POINT OF VIEW

Type IA topoisomerases can be “magicians” for both DNA and RNA in all domains of life

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ABSTRACT

Topoisomerases solve critical topological problems in DNA metabolism and have long been regarded as the “magicians” of the DNA world. Here we present views from 2 of our recent studies indicating that Type IA topoisomerases from all domains of life often possess dual topoisomerase activities for both DNA and RNA. In animals, one of the 2 Type IA topoisomerases, Top3 β , contains an RNA-binding domain, possesses RNA topoisomerase activity, binds mRNAs, interacts with mRNA-binding proteins, and associates with active mRNA translation machinery. The RNA-binding domain is required for Top3 β to bind mRNAs and promote normal neurodevelopment. Top3 β forms a highly conserved complex with Tudor-domain-containing 3 (TDRD3), a protein known to interact with translation factors, histones, RNA polymerase II, single stranded DNA and RNA. Top3 β requires TDRD3 for its association with the mRNA translation machinery. We suggest that Type IA topoisomerases can be “magicians” for not only DNA, but also RNA; and they may solve topological problems for both nucleic acids in all domains of life. In animals, Top3 β -TDRD3 is a dual-activity topoisomerase complex that can act on DNA to stimulate transcription, and on mRNA to promote translation.

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Introduction

The first DNA topoisomerase was discovered in *E. coli* by Dr. James Wang more than 4 decades ago (topoisomerase 1, abbreviated below as EcoTop1).¹ Since then, DNA topoisomerases of different types been found in species from all domains of life.^{2–5} Numerous studies have shown that these enzymes can catalyze DNA strand passage reactions; and are essential to resolve topological stress produced during various DNA metabolic reactions, including replication, transcription, recombination, repair, and chromosome segregation. Their unique attribute of cutting and re-ligating DNA strands without leaving a trace makes them true “magicians of the DNA world”.² Their defects can lead to reduced cell proliferation, shortened life-span,⁶ disease,⁷ and lethality.^{3,8}

Topoisomerases have been classified in 2 major families based on how they cleave the DNA.^{2,5} Type I topoisomerases transiently cut one strand of the duplex, whereas Type II enzymes simultaneously cut both. These families are further divided into Type IA, IB, IIA and IIB. Type IA enzymes follow the stand-passage mechanism where they cut one strand, pass the unbroken strand through the broken ends of the other strand, and re-ligate the broken ends, resulting in altered linking numbers in DNA duplex. Type IB enzymes differ from Type IA in that they allow swiveling of broken ends around the intact stand to relieve the topological stress before re-ligating the broken ends. Specifically, Type IA topoisomerases can act on both double and single stranded (ds and ss) DNA to

generate products with altered topology.⁹ Their reactions include: relaxation of supercoiled dsDNA;¹ catenation or decatenation of dsDNA rings that contain at least one pre-existing ssDNA scission in one of the 2 rings;^{10–12} interconversion between ssDNA rings without knots and those with knots;^{13,14} intertwining of 2 ssDNA rings of complementary sequences into a closed duplex ring;¹⁵ and catenation or decatenation ssDNA rings.^{16–19} This ability to act on both double and single strand substrates makes Type IA enzymes ideal topoisomerases for RNA because typical RNA molecules (mRNA, tRNA, rRNAs) consist of both double and single-stranded regions.

The first hint that topoisomerases may act on RNA came from the study by DiGate and Marriars in 1992, in which they reported that *E. coli* topoisomerase 3 (EcoTop3) can catalyze cleavage of RNA.²⁰ EcoTop3 is one of the 2 Type IA topoisomerases in this species (the other is EcoTop1). An elegant study by Seeman and colleagues in 1996 demonstrated that EcoTop3 can indeed catalyze an RNA strand passage reaction that converts a synthetic RNA circle without a knot to a trefoil knot, indicating that EcoTop3 does have RNA topoisomerase activity.²¹ However, unlike DNA topoisomerases that have been intensively investigated ever since their discovery, the RNA topoisomerase activity has attracted little attention for many years—there are no follow-up studies to link this activity to RNA metabolism, and no RNA topoisomerase activity has been reported in other species. A common thought has been

that typical RNA molecules are single-stranded linear and not topologically constrained, so that they may not need topoisomerases.

