Active DNA Topoisomerase IIα Is a Component of the Salt-stable Centrosome Core*

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Recently, we reported that the monoclonal antibody specific for human DNA topoisomerase II, Ki-S1, stains not only the nuclei of human A431 cells but also extranuclear structures suggestive of centrosomes (Meyer, K. N., Kjeldsen, E., Straub, T., Knudsen, B. K., Kikuchi, A., Hickson, I. D., Kreipe, H., and Boege, F. (1997) J. Cell Biol. 136, 775–788). Here, we confirm colocalization of Ki-S1 with the centromosomal marker γ-tubulin. In addition, we show labeling of centrosomes by peptide antibodies against the N and C termini of human topoisomerase IIα. Probing Western blots of isolated centrosomes with topoisomerase IIα antibodies, we demonstrate a protein band of 170 kDa. Moreover, isolated centrosomes exhibited DNA decatenation and relaxation activity correlated to the amount of topoisomerase IIα protein in the same way as seen in the pure recombinant enzyme. Topoisomerase IIα epitopes could not be removed from centrosomes by salt extraction, DNase treatment, or RNase treatment, procedures that completely removed the enzyme from nuclei. Taken together, these observations suggest that active topoisomerase IIα is bound tightly to the centrosome in a DNA-independent manner. Because such centrosomal topoisomerase IIα was also present in quiescent lymphocytes devoid of topoisomerase IIα in the nuclei, we assume that it might be a long-lived storage form.

We have observed (1, 2) that the monoclonal antibody Ki-S1 directed against DNA topoisomerase IIα (3) labels not only the cell nuclei (a long-standing observation) but also labels small globular structures located at the poles of mitotic spindles (a new finding). Ki-S1 labeling of extranuclear globules was reproducible in two human cell lines, whereas a similar observation was not made with antibodies directed against DNA topoisomerase I or IIβ. We assumed that these Ki-S1-positive extranuclear structures would most likely be centrosomes. However, they could just as well be chromosome fragments or micronuclei, or the staining could be due to a spurious cross-reaction of the Ki-S1 antibody with some centrosomal protein other than topoisomerase IIα. However, if such artifacts were excluded, our observation might indicate topoisomerase IIα as a possible component of centrosomes. We found our chance observation interesting enough to be followed up. Here, we carried out immunocytochemical colocalization studies combin-

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Experimental Procedures

Cells—Human A431 epidermoid cells (ATCC #1555) were cultured as described previously (2). Human lymphocytes were isolated from peripheral venous blood samples by density gradient centrifugation (Ficoll-Paque; Amersham Pharmacia Biotech, Freiburg, Germany) and subsequently cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum.

Immunocytochemistry—A431 cells were grown directly on microscopic slides, whereas lymphocytes were subjected to cytopsins (500 × g, 15 min). Fixing and immunostaining of the cells have been described previously (1). Topoisomerase IIα was detected with the mouse monoclonal antibody Ki-S1 or rabbit peptide antibodies against the very C or N termini of the enzyme (3). For preabsorption of topoisomerase IIα antibodies, pure recombinant human topoisomerase IIα (100 μg/ml) was inactivated at 80 °C for 10 min and added to the final incubation solution containing the primary antibodies. After incubation at 37 °C for 30 min, the samples were centrifuged (15,000 × g, 4 °C, 15 min), and the supernatant was used for immunostaining. Centrosomes were labeled with mouse monoclonal (clone GTU-88) or rabbit peptide antibodies, both directed against amino acids 38–53 of human γ-tubulin. These antibodies were diluted according to the instructions of the supplier (Sigma-Chemie, Deisenhofen, Germany). Stained cells were inspected with an epifluorescence microscope (Axioskop 25; Carl Zeiss, Göttingen, Germany) at ×630 magnification. Microscopic images were documented with a cooled charge-coupled device camera (Sensys; Photometrics, München, Germany) equipped with an additional x4 magnification lens providing a final magnification of ×2520.

