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Cell cycle dependent localization of the telomeric PARP, tankyrase, to nuclear pore complexes and centrosomes

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SUMMARY

Tankyrase is a human poly(ADP-ribose) polymerase that was initially identified through its interaction with the telomeric protein TRF1, a negative regulator of telomere length. In vitro poly(ADP-ribosyl)ation by tankyrase inhibits TRF1 binding to telomeric DNA suggesting a role for tankyrase in telomere function. We previously demonstrated that tankyrase co-localizes with TRF1 at the ends of human chromosomes in metaphase. Here we show that tankyrase localizes to additional subcellular sites in a cell cycle dependent manner. In interphase, tankyrase co-localized with TRF1 to telomeres, but in addition was found to reside at nuclear pore complexes, as evidenced by indirect immunofluorescence, subcellular fractionation and immunoelectron microscopy. At mitosis, concomitant with nuclear envelope breakdown and nuclear pore complex disassembly, tankyrase was found to relocate around the pericentriolar matrix of mitotic centrosomes. This complex staining pattern along with the observation that tankyrase did not contain a nuclear localization signal suggested that its telomeric localization might be regulated, perhaps by TRF1. Indeed, localization of exogenously-expressed tankyrase to telomeres was dependent upon co-transfection with TRF1. These data indicate that the subcellular localization of tankyrase can be regulated by both the cell cycle and TRF1.

Key words: Telomere, Centrosome, Nuclear pore complex, Tankyrase, PARP

INTRODUCTION

Telomeres function to ensure the complete replication of chromosome ends, a task which can not be accomplished by conventional DNA polymerases. Telomeres are maintained by telomerase, a reverse transcriptase that adds TTAGGG repeats onto the 3′ ends of vertebrate chromosomes (Greider and Blackburn, 1985; reviewed by Nugent and Lundblad, 1998). Cells have mechanisms to monitor telomere length and to ensure that telomeric repeats are added to chromosome ends in a controlled fashion (reviewed by Shore, 1997).

Human telomere function requires two telomere specific DNA binding proteins, TTAGGG repeat binding factors, TRF1 and TRF2 (Bilaud et al., 1997; Broccoli et al., 1997; Chong et al., 1995; reviewed by Smith and de Lange, 1997). TRF1 functions as a negative regulator of telomere length maintenance (van Steensel and de Lange, 1997). Long-term overexpression of TRF1 in a telomerase-positive tumor cell line results in progressive telomere shortening, whereas inhibition of TRF1 induces telomere elongation (van Steensel and de Lange, 1997). TRF1 does not control the expression of telomerase itself, but is thought to act in cis by inhibiting the action of telomerase at individual telomere termini. The mechanism by which TRF1 controls telomere synthesis by telomerase is unclear, but in vitro studies indicate that it is likely to involve additional proteins (A. Smogorzewska et al., unpublished).

A yeast two-hybrid screen using human TRF1 as bait identified tankyrase, a 142 kDa protein with homology to ankyrins and to the catalytic domain of poly(ADP-ribose) polymerase (PARP) (Smith et al., 1998). Tankyrase was found to co-localize with TRF1 to human telomeres indicating that it is a component of the human telomeric complex (Smith et al., 1998). Tankyrase is a new member of the ankyrin family, a group of structural proteins that link integral membrane proteins to the cytoskeleton (reviewed by Bennet, 1992). Like ankyrins, tankyrase contains 24 copies of the ANK repeat, a protein-protein interacting motif, in a domain responsible for its interaction with TRF1 (Smith et al., 1998). Outside of the ankyrin domain there is no homology between tankyrase and the ankyrins. Instead, tankyrase contains in its carboxy terminus a region with homology to the catalytic domain of PARP.

PARP is a nuclear enzyme that in response to DNA damage uses NAD^+ to synthesize ADP-ribose polymers onto protein acceptors (reviewed by Jeggo, 1998; Lindahl et al., 1995). Poly(ADP-ribosyl)ation is a dramatic, short-lived post-translational modification that is believed to function in the maintenance of genome integrity, although the molecular mechanism is unknown. Recombinant tankyrase was found to have PARP activity in vitro with both TRF1 and tankyrase

functioning as acceptors for poly(ADP-ribosyl)ation (Smith et al., 1998). Poly(ADP-ribosyl)ation of TRF1 diminishes its ability to bind to telomeric DNA in vitro indicating a role for tankyrase and ADP-ribosylation in telomere function (Smith et al., 1998).

Although tankyrase is located at human telomeres, it does not bind to telomeric repeats directly, but rather, it is positioned there by TRF1. In addition, tankyrase does not contain a nuclear localization signal (NLS) and therefore its mechanism for localization to telomeres is unknown. In order to understand how tankyrase localizes to telomeres and interacts with TRF1 in vivo, we investigated its subcellular localization. In addition to its telomeric location, tankyrase showed a complex pattern of subcellular localization that varied across the cell cycle. This complexity raises the possibility that tankyrase has additional functions. The regulation of its telomeric localization is proposed to be important for the function of tankyrase at telomeres.

