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Role of Pin2/TRF1 in Telomere Maintenance and Cell Cycle Control

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Abstract Telomeres are specialized structures found at the extreme ends of chromosomes, which have many functions, including preserving genomic stability, maintaining cell proliferative capacity, and blocking the activation of DNA-damage cell cycle checkpoints. Deregulation of telomere length has been implicated in cancer and ageing. Telomere maintenance is tightly regulated by telomerase and many other telomere-associated proteins and is also closely linked to cell cycle control, especially mitotic regulation. However, little is known about the identity and function of the signaling molecules connecting telomere maintenance and cell cycle control. Pin2/TRF1 was originally identified as a protein bound to telomeric DNA (TRF1) and as a protein involved in mitotic regulation (Pin2). Pin2/TRF1 negatively regulates telomere length and importantly, its function is tightly regulated during the cell cycle, acting as an important regulator of mitosis. Recent identification of many Pin2/TRF1 upstream regulators and downstream targets has provided important clues to understanding the dual roles of Pin2/TRF1 in telomere maintenance and cell cycle control. These results have led us to propose that Pin2/TRF1 functions as a key molecule in connecting telomere maintenance and cell cycle control. J. Cell. Biochem. 89: 19–37, 2003.

Key words: Pin2/TRF1; Pin2/TRF1-interacting proteins; telomeres; the cell cycle; telomerase inhibitor

INTRODUCTION

Telomeres consist of simple DNA repeats, TTAGGG in vertebrates, and associated proteins. Telomeres have many functions, including preventing the degradation, fusion, and recombination of chromosome ends; maintaining cell proliferative capacity; and blocking the activation of DNA-damage cell cycle checkpoints [for reviews see Bryan and Cech, 1999; Artandi and DePinho, 2000; Evans and Lundblad, 2000; Stewart and Weinberg, 2000; Blackburn, 2001; Shay and Wright, 2001]. With a few exceptions, most human somatic tissues and primary cultured cells do not have active telomerase activity [Broccoli et al., 1995; Taylor et al., 1996]. Telomeres in these cells undergo shortening following each cell division [Cooke and Smith, 1986; Harley et al., 1990; Hastie et al., 1990], due to the inability of the conventional DNA replication machinery to replicate the extreme 3′ ends of linear chromosomes [Watson, 1972; Olovnikov, 1973]. It has been hypothesized that senescence occurs when the telomere length of one or more chromosomes reaches a critical point, at which cells are signaled to exit the cell cycle [Cooke and Smith, 1986; Harley et al., 1990; Hastie et al., 1990; Harley, 1991; Vaziri et al., 1994].

Telomeric DNA sequences can be replenished by at least two mechanisms: telomerase activity [Greider and Blackburn, 1985] or alternative lengthening of telomeres [Bryan et al., 1995]. Telomerase is a reverse transcriptase using a small segment of an integral RNA component as a template for the synthesis of the G-rich strand of telomeres [Greider and Blackburn, 1985, 1989; Shippen-Lentz and Blackburn, 1990; Yu et al., 1990; Singer and Gottschling, 1994; Cohn

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and Blackburn, 1995; Feng et al., 1995; Counter et al., 1997; Lingner et al., 1997; Meyerson et al., 1997; Nakamura et al., 1997]. Importantly, expression of hTERT in many normal primary human cells has been shown to halt telomere erosion and prevent them from entering into senescence [Bodnar et al., 1998; Kiyono et al., 1998; Vaziri and Benchimol, 1998]. Telomerase activity is readily detected in most human tumor tissues and immortalized and/or transformed cell lines [Counter et al., 1992; Kim et al., 1994; Broccoli et al., 1995; Taylor et al., 1996] and is sufficient in most cases to allow transformed cells to escape from crisis [Bodnar et al., 1998; Vaziri and Benchimol, 1998; Halvorsen et al., 1999]. Furthermore, telomerase is critical for transforming primary human cells [Hahn et al., 1999a] and its transgenic overexpression causes tumor formation in mice [Gonzalez-Suarez et al., 2001]. In contrast, suppressing telomerase function causes telomere shortening, and often forces transformed cells to enter crisis [Feng et al., 1995; Hahn et al., 1999b; Herbert et al., 1999; Zhang et al., 1999]. Gene knockout experiments also reveal an essential role of telomerase in highly proliferative organs in mice [Blasco et al., 1997; Lee et al., 1998; Rudolph et al., 1999]. These results indicate that telomerase activity is pivotal for immortalization and transformation.

There is growing evidence suggesting that both the shielding of telomeric ends and their elongation by telomerase is dependent on telomere binding proteins. Telomere length homeostasis in budding and fission yeast cells is regulated by the telomere binding proteins Rap1p and Taz1, respectively [Krauskopf and Blackburn, 1996; Cooper et al., 1997; Marcand et al., 1997]. Mutagenesis analyses of the telomeric sequence of *Kluyveromyces lactis* also suggest that telomere length is modulated by proteins that bind telomeric DNA [McEachern and Blackburn, 1995]. Telomere maintenance in mammals is also regulated by telomere binding proteins such as Pin2/TRF1 [van Steensel and de Lange, 1997]. However, none of these telomere-binding proteins has been shown to directly affect telomerase activity per se either in vitro or in vivo. Therefore, the mechanisms by which these proteins regulate telomere maintenance remain to be elucidated.

Furthermore, there is compelling evidence to suggest a strong link between telomere maintenance and cell cycle control, specifically mitotic regulation. For example, elimination of a telomere causes a Rad9p-mediated cell cycle arrest in G2 in budding yeast [Sandell and Zakian, 1993]. In fission yeast, telomeres are clustered at the nuclear periphery in G2, but this association is disrupted in mitosis [Funabiki et al., 1993], and telomeres have been shown to mediate the attachment of chromosomes to spindle bodies and lead chromosome movement in meiotic prophase [Chikashige et al., 1994]. In *Drosophila* eyes, deletion of telomeres also induces mitotic arrest and apoptosis in vivo [Ahmad and Golic, 1999]. Mutations in the Tetrahymena telomeric DNA sequence have been shown to cause a block in anaphase chromosome separation [Kirk et al., 1997]. Furthermore, in yeast, mutations in TEL1 and its related gene MEC1 result in shortened telomeres, genomic instability, and mitotic checkpoint defects [Weinert et al., 1994; Greenwell et al., 1995; Paulovich and Hartwell, 1995; Sanchez et al., 1996a]. Moreover, mutations in the ATM gene, a human homologue of the TEL1 and MEC1 genes, cause the genetic disorder ataxia-telangiectasia (A-T). A-T patients display a wide range of abnormalities, including hypersensitivity to radiation, cell cycle checkpoint defects, premature ageing, and predisposition to cancer [Savitsky et al., 1995; Barlow et al., 1996; Elson et al., 1996; Xu and Baltimore, 1996; Xu et al., 1996]. More interestingly, cell lines derived from A-T patients have shortened telomere lengths [Pandita et al., 1995] and display high levels of mitotic chromosome fusion and mitotic checkpoint defects [Rudolph and Latt, 1989; Beamish et al., 1994]. Significantly, the hypersensitivity to ionizing radiation is correlated with telomere loss [Pandita et al., 1995; Metcalfe et al., 1996; Xia et al., 1996; Smilenov et al., 1997]. Collectively, these results suggest that there are telomere-mediated checkpoints that regulate progression through cell cycle, especially mitosis. However, little is known about the identity and function of the signaling molecule(s) connecting telomere maintenance and mitotic regulation.

