Biphasic recruitment of TRF2 to DNA damage sites promotes non-sister chromatid homologous recombination repair

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Summary Statement

TRF2 is recruited to damage sites by a two-step mechanism and functions in non-sister chromatid homologous recombination repair

Abstract

TRF2 is a shelterin component critical for telomere integrity. While TRF2 directly recognizes and binds telomeric repeats, evidence suggests that it also localizes to non-telomeric DNA damage sites. However, this recruitment appears to be precarious and functionally controversial. We find that TRF2 recruitment to damage sites occurs by a two-step mechanism: the initial rapid recruitment (phase I) and stable and prolonged association with damage sites (phase II). Phase I is poly(ADP-ribose) polymerase (PARP)-dependent and requires positively charged amino acid residues in the N-terminal domain. The phase II recruitment is through the C-terminal MYB/SANT domain and requires the iDDR region in the hinge domain of TRF2. Phase II is mediated by the MRE11 complex and is stimulated by hTERT. PARP-dependent recruitment of intrinsically disordered proteins contributes to transient displacement of TRF2 that separates phases I and II. TRF2 depletion specifically affects non-sister chromatid homologous recombination (HR) repair, but not HR between sister chromatids or classic and alternative non-homologous endjoining. Our results demonstrate a unique recruitment mechanism and function of TRF2 at non-telomeric DNA damage sites.

Introduction

TRF2 is an integral component of the telomere shelterin complex that protects telomere integrity (Bilaud, et al., 1997, Feuerhahn, et al., 2015, Okamoto, et al., 2013, van Steensel, et al., 1998). TRF2 recognizes telomere repeat sequence (TTAGGG) directly through its C-terminal MYB/SANT domain, and protects telomeres by both promoting T-loop formation and inhibiting the DNA damage checkpoint kinase, ataxia-telangiectasia-mutated (ATM) (de Lange, 2002, Griffith, et al., 1999, Karlseder, et al., 2004, van Steensel, et al., 1998). TRF2 was also shown to be recruited to non-telomeric DNA damage sites and promotes DNA double-strand break (DSB) repair, though its exact role in the process remains controversial. TRF2 recruitment was observed at high-irradiance laser-induced DNA lesions, but not at damage sites induced by low-irradiance ultraviolet (UV) radiation or ionizing radiation, despite the presence of DSBs in both cases (Bradshaw, et al., 2005, Huda, et al., 2012, Williams, et al., 2007). TRF2 was linked to homologous recombination (HR) repair (Mao, et al., 2007), but its phosphorylation by ATM appears to be important for fast repair (suggested to be non-homologous endjoining (NHEJ)) (Huda, et al., 2009). Thus, TRF2 recruitment and function at non-telomeric DNA damage sites remain enigmatic.

PARP1 is a DNA nick sensor activated rapidly and transiently in response to DNA damage (for reviews (Ball and Yokomori, 2011, Beck, et al., 2014, Daniels, et al., 2015, Kalisch, et al., 2012)). There are multiple PARP family members, but PARP1 plays a major role in PAR response at damage sites (Cruz, et al., 2015, Kong, et al., 2011). Activated PARP1 uses NAD^+ as a substrate to ADP-ribosylate multiple target proteins,

including itself. Although PARP1 was initially thought to function together with XRCC1 to specifically facilitate base excision repair (BER)/single-strand break (SSB) repair, recent studies reveal its role in multiple DNA repair pathways, including DSB repair (Beck, et al., 2014). Furthermore, PAR modification at DNA damage sites is critical for the recruitment of chromatin modifying enzymes that promote DNA repair (Ahel, et al., 2009, Ayrapetov, et al., 2014, Ball and Yokomori, 2011, Chou, et al., 2010, Gottschalk, et al., 2009, Izhar, et al., 2015, Khoury-Haddad, et al., 2014, Larsen, et al., 2010, Polo, et al., 2010, Smeenk, et al., 2010, Sun, et al., 2009). Thus, PARP1 is not only a sensor of DNA damage, but also has an emerging role as a regulator of damage site chromatin environment and multiple DNA repair pathways in higher eukaryotes.

Recently, we obtained evidence that TRF2 recruitment to damage sites is dependent on PARP1 activity, distinct from telomere targeting (Cruz, et al., 2015). This raised the possibility that TRF2 is a new downstream effector of PARP signaling in DNA repair. In the current study, we determined the unique mechanism of DNA damage recognition and repair function of TRF2 at non-telomeric DNA damage sites, providing important insight into the significance of TRF2 in DNA repair.