MATERIALS AND METHODS

Indirect immunofluorescence

HeLaI (Saltman et al., 1993) or HeLaI.2.11 cells, a subclone of HeLaI containing telomeres of 15-25 kb (van Steensel et al., 1998), were fixed with ice-cold methanol at –20°C for 10 minutes or 3.7% formaldehyde in phosphate-buffered saline (PBS) for 10 minutes followed by permeabilization with 0.5% NP-40 in PBS for 10 minutes. For hypotonic treatment cells were swollen for 15 minutes in 25% PBS, then fixed in 3.7% formaldehyde in 25% PBS for 10 minutes, followed by permeabilization with 0.5% NP-40 in 25% PBS for 10 minutes. Samples were blocked with 1% bovine serum albumin (BSA) in PBS followed by incubation with primary antibodies as indicated in the figure legends. The following polyclonal primary antibodies were used: affinity-purified rabbit anti-tankyrase 465 (Smith et al., 1998) (1-4 μg/ml), mouse serum to full-length baculovirus-derived TRF1 (S. Smith and T. de Lange, unpublished) (serum 1:10,000), or rabbit anti-TRF1 antibody 371 (van Steensel and de Lange, 1997) (0.4 μg/ml). The following mouse monoclonal primary antibodies were used: anti-FLAG M2 (Eastman-Kodak) (2-10 μg/ml), MAb414 (Davis and Blobel, 1986) (supernatant, 1:100), anti-NuMA 1F1 (Compton et al., 1991) (ascites 1:100), anti-centrin 20H5 (Sanders and Salisbury, 1994) (ascites 1:2000), or anti-γ-tubulin GTU-88 (ascites 1:2000) (Sigma). Primary antibodies were detected with FITC- or TRITC-conjugated donkey anti-mouse or rabbit antibodies (1:100) (Jackson Laboratories). DNA was stained with 4,6-diamino-2-phenylin-dole (DAPI) (0.2 μg/ml). Images were acquired on a Zeiss Axioplan 2 microscope with a Photometric CCD camera. Images were processed and merged using Adobe Photoshop.

Immunoblot analysis of rat liver fractions

Rat nuclei were prepared as described (Blobel and Potter, 1966). Nuclear envelopes were prepared according to the method of Dwyer and Blobel (1976). Urea extracted nuclear envelopes were prepared as described (Foisner and Gerace, 1993). Proteins samples were fractionated on SDS-polyacrylamide gels, transferred to nitrocellulose electrophoretically, and blocked in 5% milk in PBS containing 0.1% Tween-20. Antibody incubations were in 1% milk in PBS containing 0.1% Tween-20. Blots were incubated with affinity purified rabbit anti-tankyrase 465 (4 μg/ml), followed by horseradish peroxidase-conjugated donkey anti-rabbit IgG (Amersham) (1:2,500). Bound antibody was detected using the enhanced chemiluminescence kit (Amersham).

Preparation of WI38 cell extracts extracts

Extracts were generously provided by Dr J. Karlseder and details of their preparation will be described elsewhere (J. Karlseder and T. de Lange, unpublished). Briefly, WI38 cells were grown to confluence and maintained at confluence for 5 days, and released from arrest by subculturing and harvested at different time points. Cells were subjected to FACs analysis and whole cell extracts were prepared as described (van Steensel et al., 1998). Protein samples (15 μg per lane) were fractionated on 10% SDS-PAGE and processed for immunoblotting as described above.

Immunoelectron microscopy

HeLaI cells in tissue culture dishes were permeabilized for 15 seconds in 0.5% Triton X-100 in PBS, washed 2× in PBS, fixed for 10 minutes in 3% formaldehyde in PBS and blocked in 1% BSA/PBS. Cells were incubated with affinity purified rabbit anti-tankyrase antibody 465 (5 μg/ml), followed by 5 nm gold-conjugated anti-rabbit antibodies. Samples were processed for ultrathin (70-90 nm) sectioning and electron microscopic analysis as described (Pain et al., 1990).

Transfection

HeLaI cells were transfected by electroporation of FLAG-tankyrase (FLAG-tankyrase (aa 2-1327) was generated by PCR amplification and cloned into the *Not*I-*Apa*I cloning sites of a modified pRc/CMV expression vector (Invitrogen) carrying a FLAG epitope 5′ of the cloning sites) and pcDNA3-hTRF1, full length human hTRF1 cloned into the expression vector pcDNA3 (Invitrogen) (J. Karlseder and T. de Lange, unpublished). Cells were grown for 16 hours and then processed for indirect immunofluorescence as described above.

RESULTS

Tankyrase is located at telomeres in interphase

Previous work demonstrated the presence of tankyrase at the ends of metaphase chromosomes (Smith et al., 1998). To

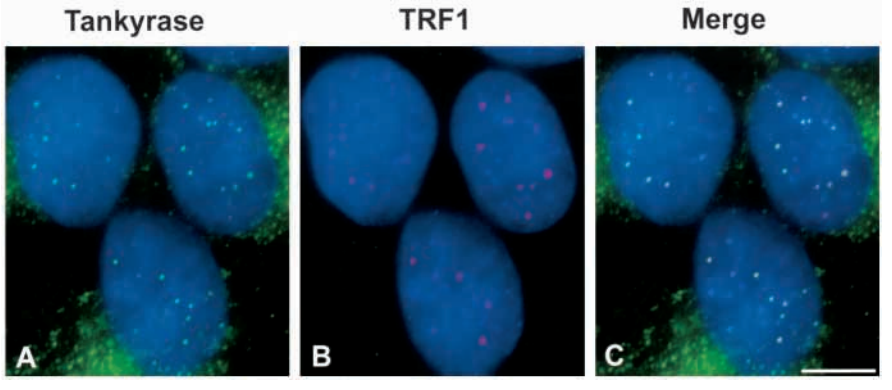

Fig. 1. Tankyrase co-localizes with TRF1 to telomeres in interphase. Indirect immunofluorescence analysis of methanol-fixed HeLaI.2.11 cells by double-staining with anti-tankyrase antibody 465 (A) (green) and mouse anti-TRF1 antibody (B) (red). (Merge) (C) indicates superimposition of the red and green images and light blue indicates co-localization of the red and green signal. DAPI staining of DNA is shown in blue. Bar, 5 μm.

