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REVIEW
Induction of endoreduplication by topoisomerase II catalytic inhibitors

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The striking phenomenon of endoreduplication has long attracted attention from cytogeneticists and researchers into cell cycle enzymology and dynamics alike. Because of the variety of agents able to induce endoreduplication and the various cell types where it has been described, until now no clear or unique mechanism of induction of this phenomenon, rare in animals but otherwise quite common in plants, has been proposed. Recent years, however, have witnessed the unfolding of a number of essential physiological roles for DNA topoisomerase II, with special emphasis on its major role in mitotic chromosome segregation after DNA replication. In spite of the lack of mammalian mutants defective in topoisomerase II as compared with yeast, experiments with inhibitors of the enzyme have supported the hypothesis that this crucial untangling of daughter DNA molecules by passing an intact helix through a transient double-stranded break carried out by the enzyme, when it fails, leads to aberrant mitosis that results in endoreduplication, polyploidy and eventually cell death. Anticancer drugs that interfere with topoisomerase II can be classified into two groups. The classical poisons act by stabilizing the enzyme in the so-called cleavable complex and result in DNA damage, which represents a problem in the study of endoreduplication. The true catalytic inhibitors, which are not cleavable complex stabilizers, allow us to use doses efficient in the induction of endoreduplication while eliminating high levels of DNA and chromosome damage. This review will discuss the basic and applied aspects of this as yet scarcely explored field.

Introduction

DNA topoisomerases are conserved nuclear enzymes that catalyze a variety of topological changes in DNA during many cellular processes, such as replication, transcription and recombination, through transient cleavage of the molecule, strand passage and religation (for a review, see Wang, 1996). There are two classes of topoisomerases according to their catalytic mechanisms. While type I topoisomerases break and rejoin one DNA strand at a time, the type II enzymes are able to break and rejoin the two strands that make up duplex DNA. Since topoisomerase-induced breaks in DNA are fleeting intermediates in the strand passage reaction, they are present at low steady-state levels and hence are well tolerated by the cell as a necessary, though otherwise potentially dangerous, process that proves mutagenic and even lethal when the enzyme is poisoned (Liu, 1989; Burden and Osheroff, 1998).

Both the type I and type II enzymes are proficient in relaxing supercoiled DNA in order to relieve torsional tension generated during replication and transcription, while only topoisomerase II can decatenate intertwined DNA molecules. This unique decatenating as well as unknotting activity of DNA topoisomerase II is essential in the segregation of replicated daughter chromosomes. Apart from its important functional roles in chromosome condensation and segregation, topoisomerase II is a basic structural protein present at high levels in the nuclear matrix and chromosome scaffold (Laemli *et al*., 1992).

Diplochromosomes, made up of four chromatids held together, instead of the normal two, are the visible mitotic manifestation of the rare, although sometimes spontaneous, phenomenon of endoreduplication. This results from two successive rounds of DNA replication without intervening mitosis, i.e. segregation of daughter chromatids (Cortés *et al*., 1987; Sumner, 1998).

A variety of agents have been reported to induce endoreduplication to different degrees, either by disrupting cytoskeleton assembly, such as the spindle poisons colcemid, colchicin and concanavalin A (Rizzoni and Palitti, 1973; Sutou, 1981), or damaging DNA (Sutou and Tokuyama, 1974; Kusyk and Hsu, 1979; Huang, *et al*., 1983; Lüke-Huhle, 1983). More recently, agents that interfere with topoisomerase II have been used to provide further evidence that the enzyme is required for separation of daughter chromosomes. As a result of these studies, it has been shown that both topoisomerase 'poisons', i.e. chemicals that cause DNA strand breaks through stabilization of topoisomerase II covalently bound to DNA in the intermediate form termed the cleavable complex (Liu, 1989), and true catalytic inhibitors (Andoh and Ishida, 1998) are able to induce endoreduplication (Ishida *et al*., 1994; Sumner, 1998) due to prevention of decatenation of replicated chromosomes by topoisomerase II with subsequent failure to complete normal mitosis.

