A New Connection at Human Telomeres: Association of the Mre11 Complex with TRF2

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It is generally assumed that the DNA damage response pathways operating in eukaryotic cells are not activated by natural chromosome ends. Eukaryotic chromosomes terminate in telomeres, nucleoprotein complexes thought to mask chromosome ends from the machinery that detects and repairs damaged DNA. Indeed, in yeast and human cells, deteriorated telomeres signal to factors that have also been implicated in the cellular response to DNA damage (Sandell and Zakian 1993; Chin et al. 1999; Karlseder et al. 1999). The recent finding that the Mre11 complex, a mediator of the cellular DNA damage response, is present at intact human telomeres (Zhu et al. 2000) contradicts the simple view that telomeres mask the chromosome end from recognition as a DNA break. Here, we discuss how the Mre11 complex could collaborate with telomeric proteins to protect chromosome ends and regulate the maintenance of telomeric DNA.

THE PROTEIN COMPONENTS OF HUMAN TELOMERES

Human telomeres are maintained by telomerase, which adds TTAGGG repeats onto the 3′ end of the chromosome, thus balancing the loss of terminal sequences that accompanies replication of linear double-stranded DNA (for review, see Greider 1996). As a consequence of their telomerase-mediated maintenance, the termini of all human chromosomes carry an array of duplex TTAGGG repeats varying in length from 2 to 30 kb. The end of this array features 75–200 nucleotides of single-stranded TTAGGG repeats forming a 3′ overhang (Makarov et al. 1997; McElligott and Wellinger 1997).

A number of telomere-specific proteins have been identified (Fig. 1) (for review, see Collins 2000). TRF1 and its paralog TRF2 are TTAGGG repeat-binding factors that carry a Myb-type helix turn helix (HTH) DNA-binding motif in the carboxyl terminus and bind double-stranded telomeric DNA as homodimers or higher-order oligomers (Chong et al. 1995; Bianchi et al. 1997; Bilaud et al. 1997; Broccoli et al. 1997; M. van Breugel et al., unpubl.). Dimerization is achieved by a large TRF-specific domain (the TRF homology domain, TRFH) in the middle of these proteins. The amino terminus of the TRFs is highly charged and contains predominantly basic amino acids in TRF2, whereas the amino terminus of TRF1 is acidic. Many copies of these ubiquitous and abundant telomere-binding proteins reside along the duplex TTAGGG repeat array in all phases of the cell cycle. TRF1 binds to TIN2 (Kim et al. 1999) and is regulated by tankyrase, a telomeric poly(ADP-ribose) polymerase (Smith et al. 1998; Smith and de Lange 2000). TRF2 is known to interact with at least one other telomeric protein, hRap1 (Li et al. 2000), a highly diverged ortholog of the yeast telomeric protein Rap1p. Proteins that bind the telomeric 3′ overhang have not been identified, although hnRNPA/UP1, which binds to single-stranded TTAGGG DNA (Ishikawa et al. 1993), has been implicated in telomere function (LaBranche et al. 1998).

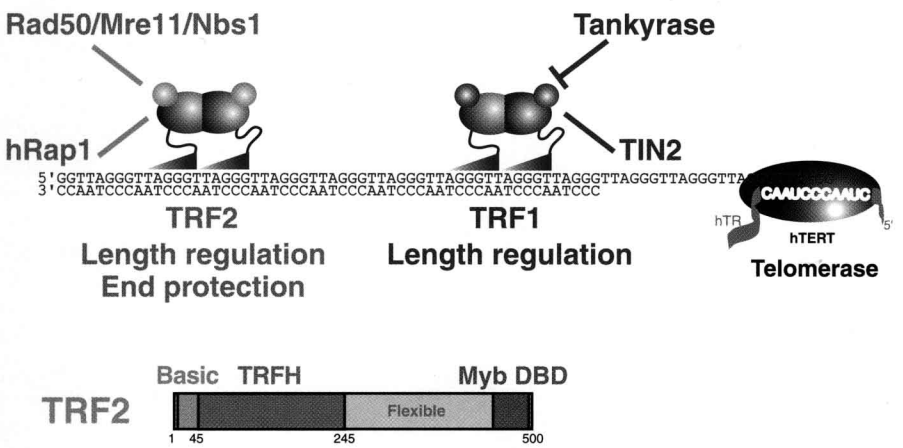

Figure 1. Components of the mammalian telomeric complex and the domain structure of TRF2. See text for details.