Cell Fractions—As outlined briefly in the legend to Fig. 5a, A431 cells were treated (4 °C, 10 min) and washed twice with lysis buffer (15 mM HEPES, pH 7.5, 60 mM KCl, 15 mM NaCl, 0.5 mM EGTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 30 μg/ml spermene, 7.5 μg/ml spermidine, 1 μg/ml aprotinin, 5 μg/ml leupeptin, 5 μg/ml pepstatin, 1 mM Pefablock, 1 mM benzamidine, and 0.5% Triton X-100) while still attached to the culture dish. Cell remnants were scraped off, suspended in lysis buffer supplemented with 10 units/ml RNase A, and subjected to differential centrifugation at 3,000 × g (4 °C, 15 min) and 35,000 × g (4 °C, 120 min) to sediment nuclei and microsomes, respectively. Nuclei were salt-extracted (4 °C, 5 min, 3.3 × 106 nuclei/ml) with 500 mM NaCl, 5 mM HEPES, pH 7.5, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 50 μg/ml leupeptin, 5 μg/ml pepstatin, 10% glycerol, 1 mM benzamidine, and 1 mM Pefablock, followed by a second centrifugation (13,000 × g, 4 °C, 15 min). The supernatant (nuclear extract) was used for further assays. Centrosomes were isolated from microsome fractions by isopycnic sucrose gradient density centrifugation following the procedures described in Ref. 4. Briefly, microsome pellets were digested with DNase I (50 units/ml, 20 min, 30 °C), resedimented, resuspended in lysis buffer, and layered on top of sucrose gradients created by stacking 1-ml portions of lysis buffer supplemented with 70, 60, 50, 40, 30, 20, or 10% (w/v) sucrose inside a 14 × 95-mm polycarbonate ultracentrifuge tube, followed by equilibrium (4 °C, 48 h). After ultracentrifugation (200,000 × g, 4 °C, 24 h) (SW 40 Ti; Beckman Instruments Inc., Palo Alto, CA), the gradients were collected from the bottom of the tubes (10 fractions at 1.2 ml). The
actual sucrose concentration of each fraction was measured by optical diffraction. The fractions were diluted 20-fold with lysis buffer, sedimented (20,000 × g, 4 °C, 30 min), and subjected to immunoblotting or topoisomerase activity assays.

**Cell Extraction on Slides**—A431 cells were grown on microscopic slides and lysed in situ (see the previous paragraph). Cell remnants were then subjected to a series of extraction procedures and finally fixed and immunostained. Extraction procedures included treatment with 500 mM NaCl (conditions were the same as those used for nuclear extraction, see the previous paragraph), followed by DNase I (1 unit/slide), followed by RNase A (10 μg/slide). Digestions were carried out at 20 °C for 30 min in 20 mM Tris-HCl, pH 7.8, 100 mM NaCl, 2 mM MgCl₂, 0.1 mM CaCl₂, 1 mM phenylmethylsulfonyl fluoride, 1% (w/v) bovine serum albumin, and 0.2% dithiothreitol. Between treatments, slides were washed (three times) with phosphate-buffered isotonic saline solution.

**Immunoblotting**—Analysis of topoisomerase IIα was essentially performed as described in Ref. 2. However, microscope pellets and isolated centrosomes were digested with DNase I (50 units/ml, 30 min, 20 °C) and resedimented before electrophoresis. Blots were probed with peptide antibodies against C and N termini of human topoisomerase IIα (3). For analysis of γ-tubulin, the samples were separated in 10% gels, and the blots were probed with the same antibodies used for immunofluorescence microscopy. Antibody-labeled protein bands were visualized with peroxidase-labeled goat secondary antibodies and the chemiluminescence system (both from Amersham Pharmacia Biotech, Little Chalford, United Kingdom). Signals of immunoblots were quantified by densitometry using a transilluminating flat-bed scanner and NIH Image 1.61 software.

Topoisomerase IIα-specific activity was determined by decatenation of *Crithidia fasciculata* catenated kinetoplast DNA (kDNA) obtained from TopoGen Inc. (Columbus, OH). Centrosomes or pure recombinant human DNA topoisomerase IIα were suspended/diluted with assay buffer (50 mM Tris-HCl, pH 8, 100 mM KCl, 50 mM NaCl, 3 mM MgCl₂, 1 mM dithiothreitol, 0.03 mg/ml bovine serum albumin, and 1 mM ATP) and incubated with 360 ng of kDNA in a final volume of 30 μl. Reactions at 37 °C were stopped after 4 h with 1% SDS followed by proteinase K digestion. Samples were then subjected to submarine gel electrophoresis in the presence of 0.5 μg/ml ethidium bromide. Gels were documented by digital photography (EDAS 120; Kodak, Frankfurt, Germany). For a more precise quantitation of catalytic activity, relaxation kinetics of 120 ng of μC18 plasmid DNA were determined in a similar fashion. In this case, electrophoresis was carried out in the absence of ethidium bromide, and gels were stained after the run.

