Antonie van Leeuwenhoek **62**: 15–24, 1992.

Topoisomerase II: its functions and phosphorylation

Susan M. Gasser, Robin Walter, Qi Dang[1] & Maria E. Cardenas[2]
Swiss Institute for Experimental Cancer Research (ISREC), CH-1066 Epalinges s/Lausanne, Switzerland
Present addresses: [1] University of California Medical School, San Francisco, CA, USA;
[2] Rockefeller University, New York, NY 10021, USA

Key words: chromosomes, nuclear scaffold, phosphorylation, topoisomerase II, yeast

Abstract

The gene encoding topoisomerase II in yeast is unique and essential, required for both mitotic and meiotic proliferation. The use of temperature-sensitive mutants in topoisomerase II have demonstrated roles in the relaxation of tortional stress, reduction of recombination rates, and in the separation of sister chromatids after replication. In vertebrate cells, topoisomerase II was shown to be the most abundant component of the metaphase chromosomal scaffold, and has been shown to play a role in chromosome condensation *in vitro*. The cell cycle control of chromosome condensation may well require phosphorylation of topoisomerase II, since the enzyme is more highly phosphorylated in metaphase than in G1. Recent studies have identified casein kinase II as the major enzyme phosphorylating topoisomerase II in intact yeast cells. The target sites of CKII are exclusively in the C-terminal 400 amino acids of topoisomerase II, the region that is most divergent among the eukaryotic type II enzymes and which is absent in the bacterial gyrase homologues.

Abbreviations: topoII – topoisomerase II; CKII – Casein Kinase II; SV40 – Simian Virus 40

Introduction

Eukaryotic chromosomes undergo major structural changes that reflect their varied functions through the cell cycle. These changes are mediated in part by DNA topoisomerases. To relieve both positive and negative supercoiling, eukaryotic organisms have two types of topoisomerases: DNA topoisomerase I and II. The type I enzymes alter the DNA linking number in steps of one by means of a transient single strand cut, while topoisomerase II makes a double stranded break, passes the same or another DNA molecule through the break and reseals the strands, changing the linking number in steps of two (reviewed by Wang 1985).

Because each has the capacity to relax supercoiled DNA, topoisomerases I and II can substitute for each other in many biological functions. Studies of yeast mutants suggest that both topoisomerases I and II are able to relieve DNA torsional stress or supercoiling introduced by chain elongation during transcription (Brill & Sternglanz 1988) and DNA replication (Kim & Wang 1989a; Brill et al. 1987). Similarly, studies with topoisomerase I-topoisomerase II double mutants demonstrated that either enzyme can suppress high levels of mitotic recombination in the ribosomal DNA repeated units (Christman et al. 1988; Kim & Wang 1989b).

Although both topoisomerases appear to compensate for each other in many instances, topoisomerase II is an essential enzyme while topoisomerase I is not. This reflects the unique ability of topoisomerase II to decatenate covalently closed DNA circles or intertwined helices, rendering the enzyme essential for the proper disjunction of sister chromatids in mitosis (Dinardo et al. 1984;

Holm et al. 1985; Uemura & Yanagida 1984, 1986) and for the segregation of chromosomes in meiosis (Rose et al. 1990). The requirement of topoisomerase II at mitosis may also reflect a role in chromosome condensation and the higher order folding of chromatin (Uemura et al. 1987).

Studies of chromosome structure suggest that topoisomerase II is involved in the organization of looped chromatin domains. Antisera raised against the most abundant component of the metaphase scaffold isolated from either human or chick chromosomes, a 170 kDa protein called SC-1, showed unequivocally that SC-1 is identical to topoisomerase II (Earnshaw et al. 1985; Gasser et al. 1986). Topoisomerase II was immunolocalized to the axial core of mitotic chromosomes (Earnshaw & Heck 1985; Gasser et al. 1986) and to the synaptonemal complex in pachytene cells from species as varied as chicken, mouse and yeast (Moens & Earnshaw 1989; Klein et al. 1992). Topoisomerase II also cofractionates with the interphase nuclear matrix fraction of *Drosophila* and yeast cells (Berrios et al. 1985; Berrios & Fisher 1988; Cardenas et al. 1990). This does not depend upon the enzymatic activity of the enzyme, since temperature-sensitive mutants in topoisomerase II show the same fractionation pattern in yeast at either permissive or non-permissive temperatures (Cardenas et al. 1990). Electron microscopic studies have suggested that the enzyme preferentially recognizes sites where two DNA molecules cross over each other, in either positively or negatively supercoiled DNA (Zechiedrich & Osheroff 1990). The role of topoisomerase II in chromosome condensation, its binding sites in AT-rich DNA, and its post-translational modification are discussed below.

A role in chromatin condensation

A study of a cold sensitive topoisomerase II mutant in *Schizosaccharomyces pombe* provided the first functional evidence that topoisomerase II was required for proper chromosome condensation (Uemura et al. 1987). In a cold-sensistive (cs) β-tubulin mutant at nonpermissive temperature, the microtubules are destabilized and one observes by DAPI fluorescence the highly condensed *S. pombe* mitotic chromosomes (Umesono et al. 1983). When this mutation is coupled with a top2 cs mutant, chromosomes do not condense properly, remaining in an extended organization reminiscent of prophase (Uemura et al. 1987).

