Minireview

Toward a comprehensive model for induced endoreduplication

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Abstract

Both the biological significance and the molecular mechanism of endoreduplication (END) have been debated for a long time by cytogeneticians and researchers into cell cycle enzymology and dynamics alike. Mainly due to the fact that a wide variety of agents have been reported as able to induce endoreduplication and the diversity of 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. DNA topoisomerase II (topo II), plays a major role in mitotic chromosome segregation after DNA replication. The classical topo II poisons act by stabilizing the enzyme in the so-called cleavable complex and result in DNA damage as well as END, while the true catalytic inhibitors, which are not cleavable-complex-stabilizers, do induce END without concomitant DNA and chromosome damage. Taking into account these observations on the induction of END by drugs that interfere with topo II, together with our recently obtained evidence that the nature of DNA plays an important role for chromosome segregation [Cortés, F., Pastor, N., Mateos, S., Domínguez, I., 2003. The nature of DNA plays a role in chromosome segregation: endoreduplication in halogen-substituted chromosomes. DNA Repair 2, 719–726.], a straightforward model is proposed in which the different mechanisms leading to induced END are considered.

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Keywords: DNA topo II; Chromosome segregation; Mitosis; Endoreduplication; Diplochromosomes

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Diplochromosomes are the visible mitotic manifestation of the striking and rare, although sometimes spontaneous, phenomenon of endoreduplication (END). As first defined and coined by Levan and Hauschka (1953), they are made up of four chromatids held together, instead of the normal two, as a result of the occurrence of two successive rounds of DNA replication without intervening mitosis, i.e. segregation of daughter chromatids (Cortés et al., 1987; Sumner, 1998). A rather common event in plants (Sun et al., 1999), spontaneous END is a phenomenon more rarely observed in animals, being a characteristic feature of specific tissues such as dipteran salivary glands (Weiss et al., 1998), in which a series of successive ENDS leads to the development of polytene chromosomes (Fig. 1), and 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).

The rather uncommon observation of END among metazoa notwithstanding, it has been proposed that the acquisition by tumor cells of high chromosome numbers may be due to END (Larizza and Schirrmacher, 1984), pointing to a possible link between both END and tumorigenesis. Besides, a typical response of liver cells to chemical damage potentially carcinogenic is an increased yield of endoreduplicated hepatocytes (Sargent et al., 1994; Madra et al., 1995). Ionizing radiation, on the
other hand, has also been reported as capable of inducing END in both human lymphocytes (Weber and Hoegerman, 1980) and cultured Chinese hamster cells (Lücke-Huhle, 1983).

While the phenomenon of END has drawn a lot of attention from both cytogeneticists and those involved in the investigation of the genetics and biochemistry of the cell cycle, no clear or unique mechanism of induction of END has been proposed, due to the variety of agents able to induce it and the various cell types where it has been described so far. As to the specific cell cycle stages sensitive to induction of END by chemicals, the G2-mitosis period appears to be the most sensitive (Speit et al., 1984; Giménez-Abián et al., 1995; Matsumoto and Ohta, 1992, 1995). Recently, it has been reported that p21\textsuperscript{waf1/Cip1/Sdi1}-induced growth arrest is associated with depletion of mitosis-control proteins, leading to abnormal mitosis and END in recovering cells (Chang et al., 2000). This observation seems to be consistent with the role of the cyclin-dependent kinase (CDK) inhibitor p21 as an integral part of cell growth arrest associated with DNA damage that in turn often involves the triggering of END. One of the essential proteins whose expression might be inhibited by p21 is likely to be topoisomerase (topo) II, which as will be discussed in the present review, plays a central role in chromosome segregation. In mammalian cells, the existence of a temporary G2 topo II-dependent checkpoint that regulates entry into mitosis has been proposed (Downes et al., 1994). We will deal with the role played by topo II in chromosome segregation in regard of the as yet poorly understood mechanism(s) leading to END. While spontaneous END is a phenomenon that deserves a lot of interest, mainly as a process that results in a highly increased transcription of different genes which play fundamental roles in development as a whole, in the present work we will mainly focus on the data available on the induction of this outstanding mechanism by chemicals in mammalian cells.

