Regulation of catalytic activity and nucleolar localization of rat DNA topoisomerase IIα through its C-terminal domain

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(Received 25 July 2020, accepted 21 September 2020; J-STAGE Advance published date: 6 February 2021)

Type II DNA topoisomerase (topo II) catalyzes double-stranded DNA cleavage and re-ligation, thus solving problems in DNA topology. Vertebrates have two isozymes (α and β). Recently, the C-terminal regulatory domain (CRD), which regulates catalytic activity and subnuclear localization by associating with RNA, was identified within the C-terminal domain (CTD) of rat topo IIβ. In contrast, it is unclear whether a β CRD-like domain is present in the CTD of topo IIα. In this study, we aimed to identify an RNA-mediated regulatory domain in the rat topo IIα CTD. First, we exchanged the CTDs of rat topo IIα (amino acids 1,192–1,528) and β (1,201–1,614) and examined the two chimeras’ in vitro catalytic activities. Interestingly, the relaxation activities of topo IIα WT enzyme and both of the CTD-swapped mutants were inhibited in the presence of isolated cellular RNA, suggesting that the α CTD is involved in the RNA-mediated regulation of catalytic activity in topo IIα. The results of on-bead assays using a CTD-deleted mutant of rat topo IIα indicated that the RNA-mediated inhibition of the relaxation activity was caused by an interaction between the α CTD and RNA. Further, to identify the domain within the CTD that is associated with subnuclear localization of rat topo IIα, we transiently expressed EGFP-tagged CTD deletion mutants in human cells. The data indicated that the 1,192–1,289 region of rat topo IIα was required for targeting the enzyme to nucleoli. Finally, a relaxation assay using 1–1,289 and Δ1,192–1,289 truncated mutants indicated that the 1,192–1,289 region is involved in RNA-mediated inhibition. These results indicated that the CTD of rat topo IIα, containing the 1,192–1,289 region, is involved in the regulation of catalytic activity by associating with RNA, as well as in the localization to nucleoli in interphase cells.

Key words: C-terminal domain, DNA binding, RNA, subnuclear localization, topoisomerase IIα

INTRODUCTION

DNA topoisomerases are essential enzymes that solve problems caused by DNA metabolic processes, such as transcription, replication and chromosome segregation (Wang, 1996; Champoux, 2001). Type II DNA topoisomerases (topo IIs) act by passing a duplex DNA from the same or a different molecule through a transient double-stranded break generated by the enzymes in DNA. These strand passage reactions are able to promote the catenation and decatenation of circular DNAs or the disentanglement of intertwined chromosomes. In eukaryotes, functional topo IIs form a heart-shaped homodimer, giving rise to a structure that consist of three domains: an N-terminal ATPase domain, a DNA cleavage/catalytic core domain and a C-terminal domain (CTD). The structure of the N-terminal ATPase domain and the DNA cleavage/catalytic core domain have been previously solved by X-ray crystallography (Berger et al.,
nucleoli in interphase cells (Linka et al., 2007). However, the EGFP-tagged human topo II
nucleoli and nucleoplasm (Christensen et al., 2002), while respectively (Supplementary Fig. S1). It has been shown and hydrophobic amino acids, and four lysine residues, posed of a 15-amino acid stretch with alternating lysines containing a Phi-K motif and a K-stretch, which are com-

1991; Capranico et al., 1992). It is known that topo II is also expressed in non-dividing cells (Woessner et al., relatively constant throughout the cell cycle, and topo II ing DNA replication and mitosis (Carpenter and Porter, 2004). In contrast, the expression level of topo IIα is relatively constant throughout the cell cycle, and topo IIβ is also expressed in non-dividing cells (Woessner et al., 1991; Capranico et al., 1992). It is known that topo IIβ regulates the expression of subsets of genes (Tsutsui et al., 2001b; Ju et al., 2006; Lyu et al., 2006; Wong et al., 2009; Trotter et al., 2015). The amino acid sequences of topo IIα and β are highly conserved, except for the CTD. Recently, the amino acid sequence diversity in the CTD was found to recognize post-translational modifications of histones, allowing topo IIα to localize to mitotic chromatin (Lane et al., 2013).

The β CTD is involved in the nuclear dynamics of topo IIs. Analysis using EGFP-tagged human topo IIβ WT and its CTD showed that topo IIβ shuttles between the nucleoplasm and nucleoli, whereas the CTD is localized to nucleoli (Christensen et al., 2002; Linka et al., 2007). Furthermore, the nuclear dynamics of topo IIβ reflects its catalytic activity, and the C-terminal regulatory domain (CRD) is involved in the regulation of catalytic activity and subnuclear localization through its association with RNA (Onoda et al., 2014). The CRD contains a Phi-K motif and a K-stretch, which are composed of a 15-amino acid stretch with alternating lysines and hydrophobic amino acids, and four lysine residues, respectively (Supplementary Fig. S1). It has been shown that EGFP-tagged human topo IIα also shuttles between nucleoli and nucleoplasm (Christensen et al., 2002), while the EGFP-tagged human topo IIα CTD accumulates in nucleoli in interphase cells (Linka et al., 2007). However, it is unclear whether rat topo IIα has an RNA-mediated regulatory domain within its CTD, as in topo IIβ. In the present study, we aimed to identify whether a regulatory domain similar to the topo IIβ CRD exists in the CTD of rat topo IIα. Interestingly, in vitro catalytic assays using rat topo IIα and β CTD-swapped mutants showed that the α CTD inhibits the relaxation activity of the enzyme in an RNA-dependent manner, as does the β CTD. Using deletion mutants of α CTD and a truncation mutant of topo IIα, we identified that the 1,192–1,289 region of α CTD is involved in RNA-mediated nucleolar localization and in the RNA-mediated inhibition of enzyme catalytic activity in vitro. These results suggest that a β CRD-like domain that regulates the catalytic activity of the enzyme and subnuclear localization in interphase cells exists in the N-proximal region (1,192–1,289) of the topo IIα CTD and functions by associating with RNA.