The silence in the RNA topoisomerase field may finally come to an end in 2013 when our group and the group lead by Dr. Palotie have independently reported that a human Type IA topoisomerase, Top3 β , can catalyze RNA strand-passage¹⁷ and cleavage reactions.⁷ The observed RNA topoisomerase activity is specific, because it is absent in the other Type IA topoisomerase in human, Top3 α .¹⁷ Our group further showed that this difference in RNA topoisomerase activity is largely due to presence of an RNA binding domain, RGG-box, in Top3 β but not in Top3 α , as deletion of this domain diminished the RNA topoisomerase activity of Top3 β .¹⁷ Importantly, the 2 studies provided several lines of evidence linking Top3 β to mRNA translation. For example, both groups showed that Top3 β forms a complex with Tudor domain-containing 3 (TDRD3),^{7,17} which interacts with a known mRNA-binding protein (RBP), FMRP (Fragile-X mental retardation protein)²² (Fig. 1, see the Animal Section). This is in contrast to Top3 α which forms a structurally similar complex with RMI1, RMI2, and a well-characterized DNA helicase, BLM.^{23–25} Thus, these data suggest that human cells contain 2 homologous Type IA topoisomerase complexes—Top3 α -RMI1-RMI2-BLM complex that functions in DNA repair, and the Top3 β -TDRD3 complex that interacts with FMRP to participate in RNA metabolic reactions (Fig. 1).¹⁷ In support of the notion that the Top3 β complex can act on RNA, Top3 β has been found to co-localize in RNA stress granules with FMRP and TDRD3 in the cytoplasm,^{7,17} interact with the mRNA exon-junction complex,⁷ bind many mRNA targets of FMRP *in vivo*,¹⁷ associate with the active translation machinery (polyribosomes),^{7,17} and promote expression of a protein at synapse.¹⁷ Moreover, Top3 β gene

deletion has been linked to schizophrenia and intellectual disability,⁷ which is a mental disorder similar to the Fragile X syndrome caused by silencing of FMRP. Furthermore, Top3 β -knockout mice and flies are defective in synapse formation,¹⁷ which mimics FMRP-knockout animals.^{26,27} Together, these data suggest that Top3 β may work with FMRP and TDRD3 to regulate expression of mRNAs important for neurodevelopment and mental health.

In this review, we summarize several major points from our 2 recent studies^{28,29} which address several fundamental questions regarding RNA topoisomerases. These include: (i) their broad prevalence in all domains of life; (ii) their structural requirement that is common or different from that of DNA topoisomerases; (iii) their conserved association with mRNA translation machinery in animals; (iv) how they are targeted to mRNAs in animals; and (v) the evidence supporting their roles in neural development and diseases. We also discuss the possible origin and evolution of RNA topoisomerase activity and the Type IA topoisomerase complexes.

RNA topoisomerase activity is prevalent in Type IA topoisomerases from all domains of life

The first basic question addressed by our studies is how prevalent RNA topoisomerases are. Prior to our studies, RNA topoisomerase activity has been detected in only 2 Type IA topoisomerases, EcoTop3²¹ and human Top3 β .¹⁷ Because Type IA enzymes are the most ubiquitous topoisomerases present in nearly all species,^{4,30} these data raised a possibility that the RNA topoisomerase activity may be prevalent in broad species similarly as is the DNA topoisomerase activity. In a recent study from our group,²⁸ we screened multiple published Type IA DNA topoisomerases from all 3 domains of life (bacteria,

Figure 1. Type IA topoisomerases have evolved from single proteins with dual activities in microorganisms to multi-protein complexes with restricted activities in animals. Schematic representation of evolution of Type IA topoisomerases in DNA and RNA metabolism. In *E. coli*, both Type IA enzymes (Top1 and Top3) have dual activities for DNA and RNA. In yeast, the only Type IA enzyme is part of a complex (Top3-Rmi1) that also has dual activities for DNA and RNA. In animals, only one of the 2 Type IA paralogs, Top3 β , has dual activity, whereas Top3 α has activity for only DNA. Interestingly, Top3 β , but not Top3 α , contains an RNA-binding domain (RGG box) that is required for its RNA topoisomerase activity. Moreover, the 2 Top3 paralogs comprise 2 distinct complexes, with the Top3 β complex containing a RNA binding protein (FMRP), whereas the Top3 α complex containing a DNA helicase (BLM). These data argue that Type IA topoisomerases have evolved into 2 functional distinct complexes in animals, one for RNA and DNA (Top3 β -TDRD3-FMRP), and one for DNA only (Top3 α -Rmi1-Rmi2-BLM). This figure is adapted from Fig. 7A of a previous publication.²⁸