**Statistics**—Because quantitative statistics could not be applied to the data, representative examples of experimental results are shown. Microscopic images of cells are representative of the whole cell population on the slide inspected in at least 10 separate fields of view. For all data shown (microscopy, catalytic assays, and immunoblotting), similar results were obtained in at least three independent experiments done on different days and with different sets of cells.

**RESULTS**

**Three Distinct Topoisomerase IIα Epitopes Colocalize with γ-Tubulin**—Fig. 1 shows immunofluorescent images of human A431 cells stained with the topoisomerase IIα-specific monoclonal antibody Ki-S1 (top) and the corresponding DNA patterns obtained with DAPI (bottom). In mitotic cells, the Ki-S1 antibody labeled a dotted structure along the central axes of the chromosome arms that represents an established finding (1, 2, 5–8). In addition, it labeled small globular structures located at the spindle poles of mitotic cells that did not show up in the DAPI staining. Such apparently DNA-free globules were also labeled in the extranuclear space of A431 cells during interphase (Fig. 1, arrow). To obtain a definite assignment of these structures, we stained the cells simultaneously with antibodies against γ-tubulin, a specific marker of centrosomes (Fig. 1, middle). Apparently, γ-tubulin and Ki-S1 antibodies labeled the same globular structures, thus establishing their identity as centrosomes (Fig. 1, compare the top with the middle). It should be noted that all centrosomes were labeled by both antibodies. This was also the case in polyploid cells with multiple sets of centrosomes (Fig. 1, large cell on the left). Ki-S1 labeling of centrosomes could be abolished by preabsorption with purified recombinant human topoisomerase IIα (Fig. 2a, top), whereas the γ-tubulin signal serving as an internal control of the preabsorption procedure was not diminished (Fig. 2a, middle). These results confirmed that Ki-S1 antibodies labeled centrosomes in a topoisomerase IIα-specific manner. Fig. 2b shows a series of triple labelings similar to those described in Fig. 1 but carried out on cytopsins of quiescent human lymphocytes isolated from peripheral venous blood and subsequently cultured for 12 h. The nuclei of such cells were not labeled by Ki-S1 antibodies (Fig. 2b, top), which is in agreement with previous data (9). In contrast, the centrosomes were strongly stained, which represents a novel finding. Again, the Ki-S1 epitope clearly colocalized with γ-tubulin (Fig. 2b, middle). These observations suggest that labeling of centrosomes by Ki-S1 antibodies is not a peculiarity of tumor cell lines and that expression of the Ki-S1 epitope in centrosomes is not restricted to proliferating cells, which is in clear contrast to expression in the nuclei (9, 10). The results shown in Fig. 1 could indicate that topoisomerase IIα is a component of centrosomes, provided that the centrosomal Ki-S1 epitope was associated or related to topoisomerase IIα. On the other hand, they could just be due to a spurious cross-reaction of the Ki-S1 antibody with a centrosomal protein other than topoisomerase IIα. We tried to distinguish between these alternatives by another series of immunofluorescent double labelings combining the Ki-S1 antibody, which targets an epitope adjacent to the C terminus of human topoisomerase IIα, with rabbit peptide antibodies directed against the very C- or N-terminals of the enzyme (9). It turned out that both peptide antibodies (Fig. 3a and b, middle) labeled the centrosomes of A431 cells in the same way as the Ki-S1 antibody (Fig. 3a and b, top). Because the three sets of antibodies target distinct and distant epitopes of human topoisomerase IIα, spurious cross-reactions with centrosomal proteins unrelated to topoisomerase IIα seemed unlikely. Consequently, we adopted the hypothesis that topoisomerase IIα might indeed be present at the centrosome.