Results and Discussion

TRF2 recruitment to damage sites is determined by the degree of PARP activation

In our previous study, TRF2 was rapidly recruited to high input-power laser damage sites that contained complex DNA damage in a PARP-dependent manner (Cruz, et al., 2015). In contrast, low input-power laser that induced relatively simple strand-breaks and no significant PAR response failed to recruit TRF2 to damage sites (for laser power measurements, see Experimental Procedures). Importantly, stimulation of PARylation at low input-power laser damage sites by a PARG inhibitor promotes TRF2 recruitment (Fig. 1A). The results demonstrate that the level of PARP activation, rather than the nature of damage per se, is the deciding factor for TRF2 recruitment to non-telomeric DNA damage sites.

Biphasic recruitment of TRF2 to damage sites

We and others observed rapid and transient TRF2 recruitment to damage sites within the first 5 min post irradiation (p.i.) (Bradshaw, et al., 2005, Cruz, et al., 2015) (Fig. 1). Upon inspection of later time points (20-30 min), however, we found that TRF2 re-appears at damage sites (Fig. 1B and C). Similar recruitment patterns were observed with both the recombinant and endogenous TRF2 (Fig. 1B and D, respectively). The initial recruitment of GFP-TRF2 peaks at ~1-3 min p.i. (termed “phase I”), which decreases once but returns peaking at ~30 min to 1 hr (termed “phase II”) (Fig. 1A-C). This phase II recruitment persists for ~two hrs (data not shown). Interestingly, the PARP inhibitor NU1025 and olaparib completely suppressed phase I, but not phase II, recruitment of both endogenous and recombinant TRF2 (Fig. 1D and

E). Distinct sensitivity to PARP inhibition suggests that two phases of TRF2 recruitment are mediated by different mechanisms.

IDPs compete with TRF2 for PARylated DNA lesions

We found that transient GFP-TRF2 displacement is inversely correlated with the appearance of a prominent dark line at damaged lesions readily visible using bright field microscope imaging (Fig. 1B and F). Close examination at damage sites revealed that GFP signals not only decrease, but are often transiently pushed to the periphery of the damage sites, suggesting that it may be displaced by the constituents of the dark line (Fig. 1B and F). Intrinsically disordered proteins (IDPs), FUS, EWS and TAF15 (FET), were shown to accumulate at damage sites in a PAR-dependent manner and are the major components of the dark line (Altmeyer, et al., 2015). We found that depletion of FET by siRNA resulted in a more even distribution of the GFP-TRF2 signal at damaged lesions, correlating with disappearance of the dark line (Fig. 1F). The results reveal that although both TRF2 and IDPs are recruited to damage sites in a PAR-dependent manner, there is a distinct order of appearance and competition between them, which contributes to the rapid disappearance of TRF2 from damage sites.

Phases I and II recruitments are mediated by distinct domains and mechanisms

TRF2 protein domains have been characterized extensively in the context of telomeres. The C-terminal MYB/SANT DNA binding domain of TRF2 specifically recognizes and binds telomere DNA whereas the N-terminal basic domain is not required for telomere targeting (Fig. 2A) (Karlseder, et al., 1999, Okamoto, et al., 2013).

We found that the phase I recruitment is completely abolished by deletion of the N-terminal basic domain similarly to that previously reported (Bradshaw, et al., 2005) (Fig. 2B and C). In contrast, phase II, but not phase I, recruitment was significantly inhibited by deletion of the C-terminal MYB/SANT domain. Deletion of both N- and C-terminal domains abolished both phases of damage site recruitment. Thus, the N-terminal basic domain is critical for rapid and transient phase I, whereas the C-terminal MYB/SANT domain is required for slow and stable phase II, recruitment to damage sites. Unlike the previous report (Bradshaw, et al., 2005), we found that deletion of the MYB/SANT domain partially reduced phase I recruitment, suggesting that the phase I recruitment is further stabilized by DNA binding of the MYB/SANT domain (Fig. 2C).

Positive charge of the basic domain is required for phase I recruitment

The above results indicate that the N-terminal basic domain is involved in the PARP-dependent phase I recruitment. Since this domain harbors multiple arginine residues, it is possible that these positively charged amino acids may interact with negatively charged PAR residues clustered at damage sites. Indeed, arginine to alanine mutations completely abolished the phase I recruitment (Fig. 2D, “RA”). In contrast, this recruitment is sustained albeit weaker by arginine to lysine mutations that preserve the positive charge (Fig. 2D, “RK”). The basic domain was also shown to bind to the holiday junction (HJ) (Fouché, et al., 2006). However, HJ binding-defective mutation (H31A) (Poulet, et al., 2009) showed no inhibitory effect on TRF2 recruitment to damage sites (Fig. 2D). The results reveal that the positive charge is essential for the PARP-dependent TRF2 recruitment to non-telomeric DNA damage sites.