In the present review we will deal with the possible role played by topoisomerase II in chromosome segregation as well as with regard to the as yet poorly understood mechanism(s) leading to endoreduplication.

Type II topoisomerases: those amazing enzymes

Topoisomerase IIα and β isoforms

Two closely related topoisomerase II isoforms, designated α and β (Drake *et al.*, 1987, 1989b) have been distinguished from each other in higher eukaryotes. Lower eukaryotes such as yeast (Goto and Wang, 1984) and *Drosophila* (Wyckoff *et al*., 1989) appear to contain only one form of the enzyme. Both the α and β isoforms function as homodimeric enzymes and share extensive amino acid sequence identity (~70%) (Austin *et al*., 1993), but they are encoded by genes located on different chromosomes (chromosomes 17 and 3, respectively, in the human) and can be identified by their characteristic protomer molecular masses (~170 and ~180 kDa, respectively) (Capranico *et al.*, 1992; Austin *et al.*, 1993; Burden and Osheroff, 1998).

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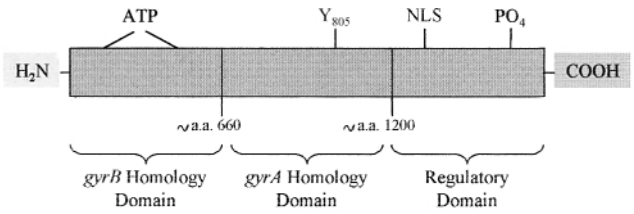

Fig. 1. The three domain structure of each topoisomerase II monomer showing regions of homology to the A and B subunits of bacterial gyrase. The three major domains of eukaryotic topoisomerase II are illustrated, as well as the site of ATP binding (ATP), the active site tyrosine (Y), the nuclear localization sequence(s) (NLS) that determines importation to the nucleus and the sites of phosphorylation (PO_4). The N-terminal domain extends from amino acid 1 to approximately amino acid 660, the central domain extends from approximately amino acid 660 to approximately amino acid 1200 and the C-terminal domain extends from approximately amino acid 1200 to the C-terminus of the enzyme.

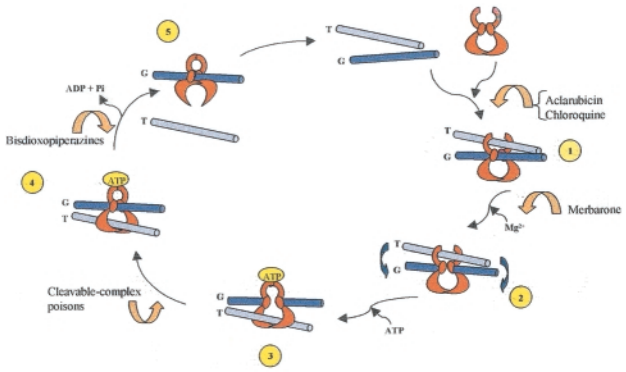

Fig. 2. Catalytic cycle of topoisomerase II (adapted from Burden and Osheroff, 1998). Enzyme catalysis is depicted as a series of five steps. Step 1, topoisomerase II DNA recognition and binding; step 2, pre-strand passage DNA cleavage/religation; step 3, ATP binding and DNA strand passage; step 4, post-strand passage DNA cleavage/religation; step 5, ATP hydrolysis and release of DNA and enzyme recycling.

In contrast to topoisomerase IIβ concentrations, which are relatively constant over the cell and growth cycles, topoisomerase IIα is highly regulated in a cell cycle- and proliferation-dependent fashion (Larsen *et al*., 1996). The level of topoisomerase IIα peaks during G_2/early mitosis, indicative of its major role in chromosome segregation, and is at its lowest during early G_1. A strongly decreased level of topoisomerase IIα is also characteristic of growth-arrested cells. This contrasts with the higher levels and activity of this isoform in cancer cells regardless of their cell cycle stage.