Several components of the telomeric complex shown in Figure 1 contribute to the regulation of telomere length. Telomere maintenance by telomerase is controlled by a homeostasis mechanism that results in a stable length setting. TRF1 and TRF2, as well as TIN2 and possibly hRap1 act as negative regulators of telomere length (van Steensel and de Lange 1997; Kim et al. 1999; Li et al. 2000; Smogorzewska et al. 2000; B. Li and T. de Lange, unpubl.), whereas tankyrase appears to be a positive regulator (Smith and Lange 2000).

TRF2 is essential for the protection of chromosome ends. Inhibition of TRF2 with a dominant-negative allele that prevents the accumulation of TRF2 at telomeres results in immediate deprotection of chromosome ends. In this context, telomere malfunction is evidenced by the loss of the 3′ overhang and the formation of end-to-end chromosome fusions (van Steensel et al. 1998). Furthermore, inhibition of TRF2 leads to the activation of the ATM/p53 DNA damage response pathway, resulting in cell cycle arrest and apoptosis (Karlseder et al. 1999). Overexpression of TRF2 can induce gradual shortening of telomeres even in the presence of telomerase, suggesting that TRF2 also functions in the regulation of telomere maintenance (Smogorzewska et al. 2000).

T-LOOPS

Inspection of the structure of telomeric DNA in mammals by electron microscopy has revealed the presence of a specific higher-order DNA structure, referred to as the t-loop (Fig. 2) (Griffith et al. 1999). Mammalian t-loops are large lasso structures composed of many kilobases of duplex TTAGGG repeats. t-loops also occur in trypanosomes (Munoz et al. 2001), and loops were demonstrated at chromosome ends in *Oxytricha* (Murti and Prescott 1999). The t-loop configuration appears to be created by the invasion of the single-stranded TTAGGG repeat tail into the duplex part of the telomere. The base of the loop contains a short region of single-stranded DNA, consistent with the displacement of the G-rich telomeric repeat strand by invasion of the 3′ overhang. It is predicted that the formation of t-loops depends on the repetitive nature of the telomeric DNA and on the remodeling of this DNA by architectural proteins. TRF2 binds to tail-loop junctions in vitro and increases the frequency of t-loop formation with telomeric DNA substrates. It is unclear whether TRF2 actually stimulates t-loop formation in this setting. Perhaps TRF2 stabilizes t-loops by blocking their resolution, for instance, by preventing branch migration. Regardless, it is tempting to speculate that inhibition of TRF2 in vivo leads to an opening of t-loops, effectively unfolding the telomere into a structure that resembles damaged DNA (Griffith et al. 1999).

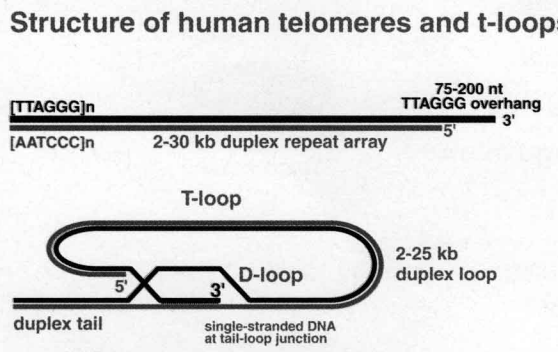

Figure 2. Structure of t-loops. See text for details.

t-loops have also been proposed to contribute to the regulation of telomere maintenance by telomerase (Griffith et al. 1999). Mounting evidence suggests that telomere length homeostasis is in part achieved via regulation of the access of telomerase to the telomere terminus (for review, see Nugent and Lundblad 1998). For instance, altered levels of several telomere-binding proteins affect telomere length maintenance, and this resetting of telomere length occurs without a change in either the expression level or the activity of telomerase (van Steensel and de Lange 1997; Kim et al. 1999; Li et al. 2000; Smith and Lange 2000; Smogorzewska et al. 2000). The simplest interpretation of these results is that the access of telomerase to the telomere terminus is controlled by the telomeric complex. In vitro, telomerase requires a protruding 3′ end, predicting that the remodeling of telomeres into t-loops might block telomerase from acting on the telomere terminus. According to this view, telomere maintenance would require the opening of t-loops, and factors that stimulate t-loop formation (possibly TRF2) should act as negative regulators of telomere length.

