Supplementary Information

Supplemental Methods

Plasmids
FlagCPAP containing full length CPAP (amino acids 5-1338) cloned into the FlagA EFrpuro vector was kindly provided by Jane Visvader (4). MycCPAP and MycCPAP-N contain amino acids 5-1338 and 5-1071, respectively, cloned into the pCMV-3Tag-2 vector containing 3 N-terminal Myc tags (Sigma). GST-CPAP-N and GST-CPAP-C contains amino acids 5-1338 and 1075-1338, respectively, cloned into pGex-5-1. CPAP.AA mutation was created by substituting the glutamic acid (D) and glycine (G) residues at position 1302 and 1303, with alanine (A) residues, by site-directed mutagenesis of MycCPAP using the oligonucleotide 5'-AGAGACGGGAATACCCAGCTGCCACTGTTAAAACCGTATA-3' and the Stratagene QuikChange site directed mutagenesis kit according to the manufacturer's instructions.

TNKS1 is plasmid TT20 containing full length tankyrase 1 (amino acids 1-1327) cloned in the vector pBK-CMV (7). TNKS1.PD was generated by replacing a C-terminal Xba I fragment of TT20 with the corresponding fragment from FN-tankyrase 1.HE/A where amino acids Histidine (aa 1184) and Glutamic acid (aa 1291) were replaced with Alanine (1). N-terminally Flag-tagged full-length tankyrase 1 (amino acids 2-1327) was cloned in the vector pRc/CMV (Invitrogen) (6) to generate FlagTNKS1 and in the vector pUDH10-3 (2) under the control of the tetracycline-controlled promoter to generate pTetFLTNKS1.

PARP assay
Samples containing recombinant baculovirus-derived tankyrase 1 (0.2 µg) (7) and recombinant GST-CPAP-C or GST-CPAP-N (2 µg) (expressed and purified from E.coli BL21 cells according to standard protocols) or baculovirus-derived TRF1 (2 µg) (7) were incubated for 30 min at 25°C in 10 µl PARP reaction buffer (20mM Tris, pH 8.0, 4mM MgCl₂, 0.2 mM dithiothreitol) containing 25 µM Biotinylated NAD⁺ (Trevigen). For PARP inhibition in vitro we added 10 mM 3AB, 300 µM PJ34, or 20 µM XAV939. We used relatively high concentrations of inhibitor; at lower concentrations of inhibitors tankyrase...
autoPARsylation was fully inhibited, but some residual CPAP PARsylation remained. Reactions were terminated by addition of 2X sample buffer, fractionated by SDS/PAGE, transferred electrophoretically to nitrocellulose, and probe with anti-biotin-HRP (1:1000) (Cell Signaling).

**Plasmid Transfection**

293T cells were transfected with FlagCPAP, TNKS1 (TT20), TNKS1.PD, FlagTNKS1, MycCPAP, MycCPAP-N, HA-ubiquitin (pMT123; kindly provided by Dirk Bowmann) (8), or Vector pcDNA3 (Invitrogen) using Lipofectamine 2000 reagent (Invitrogen) for 16 hr according to the manufacturer’s protocol. Where indicated, MG132 (10 µm) was added 4 hr prior to harvest.

**Immunoprecipitation**

Cells were lysed in 0.5 ml (per one 15-cm-diameter dish) TNE buffer [10 mM Tris (pH7.8), 1% Nonidet P-40, 0.15 M NaCl, 1 mM EDTA, and protease inhibitor cocktail (Sigma)] on ice for 1 hr, then pelleted at 8,000 x g for 10 min. Supernatants were precleared with normal mouse serum and protein G-Sepharose rotating at 4°C for 30 min. Nonspecific protein aggregates were removed by centrifugation and the supernatant was used for immunoprecipitation analysis or fractionated directly on SDS-PAGE (indicated as input, approximately 1 % of the amount used in the immunoprecipitation). Supernatants were incubated with 35 µl of mouse anti-Flag or rabbit anti-myc agarose bead conjugates (Sigma) for 3 hr, washed three times with 1 ml TNE buffer, fractionated on a 10% SDS-PAGE gel, and processed for immunoblotting as described below.

