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Local opening of the DNA double helix in eukaryotic cells detected by osmium probe and adduct-specific immunofluorescence

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SUMMARY

The structure of DNA in mouse fibroblast 3T3 cells has been investigated with the single-strand-selective probe, the complex of osmium tetroxide and 2,2′-bipyridine (Os,bipy). DNA-Os,bipy adducts in the cells were detected by immunofluorescence using a highly specific, affinity-purified polyclonal antibody. Treatment of living cells with the chemical probe led to a distinct but nonuniform nuclear staining. We attribute the positive nuclear staining to the existence of single-stranded and distorted DNA regions in the living cell. Confocal laser scanning microscopy revealed dark areas corresponding to nucleoli and regions of condensed chromatin. These conclusions were supported by the results of experiments in which the chemical probe was applied to fixed cells treated with 45% acetic acid or with acidic buffers (pH 1.8-2.5). An inverse staining pattern was obtained, characterized by intense immunofluorescence of the condensed chromatin regions. Thus, the structural transitions and/or chemical alterations (e.g. depurination) induced by acid treatment increase the accessibility of normally unreactive DNA bases. We conclude that open DNA structures recognized by the chemical probe in the cells prior to their fixation are contained mainly in decondensed and transcriptionally active chromatin, but are virtually absent from nucleoli and condensed chromatin.

Key words: chemical probing of DNA conformation, DNA structure in cells, DNA immunofluorescence, osmium tetroxide, 3T3 cells, confocal laser scanning microscopy

INTRODUCTION

The predominant conformation of the relatively polymorphic DNA double helix is the classical *B*-form, the fine structure of which depends on the nucleotide sequence (Kennard and Hunter, 1989; Lilley, 1989; Dickerson, 1991; Paleček, 1991). In negatively supercoiled DNA alternative non-*B* local structures such as cruciforms, triplexes and segments of left-handed *Z*-DNA may be stabilized. Regions with single-stranded character are usually contained within these local structures and/or at their junctions with contiguous *B*-DNA. They can be detected in vitro by means of single strand-selective enzymatic and chemical probes (reviewed by Paleček, 1991). DNA loci with a single-stranded character (open DNA structures) also form during biological processes such as DNA replication, transcription and recombination (Paleček, 1991; Yagil, 1991).

Despite the extensive knowledge about DNA conformation in vitro, information about the occurrence of the above mentioned structures inside the cell is, as yet, very limited. A number of local structures stabilized by negative supercoiling have been demonstrated recently in prokaryotic cells by chemical probing and molecular genetic methods (Jaworski et al., 1987; Paleček et al., 1987, 1988, 1990; Panayotatos and Fontaine, 1987; Rahmouni and Wells, 1989; Karlovsky et al., 1990; McClellan et al., 1990; Paleček, 1991). However, direct evidence for the existence of local open DNA structures in eukaryotic cells has been lacking.

Immunochemical methods have been valuable in the search for particular DNA structures in eukaryotic cells. In the early 1980s, indirect immunofluorescence was used to reveal left-handed Z-DNA in fixed preparations of insect polytene chromosomes (Jovin et al., 1982; Pardue et al., 1982; Rich et al., 1984). Mono- and polyclonal antibodies against Z-DNA produced different patterns of staining in the polytene chromosomes, depending on the type of fixation used (Hill and Stollar, 1983; Rich et al., 1984; Robert-Nicoud et al., 1984). These studies were extended to other organisms and cells and provided evidence for the presence of sequences with the potential for undergoing the *B-Z* transition in the genomic DNA (for recent accounts see Hill, 1990; Wittig et al., 1990; Paleček, 1991). However, the results obtained by immunofluorescence did not provide conclusive proof of the presence of left-handed DNA in living cells and the question is still open.

Recently we have shown that the complex of osmium tetroxide with 2,2′-bipyridine (Os,bipy; Fig. 1) is able to

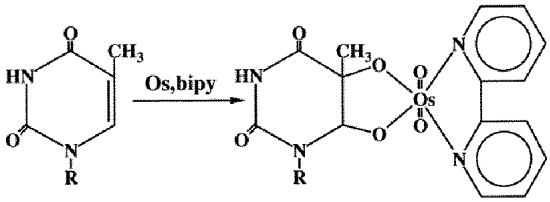

Fig. 1. Formation of the adduct between an exposed thymine and osmium tetroxide-2,2′-bipyridine (Os,bipy). The target C5-C6 double bond of thymine in the *B*-DNA double helix is located in the major groove and is inaccessible to the osmium probe.

penetrate into bacterial cells and to react specifically with bases in DNA segments that have non-*B*-DNA conformation (Paleček et al., 1987; Paleček, 1991). The Os,bipy-modified bases can be detected after DNA isolation from the cells. However, the techniques that have been applied so far to intracellular plasmid DNAs (Paleček, 1991) cannot yield information about the location of the DNA-Os,bipy adducts in chromosomal DNA. With the advent of polyclonal antibodies with specificity for the DNA-Os,bipy adducts (Paleček et al., 1989; Kuderova-Krejcova et al., 1991), the means are now available for detecting and localizing open DNA structures in the chromatin and chromosomes of eukaryotic cells.

In the present study we used Os,bipy as a probe for open DNA structures in mouse fibroblasts and specific antibodies to reveal the localization of the probe adducts. The Os,bipy probe applied to living cells produced a specific staining pattern in the cell nucleus, which was then analysed by conventional and laser scanning microscopy (LSM). This pattern differed substantially from that obtained in cells treated by Os,bipy after fixation and exposure to acid conditions.