archaea, and eukarya) for RNA topoisomerase activity, using the same trefoil knot conversion assay described in the previous studies^{17,21} (Fig. 2A). To our satisfaction, we detected RNA topoisomerases activity in multiple Type IA enzymes from all 3 domains (Fig. 2B). These include EcoTop1, the prototype of Type 1A family and the first topoisomerase discovered;¹ Tma-Top1 from the thermophilic bacteria *Thermotoga maritima*; 2 enzymes from archaea *Nanoarchaeum equitans* (NeqTop1) and *Sulpholobus solfataricus* (SsoTop1); and Top3 of yeast *saccharomyces crevisiae* (a eukarya). Consistent with our findings, Hsieh and his colleagues have developed a different RNA topoisomerase assay, which is based on intertwining of 2 single stranded complementary RNA circles into a duplex RNA ring; and independently detected RNA topoisomerase activity in EcoTop1, NeqTop1, and *Drosophila* Top3³¹ (See Fig. 2B for summary). More recently, Weizmann and colleagues have constructed new ssRNA knots as topological probes, and detected

RNA topoisomerase activity in EcoTop1.³² These combined data suggest that the RNA topoisomerase activity is prevalent in Type IA topoisomerases from all domains of life. Interestingly, the enzymes with dual activities are mostly observed in unicellular organisms (bacteria, archaea and yeast) (Figs. 1 and 2B). This is in contrast to human, as only one of the 2 Type IA enzymes (Top3 β) has dual activities, whereas the other one (Top3 α) has only DNA activity.¹⁷ Specialization of the topoisomerase activity for each nucleic acid may be beneficial to eukaryotes that have nuclear and cytoplasmic compartments, because it can prevent the DNA topoisomerases from binding to mRNAs and being mis-localized in the cytoplasm where mRNA translation occurs (see Discussion below on targeting of topoisomerase to mRNAs). Do all Type IA topoisomerases have RNA topoisomerase activity? Our screen revealed that some but not all Type IA enzymes possess RNA topoisomerase activity even in

Figure 2. RNA topoisomerase activity is prevalent in Type IA topoisomerases from all 3 domains of life. (A) Schematic representation of the RNA topoisomerase assay. A synthetic circular RNA substrate contains 2 pairs of complementary regions (red and green) separated by single-stranded spacers (black). Through strand passage reactions, this substrate is converted to a trefoil knot in which the 2 pairs of complementary regions can form normal double helices. (B) Schematic representation of the domain structures of Type IA topoisomerases from different species (left) and their RNA topoisomerase activity.^{17,21,28,31} The conserved core domains and the non-conserved CTDs, including Zn-fingers (orange boxes), are indicated. Their RNA topoisomerase activity was shown on the right. Fig. 2A is adapted from Fig. 4 of the previous paper.¹⁷ Fig. 2B is adapted from 4 figures of another paper.²⁸

microorganisms. For example, we failed to observe RNA topoisomerase activity in Top1 from *Mycobacterium tuberculosis* and *Mycobacterium smegmatis*, as well as in 2 reverse gyrases from archaea.

RNA and DNA topoisomerase activities have common and unique structural requirement

The second question addressed by our studies is that as both RNA and DNA topoisomerase activities reside in the same Type IA enzymes, do these activities have common or different structural requirement. All Type IA enzymes utilize a strictly conserved Tyr residue to cleave DNA and form a transient covalent DNA-protein complex; and they absolutely require this Tyr residue for their DNA topoisomerase activity. We found that the same Tyr residue is also necessary for RNA topoisomerase activity for every recombinant Type IA enzyme which we tested.²⁸ Thus, Type IA topoisomerases likely utilize the same catalytic residue to catalyze both DNA and RNA during topoisomerase reactions.

All Type IA enzymes have a group of highly conserved core domains, as well as a non-conserved C-terminal domain (CTDs) that is variable in lengths and numbers of Zinc-fingers (Fig. 2B). Inspection of the Type IA enzymes that carry RNA topoisomerase activity revealed that their CTDs are variable in lengths and may lack the Zinc-fingers (Fig. 2B), which imply that the conserved core domains are likely to be sufficient for the RNA topoisomerase activity. Consistent with this notion, we examined various mutants of EcoTop1, and found that its core domains are both necessary and sufficient to catalyze the RNA topoisomerase reaction, whereas its CTD is completely dispensable.²⁸ This feature is similar to that of EcoTop1 in catalyzing DNA decatenation reaction,³³ but different from the same enzyme in catalyzing relaxation of supercoiled DNA,³⁴ as the core domains alone can catalyze the former reaction, whereas both core domains and CTD are needed for the latter reaction. Thus, bacterial Type IA enzymes have both common and different structural requirement for their dual activities. For simple DNA or RNA strand-passage reactions, such as conversion of a circle to a knot and decatenation, the core domains themselves are sufficient to perform the tasks. However, for a complex reaction on DNA duplex, such as supercoiled DNA relaxation, the CTD domain is required.

