1 Introduction

1.1 DNA-topology

For maintaining the genetic stability of most organisms, a proper replication and a successful disjunction of chromosomes are required (Holm et al, 1989; Sumner, 1995). To ensure the accuracy of these processes, manipulation of the DNA topology is necessary. Due to the structure of the double helix and the organization of the DNA in the nucleus, torsional stresses will be generated in the DNA during a number of cellular processes, including replication, transcription, recombination, chromosome (de)-condensation, and segregation (Watt & Hickson, 1994; reviewed in Wang, 1996a). Enzymes capable of altering the DNA topology play a fundamental role in the regulation and control of the physiological function of the DNA. The enzyme family, DNA topoisomerase are able to change the topology of the DNA, hence the name of the enzymes (Wang & Liu, 1979). The cells have to resolve topological problems during cell division to avoid chromosomal breakage and non-disjunction. In mitosis it is believed that the function of DNA topoisomerases is required in chromosome condensation together with the untangling of intertwined sister chromatids at the metaphase-anaphase transition (Dinardo et al, 1984; Uemura & Yanagida, 1984; Holm et al, 1985). In addition the enzyme is also required to relieve torsional stress or supercoiling in the DNA introduced by chain elongation during replication or transcription (Brill et al, 1987; Kim & Wang, 1989). Furthermore, DNA topoisomerase is able to suppress hyper-recombinations in the tandemly repeated ribosomal RNA genes (Christman et al, 1988). The enzyme is found to play several independent roles in the DNA metabolism. *In vivo*, DNA is constrained into a closed structure. Either the chromosomes are organized into a series of loops anchored at defined sites to a nuclear matrix or as circular DNA

molecules. The topology feature of the DNA is characterized by its linking number Lk, which is the number of times a strand in a double helix crosses the other. Briefly, *Lk* is defined by the equation *Lk=Tw +Wr*, where twist (*Tw*) is the frequency of which one strand in a double helix crosses the other, and therefore describes the total number of helical repeats (Crick, 1976). *In vitro,* the value is found to be 10.4 basepar per turn (Wang & Liu, 1979). Writhe (*Wr*) represents the overall structure of the DNA helix and measures the coiling of the helix in space. The values of the components *Tw* and *Wr* are variable, whereas the linking number is fixed, unless reversible cleavages in the DNA strand are introduced. These cleavages can be performed by topoisomerases, which introduce reversible breaks in the DNA strand without changing the genetic information (Wang & Liu, 1979). Topoisomerases are found in two different types. If the breaks in the DNA are catalysed by type I, the linking number changes in one step, whereas for type II the linking number changes in two steps (Gellert, 1981; Wang, 1985).

1.2 DNA topoisomerases

In the early 1970's the first known enzyme which relaxes supercoiled DNA was purified and identified from *Escherichia coli* (Wang, 1971). A few years later an ATP dependent enzyme (gyrase), also derived from the *Escherichia coli* was found, which can not only unwind supercoiled DNA but also introduce supercoiling in the relaxed DNA (Gellert et al, 1976). The discovery of these two enzymes, which are designated topoisomerases, was followed by findings of other members of the enzyme family (reviewed in Vosberg 1985; Wang 1991; 1996a). On the basis of the alignment of their amino acid sequences, topoisomerases can be grouped into three subfamilies: Type IA, Type IB and Type II, each exhibiting different biochemical characteristics and different catalytic

mechanisms of action (Caron & Wang, 1994; Roca, 1995). The two forms of type I introduce transient single-stranded breaks into the DNA, allowing an intact single strand of the DNA to transfer through the break, following religation (Champoux, 1978; Maxwell & Gellert, 1986). Type II enzymes, in contrast, in the presence of ATP introduce transient double-strand breaks into DNA, passing an intact DNA helix through the opening before the break is resealed (Osheroff, 1989). In my thesis I focus on human DNA topoisomerase of type II, so in the following sections I only give a brief review of the different enzymes which belong to the group of type II.