Telomeres need to carry out two apparently irreconcilable tasks. Telomere maintenance requires that telomerase gain access to the telomere terminus, whereas protection of chromosome ends presumably involves the sequestration of the same DNA end from factors that execute genome surveillance and repair. If t-loops are the main mechanism by which chromosome ends are protected, t-loop dynamics would have to be carefully orchestrated to allow telomerase access to the telomere terminus without a general unmasking of chromosome ends. Here, we propose that the Mre11 complex contributes to the regulation of t-loop dynamics.

THE COMPONENTS OF THE Mre11 COMPLEX

The Mre11 complex is a trimeric protein that has a key role in the maintenance of genome integrity and the processing of double-strand breaks (DSBs) (Fig. 3) (for review, see Haber 1998; Petrini 1999). Two of its components, Mre11 and Rad50, are conserved in mammals and yeast (Petrini et al. 1995; Dolganov et al. 1996), and there is good evidence for their origin in the bacterial SbcCD nuclease complex and the related T4 gp46/47 nuclease (Sharples and Leach 1995; for review, see Kreuzer 2000). Mre11 has three phophoesterase motifs at its amino terminus as well as a fourth domain shared by the nucleases SbcD and T4 gp47 (Sharples and Leach 1995). Two DNA-binding domains distal to the amino-terminal nuclease domain have been identified (Furuse et al. 1998;

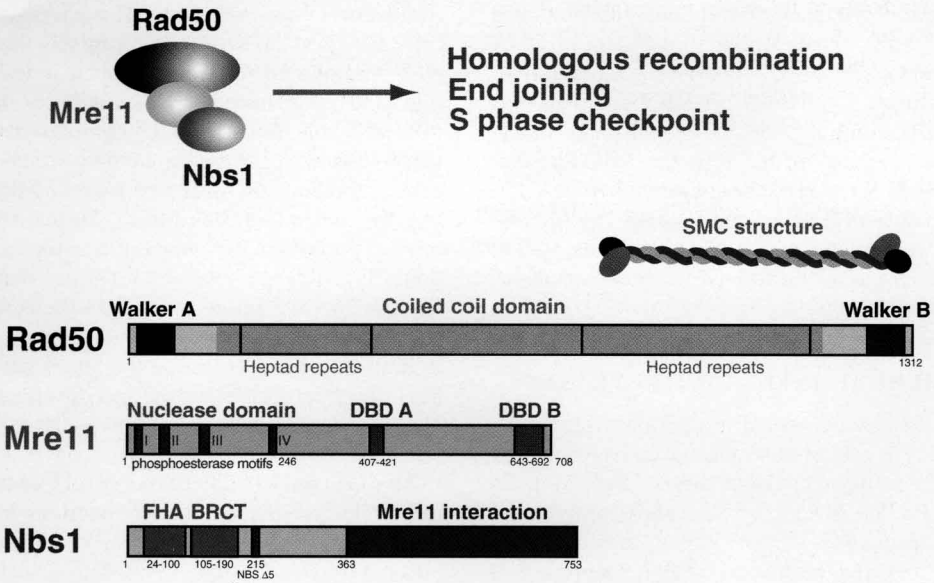

Figure 3. Core components of the mammalian Mre11 complex. See text for details.

Usui et al. 1998). In vitro, Mre11 is a 3′ to 5′ exonuclease, digesting both double- and single-stranded DNAs, and it has single-stranded endonuclease activity that can also cleave hairpins (Ogawa et al. 1995; Paull and Gellert 1998, 1999, 2000; Trujillo et al. 1998; Moreau et al. 1999).

Rad50 is an ATP-dependent DNA-binding protein that can unwind DNA, and its structure has some similarity to the SMC family of chromosome condensation factors (Sharples and Leach 1995; Connelly et al. 1999; Paull and Gellert 1999; Hopfner et al. 2000). The crystal structure of part of the *Pyrococcus* Rad50 ortholog suggests that this dimeric protein could hold onto two DNA molecules (or two DNA ends) in a reaction that is controlled by ATP hydrolysis (Hopfner et al. 2000).