"Classical" inducers of endoreduplication

To begin with, it is important to make a distinction between true induction of END and some instances in which the treatment merely stimulates already endoreduplicated cells that exist in organs such as liver or plant root to go into mitosis and show up with their characteristic diplochromosomes at metaphase. A clear example was our observation of previously endoreduplicated \textit{Allium cepa} root meristem cells, normally non-dividing, that entered mitosis after a short treatment with acetaldehyde (Fig. 2) (Cortés et al., 1987).

A variety of agents either by disrupting cytoskeleton assembly, such as the spindle poisons colcemid, colchicin or concanavalin A (Rizzoni and Palitti, 1973; Sutou, 1981), or damaging DNA (Sutou and Tokuyama, 1974; Kusyk and Hsu, 1979; Huang et al., 1983; Lücke-Huhle, 1983) have been reported to induce END to different degrees. The list of physical, and mainly chemical agents so far reported as able to induce END in a variety of eukaryotic cells is rather long, so we will only discuss the most representative ones (Table 1) according to their mode of action, mainly on DNA. Concerning physical agents, ionizing radiation treatments such as X-ray (Sutou and Arai, 1975; Weber and Hoegerman, 1980) and also α-radiation (Lücke-Huhle, 1983) have been found to induce END. DNA-damaging chemicals of different nature, on the other hand, have been shown as able to induce END to different degrees. For example, high frequencies of END in mammalian cell cultures from both mouse and Chinese hamster ovary (CHO) were observed as a result of treatment with the intercalative bisbenzimide fluorochrome 33258 Hoechst and the anthracycline zorubicin (Kusyk and Hsu, 1979). Also treatment with the DNA synthesis inhibitor aphidicolin was reported to lead to the observation of metaphases showing
diplochromosomes in Chinese hamster cells (Huang et al., 1983). Results from other investigations have shown that the bifunctional alkylating agent mitomycin C, a DNA crosslinker, induces END in cultured human lymphocytes (Takanari and Izutsu, 1981), while the radical-forming drug hydrazine is able to induce it in Chinese hamster V79 cells (Speit et al., 1984). Trying to cut a long list short, sodium arsenite, a unique human carcinogen that induces many types of cytogenetic alterations, such as sister chromatid exchanges and chromosomal aberrations, has also been reported to induce END in CHO cells (Kochhar et al., 1996) and human skin fibroblasts (Huang et al., 1995; Yih et al., 1997).

A different consideration seems to deserve those reports dealing with chemical agents that interfere with cytoskeleton assembly, as mentioned above. The anti-tubulin alkaloid agent colchicine was first reported to induce END in Chinese hamster embryonic cells (Rizzoni and Palitti, 1973; Palitti et al., 1976). Also the T lymphocyte mitogen concanavalin A, a lectin reported as responsible of cytoskeletal reorganization (Wu et al., 1998), has been shown as an END-inducer in the Don line of Chinese hamster cells (Sutou, 1981). More recently, the spindle depolymerizing drugs colcemid and nocodazole have been used to induce DNA END in primary human fibroblasts (Hixon et al., 1998). Microtubule inhibitors Taxol and vincristine, on the other hand, have been also reported to induce END in p21-deficient human tumor cells (Stewart et al., 1999a,b). Finally, the tubulin-targeting natural drug dolastatin-10, a marine-derived anticancer agent, has been reported to induce END (Pathak et al., 1998), in good agreement with that observed for other agents that interfere with tubulin polymerization.