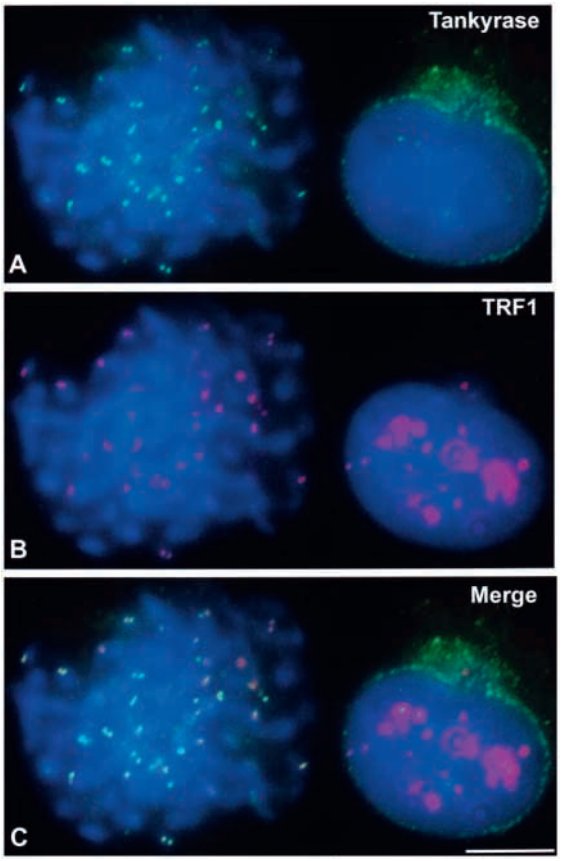

Fig. 2. Tankyrase co-localizes with TRF1 to telomeres in mitosis. Indirect immunofluorescence analysis of swollen, formaldehyde-fixed HeLaI.2.11 cells by double-staining with anti-tankyrase antibody 465 (A) (green) and mouse anti-TRF1 antibody (B) (red). (Merge) (C) indicates superimposition of the red and green images and light blue indicates co-localization of the red and green signal. DAPI staining of DNA is shown in blue. Shown is a mitotic cell on the left and an interphase cell on the right. Bar, 5 μm.

examine the staining pattern of tankyrase in interphase, HeLa cells were fixed with methanol and stained with affinity purified anti-tankyrase antibody 465. The specificity of this antibody had been established previously. Immunoblot analysis with anti-tankyrase antibody detected a single polypeptide of the predicted molecular mass (142 kDa) in whole cell HeLa extracts and indirect immunofluorescence analysis of metaphase chromosomes with anti-tankyrase antibody revealed co-localization of tankyrase with TRF1 to telomeres (Smith et al., 1998). Staining of methanol-fixed cells with anti-tankyrase antibody revealed a punctate cytoplasmic stain as well as a nuclear punctate pattern (Fig. 1A). Although the nuclear signal was relatively weak, it co-localized precisely with TRF1 (Fig. 1C) in a pattern consistent with a telomeric localization. These results demonstrate that a fraction of tankyrase co-localizes with TRF1 to telomeres in interphase.

Tankyrase localizes to telomeres during mitosis

As described above tankyrase localized to telomeres in interphase in methanol-fixed HeLa cells. Under these fixation conditions, however, tankyrase was not observed on mitotic chromosomes (data not shown; and see below Fig. 6A). Tankyrase had been observed previously at the ends of mitotic chromosomes, but under different conditions (Smith et al., 1998). In the previous study immunostaining was performed on metaphase chromosomes isolated from HeLa cells that were arrested in mitosis with colcemide. In addition, detection of tankyrase at telomeres required special fixation conditions; metaphase chromosomes were pre-swollen and formaldehyde-fixed in hypotonic buffer. We now sought to determine the subcellular localization of tankyrase using these same fixation conditions, but on an asynchronous population of HeLa cells that was not arrested with colcemide. Under these conditions, staining with anti-tankyrase antibodies revealed a punctate pattern in mitotic cells (Fig. 2A) that co-localized with TRF1 (Fig. 2C) and was consistent with a telomeric localization.

Under these fixation conditions the chromosomes appear more spread out and swollen than when cells are fixed under standard fixation conditions with methanol or formaldehyde (see below) and visualization of tankyrase at telomeres of mitotic chromosomes is facilitated.

Tankyrase localizes to the nuclear envelope at interphase

In addition to the expected telomeric staining pattern for tankyrase in mitosis, we observed an unexpected localization of tankyrase in interphase cells to the nuclear rim (Fig. 2A), a site lacking detectable TRF1 (Fig. 2C). To further analyze this nuclear rim-staining pattern, HeLa cells were fixed under standard formaldehyde-fixation conditions and stained with anti-tankyrase antibody. As shown in Fig. 3A tankyrase localized to the nuclear envelope in interphase cells and to what appeared to be centrosomes at mitosis. This staining pattern was blocked if the antibodies were preincubated with the

