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Short communication

Mapping of the Interaction Domain of DNA Topoisomerase IIα and IIβ with Extracellular Signal-Regulated Kinase 2

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Both topoisomerase IIα and IIβ exist as phosphoproteins in the cells. Recently it was reported that DNA topoisomerase IIα associates with and is phosphorylated by the extracellular signal-regulated kinase 2 (ERK2). Also, ERK2 stimulates the activity of topoisomerase II by a phosphorylation-independent manner [Shapiro *et al.*, (1999) Mol. Cell. Biol. 19, 3551-3560]. In this study, a yeast two-hybrid system was used to investigate the binding site between topoisomerase IIα or IIβ and ERK2. The two-hybrid test clearly showed that topoisomerase IIβ residues 1099-1263, and topoisomerase IIα residues 1078-1182, mediate the interaction with ERK2, and that the leucine zipper motifs of topoisomerase IIα and IIβ are not required for its physical binding to ERK2. Our results suggest that topoisomerase IIβ residues 1099-1263, and topoisomerase IIα residues 1078-1182, may be common binding sites for activator proteins.

Keywords: DNA topoisomerase II, Extracellular signal-regulated kinase 2, Interaction domain, Yeast two-hybrid system.

Introduction

DNA topoisomerase II is an essential nuclear enzyme, which regulates the various topological conformations of DNA (Watt and Hickson, 1994; Wang, 1996). The enzyme plays a major role in the segregation of sister chromatids during both mitosis and meiosis, and relieving DNA supercoils generated during DNA replication and transcription. In addition, this enzyme is a prominent component of both the interphase nuclear matrix and the mitotic chromosomal scaffold (Earnshaw *et al.*, 1985; Gasser and Laemmli, 1986; Brill and Sternglanz, 1988; Rose *et al.*, 1990; Adachi *et al.*, 1991). In cells from vertebrate species, topoisomerase II exists as two isoforms; the 170-kDa topoisomerase IIα and the 180-kDa topoisomerase IIβ (Chung *et al.*, 1989; Jenkins *et al.*, 1992; Tan *et al.*, 1992). Despite there being a strong sequence homology between the two proteins, they have quite different biochemical and pharmacological properties (Drake *et al.*, 1989; Chang, 1998). Topoisomerase IIα is expressed at a high level during the period immediately prior to cell division and is associated with condensed chromatin during metaphase (Hsiang *et al.*, 1988). In contrast, topoisomerase IIβ is excluded from the chromosomes around the period of chromosome condensation. The expression of topoisomerase IIα is restricted to the proliferative compartment of the tissue, whereas topoisomerase IIβ is expressed ubiquitously in human tissue (Swedlow *et al.*, 1993).

Both topoisomerase IIα and IIβ are phosphorylated throughout the cell cycle, but become more highly phosphorylated during the G_2 and M phases of the cell cycle (Heck *et al.*, 1989; Kroll and Rowe, 1991; Burden and Sullivan, 1994; Ishida *et al.*, 1996; Kimura *et al.*, 1996). Several protein kinases, including protein kinase CKII (CKII), protein kinase C (PKC), and CDC2 kinase, have been shown to phosphorylate topoisomerase II (Ackerman *et al.*, 1985; Sahyoun *et al.*, 1986; Corbett *et al.*, 1992; Wells and Hickson, 1995). Recently, it was shown that topoisomerase IIα associates with and is phosphorylated by the extracellular signal-regulated kinase 2 (ERK2). ERK2 stimulates the activity of topoisomerase IIα by a phosphorylation-independent manner (Shapiro *et al.*, 1999). ERK2 is one of the mitogen-activated protein (MAP) kinases and has an essential role in promoting S phase entry through the phosphorylation of nuclear transcription factors (Lewis *et al.*, 1998; Park *et al.*, 1998).

In this study, we used a yeast two-hybrid system to map the interaction sites of human topoisomerase IIα and IIβ with ERK2. The present results indicate that topoisomerase IIβ residues 1099-1263, and topoisomerase IIα residues 1078-1182, can bind to ERK2. Also, the leucine zipper motifs of topoisomerase IIα and IIβ are not required for its physical binding to ERK2.