### Topo II inhibitors and endoreduplication

As shown in yeast mutants defective in one or more topoisomerases, type I and type II enzymes, the only topoisomerase that is essential for cell viability as a whole seems to be the type II enzyme (Nitiss,
1998), which is necessary to separate replicated chromosomes before cell division. Topoisomerase type I, given its molecular mechanism breaking and rejoining just one DNA strand, is unable to fully separate double stranded DNA molecules. Without topo II activity in vivo, as expected, fully replicated yeast plasmids accumulate as unresolved catenated dimers (DiNardo et al., 1984). Concerning higher eukaryotes, the unavailability of a similar tool, i.e. topo II mutants, has made it difficult to reach conclusions on a similar mitotic role of the enzyme. An alternative approach, has been the use of topo II poisons, i.e. chemicals that cause DNA strand breaks through stabilization of topo II covalently bound to DNA in the intermediate form so-called cleavable-complex (Liu, 1989), as well as true catalytic inhibitors (Andoh and Ishida, 1998). The possible induction of polyploidy in its different manifestations such as END (Ishida et al., 1994; Sumner, 1998) as an endpoint has been considered as a proof of the prevention of decatenation of fully replicated chromosomes by topo II by the enzyme inhibitors, with the subsequent failure to complete a normal segregation at mitosis.

Reports from studies carried out in Drosophila (Buchanau et al., 1993), amphibia (Shamu and Murray, 1992) and mammals (Giménez-Abián et al., 1995; Sumner, 1995, 1998; Downes et al., 1991)

<table>
<thead>
<tr>
<th>Agent</th>
<th>DNA damage/modification</th>
<th>Cytoskeleton disturbance</th>
<th>Topoisomerase poisoning or catalytic inhibition</th>
<th>References</th>
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<tr>
<td>Colcemid, colchicine</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Rizzoni and Palitti, 1973</td>
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<td>Concanavalin A</td>
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<td>Colcemid, nocodazole</td>
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<td>Hixon et al., 1998</td>
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<td>Taxol; vincristine</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Stewart et al., 1999a,b</td>
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<td>Dolastatin-10</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Pathak et al., 1998</td>
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<tr>
<td>33258 Hoechst; zorubicin</td>
<td>+</td>
<td>–</td>
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<td>Kusyk and Hsu, 1979</td>
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<td>Aphidicolin</td>
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<td>Cortés et al., 2003</td>
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<td>Mitomycin C</td>
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<td>Takanari and Izutsu, 1981</td>
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<td>Hydrazine</td>
<td>+</td>
<td>–</td>
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<td>Speit et al., 1984</td>
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<td>Sodium arsenite</td>
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<td>Kochhar et al., 1996; Huang et al., 1995; Yih et al., 1997</td>
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<td>α-radiation</td>
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<td>–</td>
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<td>Lücke-Huhle, 1983</td>
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<td>Amsacrine, adriamycin, teniposide</td>
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<td>–</td>
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<td>Zucker et al., 1991a</td>
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<tr>
<td>Merbarone</td>
<td>–</td>
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<td>+</td>
<td>Kallio and Lahdetie, 1997a; Chen and Beck, 1993a; Sumner, 1998</td>
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<tr>
<td>Mitoxantrone, etoposide, amsacrine</td>
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<td>Pastor et al., 2002</td>
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<td>ICRF-193</td>
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An overview.

* Polyploidy reported. Possible endoreduplication not determined.
have confirmed this major role of topo II in good agreement with the observations made in yeast (Uemura et al., 1987). An observation that lends further support to the idea of the importance of the enzyme for anaphase segregation in higher eukaryotes is that if topo II function is blocked after chromosome condensation chromatids fail to separate, and the cells remain arrested at metaphase (Uemura et al., 1987; Sumner, 1995, 1998; Uemura and Yanagida, 1986; Clarke et al., 1993). These results seem to support the idea that the final step of decatenation of intertwined daughter molecules can only be carried out by topo II. Levels of topo IIα mRNA peak in late S and G2/M several-fold (normally over 10 times) over the amount detected in G1 cells. These high levels are in good agreement with the idea of a requirement of topo IIα mainly during the final stages of DNA replication to facilitate chromosome untangling, condensation and mitotic segregation. As mentioned earlier, drugs of different chemical nature that interfere with topo II have been tested and reported to induce polyploidy and END to different degrees (Zucker et al., 1991; Chen and Beck, 1993; Cummings et al., 1995; Sumner, 1995, 1998; Ferguson et al., 1996; Kallio and Lahdetie, 1997), providing indirect evidence that the enzyme is necessary for separation of sister chromatids.