Based on the above evidence, the β isoform is considered as likely responsible for the 'housekeeping' functions of topoisomerase II, while the role of the α isoform is thought to be essential as the type II enzyme that unlinks daughter chromosomes following replication (Burden and Osheroff, 1998). Nevertheless, in spite of the differences mentioned regarding the regulation of both isoforms of topoisomerase II, the enzymological characteristics of all eukaryotic type II topoisomerases appear to be similar and so far no clear definition of the physiological roles of topoisomerases IIα and IIβ have been incontrovertibly determined (Burden and Osheroff, 1998).

Protein structure

The homodimeric eukaryotic enzyme topoisomerase II is generally compared with bacterial DNA gyrase, the best characterized prokaryotic type II topoisomerase, as to their amino acid sequences. On this basis, focusing on homology with the subunits A and B of gyrase, each topoisomerase II monomer can be divided into three distinct protein domains (Figure 1) (Lynn *et al*., 1986; Wyckoff *et al*., 1989; Burden and Osheroff, 1998; Berger, 1998). The N-terminal domain, which extends from amino acid 1 to approximately amino acid 660, is homologous to the B subunit of bacterial DNA gyrase and contains consensus sequences for ATP binding (Corbett and Osheroff, 1993; Wang, 1996; Berger, 1998; Burden and Osheroff, 1998). The central domain of the topoisomerase II monomer, comprising approximately amino acid 660 to amino acid 1200, presents homology to the A subunit of DNA gyrase. This central domain of the enzyme contains the active site tyrosine residue that binds covalently to DNA during scission (Corbett and Osheroff, 1993; Wang, 1996; Berger, 1998; Burden and Osheroff, 1998). Finally, the C-terminal domain of the eukaryotic enzyme does not appear to have a corresponding region of homology with DNA gyrase (Wyckoff *et al*., 1989; Corbett and Osheroff, 1993; Wang, 1996; Berger, 1998; Burden and Osheroff, 1998) and it is the most variable region in eukaryotes studied so far. Regardless of its variability, this C-terminal domain shows consistent features, such as being highly charged and containing nuclear localization sequences (Shiozaki and Yanagida, 1992; Crenshaw and Hsieh, 1993) as well as sites that are phosphorylated *in vivo* (Cárdenas *et al.*, 1992; Wells *et al.*, 1994). Other than its role in nuclear targeting, the physiological function of this topoisomerase II C-terminus is as yet poorly understood (Burden and Osheroff, 1998).

Topoisomerase II catalytic cycle

As stated earlier, topoisomerase II catalyzes DNA double-strand breakage and rejoining in an ATP-dependent fashion to remove superhelical twists as well as intramolecular DNA knots and intermolecular tangles for chromosome segregation (Wang, 1996). To begin with, it is important to bear in mind that the enzyme can assume two alternative forms, the open and closed clamp, in the absence and presence of ATP bound to the monomers, respectively.

The enzyme's catalytic cycle of topoisomerization of DNA begins with binding of the homodimer to its double-stranded substrate (Figure 2, step 1). While binding does not seem to require any cofactor, the presence of divalent cations has been reported to stimulate it (Osheroff, 1987). As to the double helix properties influencing topoisomerase II–DNA interactions, both nucleotide sequence and topology seem to play a role. Topoisomerase II cleaves DNA at preferred sequences within its recognition/binding sites, but there is no clear specificity (Sander *et al*., 1987; Spitzner and Muller, 1988; Lee *et al*., 1989; Pommier *et al*., 1991).

Concerning the influence of the topological structure of DNA on binding and cleavage by topoisomerase II, on the other hand, it has been reported that the enzyme interacts preferentially with supercoiled DNA over relaxed molecules (Burden and Osheroff, 1998). This behaviour of topoisomerase II provides a plausible explanation for its strong interaction with supercoiled DNA as well as its release of the relaxed reaction product. As to segregation of replicated daughter molecules, to be discussed later (see below), topoisomerase II is thought to locate DNA crossovers such as those present in catenated molecules.