MATERIALS AND METHODS

Cell culture

Mouse 3T3 fibroblasts were routinely grown in Dulbecco's MEM supplemented with 10% fetal bovine serum (Biochrom, Berlin, FRG) in a 37°C, humidified CO_2 incubator.

Treatment of cells with Os,bipy

Cells on coverslips were placed in a wet chamber, the medium was then aspirated and immediately replaced with 10 µl of fresh medium. 100 µl of freshly prepared 1.1 mM osmium tetroxide, 1.1 mM 2,2′-bipyridine in Ringer's solution (87 mM NaCl, 3.2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.3) were pipetted onto each coverslip. Cells were incubated for 30 min at room temperature. After incubation, the Os,bipy solution was aspirated and 100 µl of Ringer's solution were added. Cells were washed 3× in Ringer's solution and transferred to a Petri dish containing Ringer's solution. The cells were then fixed by dipping the coverslips into methanol precooled to −20°C; the cold methanol was changed 3× within 20 min. The cells were washed 3× by dipping into Ringer's solution, followed by application of the first antibody. In some experiments, fixed cells were treated with enzymes or acid solutions as described below.

Antibody labelling

Polyclonal antibodies against the DNA-Os,bipy adduct (anti-DNA-Os,bipy) were raised in rabbits as described (Paleček et al., 1989). Antisera were purified on an immunoaffinity column (Kuderova-Krejcova et al., 1991). After methanol fixation, cells were washed 3× in Ringer's solution and 50 µl of anti-DNA-Os,bipy (in most experiments, the S89-II antibody fraction) in Ringer's solution containing 1 mg ml^{-1} BSA were added. After 60 min of incubation at 37°C, cells were washed 3× in Ringer's solution. The secondary antibody used was fluorescein-labelled, goat anti-rabbit IgG (Dianova, Hamburg, FRG) diluted 100-fold in Ringer's solution with 1 mg ml^{-1} BSA. After 90 min incubation at 37°C, cells were washed 3× in Ringer's solution and stained for DNA with 3 µM 4,6-diamidino-2-phenylindol (DAPI) for 10 min at room temperature. After rinsing thoroughly, the coverslips were mounted on glass slides in a glycerol solution containing the oxygen scavenger Mowiol 4-88 (Hoechst, Frankfurt, FRG) and stored at 4°C (Osborn and Weber, 1982).

Competition experiments

For competition experiments, thermally denatured calf thymus DNA, yeast RNA, or BSA at a concentration of 150 µg ml^{-1} in 50 mM sodium phosphate, pH 7.0, was incubated with 2 mM osmium tetroxide, 2 mM 2,2′-bipyridine for 15 h at 37°C and extensively dialysed at 4°C. The Os,bipy-modified biopolymer, at a concentration of 25 µg ml^{-1}, was incubated with the S89-II antibody for 60 min at 37°C, centrifuged for 20 min at 10^5 ***g*** in a Beckman Airfuge and the supernatant used as the primary antibody in the cell staining procedure described above.

Treatment of fixed cells

In some experiments, methanol-fixed cells were treated with 50 µl of the following enzymes: nuclease S_1, 6000 i.u. ml^{-1}, DNase I, 100-300 µg ml^{-1} (both Boehringer, Mannheim, FRG), or proteinase K (Merck, Darmstadt, FRG), 50-125 µg ml^{-1}. Incubations were for 30 min at 37°C under conditions optimal for the respective enzymes. After the incubations, cells were washed and processed further in the usual way. In another experiment, methanol-fixed cells were treated with acid solutions (including 45% (v/v) acetic acid or glycine-HCl buffers with 50 mM NaCl ranging from pH 1.8 to 3.3, and 50 mM sodium acetate, 50 mM NaCl, pH 3.8) for 20 min at room temperature. After this treatment, cells were thoroughly washed with Ringer's solution to restore the pH to neutrality and treated with Os,bipy under the same conditions as the unfixed cells (described above).

Microscopy and image analysis

Digital images were acquired with a Zeiss (Göttingen, FRG) Universal microscope equipped with a Photometrics (Tucson, Az) CH220 slow-scan scientific camera system incorporating a thermoelectrically-cooled CCD sensor with 384×576 23×23 µ pixels, a 14-bit digital resolution and an IEEE interface to a DEC Microvax II computer. A 63×1.3 NA Neofluar oil immersion objective was used. The excitation, dichroic and emission filter combinations were: fluorescein: 485/20 nm bandpass, 510 nm dichroic, 515-565 nm bandpass; DAPI: 365/11 nm bandpass, 395 nm dichroic, 420 nm longpass. Bias and dark current were subtracted from each image and a flat field correction was applied. Digital images of optical sections through the cells were collected with a Zeiss confocal laser scanning microscope (Model LSM44) using a 63×1.4 NA Planapo oil immersion objective for high resolution. The CLSM system has been described previously (Robert-Nicoud et al., 1989). Fluorescein was excited at 488 nm with an internal, air-cooled argon-ion laser. DAPI was excited at 351, 364 nm with an external, water-cooled argon-ion laser (Spectra Physics 2025) using special optics. The laser beam was attenuated with neutral density filters. Excitation, dichroic and emission filters were fluorescein: 488 nm bandpass, 510 nm dichroic, 515 nm

longpass and 525/15 nm bandpass; DAPI: none, half-silvered mirror, 410 nm longpass. All images of a given cell were taken with the same gain factor, and sequential optical sections were either 0.5 μm or 1 μm apart along the Z axis. The fluorescein, but not the DAPI images, were acquired in the confocal mode. Image processing, quantitation and display were carried out on a DEC Microvax II system or a DEC 3200 workstation using TCL-Image (Multihouse TSI, Amsterdam, The Netherlands). Three-dimensional stereopair images were generated from the set of two-dimensional serial optical sections, according to the method of Brakenhoff (1986), following thresholding and uniform filtering operations to remove background and noise.