Through the stabilization of covalent complexes between the enzyme and DNA known as cleavable complexes, topo II “poisons” do induce DNA double-strand breakage, mutations and eventually cell death. Clinically important antitumor drugs targeting topo II and poisoning the enzyme are anthracyclines, (e.g. adriamycin), epipodophyllotoxins, (e.g. etoposide and teniposide), anthracyclines, (e.g. mitoxantrone), and aminoacridines, (e.g. m-AMSA) (Liu, 1989; Chen and Liu, 1994; Froehlich-Ammon and Osheroff, 1995). More recently, a group of drugs of diverse chemical nature have been reported as non-classical “true” catalytic inhibitors of mammalian DNA topo II. These compounds either bind to DNA and alter the relationship DNA-topo II, or target the nuclear enzyme within the cell. Through any of these mechanisms, there is a loss of topo II activity that in turn results in an interference with various fundamental genetic processes such as replication and transcription as well as, more specifically, chromosome dynamics. Some examples of these drugs include merbarone (Drake et al., 1989), fostriecin (Boritzki et al., 1988), aclazarubicin (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 group of bisdioxopiperazines (ICRF-154, ICRF-193 etc.) (Ishida et al., 1991; Tanabe et al., 1991) although these latter have been recently questioned as to their possible behavior as topo II poisons (Jensen et al., 2000; Huang et al., 2001). Anyway, while the exact manner by which they inhibit the nuclear enzyme and their mechanism of toxicity are as yet poorly understood (Andoh and Ishida, 1998), contrasting with topo II poisons, these drugs lack in general the ability to stabilize the cleavable complex.

Using topo II poisons such as etoposide, to study END, presents the negative aspect that high doses have to be used to hinder enzyme function, leading to undesirable cytotoxic effects and induction of DNA and chromosome damage with subsequent G2 delay (Lock and Ross, 1990; Sumner, 1992; Chen and Beck, 1995). Taking into account this setback, the use of topo II catalytic inhibitors instead turns out to be the immediate alternative, provided that they 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). The above mentioned drawbacks notwithstanding, either END induced by topo II poisons as assessed by the presence of diplochromosomes at metaphase (Sumner, 1998) or, in most instances, a doubling of DNA content of postmitotic nuclei, without a direct observation of diplochromosomes (Zucker et al., 1991; Sumner, 1995; Cummings et al., 1995; Ferguson et al., 1996) has been reported. It is also worth mentioning here that down-regulation of topo IIα gene
expression in etoposide selected clonal cell lines has also been shown to be associated with an enhanced spontaneous polyploidization (Melixetian et al., 2000).

When it comes to topo II catalytic inhibitor-induced polyploidization and END, despite their foreseen usefulness and advantages versus drugs that poison the enzyme, somehow surprisingly the reports are rather scarce. Exposure of human leukemic CEM cells to merbarone and SN22995, reported as catalytic inhibitors (Andoh and Ishida, 1998), first resulted in accumulation in G2 but then cells escaped the G2 block to proceed into mitosis. Nevertheless, failure to divide eventually led to re-replication, and the cells accumulated at the 8C DNA stage (Chen and Beck, 1993). Unfortunately however, the possible appearance of endoreduplicated cells in next mitosis was not assessed. Inactivation of topo II by merbarone also resulted in polyploidy in male mouse meiotic cells (Kallio and Lahdetie, 1997).

Since Andoh et al. (Andoh et al., 1993; Ishida et al., 1994) did pioneer work with ICRF-193, first considered a catalytic non-cleavable-complex-forming-type topo II inhibitor (Ishida et al., 1991), and observed an accumulation of polyploid cells, the class of the bisdioxopiperazines (ICRF-154, ICRF-187, ICRF-193 etc) has been the more frequently studied as to their effects on chromosome segregation (Morita et al., 1994). While 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 observations on END resulting in large highly polyploid cells have been recently obtained in human leukemia cells treated with dexrazoxane (ICRF-187) (Hasinoff et al., 2001).