MATERIALS AND METHODS

Plasmids  FLAG-tagged rat topo IIα expression plasmids (WT and ΔCTD) were used as described previously (Kawano et al., 2016). DNA fragments encoding the 1,193–1,238, 1,193–1,289, 1,239–1,528 and 1,290–1,528 regions of rat topo IIα were amplified using the primers listed in Supplementary Table S1. A high-fidelity DNA polymerase, KOD -Plus- (Toyobo), was used throughout. The PCR product was inserted in-frame into the SmaI site of pmCherry-C23. The dual-tagged protein expression vector (FLAG-tag on the N-terminus and EGFP-tag on the C-terminus), pFLAG-CMV-2-EGFP, encoding topo IIα 1–1,191 (ΔCTD) was kindly provided by Dr. K. M. Tsutsui and Dr. K. Tsutsui (Okayama University). DNA fragments were amplified with primers containing restriction overhangs (Smal) listed in Supplementary Table S1. The PCR product was inserted in-frame into the Smal site of pFLAG-CMV-2-EGFP.

The expression vectors for the CTD-swapped mutants were constructed using pFLAG-CMV-2-EGFP, encoding topo IIβ 1–1,199 and topo IIα 1–1,191, and pFLAG-CMV-2, encoding topo IIβ 1–1,199 and topo IIα 1–1,191. DNA fragments were amplified with primers containing the restriction overhangs (Smal) listed in Supplementary Table S1. The PCR product was inserted in-frame into the Smal site of pFLAG-CMV-2-EGFP and pFLAG-CMV-2.

The plasmid pmCherry-C23, which encodes full-length human nucleolar protein C23 (NM_005381) with the mCherry sequence fused at the C-terminus, was constructed. cDNA was synthesized from HEK293 total RNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) with an oligo(dT) primer. C23 cDNA was amplified with primers containing the restric-
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Protein purification  FLAG-tagged rat topo IIα and its truncated mutants were purified by immunoprecipitation and peptide elution (Onoda et al., 2014; Kawano et al., 2016). The cells were washed once with PBS, and then lysed in extraction buffer containing 50 mM HEPES-NaOH (pH 7.4), 0.5 M NaCl, 1 mM EDTA, 1 mM dithiothreitol, 0.1% Nonidet P-40 and 1× protease inhibitor cocktail (PIC, EDTA-free; Roche). After incubation on ice for 30 min, the supernatant was separated by centrifuging at 20,000 × g for 10 min at 4 °C. The supernatant was mixed with FLAG M2 mouse monoclonal antibody (Sigma) immobilized on Dynabeads Protein G (Veritas) and incubated at 4 °C for 2 h. After incubation, the beads were washed three times with extraction buffer. FLAG-tagged proteins were eluted from the beads using elution buffer (150 μg/ml 3× FLAG peptide (Sigma), 50 mM Tris-HCl (pH 8.0), 120 mM KCl, 10 mM MgCl₂, 1 mM dithiothreitol, 0.5 mM EDTA and PIC) and incubated on ice for 30 min. The purified proteins were frozen in liquid nitrogen and stored at −80 °C until further use. For the on-bead assays, after the washing step described above, the beads were suspended in bead storage buffer (50 mM Tris-HCl (pH 8.0), 120 mM KCl, 10 mM MgCl₂, 1 mM dithiothreitol, 0.5 mM EDTA, PIC and 50% glycerol) and stored at −80 °C until further use.

RNA purification  Total RNA was purified from HEK293 cells grown in a 100-mm dish. The cells were washed once with PBS and harvested in 1 ml of TRIzol (Invitrogen). RNA purification was performed according to the manufacturer’s instructions. The purified RNA was treated with RNase-free DNase I (Sigma). After treatment, RNA was further purified by phenol/chloroform extraction and ethanol precipitation. The RNA was quantified using a Qubit RNA HS Assay Kit (Invitrogen).

Relaxation assay  The relaxation assay was performed as described previously (Onoda et al., 2014; Kawano et al., 2016). Purified enzyme and supercoiled pUC18 DNA were mixed in 10 μl of relaxation buffer (50 mM Tris-HCl (pH 8.0), 120 mM KCl, 10 mM MgCl₂, 0.5 mM EDTA, 1 mM dithiothreitol, 0.5 mM ATP and 30 μg/ml bovine serum albumin) and incubated at 30 °C for 30 min. The reaction was stopped by adding SDS and proteinase K (Roche). After incubation at 55 °C for 1 h, the samples were subjected to 1% agarose gel electrophoresis. DNA was detected by staining with GelRed Nucleic Acid Gel Stain (Biotium). Images were captured using a MediCap USB170 (MEDI CAPTURE) equipped with a CCD camera or FAS-V (NIPPON Genetics).

