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Emerging Roles for Plant Topoisomerase VI

Minireview

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Topoisomerase VI is a unique type II topoisomerase originally identified in archaea. Although lacking in most eukaryotic phyla, topoisomerase VI homologs have been recently identified and characterized in the plant *Arabidopsis thaliana*. Three new studies of *Arabidopsis* topoisomerase VI show that this enzyme is important to several processes involving DNA replication and gene expression.

Type II topoisomerases are enzymes capable of passing one DNA duplex through another. This remarkable ability allows these enzymes to solve a myriad of topological problems presented to cells by the processing of double-helical DNA (Figure 1). For example, transcription and DNA replication generate superhelical twist, which if not removed, can lead to perturbed gene expression and cell division [1]. Additionally, problems with knots and tangles resulting from DNA replication and recombination can have tragic consequences for cells if not appropriately resolved (see [2] for an excellent overview of DNA topological problems in cells). Three recent reports now help establish the existence in plants of a new type II topoisomerase previously thought to be confined to archaea, and highlight critical roles in DNA processing for this enzyme [3–5].

All type II topoisomerases characterized to date carry out DNA passage via a conserved mechanism. These enzymes first cleave the phosphodiester backbone of a substrate DNA duplex through the formation of covalent phosphotyrosyl linkages with the 5′ ends of the broken DNA strands. The topoisomerase then captures a second DNA duplex, passes it through the break in the first, and reseals the broken DNA after passage (Figure 2A). ATP binding and hydrolysis are used by type II topoisomerases to control enzyme activity by coordinating the large-scale conformational changes necessary for this reaction [2, 6].

For several decades, all type II topoisomerases were thought to belong to a single protein family, with members highly related in both sequence and structure. These "classic" type II enzymes (known as type IIA topoisomerases) include eukaryotic topoisomerase II (topoII) as well as bacterial DNA gyrase and topoisomerase IV. Type IIA topoisomerases typically assemble into homodimers or heterotetramers and retain distinct regions that are dedicated to either DNA binding and cleavage, ATP turnover, or the amplification of protein structural changes critical for duplex passage (Figure 2B). These enzymes have been extensively characterized genetically, biochemically, and structurally, and their mechanisms are becoming fairly well understood [2, 6, 7].

Topoisomerase VI: An Archaeal Curiosity

Given the ubiquity with which type IIA topoisomerases exist throughout bacteria and eukaryotes, it came as a surprise when the first complete genome sequence of an archaeon (*Methanococcus jannaschii*) failed to reveal any homologs of this enzyme family [8]. This finding was especially surprising because a type II topoisomerase activity had previously been purified directly from another archaeal organism, *Sulfolobus shibatae* [9]. Subsequent cloning of the *S. shibatae* genes coding for this enzyme revealed the presence of topoisomerase VI (topoVI), which is assembled as a heterotetramer with two A subunits dedicated to DNA cleavage (topoVI-A) and two B subunits dedicated to ATP hydrolysis (topoVI-B) (Figure 2B) [9, 10]. Topoisomerase VI proteins have since been found in all fully sequenced archaeal species.

Curiously, although topoisomerase VI shares several functional domains and seemingly all enzymatic functionality with type IIA topoisomerases [9], there are substantial differences between the two enzyme families. The topoVI B subunit is highly structurally similar to the ATPase domains of type IIA topoisomerases, and this ATPase module appears to play an equivalent role in both enzymes [11]. In contrast, though the topoVI A subunit shares two domains involved in DNA cleavage with the type IIA family, its overall structure is distinct from them [12] and is instead generally homologous to another protein, Spo11 [10]. Spo11 is ubiquitous throughout eukaryotes and mediates the double-strand DNA breaks that initiate recombination in meiosis, cleaving DNA in a manner very similar to type II topoisomerases [13, 14]. So, while topoVI is clearly related to type IIA topoisomerases in terms of structure and function, it appears that its physical mechanism of strand passage may be distinct (Figure 3). This possibility has raised questions regarding topoVI's unique biochemical and physical properties, including whether differences in activities or substrate specificities may exist between the type IIA and IIB topoisomerases.