**Topoisomerase IIα Epitopes at the Centrosome Are Salt- and DNase-stable**—From this assumption followed the question of whether topoisomerase IIα is an integral centrosomal protein (i.e. a component of the salt-stable centrosomal core), and, if so, whether it is bound there in a manner involving DNA or RNA. To address these issues, we applied various extraction procedures to A431 cells grown on microscope slides, and immunostained residual topoisomerase IIα still attached to cellular structures after the treatment. These experiments are summarized in Fig. 4 showing representative cells in interphase (Fig. 4a) or mitosis (Fig. 4b). In each column, the same cell is visualized by phase-contrast (row 1) or triple fluorescence labeling of topoisomerase IIα (row 2), γ-tubulin (row 3), and DNA (row 4). The first column on the left of each panel shows the control, i.e. cells not extracted before staining (similar to those in Fig. 1). Extraction with 500 mM NaCl (Fig. 4, 0.5 mM NaCl) removed most of the topoisomerase IIα from the cell nuclei, whereas the centrosome-bound enzyme was not extracted. Subsequent DNase I digestion of salt-extracted cells (Fig. 4, DNase I) effectively removed all of the DNA from the nuclei. It also removed that tiny portion of nuclear topoisomerase IIα that had resisted previous salt extraction. However, the centrosome-bound portion of the enzyme was again unaffected. It remained bound to the salt- and DNase-stable centrosome core. Finally, the centrosome-bound fraction of topoisomerase IIα was also resistant to subsequent RNase A digestion (Fig. 4, RNase), excluding the possibility that it might be incorporated into salt-stable ribo-
FIG. 1. Fluorescent images of A431 cells in logarithmic growth labeled simultaneously with the mouse monoclonal antibody Ki-S1 directed against topoisomerase IIα (top), rabbit peptide antibodies directed against γ-tubulin (middle), and DAPI (bottom). Representative cells are visualized at ×2520 magnification. Centrosomes are indicated by arrows. The scale bar in the DAPI image (bottom) applies to all three panels.
nucleoprotein complexes, as has been reported of *Drosophila melanogaster* topoisomerase II (11). In summary, these findings suggest that topoisomerase IIα is an integral component of the centrosome and that it is tightly bound in a manner that does not involve DNA or RNA.

Fig. 2. *a*, images corresponding to those described in Fig. 1 were obtained after preabsorption of the mixture of both primary antibodies with pure recombinant human topoisomerase IIα. Centrosomes are indicated by arrows. *b*, images corresponding to those described in Fig. 1 were obtained by staining cytopsin of quiescent peripheral human blood lymphocytes. In both cases (*a* and *b*), representative examples of cells are visualized at ×2520 magnification. The scale bars in the DAPI images (bottom) apply to all panels of the respective columns.

Fig. 3. *a*, fluorescent images of A431 cells in logarithmic growth labeled simultaneously with the mouse monoclonal antibody Ki-S1 directed against topoisomerase IIα (top), rabbit peptide antibodies directed against the last 18 N-terminal amino acid residues of human topoisomerase IIα (middle), and DAPI (bottom). *b*, fluorescent images of A431 cells in logarithmic growth labeled simultaneously with the mouse monoclonal antibody Ki-S1 directed against topoisomerase IIα (top), rabbit peptide antibodies directed against the first 12 C-terminal amino acid residues of topoisomerase IIα (middle), and DAPI (bottom). In both cases (*a* and *b*), representative cells are visualized at ×2520 magnification. The scale bars in the DAPI images (bottom) apply to all panels of the respective columns.
**Fig. 4. Salt, DNase, and RNase stability of centrosomal topoisomerase IIα.** A431 cells were grown on slides, permeabilized with Triton X-100, subjected to various extraction procedures, and finally fixed and stained. Representative cells in interphase (a) and mitosis (b) are visualized at ×2520 magnification. Each column shows the same cell by phase-contrast (row 1) or immunofluorescence of triple staining with the Ki-S1 monoclonal antibodies directed against topoisomerase IIα (row 2), rabbit peptide antibodies directed against γ-tubulin (row 3), and DAPI (row 4). Control, cells without extraction (i.e. the same as those shown in Fig. 1); 0.5 M NaCl, extraction with 500 mM NaCl; DNase I, extraction with 500 mM NaCl, followed by DNase I digestion; RNase, extraction with 500 mM NaCl, followed by DNase I digestion, followed by RNase A digestion.
probed Western blots of microsomes prepared from A431 cells with the same set of antibodies used in Fig. 3. As outlined in Fig. 5a, lipids and cytosol of A431 cells were first removed by in situ lysis with 0.5% Triton X-100. Residual cell structures were fractionated into nuclei (N) and microsomes (M). Nuclei were subsequently salt-extracted, whereas microsomes were DNase I-digested (to dissolve contaminating chromatin fragments). Finally, Western blots of nuclear extracts and microsomes were probed with polyclonal peptide antibodies against the C and N termini of topoisomerase IIa (Fig. 5b, panels 1 and 2, respectively), with monoclonal Ki-S1 antibodies (Fig. 5b, panel 3), or with polyclonal peptide antibodies against the C terminus of topoisomerase IIb (Fig. 5b, panel 4). Topoisomerase IIa was clearly detected at its usual position (equivalent to a molecular size of 170 kDa) in the nuclear extracts and in the microsomes, whereas topoisomerase IIb (serving as internal control) was only detected in the nuclear extract and was not detected in the microsome fraction. It should be noted that topoisomerase I was also not detectable in the microsome fraction (data not shown). These latter observations exclude a contamination of the microsome fraction with nuclei or chromatin fragments. Thus, the data in Fig. 5b confirm our immunocytological observations of the nuclear and microsomal components. Microsomes (M) were digested with DNase I and resedimented. The final supernatant (S) were DNase I-digested (to dissolve contaminating chromatin fragments), washed with 0.5% Triton X-100 while still attached. Microsomes (M) were digested with DNase I and resedimented. The final supernatant (S) was precipitated with trichloroacetic acid. Microsomes (M) were digested with DNase I and resedimented. The final supernatant (S) was precipitated with trichloroacetic acid.