hTERT contributes to MYB/SANT-dependent phase II recruitment

Human telomerase reverse transcriptase (hTERT) is responsible for addition of telomere sequences. Although hTERT localizes specifically at telomeres, several studies indicated that it can also polymerize DNA at non-telomeric DNA ends de novo and has a distinct role in DNA damage response (DDR) and repair (Flint, et al., 1994, Gao, et al., 2008, Majerská, et al., 2011, Masutomi, et al., 2005, Morin, 1991, Ribeyre and Shore, 2013). Since the MYB/SANT domain, which is responsible for telomere repeat recognition, is pertinent to phase II recruitment, we tested the possible contribution of hTERT in the TRF2 association at DSB sites. HeLa cells, which express hTERT, were treated with siRNA specific for hTERT (Fig. 3A and Supplemental Fig. S1A). We found that the phase II recruitment of TRF2 was partially inhibited by this treatment (Fig. 3C). TRF2, however, can also be recruited to damage sites even in telomerase-negative ALT cells albeit at a lower level (e.g., U2OS cells) (Supplemental Fig. S1B). Thus, the results indicate that hTERT contributes to, but is not essential for, the MYB/SANT domain-dependent TRF2 recruitment to damage sites.

Distinct domain requirements for phase I and II recruitment

Chimeric mutants between TRF1 and TRF2 have provided important insight into the unique TRF2 function in telomere protection (Okamoto, et al., 2013). The N-terminal basic domain is unique to TRF2 (TRF1 contains the acidic domain in the N-terminus), indicating that phase I recruitment is specific to TRF2. Similarly, we found that the TRF2 TRFH domain required for dimerization (<30% homology with TRF1) is essential

for both phase I and II recruitment (Fig. 3B and C; TRFcT). Interestingly, the TRFcH mutant replacing the TRF2 hinge domain with that for TRF1 exhibited intact phase I recruitment, but failed for phase II, indicating that the hinge domain (11% homology to TRF1) is uniquely required for the latter recruitment. In contrast, the TRF2 MYB/SANT domain was found to be interchangeable with that for TRF1 for phase II recruitment (both recognize telomere repeats) (Fig. 3B and C; TRFcM).

The hinge domain contains binding sites critical for several different factors (Chen, et al., 2008, Okamoto, et al., 2013). Deletion mutant of the TIN2 binding region (a.a. 352–367) within the hinge domain (ΔTIN2) critical for TRF2 incorporation into the shelterin complex (Kim, et al., 2004, Liu, et al., 2004, Ye, et al., 2004) had no effect on damage site recruitment, suggesting that TRF2 recruitment to damage sites is independent of the shelterin complex (Fig. 3D). The hinge domain also contains a region critical for suppression of DNA damage response and telomere maintenance (termed “inhibitor of DDR (iDDR)” (a.a. 406-432)) (Okamoto, et al., 2013). Deletion of this domain (ΔiDDR) recapitulated the phenotype of the TRFcH mutant, inhibiting only the phase II recruitment, indicating that the iDDR region is pertinent to MYB/SANT-dependent TRF2 recruitment to damage sites (Fig. 3D). The iDDR region was shown to be necessary and sufficient for the TRF2 interaction with the MRE11 complex (Okamoto, et al., 2013). We found that siRNA depletion of MRE11 and NBS1, the two components of the complex, effectively reduced the phase II recruitment (Fig. 3E). The results indicate that the MYB/SANT domain-dependent phase II association of TRF2 to damage sites requires the interaction of the MRE11 complex with the TRF2 iDDR region.

TRF2 facilitates intra-chromosomal HR repair

To determine the significance of TRF2 recruitment to damage sites, the effects of TRF2 depletion were examined using the cell-based assays for different pathways of DSB repair (Hu and Parvin, 2014). TRF2 was depleted for 48 hrs before repair assays in order to minimize telomere erosion, which was typically assayed 4-7 days after depletion (Okamoto, et al., 2013, Rai, et al., 2016) (Supplemental Fig. S2). TRF2 was implicated previously in both HR repair (Mao, et al., 2007) and fast repair (suggestive of NHEJ) (Huda, et al., 2009). Interestingly, we found that TRF2 depletion reduced the efficiency of HR in the I-SceI-dependent HR assay, but not in the sister chromatid exchange (SCE) assay (Fig. 4A). The I-SceI assay selectively captures intra-chromatid or unequal sister chromatid HR whereas the SCE assay specifically detects sister chromatid conversion (Potts and Yu, 2005). Furthermore, TRF2 depletion had no significant effect on either classical or alternative NHEJ repair (Fig. 4A). Thus, the results indicate that TRF2 plays a specific role in non-sister chromatid HR. The effect of TRF2 depletion on I-SceI HR can be complemented by the wild type TRF2.

