Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 8407–8411, September 1993
Cell Biology

DNA topoisomerase IIα is the major chromosome protein recognized by the mitotic phosphoprotein antibody MPM-2

(cell cycle/phosphorylation/chromosome scaffold/kinase/mitosis)

SALME TAAGEPERA*, POTU N. RAO†, FRED H. DRAKE‡, AND GARY J. GORBSKY*§

*Department of Anatomy and Cell Biology and §University of Virginia Cancer Center, University of Virginia, Box 439 Health Sciences Center, Charlottesville, VA 22908; †Department of Medical Oncology, University of Texas, M.D. Anderson Cancer Center, 1515 Halcombe Boulevard, Houston, TX 77030-4095; and ‡Department of Cellular Biochemistry, SmithKline Beecham Pharmaceuticals, P.O. Box 1539, King of Prussia, PA 19406

Communicated by Hans Ris, June 3, 1993 (received for review June 20, 1992)

ABSTRACT **We have determined that the major mitotic phosphoprotein in chromosomes recognized by the anti-phosphoprotein antibody MPM-2 is the 170-kDa isoform of topoisomerase II (topo II), the isoform predominant in proliferating cells. As a prerequisite to making this discovery, it was necessary to develop protocols to protect chromosomal proteins from dephosphorylation during cell extraction and chromosome isolation procedures. Immunofluorescence analysis of the large chromosomes prepared from Indian Muntjac cells revealed colocalization of MPM-2 and anti-topo II antibodies to the chromosomal centromeres and to the axial regions of the chromosomal arms. For biochemical fractionation studies, large quantities of chromosomes from the P388D1 mouse lymphocyte cell line were isolated and treated to remove DNA and histone proteins. Immunoblot and immunoprecipitation experiments with this chromosome scaffold fraction identified the major MPM-2-reactive phosphoprotein to be DNA topo II. Using a panel of anti-peptide antibodies specific to the isoforms of topo II, we determined that the major phosphoprotein recognized by MPM-2 is the 170-kDa isoform of topo II, topo IIα. The 180-kDa isoform, topo IIβ, present in the isolated chromosomes in much smaller quantities, is also recognized by MPM-2. The mitotic phosphorylation of the topo II proteins may be critical for proper chromosome condensation and segregation.**

During the $G_2 \rightarrow M$ transition of the cell cycle, the interphase chromatin condenses ≈10,000-fold into compact mitotic chromosomes containing DNA, histones, and a variety of nonhistone proteins. Extraction of isolated chromosomes with high salt buffers leaves a fraction of nonhistone proteins termed the chromosome scaffold fraction (1). This fraction contains two major proteins, Sc1 (170 kDa) and Sc2 (135 kDa) (2, 3). Sc1 was identified as the protein DNA topoisomerase II (topo II) (4, 5), an essential highly conserved protein present in the nuclei of most eukaryotic cells (6). Topo II has been implicated in the regulation of gene structure and function through its ability to alter DNA topology. Functioning as a dimer, topo II catalyzes the passage of one intact DNA helix through a transient double-stranded break in a second DNA helix in an ATP-dependent manner. Topo II appears to be involved in many aspects of nucleic acid metabolism, including initiation of DNA replication (7, 8), DNA repair (9), recombination (10), and transcription (11, 12). Additionally, topo II is essential for the restructuring of chromatin in mitosis (13). The condensation of chromatin into chromosome-like structures in cell-free mitotic extracts is dependent on the activity of topo II (14–16). Genetic studies of the yeast *Schizosaccharomyces pombe* demonstrated that topo II is required for the later stages of chromosome condensation during mitosis (17) and for separation of sister chromatids during anaphase (18, 19). Topo II may also play a karyoskeletal role in both interphase nuclei and mitotic chromosomes. Some immunofluorescence and immunoelectron microscopy studies of mitotic chromosomes indicate that topo II is concentrated along a dense axial core in the chromosome arms. This localization has been cited as evidence that topo II plays a structural role in the mitotic chromosome. However, this view has recently been challenged by evidence that topo II is evenly distributed throughout the chromosome arms and that its continuous presence is not essential in maintaining the structural integrity of mitotic chromosomes after they have been assembled (20, 21). Recent work has demonstrated the existence of two topo II isoforms in mammalian cells (22)—the 170-kDa isoform (topo IIα) and the 180-kDa isoform (topo IIβ) (23). These topo II isoforms are products of separate genes (22) and are differentially regulated during cellular proliferation (24).

MPM-2 is a monoclonal antibody originally prepared to mitotic HeLa cell extracts (25). MPM-2 recognizes a phosphorylated epitope found predominantly in proteins that are phosphorylated at mitosis (26). Immunofluorescence and immunoelectron microscopy analyses of mitotic cells revealed MPM-2 labeling of soluble cytoplasmic proteins and structural components of microtubule-organizing centers, including the centrosome, midbody, centromeres, and chromosomal arms (27).

