SHORT REPORT

TRAIP is a regulator of the spindle assembly checkpoint

Christophe Chapard\(^1\), Patrick Meraldi\(^2\), Tobias Gleich\(^1\), Daniel Bachmann\(^1\), Daniel Hohl\(^1\) and Marcel Huber\(^1,\ast\)

ABSTRACT

Accurate chromosome segregation during mitosis is temporally and spatially coordinated by fidelity-monitoring checkpoint systems. Deficiencies in these checkpoint systems can lead to chromosome segregation errors and aneuploidy, and promote tumorigenesis. Here, we report that the TRAF-interacting protein (TRAIP), a ubiquitously expressed nucleolar E3 ubiquitin ligase important for cellular proliferation, is localized close to mitotic chromosomes. Its knockdown in HeLa cells by RNA interference (RNAi) decreased the time of early mitosis progression from nuclear envelope breakdown (NEB) to anaphase onset and increased the percentages of chromosome alignment defects in metaphase and lagging chromosomes in anaphase compared with those of control cells. The decrease in progression time was corrected by the expression of wild-type but not a ubiquitin-ligase-deficient form of TRAIP. TRAIP-depleted cells bypassed taxol-induced mitotic arrest and displayed significantly reduced kinetochore levels of MAD2 (also known as MAD2L1) but not of other spindle checkpoint proteins in the presence of nocodazole. These results imply that TRAIP regulates the spindle assembly checkpoint, MAD2 abundance at kinetochores and the accurate cellular distribution of chromosomes. The TRAIP ubiquitin ligase activity is functionally required for the spindle assembly checkpoint control.

KEY WORDS: TRAF-interacting protein, TRAIP, Mitosis, Chromosome mis-segregation, Spindle assembly checkpoint

INTRODUCTION

The equal distribution of chromosomal DNA during mitosis is ensured by a highly complex process in which the control of APC/C (anaphase-promoting complex/cyclosome) activity is crucial (Foley and Kapoor, 2013; Kops et al., 2005; Pines, 2011). APC/C activity is regulated by the spindle assembly checkpoint (SAC) proteins MAD1, MAD2 (also known as MAD1L1, MAD2L1, respectively), BUBR1 (also known as BUB1B), BUB1, BUB3 and MPS1 (also known as TTK), which sense whether or not chromosomes are connected in a bi-oriented manner to opposite spindle poles through microtubules (Foley and Kapoor, 2013). The strength of the SAC crucially depends on the existence of a role for TRAIP in SAC regulation.

RESULTS AND DISCUSSION

TRAIP preferentially localizes at the periphery of chromosomes in early mitosis

Analysis of endogenous TRAIP expression by immunofluorescence detection in mitotic cells showed localization around prometaphase chromosomes and metaphase, and on chromosomal DNA in anaphase. Its functional inactivation accelerates the progression through early phases of mitosis, reduces MAD2 recruitment to kinetochores and leads to increased mitotic defects. Our findings strongly support the existence of a role for TRAIP in SAC regulation.

Received 4 March 2014; Accepted 10 October 2014

\(^{1}\)Service of Dermatology, Lausanne University Hospital, CHUV, 1011 Lausanne, Switzerland. \(^{2}\)Department of Cell Physiology and Metabolism, University of Geneva, 1211 Geneva, Switzerland.

\(^{\ast}\)Author for correspondence (marcel.huber@chuv.ch)
Fig. 1. See next page for legend.
et al., 2013), the signal became nucleolar in controls and disappeared from TRAIP-depleted cells. The signal in mitotic cells was localized close to congressing chromosomes in prometaphase and was associated with condensed chromosomes in anaphase in controls but was strongly reduced in siTRAIP-treated cells (Fig. 1C).