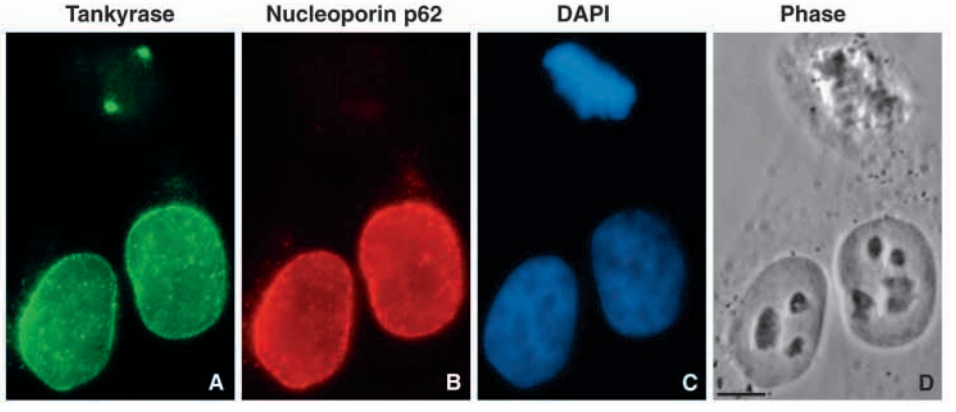

Fig. 3. Tankyrase localizes to the nuclear envelope in interphase. Indirect immunofluorescence analysis of formaldehyde-fixed HeLaI cells by double-staining with anti-tankyrase antibody 465 (A) (green), anti-Nucleoporin p62 antibody MAb414 (B) (red) or DAPI (C) (blue). The phase contrast image is shown in D. Bar, 5 μm.

recombinant tankyrase fusion protein against which the antibody was raised (Smith et al., 1998; and data not shown). The punctate nuclear rim staining was reminiscent of nuclear pore complex staining. Indeed, co-staining of cells with MAb414 (Fig. 3B), a monoclonal antibody that recognizes a family of nuclear pore complex proteins including Nucleoporin p62 (Davis and Blobel, 1986), revealed an identical staining pattern at the nuclear rim, but not at centrosomes (Fig. 3A and B).

In contrast to methanol fixation, formaldehyde-fixation did not allow detection of tankyrase at telomeres at interphase. Possibly, the telomeric tankyrase pattern is masked by the prominent nuclear pore-staining. Consistent with this idea, the tankyrase nuclear pore complex staining pattern was not preserved in methanol-fixed cells in which tankyrase was detected at telomeres (Fig. 1A). This lack of rim staining in methanol-fixed cells could be due to loss or masking of tankyrase epitopes or preferential loss of nuclear pore complex associated tankyrase during methanol fixation.

Tankyrase co-fractionates with nuclear envelopes

To confirm the localization of tankyrase at the nuclear envelope we used subcellular fractionation. Cytosol, nuclei and nuclear envelope fractions were prepared by standard procedures (Dwyer and Blobel, 1976). Subcellular fractions were analyzed by immunoblot using anti-tankyrase antibody (Fig. 4). Tankyrase was highly enriched in the nuclear envelope fraction (Fig. 4, lane 5) and remained bound to nuclear envelopes even after extraction with 0.5 M NaCl and 8 M urea (Fig. 4, lane 8), indicating a tenacious association with nuclear envelopes. Resistance to extraction by 8 M urea (which removes tightly associated, peripheral membrane proteins including the nuclear lamins; see Fig. 4, top panel, lane 7) is usually a property of integral membrane proteins. However, tankyrase is unlikely to be an integral membrane protein since its predicted amino acid sequence does not indicate a strong transmembrane domain and since it does not associate with microsomal membranes when co-translated in vitro (data not shown). The tight association between tankyrase and nuclear envelopes could reflect an unusual property of the ANK repeat domain. These results indicate that a major fraction of cellular tankyrase is associated with the nuclear envelope.

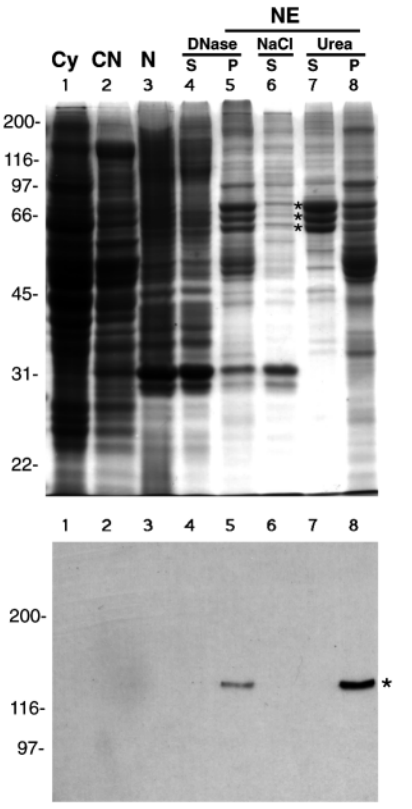

Fig. 4. Tankyrase co-fractionates with nuclear envelopes. Subcellular fractions of rat liver are: cytosol (Cy) (lane 1), crude nuclei (CN) (lane 2), nuclei (N) (lane 3), supernatant containing nuclear contents (S) (lane 4) and pellet containing nuclear envelopes (P) (lane 5) after Dnase I digestion of nuclei, supernatant (S) (lane 6) after extraction of nuclear envelopes with 0.5 M NaCl, and supernatant (S) (lane 7) and pellet (P) (lane 8) after extraction of salt-washed nuclear envelopes with 8 M urea. The amount of sample loaded for each fraction was based upon cell equivalents with an arbitrary value (x) for the starting number of cells: 1x (lanes 1 and 2), 100x (lanes 3 and 4) and 1000x (lanes 5-8). Samples were either fractionated by 10% SDS-PAGE and proteins visualized by staining with coomassie blue (top panel) or fractionated by 6% SDS-PAGE, transferred to nitrocellulose and probed with anti-tankyrase antibody 465 (bottom panel). Asterisks in the top panel indicate lamins A, B and C. Immunoreactive tankyrase is indicated by an asterisk in the bottom panel.