RESULTS

Probing DNA structures in unfixed cells with Os,bipy

Treatment of 3T3 cells with 1 mM Os,bipy for 30 min at room temperature resulted in strong immunofluorescent staining with antibodies (whole antisera or purified fractions) specific for DNA-Os,bipy adducts. The staining was intense over the nucleus and weaker in the cytoplasm (Fig. 2A and B). Cytoplasmic staining was largely due to nonspecific binding of the primary antibody, as shown in cells that had not been exposed to Os,bipy where practically no nuclear staining was observed (Fig. 2C and D). The extent of this nonspecific staining was reduced by preabsorbing the antibody on methanol-fixed cells (data not shown).

The antibodies used in this study had been shown to exhibit no crossreactivity in vitro with unmodified DNA, RNA, proteins and with Os,bipy-modified proteins (Kuderova-Krejcova et al., 1991). A low degree of cross-reactivity with Os,bipy-modified RNA was found, which was lowest with affinity-purified fractions S89-II and S89-III. S89-II was used in the majority of the experiments described here.

Specificity of S89-II anti-adduct antibody

In order to verify whether the specificity of S89-II observed in vitro was retained in experiments with cells, competition experiments with DNA-Os,bipy, RNA-Os,bipy and

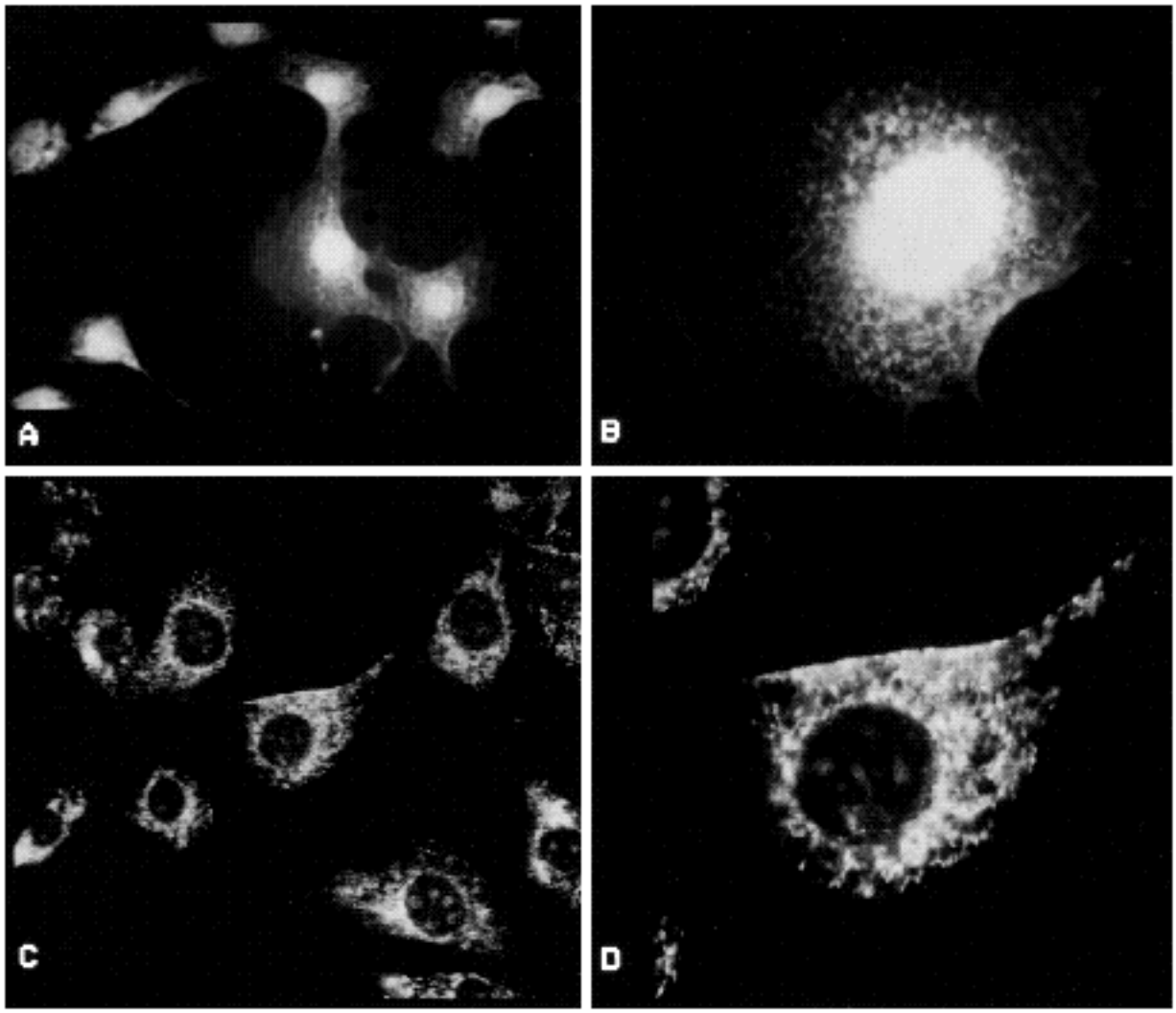

Fig. 2. Immunofluorescence of mouse 3T3 fibroblasts. (A,B) cells treated with Os,bipy prior to methanol fixation; (C,D) untreated control cells. (A,C) images taken with CCD camera (low magnification); (B,D) nonconfocal microscopy (high magnification).