It needs to be pointed out that in human, the Type IA core domains alone are insufficient for RNA topoisomerase reactions. This is supported by findings that Top3 α , which has intact core domains, lacks the RNA topoisomerase activity¹⁷ (Fig. 2B). It is also evidenced by data that the RNA topoisomerase activity of Top3 β is strongly reduced when its C-terminal RGG box is deleted;¹⁷ and the activity is completely lost when its entire CTD domain is removed.²⁸ The data suggest that during evolution, the Type IA core domains of animals have lost the ability to catalyze the strand-passage reactions by themselves. Instead, they have developed strong dependency on their unique CTDs for these reactions. One potential advantage of this dependency is that it enables regulation of topoisomerase activity by CTDs. For example, the CTD of Top3 β can target the protein to mRNAs, whereas the CTD of Top3 α cannot (see below).

RNA topoisomerases associate with polyribosomes in animals but not yeast or bacteria

The third question addressed by our studies is whether the association between the RNA topoisomerases and polyribosomes is conserved. We and others have previously shown that Top3 β , along its interacting partners TDRD3 and FMRP, associate with polyribosomes in human cells,^{7,17} suggesting that Top3 β may mediate mRNA translation together with its partners in eukaryotes. Because RNA topoisomerase activity is prevalent in Type IA topoisomerases from all 3 domains, we investigated whether the polyribosome association is a feature conserved in these enzymes across different domains. No polyribosome association was observed for 2 bacterial topoisomerases (EcoTop1 and EcoTop3), as well as yeast Top3.²⁸ However, the polyribosome association was observed for Top3 β from chicken DT40 cells and *Drosophila* S2 cells, both of which are animal species.²⁸ The data provide evidence that the Type IA topoisomerases may participate in mRNA translation in animals.

The RNA topoisomerase Top3 β depends on TDRD3 to associate with polyribosomes

The polyribosome association of human Top3 β is dependent on its partner, TDRD3, as deletion of TDRD3 or mutations of its protein interaction domains disrupt the association.⁷ We found that this TDRD3-dependence is conserved in chicken and flies, as depleting TDRD3 from these cells strongly reduced the amount Top3 β that associate with polyribosomes.²⁸ Thus, TDRD3 may play a conserved role in targeting Top3 β to the mRNA translation machinery in animals. Current evidence suggests that TDRD3 in animals can interact with multiple RNA binding proteins through its different protein-binding modules, such as its Tudor domain, FMRP-binding motif, and EBM (exon-junction-complex-binding) motif.^{7,17,k22,35} The Tudor domain can specifically recognize arginine-methylated RNA-binding proteins,^{35,36} including FMRP.¹⁷ Mutations in either Tudor or EBM motif of TDRD3 reduces the association between Top3 β and polyribosomes, indicating that TDRD3-mediated protein-protein interactions recruit Top3 β to the translation machinery.

Hsieh and colleagues have recently shown that TDRD3 has binding activities for both single-stranded RNA and DNA.³¹ Moreover, it can stimulate the topoisomerase activity of Top3 β for both nucleic acids.³¹ Thus, TDRD3 is a perfect partner for the dual activity enzyme, Top3 β , because it can help Top3 β to bind and react with either ssDNA or ssRNA. We propose that TDRD3 plays 2 similar roles for Top3 β on DNA or RNA: one, it helps to target Top3 β to its site of action by protein-protein or protein-nucleic acid interactions; and 2, it stimulates the subsequent topoisomerase reaction on either RNA or DNA. In the case of RNA, TDRD3 can recruit Top3 β to polyribosomes, RNA stress granules, and other translation factors. For reactions on DNA, TDRD3 has been shown to bind arginine-methylated histone tails through its Tudor domain;³⁷ and this binding has been shown to recruit Top3 β to chromatin to relax negatively supercoiled DNA and reduce R-loops formed during transcription.³⁸ Moreover, TDRD3 has been reported to

bind methylated carboxyl terminal domain of RNA polymerase II.³⁹ Together, these data argue that Top3 β -TDRD3 complex acts as a dual-activity complex that can promote biologic processes on either DNA or RNA.