1.3 Type II

Type II enzymes identified so far are: gyrase (Gellert et al, 1976), topoisomerase II' (Brown et al, 1979), topoisomerase IV (Kato et al, 1990), phage T4 topoisomerase (Huang et al, 1990), and eukaryotic topoisomerases (Hsieh et al, 1992). For the catalytical activities, type II enzymes require divalent cations and ATP (Vosberg, 1985). The catalytical mechanism can be divided into discrete steps (Osheroff, 1989; Andersen et al, 1991; 1994). These steps involve non-covalent binding of the enzyme to the DNA, following a double-stranded DNA cleavage. The cleaved topoisomerase-DNA complex consists of 4 bp staggered cuts and the enzyme becomes covalently attached to the protruding 5'-end (Liu et al, 1983; Sander & Hsieh, 1983). Through the opening in the DNA strand an intact double helix can be transported. Following the transport the breaks are resealed. The strand passage may involve a DNA strand from either the same DNA segment as seen for relaxing/supercoiling or for un-knotting/knotting (Liu et al, 1983), or from another DNA duplex, as seen for decatenation/catenation (Hsieh & Brutlag, 1980; Goto & Wang, 1982). The reaction catalysed by type II DNA topoisomerase is shown in

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figure 1. All type II enzymes are able to relax supercoiled DNA, but gyrase from *Escherichia coli* is unique, because the enzyme can introduce negative supercoiling, not only in relaxed DNA but also in positively supercoiled DNA (Osheroff et al, 1983; Schomburg & Grosse, 1986). As a model for this supercoiling activity, it has been suggested that the DNA is wrapped around the enzyme-DNA complex forming a gate through which the strand passage of a DNA helix is possible (Liu & Wang, 1978). Using DNn'ase I footprinting, it has been found that the binding of gyrase spans a 140 bp on DNA. The binding to DNA for other members of type II covers only 20-30 bp (Lee et al, 1989; Thomsen et al, 1990) and is too short for the DNA to wrap around the complex (Fisher et al, 1992). It is not known how gyrase is able to distinguish between the positive and negative supercoiling. Besides the relaxation of supercoiled DNA, type II enzyme is able to resolve topological problems in more complicated DNA conformations, using either decatenation or unknotting. DNA gyrase is thus responsible for controlling the superhelical tension in bacterial DNA, and topoisomerase IV (rather than the gyrase) is reported to decatenate the interlinked chromosomes (Drlica and Zhao, 1997; Levine et al, 1998). This is in agreement with other studies, which also found that type II separates new replicated daughter chromatides in mitosis (DiNardo et al, 1984; Uemura & Yanagida, 1984) as well as in meiosis by decatenation (Rose & Holm, 1993). Prior to chromosome segregation the enzyme may be involved in catenating the chromatin to a compact form (Uemura et al, 1987). To unwind or to generate topological knots in double stranded circular DNA, the enzyme will catalyse these reactions by unknotting or knotting. It is believed that the knotted DNA molecules are intermediates generated during relaxation of supercoiled DNA (Liu et al, 1980). *In vivo,* an accumulation of these complicated knots arises during replication of viral DNA structures (Vosberg, 1985).

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However, *in vitro* this catalytical mechanism of unknotting/knotting is mostly used in assays for testing the specific activity of DNA topoisomerase II (Barret et al, 1990).

Type II enzymes, from various sources ranging from viruses to eukaryotes are evolutionarily and structurally related (Austin et al, 1993; Caron & Wang, 1994). The homology between the species is greatest in the NH_2-terminal, and in the central regions of the enzyme, whereas the COOH-proximal region tends to be divergent. The highly conserved regions in DNA topoisomerase II possess two catalytical centres: a site for ATP-hydrolysis and a DNA cleavage and a rejoining centre site (Watt & Hickson, 1994). Thus, type II enzymes differ in the subunits composition. Gyrase, topoisomerase II', topoisomerase IV, and phage T4 topoisomerase consist of two different subunits, which are associated as a tetramer (Gellert et al, 1976; Adachi et al, 1987; Kato et al, 1990). The viral T4 topoisomerase enzyme is a hexamer composed of two of each of the three proteins (Liu et al, 1979). The enzyme from eukaryotes exists in a solution as a homodimer consisting of two monomers. In lower eukaryotes only one form of DNA topoisomerase II is found (Shelton et al, 1983; Sander & Hsieh, 1983), whereas in mammalian cells two isoforms for DNA topoisomerase II with a molecular weight of 170 kDa and 180 kDa respectively, have been found (Drake et al, 1989). It has been suggested that the isoform IIα is the true homologue of topoisomerase II from other species, but no evidence supports this. Both isoforms expressed in yeast can functionally substitute yeast topoisomerase II and complement yeast TOP2 mutations (Jensen et al, 1996a).