Os,bipy-modified BSA were performed (Paleček, 1990). Prior incubation of S89-II with DNA-Os,bipy resulted in a substantial decrease in the nuclear staining of the cells whereas prior incubation with RNA-Os,bipy led to a slight decrease in the intensity of both nuclear and cytoplasmic staining. Exposure of the antibody to Os,bipy-modified BSA had no detectable effect on the staining pattern of the cells.

The specificity of the S89-II staining was also tested by treatment with various enzymes. Exposure of fixed cells to nuclease S1 resulted in a substantial decrease in nuclear staining. An even greater decrease was produced by DNase I, which at a concentration of 300 μg ml^{-1} almost eliminated the immunofluorescence in the nucleus. In contrast, application of RNase at a concentration of 125 μg ml^{-1} led only to a small diminution of the overall nuclear staining. No changes were observed after incubation with proteinase K.

Confocal laser scanning microscopy

Conventional epifluorescence microscopy did not permit determination of the intranuclear distribution of Os,bipy-specific immunofluorescence, because of the classical problem posed by overlapping out-of-focus contributions (Figs 2A,B and 3C). The confocal laser scanning microscope (CLSM) largely eliminates this problem (Robert-Nicoud et al., 1989; Fox et al., 1991). The CLSM was used to analyse the distribution of fluorescence in successive optical sections of a nucleus (Fig. 4). It was clear that the immunofluorescence was not distributed homogeneously inside the nucleus; some regions were brightly stained while others