The present studies were focused on identifying nonhistone chromosomal proteins that are the targets of important mitotic phosphorylations. By developing a protocol that enabled us to maintain the phosphorylation of chromosomal proteins during cell extraction and chromosome isolation procedures, we were able to identify chromosomal scaffold proteins that are phosphorylated in a mitosis-specific manner, as detected by MPM-2. Our results show that the 170-kDa isoform of topo II, topo IIα, is the major MPM-2-reactive phosphoprotein in mitotic chromosome scaffolds. Topo IIβ, the minor topo II isoform in chromosome scaffolds, also contains the MPM-2 phosphoepitope. Cell-cycle-dependent phosphorylation of topo IIα and -β at the $G_2 \rightarrow M$ phase transition may be critical for proper chromosomal condensation and segregation.

MATERIALS AND METHODS

Cell Culture and Synchronization. P388D1 cells (ATCC CCL 46) were grown in Dulbecco's modified Eagle's medium (DMEM) enriched with nonessential amino acids and supplemented with 10% (vol/vol) defined equine serum (HyClone). Ten liters of cells were grown to a concentration of 1.5×10^6 cells per ml, synchronized in S phase with a 12-h incubation in aphidicolin (1 μg/ml), washed, and synchro-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: topo II, DNA topoisomerase II.

nized in M phase with a 10-h incubation in Colcemid (0.15 μg/ml). Indian Muntjac cells were grown in enriched DMEM, supplemented with 5% (vol/vol) fetal bovine serum (HyClone) and 5% (vol/vol) NuSerum (Collaborative Research). Cells were grown to 75% confluency and blocked in M phase with Colcemid (0.15 μg/ml) for 15 h.

Chromosome Isolation. Chromosome isolation procedures were modified from those of Laemmli (28). Mitotic P388D1 or Indian Muntjac cells were washed twice at 37°C with 15 mM Tris·HCl, pH 7.4/80 mM KCl/2 mM EDTA/0.2 mM spermine/0.5 mM spermidine/5 mM 5,5′-dithio-bis(2-nitrobenzoic acid)/1 mM sodium orthovanadate/10 nM microcystin/protease inhibitors (aprotinin, leupeptin, antipain, pepstatin, and chymostatin; each at 5 μg/ml) (extraction buffer), diluted to half strength. All the following steps were done at 4°C. Swollen cells were lysed with 1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) in extraction buffer. Muntjac cell lysates were frozen in liquid nitrogen and stored at −80°C. P388D1 cell lysates were further purified by removing interphase nuclei with a 3-min centrifugation at 200 × g. Contaminants in the crude chromosomal lysate were removed by centrifugation in polypropylene tubes through a 40/80% (vol/vol) glycerol step gradient made with extraction buffer (20 min at 4000 × g). Chromosomes were collected from within the 80% glycerol fraction, frozen in liquid nitrogen, and stored at −80°C. For the P388D1 chromosomes pictured in Fig. 1 *A* and *B*, crude chromosomal lysates were layered over a 20–50% (wt/vol) metrizamide gradient made with extraction buffer and centrifuged (20 min at 16,500 × g).

Immunofluorescence. Isolated P388D1 chromosomes were pelleted onto coverslips by centrifugation at 1200 × g for 5 min in 15 mM Pipes, pH 7.1/120 mM KCl/20 mM $MgCl_2$ and fixed with 2% (vol/vol) formaldehyde in the same buffer. The coverslips were rinsed in 10 mM Mops, pH 7.4/150 mM NaCl/0.05% Tween 20 (MBST), blocked with 20% (vol/vol) normal goat serum in MBST, double-labeled with antibodies MPM-2 and ACA, rinsed, and labeled with fluorescein-conjugated goat anti-mouse and Texas red-conjugated anti-human secondary antibodies, respectively. In all cases, the antibodies were in MBST containing 5% normal goat serum. Coverslips were counterstained with the DNA dye 4,6-diamidino-2-phenylindole at 5 μg/ml and mounted on slides with Vectashield mounting media (Vector Laboratories) containing 10 mM $MgSO_4^{2+}$. Isolated Muntjac chromosomes were spun onto coverslips by centrifugation for 5 min at 2500 × g. Chromosomes, fixed and processed as above, were double-labeled with the monoclonal MPM-2 and the polyclonal anti-topo II-29 antibodies, followed by fluorescein-conjugated anti-mouse and Texas red-conjugated anti-rabbit antibodies, respectively, and counterstained with 4,6-diamidino-2-phenylindole.