Decatenation assay  The decatenation assay was performed as described in the relaxation assay, except that kinetoplast DNA (Inspiralis) was used as the substrate. The deproteinized samples were separated on a 1% agarose gel containing GelRed Nucleic Acid Gel Stain (Biotium). Images were captured as described above.

Catenation assay  The catenation assay was performed as described previously (Kawano et al., 2016). Histone H1.0 (New England Biolabs) was used as a DNA condensing agent. To condense DNA with histone H1.0, pre-incubation was performed with histone H1.0 and 50 ng of supercoiled pUC18 in relaxation buffer at 30 °C for 5 min. The reaction was started by adding 100 fmol of purified FLAG-tagged protein. After incubation at 30 °C for 30 min, the reaction was terminated by adding SDS and proteinase K. The samples were subjected to 1% agarose gel electrophoresis. DNA bands were detected by GelRed Nucleic Acid Gel Stain (Biotium). The catenation assay using polyethylene glycol was performed as described above (Kawano et al., 2016).

On-bead relaxation assay  The on-bead relaxation assay was performed as described previously (Onoda et al., 2014), using protein immobilized on the beads. The proteins (100 fmol) were mixed with HEK293 total RNA in 10 μl of relaxation buffer and incubated at 30 °C for 5 min. After incubation, the reaction was started by adding 10 ng of pUC18 and incubated at 30 °C for 30 min. The reaction was stopped by adding SDS and proteinase K. The bound and unbound fractions were separated before stopping the reaction. The samples were subjected to 1% agarose gel electrophoresis, and the DNA was detected by staining with GelRed Nucleic Acid Gel Stain (Biotium).
On-bead RNA-binding assay The on-bead RNA-binding assay was performed as described previously (Onoda et al., 2014). The proteins immobilized on magnetic beads were mixed with HEK293 total RNA in 10 μl of binding buffer (50 mM Tris-HCl (pH 8.0), 120 mM KCl, 10 mM MgCl₂, 0.5 mM EDTA, 1 mM dithiothreitol and 30 μg/ml bovine serum albumin) and incubated at 30 °C for 30 min with occasional mixing. After incubation, the bound fraction was separated by magnetic separation. The bound RNA was directly quantified using RiboGreen (Invitrogen).

On-bead DNA-binding assay The on-bead DNA-binding assay was performed as described previously (Onoda et al., 2014; Kawano et al., 2016). FLAG- and EGFP-tagged proteins immobilized on Dynabeads Protein G (Veritas) through FLAG M2 mouse monoclonal antibody (Sigma) were mixed with 5 ng of supercoiled pUC18 in 10 μl of binding buffer and incubated at 30 °C for 30 min. After incubation, the bound and unbound fractions were separated by magnetic separation, and treated with SDS and proteinase K. The samples were separated on a 1% agarose gel containing GelGreen Nucleic Acid Gel Stain (Biotium). Images were captured using the FAS-V system (NIPPON Genetics). Band densitometry was performed with ImageJ software.

Fluorescence microscopy HEK293 cells were grown on a 35-mm glass bottom dish (MATSUNAMI) and transfected with FLAG- and EGFP-tagged rat topo IIα expression vectors. An Axiovert 135 TV (Zeiss) equipped with a SOLA light engine (Lumencor) was used for observation. The images were captured using a microscope digital camera (FLOYD, Wraymer). An Olympus FV3000 (Okayama University of Science) was used for confocal imaging.

RESULTS

Nuclear localization pattern and enzymatic activity of CTD-swapped mutants of topo IIα and β We first analyzed the effect of CTD swapping between rat topo IIα and β on the subnuclear localization of the enzymes. In this study, we defined the 1,192–1,528 region of rat topo IIα and the 1,201–1,614 region of rat topo IIβ as CTDs (Fig. 1A) (Onoda et al., 2014; Kawano et al., 2016). Dual FLAG- and EGFP-tagged proteins were transiently expressed in HEK293 cells, and their localization patterns were then observed. The WT protein of rat topo IIα and β localized to both the nucleoplasm and nucleoli (Fig. 1B), consistent with previous studies (Christensen et al., 2002; Linka et al., 2007; Onoda et al., 2014). Both of the CTD-swapped mutants (α/β CTD and β/α CTD; see Fig. 1A) also localized to the nucleoplasm and nucleoli, suggesting that CTD swapping between α and β does not affect subnuclear localization in interphase cells. The results were consistent with a previous study using human enzymes (Linka et al., 2007), although the definition of the regions of CTD differed.

Next, we analyzed the effect of CTD swapping on in vitro catalytic activities (relaxation, decatenation and catenation activities). In the relaxation assay, topo II relaxes supercoiled DNA, and the relaxed DNA bands are detected as a DNA ladder by agarose gel electrophoresis. We used a 2-fold serial dilution of the enzyme for this assay to compare the activities between WT and CTD-swapped enzymes. In topo IIα, similar DNA ladder patterns were observed between WT and α/β CTD (Fig. 1C). Similarly, the βα CTD showed almost the same ladder pattern as the topo IIβ WT enzyme. These data indicated that CTD swapping between topo IIα and β has little to no effect on the in vitro relaxation activity. In the decatenation assay, topo II decatenates a mini circle (arrows in Fig. 1D) from a catenated DNA, such as a kinetoplast DNA. We used a 2-fold serial dilution of the enzyme and obtained results similar to the relaxation assay. The decatenation activity was not significantly different between the WT enzymes and the CTD-swapped mutants (Fig. 1D). These results suggest that the in vitro decatenation activity of rat topo IIα and β was not affected by the CTD swapping. In the catenation assay, topo II produces DNA catenanes from circular plasmid DNA in the presence of a DNA aggregation factor, such as histone H1.0 (Fortune and Osheroff, 2001; Kawano et al., 2016). DNA catenanes were observed as slow-migrating higher-molecular-weight DNA on the gel (bracket in Fig. 1E). When we used histone H1.0 as a DNA aggregation factor, WT topo IIα produced DNA catenanes, consistent with a previous study (Kawano et al., 2016). WT topo IIβ also produced DNA catenanes (Fig. 1E). Both of the CTD-swapped mutants, α/β CTD and β/α CTD, produced DNA catenanes, indicating that in vitro catenation activity is not affected by the CTD swapping of the enzymes.