Top3 β is the major topoisomerase for mRNAs

The fourth question addressed by our studies is which topoisomerase works on mRNAs. Human cells contain 5 topoisomerases that are present in nucleus and/or cytoplasm, so that each of them could potentially bind mRNAs and regulate their topology. Three of the 5 topoisomerases (Top1, Top2 α and Top3 β) have previously been shown to bind directly to mRNAs in human cells using a method of UV-crosslinking coupled with mass spectrometry.^{40,41} These data raised a possibility that these 3 topoisomerases may bind mRNAs and regulate their topology. We used a variation of the above method—UV-crosslinking coupled with immunoblotting—to investigate which of the 5 topoisomerases directly binds mRNAs in human HEK293 cells.²⁹ We found that Top3 β is the only topoisomerase that is detectable in the mRNA-bound RNA-binding proteins (RBPs), whereas the other topoisomerases (Top1, Top2 α , Top2 β , and Top3 α) are undetectable. In addition, we detected the presence of both interacting partners of Top3 β -TDRD3 and FMRP. Together, these data suggest that Top3 β is the major topoisomerase working on mRNAs in human cells.

Top3 β is targeted to mRNAs by its unique RGG RNA-binding domain

An obvious question is why Top3 β is the most abundant mRNA-binding topoisomerase. We found that only Top3 β , but not any other topoisomerases, contains a recognizable RNA-binding domain, an RGG-box. This implies that Top3 β is likely to be targeted to mRNAs by this domain. Consistent with this prediction, deletion of the RGG-box strongly reduced the amount of Top3 β that associates with the mRNAs.²⁹ This result is reminiscent of earlier data that deletion of RGG-box diminishes the RNA topoisomerase activity of Top3 β .¹⁷ Together, these data reveal a crucial role of the RGG-box in targeting Top3 β to RNAs and catalyzing topoisomerase reactions.

If the RGG-box is so important for Top3 β to bind mRNAs, one would predict that it is highly conserved during evolution. Indeed, we found that the RGG-box is conserved in Top3 β from not only animals, but also plants and fungi²⁸ (Fig. 3A). Interestingly, the RGG-box is also present in Top3 α of plants and several fungi species²⁸ (Fig. 3A), which predicts that Top3 α from these species may also be able to act on RNA. Future work is needed to explore this possibility.

Top3 β requires its RNA-binding and catalytic activity to promote neurodevelopment

An important issue addressed by our studies is whether Top3 β requires its catalytic and RNA binding activity to function *in vivo*. Our previous studies have shown that Top3 β is required for normal synapse formation using mouse and *Drosophila* as model systems.¹⁷ However, the study used gene-knockout animals that completely lack the Top3 β protein, so that they did

not provide answers to this question. In our new study, we rescued the *Drosophila* Top3 β mutant by ectopically expressing wildtype and 2 mutants of Top3 β that are either substituted of its catalytic Tyr residue to Phe, or deleted of its RGG-box. The first mutant completely lacks the topoisomerase activity, but has near normal mRNA-binding activity; whereas the second mutant has defective mRNA-binding activity, and reduced topoisomerase activity. We found that whereas the wildtype Top3 β largely rescued the defective synapse formation of the Top3 β mutant flies, neither mutants rescued the defect.²⁹ Our data thus suggest that both RNA-binding and topoisomerase activities are needed by Top3 β to promote neurodevelopment.

Top3 β function is linked to mental disorders

Top3 β genomic deletion has been linked to one of the most common mental disorders, Schizophrenia.⁷ We and others have previously found that the interaction between Top3 β -TDRD3 complex and FMRP is disrupted by a Fragile X patient-derived point mutation.^{17,22} Because Fragile X syndrome is the most common known cause of autism spectrum disorders (ASD), these data argue that Top3 β mutation could be a risk factor for schizophrenia and ASD. Consistent with this notion, Top3 β genomic deletion has recently been observed in a patient with autism, cognitive impairment, facial dysmorphism and behavior concerns.⁴²