In a genetic screen for proteins that are involved in mitotic regulation, we identified three human proteins, Pin1-3 [Lu et al., 1996]. Characterization of these Pin proteins shows that they are all involved in mitotic regulation [Lu, 2000]. Pin1 binds and regulates the function of mitosis-specific phosphoproteins by phosphorylation-dependent prolyl isomerization

[Yaffe et al., 1997; Shen et al., 1998; Lu et al., 1999a,b; Winkler et al., 2000; Zhou et al., 2000; Stukenberg and Kirschner, 2001; Lu et al., 2002a,b]. Pin2 is identical in sequence to TRF1 apart from an internal deletion of 20 amino acids [Shen et al., 1997]. Pin2 and TRF1 are likely derived from the same gene *PIN2/TRF1* [Young et al., 1997]. However, in the cell Pin2 is much more abundant than TRF1 [Shen et al., 1997]. Significantly, the level and localization of Pin2/TRF1 is tightly regulated during the cell cycle, with its levels being increased at late G2 and M, and colocalization to mitotic spindles during mitosis [Shen et al., 1997; Kishi et al., 2001a; Nakamura et al., 2001a]. Furthermore, Pin2/TRF1 specifically affects mitotic progression [Shen et al., 1997; Kishi et al., 2001a]. Moreover, Pin2/TRF1 is an ATM kinase substrate that plays a crucial role in mediating the function of ATM in telomere regulation and mitotic checkpoint control [Kishi et al., 2001b; Nakamura et al., 2001a; Kishi and Lu, 2002; Nakamura et al., 2002]. Finally, recent identification of many other Pin2/TRF1-interacting proteins has provided important clues to understanding the dual roles of Pin2/TRF1 in telomere maintenance and cell cycle control [Lu et al., 1996; Smith et al., 1998; Kim et al., 1999; Wu et al., 2000; Kishi et al., 2001b; Nakamura et al., 2001a, 2002; Zhou and Lu, 2001]. Here, we review recent studies demonstrating roles of Pin2/TRF1 in telomere maintenance and cell cycle progression primarily in human cells, which lead us to propose that Pin2/TRF1 functions as a key molecule in connecting telomere maintenance and cell cycle control.

ORIGINAL IDENTIFICATION OF Pin2/TRF1 USING TWO DIFFERENT APPROACHES

Pin2/TRF1 was originally cloned at about the same time using two completely different approaches [Chong et al., 1995; Lu et al., 1996]. One approach was to purify telomere proteins biochemically using telomeric DNA repeats as an affinity column, which led to the cloning of TRF1 [Chong et al., 1995]. The other involved searching for proteins involved in mitotic regulation using a genetic screen for human proteins that bind the mitotic kinase NIMA and functionally suppress its ability to induce lethal mitotic catastrophe [Lu and Hunter, 1995; Lu et al., 1996]. This latter approach led to the identification of three proteins, Pin1-3 [Lu et al., 1996]. Pin2 is identical to TRF1 apart from an internal deletion of 20 residues (296 to 316 in TRF1) [Chong et al., 1995; Lu et al., 1996; Shen et al., 1997] (Fig. 1A). Subsequent chromosome localization and genomic sequence analyses suggest that Pin2 and TRF1 are likely generated from the same gene Pin2/TRF1 due to alternative splicing [Young et al., 1997]. Since there are so far no detectable functional differences between these two isoforms, the biological significance of this alternative splicing, if any, remains unclear. Importantly, Pin2 expression is much higher than TRF1 both at mRNA and protein levels in various cell lines examined, indicating that Pin2 is the major isoform in the cell [Shen et al., 1997]. For clarity, we will here use TRF1 for the 20 amino acid containing isoform and Pin2 for the 20 amino acid deletion isoform, as they were originally identified [Chong et al., 1995; Lu et al., 1996], but refer to endogenous proteins as Pin2/TRF1 given the difficulty in separating these isoforms physically and functionally [Zhou and Lu, 2001].

Analysis of the deduced Pin2/TRF1 peptide sequence indicates that it contains an N-terminal Asp/Glu-rich acidic domain, a C-terminal Myb-type helix-turn-helix (HTH) DNA-binding domain, a potential bipartite nuclear localization signal (NLS), and a putative mitotic destruction box, which is similar to those present in mitotic cyclins (Fig. 1A) [Chong et al., 1995; King et al., 1996; Shen et al., 1997]. Telomeric DNA-binding proteins have been isolated from several different species. Despite the diversity in their sequences, the Myb-type HTH DNA-binding motif is a common conserved domain in telomere binding proteins [Bilaud et al., 1996]. Interestingly, Pin2 and TRF1 form homodimers and heterodimers via the N-terminal domain in vitro and in vivo, and these dimers bind telomeric DNA duplexes [Bianchi et al., 1997; Shen et al., 1997; Bianchi et al., 1999]. Recent structural analysis of the Pin2/TRF1 dimerization domain has shown it to be composed of 200 amino acid residues (between residues 65 and 265) [Fairall et al., 2001]. The dimerization domain structure of the Pin2/TRF1 monomers consists of nine helices, forming an elongated helix bundle. Each dimer is formed by two monomers interacting in an antiparallel arrangement forming a symmetrical dimer whose overall structure resembles a twisted horseshoe [Fairall et al., 2001]. This structural analysis reveals that the architecture of

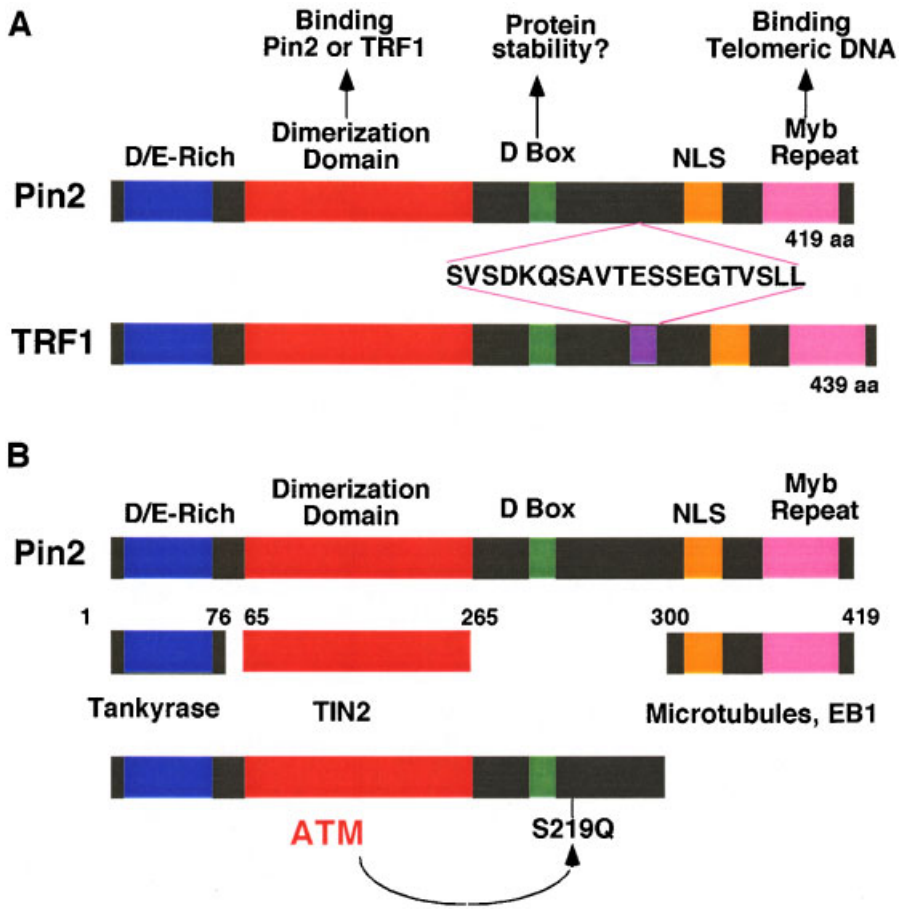

Fig. 1. Pin2/TRF1 domain structure and function. **A**: Schematic presentation of Pin2/TRF1 domains and their function as well as the sequence of the 20 amino acid insert in TRF1. **B**: Domains in Pin2/TRF1 that have been shown to interact with identified interacting proteins, with residue numbering based on the deduced Pin2 peptide sequence. ATM also phosphorylates Ser219 residue. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the dimerization domain of Pin2/TRF1 gives rise to a large surface for interaction with other proteins. Indeed, several proteins have been identified to bind this domain, including Tin2 [Kim et al., 1999] and ATM [Kishi et al., 2001b] (Fig. 1B). The structure of the DNA binding domain of Pin2/TRF1 bound to telomeric DNA has also been determined by NMR [Nishikawa et al., 2001]. Interestingly, although Pin2/TRF1 is dimers, only the single Myb domain, which consists of three helices, appears to be sufficient for the sequence-specific recognition. The third helix of Pin2/TRF1 recognizes the TAGGG part in the major groove, and the N-terminal arm interacts with the TT part in the minor groove [Nishikawa et al., 2001]. It has been suggested that, in the dimer of Pin2/TRF1, two DNA binding domains may bind independently in tandem arrays to two binding sites of telomeric DNA that is composed of the repeated AGGGTT motif [Nishikawa et al., 2001]. Alternatively, Pin2/TRF1 dimerization provides a mechanism to bring two DNA binding domains together, thereby increasing the affinity of Pin2/TRF1 for the telomeric DNA.