The third player in this complex is remarkably divergent. The amino acid sequence of Xrs2, the only known partner of Rad50/Mre11 in yeast (Johzuka and Ogawa 1995), is very different from its mammalian counterpart, Nbs1 (Carney et al. 1998), although there is a short stretch of modest sequence similarity at the amino termini of these proteins. Structural studies will be required to reveal whether Nbs1 and Xrs2 are related. Nbs1 has a Forkhead-associated (FHA) phosphopeptide-binding domain in its amino terminus, located next to a BRCA1 carboxy-terminal (BRCT) protein interaction domain. Neither Xrs2 nor Nbs1 has a recognized activity in vitro, although the presence of Nbs1 potentiates ATP-dependent unwinding and nuclease activities of baculovirus-produced Mre11 and Rad50 (Paull and Gellert 1999).

The native Mre11 complex extracted from human cells is large (>1500 kD) in part due to multimerization of the core components. The complex also contains an unidentified protein of approximately 400 kD (Carney et al. 1998), and interactions have been demonstrated with BRCA1 (Zhong et al. 1999), TRF2 (Zhu et al. 2000) (see below), TRF1 (G. Wu et al. 2000), E2F1 (see Petrini, this volume), and the ATM kinase (Gatei et al. 2000; Lim et al. 2000; X. Wu et al. 2000b; Zhao et al. 2000).

RECOMBINATIONAL DNA REPAIR

Genetic studies in yeast have implicated the Mre11 complex in diverse aspects of DSB repair and in meiotic recombination (Haber 1998; Petrini et al. 2000). The repair of DSBs in yeast occurs predominantly by homologous recombination (HR). Nonhomologous end-joining (NHEJ) activity is also present in *Saccharomyces cerevisiae*, but this mechanism does not contribute substantially to cell survival after the induction of DSBs (Mages et al. 1996; Moore and Haber 1996; Tsukamoto et al. 1996; Bressan et al. 1999). DSBs are resected in the 5′ to 3′ direction to create a 3′ single-stranded DNA tail prior to HR (Paques and Haber 1999). Although the Mre11 complex exonuclease activity has the opposite polarity, it influences the kinetics of 5′-3′ resection at HO endonuclease-induced DSBs (Ivanov et al. 1994; Lee et al. 1998).

The complex also effects subsequent steps in HR. In cells treated with ionizing radiation or methylmethane sulfonate (MMS), which is a radiomimetic in *S. cerevisiae*, HR-based repair is profoundly impaired (Bressan et al. 1999), and Mre11 complex mutants are unable to effectively utilize sister chromatids as templates for recombinational DNA repair in both haploid and diploid cells. Although this defect is most pronounced with regard to the use of sister chromatids, homologous chromosomes are also less effective as templates for HR in Mre11-deficient strains (Ivanov et al. 1992; Bressan et al. 1999). Finally, studies on chicken cells lacking normal Mre11 function are consistent with a role for this complex in HR (Yamaguchi-Iwai et al. 1999) (see below).

It has been proposed that the Mre11 complex promotes end-joining through exonucleolytic processing of DNA ends (Paull and Gellert 1999, 2000; but see Moreau et al. 1999). In addition, the Mre11 complex could have a structural role (holding together DNA ends or recombination partners) perhaps mediated by the SMC-like features of Rad50. In agreement, the contribution of RAD50 to NHEJ is greater in S phase and G_2 cells (Moore and Haber 1996), presumably because in these cells, Rad50 can use the sister chromatid to hold the broken ends together.

TELOMERE MAINTENANCE IN YEAST

Studies in yeast have revealed an additional role for the Mre11 complex at natural chromosome ends, where it is required for the maintenance of telomeric DNA. Mutants deficient for Rad50, Mre11, or Xrs2 show progressive telomere shortening and, in some strains, cellular senescence (Kironmai and Muniyappa 1997; Boulton and Jackson 1998; Nugent et al. 1998). Loss of Rad50 does not exacerbate the rate of telomere loss in telomerase-deficient strains, indicating that the Mre11 complex functions by facilitating the elongation of telomeres by telomerase (Nugent et al. 1998). Perhaps the Mre11 complex mediates exonucleolytic processing of the telomere terminus. Formation of a 3′ overhang is a presumed requirement to allow telomerase to act on the DNA end formed by leading-strand synthesis (see Fig. 4A). As a blunt end, the product of leading-strand synthesis is probably not a substrate for telomerase, which has been shown to require a 3′ overhang in vitro. However, *rad50*Δ mutants have no obvious defect in the formation of 3′ telomeric overhangs (Dionne and Wellinger 1998), and the in vitro activity of the Mre11 complex predicts formation of 5′ overhangs, a wrinkle that also has been noted in the context of DSB resection.

