Unique functions of mammalian DNA-topoisomerases IIα and IIβ are suggested by their distinct cellular distribution and chromatin binding at mitosis. Here, we studied H69-VP cells that, due to a homozygous mutation, express topoisomerase IIα mostly outside the nucleus. In these cells topoisomerase IIβ showed a normal nuclear localization. However, at mitosis it diffused away from the chromatin despite the nuclear lack of the α-isomorph. 80% of these cells performed chromosome condensation and disjunction with the aid of cytosolic topoisomerase IIα, which bound to the mitotic chromatin with low affinity. However, the genotype of these cells was highly polyploid indicating an increased rate of non-disjunction. In 20% of the mutant cells neither topoisomerase II isoform was bound to the mitotic chromatin, which appeared as an unstructured DNA spheroid unable to undergo disjunction and cytokinesis. Parental H68 cells expressing topoisomerase IIα inside the nucleus exhibited high affinity binding of the enzyme to the mitotic chromatin. Their genotype was mostly diploid and stable. We conclude (i) that high affinity chromatin binding of topoisomerase IIα is essential for chromosome condensation/disjunction and (ii) that topoisomerase IIβ does not adopt these functions.

Type II DNA topoisomerases catalyze complex topological changes in the genome by passing intact DNA double helices through transient breaks in another DNA double strand. They play a crucial role in chromosome condensation and disjunction, although their precise function in these processes is still somewhat controversial (1). In contrast to yeast and insects, which have only one form of DNA topoisomerase II, there exist two isoforms (α and β) in mammals, which are encoded by separate genes (2, 3). The two isoenzymes share a high degree of structural homology and have similar enzymatic properties (4). However, they show different patterns of spatial organization both on the level of the cell (5, 6) and in tissues (7). In a given tissue the β-isoenzyme is found in virtually every cell, whereas the α-isoenzyme is restricted to proliferative compartments (7). Cells in the cell cycle express both isoenzymes, but the subcellular localization is very different. The α-form localizes to the inside of nucleoli (6) and clusters at centromeric regions (8), whereas the β-isoenzyme shows a reticular pattern in the vicinity but mostly outside of nucleoli (6). In mitosis the β-isoenzyme diffuses away from the chromatin, whereas the α-isoenzyme becomes up-regulated (9–11) and binds tightly to the centromeres and the axes of the chromosome arms (5, 6, 12), where it displays a dynamic pattern, which changes as cells progress through mitosis (13). There are indications that the α-isoenzyme in its chromosome-bound state is mostly catalytically inactive, whereas the β-isoenzyme sustains a diffusible type II topoisomerase activity throughout the cell cycle (6). Taken together, these observations strongly suggest that the two isoforms of mammalian DNA-topoisomerase II serve different functions during the cell cycle and particularly in mitosis. However, it is unknown whether these functions are unique.

The most straightforward approach would be to block expression of one isoenzyme. However, it has turned out that at least for the α-form this cannot be done without abrogating cell proliferation. Therefore, we have chosen an indirect approach studying cells, which express DNA-topoisomerase IIα outside the nucleus. Such a model is provided by the cell line H69-VP, which was selected from the human small cell lung cancer cell line NCI-H69 by continuous treatment with etoposide (VP-16) (14). The subclone has a homozygous deletion of 9 nucleotides in the gene of human topoisomerase IIα encoding amino acid residues 1490–1492 (KSK), which are part of a cluster of potential nuclear localization sequences in the carboxyl terminus of the enzyme (15). The deleted sequence seems to be essential for nuclear translocation of the enzyme (16). Among several other cell lines with large truncations or deletions in the carboxyl terminus of topoisomerase IIα and extranuclear enzyme expression (15, 17–21), H69-VP cells are particularly suited for this study, because their mutation is homozygous and does not affect functional properties of the enzyme (16). Our data indicate that in these cells the mitotic functions of topoisomerase IIα are not adopted by topoisomerase IIβ.

