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DNA TOPOISOMERASE: THE KEY ENZYME THAT REGULATES DNA SUPER STRUCTURE

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ABSTRACT

DNA is a macromolecule carrying all genetic information and it must be packed in the cell nucleus. DNA is a double helix where one strand coils around the other. The helix is unwound and rewound every now and then to take out its information, which requires local alteration of the helical structure, resulting in the supercoiling of DNA. For replication, all the coils must be unwound at least once, and two daughter molecules are often catenated to each other. To solve these problems caused by the helical structure of DNA, topoisomerase activity introducing the transient breaking and rejoining of DNA strand is essential to perform each DNA transaction. In this article, we first review the mechanistic aspect of topoisomerase activity, and then discuss its basic and clinical importance.

Key Words: topoisomerase, supercoil, anti-cancer drugs

INTRODUCTION

DNA is one of the most important molecules in cell biology. As it encodes genetic information, it is not surprising that DNA molecules are by far the largest macromolecules in cells. A single chromosome typically contains thousands of individual genes. The size of DNA molecules represents an interesting biological problem in itself. Linearized chromosomal DNA molecules are often many orders of magnitude longer than the cells in which they are included. In mammals the total contour length of all the DNA in a single cell is about 2 m. It becomes clear that DNA packaging must involve an extraordinary degree of organization and compaction. Chromosomal DNA must be very tightly packaged just to fit into a nucleus, which is only a few μm in diameter. However, it is not enough to fold the DNA into a small space. The chromosomal DNA is extremely long and is occasionally fixed to the nuclear matrix, so a free rotation of DNA is not allowed and a helical constraint is created in each domain. Moreover, the packaging must permit access to the information on the DNA for processes such as replication and transcription. These processes require transient separation of the strands of DNA, and separation is not a simple process in a DNA structure in which the two strands are helically intertwined.[1] In cases of replication as well as transcription, positive and negative supercoils accumulate in front of and behind of the replication fork, respectively. The term "supercoiling" means literally the coiling of a coil. If there is nothing to relieve the helical stress of DNA, no more replication and transcription will proceed.

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Topoisomerases are key enzymes affecting the dramatic changes in DNA structure. As a working model, we could view a chromosome as a double stranded circular DNA, where the two ends are covalently joined. In this circular molecule, the number of coil of one strand arround another is fixed and can never be altered unless the DNA strands are transiently cleaved. If we take a canonical B form DNA, the number of coils is an integral number equal to the number of total base pairs of DNA divided by approximately 10.5 (the average number of base pairs per one helical turn). This number is designated as the linking number (L), and deviation from this value causes the DNA axis to twist or writhe (DNA coils get coiled around themselves).[2] When ΔL increases, the helical pitch becomes tighter, resulting in a positive supercoil; and when ΔL decreases, the result is a negative supercoil, which will facilitate unwinding of the double helix.[3] In the topological property of DNA, linking is not affected by twisting and turning of the DNA axis (writhing). Linking could be changed by transient scission of one (or both) strand(s) through which the other strand passes before rejoining. Conversely, DNA topology can only be changed by breaking and rejoining of the DNA backbone.[4] The enzyme called topoisomerase catalyzes this type of reaction and causes an alteration in the linking number. Usually the direction of the enzyme action is toward $\Delta L = 0$, which is the most stable form of DNA. Two exceptional enzymes in the topoisomerase group drives this reaction toward an unfavorable structure either forming a negative supercoil (DNA gyrase) or forming positive supercoil (reverse gyrase).

DNA topoisomerases are classified into two categories as shown in Figure 1; type I topoisomerases, which transiently cut one of the two strands, resulting in one linking change in every step of the reaction, and type II topoisomerases, which act by creating a transient double-stranded break, thus effecting two linking changes in one step. In most of the cells,both type I and type II enzymes are present. The eukaryotic type I enzyme acts on both negatively and positively supercoiled DNA, removing their superhelical turns. ATP-requiring type II topoisomerase was first discovered by its particular ability to catenate and decatenate or to unknot the entangled DNA rings.[5,6] At the end of the DNA replication, two daughter molecules are thought to be intertwined with each other. Type II topoisomerase is essential for separating the daughter DNA strands[7] because this reaction cannot be done with the type I topoisomerase. The type II enzyme can also remove both negative and positive superhelical turns from DNA.