Furthermore, we examined whether cellular RNA represses the in vitro relaxation activity of rat topo IIα. It has been reported that the in vitro relaxation activity of human topo IIα is inhibited by synthetic polyribonucleotide (Park et al., 2008), while that of rat topo IIβ is inhibited by cellular RNA (Kawano et al., 2010; Onoda et al., 2014). Therefore, we speculated that the relaxation activity of rat topo IIα would be inhibited by cellular RNA. We performed the relaxation assay in the presence of an increasing amount of HEK293 total RNA. In both WT topo IIα and β, the relaxed bands decreased in an RNA dose-dependent manner (Fig. 1F). Interestingly, the relaxation activity of both of the CTD-swapped mutants α/β CTD and β/α CTD was also inhibited in the presence of RNA. These results suggest
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Fig. 1. Subnuclear localization pattern and in vitro catalytic activity of the CTD-swapped mutants of rat topo IIα and β. (A) Schematic indicating the position of CTD in rat topo IIα and β. (B) Live images of FLAG- and EGFP-tagged rat topo IIα and β and the CTD-swapped mutants (α/β CTD and β/α CTD). Scale bar, 5 μm. DIC: differential interference contrast image. (C) A relaxation assay was carried out using 2-fold serial dilutions of the enzyme (2^0 = 100 fmol). The proteins were mixed with 50 ng of pUC18 and incubated at 30 °C for 30 min. The reaction was terminated by adding SDS and proteinase K. Deproteinized samples were subjected to 1% agarose gel electrophoresis. DNA bands were detected by GelRed Nucleic Acid Gel Stain. (D) A decatenation assay was carried out as described in (A), except for the use of kinetoplast DNA as a substrate. The samples were electrophoresed in a 1% agarose gel containing GelRed Nucleic Acid Gel Stain. Arrows indicate decatenated mini circles. CC: closed circular DNA. OC: open circular DNA. (E) A catenation assay was performed in the presence of histone H1.0 (1, 2 and 4 μg/ml). Histone H1.0 and 50 ng of pUC18 were mixed and incubated at 30 °C for 5 min. The reaction was started by adding 100 fmol of topo II. After incubation for 30 min, the reaction was terminated by adding SDS and proteinase K. The samples were subjected to 1% agarose gel electrophoresis. DNA bands were detected by GelRed Nucleic Acid Gel Stain. Lanes marked '-' lack H1.0. Brackets indicate catenanes. (F) A relaxation assay was performed in the presence of HEK293 total RNA (10, 100 and 1000 ng). Except for the use of 10 ng of pUC18 as a substrate, the assay was carried out as described in (C). S: substrate. I: supercoiled DNA. II: nicked circular DNA.
that the CTD of rat topo IIα inhibits relaxation activity by associating with RNA, as well as the β CTD. Taken together, our findings suggested that the CTDs of topo IIα and β play similar roles in regulating subnuclear localization in interphase cells and catalytic activities.

The CTD of rat topo IIα regulates enzymatic activity through RNA binding

To determine how RNA inhibits the relaxation activity of rat topo IIα, we performed on-bead assays. To this end, we used the FLAG-tagged rat topo IIα WT enzyme and its CTD-deleted mutant ΔCTD (Fig. 2A). The relaxation activity of rat topo IIα WT was inhibited in the presence of RNA, while that of ΔCTD was not inhibited under the same condition (Fig. 2B), suggesting that the CTD of the enzyme is involved in the RNA-mediated inhibition of relaxation activity. To determine whether RNA binds to the WT enzyme and ΔCTD, we performed an on-bead RNA binding assay (Fig. 2C). Beads immobilized with FLAG M2 mouse monoclonal antibody were used as a negative control (Protein (−)). For both WT and ΔCTD, the amount of bound RNA increased depending on the amount of input RNA (Fig. 2C). Relative to the negative control, there was about a 2-fold difference between WT and ΔCTD at the plateaux of the bound RNA (input RNA = 50 ng), suggesting that the RNA binds to both the catalytic core region (N-terminal domain and cleavage/catalytic core domain) and the CTD.

Taking advantage of the on-bead assay, we separated the bound and unbound fractions immediately after the relaxation reaction, as indicated in Fig. 2B (Fig. 2D). In WT, the unreacted supercoiled DNAs increased in the unbound fractions in an RNA dose-dependent manner (Fig. 2D). In contrast, the DNA ladder patterns in both the bound and unbound fractions of ΔCTD were not affected by RNA. These results are consistent with those of a previous study using the WT and ΔCTD of rat topo IIβ (Onoda et al., 2014). Therefore, as with topo IIβ, it was considered that RNA affected the initial DNA-binding step of WT topo IIα before the enzyme entered the catalytic cycle. To determine whether RNA represses the initial DNA-binding step of rat topo IIα, we performed an on-bead DNA-binding assay in the absence of ATP, without which the enzyme behaves as a simple DNA-binding protein. In WT, bound DNA was markedly reduced in an RNA dose-dependent manner (Fig. 2E). In contrast, the ΔCTD showed only a slight reduction in the amount of bound DNA. These results suggest that RNA affected the initial DNA-binding step of rat topo IIα in the full-length WT enzyme, resulting in the inhibition of the relaxation activity of the enzyme.