1.4 Human topoisomerase II

Human DNA topoisomerase IIα from Hela cells was the first isoform to be purified and characterized (Miller et al, 1981). Later on, another isoform was identified from murine P388 cells, and was designated topoisomerase IIβ (Drake et al, 1987). DNA topoisomerase IIα is a 1531-residue polypeptide, whereas DNA topoisomerase IIβ is 90 amino acids longer than the IIα-form (Austin et al, 1993). The alignment of the sequence of the IIα and IIβ isoform showed a strict homology of 86%, except in the region encoding the extreme NH_2- and COOH-terminal of the protein (Chung et al, 1989; Austin et al, 1993). Recently, the whole human DNA topoisomerase IIα gene and the 3' half of human DNA topoisomerase IIβ, covering the COOH-terminal region have been cloned (Sng et al, 1999). Although the COOH-terminal end differs for the two isoforms, a very similar gene structure was obtained by comparison of the intron-exon arrangement of the two genes, which implies that the isoforms might have arisen by gene duplication (Sng et al, 1999). Genetic analysis showed that the DNA topoisomerase IIα and IIβ are products of two separate genes. The isoform IIα has been mapped to chromosome 17q21-22 (Tsai-Plugfelder et al, 1988), whereas the isoform IIβ is located to chromosome 3p24 (Tan et al, 1992; Jenkins et al, 1992). Although structural similarity and equal catalytical mechanisms were reported for the two isoforms, they differ in many respects in biochemical and pharmacological properties (Drake et al, 1987; 1989), differences in protein levels at various stages of the cell cycle (Woessner et al, 1991; Hickson et al, 1996), and in subcellular localization (Zini et al, 1992; Chaly, 1996; Meyer et al, 1996). These differences for the two isoforms indicate that the larger isoform IIβ is not a variant of topoisomerase IIα as first suggested, and, therefore, separate functions in the cell for the two isoforms should be assumed. However, no evidence of this has yet been

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reported. From mammalian cells, cDNA of the two isoforms has been cloned (Tsai-Plugfelder et al, 1988; Jenkins et al, 1992; Austin et al, 1993). For DNA topoisomerase IIβ there exist two splice-variants β-1 (the form found by Tan et al, 1992 and Jenkins et al, 1992) and β-2, the latter of which has 5 additional amino acids inserted into its NH_2-terminal end (Davies et al, 1993). It is unknown whether the addition of this pentapeptide effects the localization of the enzyme in the cell or whether its regulation differs from the β-1 form. However, both splice variants appear to have similar enzymatic activity (Austin et al, 1995).

1.5 Structural and functional domains of human topoisomerase II

The structural and functional domains of the two isoforms have been investigated, and proteolytic studies of DNA topoisomerase II show that the enzymes are composed of 3 major functional domains (Lindsley & Wang, 1991).

Subdomains: The NH_2-terminal domain of DNA topoisomerase contains the ATP binding pocket, which is responsible for ATP binding and hydrolysis (Mizuuchi, 1978; Sugino et al, 1980). In yeast the specific binding sequence is reported in amino acid residue 140 –165 (Lindsley & Wang, 1993). The cleavage-reunion domain, including the active site, is mapped in the central domain of DNA topoisomerases. The active tyrosine (TYR) binds covalently and forms an O^4-phosphotyrosine bond with the cleaved DNA strand. It has been mapped to TYR^{805} for human topoisomerase IIα (Sander & Hsieh, 1983) and to TYR^{821} for human topoisomerase IIβ (Austin et al, 1993). A large body of literature has been published about mutations within or near the putative ATP-binding sites, in the conserved nucleotide binding motif (residue 472-477), or at the active tyrosine site (review in Vassetzky et al, 1995). In contrast to the conserved regions the homology between the two isoforms in the COOH-