In prokaryotes, type I topoisomerase, which was described as the first example of topoisomerase,[8] exclusively relaxes the negatively supercoiled DNA, but not the positively supercoiled one. Since the negatively supercoiled DNA is favorable to the biological effect of DNA such as initiation of transcription and replication, the function of bacterial type I enzyme remained unclear until DNA gyrase was discovered. *E. coli* DNA gyrase is able to introduce negative supercoils in closed circular DNA,[9] as well as to remove positive supercoils, whereas the eukaryotic type II topoisomerase does not exhibit supercoiling activity but relaxes both negative and positive supercoils.[10]

In bacterial cells, Topo I and DNA gyrase are antagonized to equilibrate the extent of supercoiling of chromosomal DNA. DNA gyrase as the type II enzyme also catalyzes decatenation but ineffectively. Later, another type II enzyme, Topo IV was discovered as an enzyme particularly functional for chromosome partition during cell division.[11] The bacterial type II enzyme, DNA gyrase and TopoIV, and eukaryotic type II enzyme, Topo II, show an extensive amino acid homology. The bacterial enzyme consists of two parts (gyrA, gyrB for DNA gyrase and parC, parE for Topo IV) while Topo II is composed of a single polypeptide with two domains corresponding to its bacterial counterparts.[12] On the other hand, the bacterial and eukaryotic type I enzymes share no homology. So from a mechanistic point of view, they are quite different. The Topo III enzyme, found in many bacterial and eukaryotic genomes is a relative of the

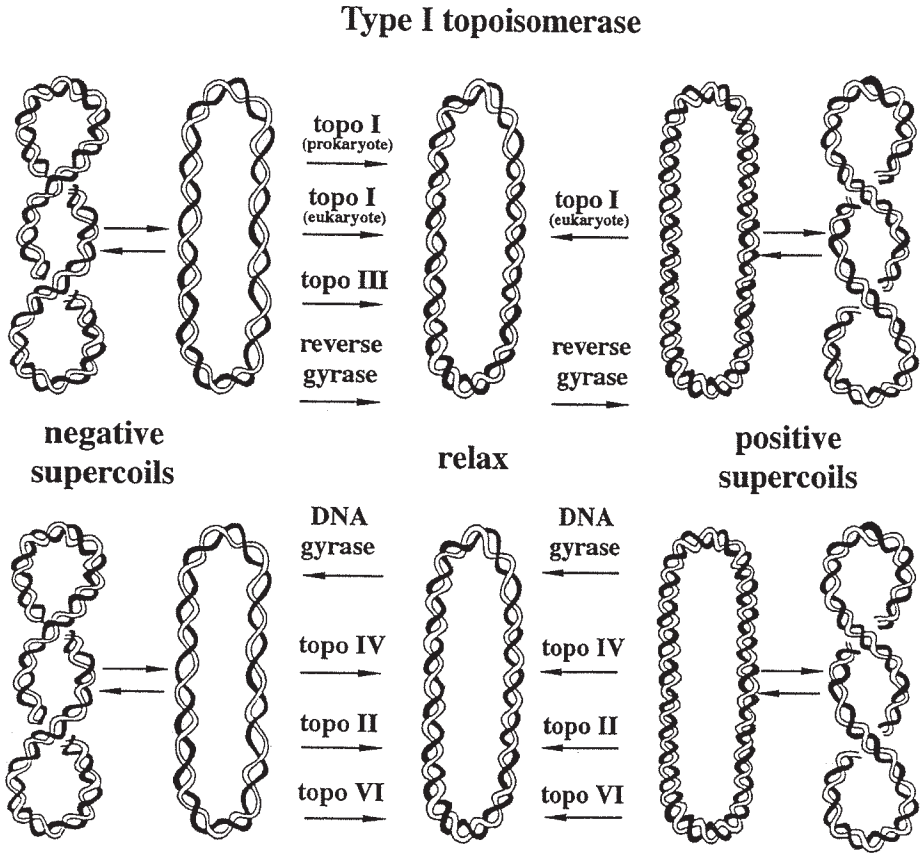

Fig. 1

bacterial type I enzyme.

REVERSE GYRASE – THE NEW TOPOISOMERASE

There is controversy over whether or not prokaryotes should be divided into two separate kingdoms: Eubacteria and Archaebacteria. Archaebacteria have several distinct features, but most of these might be a consequence of their growth in extreme habitats, such as at high temperature (75°C) for acidothermophiles, in high salt (3–4M NaCl) for extreme halophiles, and in an obligate anaerobic environment for methanogens.

It has often been suggested that some archaebacteria are more closely related to eukaryotes than prokaryotes, although nuclear structures have never been seen in archaebacterial cells. An important issue in the study of archaebacteria is how chromosome structures are maintained. Under such extreme conditions, many proteins of other kingdoms dissociate from DNA, and the DNA itself would be expected not to have the canonical B-form configuration. To keep

chromosomal structure intact, histone-like proteins or other DNA-binding proteins might play important roles.[13,14,15] Accordingly, specific topoisomerase enzyme might be needed to change the local constraints on DNA and to construct tight DNA/protein complexes in obnoxious conditions. It is worthwhile nevertheless to determine what kinds of topoisomerases are present in archaebacteria that function well at high temperatures or in high salt concentrations.