**Cell synchronization**

GFP or TNKS1 shRNA-11 cells were grown in the presence of 2 mM thymidine for 16 h, washed 3 times with PBS and released into fresh medium for 11 h, treated again with 2 mM thymidine for 16 h, washed 3 times with PBS and released into fresh medium. Cells were then harvested by trypsinization at 2 hr intervals from 0 to 14 hr for analysis by immunoblot, FACS, and immunofluorescence.
FACS analysis
Cells were resuspended in PBS containing 2 mM EDTA, fixed with cold 70% (v/v) ethanol, stained with propidium iodide (50 µg/ml) and analyzed with a Becton-Dickinson FACScan and Modfit 3.0 software to determine relative DNA content.

Stable cell lines
F7 cells were generated by stable cotransfection of pTetFLTNKS1 and the neomycin-resistance plasmid pNY-HI into HTC75 cells [a hygromycin-resistant HT1080-derived clonal cell line that stably expresses the tetracyclin-controlled transactivator (tTA) (9) using calcium phosphate coprecipitation. F7, a G418-resistant clone, was expanded in the presence of doxycyclin (Sigma) (100 ng/ml). For induction of FlagTNKS1, F7 cells were grown in parallel under uninduced (with doxycyclin) and induced (without doxycyclin) conditions for 16 hr.

TNKS1 shRNA cell lines (11,12, and 13) and the GFP shRNA line were generated by lentiviral infection of HTC75 cells as previously described (3).

Cell extracts
Cells were resuspended in 4 volumes of TNE buffer and incubated for 1 h on ice. Suspensions were pelleted at 8000 x g for 15 min. Twenty-five µg (determined by Bio-Rad protein assay) of supernatant proteins were fractionated by SDS-PAGE and analyzed by immunoblotting.

Immunoblot analysis
Immunoblots were incubated with the following primary antibodies: rabbit anti-tankyrase 1 762 (1 µg/ml) (5), mouse anti-Flag (2.2 µg/ml) (Sigma), rabbit anti-myc (0.08 µg/ml) (Santa Cruz Biotechnologies), mouse anti-α-tubulin ascites (1:10000) (Sigma), rabbit anti-cyclin B1 (0.2 µg/ml) (Santa Cruz Biotechnologies), rabbit anti-Cdc20 (0.6 µg/ml) (Abcam), rabbit anti-HA (0.25 µg/ml) (Abcam), or rabbit anti-PAR (1:4000) (Enzo Life Sciences), followed by horseradish peroxidase-conjugated donkey anti-rabbit or anti-mouse IgG (1:2500) (Amersham). Bound antibody was detected by Super Signal West Pico (Thermo Scientific).
Indirect immunofluorescence

Cells were fixed in methanol at -20°C for 10 min, blocked in 1% bovine serum albumin (BSA) in PBS, and incubated with the following antibodies: mouse anti-CPAP (2.3 µg/ml) (Proteintech), mouse anti-acetylated a-tubulin 6-11B-1 (1 µg/ml) (Abcam); rabbit anti-Flag (0.43 µg/ml) (Sigma), mouse anti-γ-tubulin ascites (1:5000) (Sigma), mouse anti-centrin ascites 20H5 (1:20000) (kindly provided by Jeff Salisbury), rabbit anti-CP110 (1:10000) (kindly provided by Brian Dynlacht), mouse anti-α-tubulin ascites (1:10000) (Sigma), rabbit anti-tankyrase 1 763 (0.1 µg/ml) (5). Primary antibodies were detected with fluorescein isothiocyanate-, tetramethyl rhodamine isothiocyanate-, or coumarin AMCA-conjugated donkey anti-rabbit or anti-mouse antibodies (1:100) (Jackson Laboratories). DNA was stained with 4,6-diamino-2-phenylindole (DAPI) (0.2 µg/ml).

For analysis of elongated centrioles cells were cold-treated for 1 h at 4°C, pre-extracted with Triton X-100 (0.5%) in PBS for 1 min, and then fixed in methanol at -20°C for 10 min.

Image Acquisition

Images were acquired using a microscope (Axioplan 2; Carl Zeiss, Inc.) with a Plan Apochrome 63x NA 1.4 oil immersion lens (Carl Zeiss, Inc.) and a digital camera (C4742-95; Hamamatsu Photonics). Images were acquired and processed using Openlab software (Perkin Elmer).

Supplemental References