proximal region is only 34% (Austin et al, 1993). The COOH-terminal is species specific, and in some of the prokaryotes the region is even missing (Huang, 1990; Carcia-Beato et al, 1992). The biological role of this domain is still unclear, but current data indicate some potential roles: nuclear localization, regulation of enzymatic activity and dimerization. From deletion analysis the domain is found dispensable for enzymatic activity *in vitro,* (Watt and Hickson, 1994) but it is reported to be required for nuclear localization *in vivo* (Jensen et al, 1996b). Heterozygous deletion or truncation in putative bipartite nucleus localization signals (NLS), spanning amino acids 1422-1489 or 1454-1497, have been identified in etoposide-resistant cell lines (Takano and Fojo, 1995). At present, these mutations in NLS sequences are only found in IIα isoforms, and consequently the enzyme is extra-nuclear (Feldhoff et al, 1994; Mirski et al, 1995). Moreover, multiple phosphorylation sites have been reported in this non-conserved domain, whereas in *Schizosaccharomyces pombe* and in human cells the IIα-isoform is also phosphorylated within the NH$_2$-terminal domain. It is believed that the enzymatic activity as well as the mitotic function of DNA topoisomerase II can be regulated by numerous protein-kinases (reviewed in Cardenas & Gasser, 1993; Austin & Marsh, 1998). Deletions in 1053-1069 and in 1124-1143 are found to have an effect on the catalytical activity, although it does not comprise the breakage-reunion domain, indicating that the region is essential for homodimerization (Bjergbæk et al, 1999).

1.6 Human topoisomerase II in various stages in cell cycle

The two isoforms are immunologically distinct, and specific peptide-antibodies can therefore be raised against the two isoforms (Chung et al, 1989). This has been used to extend the understanding of the two isoforms in cell cycle manner and in the localization of the enzymes in

cells and tissues. The isoform IIα is found to be cell cycle dependent and there is a correlation between the expression of the enzyme and the proliferative state of the cell cycle (Woessner et al, 1991). In quiescent and differentiated cell populations the isoform IIα is not found expressed in an amount detectable by immunoblotting or immunohistochemical studies (Woessner et al, 1991; Boege et al, 1995). However, after a release of cells from a serum-deprived state or upon stimulation of the lymphocytes with the mitogen phytohemagglutinin-C the level of the IIα-isoform is dramatically increased (Kaufmann et al, 1994; Isaacs et al, 1996). The gene for the IIα isoform gets transcriptionally activated in the late S-phase to a level approximately 10-fold higher than its level in the G1-phase. Its expression rises to a maximum toward mitosis and subsequently the level falls rapidly. The isoform IIβ rises 2-fold in the early S-phase and is relatively constant throughout all the stages of the cell cycle (reviewed in Isaacs et al, 1998). When proliferating cells go back to the Go-phase the transcription of the IIα gene is shut down, whereas the IIβ isoform is still detectable (Isaacs et al, 1996). This observation implies that the topoisomerase IIα is required in cell division (Heck and Earnshaw, 1986), whereas the isoform IIβ may only play a minor role (Meyer et al, 1996). Furthermore, the appearance of the IIα-isoform can be used as a specific marker for proliferation (Heck and Earnshaw, 1986; Boege et al, 1995). DNA topoisomerase IIα is expressed predominantly in proliferating cells and is barely detectable in resting and differentiating cells, whereas topoisomerase IIβ is present in most if not all cells (Turley et al, 1997).

1.7   Regulation and control of human topoisomerase II

The mechanism of regulation of DNA topoisomerase IIα and β protein is still not throughly understood. It may be regulated by changes in DNA-

topoisomerase mRNA stability, alteration in transcription rates, or post-translational modifications (Heck et al, 1988; Goswami et al, 1996). The isoforms are mapped on two distinct genes, which allow differences in the regulation of the transcription of the enzymes. Using functional analysis, it has been possible to identify the promoter region of the two isoforms, and no significant homology has been found (Hochhauser et al, 1992; Ng et al, 1997). More significantly, the up-regulation of DNA topoisomerase IIα has been examined. It is important to find the binding of transcription factors to cis elements in the 5-flanking region of the gene, which have an influence of the transcription rates. It has been reported that topoisomerase IIα gets transcriptionally activated by the binding of the heterotrimeric transcription factor NF-Y in tumour cells (Isaacs et al, 1996; Herzog & Zwelling, 1997) as well as in lymphocytes (Kneitz, unpublished data). Furthermore, a decrease in the binding affinity is correlated with down-regulations of the IIα promoter activity in confluence-arrested fibroblast cells (Isaac et al, 1996). Independently of NF-Y other proteins P53 (Wang et al, 1997) and Sp3 (Hickson et al, 1996) exert negative regulatory effects on the promoter activity.