The above result is an interesting contrast to cohesin, which only promotes sister chromatid HR but not other types of HR (Kong, et al., 2014, Potts, et al., 2006). These can be explained by their mechanisms of actions. While cohesin promotes sister chromatid cohesion, therefore promoting pairing of damaged and undamaged sister chromatids for HR, TRF2 primarily promotes loop formation and strand invasion not only at telomeres T-loop, but also with non-telomeric templates in vitro, suggesting that

TRF2 has an intrinsic ability to promote intra-chromatid HR (Amiard, et al., 2007, Doksani, et al., 2013, Griffith, et al., 1999, Stansel, et al., 2001) (Fig. 4B). Furthermore, TRF2 was shown to inhibit Rad51-mediated D-loop formation with a telomeric, but not non-telomeric, template in vitro (Bower and Griffith, 2014). Thus, the cell may hijack the ability of TRF2 by clustering the protein to damage sites by PAR to promote intra-chromatid HR. Our results further indicate that this intra-chromatid HR activity is specifically promoted in the context of complex damage that robustly activates the PARP response.

Accumulating studies reveal that PARylation at damage sites plays critical roles in recruiting a multitude of chromatin modifiers and DNA binding factors that may dictate local chromatin states at damage sites, strongly suggesting that damaged chromatin is not simply modified in one way, but rather, undergoes dynamic structural changes over time. Our results demonstrate that TRF2 is one such factor whose recruitment is regulated by PARP signaling, and sequentially utilizes two different mechanisms to associate with DNA lesions (Fig. 4C), which are interrupted by PAR-dependent accumulation of IDPs. Thus, PARP triggers the cascade of dynamic recruitment of factors, some of which compete with each other, to fine-tune repair pathway choice. Interestingly, a recent study showed that BLM is also recruited to damage sites in a biphasic fashion, initially by the ATM signaling, and subsequently by the MRE11 complex (Tripathi, et al., 2018). Thus, TRF2 provides an example of such bimodal recruitment involving PARP signaling which suggests that this type of two-step mechanism may be more common than previously recognized.

Conclusion

Using well-characterized laser microirradiation conditions, we demonstrate that PARP signaling, and not the nature of DNA damage, is the critical determinant for TRF2 recruitment. We found that TRF2 is recruited to damage sites by two distinct mechanisms involving different domains and factors, and specifically functions in non-sister chromatid HR repair. Our results reconciled previous controversies and reveal uniquely regulated non-telomeric and shelterin-independent function of TRF2 in DNA repair.

Materials and Methods

Cell lines and synchronization

HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with L-Glutamine, 10% fetal bovine serum (FBS) and antibiotics. HeLa DR-GFP (for HR assay) (Hu and Parvin, 2014, Pierce, et al., 2001) and EJ2-GFP (for Alt-NHEJ assay) (Bennardo, et al., 2008) stable cells were grown in DMEM with high glucose (4500 mg/l), supplemented with 10% (v/v) FBS, 1% Pen/Strep, 1% GlutaMAX and 1.5 µg/ml Puromycin. 293/HW1 cells (for c-NHEJ assay) (Zhuang, et al., 2009) were grown in DMEM (high glucose), 1% Pen/Strep, 1% Sodium pyruvate, 1% GlutaMAX, 10% FBS, and 2 µg/ml Puromycin. HeLa cells were synchronized as previously described (Kong, et al., 2014).

TRF2 mutants

The expression plasmids containing GFP-TRF2 full-length, deletion or TRF1 chimeric mutants were kindly provided by Dr. Eros Lazzerini Denchi (The Scripps Research Institute, La Jolla, California) (Okamoto, et al., 2013). These cDNAs were also re-cloned into a pIRES vector containing N-terminal FLAG epitope. Those include TRF2ΔN (45a.a-500a.a), TRF2ΔC (1a.a -454a.a), TRF2ΔNΔC (45a.a-454a.a), ΔTIN2, and ΔiDDR (Okamoto, et al., 2013). RA and RK mutants were described previously (Mitchell, et al., 2009).

Antibodies

Mouse monoclonal antibodies specific for PAR polymers (BML-SA216–0100, Enzo Life

Sciences, Inc.), TRF2 (NB100–56506, Novus Biologicals), MRE11 (GTX70212, GeneTex, Inc.).

Immunofluorescent staining

At different time points after damage induction, cells were fixed in 4% paraformaldehyde (15 min at 4°C), permeabilized in 0.5% Triton X-100 for five min (4°C), and stained with antibodies. The staining procedure was described previously (Kim, et al., 2002). Fluorescent images were captured through a 100× Ph3 UPlanFI oil objective lens (NA, 1.3; Olympus) on a model IX81 Olympus microscope with a charge-coupled device camera.