DUAL ROLES OF Pin2/TRF1 IN TELOMERE MAINTENANCE AND CELL CYCLE CONTROL

Role of Pin2/TRF1 in Telomere Maintenance

In addition to binding telomeric DNA in vitro and in vivo [Chong et al., 1995; Bianchi et al., 1997; Shen et al., 1997; Griffith et al., 1998], Pin2/TRF1 plays an important role in controlling telomere length [van Steensel and de Lange, 1997; Smogorzewska et al., 2000; Kishi and Lu, 2002]. Stable overexpression of TRF1 in the telomerase-positive fibrosarcoma cell line

HT1080 leads to gradual and progressive telomere shortening [van Steensel and de Lange, 1997]. In contrast, overexpression of a TRF1 mutant that contains the dimerization domain and inhibits binding of endogenous Pin2/TRF1 to telomeres, presumably by acting in a dominant-negative fashion results in telomere elongation in HT1080 [van Steensel and de Lange, 1997]. Furthermore, overexpression of similar dominant-negative Pin2 mutants also induces telomere elongation in telomerase-positive ATM-negative cells, which contain much shorter telomeres due to ATM mutations [Kishi and Lu, 2002]. In addition, Rap1p in budding yeast [Marcand et al., 1997] and Taz1 in fission yeast have been shown to negatively regulate telomere length [Cooper et al., 1997]. In the case of Rap1p, the negative regulation has been shown to function independently of the orientation of the telomere repeats [Marcand et al., 1997]. Furthermore, when the Rap1p carboxyl terminus is specifically targeted to an individual telomere, the number of repeats at the targeted telomere is reduced, which is proportional to the number of targeted molecules [Marcand et al., 1997]. These results suggest that the precise number of RAP1p protein bound to telomeres may function as a mechanism by which cells sense telomere length in a feedback mechanism of telomere length regulation [Marcand et al., 1997]. However, none of these telomere proteins including Pin2/TRF1 have been shown to directly inhibit telomerase activity in vitro or in cells. Therefore, it remains to be determined how these binding proteins negatively regulate telomere elongation.

Role of Pin2/TRF1 in Cell Cycle Control

Although the function of Pin2/TRF1 in telomere regulation has received a lot of attention, its role in the cell cycle has been mostly ignored and therefore needs to be discussed in some more detail. There is now compelling evidence to support that Pin2/TRF1 is not only tightly regulated during the cell cycle, but also plays an important and specific role in cell cycle progression, especially during mitotic progression [Shen et al., 1997; Kishi et al., 2001a,b; Nakamura et al., 2001a, 2002; Kishi and Lu, 2002]. These new findings have further validated the original identification of Pin2/TRF1 as a protein functionally important for mitotic regulation [Lu et al., 1996].

Pin2/TRF1 protein levels during the cell cycle. The level of Pin2/TRF1 protein is tightly regulated during the cell cycle, which can be demonstrated for both endogenous Pin2/TRF1 and exogenously expressed Pin2 in multiple cell lines [Shen et al., 1997; Kishi et al., 2001a]. Pin2/TRF1 levels remain relatively low during G1 and S, but are significantly increased when cells progress through G2 and M phases, followed by a decrease as cells move into the next G1 [Shen et al., 1997]. If cells are prevented from completing mitosis, Pin2 protein levels remain elevated [Shen et al., 1997]. Since exogenous Pin2 is expressed under the control of the constitutively active CMV promoter [Shen et al., 1997], the cell cycle-dependent fluctuation of Pin2/TRF1 protein levels must be regulated at the post-transcriptional level. Consistent with this idea, Pin2/TRF1 contains a motif related to the destruction D-box [Shen et al., 1997], which has been shown to mediate degradation of many mitotic cyclins [King et al., 1996]. Thus, the most likely mechanism for the fluctuation of Pin2/TRF1 protein is an increase in protein stability in G2 and M, followed by a decrease in stability in G1. The accumulation of Pin2/TRF1 in late G2 and M phases, followed by degradation as cells enter G1, is reminiscent of the degradation of other cell cycle regulatory proteins, such as the mitotic cyclins [King et al., 1996]. In these cases, accumulation and degradation is required for entry into and exit from M. Therefore, it is possible that accumulation and degradation of Pin2/TRF1, possibly the fraction of Pin2/TRF1 that is not bound to telomeres (telomere-unbound fraction), is also needed for cells to enter and exit from M phase. However, molecular mechanisms underlying this cell cycle-dependent fluctuation of Pin2/TRF1 remain to be elucidated.

Pin2/TRF1 subcellular localization during the cell cycle. In addition to protein levels, the subcellular localization of Pin2/TRF1 is tightly regulated during the cell cycle, which was first uncovered using a fluorescent GFP-Pin2 fusion protein [Nakamura et al., 2001a]. Although GFP is widely distributed both in interphase and mitotic cells, GFP-Pin2 is localized at the telomere speckles in interphase cells (Fig. 2) [Nakamura et al., 2001a], as previously shown [Chong et al., 1995; Shen et al., 1997; Kishi et al., 2001b]. Surprisingly, in mitotic cells, GFP-Pin2 is localized at fibrous structures known as mitotic spindles, in