**Fig. 5. Immunoblotting of topoisomerase IIa.** a, cell fractionation. Cells were lysed and washed with 0.5% Triton X-100 while still attached to the culture dish. Remnants were scraped off and fractionated into nuclei and microsomes by differential centrifugation. Nuclei (N) were salt-extracted. Microsomes (M) were digested with DNase I and resedimented. The final supernatant (S) was precipitated with trichloroacetic acid. b, Western blots of nuclear extracts equivalent to 106 cells (N), microsomes equivalent to 106 cells (M), and final supernatant equivalent to 106 cells (S) were probed with peptide antibodies against the C terminus (panel 1) or the N terminus (panel 2) of human topoisomerase IIa, with the monoclonal antibody Ki-S1 (panel 3), or with peptide antibodies against the C terminus of human topoisomerase IIb (panel 4). Molecular masses (in kDa) denoted on the right margin were derived from comparison with marker proteins.

**Fig. 6. Isolation of centrosomes by isopyknic sucrose gradient density centrifugation.** a, microsome fractions of A431 cells were sedimented through a sucrose gradient (10–70%, w/v). The gradient was fractionated from the bottom. The fractions (lanes 1–10) were analyzed by immunoblotting using peptide antibodies against the C terminus of human topoisomerase IIa (top) or human γ-tubulin (middle). The corresponding sucrose concentration (% w/v) of the fractions (bottom) was determined by optical diffraction. The outmost lane on the right (lane T) shows the material on top of the gradient, which was collected after centrifugation and before fractionation. Molecular mass values (in kDa) denoted on the right margin were derived from comparison with marker proteins. b, fraction 2 of the experiment shown in a was sedimented onto a microscopic slide and double-stained with peptide antibodies against the C terminus of topoisomerase IIa (top) and mouse monoclonal antibodies against γ-tubulin (bottom). Centrosomes Have DNA Decatenation and Relaxation Activity—These observations raised the obvious question of whether centrosomal topoisomerase IIa actually has a type II DNA topoisomerase activity. Unfortunately, the most straightforward approach to this question was blocked because the topoisomerase IIa protein could not be extracted from centrosome particles (see Fig. 4) and consequently could not be purified in...
against the C terminus of human topoisomerase II contain ethidium bromide. Mers were separated by electrophoresis in 1% agarose gels that did not periods indicated. Samples were treated with proteinase K. Topoisomerase II amounts was as shown inset. lane E). Normalization of enzyme amounts was as shown the inset of a. Incubations were stopped with 1% SDS after the time periods indicated. Samples were treated with proteinase K. Topoiso-
mers were separated by electrophoresis in 1% agarose gels that did not contain ethidium bromide.