The 1,192–1,289 region is involved in the subnuclear localization of rat topo IIα in interphase cells

Live images of EGFP-tagged human topo IIα and β have previously shown that both enzymes shuttle between the nucleoplasm and nucleoli in interphase cells (Christensen et al., 2002). Furthermore, the EGFP-tagged CTD of both topo IIα and β localizes to the nucleoli in interphase cells (Linka et al., 2007; Onoda et al., 2014). In the case of topo IIβ, the CRD in the N-terminal region
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(1,201–1,250) of the CTD is involved in its localization to nucleoli by associating with nucleolar RNA (Onoda et al., 2014). A characteristic feature of the β CRD is that lysine residues are enriched. The α CTD also has several lysine clusters in its N-terminal region (Supplementary Fig. S1). Therefore, we speculated that part of the CTD is involved in nucleolar localization in interphase cells. To determine which region is required for the nucleolar localization of rat topo IIα, we used deletion mutants of the topo IIα CTD (Fig. 3A) and observed their localization pattern. We co-expressed C23-mCherry as a nucleolar marker protein. The full-length topo IIα CTD was found to localize to the nucleoli, consistent with a previous study (Linka et al., 2007). First, we observed the localization pattern of the 1,192–1,238 and 1,239–1,528 regions (Fig. 3B), because the amino acid sequence and the positions of positively-charged amino acids in the 1,192–1,238 region of rat topo IIα are similar to those in the CRD of rat topo IIβ (Supplementary Fig. S1). The 1,192–1,238 region was localized to both the nucleus and cytoplasm. The 1,239–1,528 region localized to the nucleus. Nucleolar localization was observed in both the 1,192–1,238 and 1,239–1,528 regions, indicating that they both contain a sequence of amino acids involved in nucleolar localization. Next, we observed the localization pattern of the 1,192–1,289 and 1,290–1,528 regions. The nuclear localization pattern of 1,192–1,289 was just like that of the CTD. In contrast, 1,290–1,528

![Fig. 3. The CTD of rat topo IIα affects subnuclear localization of the enzyme.](image-url)

(A) The truncated mutants (Δ1,192–1,238 and Δ1,192–1,289) and CTD deletion mutants of rat topo IIα used in this study. (B) Live images of the CTD deletion mutants of rat topo IIα. C23-mCherry was co-transfected as a marker protein of the nucleoli. Scale bar, 5 μm. (C) Live images of WT and CTD truncated mutants. C23-mCherry was co-transfected as a marker protein of nucleoli. Scale bar, 5 μm. (D) HEK293 cells were permeabilized with 50 μg/ml digitonin and treated with 400 μg/ml RNase A for 20 min. Live images were captured by confocal microscopy. Scale bar, 5 μm. DIC: differential interference contrast image.
did not show any clear nucleolar localization. Therefore, we speculated that the 1,192–1,289 region of rat topo IIα is required for the localization of the enzyme to the nucleoli. We further examined whether the 1,192–1,289 region is involved in the nucleolar localization of rat topo IIα using 1,192–1,238 and 1,192–1,289 truncated mutants (Fig. 3C). While Δ1,192–1,238 was found to localize to the nucleoli, Δ1,192–1,289 did not. Therefore, we concluded that the 1,192–1,289 region of rat topo IIα is involved in the nucleolar localization of the enzyme in interphase cells.

In the case of rat topo IIβ, the association between the CRD and nucleolar RNA is involved in the nucleolar localization of the enzyme in interphase (Onoda et al., 2014). Thus, we examined whether an association between the 1,192–1,289 region and nucleolar RNA contributes to nucleolar localization. We used digitonin to permeabilize the cells, and then treated them with RNase A to degrade the nuclear RNA, as described by Onoda et al. (2014). Both WT and 1,192–1,289 were found to localize to the nucleoli without RNase A treatment (Digitonin in Fig. 3D). However, the nucleolar localization of WT and 1,192–1,289 disappeared after treatment with RNase A (Digitonin + RNase A in Fig. 3D). These results suggest that at least RNA is needed to localize rat topo IIα to nucleoli in interphase cells.

**The 1,192–1,289 region of rat topo IIα regulates catalytic activity in the presence of RNA**

It has been shown that the β CRD is involved in the regulation of both nucleolar localization and RNA-mediated catalytic activity inhibition (Onoda et al., 2014). Therefore, we considered that the 1,192–1,289 region of rat topo IIα, which is required for subnuclear localization of the enzyme, might also contribute to the regulation of RNA-mediated catalytic activity inhibition, as in the β CRD. To test this idea, we performed a relaxation assay using FLAG-tagged truncated and CTD-deleted mutants (Fig. 4A) in the presence of an increasing amount of HEK293 total RNA. The relaxation activity of ΔCTD was not inhibited by RNA (Fig. 4B), consistent with the results of the on-bead relaxation assay (Fig. 2B). Whereas the relaxation activity of 1–1,289 was inhibited by RNA, that of Δ1,192–1,289 was not inhibited. These results suggest that the 1,192–1,289 region of rat topo IIα is involved in the regulation of the RNA-mediated catalytic activity inhibition of the enzyme.