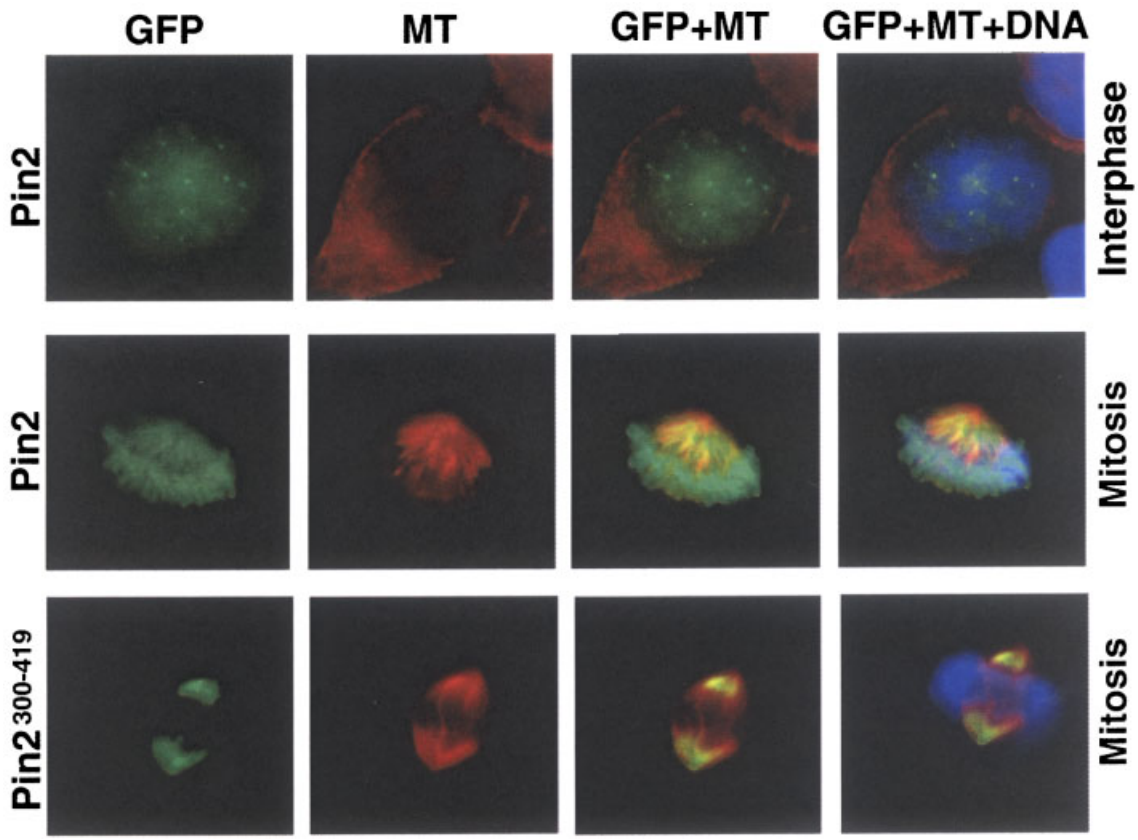

Fig. 2. Cell cycle-specific localization of Pin2. Subcellular localization of GFP-Pin2 or GFP-Pin2$^{300-419}$ in HeLa cells during both interphase and mitosis. Green, GFP-Pin2; Red, microtubules (MT); Yellow, co-localization of the GFP and microtubule signals; blue, DNA. This table is adapted from Nakamura et al. [2001a]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

addition to chromosomes (Fig. 2) [Nakamura et al., 2001a]. Further experiments revealed that a microtubule-targeting domain is located at the C-terminal domain of Pin2 (Fig. 2). To confirm that endogenous Pin2/TRF1 is localized to mitotic spindles, localization of endogenous Pin2/TRF1 was determined using anti-Pin2/TRF1 antibodies [Nakamura et al., 2001b]. In interphase, while microtubules are in the cytoplasm, Pin2/TRF1 is detected at discrete telomere speckles in the nucleus [Nakamura et al., 2001a]. However, in metaphase and anaphase, endogenous Pin2/TRF1 is again co-localized with microtubules at mitotic spindles [Nakamura et al., 2001a]. These data indicate that both exogenous and endogenous Pin2/TRF1 proteins specifically localize to mitotic spindles during mitosis. It is noteworthy that in order to observe the localization of Pin2/TRF1 at the mitotic spindles, methanol fixation is needed, which is routinely used to visualize the microtubule network in cells [Nakamura et al., 2001a,b, 2002]. Since this method was not used in other previous studies [Chong et al., 1995; Shen et al., 1997], this may explain why cell cycle-specific localization of Pin2/TRF1 at mitotic spindles was not previously reported.

Pin2/TRF1 cell cycle function. Cell cycle-specific fluctuation and localization of Pin2/TRF1 suggest that Pin2/TRF1 might affect the cell cycle. Indeed, overexpression of Pin2 or TRF1 affects cell cycle progression [Shen et al., 1997; Kishi et al., 2001a]. Specifically, cells overexpressing Pin2 enter into mitosis, but cannot progress through normal mitosis. Instead, they contain activated caspase 3 and display many other apoptotic markers [Kishi et al., 2001a]. These findings are especially significant because the fragment of Pin2 that was originally identified in the genetic screen contains only the N-terminal 332 residues [Lu et al., 1996; Shen et al., 1997]. This fragment is likely to act in a dominant-negative manner to suppress endogenous Pin2/TRF1 function, as

mentioned above. Therefore, inhibition of Pin2/TRF1 suppresses the ability of the mitotic kinase NIMA to induce premature mitotic entry and apoptosis [Lu and Hunter, 1995; Lu et al., 1996], whereas overexpression induces mitotic entry and apoptosis [Shen et al., 1997; Kishi et al., 2001a]. Interestingly, deletion of the C-terminal telomeric DNA-binding domain completely abolishes the ability of Pin2/TRF1 to induce apoptosis, indicating that the C-terminal domain of Pin2 is required for apoptosis induction [Kishi et al., 2001a]. However, point mutations in the telomere DNA-binding domain of Pin2 that completely disrupt its ability to bind telomeres in cells, has no effect on its apoptotic phenotype [Kishi et al., 2001a]. These results indicate that although telomeric binding per se is not necessary, the C-terminal domain of Pin2 is required for Pin2 to affect cell cycle progression, suggesting that this domain might have other functions in addition to binding to telomeric DNA. Indeed, this domain is essential for targeting Pin2 to the mitotic spindle during mitosis (Figs. 1B and 2), as discussed below [Nakamura et al., 2001a, 2002].

The above results suggest that the concentration of Pin2/TRF1 that is not bound to telomeres might be important for cell cycle progression. This idea is further supported by the findings that the ability of Pin2 to induce mitotic entry and apoptosis depends on the average telomere length in the cell [Kishi et al., 2001a]. Whereas Pin2 potently induces apoptosis in many cell lines containing short telomeres, including HeLa cells, A-T22IJE-T and A431, it fails to induce apoptosis in cells with long telomeres, such as 293, HT1080, and HeLa1.2.11, a HeLa subclone containing longer telomeres. Interestingly, Pin2 is highly concentrated at long telomeres in these cells [Kishi et al., 2001a]. This finding that the ability of Pin2 to induce apoptosis depends on telomere length may provide an explanation for why TRF1 has not been shown to induce apoptosis in some cells, including HT1080 cells cells [van Steensel and de Lange, 1997]. It is also consistent with the recent demonstration that the ability of telomerase inhibition to induce apoptosis highly depends on telomere length [Zhang et al., 1999]. Expression of dominant-negative telomerase mutants induces apoptosis only in cells that contain short telomeres, although it does not induce further shortening of telomeres [Zhang et al., 1999]. Similarly, expression of Pin2 in those cells containing short telomeres does not further shorten telomeres [Kishi et al., 2001a]. Since telomere length is sensed by the concentration of bound telomeric proteins, as shown in the case of Rap1p [Marcand et al., 1997], a high concentration of telomere-bound Pin2/TRF1 in long telomere cells could be a signal that the telomeres are long enough for cells to continue dividing. Conversely, a high concentration of telomere-unbound Pin2/TRF1 in short telomere cells could indicate that the telomeres are too short for the cell to divide. This latter possibility is supported by our findings that Pin2 containing point mutations in the DNA-binding domain does not bind to the telomeric DNA but still potently induces apoptosis [Kishi et al., 2001a]. Therefore, telomere length and the concentration of unbound Pin2 may be important signals for cell proliferation.

UPSTREAM REGULATORS AND DOWNSTREAM TARGET PROTEINS OF Pin2/TRF1

How is Pin2/TRF1 involved in the coordination of telomere maintenance and cell cycle progression? Our understanding of this question has increased due to the recent identification of an increasing number of Pin2/TRF1 regulators and/or interacting proteins using various approaches (Table I). Although these proteins are functionally quite diverse, they provide further evidence for the critical dual roles of Pin2/TRF1 in telomere maintenance and cell cycle control (Table I). Based on their known functions, Pin2/TRF1 regulators and/or interacting proteins can be roughly divided into five categories: (1) those that function in telomere maintenance without affecting telomerase activity, (2) those that function in telomere maintenance by regulating telomerase activity, (3) those that function in telomere maintenance and cell cycle control, (4) those that function in cell cycle control, and (5) others whose functions remained to be elucidated (Table I). Here we shall focus our discussion on the categories 1–4.