Microscopy. Images were obtained with a Nikon Diaphot microscope equipped for epifluorescence, coupled to an intensified charge-coupled device camera (Dage–MTI, Michigan City, IN) and an Image 1 image processor (Universal Imaging, Media, PA). Fluorescent filters that permitted simultaneous triple-label fluorescence were obtained from Omega Optical (Brattleboro, VT). Photographs were recorded from a video monitor on Kodak technical pan film 2415 or Kodak Tmax100 film and developed with Kodak Technidol or Kodak Tmax developer, respectively.

Chromosome Scaffold Fraction Preparation. Chromosomes were washed with extraction buffer and treated at 37°C with DNase I (40 μg/ml) in 20 mM Tris, pH 7.9/3 mM $MgCl_2$/3 mM $CaCl_2$/1 mM dithiothreitol/10 nM microcystin/protease inhibitors (5 μg/ml). All the following steps were done at 4°C. Nuclease-treated chromosomes were pelleted by centrifugation at 1100 × g for 10 min. Chromosomal scaffolds were prepared by extracting the nuclease-treated chromosomes twice with 10 mM Tris, pH 9.0/2 M NaCl/10 mM EDTA/0.1% CHAPS/1% thiodiglycol (3) containing microcystin and protease inhibitors. Scaffolds were pelleted by centrifugation for 15 min at 8100 × g and prepared for immunoprecipitation (see below) or for electrophoresis. Chromosome and scaffold gel samples were solubilized by sonication, boiled in SDS sample buffer, and analyzed by electrophoresis on SDS/5–20% gradient polyacrylamide or 8 M urea/SDS/4% polyacrylamide gels.

Immunoblot Analysis. After electrophoresis, proteins were transferred to nitrocellulose and blocked overnight in 10 mM Tris, pH 8/150 mM NaCl/0.05% Tween 20 (TBST) containing 5% (wt/vol) dried milk or 3% (wt/vol) bovine serum albumin. Nitrocellulose blots were incubated at 24°C in MPM-2 or in anti-topo II antibodies. These polyclonal anti-peptide antibodies were made against a sequence common to both topo II isoforms (topo II-29) or against sequences unique to topo IIα (topo II-22) or topo IIβ (topo II-21) (22). Topo II-21 was used in serum form, whereas topo II-29 and topo II-22 were purified by affinity chromatography. Blots were rinsed in TBST, incubated with anti-mouse or anti-rabbit horseradish peroxidase secondary antibodies and treated with the Enhanced Chemiluminescence Western blot detection system (Amersham).

Immunoprecipitation of Chromosomal Scaffold Proteins. All of the buffers below contain 10 nM microcystin and protease inhibitors (5 μg/ml). Chromosomal scaffolds were solubilized with 8 M urea in 50 mM Tris, pH 8/150 mM NaCl/1% Nonidet P-40/0.5% deoxycholate/0.1% SDS (RIPA buffer) and dialyzed overnight against TBST. For the experiments involving the immunoprecipitation of denatured proteins, chromosomal scaffold fractions were solubilized in 2% SDS in 50 mM Tris, pH 8/150 mM NaCl, boiled, sonicated, and then treated with 5% Triton X-100/RIPA buffer to sequester the SDS. Samples were immunoprecipitated with the MPM-2 antibody and recombinant protein G beads (Zymed). The beads were washed with TBST, boiled in SDS sample buffer, and analyzed by electrophoresis on SDS/5–20% gradient polyacrylamide gels. Proteins were transferred to nitrocellulose and labeled as described above.

RESULTS

Maintenance of Phosphorylation in Isolated Chromosomes. Conventional cell extraction and chromosome isolation procedures resulted in substantial loss of MPM-2 reactivity (data not shown), presumably due to the action of endogenous phosphatases. In preliminary experiments, we found that inorganic compounds, commonly used to inhibit phosphatase activity, worked poorly to preserve immunoreactivity with MPM-2 and resulted in the destabilization of chromosomes during extraction procedures. We developed a cell extraction and chromosomal isolation protocol that allows us to maintain phosphorylation of chromosomal proteins, as seen by immunofluorescence labeling with MPM-2. This protocol includes the use of 5,5′-dithio-bis(2-nitrobenzoic acid), a sulfhydryl reagent known to protect histones H1 and H3 from dephosphorylation during chromosomal isolation procedures (29), and microcystin, a potent inhibitor of type PP1 and PP2A protein phosphatases. Using P388D1 mouse lymphocyte cells, we routinely achieved a mitotic synchrony of 90–95% with a two-step synchronization in aphidicolin and Colcemid. A typical 10-liter culture of cells yielded ≈60 mg of chromosomal protein. The isolated P388D1 chromosomes have maintained their phosphorylation at the centromeres and chromosomal arms, as seen by immunofluorescence with MPM-2 (Fig. 1). Higher magnification of these isolated chromosomes reveals a regular pattern of MPM-2 immunoreactivity along the chromosomal arms, consistent with previous reports of the helical distribution of topo II within mitotic chromosomes (30).