Western blot

Protein samples were subjected to SDS-PAGE and then transferred to nitrocellulose membranes as described previously (Schmiesing, et al., 1998). The membranes were blocked with Pierce Protein-Free T20 (PBS) Blocking Buffer (Thermo Fisher Scientific). The primary antibody was incubated in 3% BSA–0.05% Tween 20 in PBS for 1hr at room temperature or overnight at 4C°, followed by three washes in PBS–0.05% Tween 20. The secondary antibody conjugated with HRP (Promega) was incubated in 3% BSA–0.05% Tween 20 in PBS for 1 hr at room temperature. The filter was then washed three times in PBS–0.05% Tween 20 and developed with SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific). Images were acquired using the Image Analyzer (LAS-4000, Fujifilm Co.) and analyzed using Quantity One.

Laser damage induction and cell imaging

Near-infrared (NIR) femtosecond laser irradiation was carried out using a Zeiss LSM 510 META multiphoton-equipped (3.0-W 170-fs coherent tunable Chameleon Ultra-NIR laser) confocal microscope. The Chameleon NIR beam was tuned to 780 nm, where the software bleach function was used to target linear tracts inside the cell nuclei for exposure to single laser scans (12.8 µs pixel dwell time) through the 100× objective lens (1.3 NA Zeiss Plan APO) (Cruz, et al., 2015). The Peak irradiance at focal for high input-power laser is $5.27X10^{10}$ W/cm^2, and for low input-power laser is $3.24X10^{10}$ W/cm^2 (Cruz, et al., 2015). Recruitment of GFP-TRF2 wt and mutant proteins to damage sites was analyzed by live-cell confocal scanning with the 488-nm CW argon laser on the same Zeiss META platform. Fluorescent measurement of the recruitment of GFP-tagged proteins to damage sites was performed by live-cell confocal scanning with the 488-nm CW argon laser on the Zeiss LSM 510 META platform. The signals were measured with the LSM510 software (version 4.0).

siRNA depletion

HeLa cells were transfected twice 24 h apart with siRNAs at a final concentration of 5 nM using HiPerFect transfection reagent according to the manufacturer's instructions (Qiagen). siRNAs directed against hTERT (5 -TTTCATCAGCAAGTTTGGA -3) (Masutomi, et al., 2005), MRE11 (5 -GCTAATGACTCTGATGATA-3) (Myers and Cortez, 2006), NBS1, (5'-GAAGAAACGTGAACTCAAG-3') (Myers and Cortez, 2006), hTRF2 (SI00742630, Qiagen), and a negative-control siRNA (Qiagen) were used. Cells were harvested for western blot analyses or were subjected to laser microirradiation,

approximately 48 h after the final transfection.

Inhibitor treatment

20 μM PARP inhibitor olaparib (Apexbio Technology) or 1 μM PARG inhibitor (PARGi) DEA ((6,9-diamino-2-ethoxyacridine lactate monohydrate) (Trevigen)) was added to the cell culture one hour prior to damage induction. DMSO only was added to control cells.

DSB repair assays

Homologous Recombination and Alt-NHEJ assays were performed as described previously in HeLa cells (Bennardo, et al., 2008, Hu and Parvin, 2014) with modification. Briefly, on day 1, the appropriate cell lines were seeded in 24-well plates. The next day, cells, 50% confluent, were transfected with siTRF2 or siControl. On day 3, cells were re-transfected with same siRNA for 5-6hrs, and then were transferred to 35-mm wells. On day 4, the plasmid encoding the I-SceI endonuclease was cotransfected with the mCherry-expressing plasmid to induce DSBs. The cells were examined by flow cytometry on day 7, and the ratio of GFP to mCherry was used as a measure of HR or Alt-NHEJ efficiency. The C-NHEJ assay utilizes quantitative real-time PCR and was carried out as described (Hu and Parvin, 2014, Zhuang, et al., 2009) in 293 cells with modification. The transfection procedure was as described above. For plasmid add-back in the rescue assays, the transfection procedure was the same except that at the second siRNA transfection, the blank control plasmid, plasmids encoding Flag-TRF2 and corresponding mutants were co-transfected into the cells using Lipofectamine 2000 (Thermo Fisher Scientific).

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Figure legends

Figure 1. Regulation on biphasic TRF2 recruitment to non-telomeric damage sites.

A. PAR stimulation by PARG depletion promotes GFP-TRF2 accumulation at low input-power damage sites. Scale bar = 10 μm. Quantification of the relative increase of GFP signals at damage sites is shown on the right.

B. Time course analysis of GFP-TRF2 recruitment to laser-induced DNA damage sites. Following the initial rapid recruitment (<1 min), GFP signal is transiently pushed to the damage site periphery surrounding the dark matters accumulate at damage sites (5 min), which redistributes more diffusely at damaged lesions by 30 min. Scale bar=10 μm.