To further validate TRAIP localization, a lentiviral vector driving expression of a functional (supplementary material Fig. S2; Besse et al., 2007; Zhou and Geahlen, 2009) TRAIP–GFP was used to infect HeLa cells stably expressing α-tubulin–mRFP and H2B–CFP (Beronja et al., 2010). In early mitotic phases, TRAIP–GFP was dispersed throughout the cytoplasm and converged at the chromosome periphery, whereas in later phases it colocalized with chromosomes (Fig. 1D). During telophase, a small fraction accumulated in cytoplasmic particles, probably corresponding to nucleolus-derived foci (NDF) and pre-nuclear bodies (PNB) (Dundr et al., 1997; Ma et al., 2007; Van Hooser et al., 2005).

The perichromosomal layer of TRAIP appeared in prometaphase and disappeared in telophase or cytokinesis (Hernandez-Verdun and Gautier, 1994; Ma et al., 2007; Van Hooser et al., 2005). Nucleolar proteins and RNAs are known to bind to the surface of chromosomes at the beginning of mitosis and are incorporated again into newly formed nucleoli during telophase (Dundr et al., 1997; Van Hooser et al., 2005). Different biological functions have been assigned to chromosomal peripheral proteins (CPP) forming the perichromosomal layer (Hernandez-Verdun, 2011; Hernandez-Verdun and Gautier, 1994; Ma et al., 2007; Matsunaga and Fukui, 2010). Our analysis identified the nucleolar protein TRAIP as a CPP and prompted us to investigate TRAIP function in mitosis.

TRAIP regulates progression through early phases of mitosis

We examined the consequences of silencing TRAIP expression on the duration of mitosis using siRNAs and fluorescence time-lapse imaging of HeLa cells expressing H2B–EGFP and α-tubulin–mRFP (Toso et al., 2009), taking nuclear envelope breakdown (NEB) as starting time, \( t = 0 \) min (Meraldi et al., 2004). In siCTRL-transfected cells, the median duration of mitosis was 116.7 min [95% confidence interval (CI) = 111.7–123.3 min; range, 75 to 275 min]. The high variability of duration of mitosis was mainly caused by the time between NEB to anaphase onset (Lim et al., 2013; McEdlishvili et al., 2012; Rieder et al., 1994; Toso et al., 2009), which varied from 20 to 200 min (median, 53.3 min; 95% CI = 46.7–61.7 min). The time from anaphase to cytokinesis fluctuated only moderately from 45 to 85 min (median, 63.3 min; 95% CI = 60–66.7 min) (Fig. 2A–D).

The average time taken to complete mitosis by siTRAIP1- or siTRAIP2-treated cells was reduced to 96.7 min (95% CI = 95–101 min) or 90 min (95% CI = 87.7–91.7 min), respectively (Fig. 2A). Although the time from anaphase to cytokinesis with siTRAIP1 (range, 45 to 95 min; median, 66.7 min; 95% CI = 63.3–70 min) and siTRAIP2 (range, 45 to 80 min; median, 58.3 min; 95% CI = 56.7–63.3 min) was very similar to that of control cells, the time from NEB to anaphase onset was significantly reduced to 30 min (95% CI = 28.3–33.3 min; range, 20 to 75 min) with siTRAIP1 and 26.7 min (95% CI = 26.7–28.3 min; range, 20 to 85 min) with siTRAIP2 (Fig. 2B,C). In total, 90% of TRAIP-depleted cells accomplished the onset of anaphase within 45 min after NEB compared to only 50% of control cells (Fig. 2E). In the skew-normal frequency distribution of anaphase onset times, a first population (peak of the distribution) with rapid and uniform chromosome congression and a second with longer anaphase onset times (active spindle checkpoint) could be distinguished (Meraldi et al., 2004; Rieder et al., 1994). In control siRNA-transfected HeLa cells, the skew-normal distribution of anaphase onset times had a peak (mode) at 31.8±1.2 min, whereas in TRAIP-depleted cells a shift to 28.4±0.4 min (siTRAIP1; mean±s.e.m.) or 24.2±0.4 min (siTRAIP2) was observed (Fig. 2F). The NEB to anaphase onset time in cells transduced with siRNA-resistant TRAIP before siRNA transfection was indistinguishable from that of the control, whereas a catalytically inactive TRAIP mutant (C25A) failed to rescue the siRNA-treated cells (Fig. 2G). Although MAD2 is a frequent siRNA off-target (Hübner et al., 2010; Sigiozzi et al., 2012; Westhorpe et al., 2010), the expression of MAD2, MAD1, BUB1 and BUB3 remained unchanged after TRAIP KD (supplementary material Fig. S1B,C). Taken together with the rescue data it is therefore unlikely that TRAIP-knockdown phenotypes are caused by off-target effects. These findings suggest that the ubiquitin ligase TRAIP is involved in the regulation of NEB to anaphase onset time.