Properties of reverse gyrase

We identified and characterized the property of a new topoisomerase from *Sulfolobus acidocaldarius*.[16,17,18] The enzyme was composed of a single polypeptide with a molecular weight of 120 kDa. The existence of other accessory proteins in our purified preparation for supercoiling activity was completely ruled out. Forterre et al. independently purified the enzyme,[19] and Nadal et al. found a single 128 kDa subunit.[20] These investigators also showed that the addition of 10% polyethylene glycol 6000 significantly enhanced reverse gyrase activity. Slesarev purified reverse gyrase from another extremely thermophilic anaerobic archaebacterium, *Desulfurococcus amylolytics*.[21] This enzyme is a single polypeptide with a molecular weight of 135 kDa and is active from 65°C to 100°C. More recently, reverse gyrase was detected in all thermophilic archaebacteria tested[22] and, surprisingly, also in thermophilic eubacteria.[23]

Sequence analysis suggests that reverse gyrase has two distinct domains; a carboxyl-terminal domain containing about 630 amino acids clearly related to eubacterial Topo I/Topo III and to budding yeast Topo III, and an amino-terminal domain without any similarity to other known topoisomerases but containing several helicase motifs, including an ATP-binding site.[24]

Reverse gyrase changes the linking number of a single topoisomer in steps of one. This was shown by Nakasu and Kikuchi[16] and by Zivanovic et al.[25] Nakasu and Kikuchi[16] observed that closed circular DNA was nicked by stoiciometric amounts of reverse gyrase in the absence of either ATP or Mg^{2+} when the reaction was terminated by the addition of SDS. This suggests that reverse gyrase linked through the 5′ end of the DNA and caused transient nicks during topoisomerization as expected for a bacterial type-I DNA topoisomerase.

Positive supercoils are generated by reverse gyrase

The novel aspect of *Sulfolobus* topoisomerase activity is that not only negatively supercoiled DNA was relaxed, but also positive supercoils were introduced. The introduction of positive supercoiles was shown to be catalytic, not the stoichiometric reaction of a conventional topoisomerase. As this topological change is just opposite to DNA gyrase, we named this new topoisomerase "reverse gyrase".[17] Yeats et al. found a lemon-shaped bacteriophage-like particle in one species of *Sulfolobus*.[26] This particle contained positively supercoiled, closed circular DNA.[27] This DNA was also identified in a plasmid state after exposure to UV irradiation, presumably by excision from the chromosome. The plasmid was also positively supercoiled, although less so than the DNA in the phage-like particles. These data imply that reverse gyrase is functional inside the *Sulfolobus* and can introduce positive superhelicity.

Role of ATP

It was shown that only 10μM ATP was required for reverse gyrase. Other ATP-requiring topoisomerase, including the *Sulfolobus* type II enzyme, required much more ATP (on the order of 1mM). In the presence of ATP and Mg^{2+}, negatively supercoiled DNA was relaxed at 75°C but not below 55°C. Good activity was detected at 95°C.[28] The temperature range of the enzymatic activity was just that expected from the growth temperature of *Sulfolobus*, 70°C to 90°C.

Shibata et al. showed that the ATPase activity was strictly DNA dependent and intrinsic to reverse gyrase. Little topoisomerase activity was detected in the abscence of Mg^{2+} and ATP,

conditions under which quantitively abundant type I topoisomerase activity is found in eukaryotes. ATP and dATP were hydrolyzed in the presence of DNA, but GTP, UTP and CTP were not. The nonhydrolyzed nucleoside triphosphates, however, were good co-factors for the relaxation of negative supercoils but not for introduction of positive supercoils.[28] Therefore, NTP hydrolysis is essential for the topoisomerase reaction only when the process is energetically unfavorable; that is, when there is an introduction of positive superhelical turns into DNA. The initial rate of ATP hydrolysis, monitored by high-performance liquid chromatograhy (HPLC), was much slower than that of the topoisomerase reaction: No more than 10% of the ATP was hydrolyzed before the supercoiling reaction was completed. It is not known whether ATP hydrolysis facilitated the relaxation of the negative supercoils or how many molecules of ATP are required to make one positive superhelical turn.

Nadal et al. reported the ATP-independent relaxation of the negative supercoils from *Sulfolobus shibatae*[29] and suggested that proteolysis of the full length reverse gyrase occurs. They imply that the reverse gyrase contains two domains; one is a classical Topo I (the ATP-independent activity) and the other one is an ATP-motor responsible for the extent of reaction.