**EXPERIMENTAL PROCEDURES**

**Cells and Cell Cycle Analysis—**NCI-H69 small cell lung cancer cells (HTB 119, American Type Culture Collection, Rockville, MD) and the etoposide-resistant subline H69-VP (14) carrying a homozygous nuclear localization sequence mutation of the topoisomerase IIα gene (16) were grown in liquid culture medium (RPMI, 10 g/liter penicillin/streptomycin, 1% l-glutamine) supplemented with 10% fetal bovine serum (accelerated growth conditions) in a humidified atmosphere containing 7.5% (v/v) CO2. Under normal growth conditions the mutant subclone H69-VP had a similar growth rate as the parental cell line H69-WT (14, 22). Cells were routinely checked to be free of mycoplasma, and the mutant cell line was re-established from frozen stock every 20 passages to avoid a drift of the geno- and phenotype. For indirect immunofluorescence microscopy cells were sedimented onto microscopic slides. Analysis of cell cycle phases was performed on cells...
fixed with 70% ethanol (20 min, 4 °C) and permeabilized with 0.1% Triton X-100 (5 min, 4 °C). DNA was stained with DAPI \(^1\) (0.1 µg/ml, 10 min, 20 °C), and cellular DNA content was analyzed using a PA2-2 flow cytometer (Partec, Münster, Germany) equipped with a mercury lamp and appropriate filters. Trout erythrocytes served as a DNA standard.

Cells were generated against a unique carboxyl-terminal peptide (residues 1512–1530) of human topoisomerase IIa (Genoys Biotechnologies, Cambridge, UK). Topoisomerase IIa was probed with the mouse monoclonal antibody 3H10 (indirect immunofluorescence microscopy) or rabbit antibodies (immunoblotting) both raised against peptides corresponding to unique carboxyl-terminal sequences of the β-isoenzyme (11, 23). Specificity of immunostaining was routinely controlled by preadsorption with purified recombinant topoisomerase IIa produced in yeast, as described previously (6).

**Indirect Immunofluorescence Microscopy**—Cells were sedimented at 500 × g onto microscopic slides. For analysis of chromosomal metaphase spreads, cells were blocked with demecolcine (0.26 µM, 24 h) and swollen with 75 mM KCl at 4 °C for 10 min before sedimentation. For 2000 × g microspreads, cells or demecolcine-swollen spreads were fixed with formaldehyde (3.7% in PBS, 10 min, 5 °C) and permeabilized (Triton X-100, 0.1% in PBS, 5 min, 4 °C). After washing with PBS, cells were blocked (PBS containing 5% standard goat serum, 1 h, 20 °C) and subsequently incubated for 30 min at 20 °C with primary antibodies diluted 1:1000 (3H10) or 1:1000 (rabbit anti-topoisomerase IIa) in PBS containing 1% bovine serum albumin and 1% dry method using 70 mM CAPS buffer, pH 11. Immobilized proteins were probed with the mouse monoclonal antibody 3H10 (indirect immunofluorescence microscopy or catalytic assays, immunoblotting, and cell cycle analysis), similar results were obtained in at least three independent experiments done on different days and different sets of cells. When quantitative evaluations of immunoblots are stated in the text, they result from densitometry of the x-ray films and represent mean values of at least three independent experiments. The standard errors of the mean are not mentioned, because they were less than 20% in all cases.