TRAIP loss increases the occurrence of chromosome alignment defects and lagging chromosomes

Because a reduced NEB to anaphase onset time could induce aberrant chromosome segregation (Meraldi and Sorger, 2005; Michel et al., 2001; Perera et al., 2007), we examined whether TRAIP depletion affected chromosome behavior. Following TRAIP depletion, the number of cells dividing with a bipolar spindle and giving rise to two living daughter cells was not significantly different from that of control cells. Mitotic indexes in siRNA-treated cells were examined by immunostaining with anti-phospho-histone H3(Ser28) and anti-α-tubulin antibodies. Although mitotic indexes were comparable to those of controls (Fig. 3A), the percentage of cells in prophase was reduced by 10–20% in TRAIP-depleted compared with control cells (Fig. 3B), consistent with shorter NEB to anaphase onset times. In TRAIP-depleted mitotic cells synchronized by a double-thymidine block, the percentage of non-aligned chromosomes at the metaphase plate was increased compared with that of the control (siRNA1, 38.5±4.9%; siRNA2, 37.1±7.6%; control, 19.6±7.5%; mean±s.d) (Fig. 3C,E). More chromosome segregation errors were detected in mitotic cells treated with either siTRAIP1 or 2 compared with the number observed in control cells (15.7±4.5% or 19.5±6.7% versus 9.7±2.9%; mean±s.d) (Fig. 3D,F). As expected, the number of chromosome alignment and segregation errors was increased in MAD2-depleted cells, supporting the reliability of our data (Fig. 3E,F). In summary, TRAIP downregulation in HeLa cells leads to chromosome misalignment and segregation defects.
Fig. 2. See next page for legend.
TRAIP loss decreases SAC function

Our results demonstrated that early mitosis and chromosome behavior are affected by TRAIP knockdown, suggesting a role of TRAIP in the SAC. To investigate whether TRAIP knockdown enabled HeLa cells to bypass the SAC, a taxol-induced mitotic arrest assay (Stegmeier et al., 2007a) was undertaken to assess mitotic index and nuclear morphology. CYLD- and MAD2-specific siRNAs were used as controls for mitotic entry delay and SAC bypass (Draviam et al., 2007; Meraldi et al., 2004; Stegmeier et al., 2007a; Stegmeier et al., 2007b). Only 13.6 ± 8% (mean ± s.d.) of MAD2-depleted or 51.6 ± 15% of CYLD-depleted cells were mitotic (Fig. 4A,B) (Stegmeier et al., 2007b), whereas the value was higher for control cells (74 ± 7%). In TRAIP-depleted cells, a reduced mitotic index (siRNA1, 52 ± 16%; siRNA2, 53 ± 8%) (Fig. 4B) and multilobed nuclear morphology (Stegmeier et al., 2007a; Stegmeier et al., 2007b), similar to that of cells treated with MAD2-specific siRNA (Fig. 4A,C), was observed, demonstrating that loss of TRAIP led to a checkpoint-bypass phenotype. However, TRAIP depletion led to a smaller effect than MAD2 knockdown. Whether this reflects a phenotypic difference or is due to differences in the degree of mRNA knockdown remains to be seen. Our data indicated that TRAIP depletion was less effective compared with MAD2 knockdown (supplementary material Fig. S1A). To summarize, loss of TRAIP reduced mitotic arrest in response to the spindle poison taxol, hence leading to the bypass of the SAC.