1. Pin2/TRF1-Interacting Proteins That Function in Telomere Maintenance Without Affecting Telomerase Activity

In support of a critical role for Pin2/TRF1 in regulating telomere length, several Pin2/TRF1-interacting proteins have now been shown to affect telomere elongation without affecting telomerase activity. These notably include

TABLE I. Pin2/TRF1-Interacting Proteins and Their Known Functions

Protein	Identification method	Known function	References
Function in cell cycle control			
NIMA	Combined two-hybrid and genetic screen	Mitotic kinase	Lu et al., 1996
Microtubules	Biochemical approach	Cytoskeleton, mitotic spindle checkpoint	Nakamura et al., 2001a
EB1	Two-hybrid screen	Microtubule-associated protein involved in mitotic spindle checkpoint	Nakamura et al., 2001b
Function in telomere maintenance without affecting telomerase activity			
Tankyrase 1	Two-hybrid screen	Poly(ADP-ribose) polymerase promoting telomere elongation	Smith et al., 1998
Tin 2	Two-hybrid screen	Inhibiting telomere elongation	Kim et al., 1999
Function in telomere maintenance by regulating telomerase activity			
PinX1	Two-hybrid screen	Telomerase inhibitor and putative tumor suppressor	Zhou et al., 2001
Function in telomere maintenance and cell cycle control			
Ku	Biochemical approach	A component of the DNA-dependent protein kinase	Hau et al., 2000
NBS1	Two-hybrid screen	Gene mutated in Nijmegen breakage syndrome	Wu et al., 2000
ATM	Biochemical approach	Protein kinase mutated in ataxia telanglectasia syndrome	Kishi et al., 2001
Function to be determined			
Nm23-H2	Two-hybrid screen	Nucleoside diphosphate kinase	Nosaka et al., 1998
Tankyrase 2	Two-hybrid screen	Poly(ADP-ribose) polymerase	Netzer et al., 2001
SALL 1	Two-hybrid screen	Transcriptional factor mutated in townes–brocks syndrome	Kaminker et al., 2001
PinX2-4	Two-hybrid screen	To be determined	Zhou et al., 2001

tankyrase-1 and Tin2 [Smith et al., 1998; Kim et al., 1999]. Tankyrase-1 is a poly(adenosine diphosphate-ribose) polymerase that binds to the N-terminal Asp/Glu-rich acidic domain of Pin2/TRF1 and causes adenosine diphosphate (ADP)-ribosylation of TRF1 [Smith et al., 1998]. This modification appears to diminish the ability of TRF1 to bind to telomeric DNA in vitro [Smith and de Lange, 2000]. Furthermore, overexpression of tankyrase-1, but not its poly(adenosine diphosphate-ribose) polymerase-deficient mutant, leads to telomere elongation in telomerase-positive cells [Smith and de Lange, 2000; Cook et al., 2002]. These results indicate that tankyrase-1 promotes telomere elongation. Recently, a closely related protein, tankyrase-2 was identified through its interaction with Pin2/TRF1 [Kaminker et al., 2001]. Furthermore, tankyrase-2 has been shown to oligomerize with tankyrase-1 and may have similar properties to those of tankyrase-1 [Kaminker et al., 2001; Cook et al., 2002]. In contrast to tankyrase-1, however, tankyrase-2 has been shown to cause rapid cell death when highly overexpressed [Kaminker et al., 2001]. These results indicate that although these two tankyrases may share some common functions, they may also have distinct roles in telomere regulation. Further experiments are needed to elucidate their functions and underlying mechanisms.

In contrast to tankyrase-1, which promotes telomere elongation, Tin2 has been shown to inhibit telomere elongation [Kim et al., 1999]. Tin2 interacts with the dimerization domain of Pin2/TRF1 and colocalizes with Pin2/TRF1 at telomeres [Kim et al., 1999]. Furthermore, a mutant Tin2 that lacks amino-terminal sequences can induce elongation of human telomeres only in telomerase-positive, but not in telomerase-negative cells. It has been proposed that this mutant Tin2 may act in a dominant-negative fashion in the cells [Kim et al., 1999]. In this case, Tin2 may be a negative regulator of telomere elongation [Kim et al., 1999], as is the case of Pin2/TRF1. However, the exact relationship between Tin2 and Pin2/TRF1 is not clear and nor is it known about how Tin2 regulates telomere elongation in a telomerase-dependent manner.

2. Pin2/TRF1-Interacting Proteins That Function in Telomere Maintenance by Inhibiting Telomerase

Most telomere proteins and Pin2/TRF1-interacting proteins characterized so far either have no detectable effect on telomere maintenance or modulate telomere length without affecting telomerase activity per se. An exception is PinX1, which represents a novel class of proteins that can regulate telomerase activity directly and has been recently characterized

[Zhou and Lu, 2001]. PinX1 is a nuclear protein that is localized at the nucleolus and at telomeres, the substrate for telomerase [Zhou and Lu, 2001]. Furthermore, PinX1 and its small C-terminal domain called TID (telomerase inhibitory domain) form stable complexes with hTERT both in vivo and in vitro, and directly inhibit telomerase activity with a high potency in vitro [Zhou and Lu, 2001]. Moreover, when overexpressed in telomerase-positive HT1080 cells, both PinX1 and TID inhibit cellular telomerase activity and induce progressive telomere shortening, eventually leading to crisis, with their ability to affect cell growth being correlated with their ability to inhibit telomerase activity and to shorten telomeres [Zhou and Lu, 2001]. However, neither PinX1 nor its TID induces telomere shortening or crisis in telomerase-negative cells [Zhou and Lu, 2001]. In contrast, depletion of endogenous PinX1 significantly increases telomerase activity and telomere length in vivo, indicating that the concentration of cellular PinX1 is a critical factor for regulating telomerase activity in vivo [Zhou and Lu, 2001]. Finally, depletion of endogenous PinX1 increased tumorigenicity of HT1080 cells, whereas overexpression of PinX1 or TID inhibits their ability to form tumors in nude mice. PinX1 is therefore the first characterized endogenous telomerase inhibitor [Zhou and Lu, 2001].

Interestingly, the human PINX1 gene localizes to chromosome 8p23 close to the microsatellite marker D8S277 [Zhou and Lu, 2001]. This region has been extensively investigated due to its frequent loss of heterozygosity (LOH) in a number of human cancers, including prostate, liver, colorectal, lung, head, and neck [Nielsen and Briand, 1989; Emi et al., 1992; Matsuyama et al., 1994; Gustafson et al., 1996; Nagai et al., 1997; Ishwad et al., 1999; Perinchery et al., 1999; Pineau et al., 1999; Sun et al., 1999; Baffa et al., 2000; Liao et al., 2000; Muscheck et al., 2000; Shao et al., 2000; Bockmuhl et al., 2001]. For example, in human hepatocellular carcinomas, about 40–50% of tumors exhibit LOH near the maker D8S277 and molecular analysis suggests the presence of tumor suppressor genes on chromosome 8p23. The significance of PinX1 in oncogenesis is substantiated by the findings that depletion of PinX1 increases tumorigenicity of HT1080 cells, whereas overexpression of PinX1 or TID inhibits their ability to produce tumors in nude mice [Zhou and Lu, 2001]. Activation of telomerase is a common and critical event for cellular transformation and this activation is important for transformed cells to continue cell division. Indeed, transgenic overexpression of hTERT induces tumor formation in mice [Gonzalez-Suarez et al., 2001]. The fact that PinX1 inhibits telomerase suggests that inactivation or down-regulation of PinX1 may contribute to activation of telomerase in cancer cells [Zhou and Lu, 2001]. These results suggest that PinX1 may be a putative tumor suppressor gene located at chromosome 8p23. Given the strong ability of the PinX1 or its TID to inhibit telomerase, induce crisis and inhibit tumor formation in nude mice [Zhou and Lu, 2001], it may well prove to be a novel reagent for cancer therapy.

3. Pin2/TRF1-Interacting Proteins That Function Both in Telomere Maintenance and Cell Cycle Control

This category of Pin2/TRF1-interacting proteins includes Ku, NBS1 [Wu et al., 2000], and ATM [Kishi et al., 2001b; Kishi and Lu, 2002]. Interestingly, all these proteins are involved in DNA damage repair and their dysfunction causes prominent defects in DNA damage-induced cell cycle checkpoint regulation and telomere function [Shiloh, 1997; Digweed et al., 1999; Gasser, 2000; Lee and Kim, 2002].