Without telomerase, yeast cells eventually succumb to the consequences of telomere attrition (Lundblad and Szostak 1989; Lundblad and Blackburn 1993; Singer and Gottschling 1994). However, frequent survivors arise from such cultures, and these cells use a *RAD52*-dependent recombination pathway either to maintain their telomeric DNA or to amplify subtelomeric elements (Lundblad and Blackburn 1993; for review, see Kass-Eisler and Greider 2000). Such survivors do not arise in *rad50/rad51* double mutants, and *RAD50* and *RAD51* appear to direct two independent pathways that allow continued growth of telomerase-deficient cells (Le et al. 1999).

MUTATIONS IN THE VERTEBRATE Mre11 COMPLEX

Although *S. cerevisiae* can survive without these proteins, at least two of the components of the vertebrate Mre11 complex are essential at the cellular level (Xiao and Weaver 1997; Luo et al. 1999; Yamaguchi-Iwai et al. 1999). Chicken DT40 cells have a reduced ability to withstand DNA damage when they are depleted of Mre11 (Yamaguchi-Iwai et al. 1999). This phenotype is worse in cells that also lack Ku70, indicating that the Mre11 complex is important for damage repair independent of its role in Ku70-mediated NHEJ. It is likely that this result reflects a role for the Mre11 complex in HR. Indeed, DT40 cells, which normally are very efficient in homologous recombination, display a profound defect in gene-targeting when they lack Mre11. Furthermore, $Mre11^{-/-}$ cells show a dramatic increase in chromosomal aberrations, specifically those abnormalities that result from DNA breaks in S phase or G_2. DT40 cells repair S phase and G_2 breaks primarily by HR; this repair is diminished in $Rad54^{-/-}$, whereas loss of the NHEJ pathway ($Ku^{-/-}$) has little effect. Therefore, the specific effect of Mre11 on the processing of breaks in S phase and G_2 again implicates this complex in HR.

Although the lethality of null Mre11 complex mutants has hampered analyses of its functions in recombinational DNA repair somewhat, the cytology of this complex has provided insight. In mammalian cells, the Mre11 complex relocates to sites of DNA damage very soon after ionizing irradiation (Nelms et al. 1998). This process results in the formation of a typical punctate pattern of numerous nuclear foci that contain Rad50, Mre11, Nbs1 (Maser et al. 1997; Carney et al. 1998; X. Wu et al. 2000a), as well as BRCA1 (Zhong et al. 1999; X. Wu et al. 2000a), Rad52 (Liu et al. 1999), and a phosphorylated form of H2A (H2AX) (Paull et al. 2000). The rapid translocation of the Mre11 complex to sites of damage suggests that it functions in early steps of DSB recognition and processing.

Further information about the function of the mammalian Mre11 complex comes from two rare human disorders, the ataxia-telangiectasia-like disorder (ATLD) and Nijmegen breakage syndrome (NBS) which are due to hypomorphic mutations in Mre11 and Nbs1, respectively (Carney et al. 1998; Stewart et al. 1999; for review, see Petrini 1999). Both syndromes are similar to ataxia-telangiectasia at the cellular level, although there are significant differences in clinical presentation. Like ataxia-telangiectasia, ATLD and NBS cells display spontaneous chromosome aberrations, often involving translocations of chromosome 14, and their frequency of clastogen-induced abnormal karyotypes is greatly increased. The activation of ATM and p53 in response to DSBs is not affected in Nbs1 or Mre11 hypomorphic mutants, indicating that signaling from DSB to the p53 pathway does not require the Mre11 complex, although JNK activation is impaired in those contexts (Stewart et al. 1999). Indeed, although cells that are mutant for Mre11 or Nbs1 have a defect in the S-phase checkpoint, their p53-dependent DSB checkpoints are intact (Yamazaki et al. 1998).

ASSOCIATION OF THE Mre11 COMPLEX WITH TRF2 AND TELOMERES

A stable interaction of the Mre11 complex with TRF2 was detected by nanoelectrospray sequencing of polypeptides that coimmunoprecipitate with human TRF2 (Zhu et al. 2000). Inspection of the TRF2 complex on SDS-

PAGE revealed polypeptides migrating at 150, 70, and 60 kD. The 70-kD doublet was found to contain TRF2, and the 60-kD band contained human Rap1, a protein that was independently isolated in a two-hybrid screen with TRF2 (Li et al. 2000). Sequencing of peptides from the 150-kD band unambiguously identified human Rad50.