In addition to the gene deletions, 2 *de novo* single nucleotide variants (SNVs) of Top3 β , C666R and R472Q, have been reported in autism and schizophrenia patients, respectively.^{43,44} We noticed that these variants mutated highly conserved residues in the CTD and core domains of Top3 β , respectively, suggesting that they may disrupt Top3 β functions. In our latest study,²⁹ we found that both mutants are defective in association with FMRP,²⁹ which is similar to earlier data that a Fragile X patient-derived mutation disrupts the association between FMRP and Top3 β -TDRD3.^{17,22} In addition, we found that the C666R variant is also defective in mRNA binding, in RNA topoisomerase activity, and in supporting normal synapse formation in *Drosophila*.²⁹ Together, these data suggest that SNVs that compromise Top3 β activities may contribute to abnormal neurodevelopment and mental dysfunction. Notably, whereas the C666R variant has not been detected in the general population, the R472Q variant has been detected with a frequency of 0.0004. It will be interesting to analyze whether individuals carrying R472Q allele have increased risk of developing mental disorders.

RNA topoisomerase activity may have arisen earlier during evolution

Type IA topoisomerases have been proposed to be present in the Last Unknown Common Ancestors (LUCAs) for the 3 domains of life, because of their near universal presence in all species^{30,45} (Fig. 3B). One evolutionary theory postulates that life originates from a pool of self-replicating RNAs, and there exists an RNA world with RNA genome before the current DNA world.⁴⁶ There are also hypotheses that LUCAs may consist of organisms with genomes of RNA, or RNA-DNA hybrid, or DNA with an RNA replication intermediate.^{30,45,47} The

Figure 3. Type IA topoisomerases in eukaryotes have evolved into 2 distinct homologous complexes in animals and plants; the RNA topoisomerase activity of Type IA topoisomerases may have arisen earlier during evolution; (A) Schematic presentations show evolution of Top3 into 2 homologous complexes in eukaryotes, Top3α-RMI1 and Top3β-TDRD3, which have distinct functions in DNA and RNA metabolism, respectively. Yeast has a single copy of Top3 and RMI, which forms a complex required for maintenance of genomic stability. In Fungi like *Mucor circinelloides* and *Rhizopus microsporus*, we predict that the Top3 enzyme has evolved into 2 paralogous complexes: Top3α-RMI1 and Top3β-TDRD3. Both complexes are also conserved in plants and animals. Top3α-RMI1 retains its function in DNA metabolism and maintenance of genomic stability, while Top3β-TDRD3 works in both DNA and RNA metabolism. The subunits of the 2 Top3 complexes, RMI1 and TDRD3, also share homology at their N-terminus, and emerged in above mentioned species of fungi. Their unique C-terminal domains provide protein-protein binding surfaces which target their respective topoisomerases to specific regulators, such as RMI2 or FMRP. Surprisingly, the C-terminal domains of TDRD3 in plants has more resemblance to those of fungi than to those of animals: they lack the UBA, TUDOR, and FMRP-interaction domains, but have the RGG-box which is also present in TDRD3 of several fungi species. Fungi and plants do not have orthologs of FMRP, which binds to the C-terminus of animal TDRD3. Accession numbers of Top3α, Top3β, RMI, TDRD3 homologs of *M. circinelloides* (Mc) and *R. microsporus* (Rm) are mentioned here. McTop3α - EPB81200, McRMI - EPB83693, RmTop3α - CEI89638, RmRMI - CEI87462, McTop3β - EPB85471, McTDRD3 - EPB83703, RmTop3β CEG78805 and RmTDRD3 GAN02671. (B) A model of origin and evolution of Type IA topoisomerases and their activity for RNA and DNA. It has been postulated that life starts with a pool of self-replicating RNAs; and there exists an RNA world with RNA genome before the current DNA world. We propose that Type IA enzymes may originate in the RNA world to solve RNA topological problems. When the RNA world evolved and was eventually replaced by the DNA world, these enzymes retained their RNA topoisomerase activity while developing a new activity for DNA. This may explain the prevalence of the RNA topoisomerase activity in Type IA enzymes from all 3 domains. Fig. 3A and 3B are reproduced from Fig. 7 and Fig. S7 of a previous publication.²⁸

discovery that RNA topoisomerase activity is prevalent in Type IA topoisomerases from 3 domains of life leads us to propose that these enzymes may have arisen earlier during evolution to solve RNA topological problems in the RNA world.²⁸ The enzymes may have been evolved later to carry DNA topoisomerase activity in LUCA when DNA-based genome replaces the RNA genome. This proposal may help rationalize the findings that Type IA enzymes from all 3 domains have largely retained their dual activities for both nucleic acids.