Immunogold localization of tankyrase to nuclear pore complexes

The immunofluorescence pattern and colocalization of tankyrase with Nucleoporin p62 strongly indicated that tankyrase localized to nuclear pore complexes. To determine precisely where in the nuclear envelope tankyrase was localized, immunogold electron microscopy was performed on formaldehyde-fixed HeLa interphase cells using affinity purified anti-tankyrase antibody. As shown in Fig. 5, tankyrase localized to the nuclear envelope, where it was readily detectable at nuclear pore complexes. Internuclear clusters of gold grains that could represent tankyrase at telomeres were not observed, most likely because telomeres are rarely captured in the thin sections analyzed here (Ludérus et al., 1996).

Evaluation of numerous images such as those shown in Fig. 5, revealed that the tankyrase antibody primarily decorated the cytoplasmic face of the nuclear pore complexes. Structural studies have indicated that the peripheral cytoplasmic and nucleoplasmic structures of the nuclear pore complex are morphologically distinct (reviewed by Pante and Aebi, 1996). The cytoplasmic face consists of a ring crowned with eight filaments that extend into the cytoplasm. Tankyrase often appeared to be located on or near the tips of these cytoplasmic filaments. In addition to the predominant cytoplasmic location, occasionally one or two gold particles appeared on the nuclear face of the nuclear pore complex.

Tankyrase localizes around the pericentriolar matrix at mitosis

In addition to its localization to telomeres and nuclear pore complexes, tankyrase localized to centrosomes during mitosis (see Fig. 3A). The centrosomal location of tankyrase was further investigated by dual-labeling cells using anti-tankyrase antibody combined with antibodies directed against well

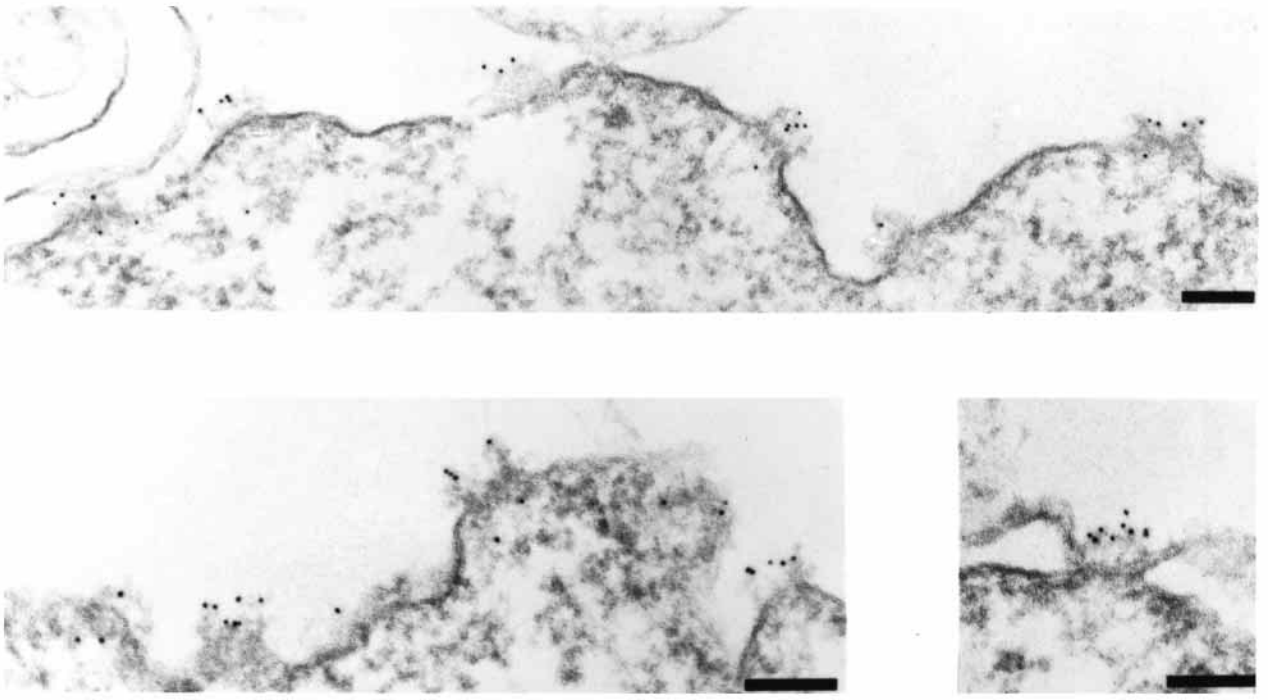

Fig. 5. Localization of tankyrase to nuclear pore complexes by immunoelectron microscopy. Triton X-100 permeabilized, formaldehyde-fixed HeLaI cells were probed with anti-tankyrase antibody 465 followed by 5 nm-gold-conjugated anti-rabbit antibodies. Samples were processed by thin sectioning followed by analysis in the electron microscope. Shown are three panels depicting typical patterns of gold labeling of nuclear pore complexes. Bars, 0.1 μm.

characterized, centrosomal proteins that could serve as markers for centrosomal subcompartments. As shown in Fig. 6, tankyrase did not co-localize with centrin, a component of the centrioles (reviewed by Salisbury, 1995) (Fig. 6C), or γ-tubulin, a pericentriolar matrix protein (Stearns et al., 1991; Zheng et al., 1991) (Fig. 6F), indicating that tankyrase is not an integral component of the centrosome per se. However, tankyrase did co-localize with NuMA (nuclear/mitotic aparatus protein), a nuclear protein that accumulates around the pericentriolar matrix at mitosis (reviewed by Cleveland, 1995) (Fig. 6I), indicating that tankyrase localizes to the periphery of mitotic centrosomes. At this location tankyrase could be acting on other centrosomal proteins.