This observation was carried further by Laemmli and coworkers, who have shown that in a *Xenopus* egg extract the conversion of nuclei into condensed mitotic chromosomes requires the presence of topoisomerase II, either as a component of the substrate nuclei, or as a component of the egg extracts in which the mitotic events occur (Adachi et al. 1991). While HeLa nuclei, which have high levels of topoII, condense properly in topoII-depleted extracts, nuclei which have low levels of endogenous topoisomerase II are unable to form fully condensed chromosomes in the depleted extracts. Addition of exogenous yeast topoisomerase II, purified from an overexpressing strain, restores chromosome condensation. Although the requirement of topoII activity was not examined in this assay, earlier studies of nuclear disassembly in *Xenopus* extracts had noted the inhibition of chromosome condensation in the presence of VM-26, a specific inhibitor of topoisomerase II (Newport and Spann 1987).

Similar results have been obtained using an extract from somatic cells blocked in mitosis (Wood & Earnshaw 1990). Again it was observed that the degree of condensation of exogenously added nuclei in a topoII-depleted extract appeared to depend on the level of endogenous topoisomerase II. However, since chick erythrocyte nuclei, with <300 copies of topoII per nucleus, showed a significant condensation of chromatin without resolution into chromosomes, these authors stress the multiplicity of events involved in chromosome condensation. Among the obvious ones are histone H1 and H3 phophorylation and lamin disassembly.

A link has been proposed to exist between topoisomerase II activity and the ability of the $p34^{cdc2}$ kinase to phosphorylate histone H1 and disassemble the nuclear lamina (Peter et al. 1990). The topoII inhibitor VM-26 was shown to block the activation of $p34^{cdc2}$ kinase at G2/M, and the cells' progression into mitosis (Roberge et al. 1990). This

might suggest that decatenation by topoisomerase II is required before entry into mitosis. An alternate explanation of these results, however, is that DNA strand breakage is induced by the inhibition of topoisomerase II, and that the damage stimulates a feedback pathway that blocks the cell in G2. There is ample evidence for such a pathway in *S. cerevisiae* (Hartwell & Weinert 1989).

While chromosome condensation in mammalian cells may be more complex than it is in yeast cells, the studies on topoisomerase II underscore the similarities in this important cell cycle event among eukaryotes. As described above, observations originally made in yeast mutants have been confirmed and extended using mammalian and vertebrate systems. In addition it is clear that *Drosophila* topoisomerase II can complement a yeast null mutant (Wyckoff & Hsieh 1988) and that in reconstituted systems from vertebrates, topoisomerase II from yeast can replace the endogenous enzyme (Adachi et al. 1991). Thus genetic and cellular approaches can be combined in the study of the mitotic function of topoisomerase II.

DNA binding properties

Many laboratories have tried to identify the preferred binding sites of topoisomerase II by mapping double strand breaks induced by the enzyme either *in vitro* or in intact cells. In some cases the sites mapped *in vitro* agree with those found *in vivo*, and in others they do not (reviewed in Liu 1989). Some of the discrepancy may be due to a certain promiscuity of the enzyme brought about by treatment with drugs (VM-26, m-AMSA, VP-16) that impede the religation step of the enzymatic reaction and thus enhance the detection of cleavages. However, it is likely that the primary cause of discrepancy is that topoisomerase II interacts differently with naked DNA in solution than with chromatin. It was noted that in reconstituted SV-40 chromatin and in the SV-40 chromosome *in vivo*, topoisomerase II cleaved primarily in linkers between nucleosomes or in DNase 1 hypersensitive sites, which generally did not coincide with preferred cleavage sites mapped on naked DNA *in vitro* (Yang et al. 1985; Capranico et al. 1990). In intact cells it was also observed that topoisomerase II cleavage sites fell either into DNase 1 hypersensitive regions, indicating an open, non-nucleosomal organization, or into nucleosomal spacers (Rowe et al. 1986; Reitman & Felsenfeld 1990; Udvardy & Schedl 1991). Although there appears no strict consensus for cleavage sites *in vivo*, sequencing of several cleavage sites found in nucleosomal linkers upstream of the *Drosophila* Histone H1 gene shows a preferential cut at GC rich stretches that are flanked by AT-rich regions (Kaes & Laemmli 1992).

In vitro the preference of topoisomerase II for AT-rich DNA is quite striking. Sander and Hsieh (1985) earlier defined a consensus for *Drosophila* topoisomerase II based on its cleavage of plasmid DNA *in vitro*. This AT-rich, 15bp sequence was but a loosely defined consensus; nonetheless, it was striking that this consensus for topoisomerase II occurred frequently in the fragments of DNA that have a high affinity for the nuclear scaffold (Gasser & Laemmli 1986a, b; Cockerill & Garrard 1986). These SARs or MARs (scaffold or matrix-attached regions) are candidates for sequences that organize the genome into looped domains, since they show a high affinity for a histone depleted nuclear and chromosomal substructure (Mirkovitch et al. 1984, 1986). In yeast these scaffold-associated regions are coincident with autonomously replicating sequences, commonly known as ARS elements (Amati & Gasser 1988, 1990). Consistent with studies in other species that map topoisomerase II cleavage sites to SARs (e.g. Udvardy et al. 1985), we show in Fig. 1 that yeast topoisomerase II also cuts preferentially in ARS sequences *in vitro*. As shown in Figure 1 for the rDNA repeat, transcribed regions have far fewer topoII cleavage sites.

