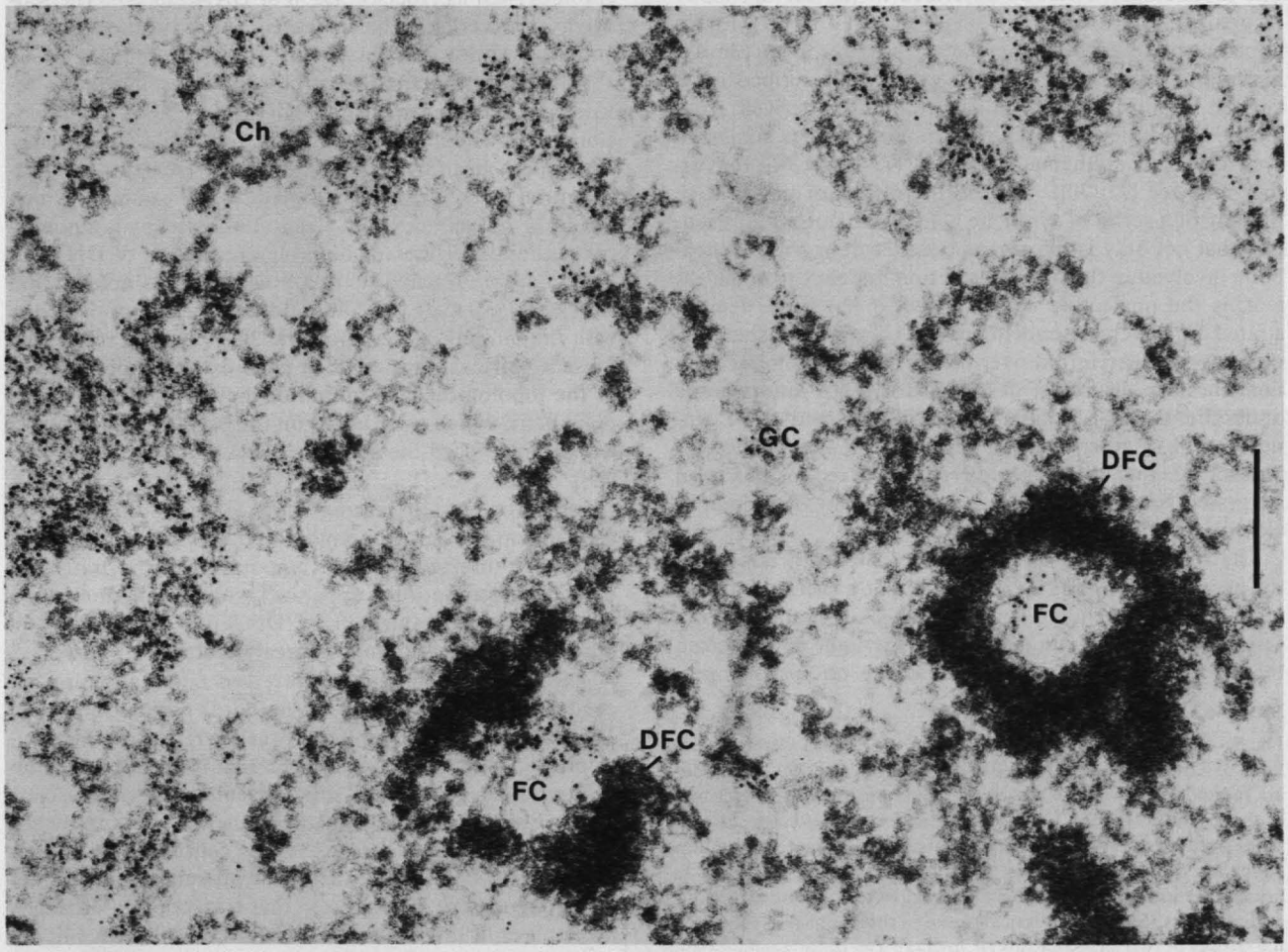

Fig. 9. Immunoelectron microscopy of intranucleolar DNA in rat hepatocytes by the preembedding technique. Gold particles (5 nm diameter), reflecting the distribution of the monoclonal antibody AK 30-10, are concentrated over the chromatin (Ch) surrounding the nucleolus and the intranucleolar fibrillar centers (FC). The granular (GC) and the dense fibrillar (DFC) components of the nucleolus are free of gold particles. — Bar 0.2 μm.