TopoVI in Plants

Because the sequences of bacterial and eukaryotic genomes initially failed to reveal topoisomerase VI homologs, this enzyme was thought to exist only in the archaeal domain of life [6, 10]. However, analysis of the *Arabidopsis thaliana* genome revealed the presence of three distantly related topoVI-A/Spo11 homologs (*AtSPO11-1*, *-2*, *-3*) and one topoVI-B homolog (*AtTOP6B*) [15, 16]. Subsequent searches of EST databases and genomic survey sequences showed that homologs of the *AtTOP6B* and *AtSPO11* genes are scattered widely throughout the plant kingdom [16]; likewise, a search of the recently deposited rice (*Oryza sativa*) genome indicates that one homolog of *AtTOP6B* and multiple homologs of the *AtSPO11* genes exist in this organism (our unpublished observations).

AtSPO11-1 has been shown to be critical for meiotic recombination, indicating that this gene codes for the

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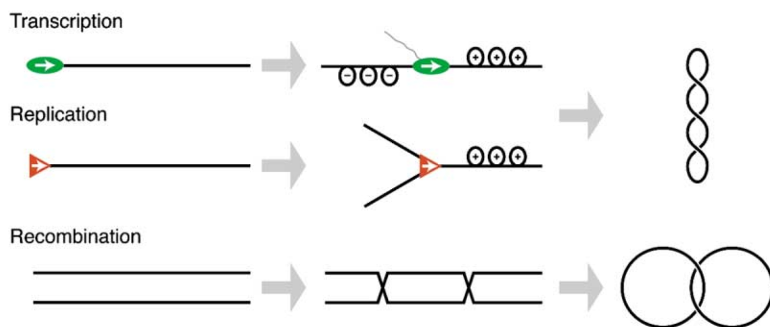

Figure 1. Problems of DNA Topology Arising in Cells

This schematic diagrams the effects of unwinding and strand-joining activities on DNAs. Processes such as transcription and DNA replication can result in excess supercoiling of DNA [1], while recombination and replication can lead to knots and tangles. For simplicity, small circular DNA molecules are used as examples; supercoiling and knotting are equally serious problems in large, linear eukaryotic genomes.

true Spo11 protein in *Arabidopsis* [17]. Additionally, yeast two-hybrid experiments examining the roles of the other topoVI subunit homologs have demonstrated that AtTop6B physically interacts with both AtSpo11-2 and -3, while gene expression studies have shown that *AtTOP6B* and *AtSPO11-3* but not *AtSPO11-2* are highly transcribed in *Arabidopsis* tissues [16]. Taken together, these distinct lines of evidence have indicated that *Arabidopsis* contains a functional topoisomerase VI enzyme made up of the AtTop6B and AtSpo11-3 proteins and suggest that this enzyme might be widely distributed among higher plants. This hypothesis has now been confirmed in recent studies by three independent groups, which together provide strong evidence for a functional link between AtTop6B and AtSpo11-3 and illustrate a new and exciting role for topoVI in DNA replication, chromosome maintenance, and gene expression in plants.

In a direct attempt to determine the role of the *Arabidopsis* topoisomerase VI homologs in vivo, Hartung et al. [3] screened a transposon insertion library for mutants of *AtTOP6B* and *AtSPO11-3*. Plants homozygous for null mutations in either gene exhibited severe dwarfism and failed to live beyond four to five weeks. Importantly, mutations in the two genes resulted in nearly identical phenotypes, and the double-mutant plant was indistinguishable from either single mutant, suggesting that the two genes function in the same process, perhaps even as part of the same protein complex.

The overall dwarf phenotype of the mutants observed by Hartung et al. was partially attributed to a defect in cell division, since the mutant plants exhibited greatly reduced mitotic indices (indicating fewer dividing cells) in actively growing meristematic tissue. In addition, the mutants were shown to have abnormally high levels of chromosomal DNA breaks, suggesting that a defect had arisen in the processing of DNA replication intermediates. Finally, flow cytometric measurement of nuclear DNA content showed that the mutant plants were defective in endoreduplication, an “alternative cell cycle” in which the chromosomal DNA of a cell is replicated several times without corresponding cellular divisions. Endoreduplication occurs in certain cell types throughout eukaryotes, one prominent example being the polytene chromosomes of *Drosophila melanogaster* salivary gland cells [18]. The process is particularly widespread and important for plants, however, where endoreduplication has been linked to the control of cell size in various tissues [19]. The endoreduplication defect observed in *AtTOP6B* and *AtSPO11-3* mutants helps explain their dwarf phenotype, since cells of the primary seedling stem (hypocotyl) require endoreduplication for their elongation in the initial period following germination. Additionally, the finding that both *AtTOP6B* and *AtSPO11-3* mutations cause lowered mitotic indices, chromosomal DNA breaks, and eventual plant death indicates that topoVI may play a more central role in DNA metabolism than in endoreduplication alone.