As shown in Figure 2, step 2, in the presence of a divalent cation the enzyme generates a double-strand break by making

two coordinated breaks on opposite strands of the gap (G) double helix, with the formation of a 5′-phosphotyrosyl covalent bond. This normally fleeting DNA cleavage intermediate that forms as a prerequisite for the passage of the other, intact transported (T) double helix is called the 'cleavable complex'.

Upon binding of an ATP molecule, topoisomerase II undergoes a conformational change from the open to the closed clamp form that somehow allows the double-stranded DNA (T) passage through the protein–DNA gate previously opened in the G molecule (Figure 2, step 3). Concerning the release of the T molecule from the enzyme, it is generally believed that this depends on ATP hydrolysis, although this is still a matter of controversy (Roca and Wang, 1994).

The broken ends of the G molecule are then rejoined in what constitutes the 'post-passage complex' (Figure 2, step 4), while hydrolysis of ATP by the intrinsic ATPase activity of the enzyme located in the region of homology to gyrase B (see Figure 1) leads to the open clamp conformation and the release of DNA from topoisomerase II (Figure 2, step 5). Finally, the enzyme proceeds to normal turnover, regaining the ability to start its catalytic cycle again.

The role of DNA topoisomerases in chromosome segregation

Requirement of topoisomerase II for anaphase separation of chromosomes

While reports on yeast mutants that are defective in one or more topoisomerases imply that the type I and type II enzymes are able to substitute for each other to some extent in many functions, only the topoisomerase type II enzyme is essential for cell viability (Nitiss, 1998).

Topoisomerase II, encoded by the *top2* gene in yeast, is absolutely required to carry out separation of completely replicated chromosomes prior to cell division. Topoisomerase type I is essentially unable to fully separate double-stranded DNA molecules. Consistent with this proposed role of the enzyme, it has been reported that with no topoisomerase II activity *in vivo*, yeast plasmids are fully replicated but accumulate as catenated dimers (DiNardo *et al*., 1984).

In agreement with these and other observations in yeast (Uemura *et al.*, 1987), reports from studies carried out in *Drosophila* (Buchenau *et al*., 1993), amphibia (Shamu and Murray, 1992) and mammals (Downes *et al.*, 1991; Giménez-Abián *et al*., 1995; Sumner, 1995, 1998) indicate that the conserved nuclear enzyme topoisomerase II plays a major role in chromosome segregation during mitosis. A consistent observation that lends support to the idea of the importance of the enzyme for anaphase segregation is that if topoisomerase II function is blocked after chromosome condensation, the cells are arrested at metaphase and the chromatids fail to separate (Uemura and Yanagida, 1986; Clarke *et al*., 1993; Sumner, 1995, 1998).

On the other hand, a role for topoisomerase II in meiotic chromosome condensation and segregation in the yeast *Schizosaccharomyces pombe* (Hartsuiker *et al*., 1998) and mice (Cobb *et al*., 1997; Kallio and Lahdetie, 1997) has also been reported, supporting a similar meiotic role for topoisomerase II as that played in mitosis in the segregation of sister chromatids after completion of DNA replication. In the case of meiosis, for separation of homologous chromosomes having one or more crossovers in metaphase I topoisomerase II has to carry out the same task as in mitosis: separation of sister chromatids that are entangled because of meiotic DNA replication. Failure of this process should lead to arrest at the first meiotic division (Cobb *et al*., 1997; Kallio and Lahdetie, 1997).

Other gene products that interact with topoisomerase II for chromosome segregation

Focusing exclusively on topoisomerase II as the enzyme responsible for chromosome segregation at mitosis is too simplistic, given the high complexity of molecular interactions during cell division. To ensure proper progression from metaphase to anaphase, a number of processes, such as ubiquitin-dependent proteolysis, protein dephosphorylation, an unknown function of the TPR repeat proteins, chromosome transport by microtubule-based motor proteins and DNA topological change by topoisomerase II, are all necessary. In addition to chromosome condensation, mitotic kinetochore function and spindle formation require a large number of proteins, which are also prerequisites for successful sister chromatid separation (Yanagida, 1995). A number of genes involved in sister chromatid separation and segregation have recently been reported in both budding (Biggins *et al*., 2001; Bhalla *et al*., 2002) and fission yeast (Leverson *et al*., 2002; Wang, *et al*., 2002). Gene products reported to interact with topoisomerase II and play a role, in association with the enzyme, in its ability to decatenate intertwined fully replicated DNA molecules before their segregation have also been described in different organisms.