The purpose of this study has been to illustrate the potential value and applicability of a monoclonal DNA antibody (AK 30–10) in cell biology; notably in immunocytochemistry. It is obvious that antibodies of this kind are not only useful reagents for the high resolution detection of DNA in cells but can also be employed in many other areas of nucleic acid research such as, e.g., detection of DNA molecules in hybridization experiments.

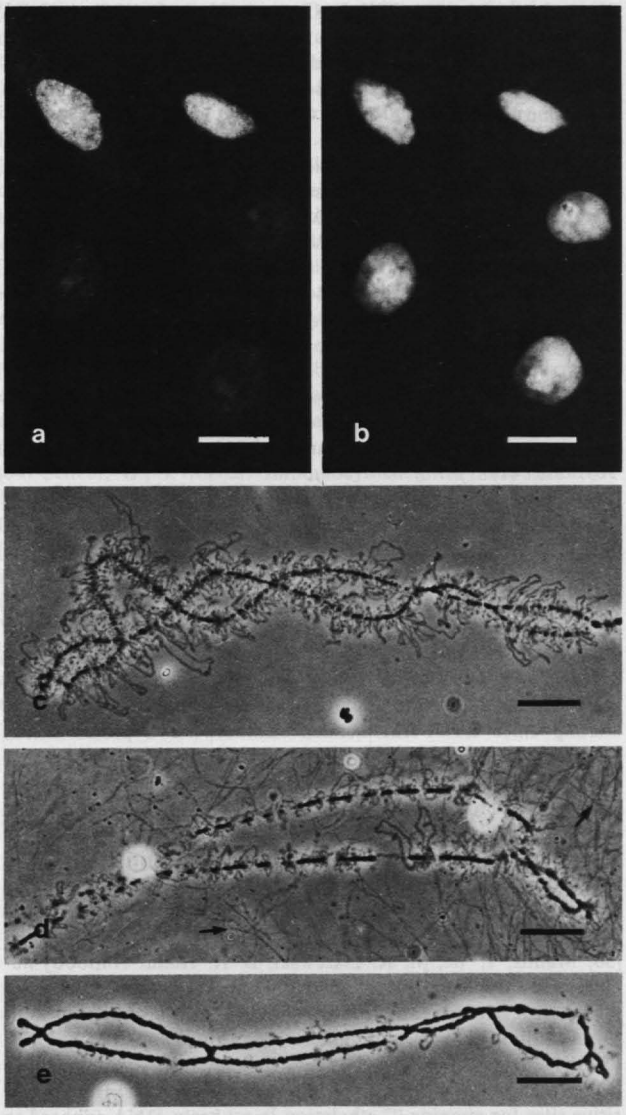

Fig. 10. Antibody AK 30-10 binding to and interaction with chromatin of living cells. — **a.** Distribution of DNA antibody AK 30-10 in postmitotic daughter cells 2 h after microinjection into a mitotic PtK_2 cell, as demonstrated by immunofluorescence microscopy. The same field is shown in (**b**) after staining of the DNA with DAPI. — Note that the injected antibodies have been concentrated, in the living postmitotic cells, on the nuclear chromatin (two uppermost cells). — **d, e.** Inhibition of transcription of lampbrush chromosomes after microinjection of monoclonal antibody AK 30-10 into nuclei of Pleurodeles oocytes. Normal appearance of isolated lampbrush chromosomes is shown after injection of nonimmune mouse IgM (**c**). Injection of the DNA antibody causes a time-dependent retraction of the lateral loops (**d,** 1 h; **e,** 3 h after injection), indicative of transcriptional inactivation. Chromosomes with partially or completely retracted loops are often embedded in a conspicuous filamentous meshwork of actin filaments (some are denoted by *arrows* in **d**). — Bars 20 μm.

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