Figure 1F shows that the relaxation activities of the CTD-swapped mutants of rat topo IIα and β (α/β CTD and β/α CTD) were inhibited in the presence of RNA. These results suggest that the β CRD affects the catalytic activity of topo IIα by associating with RNA. Since the 1,192–1,289 region of rat topo IIα inhibited the relaxation activity of the enzyme in the presence of RNA (Fig. 4B), we speculated that the 1,192–1,289 region had affected the catalytic activity of topo IIβ in the assay using the β/α CTD (Fig. 1F). To determine whether the CRD of rat topo IIβ or the 1,192–1,289 region of rat topo IIα affects the RNA-mediated inhibition of the relaxation activity of the CTD-swapped mutants (α/β CTD and β/α CTD), we used the mutants indicated in Fig. 4C (α/β1,251–1,614 and β/α1,290–1,528). As expected, the relaxation activities of both α/β1,251–1,614 and β/α1,290–1,528 were not inhibited in the presence of RNA (Fig. 4D). Therefore, in the RNA-mediated inhibition of the relaxation activity of rat topo IIα and β, the N-proximal region of the CTD is involved in the regulation of catalytic activity.

**The 1,192–1,289 region of rat topo IIα contributes to efficient in vitro catenation**

The function of the 1,192–1,289 region of rat topo IIα was further examined. We previously reported that the linear DNA-
binding activity of the topo IIα CTD contributes to efficient in vitro catenation (Kawano et al., 2016). To determine whether the 1,192–1,289 region exerts linear DNA-binding activity, we performed an on-bead DNA-binding assay using dual FLAG- and EGFP-tagged proteins (Fig. 3A). As a negative control we used FLAG-tagged EGFP, which binds very little linear DNA (Fig. 5A). The CTD was found to bind to linear DNA, consistent with a previous study (Kawano et al., 2016). The 1,192–1,289 region also bound to linear DNA. In contrast, the 1,290–1,528 region hardly bound to linear DNA. These results indicate that the 1,192–1,289 region exerts linear DNA-binding activity in the CTD of rat topo IIα, contributing to the formation of catenanes in vitro. We performed a catenation assay using the FLAG-tagged truncated and CTD-deleted mutants of the enzyme (Fig. 4A). As shown in Fig. 1E, WT produced DNA catenanes in the presence of histone H1.0 (Fig. 5B). In contrast, the mutant lacking the entire CTD (ΔCTD) produced only small amounts of DNA catenanes, consistent with a previous study (Kawano et al., 2016). While the 1–1,289 region produced DNA catenanes comparably to the WT enzyme, the catenation efficiency was reduced in Δ1,192–1,289. These results indicate that the linear DNA-binding activity of the 1,192–1,289 region contributes to the efficient formation of DNA catenanes. Previously, we have shown that the ΔCTD mutant of topo IIα, which could not produce DNA catenanes efficiently in the presence of histone H1.0, is able to produce DNA catenanes in the presence of polyethylene glycol (PEG) (Kawano et al., 2016). Under these conditions, since PEG increases the local concentration of enzymes at DNA, Δ1,192–1,289 may be able to produce the DNA catenanes, despite lacking linear DNA-binding activity. As expected, the WT and all of the mutants produced DNA catenanes (Fig. 5C). These results suggest that the DNA-binding activity of the 1,192–1,289 region of rat topo IIα concentrates the enzyme in the vicinity of condensed DNA and allows the enzyme to form DNA catenanes.

**DISCUSSION**

It has been suggested that topo IIα and β arose by gene duplication (Sng et al., 1999), and have evolved to exert isozyme-specific functions. Recently, the CTD of each isozyme was found to be involved in the exertion of isozyme-specific functions, as the conservation of the amino acid sequences between topo IIα and β is very low. In this study, we demonstrated that the 1,192–1,289 region of rat topo IIα contributes to the nucleolar localization of the enzyme in interphase cells, thus affecting its in vitro catalytic activity, as in the β CRD. Our findings suggest that common functions shared between the two isozymes exist in a part of each CTD.

We used the CTD-swapped mutants of rat topo IIα and β (α/βCTD and β/αCTD) to determine whether the CTD affects the in vitro catalytic activities of the α and β catalytic cores, consisting of N-terminal and catalytic core domains (Fig. 1). The CTD-swapped mutants
showed similar activities to those of the WT enzymes in these assays. In the relaxation assay, a previous study found that clusters of positively charged amino acid residues in the CTD of human topo IIα were required for the recognition of supercoil geometry, as well as to allow the enzyme to preferentially relax positively supercoiled DNA (McClendon et al., 2008). Interestingly, whereas the human α/βCTD showed similar relaxation activity against both positively and negatively supercoiled DNA, the human βαCTD preferentially relaxed positively supercoiled DNA, despite the fact that the WT of human topo IIβ does not preferentially relax positively supercoiled DNA. We did not observe any differences between the α and β CTDs, as we only used negatively supercoiled DNA as a substrate. However, we were able to demonstrate that the CTDs of topo IIα and β acted in a similar manner to relax negatively supercoiled DNA. In their study, Dickey and Osheroff (2005) found that the decatenation activity of human topo IIα was significantly reduced after the removal of its CTD. Consistent with this, our rat topo IIα ΔCTD mutant showed similar results (Supplementary Fig. S2). Surprisingly, the rat topo IIα/β CTD showed a similar decatenation activity to the topo IIα WT enzyme (Fig. 1D), indicating that the decatenation activity of the α ΔCTD mutant was complemented by the β CTD. Although the influence of the α CTD on the decatenation activity of topo IIα is unclear, it was clarified that the β CTD acts in a similar manner to the α CTD in the decatenation reaction. In the catenation assay, the DNA-binding activity of the CTD contributes to the efficient formation of catenanes (Kawano et al., 2016). Further, the β CTD was also found to complement the catenation activity of the α ΔCTD mutant.