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Materials and methods

Materials The transformation recipient for all plasmid constructions was *E. coli* DH5α. *S. cerevisiae* HF7c (*MATa ura3-52 his3-200 ade2-101 lys2-801 trpl-901 leu2-3,112 gal4-542 gal80-538 LYS2::GAL1*$_{UAS}$*-GAL1*$_{TATA}$*-HIS3 URA3:: GAL4*$_{17mers(x3)}$*-CyC1*$_{TATA}$*-lacZ*) was used for the yeast two-hybrid system. Yeast cultures were grown in either a YPAD media (1% yeast extract, 2% peptone, 0.004% adenine sulfate, 2% glucose), or a synthetic minimal media with appropriate supplements. The hybrid plasmids pGBT9 and pGADGH containing different regions of human topoisomerase IIα or IIβ have been previously described (Park *et al.*, 2001).

Plasmid constructions In order to insert the complete open reading frame of human ERK2 into the vectors pGBT9 and pGADGH, the entire coding sequence of ERK2 was PCR amplified using the following sequences: 5' primer, 5'-GGC CCCGGGTATGGCGGCGGCGGCGGC-3' and 3' primer, 5'-CCTGGTCGACTTAAGATCTGTATCCTGGCTG-3'. The *Sma*I and *Sal*I sites are underlined. The PCR incubations were carried out by 25 cycles of denaturation at 95°C for 1 min, annealing at 52°C for 30 sec, and extension at 72°C for 2 min. The PCR products were digested with *Sma*I and *Sal*I, purified, and cloned into the *Sma*I and *Sal*I sites of both pGBT9 and pGADGH. A reading frame of hybrid plasmids was confirmed by nucleotide sequencing.

Yeast two-hybrid assay The *in vivo* protein-protein interaction was monitored by the two-hybrid system. The reporter strain *S. cerevisiae* HF7c, which contains the reporter genes *lacZ* and *HIS3*, was cotransformed with various combinations of hybrid plasmids containing a DNA binding domain or transcriptional activation domain. Transformants were plated on a synthetic media lacking tryptophan and leucine. After 4 days of growth, the transformants were patched on selective media lacking tryptophan, leucine, and histidine, but including 0.5 mM 3-amino-1,2,4-triazole (3-AT), then incubated for 3-5 days at 30°C. Interactions between the hybrid proteins were monitored by either growth on the selective media, or β-galactosidase activity assay (Fields and Song, 1989; Yu *et al.*, 1998).

Results and discussion

Interaction between human topoisomerase IIβ and ERK2 It has been reported that topoisomerase IIα associates with ERK2 (Shapiro *et al.*, 1999). Because topoisomerase IIβ is closely homologous to topoisomerase IIα (Chung *et al.*, 1989; Jenkins *et al.*, 1992; Tan *et al.*, 1992), we investigated whether or not topoisomerase IIβ interacts with ERK2 using the yeast two-hybrid system. ERK2 was subcloned into either pGBT9, so that it was expressed as a fusion protein with a DNA binding domain, or pGADGH, so that it was expressed as a fusion protein with a transcriptional activation domain (described in Materials and Methods.) The hybrid plasmids containing different regions of human topoisomerase IIβ for the two-hybrid test have been previously described (Park *et al.*, 2001). The yeast reporter strain was co-transformed with various combinations of hybrid plasmids, and interactions between hybrid proteins were detected by the expression of the reporter genes, *HIS3* and *lacZ*. All hybrid plasmids were also tested against the empty expression vectors pGBT9 and pGADGH, in order to control for autonomous activation of the hybrid proteins. None of these controls were able to activate the transcription of the reporter genes. Fig. 1 shows that the pGBTopβ$_{1-807}$, pGATopβ$_{681-1621}$, and pGBTopβ$_{1272-1621}$ failed to activate transcription in the presence of the ERK2-fused construct. However, the pGATopβ$_{901-1621}$ constructs did. The reason why pGATopβ$_{681-1621}$ failed to activate transcription in the presence of the ERK2-fused construct seemed to be that the fusion protein of topoisomerase IIβ residues 681-1621 and GAL4 activation domain was too large to properly activate the expression of reporter genes. Similar phenomena were also observed in other two-hybrid tests (our unpublished observations). These data indicate that topoisomerase IIβ also associates with ERK2 and that topoisomerase IIβ residues 901-1621 include the binding site for ERK2.