Type IA topoisomerases have evolved into 2 distinct homologous complexes in animals and plants

We have previously observed that the 2 Type IA topoisomerases, Top3α and Top3β, are present in distinct complexes in human.^{17,23} Their direct interacting partners within each complex, RMI1 for Top3α and TDRD3 for Top3β, are also homologous to each other: they share 2 common domains at their N-terminus, DUF1767 domain and a discontinuous OB-fold with

an intervening loop^{17,23,24} (Fig. 3A). Structural and functional analyses have revealed that the OB-fold and intervening loop of RMI1 and TDRD3 directly interact with Top3 α or Top3 β , respectively.^{17,48–50} Unlike the conserved N-terminus, the C-terminal domains of RMI1 and TDRD3 in animals are divergent: that of RMI1 consists of an OB-fold, which interacts with RMI2^{23,25,50,51}, as well as a DNA translocase, FANCM;⁵² whereas that of TDRD3 consists of multiple protein-interacting modules, UBA (ubiquitin association domain), Tudor, FMRP-interacting motif, and Exon-Junction-complex-binding motif.^{7,17,22,35} The last 3 domains of TDRD3 can interact with RNA-binding proteins and translation factors,^{7,17,22,35} consistent with the notion that TDRD3 targets Top3 β to mRNA translation machinery.

Our BLAST searches of NCBI database revealed that RMI1 and TDRD3 are only present in eukaryotes, suggesting that mechanisms of Type IA enzymes in eukaryotes might be different from those in other domains of life. In addition, yeast has a single Top3 and a shortened version of RMI1 that contains only the conserved N-terminal domains (Fig. 3A). The absence of the C-terminal protein-interaction domains in yeast RMI1 may account for the lack of association between yeast Top3 and polyribosomes.²⁸

Our BLAST searches also found that Top3 α , Top3 β , and their 2 partners, RMI1 and TDRD3, are present in plants and several fungi species. This suggests that Type IA topoisomerases from these species may form Top3 α -RMI1 and Top3 β -TDRD3 complexes that are similar to their counterparts in animals. However, TDRD3 from plants and fungi have 2 structural features different from their counterparts in animals (Fig. 3A): one, they contain an RGG-box that is absent in TDRD3 of animals; and 2, they lack the Tudor domain, FMRP-binding motif, and EBM motif present in the animal TDRD3. First, these data imply that RMI1 and TDRD3 are likely derived from a common ancestor that consists of only the DUF1767 domain and the OB-fold, which may resemble the RMI1 protein in yeast. They may have acquired their distinctive C-terminal domains later in evolution through gene fusion. These C-terminal domains may provide specific interface for DNA binding proteins, RNA binding proteins, or RNA, thus targeting their respective topoisomerases to appropriate action sites. Second, the data also argue that TDRD3 in fungi and plants may target Top3 β to mRNA translation machinery differently compared with that of animals—they might directly recruit Top3 β to mRNAs through their putative RGG boxes. Overall, our data suggest that Type IA topoisomerases are evolved from single enzymes in microorganisms to 2 multisubunit complexes with more specialized activities and interface in animals and plants.

Concluding Remarks and Future Work

A major discovery of our studies is that many Type IA DNA topoisomerases across all 3 domains of life have RNA topoisomerase activity. Because these enzymes possess dual activities, we propose that they should now be considered as “magicians” for not only DNA, but also RNA. In particular, our studies have provided new evidence for participation of one animal Type IA enzyme—Top3 β —in mRNA metabolism. The evidence includes: one, Top3 β is the most abundant mRNA-binding topoisomerase in human cells; 2, it is

specifically targeted to mRNAs by its RNA-binding domain; 3, its RNA binding domain as well as the catalytic activity are required for neural development; 4, it has conserved association with the mRNA translation machinery in animals; and 5, this association requires its partner, TDRD3, which interacts many RBPs. It should be noted that Top3 β also possesses DNA topoisomerase activity,⁵³ and has been suggested to relax supercoiled DNA generated during transcription to reduce R-loop formation.³⁸ Thus, Top3 β could act as a dual-activity enzyme solving topological problems for DNA during transcription, and for mRNA during translation.