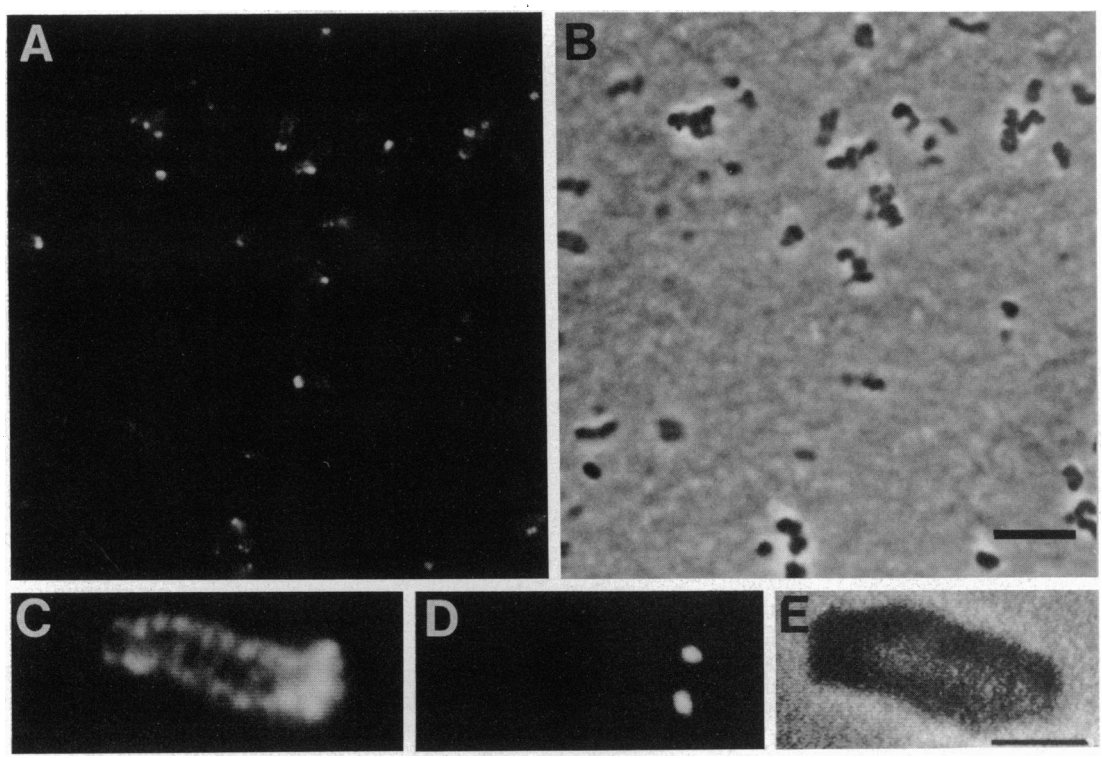

FIG. 1. MPM-2 labeling of isolated P388D1 chromosomes. P388D1 chromosomes were isolated in the presence of phosphatase inhibitors and labeled by indirect immunofluorescence with the mitotic phosphoprotein antibody MPM-2 or the anti-centromeric antibody ACA. (*A*) MPM-2-immunoreactive material was localized to chromosomal arms and centromeres. (*B*) Corresponding phase-contrast image. (Bar = 10 μm.) Higher magnification image of individual P388D1 chromosomes labeled with MPM-2 (*C*) or the anti-centromeric antibody ACA (*D*) and the corresponding phase image (*E*). (Bar = 2 μm.)

Colocalization of MPM-2 and Anti-Topo II Immunoreactive Material in Mitotic Chromosomes. Indian Muntjac chromosomes were isolated and labeled with MPM-2 and anti-topo II-29, a polyclonal anti-peptide antibody made against a sequence common to both topo II isoforms. The MPM-2 and anti-topo II-29 antibodies showed similar localization patterns (Fig. 2). In isolated chromosomes, both antibodies labeled an axial structure running the length of the chromosome arms.

Isolation and Characterization of Chromosomal Phosphoproteins. To determine which chromosome proteins were the targets of the mitotic phosphorylation recognized by MPM-2, chromosome and chromosome scaffold fraction proteins were analyzed on immunoblots with MPM-2. The major protein identified by MPM-2 was a 170-kDa protein (Fig. 3). To determine whether the major MPM-2-reactive chromosomal phosphoprotein was indeed topo II, we probed solubilized chromosome and chromosomal scaffold proteins with the anti-topo II-29 antibody. Both MPM-2 and anti-topo II-29 identified a protein with a similar electrophoretic mobility.

MPM-2 Immunoprecipitation of Chromosomal Scaffold Proteins. Topo II has at least two distinct isoforms: the 170-kDa isoform, topo IIα, and the 180-kDa isoform, topo IIβ. P388D1 cells have previously been shown to contain both topo II isoforms (23). By using antibodies that bind to the 170-kDa isoform (anti-topo II-22), the 180-kDa isoform (anti-topo II-21) or to both (anti-topo II-29), we found that both isoforms are present within the P388D1 mitotic chromosome scaffold fraction (Fig. 4*A*).