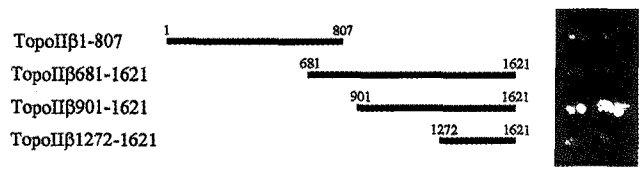

Fig. 1. Interaction between human topoisomerase IIβ and ERK2. The report strain was co-transformed with various combinations of hybrid plasmids encoding the respective GAL4 domains fused to truncated topoisomerase IIβ and full-length ERK2. Double transformants were patched for 4 days at 30°C on selective media lacking tryptophan, leucine, and histidine, but including 0.5 mM 3-AT. Interactions between the hybrid proteins were tested for their ability to support growth on the selective media. All constructs were also screened against the empty expression vectors pGBT9 and pGADGH, in order to control for autonomous activation of the hybrid proteins.

Mapping of the binding domain of human topoisomerase IIβ for ERK2 In order to map the ERK2-interacting region within topoisomerase IIβ, the C-terminal regions of topoisomerase IIβ 901-1621 were deleted and the yeast two-hybrid test was performed using ERK2 and different topoisomerase IIβ deletion constructs. As shown in Fig. 2A, the deletion of topoisomerase IIβ 901-1621 from the C-terminal end still showed an interaction when amino acids 901-1263 were present, but not when the C-terminus was further deleted until amino acid 1213. For control experiments, all hybrid plasmids were also tested against the empty expression vectors. However, these controls were unable to activate the transcription of the reporter gene (data not shown). Thus, these results indicate that residues 901-1263 of the human topoisomerase IIβ are sufficient for its binding to ERK2, and that residues 1213-1263 are necessary

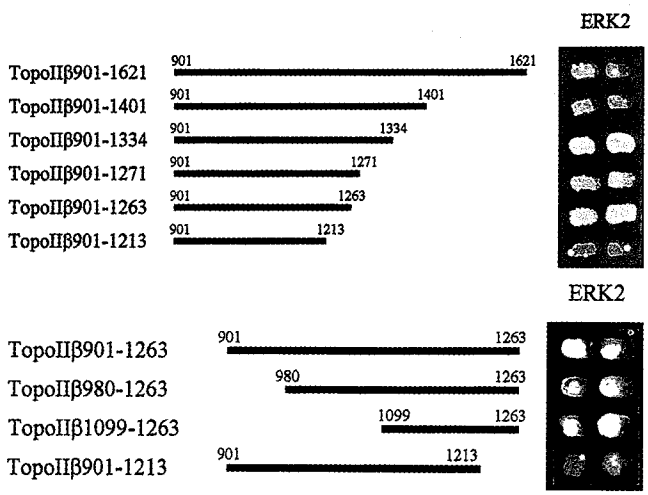

Fig. 2. Delineation of the subregion within topoisomerase IIβ required for an interaction with ERK2. The interaction between the full-length ERK2 and different topoisomerase IIβ deletion constructs was examined by the yeast two-hybrid system. The protein-protein interactions were detected by the expression of the reporter gene as described in the Fig. 1 legend. All constructs were also screened against the empty expression vectors pGBT9 and pGADGH to control for autonomous activation of the hybrid proteins.

for the binding of ERK2 (Fig. 2A).

Potential leucine zipper regions are found in many eukaryotic topoisomerase II enzymes (Zwelling and Perry, 1989). Topoisomerase IIβ also contains a similar leucine zipper motif between amino acid residues 1016 and 1098. The leucine zipper motif, originally described as a series of four or five leucine residues each separated by six amino acids, allows for the positioning of these hydrophobic residues on one face of an idealized α-helix (Landschulz *et al.*, 1988). Proteins containing this motif have been shown to selectively homo- and heterodimerize via the leucine zipper by forming a coiled coil structure (O'Shea *et al.*, 1989). Thus, we examined whether or not the leucine repeats in topoisomerase IIβ are required for its interaction with ERK2. To accomplish this, we constructed deletion mutant forms of topoisomerase IIβ, including (residues 980-1263) or lacking (residues 1099-1263) the putative leucine zipper motif. We then examined the interaction of these deletion mutants with ERK2 using the two-hybrid system. Fig. 2B shows that both constructs of the topoisomerase IIβ 980-1263 and topoisomerase IIβ 1099-1263 activated transcription of the reporter gene in the presence of an ERK2 fusion. These results indicate that the leucine zipper motif does not contribute to the protein-protein interaction between topoisomerase IIβ and ERK2, and that the residues 1099-1263 of topoisomerase IIβ are sufficient for the interaction with ERK2.