THE EUKARYOTIC TYPE I TOPOISOMERASE

Structure and function of type I topoisomerase

Type I topoisomerase is a monomeric enzyme. Its molecular weight is about 91 kDa. Human Topo I is composed of four major functional domains; the unconserved and highly charged "amino-terminal" domain, the conserved "core" domain, a poorly conserved and positively charged "linker" region and the highly conserved "carboxyl-terminal" domain which contains the active site tyrosine at the position 723.[30] The function of the N-terminal domain is yet to be proved.[31] This part is extremely labile and not essential *in vitro* activity of topoisomerase. The remaining 70 kDa protein is called SCL70 antigen, a common hallmark for scleroderma patients. The linker domain may bind the DNA directly, thereby anchoring the enzyme to its substrate.[32]

While the bacterial type I topoisomerase is linked to the 5′ end of the substrate DNA for transient cleavage reactions, the eukaryotic type I topoisomerase is linked to the 3′ end of the DNA. *top 1* null mutants of the yeast *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* are viable, and various experiments suggest that the dispensability of the enzyme is due to substitution of its cellular function by type II topoisomerase (*TOP 2*).[33] But the double mutant phenotype of *top1* and *top2* causes the immediate arrest of the cell growth and lethality. On the contrary in fruit flies and mice, type I topoisomerase is essential for their development. If the *topo I* gene is knocked out, it will result in death at an early stage of embryogenesis,[34,35] probably because the early embryo has extremely rapid cell turnover, so *topo I* disrupted clones can nolonger catch up with the superhelical stress generated by the rapid DNA replication.

Type I topoisomerase as a drug target

As described above, type I topoisomerase is required for cell proliferation. Topo I targeted drugs (camptothecins) are widely used in cancer chemotherapy. Camptothecin inhibits both DNA and RNA synthesis,[36] arrests cells in the G2 phase of the cell cycle,[37] stimulates sister chromatid exchanges and chromosomal aberations,[38] elevates p53 levels,[39] induces multiubiqutination and degradation of Topo I[40,41] and activates signal transduction molecules such as protooncogenes and NF-kB.[42,43] The precise mechanism for Topo I targeted drugs are largely unknown, but the first step of drug action would be stabilization of cleavable complex

formation. According to the collision model proposed by Liu,[44,45] the on-going replication machinary collides with covalently linked Topo I, resulting in DNA strand scission.

THE TYPE II TOPOISOMERASE

Structure and function of type II DNA topoisomerase

The structure of type II topoisomerase is highly conserved from bacteria to human beings (Figure 2). Bacteria have two different type II topoisomerases named DNA gyrase and Topo

Fig. 2

IV. Both type II topoisomerases are made of heterotypic tetrameres, A2B2 for the DNA gyrase and C2E2 for Topo IV. Subunits A and B are encoded by the *gyrA* and *gyrB* genes, respectively. Subunits C and E are encoded by *parC* and *parE* which are designated as partition defects. The X-ray crystalographic structure of DNA gyrase was obtained.[46] The Gyr B domain has a consensus sequence for ATPase and works in energy transduction. The Gyr A domain is thought of as the breakage/rejoining domain.

Eukaryotic type II topoisomerase consists of an ATPase domain, a breakage/rejoining domain and a putative regulatory domain, and these subdomains are fused to make a single molecule. The first two domains show striking similarity among all the species and are essential for enzymatic activity.[47-50] X-ray crystalography of a core region of the budding yeast Topo II was obtained and it was shown that the Topo II homodimer has a "heart-like" shaped structure.[51]

For bacterial proliferation, Topo IV is as indispensable as DNA gyrase. But DNA gyrase and topo IV play different roles. DNA gyrase can introduce negative supercoils. It counteracts the action of type I topoisomerase which removes negative supercoils. DNA gyrase and Topo I regulate the superhelicity of DNA coordinately. Topo IV is identified from the mutant clone that cannot separate the daughter DNA strands. Topo IV might be more important for DNA segregation.

Lower eukaryotes have only one type II topoisomerase. But higher eukaryotes like mammals and birds (and probably frogs or higher vertebrates) have genetically distinct isoforms of Topo II, designated Topo II α and β.[52,53] *In vitro* activity of Topo II α and β is almost equivalent. Their localizations in the nucleus and profiles of cell cycle dependent expression differ greatly. During cell cycle progression, the level of Topo II α reaches a maximum in the G2/M phase. Rapidly proliferating cells, particularly in the testis and thymus, show a high expression level, as do tumor cells. Proliferating tumor cells keep a high expression level at the interphase, so Topo II α can be used as the pathophysiological marker for tumor diagnosis.[54,55] Topo II α localizes in the metaphase chromosome and is thought to be one of the major components of the chromosome scafford.[56]

From our observation, Topo II α accumulates first in the centromere region. As soon as the chromosome condensation begins, it accumulates towards the longitudinal axis of the chromosomes. Topo II α disappears at the time of reconstruction of the daughter nuclei, followed by decondensation of the chromosome. On the other hand, Topo II β are constantly expressed throughout the cell cycle and distributes diffusely to the nucleoplasm. Accumulation on the chromosome does not occur. Topo II α might disentangle the DNA that is generated by DNA compaction and segregation. On the contrary Topo II β might play a role in resolving the superhelical constraints, which inevitably occur during replication and transcription.