Ku. The Ku heterodimer is the high-affinity DNA binding component of the DNA-dependent protein kinase and is essential for the non-homologous end-joining pathway of DNA double-strand break repair [Gasser, 2000; Lee and Kim, 2002]. During the repair of double-strand breaks, Ku binds non-specifically to DNA ends with high affinity. However, telomeric ends are capped or bound by specific telomere proteins that serve to conceal and disguise the telomeric DNA end, thereby preventing end fusion events and preventing cellular DNA damage signaling. Interestingly, Ku has been shown to be physically localized to telomeres both in yeast and in mammalian cells [Gravel et al., 1998; Hsu et al., 1999]. Furthermore, Ku is important for maintaining chromosomal DNA end structure in yeast [Gravel et al., 1998], but is released from telomeres in a RAD9-dependent response to DNA damage [Martin et al., 1999]. Further experiments indicate that Ku does not bind telomeric DNA directly but localizes to telomeric repeats via its interaction with Pin2/TRF1 in mammalian cells [Hsu et al., 2000].

Importantly, cells that are deficient for Ku80 or the DNA-dependent protein kinase catalytic subunit accumulate a large percentage of telomere fusions [Hsu et al., 2000; Gilley et al., 2001]. These results suggest that Ku plays a critical role in telomere capping.

NBS1. NBS1 is encoded by the gene mutated in Nijmegen breakage syndrome, a chromosomal instability disorder, which is characterized in part by cellular hypersensitivity to ionizing radiation [Featherstone and Jackson, 1998; Digweed et al., 1999]. NBS1 forms a complex with Rad50 and Mre11 [Haber, 1998]. Cells deficient in the formation of this complex are defective in DNA double-strand break repair, cell cycle checkpoint control, and telomere length maintenance [Digweed et al., 1999]. The function of NBS1 is unknown although there is speculation that it might recognize signals from a DNA damage-sensing complex that could be in the form of phosphorylation of serine or threonine residues that are, in turn, recognized by the forkhead-associated domain in NBS1 [Featherstone and Jackson, 1998]. Mre11 colocalizes to subnuclear regions containing DNA breaks after irradiation [Maser et al., 1997]. In NBS1 cells, a deficiency of NBS1 is correlated with an inability to form Mre11–Rad50 nuclear foci in response to ionizing radiation [Carney et al., 1998]. Together, these observations point to a major role for the Mre11–Rad50–NBS1 complex in repair of DNA double-strand breaks. Interestingly, Pin2/TRF1 has been shown to bind NBS1 and colocalize with NBS1 and Mre11 at promyelocytic leukemia (PML) nuclear bodies in immortalized telomerase-negative cell lines, but rarely in telomerase-positive cell lines [Wu et al., 2000]. Significantly, the translocation of NBS1 to PML bodies occurs specifically during late S to G2 phases of the cell cycle and coincides with active DNA synthesis in these NBS1-containing PML bodies [Wu et al., 2000]. These results suggest that NBS1 may be involved in alternative lengthening of telomeres in telomerase-negative immortalized cells. However, the biological significance of the Pin2/TRF1 and NBS1 interaction in telomere regulation and DNA damage response remains to be elucidated.

ATM. ATM encodes a protein kinase that is activated by ionizing DNA damage and is critical for maintaining genome stability, telomere maintenance, and induction of cell cycle checkpoints by double strand DNA breaks [Lavin and Shiloh, 1997; Abraham, 2001]. Double strand DNA breaks activate ATM and trigger multiple pathways to ensure that cells delay entry into mitosis following DNA damage to repair the damaged DNA before cell division. Many of these pathways ultimately lead to the inhibition of cyclin B/Cdc2, a major protein kinase that regulates entry into mitosis [Furnari et al., 1997; O'Connell et al., 1997; Peng et al., 1997; Sanchez et al., 1997; Abraham, 2001]. However, in A-T cells, the ATM-dependent mitotic checkpoint is disrupted and cyclin B/Cdc2 cannot be kept in an inactive state after DNA damage [Paules et al., 1995; Beamish et al., 1996; Rotman and Shiloh, 1999]. Therefore, A-T cells are hypersensitive to ionizing radiation [Pandita et al., 1995; Metcalfe et al., 1996; Xia et al., 1996; Smilenov et al., 1997].

Interestingly, radiation hypersensitivity of A-T cells has been shown to correlate with their telomere loss [Pandita et al., 1995; Metcalfe et al., 1996; Xia et al., 1996; Smilenov et al., 1997]. There is compelling evidence supporting an important role for ATM in the regulation of telomere metabolism. Cells derived from humans and mice with a defective ATM gene show a prominent defect related to telomere dysfunction [Savitsky et al., 1995; Barlow et al., 1996; Elson et al., 1996; Xu and Baltimore, 1996; Xu et al., 1996]. These cells have an accelerated rate of telomere loss and chromosome end-to-end associations and show premature senescence [Rudolph and Latt, 1989; Beamish et al., 1994; Pandita et al., 1995; Metcalfe et al., 1996; Xia et al., 1996; Smilenov et al., 1997]. Furthermore, ATM has recently been shown to regulate the interaction between telomeres and the nuclear matrix [Smilenov et al., 1999]. In addition, the yeast ATM homologues TEL1 and MEC1 control telomere length and the G2/M checkpoint; their mutations result in shortened telomeres, G2/M checkpoint defect, and genomic instability [Greenwell et al., 1995; Morrow et al., 1995; Sanchez et al., 1996b]. Furthermore, TEL1 substitutes for ATM in rescuing telomere shortening, radiation hypersensitivity, and the G2/M checkpoint defect in A-T cells [Fritz et al., 2000]. These results indicate that ATM plays a crucial role in regulating telomere length and the DNA damage mitotic checkpoint. However, it is not fully clear how ATM is involved in coordinating these two events.

Pin2/TRF1 as a key molecule in mediating ATM regulation of DNA damage response and telomere maintenance. ATM interacts with the NH2-terminal half of Pin2/TRF1 and forms stable complexes with Pin2/TRF1 in cells [Kishi et al., 2001b]. Furthermore, ionizing DNA damage induces phosphorylation of Pin2/TRF1 in an ATM-dependent manner and ATM directly phosphorylates Pin2/TRF1 preferentially on Ser219 both in vitro and in vivo [Kishi et al., 2001b]. The biological significance of this phosphorylation is supported by functional analyses of the phosphorylation site mutants [Kishi et al., 2001b]. The Ser219A Pin2 mutant refractory to ATM phosphorylation potently induces mitotic entry and apoptosis and increases radiation hypersensitivity of A-T cells. In contrast, Ser219Asp or Ser219Glu mutants potentially mimicking ATM phosphorylation on Ser219 completely fail to induce apoptosis, and also reduces radiation hypersensitivity of A-T cells. These results indicate that ATM binds and phosphorylates Pin2/TRF1, and likely negatively regulates its function [Kishi et al., 2001b].