**RESULTS**

**Extraneuronal Expression of Active Topoisomerase IIa**

The subclone H69-VP carrying a homozygous mutation of the nuclear localization sequence of the topoisomerase IIa gene contained similar amounts of topoisomerase IIa activity as the parental cell line H69-WT. This became apparent when serial dilutions of whole cell extracts of log cultures were assessed for DNA decatenation (Fig. 1a). Moreover, the two cell lines expressed similar amounts of topoisomerase IIa and β-antigens, as demonstrated by probing Western blots of whole cell lysates with isoenzyme-specific antibodies (Fig. 1b, lanes 1 and 2). However, major differences between the two cell clones became apparent, when the subcellular organization of topoisomerase II isoforms was assessed. We disrupted the cells by combined hypotonic treatment and mechanical shearing, separated cytosol and nuclei by centrifugation, and analyzed the amounts of topoisomerase IIa and β in the cytosolic fractions and the nuclei by immunoblotting (Fig. 1b, lanes 3–6). The parental H69-WT cells expressed both isoforms mostly in the nucleus (Fig. 1b, lane 5), whereas only minor fractions (20%) of both isoenzymes were detectable in the cytosol (Fig. 1b, lane 3). In contrast, the mutant cells expressed the α-isoenzyme mainly (>85%) in the cytosol (lane 4). Only a minor fraction (less than 5%) was detectable in the nuclei (lane 6). In contrast, the majority of the β-isoenzyme was present in the nuclei of both cell lines (Fig. 1b, lane 6). In agreement with previous data (16), this pattern could indicate that the mutant α-isoenzyme cannot gain access to the nuclei of H69-VP cells. However, it could equally well reflect an attenuation of nuclear translocalization resulting in an altered equilibrium between cytosolic and nuclear pools of the enzyme, which are both active and readily visualized. To exclude the latter possibility, we treated the cells with the topoisomerase II poison teniposide (VM-26). The drug stabilizes a covalent catalytic intermediate of topoisomerase IIa and DNA, trapping the active fracion of topoisomerase IIa in covalent DNA-complexes too large to migrate into a polyacrylamide gel. Consequently, enzymes catalytically interacting with the genome will be depleted from the immunoblot.
representative result is demonstrated in Fig. 1c. In the parental cell line VM-26 caused an almost complete immuno-band depletion of both topoisomerase II isoforms in a dose-dependent manner (Fig. 1c, lanes 5 and 6), indicating that the full cellular complement of both isoforms is engaged in catalytic DNA turn over. In contrast, in the mutant cell line (Fig. 1c, line 1–4) only the β-isoform became depleted, whereas the α-isoform was not notably targeted by VM-26 even at concentrations as high as 200 µM. A similar lack in band depletion of the mutant α-isoform has previously been observed using the catalytic topoisomerase II inhibitor ICRF-187 (16). These results could indicate that the majority of the mutant α-isoenzyme does not interact with the genome of the cell. However, similar results would also be obtained if the mutant enzyme was engaged in DNA turnover, but resistant to the drug, or if the mutant enzyme was inactive. In order to exclude these last two possibilities, we checked the activity of topoisomerase II in the cytosolic fraction of H69-VP cells and its susceptibility to VM-26 treatment. The data shown in Fig. 1d confirm that cytosolic topoisomerase IIα of H69-VP cells was catalytically active (Fig. 1d, lane 5) and could be blocked by VM-26 (Fig. 1d, lanes 6 and 7) in a cell-free system. Thus, the lack of drug-induced band depletion of the mutant topoisomerase IIα inside the cells (Fig. 1c) must be due to the fact that the major part of the mutant enzyme is unable to enter the nucleus.