Genetic approach to the type II topoisomerase

The genetic understanding of mammals has accelerated in the last decade. Transgenic mice brought us valuable information for our understanding of precise molecular mechanisms *in vivo*. For the first step, we cloned mouse Topo II α and β from the mouse cDNA library of spermatocyte and infantile brain cells, respectively. Mouse Topo II α showed 86% identity to human topo II α, with little similarity in the carboxyl-terminal region. On the other hand, mouse and human Topo II β has extensive homology from N-terminal to the 3′ non-coding region. The homology between a mouse and human Topo II β is about 96%. In general, Topo II β is more conserved than Topo II α.

Next we employed the genetic approach using yeast. As yeast *TOP 2* gene is essential, we took the *top2* conditional mutant to examine whether mouse Topo II α or Topo II β could complement the *top2* mutant. We confirmed that both of them function properly in yeast cells, using

the appropriate expression vector.[48] Subsequently we constructed several mouse Topo II mutants which exhibit the thermo sensitive phenotype in the *top2* null yeast,[57] as shown in Figure 3. In one of these clones, missense mutation of mouse Topo II α occured at the residue C2882 to T transition which would change the threonine 961 to isoleucine. Furthermore Topo II α purified from this ts clone exhibited no enzymatic activity.

Molecular dynamics of type II topoisomerase.

The cleavage site structure is identical for all type II topoisomerases; staggered cuts are introduced in each strand of double helix (four bases apart). The 3′ recessed ends have free hydroxyl groups, while the protruding 5′ phosphoryl ends are covalently attached to the tyrosine residue in the topoisomerase molecules.

A two-gate model was postulated by Roca and Wang in 1994. In this model, a dimeric enzyme cleaves a double-stranded DNA segment to create an opening gate for the enzyme-