Each of the three components of the Mre11 complex, Rad50, Mre11, and Nbs1, could be demonstrated in TRF2 immunoprecipitates derived from several tumor cell lines and primary human fibroblasts. However, the immunoprecipitation experiments indicate that only a minor fraction (1–5%) of the Mre11 complex is bound to TRF2, and vice versa, most of the TRF2 extracted in nuclear extracts appears to be free of the Mre11 complex.

Mre11 and Rad50 were detected at telomeres by indirect immunofluorescence of cells extracted with Triton X-100. Extraction of soluble nuclear factors prior to fixation is necessary because Mre11 and Rad50 are abundant nuclear proteins resulting in a bright overall nuclear staining pattern that can mask telomeric association. Using this technique, we found a minor fraction of the Mre11 and Rad50 signals at discrete sites that were identified as telomeres based on costaining for TRF1, a specific marker for telomeres. Telomeric Mre11 and Rad50 was observed in all interphase cells, and most cells had numerous telomeric sites containing these factors. However, it was not possible to determine whether all telomeres contained these proteins. The association of Mre11 with telomeric DNA was independently verified by chromatin immunoprecipitation analysis (D. Loayza and T. de Lange, unpubl.).

TELOMERIC PROTEINS DO NOT RELOCATE IN RESPONSE TO DNA DAMAGE

Upon treatment of cells with ionizing radiation, the Mre11 complex migrates rapidly to sites of DNA damage (Nelms et al. 1998). This initial relocation is eventually followed by accumulation of the complex in large aggregates (referred to as ionizing-radiation-induced foci, IRIFs) that are readily detectable by immunofluorescence at about 8 hours postirradiation. In contrast, the localization of TRF2 and other telomeric proteins is not detectably affected by DNA damage. Specifically, TRF2, TRF1, and hRap1 remained at telomeres after irradiation, and none of these factors were detectable in association with IRIFs. Furthermore, ChIP analysis revealed that TRF1 and TRF2 remained associated with telomeric DNA in cells that were γ-irradiated (E. Aanhane et al., unpubl.). Finally, induction of DNA damage did not affect the association of the Mre11 complex with TRF2 as evidenced from the persistence of Rad50, Mre11, and Nbs1 in the TRF2 immunoprecipitates. Similarly, no changes were detected in the subnuclear distribution of telomeric proteins in cells that were treated with UV (X.-D. Zhu and T. de Lange, unpubl.). Thus, several components of the human telomeric complex are stably associated with telomeres in cells with severely damaged DNA. This finding contrasts the situation in the yeast *S. cerevisiae* where two components of the telomeric complex, the Ku heterodimer and Sir3p, are released from telomeres after induction of double-stranded DNA breaks (Martin et al. 1999; Mills et al. 1999), presumably to migrate to sites of damage.

Nbs1 IS RECRUITED TO TELOMERES IN S PHASE

Although Mre11 and Rad50 could be detected at telomeres in the majority of interphase nuclei, most cells had no detectable Nbs1 at telomeres, instead showing Nbs1 signals in the nucleoli as well as throughout the nucleus. However, in approximately 10% of the nuclei, Nbs1 was found at telomeres based on colocalization with TRF1. Inspection of HeLa cells progressing through the cell cycle showed that the localization of Nbs1 was dependent on the cell cycle stage. Specifically, Nbs1 could be observed at telomeres in S-phase nuclei but not before or after DNA replication. Furthermore, the TRF2 complex contained Nbs1 primarily during DNA replication, whereas no (or less) Nbs1 appeared to be present in the TRF2 complex in G_2/M and G_1. In contrast, the interaction of Mre11 and Rad50 with TRF2 was observed throughout the cell cycle.

The association of Nbs1 with telomeres was confirmed by an analysis of meiotic chromosomes. Specifically, Nbs1 is at telomeres in spermatocytes where it colocalizes with TRF1 and Mre11 (Lombard and Guarente 2000; J.H.J. Petrini, and H. Scherthan, pers. comm.). Finally, Nbs1 interacts with TRF1 as detectable by two-hybrid analysis and such an association may further solidify the binding of Nbs1 to telomeres (G. Wu et al. 2000).