DNA topoisomerase IIα and IIβ appear to be phosphorylated throughout the cell cycle. The level of phosphorylation increases gradually during S-phase and reaches a maximum in the G_2/M-phase (Heck et al, 1989; Kroll & Rowe, 1991). The hyperphosphorylated state gives rise to a changed electrophoretic mobility. It is most pronounced for the IIβ isoform. The role of phosphorylation of DNA topoisomerase II remains controversial because some groups claim that the modulation of the overall catalytical activity of the enzyme is enhanced by hyperphosphorylation (Saijo et al, 1990; Cardenas & Gasser, 1993). However, our group recently showed that the activity of DNA topoisomerase II was decreased in mitotic cells, although the enzyme was hyperphosphorylated (Meyer et al, 1996). A

similar conclusion was reached using an etoposide resistant cell line (Takano et al, 1991). The mechanism responsible for regulating the phosphorylation state of topoisomerase is not clear but it is notable that distinct kinases are involved in phosphorylating DNA topoisomerase II during mitosis. In S-G$_2$ phases, phosphorylation stimulates the activity of DNA topoisomerase II, whereas upon entry in mitosis the enzyme is inhibited. This is due to a phosphorylation of topoisomerase II on several new serine and threonin residues as seen in the interphase (Wells et al, 1995; Ishida et al, 1996). Phosphorylation may also regulate its subcellular location, suggesting that topoisomerase IIα requires phosphorylation for nuclear localization as seen in other proteins such as P53 (Jans, 1995). It is not clear if phosphorylation of DNA topoisomerase II influences its ability to interact with other proteins in the nucleus. Using two–hybrid screening, a direct interacting of the COOH-terminal domain in yeast top2 with Sgs-1 and Pat-1 has been found (Watt et al, 1995, Wang et al, 1996b). These proteins are non-essential but a mutation in each gene leads to mis-segregation of the chromosome. Topoisomerase II might co-operate with these proteins to obtain a proper cell division. Moreover, topoisomerase II in *Drosphopila* interacts with Barren, which also plays a role in cell division (Yanagida, 1998). Another post-translational regulation, poly-ADP-ribosylation is commonly found in nuclear proteins but this modification on the topoisomerase II has only been demonstrated *in vitro* (Darby et al, 1985). Very little is known about the degradation of the enzymes. The level of IIβ isoform drops significantly when it leaves the nucleus as the cells enter mitosis. It might be degraded by ubiquitination or by other post-translational modifications (Meyer et al, 1996). In apoptotic Hl-60 cells only the isoform IIβ degrades, although not due to apoptotic proteolysis of the catalytic sites

but to loss of the COOH-terminal region at an early phase of apoptosis (Sugimoto et al, 1998).

1.8 Enzymatic mechanism for human topoisomerase II

The ATP-driven enzymatic mechanism for topoisomerase IIα and β is assumed to be identical. However, the isoform IIβ has a higher affinity for KCl compared with IIα (Drake et al, 1989) and a different binding/cleavage pattern for the two isoforms has been found (Sander and Hsieh, 1983; Drake et al, 1989). The catalytic mechanism of DNA topoisomerase involves the passing of one double stranded DNA duplex through a transient break created by the enzyme in a second DNA duplex. The mechanism is understood in some detail and the enzyme is believed to act as an ATP- modulated clamp with two molecular gates at opposite sites (Roca & Wang, 1992; 1994; Berger et al, 1996). Unfortunately, attempts to make a crystal structure of human DNA topoisomerase have failed, so the model is predominantly based on crystal structure of a 92 kDa yeast DNA topoisomerase II fragment (spanning amino acids residues 409-1201) and the ATP binding site from GYRB from gyrase (Berger et al, 1996; Morais Cabral et al, 1997). Mutagenesis studies in the enzyme might give further insight in understanding the co-ordination between ATP hydrolysis and DNA breakage/rejoining.