An advance was made in understanding this interaction by showing that topoisomerase II binds preferentially to SAR sequences *in vitro*, whether it is induced to cleave the DNA or not (Adachi et al. 1989; Sperry et al. 1989). In addition, Adachi et al. (1989) showed that topoisomerase II binds in a cooperative manner and is capable of precipitating the DNA, probably as a result of aggregation of the protein-DNA complexes. Since the binding of to-

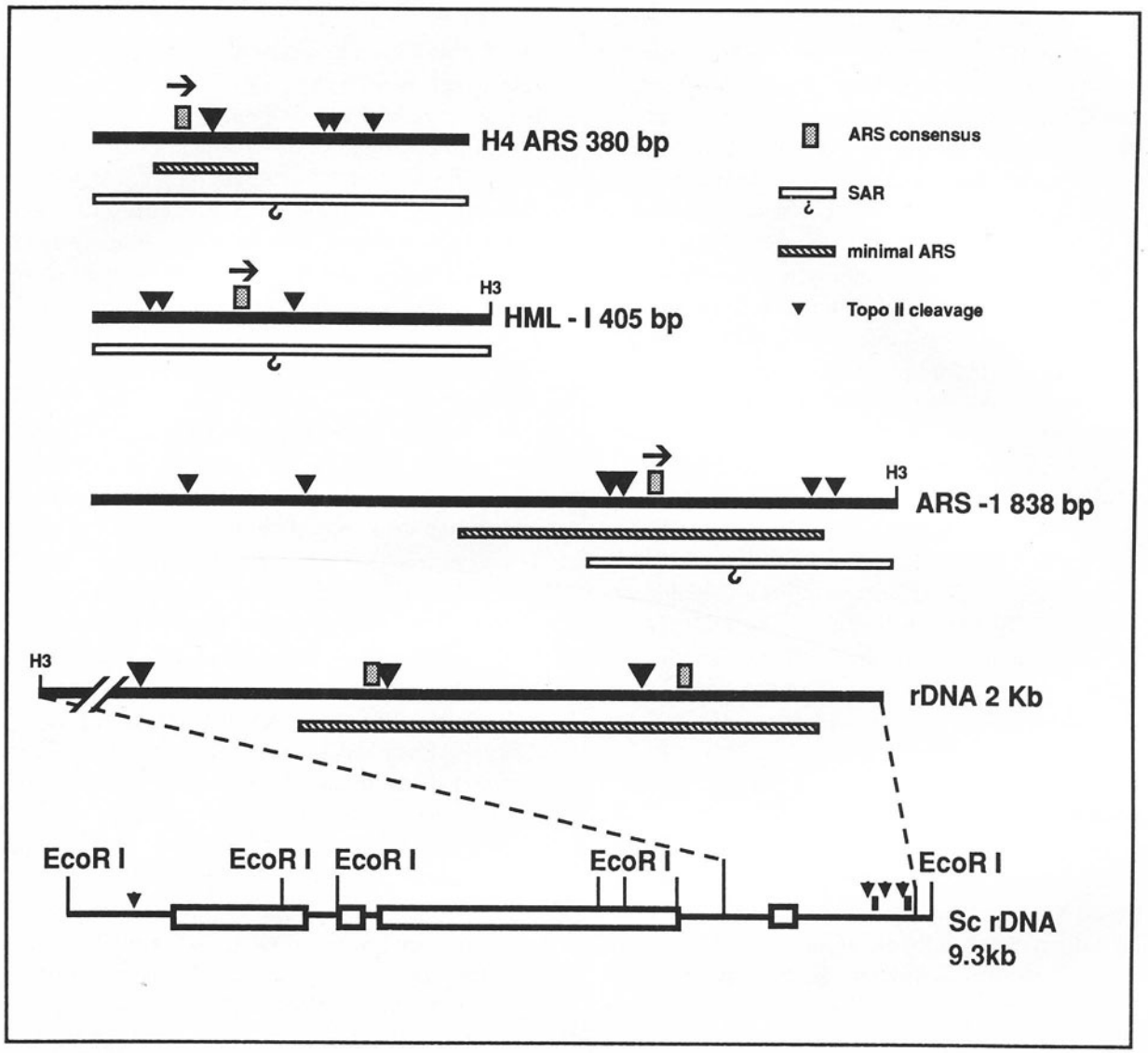

Fig. 1. Mapping of preferred sites of topoisomerase II cleavage in yeast ARS sequences.

Purified fragments of DNA containing the histone H4 ARS (H4 ARS), the HMLI silencer-ARS (HML-I), ARS-1, and the rDNA repeat (Sc rDNA), all from *S. cerevisiae*, were excised, end-labelled and incubated with purified yeast topoisomerase II. Trapping of the cleavage complex was achieved by the addition of 1% SDS in the absence of anti-topoII drugs. This leaves topoII covalently bound to the each strand of the cleaved double helix. Determination of the precise cleavage site was done on a sequencing gel, as described in Cardenas et al. (1992b). Cleavage sites are indicated by arrowheads. In the cases in which a minimal ARS domain has been identified, this is indicated by a hatched box. The location of the 11bp ARS consensus is indicated by a filled box with the arrows indicating the 5′ to 3′ direction of the T-rich strand of the consensus. The minimal fragments showing association with the nuclear scaffold of yeast is indicated below the maps of H4 ARS, ARS-1 and HML-I. The size and name of each fragment is indicated at the right.

poII is inhibited by distamycin, the interaction of topoisomerase II with SARs apparently reflects the enzyme's preference for the narrow minor groove of oligo dT-rich DNA (Nelson et al. 1987). Assuming that this interaction can occur *in vivo*, it suggests a means by which topoisomerase II could mediate chromosome condensation. If topoisomerase II were to able to complex other proteins

and/or itself while binding SARs, or the nucleosomal linkers in SARs, the bases of loops might be brought together in clusters, forming a subunit of a radially organized chromatid. One would expect any such function of topoisomerase II to be regulated through the cell cycle.