Coincident with the work of Hartung et al., Sugimoto-Shirasu et al. cloned *AtTOP6B* and *AtSPO11-3* from a screen for growth-retarded mutants potentially defective in endoreduplication [4]. The authors found that mutations in either gene halted endoreduplication during the second cycle in both leaves and hypocotyls, whereas generally up to four cycles occur in these tissues. Interestingly, the overall defect in these mutant plants was much less severe than that observed by Hartung et al., and no obvious problems in cell division were observed. The mutant plants also set fertile seeds, indicating that neither *AtTOP6B* nor *AtSPO11-3* function in meiotic recombination. Thus, the results of this study generally agree with those of Hartung et al. but differ slightly on the extent to which topoVI is required for normal cellular DNA maintenance and replication. Upon comparison, it seems likely that these differences might be attributable to variations between the strains (ecotypes) that the two groups used. There are hundreds of distinct *Arabidopsis* ecotypes currently under study, and their individual physical and biochemical characteristics can sometimes lead to strain-specific experimental differences (see [20] and references therein). Further efforts will undoubtedly be required to illuminate the specific functions of topoVI in *Arabidopsis* and to understand why this enzyme appears more important in some ecotypes than others.

The work of Hartung et al. and Sugimoto-Shirasu et al. is even more striking in light of an earlier report from J. Chory and coworkers. In this study, researchers cloned *AtTOP6B* and *AtSPO11-3* from a screen designed to find mutants with reduced responses to a class of plant growth hormones called brassinosteroids [5]. Brassinosteroids act through a multicomponent signaling pathway to induce the expression of a set of genes involved in cell wall breakdown and biosynthesis, and generally induce plant growth due to cell wall expansion [21]. Plants insensitive to brassinosteroid signals show a retarded growth phenotype much like that seen in endoreduplication-defective mutants, with the additional

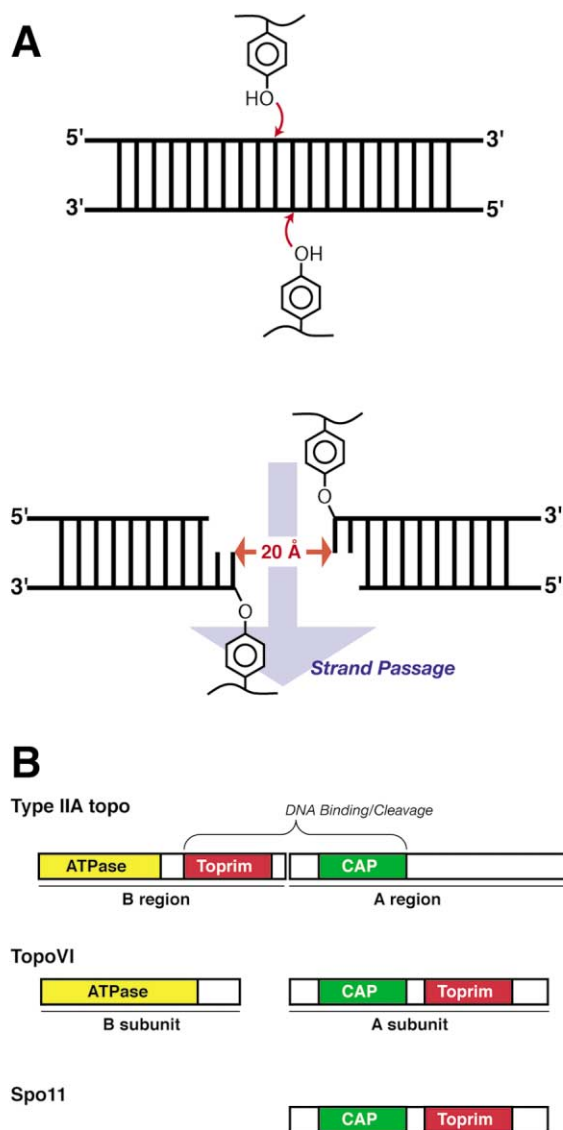

Figure 2. Organization of and DNA Cleavage by Type II Topoisomerases and Spo11

(A) Overview of DNA cleavage by type II topoisomerases. Each half of a type II topoisomerase contains an active site with a nucleophilic tyrosine residue that cleaves one strand of DNA by attacking the backbone, creating a transient, covalent phosphotyrosyl protein:DNA linkage. After cleavage, the two ends are separated to allow the passage of a second DNA duplex, then religated. ATP is required during the reaction to promote capture of the second DNA duplex by the enzyme and to stimulate DNA cleavage.