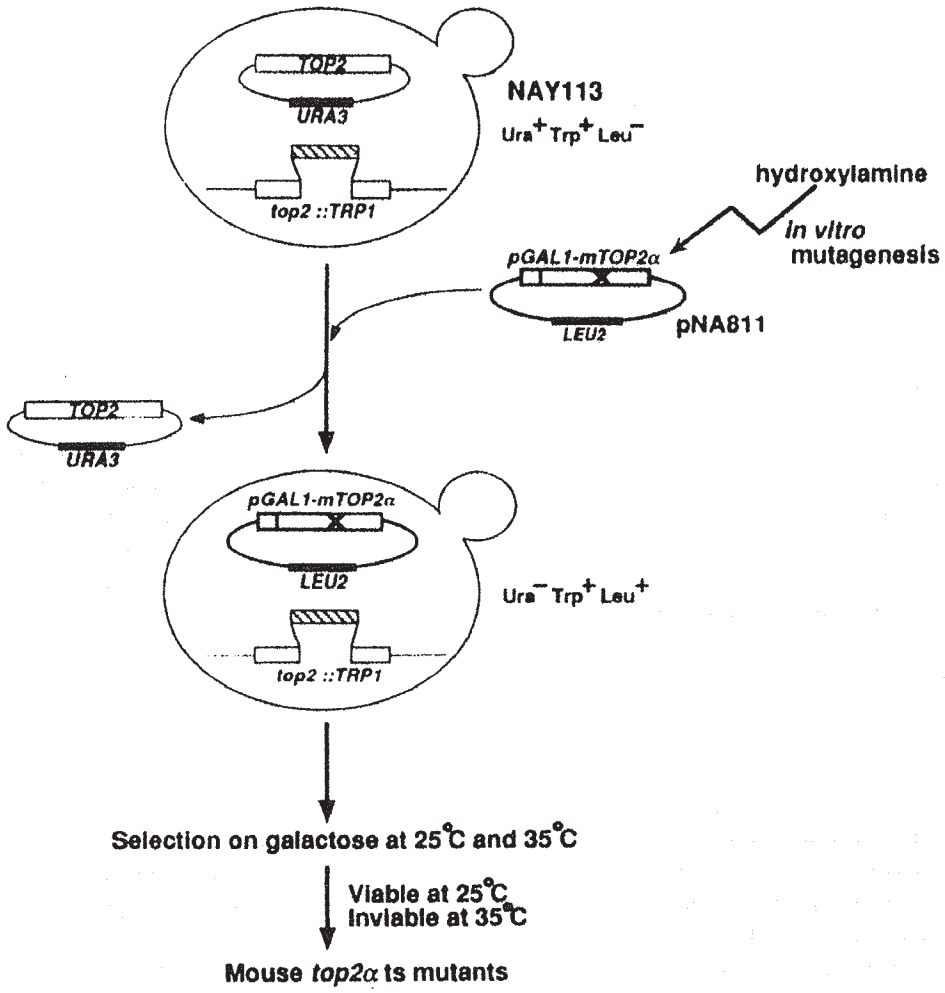

Fig. 3

mediated passage of a second double-stranded DNA segment; the enzyme rejoins the severed DNA strands after the passage of the second DNA segment to complete a reaction cycle.[53] In this reaction, ATP binding and hydrolysis is needed. But studies using a nonhydrolyzable ATP analog showed that hydrolysis of ATP and the release of the resulting ADP and orthophosphate are required only for enzyme turnover.[59] According to X-ray crystalographic analysis, *E. coli* DNA gyrase and *Saccharomyces cerevisiae* Topo II support this model. The enzyme can be viewed as an ATP-modulated clamp with two sets of jaws at opposite ends of a homodimeric protein. Recently, supportive data for the two gate model using transmission electron microscopy was obtained. Conformational changes which induced binding of ATP analogs were visualized, demonstrating the conversion of the open clamp to the closed clamp.[60]

Type II topoisomerase as a drug target

Eukaryotic Topo I and II and bacterial DNA gyrase (type II topoisomerase) are the targets for numbers of clinically important drugs.

Bacterial DNA gyrase and Topo IV are the target for quinolone synthetic antibiotics.[61,62] Quinolones potentially interact with the host topoisomerase, and their affinity to eukaryotic topoisomerase is thought to be relatively low. The precise mechanism of quinolone-topoisomerase interaction is unknown. It is supposed that the DNA and topoisomerase complex causes a conformational change in the "quinolone pocket", and drugs bound at this site stabilize the complex. The stabilization of this complex inhibits DNA rejoining and brings about the subsequent DNA damage. It is likely that quinolone resistance is not transmitted by plasmids or transposons. Rare point mutations at the quinolone pocket might decrease quinolone affinity and show drug resistance.

Type II topoisomerase is essential for cell proliferation. In rapidly dividing mammalian cells, total amount of Topo II α is dramatically increased at the G2/M phase. Hence Topo II α is a good target for cancer chemotherapy. Drugs such as etoposide, doxorubicin, daunomycin and mitoxantrone are widely used for systemic treatment of malignant neoplasms.[12] Furthermore, the chemotherapeutic regimens either include or are based on agents targeted to Topo II. Topoisomerase poisons can be categorized into three groups; intercalators (such as doxorubicin and m-AMSA), non-DNA-intercalators (etoposide,teniposide) and catalytic inhibitors of the topoisomerase (aclarubicin and bisdioxopiperidines like ICRF-193[63]). The first two groups trap Topo II on the DNA in a covalently bound state, often referred to as the "cleavable complex".[64] Catalytic inhibitors act on Topo II without forming the cleavable complex. It is not entirely clear what causes cell death following topoisomerase stabilization. DNA damage provoked by Topo II poisons was shown in some cases to result in apoptosis.[65]

What are the mechanisms of catalytic inhibitors? The inhibition mechanisms were described as a situation where the drugs inhibit the enzyme's binding to its DNA substrate (aclarubicin, chloroquine)[66,67] or lock homodimeric Topo II in the form of a closed clamp (bisdioxopiperidines).[68]

Mechanism of drug resistance

Drug resistant topoisomerase restricts the therapeutic choices in many cases. Drug resistance is sometimes caused by the overexpression of membrane P-glycoprotein (*MDR1* gene product) which discharge the wide range of drugs from cells.[69] Others are produced by the *MDR1* independent mechanisms, which in many cases are correlated with alterations of Topo II, for example, the expression level or mutation of Topo II. The expression level of Topo II is affected by several factors, especially, down regulation of the Topo II mRNA,[70] instability of mRNA[71] and destruction of the Topo II protein.[72]

Down regulation of Topo II is controlled by the inverted CCAAT box (ICB) in the 5′ untranslated region of the Topo II. The ICB site is recognized by the ubiquitous transcription factor NF-Y (also called CBF, ACF and CP1). Instability of the Topo II mRNA increases as a result of ionizing radiation or heat shock.[71]

Mutations that confer the drug resistance are divided into three groups[73] (Figure 4): mutations of the topoisomerase hinge region, mutations around the active site and carboxyl-terminal deletion. The Topo II hinge region, which is between the ATPase domain and the active site, is predominant among most mutations. Several laboratories independently reported mutations between A429 to R486.[74-77] Similar mutations around this region are identified in yeast[78] and hamster.[79] In this region, the nucleotide binding motif is present, but what this means for drug resistance is controversial. Human, mouse and fruit fly Topo II have this motif but *Saccharomyces cerevisiae* and *Arabidopsis thaliana* do not conserve this motif (Figure 2). Drug resistance might be caused by lowered topoisomerase activity or a lack of interaction with the drugs. On the other hand, the effect of the mutations around the active site is likely to be reduced topoisomerase function.