POSSIBLE ROLES OF THE Mre11 COMPLEX AT TELOMERES

The function of the Mre11 complex at human telomeres has not been established. Preliminary analysis of a limited number of Mre11 and Nbs1 mutant cells failed to reveal an obvious change in telomere structure (X.-D. Zhu and T. de Lange, unpubl.), and no indication for telomere dysfunction (e.g., end-to-end fusions) was noted in ATLD or NBS cells. Perhaps the loss of the Mre11 complex has a more subtle telomere phenotype or the function of this complex at telomeres is redundant. It is also important to note that the human cell lines with mutations in this complex are derived from ATLD and NBS patients and as such represent hypomorphic alleles, rather than null mutations. Indeed, the lethality of targeted deletions of Mre11 and Rad50 in mouse cells is fully compatible with an important telomere function (Xiao and Weaver 1997; Luo et al. 1999). Here, we discuss three possible roles for the Mre11 complex at telomeres.

One possibility is that the Mre11 complex is required for the generation of 3′ overhangs at telomeres (Fig. 4A). This idea was proposed to explain the requirement of the Mre11 complex in telomere maintenance in yeast (discussed above), and the same mechanism might hold for human telomeres. Indeed, human telomeres contain a 3′ overhang (Makarov et al. 1997; McElligott and Wellinger 1997; Wright et al. 1997; Huffman et al. 2000), and this

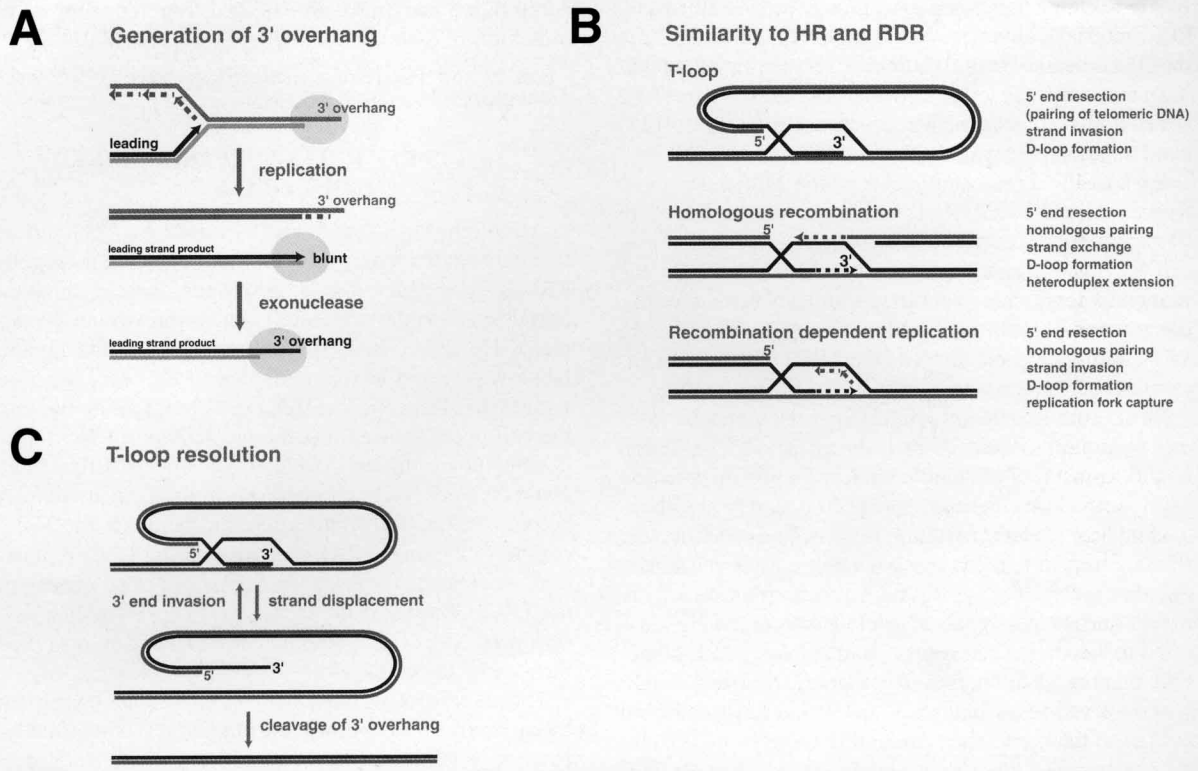

Figure 4. Three possible roles for the Mre11 complex at telomeres. (*A*) The Mre11 complex may represent or recruit a 5′ exonuclease that recreates a 3′ overhang after replication of telomeres. (*B*) t-loop formation is mechanistically similar to homologous recombination and recombination-dependent replication, and the contribution of the Mre11 complex to t-loop formation may therefore reflect its role in these recombination events. (*C*) t-loops could be resolved with the aid of the unwinding activity of the Mre11 complex, and the ability of this complex to cleave the 3′ overhang is predicted to make t-loop resolution irreversible. Both activities are enhanced by the presence of Nbs1 in the complex. See text for discussion.