Post-translational modification of topoisomerase II

In vitro phosphorylation experiments have shown that topoisomerase II is a substrate for casein kinase II (Ackerman et al. 1985), protein kinase C (Rottman et al. 1987; Sahyoun et al. 1986), Ca^{2+}/calmodulin-dependent protein kinase and $p34^{cdc2}$ kinase (Cardenas et al. 1992a). In all cases phosphorylation *in vitro* enhances topoisomerase II activity, as measured by the kinetoplast decatenation assay. In a variety of species ranging from yeast to mammalian cells, topoisomerase II has been shown to be phosphorylated *in vivo* (Ackerman et al. 1988; Rottman et al. 1987; Heck et al. 1989; Saijo et al. 1990; Cardenas et al. 1992a). Cell cycle phosphorylation studies in chicken lymphoblastoid cells have shown that topoisomerase II phosphorylation increases as cells enter mitosis (Heck et al. 1989). Similarly, studies with cell cycle-arrested yeast cells show that topoisomerase II is 6–10 times more highly phosphorylated in mitosis than in G1 (Cardenas et al. 1992a). Consistent with a role of phosphorylation in stimulating topoisomerase II activity at mitosis is the observation that soluble extracts from mitotic Hela cells contain more DNA decatenation activity than extracts obtained from S-phase cells (Estey et al. 1987). Taken together, these observations strongly suggest that *in vivo* the activity of topoisomerase II is modulated by phosphorylation. Our own studies have analyzed the pattern of modification obtained in both mitosis and G1 phase, and have identified the major kinase involved in the modification.

In studies using phosphate-labelled yeast cells, two dimensional phosphopeptide analysis of topoisomerase II showed that the phosphorylation patterns of the enzyme in G1 phase and at mitosis are related but not identical (Cardenas et al. 1992a). Among major phosphate accepting peptides, all but two comigrate with those phosphorylated by purified casein kinase II *in vitro*. Nonetheless, there are qualitative differences between the CKII-modified phosphopeptides observed in mitotic and in G1 phase cells. Some CKII sites appear to be preferentially targeted in mitosis, while others are weak in mitosis and relatively strong in G1 phase cells. Barring a non-physiological effect of the synchronization procedure, this suggests that the interaction of CKII with topoisomerase II varies through the cell cycle, the significance of which remains to be understood.

Genetic evidence using a temperature-sensitive casein kinase II mutant confirms the importance of casein kinase II in the modification of topoisomerase II. At non-permissive temperature in the casein kinase II mutant, the phosphate incorporated into topoisomerase II *in vivo* dropped to an almost undetectable level (Cardenas et al. 1992a). This role for casein kinase II in phosphorylation of topoII is likely to be conserved across species, since both topoisomerase II and CKII show high degrees of conservation in both sequence and function. The catalytic α subunit of CKII complexes the regulatory β subunit in an $\alpha_2\beta_2$ heterodimer cofiguration. The β subunit appears to be encoded by a single gene, while in yeast there are two α-subunits encoded by different genes (Padmanabha & Glover 1987; Chen-wu et al. 1988; Padmanabha et al. 1990). The disruption of both α subunits of CKII is lethal, and the *S. cerevisiae* cka1-cka2ts mutant used to study topoisomerase II phosphorylation arrests preferentially at the non-permissive temperature as large budded cells, a phenotype consistent with a G2-M arrest point (Glover CVC, unpublished results). This arrest point may be due in part to the requirement of CKII to activate topoisomerase II, which in its turn is required for the proper traversal of mitosis.

From the analysis of topoII phosphorylated *in vivo*, at least two significant phospho-acceptor sites could be identified that do not correspond to CKII sites *in vitro* (Cardenas et al. 1992a). These may indicate a difference in the specificity of CKII *in vitro* as compared to *in vivo*, or may represent acceptor sites for another kinase. In the latter case, there are two plausible explanations why non-CKII