Specific binding domain of human topoisomerase IIα for ERK2 Interaction domain of topoisomerase IIα for ERK2 was also investigated using the two-hybrid system. The yeast

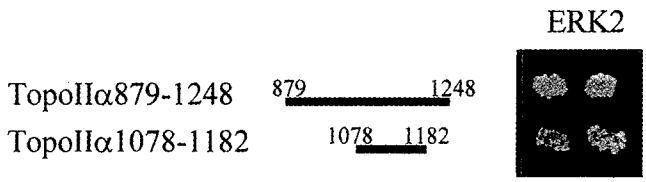

Fig. 3. Specific binding domain of human topoisomerase IIα for ERK2. The interaction between the topoisomerase IIα deletion construct and ERK2 was examined by the yeast two-hybrid system. The protein-protein interactions were detected by the expression of the reporter gene as described in the Fig. 1 legend. All constructs were also screened against the empty expression vectors pGBT9 and pGADGH to control for autonomous activation of the hybrid proteins.

reporter strain was cotransformed with pGADGH-ERK2 and either pGBTopo$\alpha_{879\text{-}1248}$ or pGBTopo$\alpha_{1078\text{-}1182}$ containing the C-terminal region of human topoisomerase IIα, and the interactions between hybrid proteins were detected by expression of the reporter genes. As shown in Fig. 3, both deletion mutant forms of topoisomerase IIα interacted with ERK2. Thus, we could conclude that the residues 1078-1182 of topoisomerase IIα are sufficient to allow binding to ERK2. Because topoisomerase IIα contains the potential leucine zipper motif between amino acids 994 and 1076, the results indicate that the leucine zipper motif does not involve the protein-protein interaction between topoisomerase IIα and ERK2.

Recently, the nuclear localization signals (NLS) of topoisomerase II isozymes (for example, amino acids 1294-1332, amino acids 1522-1548, and amino acids 1538-1573 of topoisomerase IIβ; amino acids 1259-1296 and amino acids 1454-1497 topoisomerase IIα) were mapped (Cowell *et al.*, 1998; Mirski *et al.*, 1999). The present results show that the ERK2-binding domains of topoisomerase IIα and IIβ do not contain the nuclear localization signal.

Activity of topoisomerase II can be modulated by either protein phosphorylation, or association with other proteins. For example, phosphorylation by CKII or PKC stimulates the catalytic activity of topoisomerase II as monitored by DNA relaxation, catenation, or decatenation assays (Ackerman *et al.*, 1985; Sahyoun *et al.*, 1986; Corbett *et al.*, 1992). Redwood *et al.* (1998) have shown that CKII holoenzyme stabilizes topoisomerase IIα activity during incubation at 37°C, and this effect does not depend upon the phosphorylation of topoisomerase IIα. ERK2 phosphorylates topoisomerase IIα and stimulates the enzyme activity by a phosphorylation-independent manner (Shapiro *et al.*, 1999). The p53 tumor suppressor stimulates the catalytic activity of topoisomerase IIα by specifically enhancing the rate of ATP hydrolysis (Kwon *et al.*, 2000). Recently we have shown that topoisomerase IIβ residues 1099-1263 and topoisomerase IIα residues 1078-1182 interacts with the β subunit of CKII, and the stimulation of topoisomerase IIα activity can be mediated by a physical association with the noncatalytic CKIIβ subunit

(Park *et al.*, 2001).

In summary, the present results demonstrate that topoisomerase IIβ residues 1099-1263, and topoisomerase IIα residues 1078-1182, interact with ERK2. Thus, we suggest that topoisomerase IIβ residues 1099-1263, and topoisomerase IIα residues 1078-1182, may be common binding sites for topoisomerase II activator proteins. Further identification of the precise sequence responsible for these interactions awaits site-directed mutation studies on topoisomerase IIα and IIβ. The present study will help in the elucidation of the activation mechanism of topoisomerase enzymes by association with other proteins.

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