Chromosome Condensation and Disjunction

The data in Fig. 1 corroborate previous findings (16), suggesting that H69-VP cells proliferate under conditions where the β-isoenzyme of topoisomerase II is predominantly present in the nucleus and engaged in DNA processing, whereas the mutant α-isoform (although catalytically active) appears to be mostly (>95%) excluded from the nucleus. The mutant cell line did not exhibit increased levels of topoisomerase IIα or β nor an increase in overall cellular topoisomerase II activity, as compared with the parental cells (Fig. 1, a and b), indicating that the lack of nuclear topoisomerase IIα did not stimulate up-regulation of either topoisomerase II isoform. These observations seemed to suggest that topoisomerase IIα is dispensable for cell proliferation or can at least be complemented by the β-isoform to such an extent that an up-regulation of the enzyme is not required for maintaining unattenuated cell proliferation. From our previous localization studies (6), we knew that usually during mitosis only the α-form binds to the chromosomal scaffold, whereas the β-isoenzyme becomes excluded from the condensed chromatin. If these functions were adopted by topoisomerase IIβ, we would expect the β-isoenzyme to localize to the chromosomal scaffold of the mutant cell line in a manner similar as normally seen with the α-isoenzyme. To test this assumption, we used fluorescent images of parental H69-WT cells simultaneously stained for topoisomerase IIα, IIβ, and DNA (Fig. 2a) resembled patterns previously observed in human...
A431 cells (6). In interphase, the α-isof orm had a mostly homogeneous distribution in the nucleoplasm, whereas the β-isof orm had a highly inhomogeneous reticular distribution in the nucleus. In prometaphase most of the β-isoenzyme diffused into the cytosol and remained there until anaphase, whereas the majority of the α-isoenzyme bound to the condensing chromatin. In chromosomes of parental H69-cells the α-isof orm aligned in the axes of the chromosome arms (Fig. 3b). Representative fluorescent images of the mutant H69-VP cells simultaneously stained for topoisomerase IIα, -β, and DNA (Fig. 2b) confirmed that in interphase the majority of topoisomerase IIα was localized in the cytosol and not in the nucleus, whereas the β-isoenzyme exhibited a nuclear pattern similar to interphases of the parental cell line. However an unexpected result was obtained with mitotic H69-VP cells. The β-isoenzyme did not bind to the chromosomal scaffold but rather diffused into the cytosol and remained excluded from the chromatin throughout mitosis, just like in the parental cells. These observations indicated that topoisomerase IIβ did not substitute for the lacking α-isoenzyme and suggested that none of the type II topoisomerases was required for mitosis. However, from the unusual aspects of the mitotic figures of H69-VP cells and in particular from the staining patterns of topoisomerase IIα in such cells, it became apparent that lack of topoisomerase IIα in the nucleus had a significant effect on chromatin condensation and disjunction. Two distinct types of mitotic events could be discriminated as indicated below.

Metaphase Type I (DNA-ball)—In about 20% of mitotic H69-VP cells an abortive type of mitosis was observed (Fig. 2b, Meta Type I). Neither topoisomerase IIα nor -β were bound to the chromatin, which appeared highly condensed and unstructured. Fig. 3a shows a high resolution image of such metaphases from two views. The DNA appeared to be contracted into the shape of a flattened melon, and both topoisomerase II isoforms were excluded from this DNA spheroid with the exception of two spots, which according to our previous investigations (6) probably represent cross-reactions of our topoisomerase IIα antibodies with centrosomes. When H69-VP cells were first brought into accelerated growth by increased serum supplementation and subsequently blocked in metaphase with demecolcine, this abortive type of metaphase occurred with high frequency (80% of the cells). After removal of the block these cells did not continue to grow, but died after a few days, indicating that the ball-shaped chromatin condensed in the absence of topoisomerase II could not be further processed.

Metaphase Type II (Chromosomes)—Under normal growth conditions about 80% of the metaphases of logarithmically growing H69-VP cells appeared as shown in Fig. 2b (Meta Type II). These cells contained chromosome-like DNA structures, which were apparently devoid of topoisomerase IIβ, but clearly stained for topoisomerase IIα. Metaphase spreads prepared from H69-WT and H69-VP cells blocked with demecolcine under normal growth conditions and visualized at high resolution are shown in Fig. 3b. The images are grouped in vertical pairs, the top one showing parental H69-WT cells and the bottom one the mutant cell line H69-VP. In each case a single metaphase plate viewed along the axis of the spindle poles is simultaneously stained for topoisomerase IIα (left), DNA (middle), and topoisomerase IIβ (right).
mutant α-isoenzyme lacking in the nuclei of H69-VP cells. On the contrary, mitosis appeared to be entirely sustained by cytosolic topoisomerase IIα, which in these cells probably entered the chromatin after breakdown of the nuclear envelope. Apparently under normal growth conditions in most cases (80%) chromatin condensation and disjuction were possible, because sufficient amounts of topoisomerase IIα gained access and bound to the DNA (Fig. 2b, Meta Type II and Fig. 3b, *bottom*). However, when topoisomerase IIα was not bound to the chromatin in detectable amounts, cells ended up with a DNA spheroid instead of chromosomes (Fig. 2b, Meta Type I and Fig. 3a). Time seemed to be a crucial factor for binding of cytosolic topoisomerase IIα to the mitotic chromatin, because formation of topoisomerase IIα-associated chromosomes (metaphase type II) was favored by normal growth conditions, whereas formation of DNA spheroids devoid of topoisomerase IIα (metaphase type I) was favored by accelerated cell growth.