Catalytical mechanism of topoisomerase II: DNA topoisomerase binds non-covalently to a double stranded duplex, termed the gate-segment (or G-segment). *In vivo,* the binding of the enzyme to DNA is determined by the nucleotide sequence and the topological state of DNA (Osheroff, 1986). Although the specificity of the enzyme binding is not stringent, several binding sites have been identified (Sander & Hsieh, 1985; Capranico et al, 1997). Furthermore, the enzyme is able to distinguish between different topological states of DNA because it binds supercoiled

DNA more efficiently than relaxed or linear DNA (Zechiedrich & Osheroff, 1990). The binding of the enzyme spans a region of 20-30 bp symmetrically around the cleavage site (Lee et al, 1989; Thomsen et al, 1990). The enzyme cleaves both strands of the G-segment in an asymmetrical manner (4 base pairs apart in the DNA), which is unusual because homodimers often cleave DNA within a palindrome region. Topoisomerase II binds covalently to the G-segment *via* transesterification: a tyrosine residue in the active site of each monomer becomes covalently bound to a 5'-phosphoryl end of the newly broken strand. The 3'-hydroxyl end is recessed by 4 bases (Tse et al, 1980; Liu et al, 1983). The DNA cleavage caused by topoisomerase is an equilibrium reaction, favouring the religation of the DNA, probably to control the ability of the enzyme to change the DNA topology (Osheroff et al, 1991). This equilibrium reaction has been studied intensively, and by adding sodium dodecyl sulphate it is possible to trap the transient enzyme–DNA complexes, also called cleavage complexes, for further investigation (Liu et al, 1983). The strand passage of another DNA duplex (transport segment or T-segment) is dependent on the binding of a Mg-ATP complex to the ATP domain (Roca et al, 1993). The T-segment is transported through the open entrance of the N-gate formed by the pair of NH_2-terminal domains of the two subunits (Roca & Wang, 1992). The N-gate is believed to be open in the absence of ATP, whereas the binding of ATP stimulate a conformational change in the enzyme, which leads to closure of the gate (Wigley et al, 1991; Maxwell, 1997). The T-segment is now captured in the hole made of the N-terminal regions. A cascade of events has been triggered by the ATP binding: between the entrapped T-segment and the amino terminal domains steric repulsion is generated, and the T-segment is forced through a transient opening into a large hole between the G-segment and the second gate, termed C-gate (Roca &

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Wang, 1994). The C-gate is formed by amino acid residues 1030–1045 and 1113-1129 seen in yeast topoisomerase II (Berger et al, 1996; Roca et al, 1996). To complete the transport of the T-segment, it is released through the exit gate of the enzyme. Following strand passage a second transesterification occurs (Osheroff & Zechiedrich, 1987; Wang, 1996). The hydroxyl group formed in the cleavage reaction acts as the nucleophile, breaking the phosphotyrosine link and reforming the DNA phosphodiester bond to close the G-segment gate. The religation step is also an equilibrium reaction and 2 types of religation are proposed: when rejoining occurs in the DNA duplex it is an intramolecular religation, whereas a ligation between two different DNA duplexes is designated intermolecular religation. *In vitro* studies of recombinations can be done at this step (Gale & Osheroff, 1992; Schmidt et al, 1994). After religation hydrolysis of the bound ATP to ADP and Pi triggers a conformational change in the enzyme, allowing the N-gate to open so the enzyme regains its normal active conformation. The enzyme can in the presence of ATP initiate a new round of catalysis or enzyme turnover (Osheroff, 1986).

1.9   Subcellular localization of human topoisomerase II

To learn more about the specific functions of DNA topoisomerase IIα and β, localization of the two isoforms has been investigated. Different staining patterns for the two isoforms have been reported, probably due to different fixation procedures, different antibodies and different stages in the cell cycle (Warburton & Earnshaw, 1997). Additionally, the distribution of topoisomerase II could be species specific since in insects the enzyme is distributed uniformly in the whole chromosome fibre (Swedlow et al, 1993), whereas in human cells the enzyme is found along the axis of the chromosome (Earnshaw & Heck, 1985; Sumner, 1996; Meyer et al, 1996). Topoisomerase II is found to be a major component