C. Quantification of GFP signals at damage sites as in (A). P-values are shown in comparison to the initial (phase I) recruitment signals. N=16

D. Detection of the endogenous TRF2 at damage sites. Phase II TRF2 accumulation is variable, correlating with the intensity of the dark line at damage sites. PARP inhibition suppresses phase I, but has no effect on phase II, TRF2 recruitment. Scale bar=10 μm.

E. The effects of two different PARP inhibitors on immediate (phase I) and late (phase II) GFP-TRF2 recruitment. PARP inhibition suppresses phase I recruitment, but has no significant effect on phase II recruitment.

F. The effect of IDP depletion on dispersion of TRF2 at damage sites. Right: Time course analysis of signal intensities of GFP-TRF2 (blue) and dark line (red) in control siRNA (siControl) and FET siRNA (siFET)-transfected cells. Dispersion of the dark line by siRNA depletion of FET, one of the IDPs, partially inhibited transient delocalization of

TRF2 from damage sites. Scale bar=10 μm.

Figure 2. The N-terminal basic domain of TRF2 is responsible for PARP-dependent phase I recruitment while the C-terminal MYB/SANT domain is required for the phase II recruitment.

A. Schematic diagrams of TRF2 deletion mutants.

B. Time course analysis of damage site localization of WT and deletion mutants.

C. Quantification of the GFP signals at damage sites at 1 min (phase I) and 30 min (phase II) post damage induction. The deletion of the N-terminal basic domain (ΔN) abolished phase I, but not phase II, recruitment. The C-terminal MYB/SANT domain deletion (ΔC) partially affected phase I recruitment, and abolished phase II recruitment. Deletion of both N- and C- terminal regions (ΔNΔC) completely abrogated damage site recruitment.

D. The effects of the N-terminal amino acid substitutions on damage on phase I recruitment. Arginine to alanine mutations (RA) abolished phase I recruitment whereas the recruitment can still be seen with the Arginine to lysine substitution (RK) mutation. The HJ binding mutation (H31A) failed to affect phase I recruitment. Amino acid residues are shown on the right.

Figure 3. Phase II damage site recruitment is affected by hTERT and is dependent on the iDDR region in the hinge domain of TRF2.

A. hTERT depletion by siRNA inhibits TRF2 phase II recruitment.

B. Schematic diagrams of TRF2 mutants (Okamoto, et al., 2013).

C. Representative cell images of the recruitment of chimeric mutants to damage sites at ~1 min (phase I) and 30 min (phase II) after damage induction. Scale bar=10 μm. Quantification of GFP signal intensity increase over the background signal in the nucleus at ~1 min (phase I) and 30 min (phase II) after damage induction is shown on the right. P-values are shown at the top.

D. Comparison of iDDR and TIN2 deletion mutants at 30 min after damage induction. Scale bar=10 μm.

E. TRF2 phase II recruitment requires the MRE11 complex. MRE11 and NBS1 depletion didn't affect GFP-TRF2 phase I recruitment at damage sites, but abolished the phase II recruitment. Nonspecific siRNA was used as control (siControl). Cells were fixed and stained with anti-MRE11 antibody to confirm the depletion. Scale bar=10μm. N=20.

Figure 4. TRF2 specifically promotes non-sister chromatid HR repair

A. The effect of TRF2 depletion on different DSB repair pathways was examined using I-SceI HR, SCE, NHEJ, and alt-NHEJ assays. Complementation analysis of TRF2-depleted cells in I-SceI HR assay using the wild type TRF2.

B. Similarity between strand invasion in D-loop formation at telomeres and at DSB sites, which may both be promoted by TRF2.

C. Biphasic mechanism of TRF2 recruitment to damage sites. Phase I involves PARP-dependent recruitment through the basic domain of TRF2. Phase II is mediated by the MYB/SANT domain of TRF2, which is also dependent on the iDDR region and the Mre11 complex.