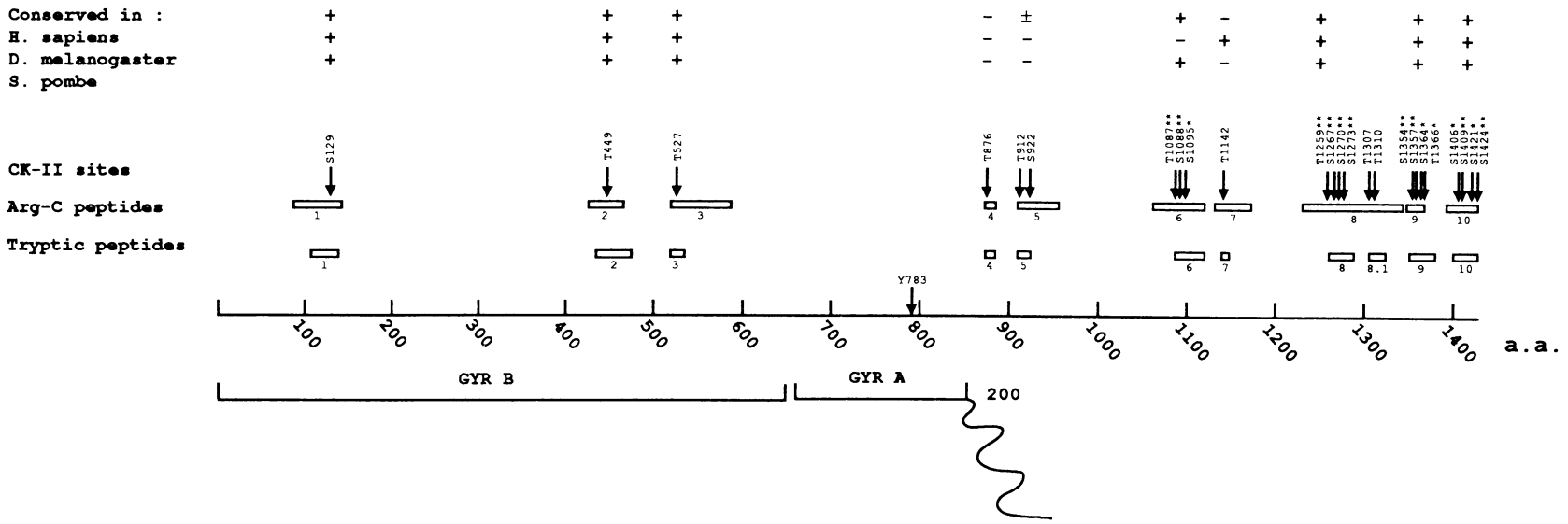

Fig. 2. Schematic representation of yeast topoisomerase II, its phoshopeptides and CKII target sites.

Topoisomerase II from *S. cerevisiae* is portrayed linearly as amino acids 1–1429 (a.a.). The tyrosine at a.a. 783 contacts DNA during catalysis. Regions of homology to the bacterial gyrase subunits A (GYR A) and B (GYR B) are shown. The amino acid residues that best fit the casein kinase II consensus for phosphoacceptor sites are indicated by arrows above the map, and the a.a. corresponding to the target serine or threonine is given. The corresponding tryptic peptides or the arginine C peptides are indicated as open bars. Single stars indicate sites which are likely to be phosphorylated by CKII *in vitro*, and two stars indicate sites are that are definitely phosphorylated by CKII. Above the corresponding peptides it is indicated whether or not the target sites are conserved (+) or not conserved (-) in topoII from *H. sapiens*, *D. melanogaster*, or *S. pombe*.

sites might not be significantly phosphorylated in the ck2 mutant. First, casein kinase II may be required to modify and activate the second kinase. Alternatively, the CKII modification may be required to render topoisomerase II a proper substrate for the second kinase. One intriguing possibility is that CKII phosphorylation of topoisomerase II is a necessary prerequisite for modification by a cell-cycle specific kinase, such as the $p34^{cdc2}$ kinase. Indeed, *in vitro* yeast topoisomerase II is a good substrate for $p34^{cdc2}$ kinase (Labbé et al. 1989; Cardenas et al. 1992b). Phosphorylation by $p34^{cdc2}$ kinase would be G2/M specific in higher eukaryotic cells, and might modify topoisomerase II in such a way to help catalyze the condensation of chromatin into metaphase chromosomes. While this question can probably not be addressed in yeast alone, a combined study of yeast and vertebrate cells may lead us to an understanding of the fine-tuning of topoisomerase II activities through the cell cycle by phosphorylation.

Mapping phosphoacceptor sites in topoisomerase II

The location of putative CKII phosphorylation sites in yeast topoisomerase II was determined by computer analysis using the minimal consensus of T/S-x-x-E/D, as described by Pinna (1990). While many potential sites could be identified, the phosphoamino acid analysis of the various of tryptic and arginine-C derived phosphopeptides showed that only sites in the C-terminus of topoisomerase II are labelled to a significant level in either G1 or M phase (Fig. 2). In fact, each of the four modified peptides contains multiple casein kinase II sites, which results in a characteristic series of diagonally aligned phosphopeptide derivatives after two dimensional peptide analysis (Cardenas et al. 1992a).

The C-terminal region of topoisomerase II diverges completely from the corresponding domain in the bacterial gyrase, suggesting that the regulation of activity by phosphorylation is an exclusive property of the eukaryotic topoisomerase II. The importance of this C-terminal domain is supported by the study of a truncated version of the yeast topoisomerase II. Truncation at a.a. 979, which removes all of the C-terminal CKII phospho-acceptor sites, renders the enzyme unable to complement a *S. cerevisiae* top2 ts mutant, even though the truncated form of the enzyme contains all regions of homology to gyrase A and B subunits including the active site tyrosine residue (MEC, unpublished results). Among the various eukaryotic type II topoisomerases that are cloned, despite significant sequence variation in this C-terminal region, sequence comparison shows that the presence and relative position of strong casein kinase II recognition sites are conserved among the *S. cerevisiae, S. pombe, Drosophila*, and human enzymes (Fig. 2). No tryptic peptide mapping data are available from species other than *S. cerevisiae*, but at some sites multiply phosphorylated peptides are predicted from the C-terminal domain, as we have observed in yeast. It will be necessary to modify the sites of phosphorylation by site-directed mutagenesis to confirm their localization and to provide further evidence concerning their proposed regulatory role.