To determine which of these topo II isoforms binds the MPM-2 antibody, chromosomal scaffold proteins were solubilized and immunoprecipitated with MPM-2. The resulting MPM-2 immunoprecipitated material was probed with MPM-2 and the topo II peptide antibodies specific to the two isoforms (Fig. 4*B*). Anti-topo II-22, which is specific for the 170-kDa isoform, recognized the MPM-2 immunoprecipitated protein. The starting material was almost completely depleted of the 170-kDa topo II isoform after MPM-2 immunoprecipitation, suggesting that most of this isoform is phos-

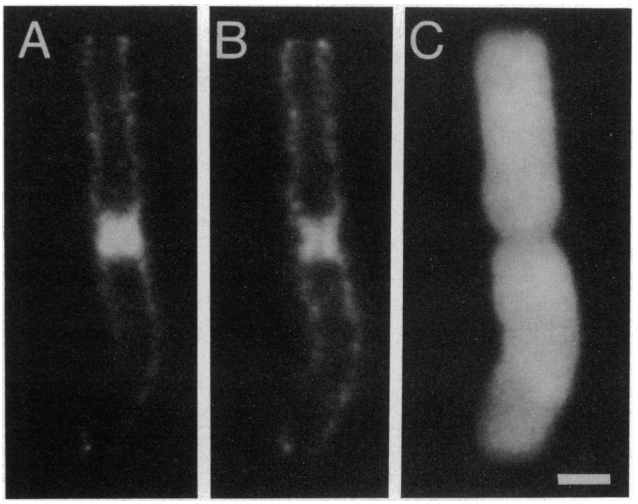

FIG. 2. Colocalization of MPM-2 and anti-topo II-29 antigens on isolated Indian Muntjac chromosomes. Indian Muntjac chromosomes were isolated in the presence of phosphatase inhibitors and labeled by indirect immunofluorescence. (*A*) MPM-2 immunofluorescence, showing labeling of the axial chromosomal scaffold and of the centromere. More than 80% of chromosomes showed clear axial and centromere labeling. (*B*) Immunofluorescence with the anti-peptide antibody that recognizes both isoforms of topo II, revealing a similar localization pattern of the immunoreactive material. (*C*) Labeling of the same chromosome with the DNA dye 4,6-diamidino-2-phenylindole. (Bar = 2 μm.)

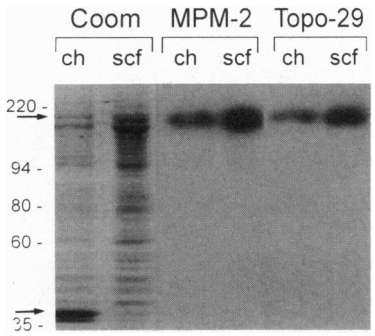

FIG. 3. Western blot analysis of P388D1 chromosome scaffold proteins. Chromosomes (lanes ch) and chromosome scaffold (lanes scf) protein samples were electrophoresed on a SDS/5–20% polyacrylamide gel. The gel was stained for total protein with Coomassie blue dye (lanes Coom) or blotted to nitrocellulose membrane and probed with the MPM-2 or the anti-topo II-29 antibody (lanes topo-29), which is specific for a peptide sequence found in both topo II isoforms. Positions of topo II and histone H1 bands are indicated with arrows.

phorylated in a MPM-2-reactive manner. Anti-topo II-21 labeling showed that the 180-kDa isoform of topo II was also immunoprecipitated by MPM-2. Most, if not all, of the topo IIβ could be removed from solubilized chromosomes by exhaustive immunoprecipitation with MPM-2 (data not shown), suggesting that, similar to the 170-kDa isoform, most of the 180-kDa topo II proteins in mitotic chromosomes contain the MPM-2 phosphoepitope.

In vivo, topo II is a dimeric protein. It has not been determined whether the two isoforms can combine to form heterodimers. To test whether the formation of heterodimers between the 170- and 180-kDa isoforms might account for the ability of MPM-2 to immunoprecipitate both isoforms, chromosome scaffold proteins were extensively denatured by boiling in SDS prior to immunoprecipitation. After diluting the SDS with Triton X-100, both isoforms were immunoprecipitated with MPM-2 (Fig. 4*C*), indicating that each isoform of topo II independently contains the MPM-2 epitope. Control antibodies or recombinant protein G beads alone did not precipitate any MPM-2-, anti-topo II-22-, or anti-topo II-21-reactive material (data not shown).