Sister chromatid segregation in mitosis, though highly dependent on topoisomerase II, also requires other gene products to varying extents. For instance, a gene product isolated from *Drosophila*, the so-called barren (*barr*), has been shown to be required for anaphasic segregation of chromosomes (Bhat *et al.*, 1996). The protein encoded by *barr* is present in proliferating cells, localizes to chromatin throughout mitosis and has homologs in yeast and human (Lavoie *et al*., 2000). Co-localization and biochemical experiments have indicated that the barren protein associates with topoisomerase II during mitosis and alters the activity of the enzyme. A cooperative role for proper chromosomal segregation by facilitating chromatid decatenation has also been proposed (Bhat *et al*., 1996). A second protein that has been proposed to interact with *Drosophila* topoisomerase II is the protein encoded by the *PAT1* gene (Wang, *et al*., 1996). This gene product does not appear to be essential for viability, but *pat1* mutants present a slow growth rate and exhibit a phenotype that resembles that of yeast cells grown under limiting amounts of topoisomerase II. The mutants show a reduced fidelity of chromosome segregation during both mitosis and meiosis, suggesting that this protein plays a key, but non-essential, role in accurate chromosome transmission during cell division. The precise function remains to be determined (Nitiss, 1998).

Contrasting with topoisomerases I and II, whose functions in eukaryotes are well established, the role of DNA topoisomerase III (a type I enzyme) remains poorly defined. It has been proposed that topoisomerase III may play a role in chromosomal segregation (DiGate and Marians, 1988). In bacteria, topoisomerase III, in conjunction with the RecQ helicase, is capable of decatenating completely double-stranded interlinked DNA molecules (Harmon *et al*., 1999), presumably via two sequential strand passage reactions. This proposal challenges the early notion that ascribed the ability to decatenate completely double-stranded interlinked circular DNA molecules solely to type II topoisomerases (Zhu *et al*., 2001).

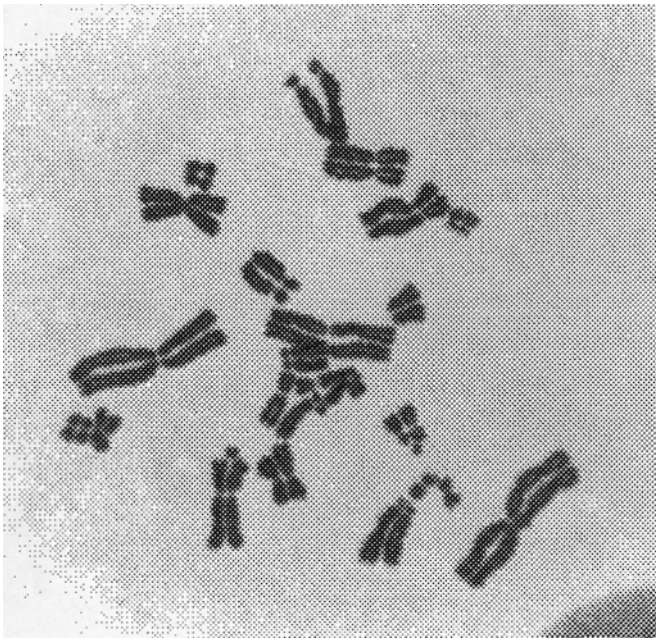

Fig. 3. Induction of endoreduplication by the topoisomerase II catalytic inhibitor ICRF-193, a bisdioxopiperazine. Chinese hamster AA8 cell showing the characteristic diplochromosomes made up of four chromatids.