It has been previously shown that the CTDs of yeast and human topo II are dispensable for the basic catalytic activity of the enzymes (Shiozaki and Yanagida, 1991; Jensen et al., 1996). On the other hand, since the nuclear localization signals of eukaryotic topo II exist in the CTD (Caron et al., 1994; Jensen et al., 1996; Mirski et al., 1999), the CTD is an indispensable region for the exertion of the in vivo functions of the enzymes. Nuclear localization signals consist of positively charged amino acids, namely lysine and arginine (Dingwall and Laskey, 1991). Thus, clusters of positively charged amino acid residues are found in the CTD of eukaryotic topo II. As described above, the clusters of positively charged amino acid residues in the CTD of human topo IIα are thought to be required for the enzyme to distinguish supercoil geometry during relaxation (McClendon et al., 2008). Interestingly, McClendon et al. found that a chimera in which the C-terminal 30 amino acids of human α CTD (1,502–1,531) were replaced with the corresponding region of the human β CTD (1,585–1,621) was unable to complement the specific recognition of supercoil geometry. In addition, the extreme C-terminal 31 residues of the human topo IIα CTD, termed the chromatin tether domain, was recently found to control the dynamics of topo IIα on mitotic chromosomes, and to be necessary for the formation of mitotic chromosomes (Lane et al., 2013). These findings suggest that the N-distal region of the topo IIα CTD is involved in isozyme-specific functions. On the other hand, we demonstrated that the N-proximal region of the rat topo IIα CTD regulates RNA-mediated in vitro catalytic activity and interphase nucleolar localization. These functions are very similar to those of the β CRD, located in the N-proximal region of its CTD, suggesting that the N-proximal region of both CTDs has a common role in the regulation of the enzymes. Thus, we speculate that the functions of the N-proximal CTD have been conserved between topo IIα and β during their divergence after gene duplication. However, it is not known whether the N-proximal CTD in the topo IIIs of other eu- karyotes has similar functions to mammalian topo II iso-zymes, indicated in this study. Therefore, it is unclear whether the functions of the N-proximal CTD that we have identified are conserved widely in eukaryotic topo IIIs or are limited to mammalian topo II isozymes.

Topo IIβ is an essential enzyme for gene regulation in the final stage of neuronal differentiation, when the expression levels of this enzyme are high (Tsutsui et al., 2001b; Lyu et al., 2006; Sano et al., 2008; Tiwari et al., 2012). The active enzyme mainly localizes to the nucleoplasm during neuronal differentiation. In contrast, in mature cells, topo IIβ is concentrated in the nucleoli and is maintained in an inactive state (Tsutsui et al., 2001a, 2001b). The CRD, comprising the N-proximal 50 amino acids in the CTD of rat topo IIβ, is involved in the nucleolar localization and repression of the catalytic activity of the enzyme (Onoda et al., 2014). In the present study, we identified a domain (1,192–1,289) corresponding to the β CRD in the N-proximal region in the CTD of rat topo IIα. EGFP-tagged 1,192–1,289 localized to the nucleoli and relocated to the nucleoplasm after RNase A treatment, suggesting that topo IIα is retained in the nucleoli through an association with RNA or RNA-binding proteins. It was previously shown that topo IIα in the salt-insoluble subnuclear fraction is mostly catalytically inert (Meyer et al., 1997). In interphase cells (from early G1 phase to G2 phase), salt-insoluble topo IIα was found to localize to nucleoli (Agostinho et al., 2004). Agostinho et al. further showed that catalytically active human topo IIα concentrates at replicating heterochromatin in late S phase in a replication-dependent manner and at centric heterochromatin during G2 phase. In other words, a proportion of topo IIα localizes to the nucleoli and is maintained in an inactive state by associating with nucleolar RNA during interphase. Our results suggest that the N-proximal CTD of topo IIα may contribute to maintaining some of the enzyme in an inactive state by associating with RNA in the nucleoli in interphase cells.
The β CRD contains two motifs, the Phi-K and the K-stretch, both of which are required for the RNA-mediated nucleolar localization of rat topo IIβ (Onoda et al., 2014). The 1,192–1,289 region of rat topo IIα has short Phi-K and K-stretch motifs in regions 1,193–1,202 and 1,287–1,289, respectively (Supplementary Fig. S1). The nuclear localization pattern of the EGFP-tagged 1,192–1,289 and 1,239–1,528 regions was more diffuse than that of region 1,192–1,289 and the CTD (Fig. 3B). In addition, the nuclear localization pattern of the 1,192–1,289 region was more diffuse than that of the WT (Fig. 3C). Therefore, similar to rat topo IIβ, both the short Phi-K and K-stretch motifs in the 1,192–1,289 region of rat topo IIα may be involved in the nucleolar localization of the enzyme. Moreover, the 1,192–1,289 region contributes to RNA-mediated repression of relaxation activity (Fig. 4B). Because the degree of repression of the relaxation activity of the 1–1,238 and 1,192–1,238 regions by RNA was lower than that of the WT and of the 1,192–1,289 region (Supplementary Fig. S3), the short Phi-K and K-stretch motifs in the 1,192–1,289 region of rat topo IIα may contribute to regulation of the catalytic activity of the enzyme.