DISCUSSION

Many of the structural changes that occur in eukaryotic cells during the $G_2 \rightarrow M$ transition are regulated by cell-cycle-specific kinases. Phosphorylation of key chromosome proteins may be responsible for the dramatic changes of chromosome structure seen at the onset of mitosis. By utilizing a combination of phosphatase inhibitors, we were able to isolate chromosomes that had maintained mitosis-specific phosphorylation of their proteins, as revealed by labeling with the anti-phosphoprotein antibody MPM-2. Immunofluorescence analysis of the isolated P388D1 mouse lymphocyte and Indian Muntjac chromosomes revealed intense MPM-2 labeling of the centromere and relatively weaker labeling on narrow tracks along chromosome arms. These results correspond to similar MPM-2 labeling reported in isolated CHO chromosomes (15). We demonstrated colocalization of the MPM-2 and topo II immunoreactive material. The precise location of this label relative to chromosomal DNA and whether it appears coiled depends on the composition of the buffers to which isolated chromosomes are exposed (30–32). Both MPM-2 and anti-topo II-29 immunolabeling were concentrated at the chromosomal centromere, with the MPM-2 labeling being relatively greater in this region. Immunoblot analysis and immunoprecipitation experiments confirmed that the major chromosomal scaffold protein recognized by MPM-2 was the 170-kDa isoform of DNA topo II, topo IIα. The minor topo II isoform, topo IIβ, which represents 20–25% of total topo II in proliferating cells (33, 34), was also present in mitotic chromosome scaffolds and contained the MPM-2 phosphoepitope.

The 170-kDa isoform of topo II exists as a phosphoprotein *in vivo* (35–38). In cycling vertebrate cells in culture, topo II initially incorporates ^{32}P during DNA replication, with the level of phosphorylation peaking at G_2 and M phases (37). *In vitro*, the purified 170-kDa isoform of topo II is a high-affinity substrate for casein kinase II (36, 39, 40), protein kinase C (35, 41), calcium/calmodulin-dependent protein kinase type II (41), and cdc2 kinase (40). The *in vitro* phosphorylation of topo IIα by casein kinase II (39) and protein kinase C (35) results in a 3-fold increase in its enzymatic activity, as measured by DNA relaxation of supercoiled bacterial plasmid pPR322 DNA. Analysis of topo II purified from unsynchronized mammalian cell lines revealed that serine was the only phosphoamino acid detected (42, 43); however, recent work indicates that topo II isolated from budding yeast contains significant levels of phosphothreonine, especially during mitosis (40). It is not yet clear which kinases catalyze the modification that renders topo II isoforms recognizable by MPM-2. Some of these experiments were done in the

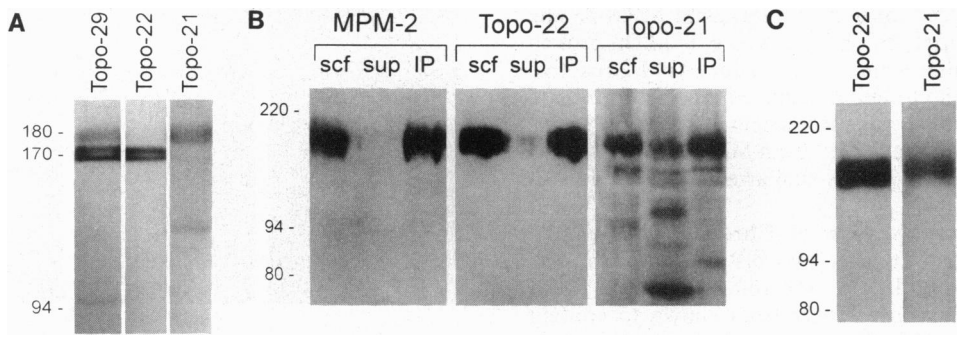

FIG. 4. Characterization of the MPM-2 reactivity of the two topo II isoforms. (*A*) Chromosome scaffold protein samples were electrophoresed on an 8 M urea/SDS/4% polyacrylamide gel to increase resolution of the two topo II isoforms. The resulting nitrocellulose blot was probed using our panel of topo II anti-peptide antibodies including: anti-topo II-29 (lane topo-29), against a peptide sequence common to both topo II isoforms; anti-topo II-22 (lane topo-22), an anti-peptide antibody specific for the 170-kDa isoform of topo II, topo IIα; anti-topo II-21 (lane topo-21), an anti-peptide antibody specific for the 180-kDa isoform, topo IIβ. Under these conditions, topo IIα runs as a doublet. (*B*) Chromosome scaffold fractions were solubilized and immunoprecipitated with the MPM-2 phosphoprotein antibody. The resulting immunoprecipitated proteins were electrophoresed on minislab SDS/5–20% gradient polyacrylamide gels, transferred onto nitrocellulose, and probed with MPM-2 or the isoform-specific topo II antibodies indicated. (*C*) Chromosome scaffold proteins were denatured by boiling in SDS and immunoprecipitated with MPM-2. The immunoprecipitated material was probed with the isoform-specific topo II antibodies. Molecular mass standards are as indicated. Lanes: scf, initial solubilized scaffold material; sup, supernatant after MPM-2 immunoprecipitation; and IP, MPM-2-immunoprecipitated material.