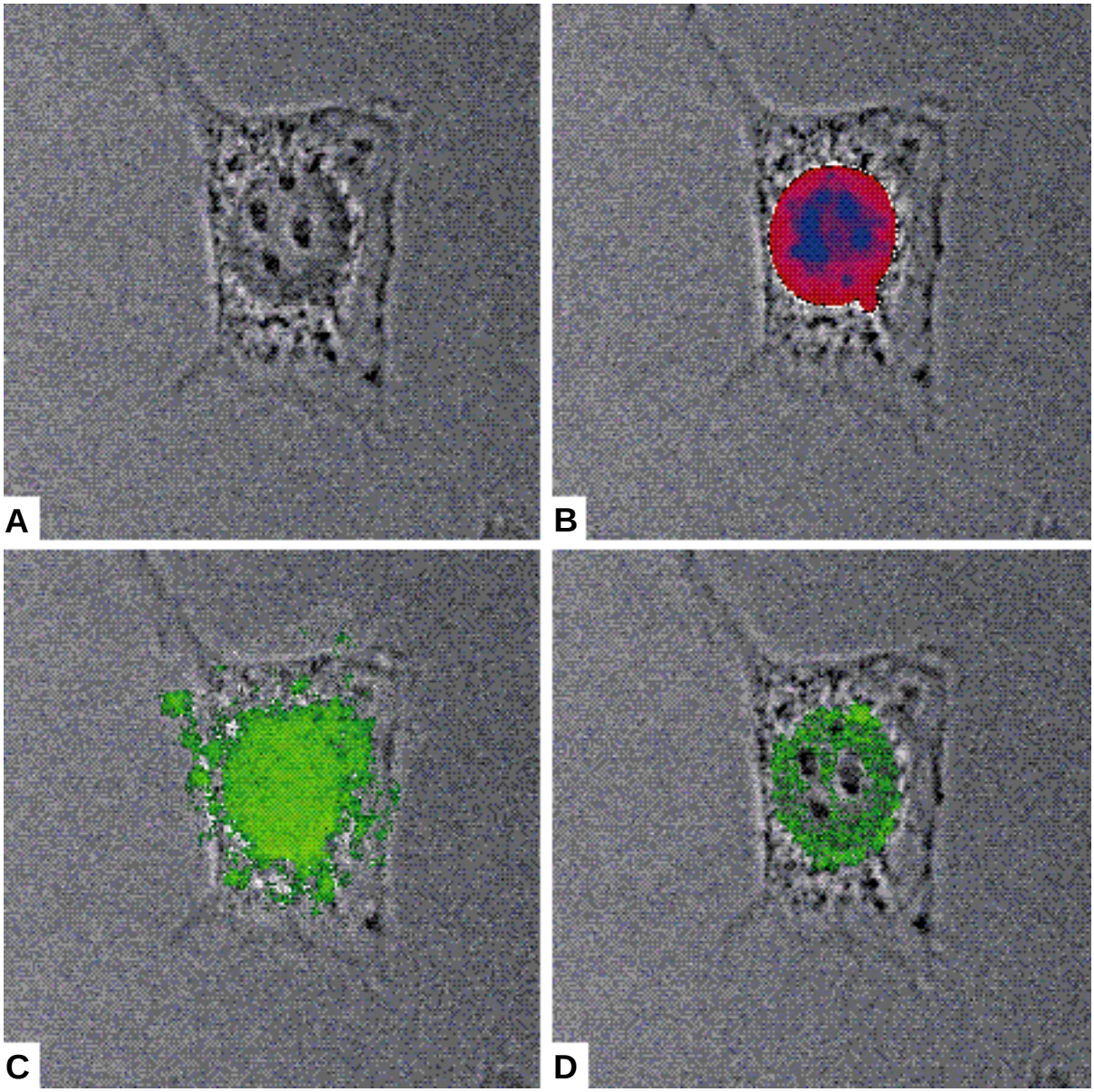

Fig. 3. 3T3 cells treated with Os,bipy prior to methanol fixation. (A) phase contrast; (B) superposition of DNA staining (DAPI) with phase contrast (A); (C) superposition of the nonconfocal immunofluorescence pattern with phase contrast (A); (D) superposition of the confocal immunofluorescence pattern with phase contrast (A).

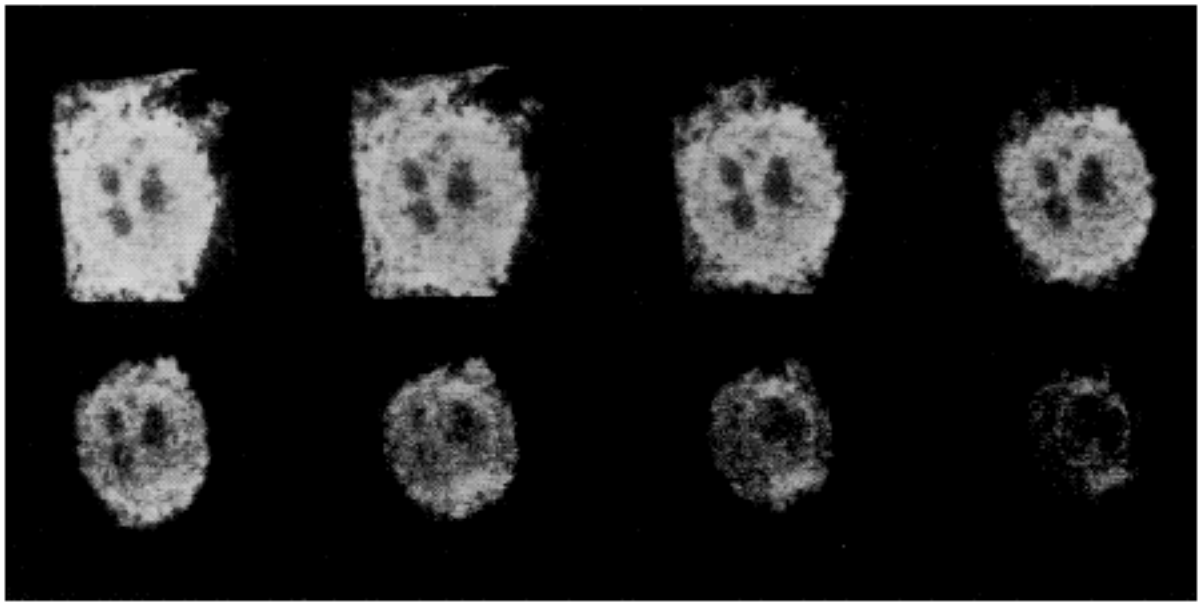

Fig. 4. 3T3 cells treated with Os,bipy prior to methanol fixation. Individual confocal optical sections showing immunofluorescence of an Os,bipy-treated cell. The sections of the cell nucleus are shown sequentially from left to right, top to bottom, in Z axis increments of 0.5 μm.

showed only weak or no fluorescence. Comparing such a fluorescence distribution with the corresponding phase-contrast image of the nucleus (Fig. 3A) and with the fluorescence pattern produced by a DNA-staining dye (DAPI) (Fig. 3B) leads to the conclusion that the unstained or weakly stained areas were nucleoli (Fig. 3D) and/or condensed A+T-rich chromatin regions (Fig. 5), and that the brightly stained regions corresponded to decondensed chromatin. This is clearly shown in the superimposed phase-contrast and fluorescence images in Figs 3 and 5. Thus, it appears that Os,bipy-DNA adducts are mainly formed in decondensed, presumably transcriptionally active chromatin, revealing the presence of open DNA structures in these regions.

Distribution of Os,bipy-DNA adducts in fixed acid-treated cells

At acidic pH, DNA is known to denature and to depurinate. Therefore, the reactivity of DNA is expected to increase in acid-treated cells. In heterochromatin containing A+T-rich DNA, acid treatment should induce the strongest increase in staining due to high DNA density in such regions and the preferential binding of Os,bipy to thymine. In fact, cells treated with 45% acetic acid after fixation in methanol showed a very intense immunofluorescent staining of condensed DAPI-positive chromatin. Similar patterns of Os,bipy-specific immunofluorescence were obtained after treatment of cells at pH 1.8 (Fig. 6C). At pH 2.5 the same distribution was observed but was weaker in intensity. Treatment of cells between pH 3.4 and 4.0 was without effect. Superpositions of the the phase contrast (Fig. 6A) image with the DAPI (Fig. 6B) and Os,bipy (Fig. 6D) patterns obtained after pH 1.8 treatment demonstrated excellent correspondence between the two probes. The spatial distribution of the brightly Os,bipy-stained spots is shown as a stereopair in Fig. 7.

