Heterogeneous Nuclear Ribonucleoprotein A1 and UP1 Protect Mammalian Telomeric Repeats and Modulate Telomere Replication in Vitro*

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Frédéric Dallaire‡, Sophie Dupuis‡§, Stéphan Fiset, and Benoit Chabot¶

From the Département de Microbiologie et d’Infectiologie, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, Québec J1H 5N4, Canada

The heterogeneous nuclear ribonucleoprotein A1 protein and a shortened derivative (UP1) promote telomere elongation in mammalian cells. To gain insights into the function of A1/UP1 in telomere biogenesis, we have investigated the binding properties of recombinant A1/UP1 and derivatives to single-stranded DNA oligonucleotides. Our results indicate that UP1 prefers to bind to DNA carrying single-stranded telomeric extensions at the 3’ terminus. The RNA recognition motif 1 is sufficient for strong and specific binding to oligomers carrying vertebrate telomeric repeats. We find that the binding of A1/UP1 protects telomeric sequences against degradation by endo- and exonucleases. Moreover, A1/UP1 binding prevents extension by telomerase and-terminal deoxynucleotidyltransferase and inhibits rNTP-dependent DNA synthesis in vitro. These observations are consistent with the hypothesis that A1/UP1 is a telomere end-binding protein that plays a role in the maintenance of long 3’ overhangs.

Telomeres are the specialized structures at the end of linear chromosomes and are essential for normal cellular function. Telomeres prevent degradation and aberrant recombination of chromosome termini and facilitate the complete replication of chromosome ends (for reviews, see Refs. 1–3). Vertebrate telomeres contain variable numbers of TAGGGT repeats in double-stranded form and terminate with a single-stranded overhang. The 3'-end of the G-rich strand, the strand making the 3' end of the chromosome (4–6). The ribonucleoprotein enzyme telomerase directs the synthesis of telomeric repeat units onto this G-rich strand, thereby counteracting the loss of sequence that occurs at each cell division (3). It is thought that the G-rich strand will then serve as substrate for the synthesis of the complementary strand by DNA primase followed by conventional DNA polymerases (7, 8).

The presence of a 3’ overhang of the G-rich strand suggests that single-stranded DNA binding activities will play an important role in telomere function. Proteins that can bind to single-stranded telomeric repeats include protein α of Oxytricha, Stylonychia, and Euplotes. The 56-kDa protein α of Oxytricha exists as an heterodimer with the 41-kDa β protein (9–11). These proteins protect single-stranded overhangs from nuclease digestion and chemical modification (for a review, see Ref. 12). Moreover, the binding of an α/α homodimer or an α/β heterodimer to telomeric DNA renders the end inaccessible to telomerase (13). The Chlamydomonas protein Gbp1p binds to single-stranded G-rich telomeric DNA (14, 15), but its role in telomere function in vivo remains to be shown. While telomerase RNA makes direct contacts with single-stranded extensions during repeat synthesis, protein components of Tetrahymena and Euplotes telomerasers can also interact with telomeric single-stranded DNA substrates by protein-DNA interactions (16–18).

In Saccharomyces cerevisiae, Est1p and Est4p/Cdc13p have properties of terminus-binding proteins, and their association with G-rich extensions may mediate recognition by telomerase. Mutant strains engineered not to express Est1p, or expressing mutated forms of Cdc13p, undergo telomere attrition despite having wild-type levels of telomerase (19, 20). While Est1p interacts with telomerase RNA in vitro and in vivo, its presence is not essential in some telomerase activity assays in vitro (20–22).

In vertebrates, several proteins can interact with single-stranded G-rich extensions in vitro. For example, the chicken MF3 protein and the Xenopus XTEF protein share this property (23, 24). However, there has been no demonstration that these proteins bind to telomeric repeats in vitro or that their expression influences the structure of telomeres. Mammalian hnRNPα proteins have been reported to associate with RNA and DNA oligonucleotides carrying telomeric repeats (25–29). The only mammalian hnRNP protein for which genetic evidence of a function in telomere biogenesis has been obtained is the hnRNP A1 protein. Ectopic expression of A1 promotes telomere elongation in mammalian cells (29). Although hnRNP A1 is a well known modulator of alternative pre-mRNA splicing (30–32), several observations are consistent with the notion that the function of A1 in telomere control may be direct and therefore independent of its role in alternative splicing. First, a shortened derivative of A1 (UP1) that has no intrinsic activity in alternative splicing (31), but which can antagonize the modulatory activity of A1 in splicing extracts (30), also promotes telomere elongation (29). Second, UP1 and A1 can interact specifically with single-stranded telomeric repeats in vitro (29). Third, UP1 may interact with telomerase, as judged by its...
ability to recover telomerase activity from a cell lysate (29). Although we do not yet know whether A1 binds to single-stranded telomeric extensions in vivo, here we present further evidence consistent with a direct role for A1 in telomere biogenesis. We show that the binding of A1 and UP1 to a long single-stranded oligonucleotide protects DNA from endonuclease and exonuclease attack. Moreover, A1/UP1 binding to telomeric repeats represses telomerase extension and rNTP-dependent DNA synthesis. Thus, our results suggest that A1/UP1 can contribute to telomere protection and can modulate telomere replication in vitro.