presence of general phosphatase inhibitors (36, 40, 42); however, none of these experiments included the more potent phosphatase inhibitors microcystin or okadaic acid. The MPM-2 phosphoepitope is particularly sensitive to dephosphorylation, and considering the transient nature of the mitotic phosphorylation recognized by MPM-2, it is likely that the phosphorylation of topo II during mitosis has been incompletely characterized. Most of the previous characterization of topo II phosphorylation was done on what was presumed to be the only topo II isoform, the 170-kDa isoform. Further work is necessary to determine whether the two topo II isoforms share other similar phosphoepitopes in addition to the MPM-2 phosphoepitope.

The fact that both isoforms of topo II found in mitotic chromosomes contain the MPM-2 phosphoepitope suggests that phosphorylation may be involved in targeting the proteins to the mitotic chromosomes. Alternatively, the phosphorylation may regulate topo II enzymatic activity, which has been shown to be essential for chromosome condensation and sister chromatid segregation. The concentration of both MPM-2- and anti-topo II-immunoreactive material at the centromere, as compared to the label seen in chromosomal arms, may have important consequences on the condensation of chromatin in this region. Concentration of the phosphorylated topo II at the centromere may result in tighter condensation of the centromeric chromatin, creating a more rigid centromeric region. Increased rigidity of the centromere may be required to withstand the mechanical stresses applied by spindle microtubules during chromosome movement. While both MPM-2 and anti-topo II antibodies are concentrated at the centromeres of mitotic chromosomes, MPM-2 exhibits a relatively greater accumulation at the centromere. We believe this may be due to other centromere-specific proteins that carry the epitope recognized by MPM-2. Extended exposures of MPM-2 immunoblots of chromosomal proteins reveal polypeptides other than topo II that are reactive with MPM-2. Phosphorylation of centromeric proteins may play a functional role in regulating chromosome movement. For example, *in vitro* studies on the movement of microtubules on isolated chromosomes have implicated two oppositely directed motor activities that may be controlled through differential phosphorylation (44).

We have used the MPM-2 antibody to detect an M-phase phosphorylation of DNA topo IIα and -β. While this phosphorylation exhibits a close correlation with important mitotic functions of topo II, further evidence for its significance will require molecular dissection of the epitope. Topo II is the target of many of the most important chemotherapeutic agents. Better understanding of its control during the cell cycle may provide additional avenues for the optimization of anticancer therapies.

We thank S. K. Tarkington and W. A. Ricketts for their valuable technical assistance. We are grateful to J. B. Rattner (University of Calgary) for providing the Indian Muntjac cells. This research was supported by funds from the University of Virginia Cancer Center and Grants 5-SO7-RRO5431-29 from the National Institutes of Health, J-192 from the Jeffress Memorial Trust, and CD-500 from the American Cancer Society to G.J.G., and CD-470 from the American Cancer Society to P.N.R.