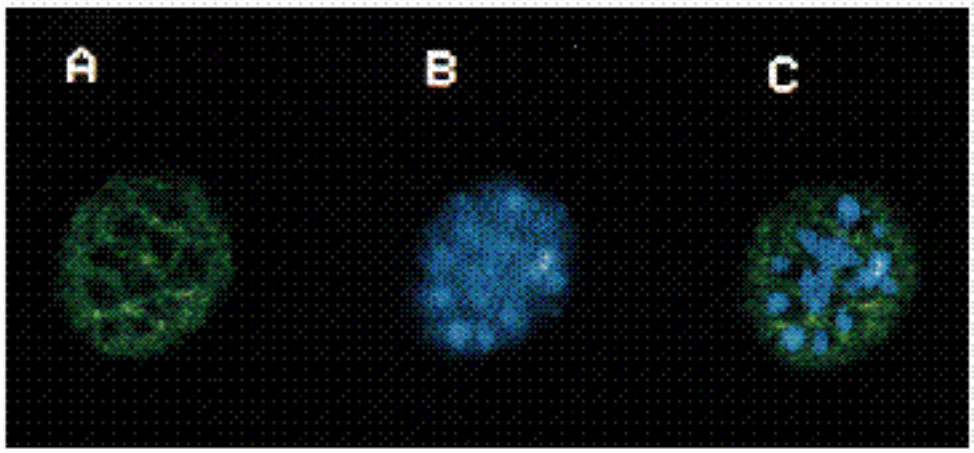

Fig. 5. 3T3 cells treated with Os,bipy prior to methanol fixation. (A) confocal immunofluorescence pattern; (B) DNA staining with DAPI; (C) superposition of the confocal immunofluorescence pattern (A) with the DNA staining (B).

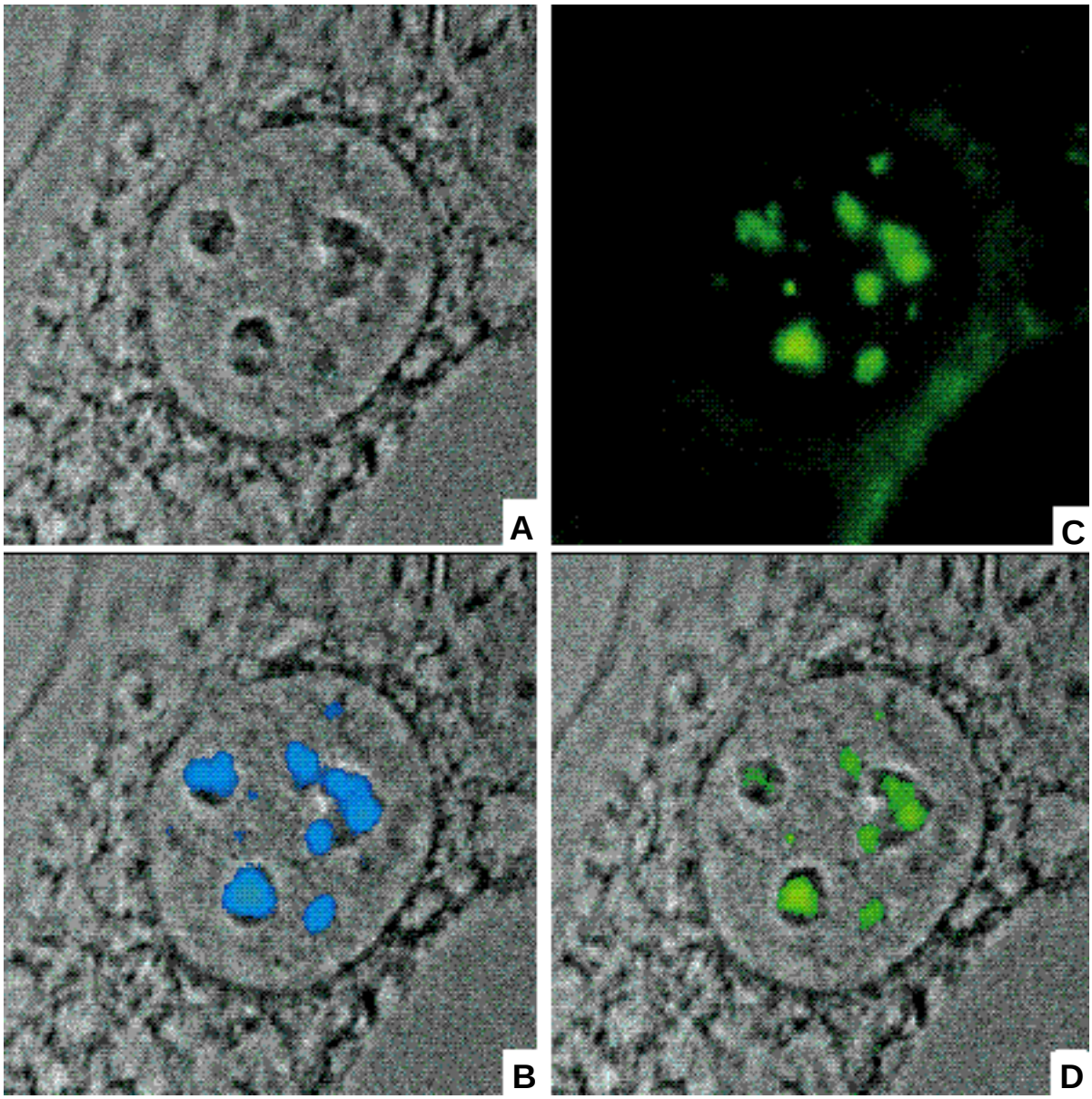

Fig. 6. 3T3 cells treated with Os,bipy after methanol fixation and exposure to pH 1.8. (A) phase contrast; (C) confocal immunofluorescence pattern; (B) superposition of the nonconfocal DAPI staining of DNA with phase contrast (A); (D) superposition of the confocal immunofluorescence pattern (C) with phase contrast (A).