The effects of phosphorylation on topoisomerase II

Many important nuclear proteins are substrates of casein kinase II. Among these are c-ErbA, E1A, the E7 protein of human papilloma virus (HPV), SV-40 T antigen, the avian c-Myb, c-Fos, p53, cAMP response element binding factor, Serum Response Factor, and two nucleolar substrates, B23 and nucleolin (reviewed in Pinna 1990; Meisner & Czech 1991). The effects of phosphorylation by CKII are not uniform among this diverse set of substrates; in the case of HPV E7, CKII phosphorylation is proposed to correlate with the transforming potential of the virus, while in the large T antigen, mutagenesis of the acceptor serine had little effect on the transforming potential of SV40 (Barbosa et al. 1990). On the other hand, mutation of the phospho-acceptor site decreases the rate of nuclear-cytoplasmic transport of β-galactosidase-T antigen fusion polypeptides (Rihs et al. 1991). In the case of yeast topoisomerase II, phosphorylation by CKII does not seem to be a prerequisite for nuclear targetting, since in the CKII ts mutants at

non-permissive temperature, topoisomerase II is properly localized to the nucleus (MEC and SMG, unpublished results).

The most clear cut effect of topoII modification by CKII is a dramatic stimulation of its decatenation activity. Dephosphorylation of topoisomerase II renders the enzyme unable to decatenate kinetoplast DNA and its affinity for DNA is greatly diminished, as measured by a gel retardation assay. Phosphorylation by purified CKII suffices to reactivate alkaline phosphatase-treated topoisomerase II (Cardenas et al. 1992b). Thus, an active yeast topoisomerase II appears to require at least a basal level of phosphorylation. On the other hand, hyperphosphorylation of topoII by casein kinase II *in vitro* has little effect on the efficiency with which topoII could be trapped in its cleavable complex (Cardenas et al. 1992b). Thus the stimulation of decatention activity by casein kinase II most likely reflects an enhanced affinity for DNA and perhaps an enhanced efficiency at a step in the catalytic process other than DNA cleavage. Consistent with a role in chromosome condensation is the enhancement of protein-protein interactions and aggregation upon hyperphosphorylation *in vitro* (QD, in preparation). To determine which of the minor or the major phosphoacceptor sites on topoisomerase II plays a role in the observed stimulation of activity requires *in vitro* mutagenesis of the acceptor sites, work that is currently in progress.

Acknowledgements

Research was supported by grants to SMG from the Swiss National Science Foundation, the Swiss Cancer League, and the Human Frontiers Research Program. MEC was supported by the Roche Research Foundation (Switzerland).

References

Ackerman P, Glover CVC & Osheroff N (1985) Phosphorylation of DNA topoisomerase II by casein kinase II: modulation of eukaryotic topoisomerase II activity *in vitro*. Proc. Natl. Acad. Sci. USA 82: 3164–3168

Ackerman P, Glover CVC & Osheroff N (1988) Phosphorylation of DNA topoisomerase II *in vivo* and in total homogenates of *Drosophila* Kc cells. J. Biol. Chem. 263: 12653–12660

Adachi Y, Kaes E & Laemmli UK (1989) Preferential, cooperative binding of DNA topoisomerase II to scaffold-associated regions. EMBO J. 8: 3997–4006

Adachi Y, Luke M & Laemmli UK (1991) Chromosome assembly *in vitro*: topoisomerase II is required for condensation. Cell 64: 137–148

Amati B & Gasser SM (1988) Chromosomal ARS and CEN elements bind specifically to the yeast nuclear scaffold. Cell 54: 967–978

Amati B & Gasser SM (1990) Drosophila scaffold-attached regions bind nuclear scaffolds and can function as ARS elements in both budding and fission yeast. Mol. Cell. Biol. 10: 5442–5454

Barbosa MS, Edmonds C, Fisher C, Schiller JT, Lowy DR & Vousden KH (1990) The region of the HPV E7 oncoprotein homologous to adenovirus E1a and SV40 large T antigen contains separate domains for Rb binding and casein kinase II phosphorylation. EMBO J. 9: 153–160

Berrios M, Osheroff N & Fisher PA (1985) In situ localization of DNA topoisomerase II, a major polypeptide component of the Drosophila nuclear matrix fraction. Proc. Natl. Acad. Sci. USA 82: 4142–4146

Berrios S & Fisher PA (1988) Thermal stabilization of putative karyoskeletal protein-enriched fractions from S. cerevisiae. Mol. Cell. Biol. 8: 4573–4575

Brill SJ & Sternglanz R (1988) Transcription-dependent DNA supercoiling in yeast DNA topoisomerase mutants. Cell 54: 403–411

Brill SJ, Dinardo S, Voelkel Meiman K & Sternglanz R (1987) Need for DNA topoisomerase activity as a swivel for DNA replication for transcription of ribosomal RNA. Nature 326: 414–416

Capranico G, Jaxel C, Roberge M, Kohn KW & Pommier Y (1990) Nucleosome positioning as a critical determinant for the DNA cleavage sites of mammalian DNA topoisomerase II in reconstituted Simian virus 40 chromatin. Nucleic Acids Res. 18: 4553–4559

Cardenas ME, Laroche T & Gasser SM (1990) The composition and morphology of yeast nuclear scaffolds. J. Cell Sci. 96: 439–450

Cardenas ME, Dang Q, Glover CVC & Gasser SM (1992a) Casein kinase II phosphorylates the eukaryotic specific C-terminal domain of topoisomerase II *in vivo*. EMBO J. 11 (in press)

Cardenas ME, Hanna DE, Walter R & Gasser SM (1992b) Topoisomerase II requires phosphorylation for efficient DNA binding and DNA decatenation: involvement of casein kinase II (submitted to J. Cell Sci.)