The carboxyl-terminal region is dispensable for topoisomerase activity.[50] But the carboxyl-terminus truncations show the drug resistance.[80] In this case, Topo II localizes not in the nucleus but in the cytoplasm. Adachi et al. identified the nuclear localizing signal at the carboxyl-terminal region[81] and this observation correlates well *in vivo*.[82]

Topoisomerase and aging

Topo III[83] belongs to type I topoisomerase that is originally identified by its suppression of mitotic recombination between repetitive sequences.[84] Yeast *top3* mutants grow more slowly. Spolulation is also known to be completely blocked in *top3*$^{-}$/*top3*$^{-}$ diploids.[83] Mutation in *top3* itself shorten telomeric repeats as well as destabilizes subtelomeric elements.[85] In human Ataxia Teleangiectasia cells, cell death caused by double strand breaks is influenced by the overexpression of the Topo III fragment or introduction of its antisense RNA.

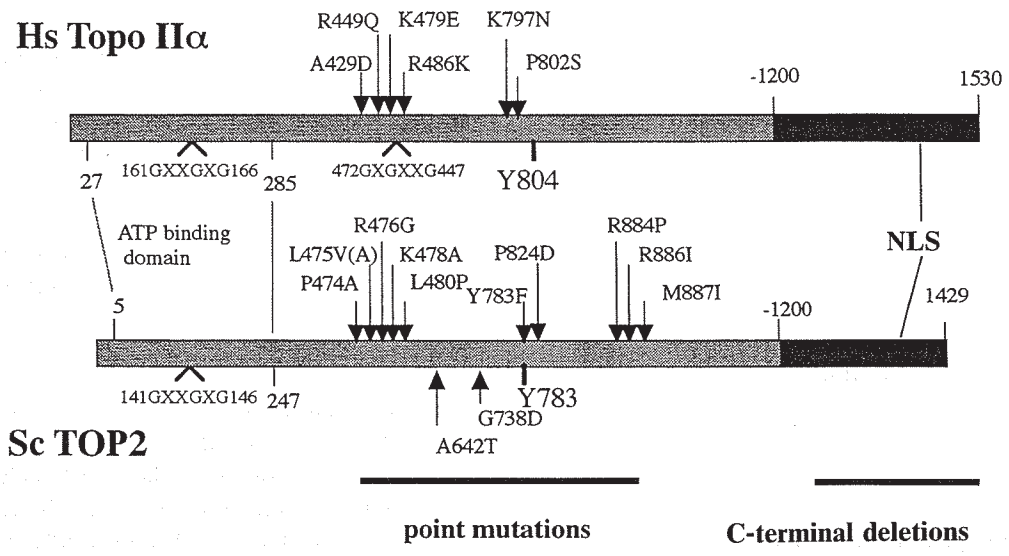

Fig. 4. Drug resistant topo II mutations.

Recently, inactivation of a yeast *SGS1* was found to suppress *top3*$^-$ phenotypes.[86] Sequence analysis indicates that the Sgs1 protein has homology with RecQ in *E. coli.* RecQ possesses the DNA helicase activity and is involved in the recombination pathway.[87] Deletion of the *sgs1* gene causes increased mitotic chromosome nondisjunction. Three human *RecQ* homologs were discovered and named Q1 helicase, *BLM* and *WRN* products. Mutation of the *BLM* and *WRN* were identified as responsible for Bloom's syndrome[88] and Werner's syndrome,[89] respectively. Bloom's syndrome shows frequent malignant neoplasms. In Werner's syndrome, acceleration of the aging are observed. In each case, chromosome translocation frequently occurs.

Moreover, the direct interaction between yeast Sgs1p and Top2p was demonstrated by two hybrid method.[90] The meaning of this interaction is largely unknown. It is the open question whether the interaction of Sgs1p with two distinct topoisomerases in *Saccharomyces cerevisiae* implicates these three proteins in forming a multienzyme complex required for chromosome segregation. Two Topo III genes have been identified in the human chromosome. Topo III α knock-out mouse was found to be lethal.