DISCUSSION

Using indirect immunofluorescence, we have demonstrated that a chemical probe of DNA structure (Os,bipy) penetrates into eukaryotic cells and produces a specific staining in the cell nucleus. The fluorescence signal depends mainly on two factors: (1) the specificity of the chemical probe, and (2) the specificity of the first antibody recognizing the products of the probe reactions.

Specificity of Os,bipy for open DNA structures in vitro

Os,bipy reacts preferentially with thymine residues in single-stranded DNA. In the *B*-DNA double helix, the target C5-C6 double bond (Fig. 1) is not accessible to attack by the bulky osmium probe (reviewed by Paleček, 1991). Local changes in the DNA helix geometry, including single-base mismatches, base unstacking, structural distortion in the vicinity of drug-binding sites, and single-strand interruptions, increase the accessibility of the double bond to attack by the chemical probe. Supercoil-stabilized local DNA structures, including cruciforms, hairpins and triplexes as well as junctions between *B*-DNA and *Z*-DNA, are site-specifically modified with Os,bipy in vitro. In addition, other open structures and DNA complexes can be transiently formed during the cell cycle, gene expression and other mechanisms of genetic processing (Paleček, 1991; Yagil 1991). An open complex of RNA polymerase with a

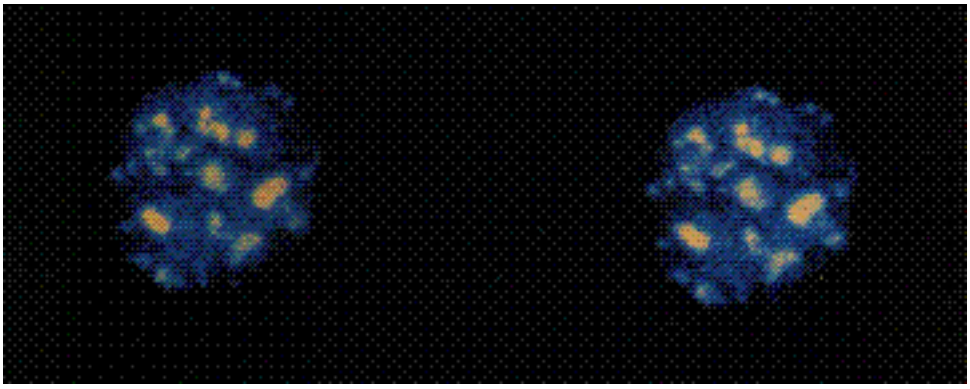

Fig. 7. Stereo images of 3T3 cells treated with Os,bipy after methanol fixation and exposure to pH 1.8.

promoter sequence may serve as an example of such a structure; it has been shown that bases at specific positions in the template strand react in vitro with Os,bipy (Heumann et al., 1992) and other single-strand-selective chemical probes (Buckle and Buc, 1989; Sasse-Dwight and Gralla, 1989). Numerous studies based on chemical probing of DNA in vitro suggest that Os,bipy efficiently recognizes open DNA structures without reacting significantly with *B*-DNA (for review see Paleček et al., 1990; Paleček, 1991, 1992a).

Reactions of Os,bipy in the cell

Recently, Os,bipy has been used to demonstrate the existence of *Z*-DNA (Paleček et al., 1987, 1988; Rahmouni and Wells, 1989), cruciforms (Paleček et al., 1988; McClellan et al., 1990) and triplexes (Paleček, 1990; Karlovsky et al., 1990) in *E. coli* cells. These structures were site-specifically modified inside the cell with Os,bipy, but the detection of the chemical modification was performed in vitro after DNA isolation using biochemical techniques. In contrast to the reactions in vitro, in the complex cell environment the chemical probe can react not only with DNA but also with other cell components. In addition to the preferential, relatively fast reaction of Os,bipy with thymine residues in DNA, slower reactions with uracil, cytosine and guanine residues (Paleček et al., 1990; Jelen et al., 1991) may take place in single-stranded regions of RNA and DNA. In proteins, tryptophan and sulphur-containing amino acids represent the main target of the osmium probe (Deetz and Behrman, 1981), unless the particular side chains are buried in the protein molecule. Therefore, the specificity of the immunofluorescent signal in cells for the DNA-Os,bipy adducts reflects primarily the corresponding specificity of the antibody used to reveal the adducts.

Antibodies to DNA-Os,bipy adducts

In vitro studies demonstrated that the affinity-purified S89-II fraction is highly specific for the DNA-Os,bipy adducts (Kuderova-Krejcova et al., 1991). Results of the competition experiments and the effect of enzymatic treatment on the staining of 3T3 cells presented here are in a good agreement with those of the specificity tests in vitro. After methanol fixation, Os,bipy-modified nuclear DNA was readily accessible to protein molecules, as judged from the marked susceptibility to nucleases and the insensitivity of the staining to hydrolysis of cellular proteins with proteinase K. Considering the specificity of the antibody and of the chemical probe (Paleček, 1991) we feel confident that the immunofluorescence pattern observed in the 3T3 cells is representative of the distribution of open DNA structures in the cell. A small contribution from RNA-Os,bipy adducts, however, cannot be excluded.