The biological importance of ATM regulation of Pin2/TRF1 has further been established by inhibiting Pin2/TRF1 function through stably expressing different dominant-negative Pin2/TRF1 mutants in A-T cells [Kishi and Lu, 2002; Nakamura et al., 2002] (Fig. 3). As shown previously in other cell lines [van Steensel and de Lange, 1997], dominant-negative Pin2/TRF1 mutants increase telomere length in A-T cells [Kishi and Lu, 2002]. More impressively, they also restore many other important phenotypes in A-T cells [Kishi and Lu, 2002; Nakamura et al., 2002]. Following ionizing radiation, most A-T cells quickly enter apoptosis and a small fraction of them that do not enter apoptosis eventually senesce. In sharp contrast, A-T cells stably expressing dominant-negative Pin2/TRF1 mutants do not enter apoptosis immediately following ionizing radiation [Kishi and Lu, 2002]. Instead, these cells delay entry into mitosis and accumulate in G2 phase [Kishi and Lu, 2002], which is a normal DNA damage response [Beamish et al., 1994]. These Pin2/TRF1-inhibited A-T cells do not senesce but continue to divide, eventually leading to the formation of many cell colonies [Kishi and Lu, 2002]. In addition, inhibition of Pin2/TRF1 function in A-T cells fully restores their mitotic spindle defect in response to microtubule disruption [Nakamura et al., 2002]. Interestingly, these phenotypes of Pin2/TRF1-inhibited A-T cells are indistinguishable from those of A-T cells re-expressing ATM [Kishi and Lu, 2002; Nakamura et al., 2002]. However, inhibition of Pin2/TRF1 completely fails to correct the S phase checkpoint defect in the same cells, in contrast to ATM, which can restore both the S and G2/M checkpoint defect in A-T cells [Kishi and Lu, 2002]. Similarly, expression of either yeast CHK1 or yeast TEL1 gene in A-T cells complements the radiosensitivity and G2/M checkpoint defect, but not the S phase checkpoint defect [Chen et al., 1999; Fritz et al., 2000].

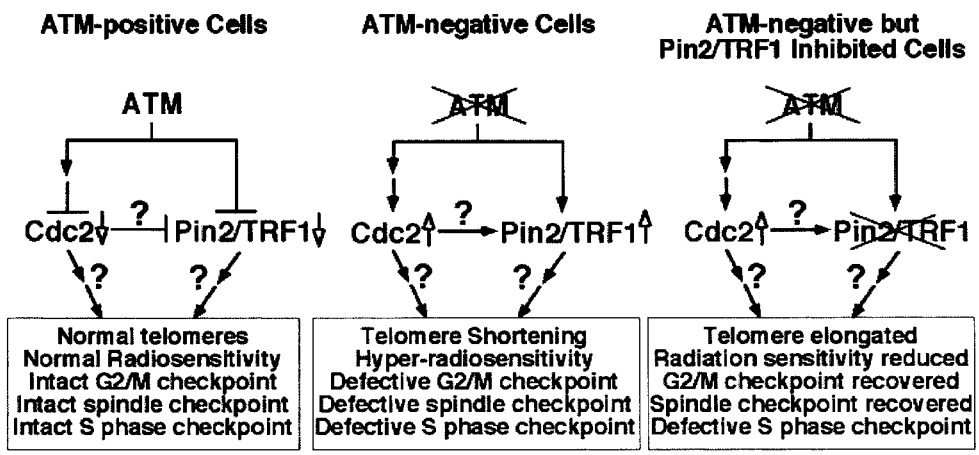

Fig. 3. Restoration of specific phenotypes in ATM-negative cells by inhibiting Pin2/TRF1. In contrast to normal ATM-positive cells, ATM-negative cells have shortened telomeres, hypersensitivity to radiation, defects in multiple cell cycle checkpoints upon either DNA damage or mitotic spindle disruption. Interestingly, stable expression of dominant-negative Pin2/TRF1 mutants in ATM-negative cells can elongate telomeres, reduce radiation sensitivity, and restore G2/M and mitotic spindle checkpoint defects, but not the S phase checkpoint defect.

These results indicate that direct inhibition of endogenous Pin2/TRF1 function can bypass the requirement for ATM in ATM-negative cells in specifically rescuing telomere shortening, radiosensitivity, and defects in the G2/M and mitotic spindle checkpoints. These results provide convincing evidence for the functional importance of Pin2/TRF1 in mediating ATM-dependent regulation and strongly argue that the negative regulation of Pin2/TRF1 by ATM, presumably via phosphorylation, plays a critical role both in maintaining telomeres and in mitotic regulation. The fact that this negative regulatory mechanism is missing in ATM-negative cells may contribute to both shortened telomeres and the hypersensitivity to ionizing radiation seen in such cells [Pandita et al., 1995; Savitsky et al., 1995; Metcalfe et al., 1996; Xia et al., 1996; Morgan et al., 1997; Smilenov et al., 1997; Ziv et al., 1997]. Thus, Pin2/TRF1 is a critical ATM downstream target in the regulation of telomeres and mitotic checkpoints.

It remains to be determined how Pin2/TRF1 is involved in the ATM-dependent G2/M checkpoint regulation. Upon double-strand DNA breaks, activation of ATM kinase in normal cells phosphorylates several downstream target proteins, including p53 and the checkpoint kinases Chks [Lavin and Shiloh, 1997; Abraham, 2001]. Chks inhibit Cdc25C and activate Wee1, which are the protein phosphatase and kinase that activate and inhibit Cdc2, respectively. In addition, Chks and ATM also phosphorylate p53, resulting in an increase in transcription of the Cdk inhibitor p21 and the Cdc2 sequester 14-3-3σ. These multiple and redundant pathways have been shown to ensure that Cdc2 is not activated and cells delay entry into mitosis following DNA damage [Lavin and Shiloh, 1997; Abraham, 2001]. However, since these G2/M checkpoint cascades are disrupted and Cdc2 cannot be kept in an inactive state after DNA damage in ATM-negative cells, they fail to delay entry into mitosis and instead enter abortive mitosis and apoptosis. Surprisingly, it appears that inhibition of Pin2/TRF1 restores the G2/M checkpoint without inhibiting Cdc2 in A-T cells after ionizing radiation, at least as assayed by Cdc2 tyrosine phosphorylation and H1 kinase activity [Kishi and Lu, 2002]. These results suggest that ATM may also regulate the G2/M checkpoint via controlling the function of Pin2/TRF1 directly. Indeed, ATM regulates the mitotic function of Pin2/TRF1 via phosphorylation on Ser219 [Kishi et al., 2001b]. These results suggest that Cdc2 and Pin2/TRF1 may be collaboratively or sequentially involved in the cascade of the G2/M checkpoint control, although their exact relation remains to be determined.

4. Pin2/TRF1-Interacting Proteins That Function in Cell Cycle Control

As further support of the role for Pin2/TRF1 in mitotic regulation, several Pin2/TRF1-interacting proteins have known roles in cell cycle regulation. These include NIMA [Lu et al., 1996], mitotic spindle [Nakamura et al., 2001a], and the microtubule plus end-binding protein EB1 [Nakamura et al., 2002]. NIMA is a mitotic kinase in *Aspergillus nidulans* that can induce a lethal mitotic catastrophic in all eukaryotic cells so far examined [Osmani et al., 1988, 1991; O'Connell et al., 1994; Lu and Hunter, 1995; Krien et al., 1998]. The original genetic screen leading to the discovery of Pin1-3 indicates that this mitotic lethal phenotype can be suppressed by a dominant-negative Pin2/TRF1 mutant [Lu et al., 1996]. This phenotype is also supported by the findings that overexpression of Pin2/TRF1 induces mitotic entry and apoptosis [Kishi et al., 2001a]. However, the biological significance of the Pin2/TRF1 and NIMA interaction remains to be elucidated, although multiple mammalian NIMA-related kinases have been identified [Fry, 2002]. Interestingly, Pin2/TRF1 directly binds microtubules via its C-terminal domain and can also promote microtubule polymerization in vitro [Nakamura et al., 2001a]. Furthermore, this microtubule-promoting activity appears to be regulated by EB1, which can also bind to the C-terminal domain of Pin2/TRF1 [Nakamura et al., 2002]. EB1, originally identified as an APC (adenomatous polyposis coli)-interacting protein, binds APC, a tumor suppressor, in a cell cycle-dependent manner [Su, 1993 #1414; Askham, 2000 #2032; Tirnauer, 2000 #2033]. EB1 is located at the mitotic spindle and is important in spindle assembly [Mimori-Kiyosue et al., 2000; Nakamura et al., 2001b] as well as for the cytokinesis/spindle checkpoint regulation [Muhua et al., 1998]. Interestingly, Pin2/TRF1 also colocalizes with the mitotic spindles specifically during M phase [Nakamura et al., 2001a]. These results suggest a new role for Pin2/TRF1 in modulating the function of microtubules during mitosis. This suggestion

is further substantiated by the findings that mitotic arrest and then apoptosis induced by overexpression of Pin2/TRF1 can be potentiated by microtubule-disrupting agents [Kishi et al., 2001a]. Furthermore, inhibition of Pin2/TRF1 function in A-T cells is able to fully restore their mitotic spindle defect in response to the disruption of mitotic spindles, as does re-expression of ATM [Nakamura et al., 2002]. These results have demonstrated that Pin2/TRF1 not only interacts with the mitotic spindle, but also plays an important role in the regulation of the mitotic spindle checkpoint, providing the convincing evidence for a role of Pin2/TRF1 in mitotic regulation.