TRAIP depletion decreases MAD2 levels at unattached kinetochores

The expression and/or localization of SAC proteins are finely modulated to regulate APC/C activity (Musacchio and Salmon, 2007) in a dose-dependent manner (Collin et al., 2013; Heinrich et al., 2013). The robustness of the SAC is, in part, determined by the amount of MAD2 recruited to kinetochores. To elucidate whether TRAIP deficiency is affecting the kinetochore localization...
Fig. 4. See next page for legend.
The spindle checkpoint, in particular, is a mechanism that monitors the proper attachment of chromosomes to the mitotic spindle. The spindle checkpoint ensures that all chromosomes are captured by the spindle before cells proceed to anaphase. This ensures the faithful segregation of genetic material into daughter cells. TRAIP, a ubiquitin ligase, has been implicated in the regulation of the spindle checkpoint. In this study, the authors investigated the role of TRAIP in the spindle checkpoint by using siRNA-mediated knockdown of TRAIP in HeLa cells.

The authors first confirmed the downregulation of TRAIP expression after siRNA treatment, as demonstrated by immunoblotting. Following siRNA treatment, the cells were arrested at mitosis by nocodazole treatment, allowing the authors to analyze the effects of TRAIP knockdown on the spindle checkpoint. They observed that cells with reduced TRAIP levels showed defects in the spindle checkpoint, as evidenced by the misalignment of chromosomes and the activation of the spindle assembly checkpoint (SAC) proteins.

The authors further analyzed the SAC machinery by examining the levels of various SAC proteins in TRAIP-depleted cells. They found that the levels of SAC proteins such as MAD1, MAD2, BUB1, BUBR1, and BUB3 were reduced in TRAIP-depleted cells, indicating that TRAIP is required for the proper function of the SAC.

The authors also used a live-cell imaging approach to visualize the SAC proteins and their interactions with chromosomes. They observed that the SAC proteins were mislocalized in TRAIP-depleted cells, suggesting that TRAIP is essential for the correct localization of the SAC proteins.

In summary, the authors have demonstrated that TRAIP plays a crucial role in the spindle checkpoint by controlling the levels of SAC proteins and their localization. This study provides insights into the molecular mechanisms underlying the spindle checkpoint and highlights the importance of TRAIP in this process.
References


**Supplementary Figures**

**Supplementary Figure S1: RNA interference in HeLa cells.** Analysis of mRNA (A-C) and protein (A-B) levels were analyzed by quantitative PCR and immunoblot 24h after siRNA-transfection of HeLa cells. Results are reported as representative mean±s.d. of 2 or more independent experiments.

**Supplementary Figure S2: TRAIP-GFP is a functional fusion protein.** (A) Western blot of TRAIP-GFP (IB: TRAIP) and HA-ubiquitin (IB: HA) after immunoprecipitation of TRAIP-GFP from 293T cells transfected with pCMV-TRAIPGFP and/or pHA-Ub. B) Confocal images of cells after immuno-staining for GFP (green) and nucleolin (red). Scale bar = 10µm.
Suppl. Figure S1

A) TRAIP protein levels

- siCTRL
- siTRAIP1
- siTRAIP2

TRAIPI

Actin

B) MAD2 mRNA levels

- siCTRL
- siTRAIP1
- siTRAIP2

MAD2 protein levels

- siCTRL
- siTRAIP1
- siTRAIP2

MAD2

Actin

C) MAD1 mRNA levels

- siCTRL
- siTRAIP1
- siTRAIP2

Bub1 mRNA levels

- siCTRL
- siTRAIP1
- siTRAIP2

Bub3 mRNA levels

- siCTRL
- siTRAIP1
- siTRAIP2