We have recently observed that a fraction of topoisomerase IIα binds to chromosomes with a higher affinity than to interphase chromatin. The increase in binding affinity is detectable by an increase in the salt concentration needed for extraction of the enzyme (6). To find out whether cytosolic topoisomerase IIα could also form such high affinity chromatin bonds in type II metaphases of H69-VP cells (compare with Fig. 3b), we investigated the salt stability of the chromatin binding of topoisomerase IIα and -β in comparison to the parental cell line. We disrupted the cells by mechanical shearing (10 passages through a 29-gauge needle), sedimented and washed the nuclei, extracted chromatin-bound proteins serially with increasing salt concentrations, and analyzed topoisomerases IIα and -β in these fractions and in the salt-insoluble remnant by immuno-blotting and densitometry (Fig. 4). In log cultures of parental H69-WT cells at normal growth conditions (WT, I) only a minor fraction of topoisomerase IIα (30%) was not bound to the chromatin (lanes 1 and 2). The majority of the α-isoenzyme (70%) was extractable by 400 mM KCl (lane 3), but only traces (≤1%) resisted extraction by 400 mM KCl and were subsequently released from a tighter chromatin binding by 600 mM KCl or more (lanes 4 and 5). When the parental cells were blocked with demecolcine in metaphase at normal growth conditions (WT, M), the bulk of topoisomerase IIα (about 85%) resisted salt extraction by KCl concentrations less than 600 mM (lanes 4 and 5), indicating recruitment of the enzyme to a high affinity chromatin-bound state (6). A markedly different pattern was observed in the mutant H69-VP cells. In log culture at normal growth conditions (VP, I) the majority of topoisomerase IIα appeared to be not bound to the chromatin (lanes 1 and 2), which confirms the data shown in Figs. 1–3. When the cells were blocked with demecolcine at normal growth conditions (VP, M) and consequently formed type II metaphases such as shown in Fig. 3b (*bottom*), a small fraction of topoisomerase IIα (5%) was shifted to the 400 mM KCl extract (Fig. 4, lane 3), but the enzyme was neither detectable in fractions extracted subsequently with higher salt concentrations (lanes 4 and 5) nor in the salt-insoluble remnant (lane 6). These data indicate that in type II metaphases of H69-VP cells (compare Figs. 2 and 3b), topoisomerase IIα did not undergo a high affinity chromatin bond in the same way as in metaphases of parental H69-WT (Fig. 4, WT, M) or A431 cells (6), although a fraction (5%) of the enzyme was bound to the metaphase chromatin with low affinity. Unfortunately, a similar experiment could not be carried out with type I metaphases of H69-VP cells, because these cells were too fragile to endure sequential salt extractions. The four *bottom* panels of Fig. 4 show chromatin binding of topoisomerase IIβ in interphase (I) and release of the enzyme from the chromatin at mitosis (M). Similar such data were obtained in H69-WT and H69-VP cells, supporting the notion that topoisomerase IIβ does not get recruited to the mitotic chromatin instead of topoisomerase IIα, when the latter is lacking in the nucleus at the onset of mitosis.