The consideration of bisdioxopiperazines as pure catalytic inhibitors of DNA topo II however, has been recently challenged (Jensen et al., 2000; Huang et al., 2001; Hajji et al., 2003), with even controversial reports on their possible poisoning mechanism (Hajji et al., 2003), we have reported on a high yield of END as a consequence of ICRF-193 treatment in Chinese hamster ovary cells at concentrations shown as efficiently inhibiting topo 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 to its parental AA8 cell line (Thompson et al., 1982), has shown to be particularly sensitive to induction of END by ICRF-193. A consistent feature of EM9 previously reported by us (Cortés et al., 1993) is its elevated spontaneous yield of metaphases showing diplochromosomes as a result of END, not observed in the parental AA8 cell line. In good agreement with these observations, we have recently found that aclorubicin, another reported topo II catalytic inhibitor (Jensen et al., 1990; Andoh and Ishida, 1998), is also an efficient inducer of END (unpublished data).

Changes in the nature of DNA lead to endoreduplication

As stated above, it is widely accepted that topo II plays a major role in segregating replicated daughter chromatids before anaphase. Concerning the relationship between DNA nucleotide sequence and topo II, though it is generally agreed upon that the former plays a role in enzyme function, the rules that determine the nucleic acid specificity of topo II are as yet far from being completely elucidated. It has been reported that topo II cleaves DNA at preferred sequences within its recognition/binding sites, but there is no report on high specificity (Sander et al., 1987; Spitzner and Muller, 1988; Lee et al., 1989; Pommier et al., 1991).

In order to study the sequence specificity of double-strand DNA cleavage by Drosophila topo II, the frequencies of the nucleotides and dinucleotides in the region near the site of phosphodiester bond
breakage was analyzed (Sander and Hsieh, 1985) and revealed a nonrandom distribution. The consensus sequence derived was 5′ GT.A/TAY decrease ATT.AT..G3′ where a dot means no preferred nucleotide, and Y stands for pyrimidine (Sander and Hsieh, 1985). On the other hand, DNase I footprint analysis has revealed that Drosophila topo II can protect a region in both strands of the duplex DNA, with the cleavage site located near the center of the protected region (Lee et al., 1989), and it has been proposed that the strong DNA cleavage sites of Drosophila topo II (Sander and Hsieh, 1985) likely correspond to specific DNA-binding sites of the enzyme (Sander et al., 1987; Spitzner et al., 1990).

Using a transcription assay (Thomsen et al., 1990) the interaction between topo II from calf thymus and DNA was also characterized. The conclusion was that topo II binds to a region of DNA located symmetrically around the enzyme-mediated cleavage site.

We have recently found that END is readily induced in AA8 Chinese hamster cells treated for two consecutive cell cycles with different halogenated nucleosides, namely 5-chlorodeoxyuridine (CldU), 5-iododeoxyuridine (IdU), and 5-bromodeoxyuridine (BrdU) (Fig. 3). Interestingly however, treatment for just one cell cycle did not lead to a similar increase in END (Cortés et al., 2003). The frequency of endoreduplicated cells was highest for CldU, intermediate for IdU and lowest for BrdU. Besides, the frequency of cells showing diplochromosomes paralleled the relative percentage established concerning the halogenated pyrimidine:deoxythymidine incorporation into DNA. Although the observation that treatment of the cells for one cell cycle with CldU did not result in END seems to indicate otherwise, we considered the unlikely hypothesis of a possible direct interaction between the exogenous halogenated nucleoside and topo II. As expected, we did not find any loss of decatenating activity of topo II that in turn might have hampered proper chromosome segregation (Cortés et al., 2003).

While the possible involvement of other proteins cannot be ruled out at present, our observations seem to favor the likely hypothesis that the nature of DNA might play a role for the recognition/binding of topo II and its subsequent cleavage of the fully replicated molecule for chromosome segregation. It has been reported that eukaryotic topo II preferentially cleaves alternating purine-pyrimidine repeats within the consensus sequence, and additionally, GT, AC and AT repeats were better substrates for