(B) Schematic showing domain organization of type II topoisomerases and Spo11. Eukaryotic type IIA topoisomerases are assembled as homodimers, with each chain possessing an ATP binding and hydrolyzing domain (yellow) and two domains responsible for DNA binding and cleavage (the helix-turn-helix CAP domain [green] and the metal binding toprim domain [red]). The homologous bacterial enzymes split each monomer into separate A and B subunits and assemble into A_2B_2 heterotetramers. TopoVI is arranged as a heterotetramer, with its two different subunits sharing the three major domains found in type IIA topoisomerases; however, the order of these domains is rearranged in topoisomerase VI. Spo11 is homologous to topoVI-A, possessing the CAP and toprim domains necessary for DNA cleavage.

feature that they exhibit little or no response to exogenously applied brassinosteroids. Mutants of *AtTOP6B* and *AtSPO11-3* are severely growth retarded, explaining why they were isolated in the screen, but these plants nonetheless respond to applied brassinosteroids. When considered with the insights gained from the other studies, it now seems that topoVI's apparent role in the brassinosteroid response is indirect and that an endoreduplication deficiency, as opposed to brassinosteroid insensitivity, may be the cause of the observed growth retardation of the topoVI mutants. It remains possible, however, that endoreduplication and the brassinosteroid response, two processes intimately involved in cell growth, may nonetheless be coordinately regulated.

Two of these three recent papers also indicate a potential second function for topoVI in *Arabidopsis*, in addition to its role in endoreduplication. Using microarray experiments with *AtTOP6B* and *AtSPO11-3* mutant plants, Yin et al. identified a set of 321 genes (out of 5500 analyzed) whose expression is downregulated at least 2-fold in both mutants. These data suggest that topoVI may play a direct role in transcriptional regulation, perhaps by modifying the state of genomic DNA [5]. Sugimoto-Shirasu et al. present similar results, stating that their preliminary microarray data reveal a large set of genes that are over- or underexpressed in plants mutant for the topoVI subunits [4]. Together, these data provide further indications that *Arabidopsis* topoVI might play a role in normal cellular DNA-processing activities beyond its specialized role in endoreduplication.

TopoVI in Endoreduplication

On the combined basis of these three recent efforts, it now appears highly likely that AtSpo11-3 and AtTop6B form a functional type IIB topoisomerase in *Arabidopsis* whose principle function is in endoreduplication. Given that endoreduplication is far more widespread in plants than in other eukaryotes, this action of topoisomerase VI provides a convenient answer to the question of why only plants, of all eukaryotes, appear to possess this enzyme. The precise relationship between topoVI and endoreduplication is, however, still quite complex and relatively unresolved.

Given that *Arabidopsis* possesses four type I topoisomerases and one type IIA topoisomerase in addition to topoVI, why should topoVI be needed in order to successfully undergo endoreduplication? One possibility is that topoVI might process DNA replication intermediates that are unique to endoreduplication. However, there is no evidence of specialized knotted or catenated DNA intermediates that form only during endoreduplication, nor has study of the archaeal enzyme revealed any activities distinct from type IIA topoisomerases. Additionally, though endoreduplication is important for plants, it is also observed in a variety of cell types in many eukaryotic organisms outside the plant kingdom. Were topoVI needed to process special intermediates resulting specifically from endoreduplication, such functions would have to have been taken over by other topoisomerases in these eukaryotic lineages.