It remains to be elucidated how Pin2/TRF1 is involved in mitotic spindle checkpoint regulation. It is possible that the interaction between Pin2/TRF1 and microtubules during mitosis helps maintain the mitotic spindle checkpoint by affecting the function of microtubules or other proteins on the mitotic spindles [Nakamura et al., 2002]. The decrease in Pin2/TRF1 as cells exit from mitosis [Shen et al., 1997] is reminiscent of the degradation of other cell cycle regulatory proteins, such as the mitotic cyclins [King et al., 1996]. In these cases, degradation is required for exit from mitosis [King et al., 1996 #710]. Therefore, it is possible that degradation of Pin2/TRF1, especially the spindle-associated Pin2/TRF1, is also needed for cells to exit from mitosis. This would be consistent with the findings that the ability of Pin2/TRF1 to affect mitosis depends on its concentration outside telomeres and is potentiated by the microtubule-affecting drug nocodazole [Kishi et al., 2001a]. It would also be consistent with findings that inhibition of Pin2/TRF1 can restore the mitotic checkpoint defect in A-T cells [Kishi and Lu, 2002] because Pin2/TRF1 levels are elevated by nocodazole arrest [Shen et al., 1997]. However, further experiments are needed to elucidate the precise mechanism by which Pin2/TRF1 is involved in the regulation of the mitotic spindle checkpoint.

A MODEL FOR THE DUAL ROLES OF Pin2/TRF1 IN TELOMERE MAINTENANCE AND CELL CYCLE CONTROL

From these observations, we propose a model in which Pin2/TRF1 functions as a key molecule in connecting telomere maintenance and cell cycle control (Fig. 4). In this model, the key aspect is the localization and concentration of Pin2/TRF1, which is tightly regulated and determines its role in both telomere maintenance and mitotic progression. When Pin2/TRF1 binds to telomeres, its bound concentration may function as a mechanism by which cells sense telomere length in a feedback control mechanism of telomere length regulation, as shown for Rap1p [Marcand et al., 1997]. Telomere-bound Pin2/TRF1 may perform this function by recruiting Pin2/TRF1-interacting proteins such as telomerase inhibitor PinX1 [Zhou and Lu, 2001] or other factors such as Tin2 [Kim et al., 1999] to inhibit telomere elongation. This is consistent with the finding that overexpression of Pin2/TRF1 accelerates telomere shortening whereas reducing Pin2/TRF1 binding to telomeres elongates telomeres [van Steensel and de Lange, 1997; Kishi and Lu,

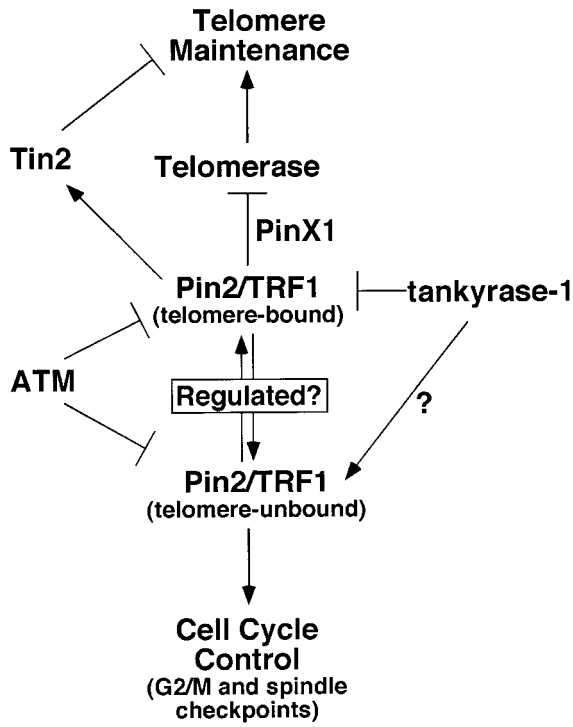

Fig. 4. A model for the dual role of Pin2/TRF1 in telomere maintenance and cell cycle control. The key aspect is the localization and concentration of Pin2/TRF1, which is tightly regulated likely by post-translation modifications and determines its role in both telomere maintenance and mitotic progression. When bound to telomeres, Pin2/TRF1 may regulate telomere maintenance by recruiting its interacting proteins such as telomerase inhibitor PinX1 or other factors such as Tin2 in a negative feedback mechanism. When not bound (unbound) to telomeres such as during late G2 and M, Pin2/TRF1 may interact with cell cycle proteins such as ATM and/or mitotic spindles to regulate entry into M phase and progression through M phase.

2002]. However, Pin2/TRF1 that is free from telomeres is increased during late G2 and M, it may interact with cell cycle proteins such as ATM, NIMA, and/or mitotic spindles to function as a mechanism that promotes entry into M phase and progression through M phase [Lu et al., 1996; Shen et al., 1997; Kishi et al., 2001a,b; Nakamura et al., 2001a, 2002; Kishi and Lu, 2002]. Finally, degradation of Pin2/TRF1 may be required for cells to exit from M phase. This is consistent with cell cycle-dependent fluctuation of Pin2/TRF1, being increased during late G2 and M and decreased as cells exit from M [Shen et al., 1997]. Furthermore, this idea is supported by the findings that overexpression of Pin2/TRF1 promotes entry into M and then apoptosis [Kishi et al., 2001a]. Furthermore, the ability of Pin2 to induce mitotic entry and apoptosis depends on telomere length in the cell [Kishi et al., 2001a]. It is possible that telomere length may determine the concentration of telomere-unbound Pin2/TRF1 [Kishi et al., 2001a]. Moreover, it is also supported by the findings that a point mutant in the DNA-binding domain of Pin2 abrogates binding to the telomeric DNA but still potently induces apoptosis [Kishi et al., 2001a]. Finally, it is consistent with the findings that inhibition of Pin2/TRF1 can restore many mitotic checkpoint defects in A-T cells [Kishi et al., 2001b; Nakamura et al., 2001a, 2002; Kishi and Lu, 2002]. Therefore, telomere-bound and unbound Pin2/TRF1 may function as important separate signals for regulating telomere length and the cell cycle, respectively.

FUTURE DIRECTIONS

A major challenge for the future will be to determine how Pin2/TRF1 affects the cell cycle, how Pin2/TRF1 modulates telomere maintenance, and how these two functions are coordinated and regulated during the cell cycle as well as during long-term cell growth. In addition, deregulation of telomeres and the cell cycle have an important role in the pathogenesis of human diseases such as cancer and ageing [Bryan and Cech, 1999; Artandi and DePinho, 2000; Evans and Lundblad, 2000; Stewart and Weinberg, 2000; Blackburn, 2001; Shay and Wright, 2001]. Furthermore, Pin2/TRF1 has been shown to be deregulated in some human cancers [Aragona et al., 2000; Yamada et al., 2000; De Divitiis and La Torre, 2001; Kishi et al., 2001a; Ohyashiki et al., 2001] and also to interact with human disease genes such as ATM and NBS1 [Wu et al., 2000; Kishi et al., 2001b; Kishi and Lu, 2002]. Another major challenge will therefore be to determine the role of Pin2/TRF1 in the development and treatment of human diseases such as cancer, premature ageing, and ataxia-telangiectasia. Answers to these questions likely come from the further identification and characterization of Pin2/TRF1 upstream regulators and downstream targets.

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