![Fig. 3. Third mitosis (M3) CldU-substituted diplochromosomes showing the differential Giemsa staining indicative that analogue substitution into DNA has taken place for two consecutive rounds of DNA replication, followed by an additional S-period in absence of CldU (the crucial one during which endoreduplication took place).](image)
cleavage than GC repeats (Spitzner and Muller, 1988; Thomsen et al., 1990). Furthermore, the
distribution of DNA cleavage sites induced by topo II in the presence or absence of enzyme poisons
were mapped in the simian virus 40 genome and the finding was that strong sites tended to occur within
A/T runs such as those that have been associated with binding to the nuclear scaffold (Pommier et al.,
A likely conclusion is that our results support the idea that the presence of anomalous bases such as
halogenated pyrimidines in DNA results in a defective function of topo II in chromosome segregation
that eventually leads to aberrant mitosis and the subsequent END (Pastor et al., 2002). Our recent
observation that treatment with the analogue 5-azadeoxycytidine (5-aza-dC), well known to induce
strong and permanent demethylation of DNA also results in END in CHO cells (unpublished results)
seems to lend further support to the hypothesis that changes in the nature of DNA might be important to
determine chromosome segregation. When this process fails, END mechanism(s) should be triggered.
Besides, the observation that analogue (CldU etc) incorporation for only one S period, i.e. only in
nascent DNA, does not result in END, contrasting with the increased yield of END when incorporation
takes place for two consecutive rounds of replication, seems to point to the apparent relative importance
of template DNA for chromosome segregation to proceed normally.

A straightforward model

Taking into account the high complexity of molecular events going on during cell division, thinking
on topo II as the unique responsible for chromosome segregation at mitosis is really a too simplistic idea. A
number of processes such as ubiquitin-dependent proteolysis, protein dephosphorylation, an unknown
function by the TPR repeat proteins, chromosome transport by microtubule-based motor proteins and
DNA topological change by topo II are all necessary to ensure a proper progression from metaphase to
anaphase. Besides, chromosome condensation, mitotic kinetochore function and spindle formation
require a larger number of proteins, which are also prerequisites for successful sister chromatid
separation (Yanagida, 1995). On the other hand, even though, as stated above, topo II seems to play a
central role, even for centromere and kinetochore function (Ouspenski and Brinkley, 1993; Rattner et al.,
1996; Strissel et al., 1996), separation of sister chromatids in mitosis seems to require, to different extent,
the concourse of a number of gene products.

This latter consideration notwithstanding, taking into account the evidence gathered in the present
review on the variety of agents so far reported as inducers of END as well as the overwhelming body of
data on the importance of the nuclear enzyme topo II for chromosome segregation, a temptative model of
induced END is presented here (Fig. 4). We consider three main mechanisms/categories of agents
capable of inducing END or, more properly speaking, able to switch on a cell cycle signal that eventually
leads to the triggering of an endocycle. It has recently been proposed, for the sake of simplicity, that such
a signal requires nothing more elaborate than a loss of M-phase CDK activity and oscillations in the
activity of S-phase CDK (Larkins et al., 2001).

As shown in Fig. 4, a first group should include those agents that, as colchicines and Colcemid,
interfere with cytoskeleton assembly (microtubule inhibitors etc). The block of mitosis at metaphase that
results in this case might, as stated above, switch on a new replication cycle (endocycle) without
anaphase segregation. The results at the chromosome level (diplochromosomes) should be visible in the
next mitosis (endomitosis).
Agents that inhibit the enzyme topo II can be considered, according to our model, to belong to a second broad category that includes both the enzyme poisons (etoposide, amsacrine, adriamycin etc) and catalytic inhibitors (merbarone, aclarubicin, ICRF-193 etc) (Fig. 4). In a whole sense, regardless of the important differences among them as to their mechanisms of interaction with topo II, the final outcome...
should be that the enzyme cannot carry out proper separation of entangled fully replicated DNA before mitosis. Again this situation might result in a suspension of anaphase and maybe switching on cell cycle “emergency” signals leading to END.

Finally, important modifications in the nucleotide sequence and/or topology of DNA are likely to result in a failure of topo II in its binding to DNA, which in turn leads to a defective chromosome segregation, with the consequences already described as to trigger END. This latter mechanism provides a likely explanation for the reported effectiveness of physical and chemical agents that damage DNA to induce END. Our recent observations that halogen-substitution into DNA also results in END (Cortés et al., 2003) also seem to lend further support to this latter hypothetical mechanism.

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