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Oligomerization of TopBP1 is necessary for the localization of TopBP1 to mitotic centrosomes

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ABSTRACT

Human TopBP1 is involved in the DNA damage checkpoint response, chromosome replication, and other functions of cell cycle control. The C-terminal region of TopBP1 (TbpCtr: amino acid residues 1222–1522) is involved in the localization of TopBP1 to the centrosomes during mitosis. Here, we showed that the amino acid residues 741–885 of TopBP1, in addition to TbpCtr, are necessary for the centrosomal localization of TopBP1. Whereas oligomeric tags fused to TbpCtr localized to mitotic centrosomes, monomeric tags fused to TbpCtr did not. Insertion of the amino acid residues 741–885 into the monomeric tag fused to TbpCtr allowed the protein to localize to the mitotic centrosome. These results suggest that the amino acid residues 741–885 are necessary for oligomerization of TopBP1 for centrosomal localization.

1. Introduction

TopBP1, containing 8 BRCT (BRCA1 C-terminus) repeats, functions as a DNA damage checkpoint mediator and regulator of DNA transcription and replication [1]. In the DNA damage checkpoint response, TopBP1 interacts with ATR in an ATRIP-dependent manner and activates ATR kinase activity [2]. In the replication stress response, TopBP1 is required for the loading of the 9-1-1 (Rad9-Hus1-Rad1) complex and DNA polymerase α onto stalled replication forks [3,4]. DNA damage leads to the formation of TopBP1 foci with NBS1, BRCA1, and 53BP1 at the sites of DNA breaks [5].

The interaction of Dpb11, the budding yeast homolog of TopBP1, with Sld2 and Sld3 leads to the recruitment of Cdc45 to the origins of DNA replication [6–9]. Treslin interacts with TopBP1 in a CDK2-dependent manner and triggers the loading of Cdc45 onto replication origins [10]. During formation of the pre-replicative complex, TopBP1 promotes chromatin binding of GEMC1, which stimulates the initiation of DNA replication [11,12]. In human cells, TopBP1 depletion showed that TopBP1 is required for the activation of cyclin E/CDK2 and loading of replication components onto chromatin [13]. TopBP1 deficiency in mouse and human primary cells induced cellular senescence, and TopBP1-knockout mice exhibited early embryonic lethality [14].

The centrosome is composed of 2 centrioles surrounded by the pericentriolar matrix, and functions as the microtubule-organizing center of the cell [15]. The centrosome performs various functions, such as cell motility, cell division, and maintenance of the cell shape [15,16,23]. During mitosis, the centrosome functions as spindle poles for the equal segregation of chromosomes. TopBP1, containing a mitosis-specific centrosome localization signal TbpCtr (the C-terminal region of TopBP1), localizes to the centrosome in prometaphase, metaphase, and anaphase during mitosis [17,18]. Elimination of endogenous TopBP1 from centrosomes by ectopic expression of TbpCtr showed that TopBP1 participates in the proper progression of mitosis at the centrosome. In this study, we showed that TopBP1 oligomerization is necessary for TopBP1 localization to the mitotic centrosome.

2. Materials and methods

2.1. Cell culture and plasmid transfection

HeLa and HeLa cells stably expressing GFP-centrin were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and antibiotics (penicillin/streptomycin). Transfection of DNA constructs was performed using a Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions. Transfected cells were incubated for 48 h and immunostained with specific primary antibodies and fluorescent-dye conjugated secondary antibodies.

Abbreviations: PBS, phosphate buffered saline; 741/885, the amino acid residues 741–885 of TopBP1; TbpCtr, the C-terminal region of TopBP1 (amino acid residues 1222–1522).

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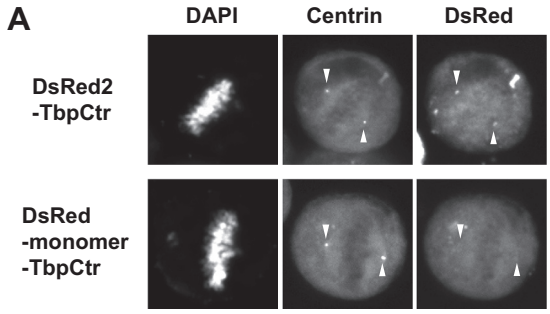

B

tag		Centrosome localization of tag fused to TbpCtr
DsRed2-C1 tag	tetramer	O
GST tag	dimer	O
myc tag	monomer	X
DsRed-monomer-C1 tag	monomer	X

Fig. 1. Oligomerization of TbpCtr is necessary for the centrosome localization of TopBP1. (A) Each DNA construct expressing the indicated tag fused to the N-terminus of TbpCtr was transfected into HeLa cells stably expressing GFP-centrin. At 48 h after transfection, cells were fixed with 4% paraformaldehyde. Centrin and TbpCtr were detected by GFP and DsRed immunofluorescence, respectively. DNA was stained with DAPI. Arrowheads indicate GFP-centrin or DsRed signals. (B) Centrosomal localizations of the corresponding tags in mitotic cells were determined using immunostaining (GST and myc tags) or DsRed fluorescence (DsRed2-C1 and DsRed-monomer-C1 tags) by co-localization with GFP-centrin fluorescence.