Observation of tankyrase-stained cells at different stages of mitosis indicated that tankyrase localization to and from mitotic centrosomes occurred concomitant with nuclear envelope breakdown and reassembly. Thus, tankyrase first appeared at centrosomes in early prophase and remained there throughout mitosis to telophase (data not shown). A similar pattern of staining was observed for NuMA (reviewed by Cleveland, 1995).

Tankyrase staining of mitotic centrosomes was observed on cells fixed under standard conditions with formaldehyde (Fig. 3A) or methanol (Fig. 6A), but not when cells were preswollen and fixed with formaldehye in hypotonic buffer (Fig. 2A).

Expression of tankyrase is constant across the cell cycle

Our data indicate that tankyrase displays a complex pattern of subcellular localization across the cell cycle. To determine if tankyrase protein expression varied across the cell cycle, immunoblot analysis was performed on staged cell extracts. WI38 cells (human fetal lung fibroblasts) were synchronized by contact inhibition (J. Karlseder and T. de Lange, unpublished). Following arrest and release, whole cell extracts were prepared from cells at the indicated stages of the cell cycle and probed with anti-tankyrase antibody. As shown in Fig. 7, the steady-state level of tankyrase protein remains relatively unchanged across the cell cycle. Similar results were obtained with with Swiss 3T3 cells synchronized by serum starvation and with HeLa cells synchronized with a thymidine/aphidicoline double block (data not shown). Thus, while dramatic changes in the subcellular localizaton of tankyrase are observed across the cell cycle, the level of tankyrase protein remains relatively unchanged.

Localization of exogenous tankyrase to telomeres is TRF1 dependent

The absence of a nuclear localization signal in tankyrase combined with its complex pattern of subcellular localization suggested that localization of tankyrase to telomeres might be regulated. One possibility is that the nuclear import of tankyrase is dependent on its interacting partner, TRF1, which is a nuclear protein harboring a candidate NLS. To investigate this possibility, the effect of TRF1 on the localization of exogenously-expressed tankyrase was determined. Full-length tankyrase was tagged with a FLAG epitope at its N terminus and expressed by transient transfection in HeLa cells. Indirect immunofluorescence with anti-FLAG antibodies indicated a cytoplasmic staining pattern for the transfected protein in interphase (Fig. 8A) and a centrosomal staining pattern in mitosis (Fig. 8G). Co-staining with TRF1 antibody showed that the transfected tankyrase did

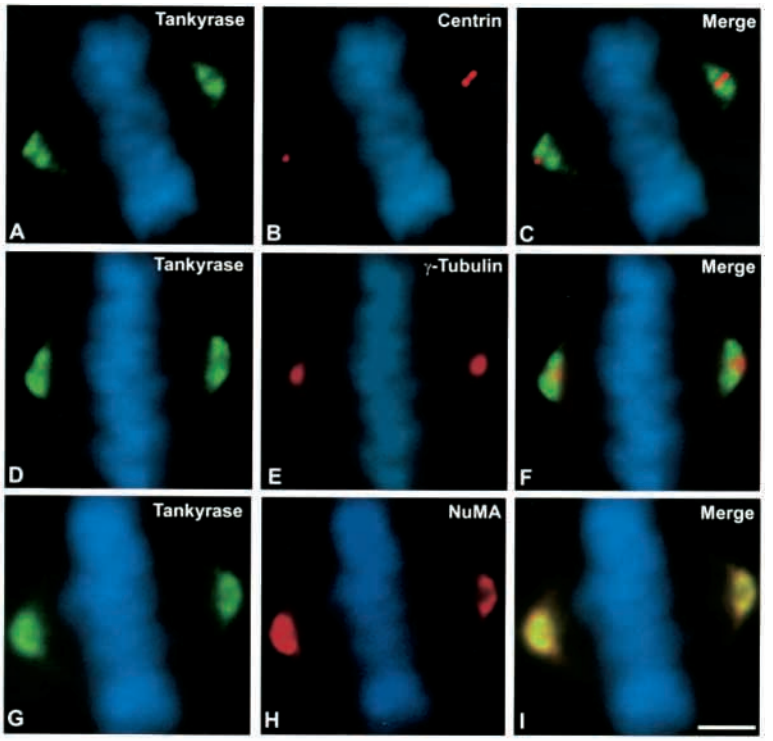

Fig. 6. Tankyrase localizes around the pericentriolar matrix in mitotic cells. Indirect immunofluorescence analysis of methanol-fixed HeLaI.2.11 cells by double-staining with anti-tankyrase antibody 465 (A, D, and G) (green) and anti-centrin antibody 20H5 (B) (red) or anti-γ-tubulin antibody GTU-88 (E) (red) or anti-NuMA antibody 1F1 (I) (red). Merge (C, F, and I) indicates superimposition of the red and green images; yellow indicates co-localization of the red and green signal. DAPI staining of DNA is shown in blue. Bar, 2 μm.

not co-localize with the endogenous TRF1 to telomeres and, in fact, was predominantly excluded from the nucleus (Fig. 8C) and mitotic chromosomes (Fig. 8I).