We frequently observed that nuclei of H69-VP cells were disfigured and of much larger average size than those of parental H69 cells, suggesting a high frequency of non-disjunction in these cells. This notion was supported by comparative analysis of DNA mass distribution of the two cell lines under normal growth conditions by flow cytometry (Fig. 5). The parental cells (H69-WT) had a normal diploid genotype with a cell cycle distribution not unusual for a tumor cell line (G1 = 41 ± 4%, S = 46 ± 7%, and G2/M = 13 ± 2%). In contrast, H69-VP cells had a complex distribution of DNA mass with a greatly increased population of aneuploid cells (Fig. 5, Lines 1 and 2). When the cells were blocked with aphidicolin at normal growth conditions (VP, M), the two cell lines showed a similar cell cycle distribution, but the relative amounts of the respective DNA mass distributions were different (Fig. 5, Lines 3 and 4). With this procedure, the majority of the H69-WT cells (lanes 3 and 4) were arrested in the S phase (44.1%) or G2/M (15.5%), whereas in H69-VP cells (lanes 5 and 6) almost one third of the cells (32.7%) were in G2/M, whereas the other two thirds (67.3%) were in the S phase (91.8%). This result suggests that topoisomerase IIα is dispensable for cell cycle progression through the S phase, whereas topoisomerase IIβ is indispensable for normal cell cycle progression through the S phase.
increased peak at the 2n position and an additional broad peak at a position of 4n or larger. Blockade in S- or G2/M-phase resolved this complex pattern into two peaks and revealed that more than 90% of the H69-VP cells were tetraploid or had genomes of higher ploidity, whereas the parental cells contained less than 5% aneuploid cells.

**DISCUSSION**

*Essential Mitotic Functions of Topoisomerase IIα Are Not Adopted by the β-Isom-form—Studies on temperature-sensitive mutants of topoisomerase II have clearly demonstrated that for the yeast *Schizosaccharomyces pombe* the attempt to carry out mitosis in the absence of topoisomerase II ends up fatally with a failure in chromosome condensation and eventually non-disjunction of the chromatin (25). Studies on higher eukaryote cells using catalytic inhibitors of topoisomerase II have essentially confirmed that mammalian cells are similar to *S. pombe* in their response to topoisomerase II inactivation during mitosis (26–29), although these studies could not clearly distinguish between direct effects of topoisomerase II inactivation and indirect effects of cell cycle checkpoint activation. In contrast to yeast, mammalian cells express two isoforms of topoisomerase II that are encoded by separate genes. These two isoforms complement temperature-sensitive topoisomerase II mutants in yeast equally well (30) indicating that both of them support mitotic topoisomerase II function in yeast. However, there exists only one form of topoisomerase II in yeast. Thus, yeasts might not be able to distinguish topoisomerase II isoforms in the same way as higher eukaryotes.

A number of observations indicate that in their native environment the two isoforms of mammalian topoisomerase II serve unique functions. (i) In cells expressing both isoforms, only the α-form binds to the mitotic chromatin, whereas the β-isoform is excluded from the chromatin during mitosis and diffuses into the cytosol. This has previously been shown in human A431 cells (6) and was also seen here with parental H69-WT cells. Moreover, topoisomerase IIα is labeled in chromosomes by the mitotic phosphoprotein antibody MPM-2 (31). (ii) Resting cells express topoisomerase IIβ but not α. The α-isoform gets up-regulated when cells enter the cell cycle, and expression rises dramatically toward mitosis (10). In cycling cells expression of the β-isoform peaks early in S-phase, whereas expression of the α-isoform peaks during late S- and G2/M-phase (9). (iii) Expression of the α-isoform is restricted to the proliferative compartment of tissues (10), whereas the β-isoform is expressed in most if not all cells (7). (iv) Expressional increase of topoisomerase IIα but not β is associated with malignant cell transformation (32). In summary, these observations suggest that topoisomerase IIα is predominantly involved in cell cycling and mitosis, whereas the β-isoenzyme serves other as yet less clearly defined purposes.