of the mitotic scaffold (Earnshaw & Heck, 1985). However, only the IIα isoform can be located in the mitotic chromosomes. The IIβ isoform is found excluded from the nucleus during mitosis (Charly et al, 1996; Meyer et al, 1996). Although a recent paper claims that the isoform IIβ is also located on the chromosomes (Sugimoto et al, 1998). The finding of topoisomerase II in the different species is assumed to be the IIα isoform. The localization of DNA topoisomerase II and its possible variation during the cell cycle has been followed in *Drosophila* embryos and the action of the enzyme appears to be involved in chromosome condensation and segregation (Swedlow et al, 1993). After these two events most of the enzyme is moved to the cytoplasma, and only 30% of the enzyme remains bound to the chromatin throughout the mitotic cycle (Swedlow et al, 1993). Topoisomerase IIα is found associated with AT-rich regions in the nuclear scaffold and some groups believe that topoisomerase IIα within the scaffold is a tightly associated structural protein (Sander & Hsieh, 1983; Gasser & Lämmli, 1987). However, as mentioned in the above, 70 % of the enzyme leaves the nucleus during mitosis. Other studies showed absence of topoisomerase II in meiotic chromosome in *Xenopus oocytes.* (Fisher et al, 1993). This information raises doubts about the structural role of topoisomerase II in maintaining the structural integrity of chromosomes. Thus, the enzyme has a dynamic pattern because a subtle difference of the staining pattern of the enzyme has been observed when it progresses through mitosis. In prophase, a high concentration of the isoform IIα throughout the body of the chromosome is found, whereas at metaphase an intense staining at the centromeric regions is seen (Rattner et al, 1996; Sumner et al, 1996). Centromeres are also the last parts of the chromosomes to separate, and it is believed that association of topoisomerase II with the centromeres is required for a proper centromere/kinetochore structure (Rattner et al, 1996). In

anaphase, however, labelling of the enzyme is seen at the axial core, whereas the staining around the centromer has been lost (Sumner, 1996). To localize the two isoforms in the interphase nucleus is much more difficult. Nevertheless, recent studies from our group, using a new set of antibodies, have shown that the α-form is accumulated inside the nucleoli, whereas the β-form is mainly found in the nucleoplasma (Meyer et al, 1996). The latter is not in agreement with previous immunohistochemical studies where the localization of IIβ has mainly been found in the nucleoli (Negri et al, 1992; Zini et al, 1992; Petrov et al, 1993). In quiescent cells topoisomerase IIβ was expressed both in the nucleoli and nucleoplasma (Turley et al, 1997). Due to the striking controversy in the staining pattern of the isoform IIβ, its role has not yet been established. However, it is believed that the IIβ-isoform manages topological problems during replication and transcription, whereas the IIα-isoform is required in cell division.

1.10 Inhibitors of the topoisomerase catalytical cycle

In vivo, DNA topoisomerase IIα is a strict proliferation marker*,* and the expression of the enzyme happens to be upregulated in rapidly proliferating tumours. Indeed, tumour cell lines contain an increased level of topoisomerases and they are therefore chosen for investigating the enzyme. It has turned out that topoisomerases are a specific target for several clinically important antitumour drugs and therefore became interesting for clinical research (Liu, 1989; Chen et al, 1994). Drug resistance might be due to an altered or a decreased level of the enzymes in the tumour cells (Pommier et al, 1986; Danks et al, 1988; Beck et al, 1994a). The drugs can be divided into two categories: most of the known drugs, e.g. amsacrine or etoposide, exert cytotoxic effects by interfering with the cleavage/religation equilibrium of the enzyme, either by

stimulating the cleavage reaction, resulting in increasing proportions of the cleavable complex, or stabilizing the cleaved intermediate through the inhibition of the religation reaction (Liu et al, 1983). The drugs convert the enzyme to a lethal DNA-cleaver, and are therefore referred to as topoisomerase poison (Corbett & Osheroff, 1993). Recently, other types of drugs that do not interfere with the enzyme-DNA-complexes have been found. These drugs, e.g. aclarubicin or ICRF187, are called catalytical inhibitors (Jensen et al, 1991; Tanabe et al, 1991). Relatively few studies have examined the pharmacological role of topoisomerase IIβ, which was found to be less sensitive to some of the topoisomerase drugs compared to the α-form (Drake et al, 1989). However, other groups showed that the isoform IIβ might also be a target of antitumour poisons (Cornarotti et al, 1996; Marsh et al, 1996). Although an extensive body of literature on topoisomerase as a drug target is available, the precise reason why the drug interactions with the enzyme-DNA is highly lethal for the cell is not quite understood (Beck et al, 1994b). Inhibition of replication and transcription, an increase in sister chromatide exchange, and chromosome aberrations have all been suggested, but the accumulation of the drug-cleavage complex is not sufficient to ensure cell death (Corbett & Osheroff, 1993; Cortes & Pinero, 1994). The biochemical changes triggered by topoisomerase drugs which lead to the apoptosis in the cell remain to be identified.