Strikingly, when tankyrase was co-transfected with a cDNA expressing full length human TRF1, its pattern of localization was altered. In interphase cells, a substantial fraction of the exogenous FLAG-tankyrase was translocated from the cytoplasm to the nucleus (Fig. 8D) where it co-localized with TRF1 in a punctate pattern (Fig. 8F) consistent with localization to telomeres. Similarly, in co-transfected mitotic cells, FLAG-tankyrase co-localized with TRF1 on mitotic chromosomes in a telomeric staining pattern (Fig. 8L). Note that in these experiments the anti-TRF1 antibodies did not distinguish between exogenous and endogenous TRF1. However, TRF1-transfected cells were easily recognized by the increased level of TRF1 expression.

These experiments indicate that localizaton of exogenously expressed tankyrase to telomeres is dependent upon co-expression of TRF1.

DISCUSSION

TRF1 recruits tankyrase to telomeres

Our study indicates that tankyrase displays a complex pattern of subcellular locations across the cell cycle. Tankyrase localizes to telomeres throughout the cell cycle, but in addition, is found at the nuclear envelope in interphase and centrosomes during mitosis. Indeed, it appears that only a minor fraction of total cellular tankyrase resides in the nucleus at telomeres. Inspection of the primary sequence of tankyrase does not reveal a convincing match to a consensus monopartite or bipartite nuclear localization signal (NLS) (Dingwall and Laskey, 1991), raising the question of how tankyrase gets into the nucleus. Our data indicate that, in fact, transfected tankyrase is excluded from the nucleus. The demonstration that co-transfection of TRF1 with tankyrase results in translocation of tankyrase to the nucleus, suggests the possibility of a 'piggy back' mechanism. Thus, newly synthesized TRF1, which contains two overlapping bipartite NLSs (Chong et al., 1995), could bind to the ANK repeat domain in tankyrase and carry the protein to telomeres. Interestingly, a recent report identified ANK repeats within several different proteins as cis-acting NLSs (Sachdev et al., 1998). Thus, perhaps a more general function of ANK repeat domains is to mediate interaction between a non NLS-containing ANK repeat protein with an NLS-containing protein, thereby allowing regulated import of the former by the latter. In this scenario, tankyrase localization to telomeres could be tightly regulated by TRF1 synthesis.

An alternative and not necessarily exclusive mechanism of tankyrase localization to telomeres could occur at mitosis, when the nuclear envelope breaks down and nuclear pore complexes are disassembled. Breakdown of the nuclear envelope would remove the barrier excluding tankyrase from telomeres and disassembly of nuclear pore complexes would release complexed tankyrase, thereby allowing access of tankyrase to telomere-bound TRF1.

Tankyrase at nuclear pore complexes

Immunogold electronmicroscopy showed that tankyrase was located specifically at the tips of the fibers that emanate from nuclear pore complexes into the cytoplasm (Fig. 5). This location is likely to be the entry site for transport of proteins through nuclear pores. Two other mammalian proteins have been localized to the tips of the cytoplasmic fibers, SUMO1-

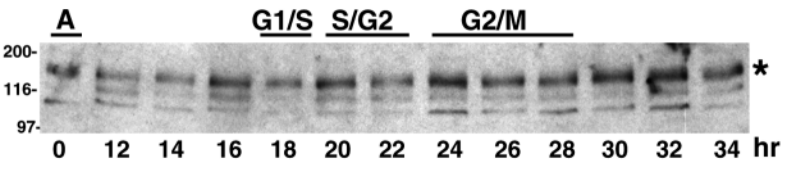

Fig. 7. Expression of tankyrase is constant across the cell cycle. Immunoblot analysis of whole cell extracts prepared from WI38 cells that were arrested by contact inhibition and released by subculturing for the indicated times in hours (hr). Samples were fractionated on 10% SDS-PAGE transferred to nitrocellulose and probed with anti-tankyrase antibody. A, indicates the arrested culture and G_1/S, S/G_2, G_2/M indicate stages of the cell cycle based upon FACS analysis at the time of harvest. Immunoreactive tankyrase is indicated by an asterisk.