In both yeast (Gangloff *et al*., 1994) and higher eukaryotes, including humans (Shimamoto *et al.*, 2000; Kobayashi and Hanai, 2001), a body of data seems to support a model in which the association of a RecQ helicase and topoisomerase III might be important to facilitate decatenation of late stage replicons to permit faithful chromosome segregation during anaphase. Interestingly, the human Bloom's and Werner's syndrome gene products, which belong to the RecQ family of DNA helicases, have been shown to be associated with topoisomerase IIIα (Johnson *et al*., 2000; Wu *et al*., 2000). Nevertheless, it has recently been proposed that type I topoisomerases such as topoisomerases I and III may primarily be involved in recombination as opposed to being directly involved in chromosome decatenation (Harmon *et al*., 1999; Wu *et al*., 2000; Zhu *et al*., 2001), and an important role for topoisomerase III in the restart of stalled DNA replication forks after DNA damage has been reported (Chakraverty *et al*., 2001). As a result, attention has again been focused on topoisomerase II as an essential enzyme for DNA segregation.

The rare phenomenon of endoreduplication

Endoreduplication consists of two successive rounds of DNA replication without an intervening mitosis, i.e. segregation of daughter chromatids (Cortés *et al*., 1987; Sumner, 1998). The visible mitotic manifestation of previous endoreduplication is the presence of diplochromosomes, made up of four chromatids held together, instead of the two normally observed in metaphase chromosomes (Figure 3). While in plants endoreduplication is generally considered a rather common event (Sun *et al*., 1999), spontaneous endoreduplication is a phenomenon more rarely observed in animals, generally as a characteristic feature of specific tissues such as dipteran salivary glands (Weiss *et al*., 1998), mammalian liver (Lu *et al*., 1993; Sigal *et al*., 1999), tonsils (Takanari and Izutsu, 1981) and trophoblast giant cells of the placenta (Bower, 1987; MacAuley *et al*., 1998; Zybina *et al*., 2000, 2001).

Interestingly, the acquisition by tumor cells of high chromosome numbers may be due to endoreduplication (Larizza and Schirrmacher, 1984), pointing to a possible link between endoreduplication and tumorigenesis. A typical response of liver cells to potentially carcinogenic chemical damage is an increased yield of endoreduplicated hepatocytes (Sargent *et al*., 1994; Madra *et al*., 1995). Ionizing radiation has also been reported to be capable of inducing endoreduplication in both cultured Chinese hamster cells (Lüke-Huhle, 1983) and human lymphocytes (Weber and Hoegerman, 1980).

While the phenomenon of endoreduplication has drawn a lot of attention from both cytogeneticists and those involved in the investigation of the genetics and biochemistry of the cell cycle, due to the variety of agents able to induce it and the various cell types in which it has been described so far, no clear or unique mechanism of induction of endoreduplication has been proposed. A variety of agents have been reported to induce endoreduplication to different degrees, either by disturbing cytoskeleton assembly, such as the spindle poisons colcemid, colchicin and concanavalin A (Rizzoni and Palitti, 1973; Sutou, 1981), or damaging DNA (Sutou and Tokuyama, 1974; Kusyk and Hsu, 1979; Huang, *et al*., 1983; Lüke-Huhle, 1983).

As to specific cell cycle stages sensitive to induction of endoreduplication by chemicals, the G_2/mitosis stage appears to be the most sensitive (Speit *et al*., 1984; Matsumoto and Ohta, 1992, 1995; Giménez-Abián *et al*., 1995). Recently, it was reported that $p21^{waf1/Cip1/Sdi1}$-induced growth arrest is associated with a depletion of mitosis-control proteins, leading to abnormal mitosis and endoreduplication in recovering cells (Chang *et al*., 2000). This observation seems to be consistent with the role of the cyclin-dependent kinase inhibitor p21 as having an integral role in cell growth arrest associated with DNA damage, which in turn often involves the triggering of endoreduplication, as stated above. As will be discussed, one of the essential proteins whose expression might be inhibited by p21 is likely to be topoisomerase II. In mammalian cells, the existence of a temporary G_2 topoisomerase II-dependent checkpoint that regulates entry into mitosis has been proposed (Downes *et al*., 1994).