2.2. Immunofluorescence microscopy

For the detection of fluorescent proteins, transfected cells grown on coverslips were fixed with 4% paraformaldehyde for 15 min. After fixation, cells were washed 3 times with phosphate buffered saline (PBS) and then mounted on glass slides with mounting media (Biomeda Corp.) containing DAPI (1 μg/ml). For immunostaining of endogenous TopBP1, fixed cells were permeabilized in PBS containing 0.3% Triton X-100 for 15 min. After incubation for 5 min in a blocking solution (PBS containing 3% BSA, 0.1% Triton X-100), cells were treated with a rabbit anti-TopBP1 antibody [13]. The cells were washed 3 times with PBS containing 0.1% Triton X-100, and incubated with a FITC-conjugated anti-rabbit secondary antibody (Jackson ImmunoResearch). DNA was stained with mounting media containing DAPI (1 μg/ml). Images were viewed at 100× magnification under an Olympus BX51 microscope.

3. Results and discussion

3.1. The centrosome localization of TopBP1 requires the 741–885 amino acid residues of TopBP1

The C-terminal region of TopBP1 (TbpCtr: amino acid residues 1222–1522) localized the fused tags, glutathione S transferase (GST), and DsRed2 to the mitotic centrosomes [17]. The centrosomal localizations suggest that TbpCtr functions as a centrosome localization signal. The indicated tags were fused to the N-terminus of TbpCtr, and their localization to the mitotic centrosomes

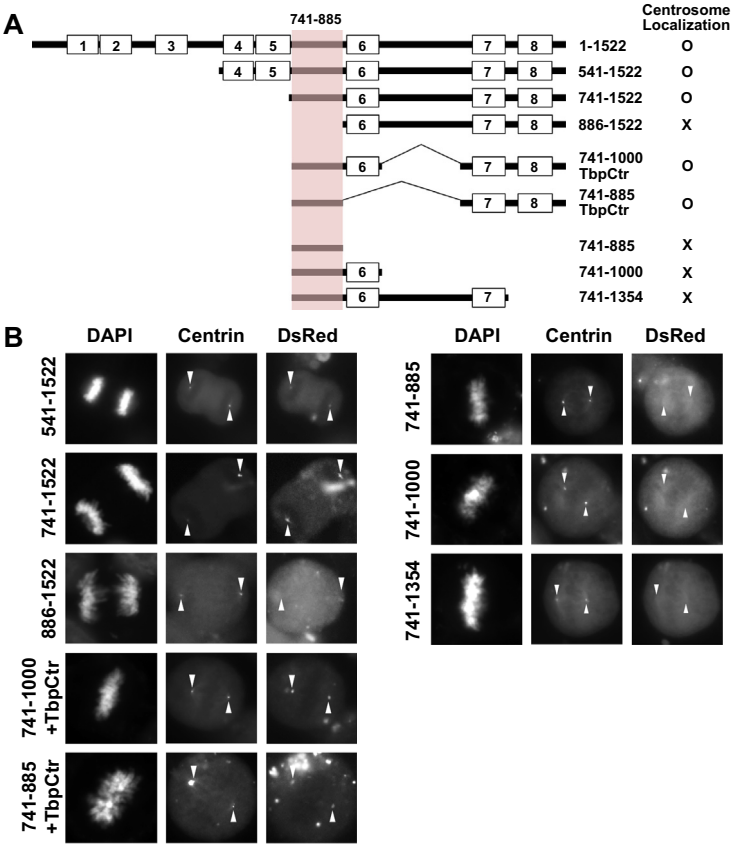

Fig. 2. The centrosomal localization of TopBP1 depends on the amino acid residues 741–885 of TopBP1. (A) The deletion fragments of TopBP1 were fused to the C-terminus of DsRed-monomer-C1 tag. Centrosomal localizations of the DsRed monomer tag in mitotic cells were determined by co-localization of DsRed fluorescence with GFP-centrin fluorescence as described in Fig. 1. BRCT domains were boxed. (B) DNA was stained with DAPI. Arrowheads indicate GFP-centrin or DsRed signal.