Here, we studied a human cell line, which in interphase expresses a mutant α-isoenzyme mostly outside and the wild type β-isoenzyme mostly inside the nucleus. Under the assumption that the mutation of the α-isoenzyme is the only mutation governing the altered behavior of DNA topoisomerases in these cells (which may not be true), they seem to be a suitable model for finding out what happens to a mammalian cell, when topoisomerase IIα is lacking in the nucleus at the onset of mitosis. Surprisingly, in this situation the cells did not recruit topoisomerase IIβ (which was present and active in the nucleus) to the condensing chromat in order to overcome the lack in topoisomerase IIα. On the contrary, chromosome formation in these cells appeared to be entirely dependent on cytosolic topoisomerase IIα entering the chromat in. These data confirm previous indications that in mammalian cells the mitotic functions of topoisomerase II are mainly performed by the α-isoenzyme. Furthermore, they show that topoisomerase IIβ does not adopt these mitotic functions of the α-isoform. Such a situation is in clear contrast to yeast, which utilizes topoisomerase II at mitosis without distinction of isoforms.

**Role of Topoisomerase IIα in Chromosome Condensation and Disjunction—**H69-VP cells attempting mitosis without sufficient amounts of topoisomerase IIα bound to the chromatin formed segmented DNA spheroids instead of chromosomes. Formation of similar spherical chromatid balls by an active mitotic process has previously been observed, when the AT-rich tracts of scaffold adhesion regions were blocked with synthetic multiple AT-hook proteins (33). Thus, it seems that interaction of topoisomerase IIα with the chain of AT-rich sequences delineating the core of the chromatid fibers (34) is essential for condensation and shaping of chromosomes. The observation that the α- but not the β-isoenzyme is selectively inhibited by AT-rich oligonucleotides (4) further supports this notion. However, DNA sequence specificity alone does not explain why in mammalian cells only the α- and not the β-isoenzyme templates chromosome condensation, because otherwise isoenzyme specificity of this process would also be seen in yeast. Additional mechanisms must be involved in the selective targeting of the α-isoenzyme to the chromosomal scaffold, for example interactions with condensins (35).

Several studies show that intact two-armed chromosomes can be formed in the presence of catalytic inhibitors of topoisomerase II, such as merbarone, ICRF 193, or aclacinomicin (29, 36), indicating that formation of the two-armed chromosomal scaffold can occur in the absence of topoisomerase II activity. However, the data presented here suggest that the α-enzyme needs to be physically present and bound to the chromat in with high affinity in order to template chromosome structure faithfully. Actually, our previous results (6) show that during mitosis only a fraction of topoisomerase IIα is involved in catalytic DNA turnover and that this catalytically active fraction is not tightly chromatin-bound. Thus, we assume that chromosome condensation essentially involves high affinity binding of topoisomerase IIα to the chromosomal scaffold, whereas catalytic DNA topoisomerization at these places seems to be dispensable for the condensation process. In contrast, activity of topoisomerase IIα is clearly required for chromosome disjunction, because treatment of mammalian cells with catalytic inhibitors of topoisomerase II causes non-disjunction, asymmetric cell division, and polyploidy (27, 37). H69-VP cells show similar abnormalities, and it is reasonable to assume that these are due to the extra-nuclear expression of topoisomerase IIα in these cells, suggesting an essential and unique role of topoisomerase IIα in chromosome segregation. However, the presence of topoisomerase IIα activity per se seems to be insufficient for supporting these functions, because after the breakdown of the nuclear envelope topoisomerase IIα should have access to the chromat in of H69-VP cells in an active form and in similar amounts as in wild type H69 cells. However, the cytosolic enzyme apparently failed to form a high affinity chromat in bond. Thus, it appears that the enzyme does not interact with all its putative substrates by free diffusion and random collision.

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