An alternative explanation for the functional specialization of topoVI could be that the two type II topoisomerases in *Arabidopsis* are differentially regulated and that this regulation is particularly important during en-

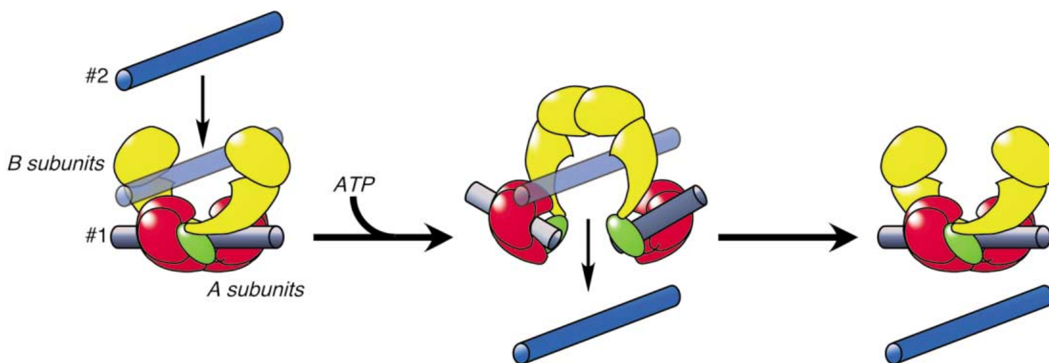

Figure 3. Proposed Reaction Mechanism for TopoVI

This model indicates how topoVI is thought to carry out DNA transport. Step 1: DNA segment #1 (gray) is bound by the topoVI A-subunits. Step 2: DNA segment #2 (blue) is trapped inside the enzyme upon closure of the ATP binding B subunit dimer. Concomitant with this capture, DNA segment #1 is cleaved and opened, and segment #2 is passed through the break. Step 3: DNA segment #1 is resealed and released, and the enzyme resets [2, 6]. Domains of the two subunits are colored as in Figure 2A: the ATP binding B subunits are yellow, the A subunit CAP-like domains are green, and toprim domains are red.

doreduplication. In support of this idea, it has been shown that differentiated *Arabidopsis* tissues have virtually no detectable topoisomerase II protein [22], whereas topoVI is highly expressed in all tissues examined [16]. It is therefore possible that the major type II topoisomerase present in tissues undergoing endoreduplication, which have halted mitotic cell division, is in fact topoVI. Further experimentation will be needed to determine which of these scenarios is correct and to help answer the question of why topoVI, as opposed to an endogenous type IIA topoisomerase, is the enzyme necessary for endoreduplication.

The Evolution of TopoVI and Spo11

Studies of topoisomerase VI have clearly shown that it is related to the type IIA topoisomerases and also that the A subunit is highly similar to Spo11. Current theories of evolution indicate that a cenancestral cell, the common ancestor of all modern cellular life, probably had a well-developed complement of DNA-processing machinery, including a classic type IIA topoisomerase [23]. It therefore seems possible that topoVI might have evolved from the duplication and subsequent reorganization/minimization of one or both subunits of a type IIA enzyme in a cell ancestral to both archaea and eukaryotes. After the archaeal/eukaryotic split, each lineage would have lost one of these duplicated components: archaea lost the type IIA topoisomerases in favor of topoVI, while eukaryotes lost the B subunit of topoVI and recruited the A subunit into the meiotic recombination machinery as Spo11.

Most eukaryotes have only one topoVI A-subunit homolog, Spo11, and no topoVI B-subunit homologs. In contrast, *Arabidopsis* possesses three homologs of topoVI-A/Spo11 and a single topoVI-B homolog. Importantly, no two homologs of topoVI-A/Spo11 in *Arabidopsis* are significantly more related to each other than they are to other Spo11 or topoVI-A proteins from other organisms [15, 16], indicating that they are ancient paralogs, as opposed to recently duplicated genes. Thus, a lateral gene transfer event of topoVI-A and -B genes from an archaeon to a primitive plant is the most likely explanation for this enzyme's presence solely in eukaryotes of the plant kingdom. Interestingly, there seems to be an extra topoVI-A/Spo11 homolog in *Arabidopsis*: while *AtSPO11-1* is a true Spo11 gene and *AtSPO11-3* codes for the topoVI A-subunit, the role of *AtSPO11-2* is still unknown. Undoubtedly, emerging genomic data on additional organisms scattered throughout the eukaryotic domain will shed more light on these complicated evolutionary relationships.

In summary, since its discovery in the mid-1990s, steady progress has been made in understanding the mechanism of topoVI and its relationship to the type IIA topoisomerases. Recent genetic studies in *Arabidopsis thaliana* have definitively shown that topoVI plays a critical role in DNA metabolism in plants, whereas even its existence had been previously debatable. This important work now sets the stage for future studies on the role, regulation, and evolution of this unique enzyme family.

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