Chen-wu JL, Padmanabha R & Glover CVC (1988) Isolation, sequencing and disruption of the CKA1 gene encoding the alpha subunit of yeast casein kinase II. Mol. Cell. Biol. 8: 4981–4990

Christman MF, Dietrich FS & Fink GR (1988) Mitotic recombination in the rDNA of *S. cerevisiae* is suppressed by the combined action of DNA topoisomerase I and II. Cell 55: 413–425

Cockerill PN & Garrard WT (1986) Chromosomal loop anchorage of the kappa imunoglobulin gene occurs next to the enhancer in a region containing topoisomerase II sites. Cell 44: 273–282

Dinardo S, Voelkel K & Sternglanz R (1984) DNA topoisomerase II mutant of Saccharomyces cerevisiae: topoisomerase II is required for segregation of daughter molecule at the termination of DNA replication. Proc. Natl. Acad. Sci. USA 82: 2616–2620

Earnshaw WC & Heck MMS (1985) Localization of topoisomerase II in mitotic chromosomes. J. Cell Biol. 100: 1716–1722

Earnshaw WC, Halligan B, Cooke CA, Heck MMS & Liu LF (1985) Topoisomerase II is a structural component of mitotic chromosome scaffolds. J. Cell Biol. 100: 1706–17150

Estey E, Adlakha RC, Hittelman WN & Zwelling LA (1987) Cell cycle stage dependent variations in drug-induced topoisomerase II mediated DNA cleavage and cytotoxicity. Biochemistry 26: 4338–4344

Gasser SM & Laemmli UK (1986a) The organization of chromatin loops: characterization of a scafffold attachment site. EMBO J. 5: 511–518

Gasser SM & Laemmli UK (1986b) Cohabitation of scaffold binding regions with upstream/enhancer elements of three developmentally regulated genes of D. melanogaster. Cell 46: 521–530

Gasser SM & Laemmli UK (1987) A glimpse at chromosomal order. Trends Genet. 3: 16–22

Gasser SM, Laroche T, Falquet J, Boy de la Tour E & Laemmli UK (1986) Metaphase chromosome structure involvement of topoisomrase II. J. Mol. Biol. 188: 613–629

Hartwell LH & Weinert TA (1989) Checkpoints: controls that ensure the order of cell cycle events. Science 246: 629–634

Heck MMS & Earnshaw WC (1986) Topoisomerase II, a specific marker for cell proliferation. J. Cell Biol. 103: 569–2581

Heck MMS, Hittelman WN & Earnshaw WC (1989) *in vivo* phosphorylation of the 170-kDa form of eukaryotic DNA topoisomerase II. J. Biol. Chem. 264: 15161–15164

Holm C, Goto T, Wang JC & Botstein D (1985) DNA topoisomerase II is required at the time of mitosis in yeast. Cell 41: 553–563

Kaes E & Laemmli UK (1992) *In vivo* topoisomerase II cleavage of the Drosophila histone and satellite III repeats: DNA sequence and structural requirements. EMBO J. 11: 705–716

Kim RA & Wang JC (1989a) A subthreshold level of DNA topoisomerases leads to the excision of yeast rDNA as extrachromosomal rings. Cell 57: 975–985

Kim RA & Wang JC (1989b) Function of DNA topoisomerases as replication swivels in Saccharomyces cerevisiae. J. Mol. Biol. 208: 257–267

Klein F, Laroche T, Cardenas ME, Hofmann JFX, Schweizer D & Gasser SM (1992) Localization of RAPI and Topoisomerase II in Nuclei and Meiotic Chromosomes of Yeast. J. Cell Biol. 117 (in press)

Labbé JC, Capony JP, Caput D, Cavadore JC, Derancourt J, Kaghad M, Lelias JM, Picard A & Dorée M (1989) MFP from starfish oocytes at first meiotic metaphase is a heterodimer containing one molecule of cdc2 and one molecule of cyclin B. EMBO J. 8: 3053–3058

Liu LF (1989) DNA topoisomerase poisons as anti-tumor drugs. Ann. Rev. Biochem. 58: 351–375

Meisner H & Czech PM (1991) Phosphorylation of transcriptional factors and cell-cycle-dependent proteins by casein kinase II. Curr. Op. in Cell Biol. 3: 474–483

Mirkovitch J, Mirault ME & Laemmli UK (1984) Organization of the higher-order chromatin loop: specific DNA attachment sites on nuclear scaffold. Cell 39: 223–232

Mirkovitch J, Gasser SM & Laemmli UK (1988) Scaffold attachment of DNA loops in metaphase chromosomes. J. Mol. Biol. 200: 101–110

Moens B & Earnshaw WC (1989) Anti-topoisomerase II recognizes meiotic chromosomal cores. Chromosoma 98: 317–322

Nelson HCM, Finch JT, Luisi BF & Klug A (1987) The structure of an oligo(dA).oligo(dT) tract and its biological implications. Nature 330: 221–226

Newport J & Spann T (1987) Disassembly of the nucleus in mitotic extracts: membrane vesicularization, lamin disassembly, and chromosome condensation are independent processes. Cell 48: 219–230

Padmanabha R & Glover CVC (1987) Casein kinase II of yeast contains two distinct polypeptides and an unusually large subunit. J. Biol. Chem. 262: 1829–1835

Padmanabha R, Chen-wu JL, Hanna DE & Glover CVC (1990) Isolation, sequencing and disruption of the yeast CKA2 gene: casein kinase II is essential for viability in *S. cerevisiae*. Mol. Cell. Biol. 10: 4089–4099