Is the RNA-binding and topoisomerase activity important for Top3 β function? We have demonstrated that mutations disrupting the RNA-binding and catalytic activity of Top3 β impair the capability of the protein to promote neurodevelopment.²⁹ However, the same mutations can also disrupt the DNA topoisomerase activity of enzyme.¹⁷ At present, there is no separation-of-function mutant that inactivates one activity but leaves the other activity intact. Thus, it remains possible that it is the DNA topoisomerase activity or both activities of Top3 β that are needed for normal neurodevelopment. Future work is needed to distinguish which activity is critical for Top3 β function *in vivo*.

One unanswered question is what types of topological problems may arise during RNA metabolism? We have previously proposed several hypothetical situations where RNA topological problems may occur, which are then resolved by Top3 β .¹⁷ For example, a linear mRNA may become topologically constrained by forming circular structures via protein-mediated interactions between the Poly-A binding proteins at the 3' end of mRNA and the translation initiation factors at the 5' end.⁵⁴ Top3 β may catalyze catenation or decatenation of 2 mRNA circles, thus enhancing or inhibiting translation (Fig. 4A) or transport of mRNAs (Fig. 4B). In addition, helical torsions may arise when a translating ribosome or a helicase unwinds a duplex region in an mRNA hairpin (Fig. 4C). If the hairpin is bound to an immobile protein or cellular matrix, the helical torsion will not be relieved by rotation, and may impede progression of ribosomes or helicases during translation or transport. Top3 β may relax the helical torsion through its strand passage activity. Moreover, a large family of circular RNAs have been identified from many species,⁵⁵ and may play roles in neurodegenerative diseases.^{56,57} Top3 β may interconvert these RNAs between circles and knots or catenane structures (Fig. 4D), thus affecting their function. Furthermore, it is possible that linear RNAs may generate topological problems—they may form intramolecular or intermolecular knots, which may block mRNA translation (Fig. 4E). It is also possible that a linear RNA can get entangled with another linear RNA or DNA (Fig. 4F). The strand-passage activity of an RNA topoisomerase may solve these topological problems, thus facilitating various RNA-based reactions. It should be indicated that topoisomerases may function other than solving topological problems. For example, eukaryotic Top1 mutants that lack DNA topoisomerase activity can stimulate transcription initiation similarly as the wildtype protein.^{58,59} Thus, it remains to be determined whether Top3 β and other Type IA enzymes

Figure 4. Models for why RNA metabolism may create topological problems that need an RNA topoisomerase to solve. (A)(B) Models to illustrate that Top3β may catalyze catenation or decatenation of 2 mRNA circles, thus enhancing or inhibiting translation (B) or transport of mRNAs (C). It has been shown that mRNA circles can form when 2 ends of mRNA interacting with a common protein. (C) A model illustrates that helical torsions may arise when a translating ribosome or a helicase unwinds a duplex region in an mRNA hairpin. If the hairpin is bound to an immobile mRNP or cellular matrix, the helical torsion will not be relieved by rotation. Such torsion may impede progression of ribosomes or RNA helicases during translation or transport. Top3β can relax the helical torsion through its strand passage activity. (D) Top3β may interconvert circular RNAs to knots or catenane structures, which may affect the function of these RNAs. (E) A linear mRNA may form an intramolecular knot, which may block progression of translating ribosomes. Top3β may catalyze knotting or unknotting of the linear mRNA, and thus affect translation. (F) Two linear mRNAs get entangled with each other, which may interfere with their functions. Top3β may catalyze entangling or segregation of these mRNAs, thus affecting their functions. This figure was adapted from Fig. S7 of a previous publication.¹⁷

function by solving RNA topological problems, or by alternative mechanisms, such as RNA binding or protein-protein interactions.

The other important questions are which mRNAs are bound and regulated by Top3 β ? Does Top3 β recognize some specific sequences or topological structures? Top3 β -knockout mice have shortened life-span.⁶ Identification of Top3 β -regulated mRNAs may shed new light on how the topoisomerases functions in promoting neurodevelopment, preventing mental dysfunction, and maintaining normal life-span.

Can other families of topoisomerases work on RNA? Several Type IB enzymes can catalyze cleavage of ribonucleotides incorporated into DNA.^{60,61} In addition, human Type II enzymes (Top2 α and Top2 β) can cleave DNA containing a single-ribonucleotide substitution.⁶² Moreover, human Type IB and Type II enzymes, Top1 and Top2 α , respectively, have been shown to bind mRNAs in cells.^{40,41} Thus, these enzymes may be potential dual-activity topoisomerases. New assays will be needed to determine if these enzymes can act on RNA.

Disclosure of potential conflicts of interest

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