overhang is thought to be maintained at all telomeres even in cells that lack telomerase. Thus, resection of the 5′ end would be required to recreate the overhang at the chromosome end formed by leading-strand synthesis (Fig. 4A). Interestingly, the presence of this overhang is severely diminished after inhibition of TRF2 (van Steensel et al. 1998), the protein that tethers the Mre11 complex to telomeres (Zhu et al. 2000).

In addition to providing a substrate for telomerase, exonucleolytic processing of telomere termini may be required to generate an overhang of sufficient length to allow t-loop formation. In this context, it is important to note that the minimal length of the 3′ overhang for t-loop formation has not been established. Exonucleolytic attack may also be relevant to the programmed shortening of human telomeres in normal somatic cells. It was understood early on that the rate of telomere shortening in primary human cells far exceeds that predicted based on the end-replication problem. Indeed, yeast cells and *Drosophila* lacking telomerase lose no more than a few base pairs from each chromosome end per cell division (for review, see Biessmann and Mason 1997); this rate matches the predicted shortening due to removal of the last RNA primer for lagging-strand synthesis. Since human cells show a rate of telo-mere shortening that often exceeds 100 bp/end/population doubling, resection of the 5′ telomere end may well be the main mechanism by which telomere shortening is effected. If the Mre11 complex controls this process, it is predicted that the length of the 3′ overhang or the rate at which 3′ overhangs are formed is diminished in cells from NBS or ATLD patients. Furthermore, the rate of telomere shortening may be altered in these syndromes.

An indirect link between telomere shortening rates and the Mre11 complex is provided by the ATM kinase. Recent data have placed the ATM kinase upstream of the Mre11 complex in the S-phase DNA damage checkpoint (Gatei et al. 2000; Lim et al. 2000; X. Wu et al. 2000b; Zhao et al. 2000; see also J.H. Petrini [this volume] for a discussion of the S-phase checkpoint). Primary cells from ataxia-telangiectasia patients have an increased rate of telomere shortening (Metcalfe et al. 1996; Xia et al. 1996; Vaziri et al. 1997), raising the possibility that the ATM kinase through its ability to phosphorylate Nbs1 could modulate the exonucleolytic degradation of telomeres.

A second proposal is that the Mre11 complex contributes to the formation and maintenance of t-loops. t-loop formation resembles initial steps in HR as well as early events in recombination-dependent replication (RDR) (Fig. 4B). In both processes, homologous sequences are brought together, and a 3′ single-strand end is invaded into duplex DNA. The Mre11 complex has been shown to facilitate HR in yeast (see above) and its

role in HR may be equivalent to its telomeric function. The bacterial counterpart of the Mre11 complex, the SbcCD nuclease, is required for the processing of stalled DNA replication forks (for review, see Kowalczykowski 2000), and recombination-dependent replication of T4 phage requires the contribution of its Mre11/Rad50 homologs, gp46/47 (for review, see Kreuzer 2000).

Finally, it is possible that the Mre11 complex contributes to the controlled resolution of t-loops. Presumably, t-loops will need to be resolved for replication forks to proceed to the end of the chromosome, and the opening of t-loops is likely to be required for telomerase-mediated telomere elongation. In vitro, the Mre11 complex has two activities that could facilitate t-loop resolution. First, the complex can unwind DNA to some extent and second, it can cleave a 3′ overhang at the junction with double-stranded DNA (Paull and Gellert 1999). Interestingly, both activities are enhanced by the addition of Nbs1. Thus, the acquisition of Nbs1 in the telomeric Mre11 complex in S phase could reflect a switch in the activity of the Mre11 complex from a protein that promotes t-loops (as in Fig. 4B) to a factor that opens this structure (as in Fig. 4C). Nbs1 and S-phase-regulated t-loop dynamics may ensure appropriate replication of the chromosome end while preventing inappropriate activation of the DNA damage response.

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