Open DNA structures in eukaryotic cells

The intensive staining observed in the cell nucleus indicates the presence of open DNA structures in the eukaryotic cell. It was shown previously that certain fractions of DNA isolated from various organisms have a single-stranded character (reviewed by Paleček, 1976). The amount of DNA cleavable with single-strand-selective nuclease S1 increases during the period of DNA synthesis in the cell cycle of human diploid fibroblasts (Collins, 1977). However, in experiments performed with isolated DNA it is difficult to exclude the possibility that regions with single-stranded character are formed secondarily during the isolation procedure. In the experiments reported here, the chemical probe was applied to living cells at a 1 mM concentration, i.e. under conditions that are known to induce site-specific modification of open DNA structures in vitro without inducing secondary changes in the DNA double helix. Thus, our results suggest that open DNA exists in the living cell. While such structures have already been demonstrated in plasmid DNA of prokaryotic cells by chemical probes (Paleček et al., 1987, 1988; Sasse-Dwight and Gralla, 1989; Karlovsky et al., 1990; McClellan et al., 1990; Paleček et al., 1990) and by molecular genetic methods (Jaworski et al., 1987; Panayotatos and Fontaine, 1987), this paper provides the first direct evidence for the existence and localization of open regions in the genomic DNA of eukaryotic cells.

The results do not, however, lead to conclusions about the nature of the open DNA structures in 3T3 cells, although one can surmise that they arise as a consequence of biological processes such as DNA replication, transcription and recombination (Yagil, 1991). On the other hand, an appreciable part of the observed staining could be due to the presence of supercoil-stabilized DNA structures such as triplexes, cruciforms and *B-Z* junctions, which have been detected by indirect immunofluorescence in fixed cells (Nordheim et al., 1981; Jovin et al., 1982; Arndt-Jovin et al., 1983; Robert-Nicoud et al., 1984; Lee et al., 1987; Ward et al., 1990).

Advantages of probing DNA structure in the cells with Os,bipy

The use of antibodies as probes of DNA structure in cells generally requires prior fixation to render the targets accessible. In contrast, chemical probes such as Os,bipy can be applied to living cells. In this case, fixation is only required after application of the chemical probe and therefore does not perturb the loci and extent of reaction. The Os,bipy adducts resist fixation based on treatment with acid and/or organic solvent. Removal of proteins accompanying fixation, and the subsequent changes in the DNA superhelix density that induce the formation of an alternative DNA structure, cannot influence the resulting staining because the primary antibody does not recognize the DNA structure per se, but only the DNA-Os,bipy adducts. In other words, if a specific DNA conformation is secondarily induced in the cell as a result of acid fixation, it will not be recognized by the antibody to DNA-Os,bipy because the chemical probe was applied prior to fixation, that is, in the absence of the new conformation. On the other hand, if nucleotides in an open DNA structure are selectively modified in the cell by Os,bipy and the structure disappears during fixation, then the Os,bipy-modified DNA will be recognized by the antibody, thereby detecting the structure present originally. Thus, an optimal fixation procedure can be chosen regardless of its influence on the DNA structure. The power of the Os,bipy probing method can be greatly enhanced by application of modern scanning optical microscopy and image analysis techniques.

Specific staining of 3T3 cells

The CLSM images of 3T3 cells to which Os,bipy was applied prior to fixation showed discrete unstained or weakly stained areas, corresponding in some cells to nucleoli (Figs 3 and 4) and to condensed chromatin regions (Fig. 5) intensively stained with DAPI. The localization of unstained areas in Os,bipy-probed cells to nucleoli (Figs 2 and 3) is an interesting finding. In nucleoli, RNA is present at high concentration and should contain single-stranded regions accessible to the Os,bipy probe. The absence of staining in nucleoli constitutes further evidence for the specificity of the immunofluorescent signals and suggests that open DNA structures are absent from nucleoli under certain conditions. Treatment of cells with 45% acetic acid or with acidic buffers up to pH 2.5 resulted in a drastic change in the immunofluorescent pattern (Figs 6 and 7). The unstained areas seen in cells not treated with acid became brightly stained and, in fact, displayed an even more intensive fluorescence than the regions of decondensed chromatin in the nucleus. We conclude from this finding that denaturation and/or depurination is/are necessary to transform the non-reactive double-helical DNA into an Os,bipy-reactive species. Furthermore, regions of native condensed chromatin with a high content of A+T-rich double-stranded DNA appear to contain little or no open DNA. Rather, the latter is located primarily in the more transcriptionally active diffuse decondensed chromatin regions.

We have demonstrated in this study that a chemical probe can be used as a powerful tool for investigating the structural states of DNA in a living cell. Efforts should be made to develop further immunogenic chemical probes applicable to living cells with different selectivities for bases and/or specific DNA structures. Chemical probing has been applied successfully in DNA structure studies in vitro (Paleček, 1991, 1992a) and in bacterial cells (Paleček, 1991, 1992b). Its more widespread use with eukaryotic cells may produce new insights into the structure and function of DNA. Preliminary experiments (M. Robert-Nicoud, T. M. Jovin and E. Paleček, unpublished data) suggest that the use of Os,bipy is not limited to cell cultures but that the probe can be applied to isolated chromatin and to the salivary glands of *Chironomus thummi*, thereby yielding specific staining of certain bands.

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