Figure 1

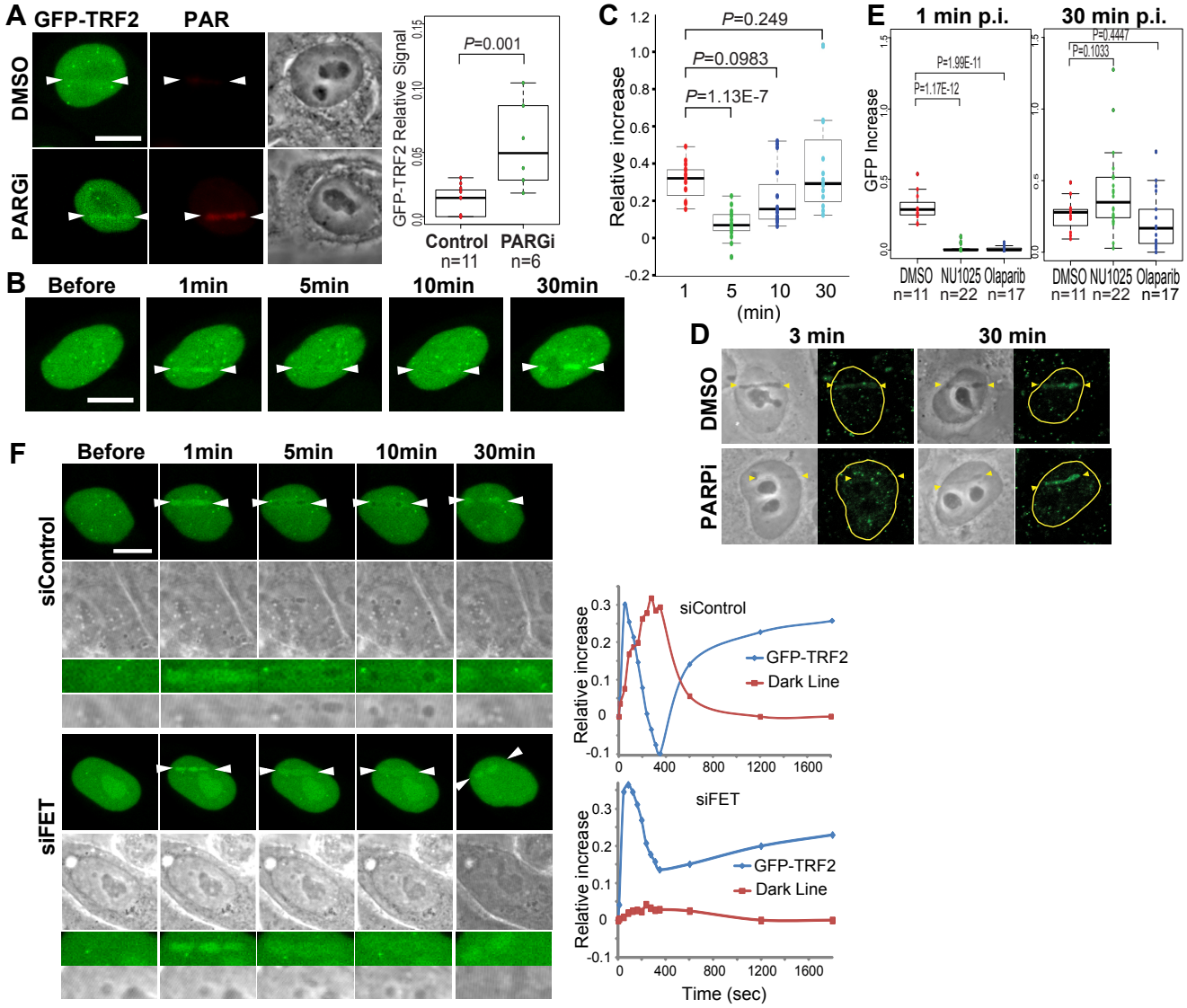

Figure 2

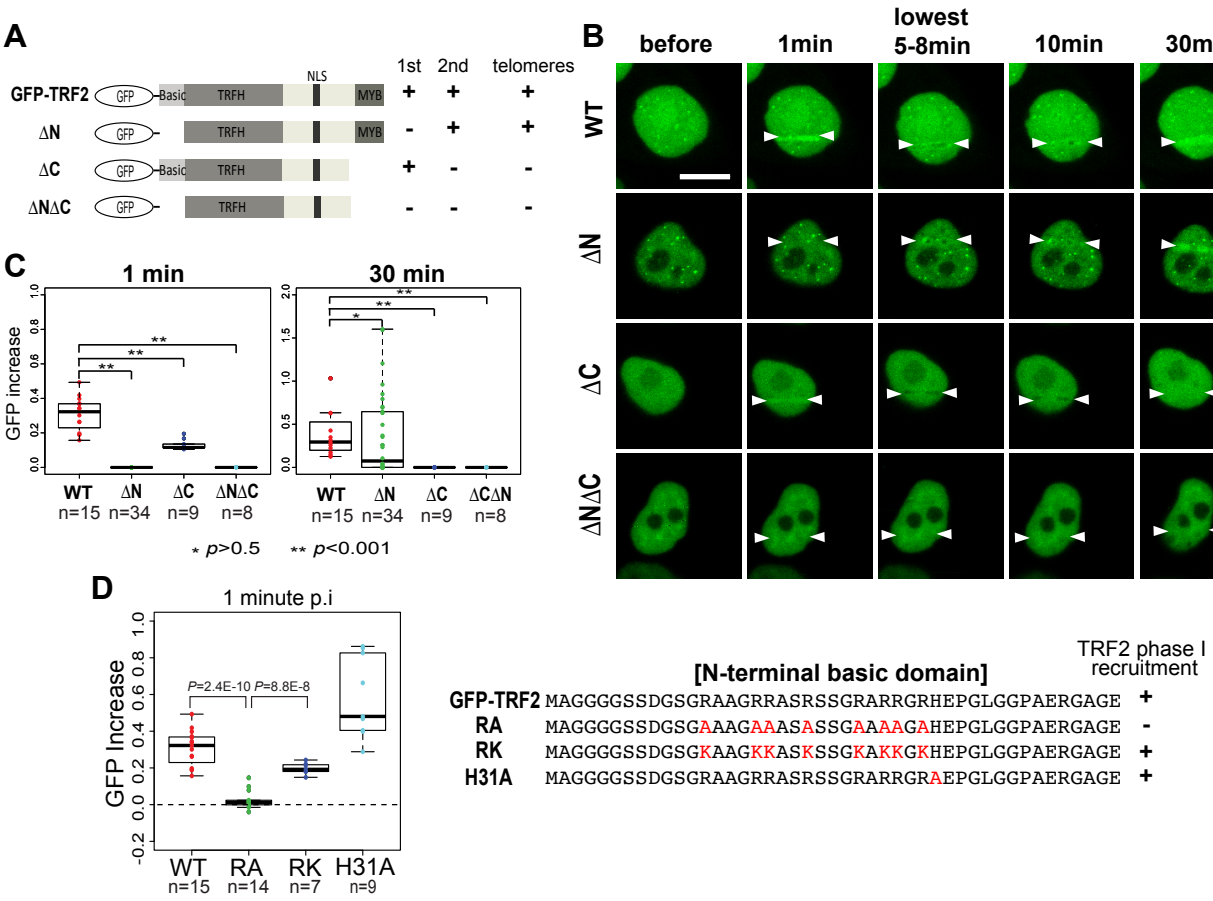