![Table](image)

| Centrosomes | rhTopo IIα | Enzyme [ng] |
|-------------|------------|-------------|
| 0 | 60 | 120 | 180 | 180 | 0 | 60 | 120 | 180 | 180 |
| Network | Network |
| Free Circles | Free Circles |
| Supercarton | Supercarton |

**FIG. 7. Activity of centrosomal topoisomerase II α assessed by DNA decatenation and relaxation.** a. Decatenation. 360 ng of kDNA were incubated for 4 h at 37 °C with 60–180 ng of pure recombinant human topoisomerase IIα (lanes 2–5) or equivalent amounts of the enzyme contained in centrosomes prepared from A431 cells (lanes 2–5). Orthovanadate (0.3 mM) was added to the assays in lanes 5 and 9 as an ATPase inhibitor. Lane 1 shows 360 ng of kDNA alone. Incubations were stopped with 1% SDS. Samples were treated with proteinase K and electrophoresed on 1% agarose gels containing 0.1 μg/ml ethidium bromide. The inset shows a Western blot stained with rabbit antibodies against the C terminus of human topoisomerase IIα, attesting to normal-
ization of the centrosome preparation (lane C) to 20 ng of pure recombinant human topoisomerase IIα (lane E). b, relaxation kinetics. 120 ng of pUC18 plasmid DNA were incubated at 37 °C with 5 ng of pure recombinant human topoisomerase IIα (lanes 2–11) or an equivalent amount of the enzyme contained in centrosomes prepared from A431 cells (lanes 2–6). Normalization of enzyme amounts was as shown in the inset of a. Incubations were stopped with 1% SDS after the time periods indicated. Samples were treated with proteinase K. Topoiso-
mers were separated by electrophoresis in 1% agarose gels that did not contain ethidium bromide.

The centrosome controls assembly of microtubules, a process that plays a central role in organizing cell structure, determining cell polarity, directing cell movement during interphase, and orchestrating formation of the bipolar spindle during mitosis (12). In mammalian cells, the centrosome is comprised of a pair of centrioles and pericentriolar material initially denoted as “differentiated cytoplasm” (13). The centrioles seem to be needed for organizing centrosomal components into a structural stable organelle (14), whereas the pericentrin, and other proteins, which control the assembly of microtubules (15–18). However, the centrosome also seems to serve as a “landing pad” for many proteins that have nothing to do with the organization of microtubuli (17), including adaptors (19, 20), kinases (21), molecular chaperones (22), components of the proteasomal machinery (23), and cell cycle-related proteins (24, 25). Some of these seem to be instrumental in centrosome replication (24–26), whereas others are believed to associate with centrosomes only as passengers (27, 28). Here we present evidence strongly suggesting that DNA topoisomerase IIα is also present at the centrosome. However, we can only speculate about the biological function of the enzyme at this location.

Centrosomal topoisomerase IIα is characterized by several features that distinguish it from the nuclear variety. Most notably, it appears to be very tightly bound to the salt-stable centrosome core in a manner not involving DNA or RNA. This raises the question of how it gets there and how it is fixed. Recent evidence suggests that the centrosomal adaptor protein 14-3-3ε believed to link mitotic signaling to centrosome duplication (20) is capable of binding strongly to topoisomerase IIα in vitro (29). Thus, it might be involved in recruiting and fixing topoisomerase IIα to the centrosome. However, it remains unclear why centrosomes should have a type II topoisomerase activity at all. It is hard to imagine that the DNA-modifying enzyme plays a role in the organization of microtubuli. Some other cell cycle-related proteins have been proposed to associate with centrosomes during mitosis to achieve appropriate distribution between the two daughter cells (27, 28). However, such a mechanism would not be meaningful for a chromosome-bound enzyme, such as topoisomerase IIα (2, 8), which gets equally distributed between daughter cells via the chromo-

some. A hint at a possible biological meaning of centrosomal topoisomerase IIα might be provided by our finding of the enzyme at centrosomes of quiescent peripheral blood lymphocytes, which are devoid of topoisomerase IIα-specific mRNA and do not have topoisomerase IIα in the nucleus (30). The finding suggests a significantly extended life span of the cen-
trosomal enzyme, as compared with the nuclear variety. Possibly, topoisomerase IIα is stored and propagated at the centrosome independently of the extensive up- and down-regulations taking place in the nucleus (2, 30–34). Such a source of spare topoisomerase IIα would be particularly meaningful for intermittently quiescent cells (such as lymphocytes) because it could safeguard cells from mitotic catastrophes otherwise likely encountered during unscheduled attempts at mitosis in the complete absence of active topoisomerase IIα (1). The argument is supported to some extent by our observation that only topoisomerase IIα is present at the centrosome, whereas topoisomerase IIβ is not. We have recently shown that essential mitotic functions of topoisomerase IIα are not adopted by topoisomerase IIβ (1), whereas topoisomerase IIα can, at least in cells, fully complement a lack of topoisomerase IIβ (35). Thus, it would make sense to keep spare topoisomerase IIα but not spare topoisomerase IIβ at the centrosome.

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