Topoisomerase II catalytic inhibitors as inducers of endoreduplication

Topoisomerase II poisons versus catalytic inhibitors

The 'classical' topoisomerase II 'poisons' are characterized by their ability to induce DNA double-strand breakage through the stabilization of covalent complexes between the enzyme and DNA known as 'cleavable complexes'. This stabilization leads to mutations and eventually to cell death. Some of the more relevant clinically important antitumor drugs targeting topoisomerase II and poisoning the enzyme are anthracyclines (e.g. adriamycin), epipodophyllotoxins (e.g. etoposide and teniposide), anthracenediones (e.g. mitoxantrone) and aminoacridines (e.g. m-AMSA) (Liu, 1989; Chen and Liu, 1994; Froehlich-Ammon and Osheroff, 1995).

In recent years a novel group of drugs of diverse chemical nature have been reported as non-classical 'true' catalytic inhibitors of mammalian DNA topoisomerase II. These include merbarone (Drake *et al*., 1989a), fostriecin (Boritzki *et al*., 1988), aclarubicin (Jensen, *et al*., 1990), SN 22995 (Chen and Beck, 1993), suramin (Bojanowski *et al*., 1992), novobiocin (Utsumi *et al*., 1990), chloroquine (Jensen *et al*., 1994) and the bisdioxopiperazines (ICRF-154, ICRF-193, etc.) (Ishida

et al., 1991; Tanabe *et al*., 1991), although possible behavior as topoisomerase II poisons of the latter has recently been questioned (Jensen *et al*., 2000; Huang *et al*., 2001). These compounds target the nuclear enzyme within the cell and interfere with various fundamental genetic processes such as replication and transcription, as well as, more specifically, chromosome dynamics.

Contrasting with topoisomerase II poisons, these drugs lack the ability to stabilize the cleavable complex. In addition, the exact manner by which they inhibit the nuclear enzyme and their mechanism(s) of toxicity is, in general, poorly understood (Andoh and Ishida, 1998).

Are both polyploidy and endoreduplication induced by drugs that interfere with topoisomerase II?

In budding and fission yeast, temperature-sensitive *Top2* mutants have been useful to demonstrate the essential role of topoisomerase II in decatenating sister chromatids before anaphase commences, while it is not needed for progression through the later stages of mitosis (Uemura and Yanagida, 1986). In higher eukaryotes, the lack of topoisomerase II mutants has made definitive proof of a similar mitotic function of the enzyme difficult to demonstrate (Clarke *et al*., 1993). As an alternative approach, topoisomerase II poisons and catalytic inhibitors have been employed.

The problem encountered when using topoisomerase II poisons such as etoposide is that high doses have to be used to hinder enzyme function, leading to cytotoxic effects and induction of DNA damage with subsequent G_2 delay (Lock and Ross, 1990; Sumner, 1992; Chen and Beck, 1995). This drawback necessarily focuses the attention on topoisomerase II catalytic inhibitors which do not cause DNA and chromosome damage in order to determine the relative importance of the enzyme in promoting chromosome segregation at the metaphase/anaphase transition (Sumner, 1998).

In spite of the above-mentioned drawbacks, a number of reports have dealt with endoreduplication induced by topoisomerase II poisons (Sumner, 1998) or, in most instances, a doubling of DNA content of post-mitotic nuclei, without a direct observation of diplochromosomes (Zucker *et al*., 1991; Cummings *et al*., 1995; Sumner, 1995; Ferguson *et al*., 1996). Down-regulated topoisomerase IIα gene expression in etoposide-selected cells has also been shown to be associated with an increased rate of spontaneous polyploidization (Melixetian *et al*., 2000).