Figure 3

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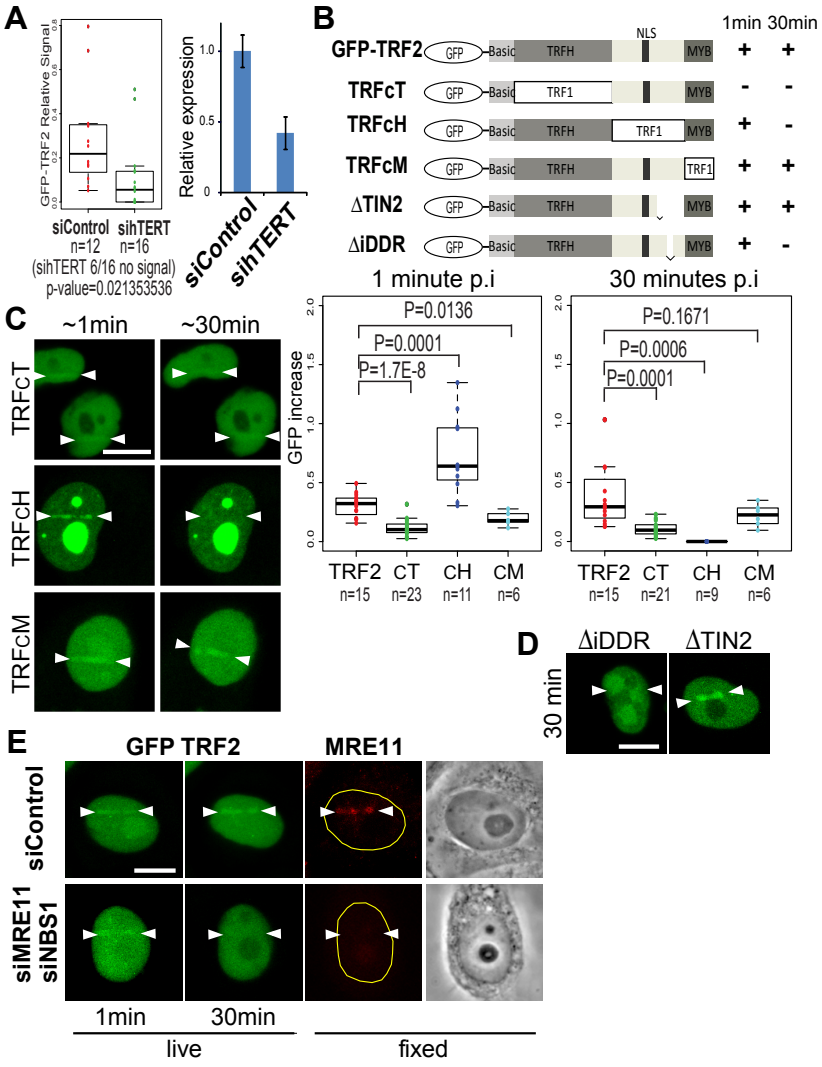

Figure 4