Genome era and a new type II topoisomerase

Recent progress in the sequencing of the whole genome of several bacterial species[91-95] supports our conclusion well, in that both type I and type II topoisomerase play important roles in the organism (Figure 5), although Topo I is indispensable in certain cases. In the smallest living genome, *Mycoplasma*, consists of 470 genes,[96] among which we could easily identify the presence of Topo I, Topo III, DNA gyrase and Topo IV. Topo III is missing from several species. In *Helicobacter*[97] although no Topo III counterpart was listed, instead three Topo I sequences were found.

In *B. subtilis*,[98] DNA gyrase like sequence was listed instead of Topo IV and *Synechocystis*[99] carries one *gyrB* gene with two *gyrA* sequences and no Topo IV (*parC*, *parE*) was found. Does one of the *GyrA* product act in place of *ParC* combined with a common *GyrB* subunit? It might be premature to call them topoisomerase until enzymatic activity is shown in each gene product.

The recent completion of whole genome sequences for several bacterial species also shows us unexpected features; such that the genome consists of a linear double stranded DNA with telomeric ends like typical eukaryotes and that several plasmids are present. In this organism topo

genome	size (Mb)	type I topoisomerase			type II topoisomerase		
		prokaryotic	eukaryotic				
Mycoplasma genitalium G37	0.58	topo I		topo III	DNA gyrase	topo IV	
Mycoplasma pneumoniae	0.81	topo I			DNA gyrase	topo IV	
Haemophilus influenzae Rd KW20	1.83	topo I		topo III	DNA gyrase	topo IV	
Escherichia coli k12	4.60	topo I		topo III	DNA gyrase	topo IV	
Bacillus subtilis 168	4.20	topo I		topo III	DNA gyrase	(DNA gyrase like)	
Helicobacter pyroli 26695	1.66	topo I×3			DNA gyrase	topo IV	
Synechocystis sp. PCC6803	3.57	topo I			DNA gyrase	(gyrAx2,gyrBx1)	
Borrelia burgdorferi B31	2.18	topo I			DNA gyrase	topo IV	
Methanococcus jannachii DSM2661	1.66	topo I		R.gyrase			topo VI
Methanobacterium thermoautotrophicum ΔH	1.75	topo I					topo VI
Archaeglobus fullgidus VC-16	2.18	topo I		R.gyrase	DNA gyrase		topo VI
Saccharomyces cerevisiae	13		topo I	topo III		topo II	Spo11
Human / Mouse	3000		topo I	topo III α,β		topo IIα,β	

Fig. 5

I, DNA gyrase and Topo IV are all present except for Topo III.

On the other hand, when surveying the archaebacterial genome Methanococcus,[100] astonishingly we found no type II topoisomerase sequence, while Topo I and reverse gyrase were present. We could not imagine how replicated DNA are segregated in the daughter cell without type II enzyme activity. Paradoxically, we have purified type II topoisomerase activity in near homogeniety from one thermophilic archaebacterium, *Sulfolobus.*[15] This enzyme consists of two subunits of 60 kDa and 40 kDa, and apparently its decatenating activity is far more proficient than supercoiling one, much like Topo IV.[18] We thought this enzyme might be a close relative to Topo II encoded by the bacteriophage T4, which consists of three small subunits of 58, 50 and 19 kDa, and that is quite homologous to the bacterial gyrase sequence.

Bergerac et al.[101] finally solved this mystery by cloning the gene of this type II topoisomerase of *Sulfolobus* and found it to be completely new and unrelated to any known topoisomerase, eventhough this sequence is actually present in the *Methanococcus* genome. The homologous sequence of one of the subunits is also found in *S. cerevisiae* as the *SPO11* gene, which is concerned in the first step of meiotic recombination. Moreover, Keeney et al.[102] demonstrated that *SPO11* is involved in the double strand cut in this process. The second subunit might be related to ATPase, but no counterpart has so far been found in other organisms. Three of the thermophilic archaebacteria have this type II enzyme, now designated Topo VI. Could we then suggest that the distribution of topoisomerase is very distinct to the phylogenic separation of species? One of the archaebacteria, *Archaeglobus*[103] was found to be rich in topoisomerase. It contains DNA gyrase in addition to Topo I, reverse gyrase and TopoVI. In fact this is the second case where the DNA gyrase sequence was discovered in archaebacteria, since in *Haloferax alicantei* the presense of the DNA gyrase sequence are reproted.[104]

Is reverse gyrase specific in thermophilic archaebacteria? Actually, the sequence analogous to reverse gyrase is missing from thermophilic *Methanobacterium autotrophicum.*[92] On the other hand, in the thermophilic eubacteria, *Thermotoga* and *Aquifex* contain reverse gyrase activity and its gene.[23,105] There might be no strict phylogenic boundary to eubacteria and archaebacteria in respect of topoisomerase!

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