In summary, the CTD of rat topo IIα is involved in the regulation of the catalytic activity of the enzyme, as well as in subnuclear localization. The 1,192–1,289 region in the CTD is required for the RNA-mediated inhibition of the in vitro catalytic activity, and it is also necessary for targeting the enzyme to the nucleoli in interphase cells. This region of topo IIα has similar functions to the rat topo IIβ CRD, suggesting that functionality in the C-terminal region is conserved between the two isoforms.

This work was supported in part by the Wescow Scientific Promotion Foundation. We are thankful to K. M. Tsutsui and K. Tsutsui (Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan) for providing rat topo IIα expression vectors and to Ami Hamada for her assistance in the cDNA cloning of human C23 and the construction of the mCherry-tagged C23 expression vector.

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| Vector          | Target | Primer | Sequence                  | Restriction site |
|-----------------|--------|--------|---------------------------|-----------------|
|                 |        |        | (Restriction site is underlined) |                  |
| pFLAG-CMV-2     | topo IIα | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        |        |                           |                 |
|                 |        | Antisense | TCCCCCGGGTATTTTTTTTCCCTTTTTTGT | Smal            |
| topo IIα        |        | Sense  | TCCCCCGGGGAACCTTTTGTCTTGATTCTGA | Smal            |
|                 |        |        |                           |                 |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
| topo IIβ        |        | Sense  | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
|                 |        | Antisense | TCCCCCGGGGAAGGGGTGAAAGCGAA | SmaI            |
Supplementary Fig. S1.  Amino acid sequences of the CTD of rat topo IIβ (1,201–1,614) and α (1,193–1,528). Lysine (K) and arginine (R) are highlighted in blue. NLSs are demarcated by orange bars. A bracket indicates the CRD in rat topo IIβ. Boxes indicate the Phi-K motif and K-stretch. Points of C-terminal truncations in rat topo IIα mutant enzymes are indicated.

**Rat topo IIβ**

| Phi-K motif | K-stretch | CRD |
|-------------|-----------|-----|
| 1201 | ELPQGGRVQGLC QLEE TMPSPYCORAI VPEITAMKAD ASRKLKQPP GDPTTVVKV | 1260 |
| 1261 | EFDEEFSGTP AEOTGEELT PSAVNMKFGK HKREKKKPEGK RVRKTPASTG RPIKTVVKKR | 1320 |
| 1321 | NPHSDDEESKS ESDEEAEPV VIPRDSLARR AAEERPQYTF DFSEEEEDDA DDDDDNNDLE | 1380 |
| 1381 | ELKVKASPI NTGDGEFVPS DGIDKDEYAF SPGSKATPE KSSHQKEKQD FGNLFSPPSY | 1440 |
| 1441 | SQKSEDSDKS FDHNSEEDTS VFAPSGFLKQ TDKVPSQTVA AEGKAKPSDA AAKPARKAPR | 1500 |
| 1501 | QRVVEPANS DSSESGSNIF RRTAAAPKRG ROQKRRKASSG SENEKDYNPG RPSKTKASKR | 1560 |
| 1561 | PKKTSFDQOS DVDIFPSDFT SIEPALFTG RARKEVYFA ESDEEEDVDF AMPF | 1614 |

**Rat topo IIα**

| Phi-K motif | K-stretch |
|-------------|-----------|
| 1192 | SISEVLPS PVKRVIPQV TMEMRAEAER KIRKIKSEN VEGTPAEDGA | 1251 |
| 1252 | RPGLQRORE KRCKKEPST AKPTTTLPEK PIGK | 1238 |
| 1292 | WDDSESMDSS NESNFDVPPR | 1331 |
| 1312 | EKFRPIAAATK AKPTADLDD DDFSGLEKED EDEDFPLDDE TPPRTEHPKP NTKKALKQK | 1371 |
| 1372 | STSVDELESD GKDSSPASPG ASAADVAPET EPSKPSKSOT VGVKRTITAG KSSLTSTAGTK | 1431 |
| 1432 | KRVPKETKS DSALNARVSE KPAKAKAGS RRKPSSSDS SDEFKKAIS KGATKPPKG | 1491 |
| 1492 | EEOPHVDDL DTVAPRAKSG RARKPIYLE ESDDDLF | 1528 |
Supplementary Fig. S2. Decatenation activity of rat topo IIα WT and ΔCTD. The decatenation assay was performed as described in Fig. 1D in the main text. Arrows indicate decatenated mini circles. S: substrate. CC: closed circular DNA. OC: open circular DNA.
Supplementary Fig. S3. Relaxation activity of rat topo IIα 1–1,238 and Δ1,192–1,238 regions in the presence of RNA. (A) The domain structure of rat topo IIα and FLAG-tagged proteins are indicated in the scheme. (B) The relaxation assay in the presence of HEK293 total RNA was performed as described in Fig. 4B in the main text. S: substrate, I: supercoiled DNA. II: nicked circular DNA.