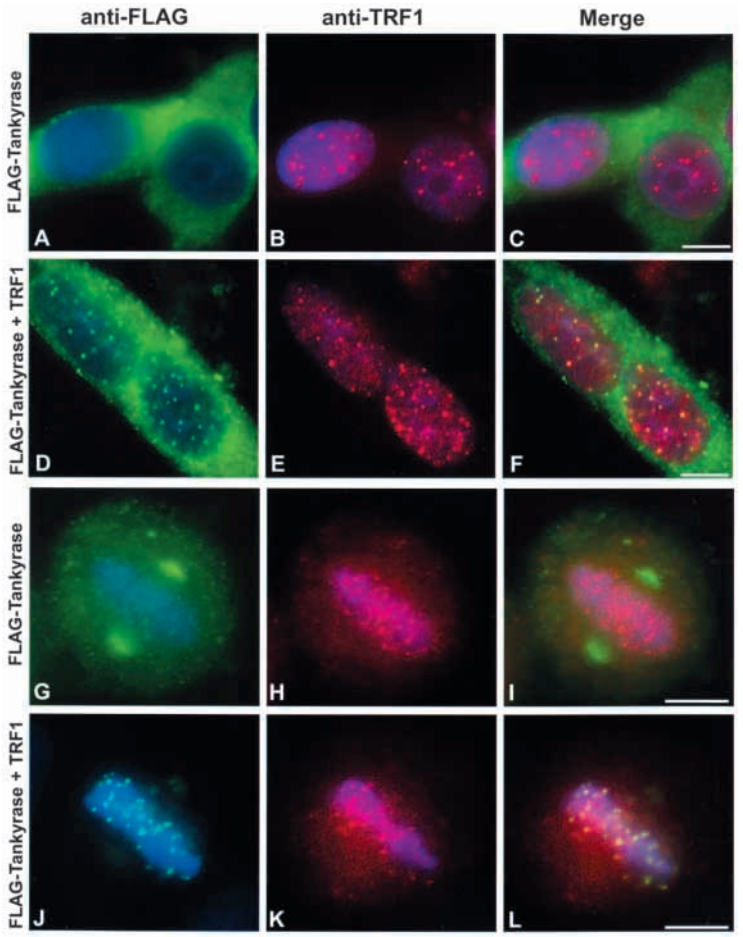

Fig. 8. Localization of exogenous tankyrase to telomeres is dependent on TRF1. Indirect immunofluorescence analysis of methanol-fixed HeLaI cells transfected with FLAG-Tankyrase (A-C and G-I) or co-transfected with FLAG-Tankyrase + TRF1 (D-F and J-L) by double-staining with anti-FLAG antibody M2 (A,D,G,J) (green) and anti-TRF1 antibody 371 (B,E,H,K) (red). (Merge) (C,F,I,L) indicates superimposition of the red and green images; yellow indicates colocalization of the red and green signal. DNA is stained with DAPI (blue). Bars, 5 μm.

modified RanGAP1 which, like tankyrase, also localizes to mitotic centrosomes (Mahajan et al., 1997; Matunis et al., 1996) and the nucleoporin Nup358 (Wu et al., 1995; Yokoyama et al., 1995).

What is the significance of tankyrase's localization to the port of entry for nuclear traffic? Tankyrase could play a structural role at this site and (like ankyrins) serve as a linker between the cytoplasmic fibers of the nuclear pore complex and the cytoskeleton. At this location, its PARP activity could play a role in regulating nuclear transport. Alternatively, tankyrase's location at nuclear pore complexes may serve to provide a ready pool of tankyrase waiting to be picked up by TRF1 and translocated through nuclear pores to telomeres, thus allowing its localization to telomeres to be tightly controlled by TRF1.

Tankyrase at the centrosome: possible relevance to meiosis

Indirect immunofluorescence analysis indicates that tankyrase is not an integral component of centrosomes per se, but rather that it is located around the pericentriolar matrix where it colocalizes with NuMA (Fig. 6I). NuMA exists in a complex with cytoplasmic dynein and dynactin and appears to be required for mitotic spindle pole assembly and stabilization (Merdes et al., 1996). Tankyrase could be modifying these or other centrosomal proteins. The role of tankyrase (if any) in mitotic spindle function or stability remains to be determined.

To our knowledge this is the first report of a protein that localizes to both telomeres and centrosomes. At first glance it is difficult to imagine a connection between these two structures. Normally it is not telomeres, but rather, centromeres that associate with the mitotic centrosome. However, association between telomeres and the centrosome does occur during meiosis. In mammalian cells during prophase of meiosis I (in a process that may be essential for the pairing and subsequent recombination of homologous chromosomes), telomeres attach to the nuclear envelope and gather at one pole of the nucleus to form the bouquet structure (Bass et al., 1998; Scherthan et al., 1996). The base of the bouquet is always juxtapositioned to the centrosome and early cytological evidence indicates a connection between the centrosome and telomeres (reviewed by Dernburg et al., 1995). Interestingly, there is also a massive clustering of nuclear pore complexes to the site of chromosome attachment (Church, 1976). Tankyrase could play multiple roles in the generation of the bouquet structure. First, tankyrase could play a structural role (like ankyrins) and mediate attachment of telomeres to the nuclear envelope. Second, tankyrase could act as a sink at the centrosome to recruit telomeres to the base of the bouquet. Consistent with a proposed role in meiosis, we observed abundant and alternative tankyrase transcripts in testis tissue. In addition, immunoblot analysis on purified cell populations from rat testis indicated that tankyrase was highly expressed in meiotic prophase I (S. Smith, P. Morris and T. de Lange, unpublished). Although it is not yet known if TRF1 functions in meiosis, its ability to promote parallel pairing of telomeric tracts in vitro (Griffith et al., 1998) would be consistent with such a role. Interestingly, Taz1p, the *S. pombe* telomeric protein with structural and functional similarity to TRF1, was recently found to play a critical role in prophase of meiosis I, during the horse tail stage. Here telomeres cluster at the spindle pole body (SPB, the yeast equivalent of a centrosome) and move the nucleus to facilitate alignment of homologous chromosomes (Chikashige et al., 1994, 1997). Taz1p is involved in connecting telomeres to the SPB, the horse-tail movement, and the subsequent segregation and recombination of homologous chromosomes (Cooper et al., 1998; Nimmo et al., 1998; reviewed by de Lange, 1998). It will be important to determine if TRF1 and tankyrase play similar roles in the formation of mammalian gametes.

Regulated localization of active tankyrase to telomeres

The function of tankyrase in vivo remains to be determined. However, based upon tankyrase's catalytic PARP activity and its ability to inhibit TRF1 binding to telomeres in vitro, we propose that tankyrase functions at telomeres to control accessibility and/or activity of telomerase. If tankyrase functions at telomeres why is most of the protein found at other

subcellular sites? One possibility is that the tankyrase that we observe on telomeres is, in fact, inactive. After all, if tankyrase is catalytically active it is likely to poly(ADP-ribosyl)ate TRF1 releasing it (and therefore itself) from telomeric DNA. Localization of catalytically active tankyrase to telomeres may, in fact, be a very transient phenomenon that occurs at a discreet time in the cell cycle, perhaps when telomerase acts. Centrosomal and/or nuclear envelope tankyrase may represent pools of tankyrase waiting for the signal to go to telomeres.

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