While they have been available for a long time and despite their usefulness and promising prospects, reports of topoisomerase II catalytic inhibitor-induced polyploidization and endoreduplication are scarce. Human leukemic CEM cells continuously exposed to merbarone and SN22995 first accumulated in G_2, but then escaped the G_2 block and proceeded into mitosis. Failure to divide leads to re-replication, and the cells accumulate at the 8C DNA stage (Chen and Beck, 1993). Unfortunately, however, the possible appearance of endoreduplicated cells in the next mitosis was not assessed. Inactivation of topoisomerase II by merbarone also resulted in polyploidy in male mouse meiotic cells (Kallio and Lahdetie, 1997).

More than any other catalytic inhibitor, the group of bisdioxopiperazines (ICRF-154, ICRF-187, ICRF-193, etc.) has been studied in order to analyze their effect on chromosome segregation. Andoh and co-workers (Andoh *et al*., 1993; Ishida *et al*., 1994) first reported that ICRF-193, considered a catalytic non-cleavable complex-forming type topoisomerase II inhibitor (Ishida *et al*., 1991), led to an absence of chromosome segregation during mitosis with further accumulation of polyploid cells with ⩾8C complements. Formation of polyploid nuclei as a consequence of failure of chromosome segregation in the presence of ICRF-193 was also reported in HeLa cells (Haraguchi *et al*., 1997). Similar results on endoreduplication resulting in large highly polyploid cells have recently been obtained after treatment of human leukemia cells with dexrazoxane (ICRF-187) (Hasinoff *et al*., 2001).

While the notion that bisdioxopiperazines are pure catalytic inhibitors of DNA topoisomerase II has recently been the focus of controversy (Jensen *et al*., 2000; Huang, *et al*., 2001), with reports on their possible poisoning mechanism, we have reported on a high yield of endoreduplication as a consequence of ICRF-193 treatment in Chinese hamster ovary cells at concentrations shown to efficiently inhibit topoisomerase II catalytic activity (Pastor *et al*., 2002). An interesting observation was that the EM9 mutant cell line, which is defective in the repair of both DNA single- and double-strand breaks, as compared with its parental AA8 cell line (Thompson *et al*., 1982), was shown to be particularly sensitive to induction of endoreduplication by ICRF-193. A consistent feature of EM9 is its elevated spontaneous yield of metaphases showing diplochromosomes as a result of endoreduplication, not observed in the parental AA8 cell line (Cortés *et al*., 1993). In addition, we have also found that mutant EM9 cells are extremely sensitive to inhibition of topoisomerase II, leading to endoreduplication (Pastor *et al*., 2002). In good agreement with these observations, we have found that aclarubicin, another reported topoisomerase II catalytic inhibitor (Jensen *et al*., 1990; Andoh and Ishida, 1998), is also an efficient inducer of endoreduplication (unpublished data).

Concluding remarks: trying to cut a long story short

While over the years it has become evident that the phenomenon of endoreduplication is far from being simple, both in terms of its molecular mechanisms and regarding its control in the eukaryotic cell cycle, the recent incorporation of type II DNA topoisomerases in the enzymology of chromosome dynamics has shed new light on this rather obscure area of research.

The first conclusion is that, although of paramount importance, type II topoisomerases are not the only eukaryotic enzymes involved in the complex endoreduplication process. For example, it has been reported that inhibition of cyclin-dependent kinases (Cdks) prevents endoreduplication and polyploidization (Motwani *et al*., 2000), while induced levels of p21 were found to lead to endoreduplication, most likely through inhibition of the expression of multiple proteins involved in the execution and control of mitosis (Chang *et al*., 2000).

Nevertheless, it is now widely accepted that failure to properly segregate daughter chromosomes by topoisomerase II leads to endoreduplication. This outcome of aberrant mitosis leads to death in many cell types, while for others it seems to be a normal process in their development, as frequently found in plants or in dipteran salivary glands.

Focusing on the proposed model for the topoisomerase II catalytic cycle (Figure 2) and on the basis of the increasing body of data about the diverse molecular mechanisms of operation of the enzyme poisons and true catalytic inhibitors, it can be proposed that both types of compounds that cause

topoisomerase misfunction frequently lead to endoreduplication.

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