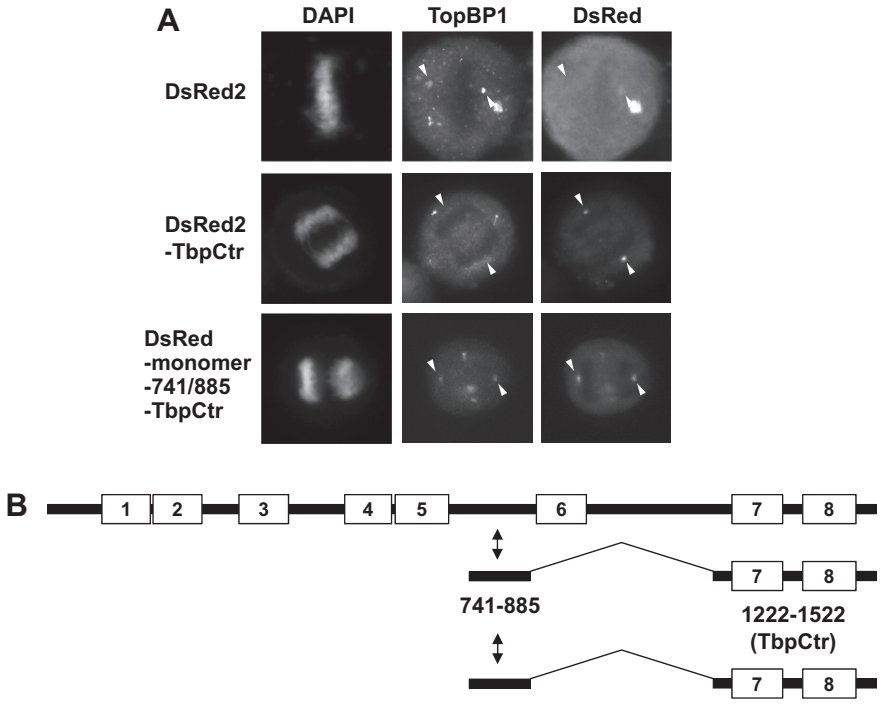

Fig. 3. Oligomerization of TopBP1 through the amino acid residues 741–885. (A) HeLa cells were transfected with DsRed2-TbpCtr or DsRed-monomer-741/885-TbpCtr. Endogenous TopBP1was immunostained with anti-TopBP1 antibody [17] and the expressed DsRed2 or DsRed-monomer-C1 tag fused to TbpCtr was detected with DsRed fluorescence. (B) The interactions between 741/885 regions were postulated.

was determined by co-localization with GFP-centrin (Fig. 1). Centrin exists in the centrioles of centrosomes [19]. The DsRed2-C1 and GST tag fused to the N-terminus of TbpCtr localized to the mitotic centrosomes (Fig. 1). On the other hand, both the myc and DsRed-monomer-C1, fused to TbpCtr, were not able to localize to the centrosomes. The GST and DsRed2-C1 tags form a dimer and a tetramer, respectively [20,21]. However, the DsRed-monomer-C1 and myc tags are monomeric [22]. The inability of TbpCtr fused to the monomeric tags for localization suggests that localization of TbpCtr to the centrosomes may require oligomerization of TbpCtr for centrosome localization.

In order to identify an element that allows the TbpCtr fused to the DsRed-monomer tag to localize to the centrosomes, we constructed TopBP1 deletions fused to the C-terminus of DsRed-monomer-C1 tag (Fig. 2A and B). N-terminal deletions up to amino acid residue 740 localized the fused DsRed-monomer tag to centrosomes. Although the fragment possessing deletion up to amino acid residue 885 contained TbpCtr, this fragment did not permit the centrosomal localization of the DsRed-monomer tag. When TbpCtr fused to the fragment of amino acid residues 741–885 (741/885), the DsRed-monomer tag was detected in the centrosomes. However, deletion of the seventh, the eighth, or both BRCT domains, in which the BRCT domains consist of TbpCtr [17], did not allow the constructs containing the 741/885 fragment to localize to the centrosomes (Fig. 2A and B). These results suggest that the 741/885 region, in addition to TbpCtr, is necessary for the fused DsRed-monomer tag to localize to the centrosomes.

Overexpression of DsRed2-TbpCtr eliminated endogenous TopBP1 from the mitotic centrosomes, suggesting that DsRed2-TbpCtr and TopBP1 compete with mitotic centrosome localization [17]. We examined whether the expression of DsRed-monomer-741/885-TbpCtr removes TopBP1 from the mitotic centrosome (Fig. 3A). As shown previously [17], endogenous TopBP1 was not detectable in the mitotic centrosome by the expression of DsRed2-TbpCtr. However, upon expression of DsRed-monomer-741/885-TbpCtr, both TopBP1 and DsRed-monomer-741/885-TbpCtr were detected in the centrosomes. The presence of both proteins can be attributed to the interaction between endogenous TopBP1 and ectopically expressed DsRed-monomer-741/885-TbpCtr (Fig. 3B). In contrast, DsRed2-TbpCtr could not interact with endogenous TopBP1, because DsRed2-TbpCtr did not contain the 741/885 fragment. These results support that the 741/885 region is involved in oligomerization of TopBP1 for centrosomal localization.

Oligomerization of TopBP1 has been found in previous studies. Oligomerization by ionizing radiation required the fifth BRCT domain of TopBP1, which resulted in the formation of foci at the sites of DNA double-strand breaks [5,24]. Phosphorylation of TopBP1 by Akt kinase at Ser 1159 between the sixth and seventh BRCT domains induces oligomerization of TopBP1, which is essential for TopBP1 to repress the transcriptional and apoptotic activity of E2F1 [25]. This study suggested that oligomerization of TopBP1 mediated by the amino acid residues 741–885 is necessary for the centrosomal localization of TopBP1 during mitosis. Therefore, variable ways of achieving the oligomerization of TopBP1 participate in exerting certain functions of TopBP1 required for physiological conditions.

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