Peter M, Nakagawa J, Dorée M, Labbé J C & Nigg EA (1990) *in vitro* disassembly of the nuclear lamina and M phase-specific phosphorylation of lamins by cdc2 kinase. Cell 61: 591–602

Pinna LA (1990) Casein kinse II: an 'eminence grise' in cellular regulation? Biochim. Biophys. Acta 1054: 267–284

Reitman M & Felsenfeld G (1990) Developmental regulation of topoisomerase II sites and DNase I-hypersensitive sites in the chicken b-globin locus. Mol. Cell. Biol. 10: 2774–2786

Rihs HP, Jans DA, Fan H & Peters R (1991) The rate of nuclear cytoplasmic protein transport is determined by the casein kinase II site flanking the nuclear localization sequence of the SV40 T-antigen. EMBO J. 10: 633–639

Roberge M, Th'ng J, Hamaguchi J & Bradbury EM (1990) The topoisomerase II inhibitor VM-26 induces marked changes in histone H1 kinase activity, histones H1 and H3 phorphorylation and chromosome condensation in G2 phase and mitotic BHK cells. J. Cell Biol. 111: 1753–1762

Rose D, Thomas W & Holm C (1990) Segregation of recombined chromosomes in meiosis I requires topoisomerase II. Cell 60: 1009–1017

Rottman M, Schröder HC, Gramzow M, Renneisen K, Kurelec B, Dorn A, Friese U & Müller WEG (1987) Specific phospho-

rylation of proteins in pore-complex-laminae from the sponge *Geodia cydonium* by the homologous aggregation factor and phorbol ester. Role of protein kinase C in the phosphorylation of DNA topoisomerase II. EMBO J. 6: 3939–3944

Rowe TC, Wang JC & Liu LF (1986) *In vivo* localization of DNA topoisomerase II cleavage sites on Drosophila heat shock chromatin. Mol. Cell. Biol. 6: 985–992

Sahyoun N, Wolf M, Besterman J, Hsieh TS, Sander M, Levine III H, Chang KJ & Cuatrecasas P (1986) Protein kinase C phosphorylates topoisomerase II: topoisomerase activation and its possible role in phorbol ester-induced differentiation of HL-60 cells. Proc. Natl. Acad. Sci. USA 83: 1603–1607

Saijo M, Enamoto T, Hanaoka F & Ui M (1990) Purification and characterization of type II DNA topoisomerase from mouse FM3A cells: phosphorylation of topoisomerase II and modification of its activity. Biochemistry 29: 583–590

Sander M & Hsieh TS (1985) Drosophila topoisomerase II double-strand DNA cleavage: analysis of DNA sequence homology at the cleavage site. Nucleic Acids Res. 13: 1057–1072

Sperry AO, Blasquez VC & Garrard WT (1989) Dysfunction of chromosomal loop attachement sites: illegitimate recombination linked to matrix association regions and topoisomerase II. Proc. Natl. Acad. Sci. USA 86: 5497–5501

Tsai-Pflugfelder M, Liu L, Liu A, Tewey KM, Whang-Peng J, Knutsen T, Huebner K, Croce CM & Wang JC (1988) Cloning and sequencing of cDNA encoding human DNA topoisomerase II and localization of the gene to chromosome region 17q21–22. Proc. Natl. Acad. Sci. USA 85: 7177–7181

Udvardy A & Schedl P (1991) Chromatin structure, not DNA sequence specificity, is the primary determinant of topoisomerae II sites of action *in vivo*. Mol. Cell. Biol. 11: 4973–4984

Udvardy A, Schedl P, Sander M & Hsieh TS (1985) Novel partitioning of DNA cleavage sites for Drosophila topoisomerase II. Cell 40: 933–941

Uemura T & Yanagida M (1984) Isolation of type I and II DNA topoisomerase mutants from fission yeast: single and double mutants show different phenotypes in cell growth and chromatin organization. EMBO J. 3: 1737–1744

Uemura T & Yanagida M (1986) Mitotic spindle pulls but fails to separate chromosomes in type II DNA topoisomerase mutants: uncoordinated mitosis. EMBO J. 5: 1003–1010

Uemura T, Ohkura H, Adachi Y, Morino K, Shiozaki K & Yanagida M (1987) DNA topoisomerase II is required for condensation and separation of mitotic chromosomes in *S. pombe*. Cell 50: 917–925

Umesono K, Hiraoka Y, Toda T & Yanagida M (1983) Visualization of chromosomes in mitotically arrested cells of the fission yeast Schizosaccharomyces pombe. Curr. Genet. 7: 123–128

Wang JC (1985) DNA topoisomerases. Ann. Rev. Biochem. 54: 665–697

Wood ER & Earnshaw WC (1990) Mitotic chromain condensation *in vitro* using somatic cell extracts and nuclei with variable levels of endogenous topoisomerase II. J. Cell Biol. 111: 2839–2850

Wyckoff E & Hsieh TS (1988) Functional expression of a Drosophila gene in yeast: genetic complementation of DNA topoisomerase II. Proc. Natl. Acad. Sci. USA 85: 6272–6276

Yang L, Rowe TC, Nelson EM & Liu LF (1985) *in vivo* mapping of DNA topoisomerase II-specific cleavage sites on SV40 chromatin. Cell 41: 127–132

Zechiedrich EL & Osheroff N (1990) Eukaryotic topoisomerases recognize nucleic acid topology by preferentially interacting with DNA crossovers. EMBO J. 9: 4555–4562