1. Earnshaw, W. C. & Laemmli, U. K. (1983) *J. Cell Biol.* **96**, 84–93.
2. Adolph, K. W., Cheng, S. M., Paulson, J. R. & Laemmli, U. K. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4937–4941.
3. Lewis, C. D. & Laemmli, U. K. (1982) *Cell* **29**, 171–181.
4. Earnshaw, W. C., Halligan, B., Cooke, C. A., Heck, M. M. & Liu, L. F. (1985) *J. Cell Biol.* **100**, 1706–1715.
5. Gasser, S. M., Laroche, T., Falquet, J., Boy de la Tour, E. & Laemmli, U. K. (1986) *J. Mol. Biol.* **188**, 613–629.
6. D'Arpa, P. & Liu, L. F. (1990) in *The Eukaryotic Nucleus: Molecular Biochemistry and Macromolecular Assemblies*, eds. Strauss, P. R. & Wilson, S. H. (Telford, Caldwell, NJ), pp. 623–638.
7. Nelson, W. G., Liu, L. F. & Coffey, D. S. (1986) *Nature (London)* **322**, 187–189.
8. Yang, L., Wold, M. S., Li, J. J., Kelly, T. J. & Liu, L. F. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 950–954.
9. Tan, K. B., Mattern, M. R., Boyce, R. A. & Schein, P. S. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7668–7671.
10. Bae, Y.-S., Kawasaki, I., Ikeda, H. & Liu, L. F. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2076–2080.
11. Cockerill, P. N. & Garrard, W. T. (1986) *Cell* **44**, 273–282.
12. Schroder, H. C., Trolltsch, D., Friese, U., Bachman, M. & Muller, W. E. (1987) *J. Biol. Chem.* **262**, 8917–8925.
13. Earnshaw, W. C. & Heck, M. M. S. (1990) in *The Eukaryotic Nucleus: Molecular Biochemistry and Macromolecular Assemblies*, eds. Strauss, P. R. & Wilson, S. H. (Telford, Caldwell, NJ), pp. 639–657.
14. Wood, E. R. & Earnshaw, W. C. (1990) *J. Cell Biol.* **111**, 2839–2850.
15. Hirano, T. & Mitchison, T. J. (1991) *J. Cell Biol.* **115**, 1479–1489.
16. Adachi, Y., Luke, M. & Laemmli, U. K. (1991) *Cell* **64**, 137–148.
17. Uemura, T., Ohkura, H., Adachi, Y., Morino, K., Shiozaki, K. & Yanagida, M. (1987) *Cell* **50**, 917–925.
18. Holm, C., Goto, T., Wang, J. C. & Botstein, D. (1985) *Cell* **41**, 553–563.
19. Uemura, T. & Yanagida, M. (1986) *EMBO J.* **5**, 1003–1010.
20. Hirano, T. & Mitchison, T. J. (1993) *J. Cell Biol.* **120**, 601–612.
21. Swedlow, J. R., Sedat, J. W. & Agard, D. A. (1993) *Cell* **73**, 97–108.
22. Chung, T. D., Drake, F. H., Tan, K. B., Per, S. R., Crooke, S. T. & Mirabelli, C. K. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9431–9435.
23. Drake, F. H., Zimmerman, J. P., McCabe, F. L., Bartus, H. F., Per, S. R., Sullivan, D. M., Ross, W. E., Mattern, M. R., Johnson, R. K., Crooke, S. T. & Mirabelli, C. K. (1987) *J. Biol. Chem.* **262**, 16739–16747.
24. Drake, F. H., Hofmann, G. A., Bartus, H. F., Mattern, M. R., Crooke, S. T. & Mirabelli, C. K. (1989) *Biochemistry* **28**, 8154–8160.
25. D'Arpa, P., Machlin, P. S., Ratrie, H., III, Rothfield, N. F., Cleveland, D. W. & Earnshaw, W. C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2543–2547.
26. Davis, F. M., Tsao, T. Y., Fowler, S. K. & Rao, P. N. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2926–2930.
27. Vandre, D. D., Davis, F. M., Rao, P. N. & Borisy, G. G. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4439–4443.
28. Mirkovitch, J., Gasser, S. M. & Laemmli, U. K. (1988) *J. Mol. Biol.* **200**, 101–109.
29. Paulson, J. R. (1980) *Biochemistry* **111**, 189–197.
30. Boy de la Tour, E. & Laemmli, U. K. (1988) *Cell* **55**, 937–944.
31. Ohnuki, Y. (1968) *Chromosoma* **25**, 402–428.
32. Earnshaw, W. C. & Heck, M. M. S. (1988) in *Chromosomes and Chromatin*, ed. Adolph, K. W. (CRC, Boca Raton, FL), Vol. 3, pp. 37–55.
33. Woessner, R. D., Chung, T. D., Hofmann, G. A., Mattern, M. R., Mirabelli, C. K., Drake, F. H. & Johnson, R. K. (1990) *Cancer Res.* **50**, 2901–2908.
34. Woessner, R. D., Mattern, M. R., Mirabelli, C. K., Johnson, R. K. & Drake, F. H. (1991) *Cell Growth Differ.* **2**, 209–214.
35. Rottmann, M., Schroder, H. C., Gramzow, M., Friese, U. & Muller, W. E. G. (1987) *EMBO J.* **6**, 3939–3944.
36. Ackerman, P., Glover, V. C. & Osheroff, N. (1988) *J. Biol. Chem.* **263**, 12653–12660.
37. Heck, M. M., Hittelman, W. N. & Earnshaw, W. C. (1989) *J. Biol. Chem.* **264**, 15161–15164.
38. Saijo, M., Enomoto, T., Hanaoka, F. & Ui, M. (1990) *Biochemistry* **29**, 583–590.
39. Ackerman, P., Glover, C. & Osheroff, N. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3164–3168.
40. Cardenas, M. E., Dang, Q., Glover, C. V. C. & Gasser, S. M. (1992) *EMBO J.* **11**, 1785–1796.
41. Sayhoun, N., Wolf, M., Besterman, J., Hsieh, T.-S., Sander, M., LeVine, H., Chang, K.-J. & Cuatrecasas, P. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1603–1607.
42. Kroll, D. J. & Rowe, T. C. (1991) *J. Biol. Chem.* **266**, 7957–7961.
43. Takano, H., Kohno, K., Ono, M., Uchida, Y. & Kuwano, M. (1991) *Cancer Res.* **51**, 3951–3957.
44. Hyman, A. A. & Mitchison, T. J. (1991) *Nature (London)* **351**, 206–211.