**EXPERIMENTAL PROCEDURES**

**Oligonucleotides**—DNA oligonucleotides (see Table I) were purified on acrylamide/urea gels. For binding assays, oligonucleotides were 5′-end-labeled by incubation with T4 polynucleotide kinase (Promega) and purified using MicroSpin G-25 columns (Amersham Pharmacia Biotech).

**Plasmids**—pGEX-A1 was constructed by inserting the mouse A1 cDNA (33) into the EcoRI site of pGEX-2T. The UP1 fragment was produced as described by LaBranche et al. (29) and was inserted into the EcoRI site of pGEX-2T. pGEX-UP1ΔRM1 was produced by deleting the Pos1–Nco1 fragment of pGEX-UP1ΔRM2 was produced by cutting pGEX-UP1 with RglII, filling with Klenow before religation.

**Production of Recombinant Proteins**—Recombinant A1, UP1, and UP1ΔRM2 were expressed in *Escherichia coli* BL21 or DH5α. Cells were grown 2–3 h at 37 °C until reaching an OD between 0.5–2.0 at 600 nm. Induction with 100 μM isopropyl-β-D-thiogalactopyranoside was performed for 4 h. Cells were washed with PBS and resuspended in 5 ml of PBS per 100 ml of culture, to which was added 10 μM of phenylmethylsulfonyl fluoride (0.5 M) and 25 μM of MgCl₂, 7.5 mM KCl, 1 mM DTT, 0.1 M Hepes (pH 7.6), 0.1 mM EDTA, 2.5 mM MgCl₂, 7.5 mM KCl, 1 mM EDTA, 0.1 μg of poly(dI-dC)/poly(dI-dC) (Amersham Pharmacia Biotech). Samples were loaded onto a 5% nondenaturing polyacrylamide gel (115 V), and complexes were visualized by autoradiography. DNase I (Amersham Pharmacia Biotech), exonuclease I (U.S. Biochemical Corp.), and Bal31 (Promega) protection assays were set up with 5′-labeled oligonucleotides incubated with recombinant proteins in the binding buffer described above. Following a 30-min incubation at 30 °C, each sample was split into two sets. One set was tested for binding in a native gel, as above. Nuclease was added to the other set (5 units of DNase I, 1 unit of exonuclease I, or 1 unit of Bal31), and the mixtures were incubated for 10 min at room temperature (DNase I), 1 min at 37 °C (exonuclease I), or 10 min at 30 °C (Bal31).

**TdT Assay**—Ten pmol of oligonucleotides was added to each of 14 wells, in the presence of G-UP1 (G-1), G-UP1 (G-2), G-A1, UP1, G-UP1ΔL121, G-UP1ΔL121, and gene 32 protein (gp32), 1 μl of [α-32p]dGTP (3 000 Ci/mmol; Amersham Pharmacia Biotech), and 5 μl of Telomix B (200 μM Tris-HCl (pH 8.2), 20 mM dATP, 20 mM dTTP, 4 μM dGTP, 4 mM MgCl₂, 8 mM EDTA, 8 mM DTT, 1 μl of RNaseA (Amersham Pharmacia Biotech), 4 mM spermidine, 0.4 mM spermine, 0.1 mg/ml of calf thymus DNA, 10 mM 3-aminopropionic acid, 10 μM telomeric repeats represses telomerase extension and rNTP-dependent DNA synthesis. Thus, our results suggest that A1/UP1 can contribute to telomere protection and can modulate telomere replication in vitro.

**RESULTS**

**A1 and UP1 Binding to Telomeric Repeats**—We report that recombinant forms of the A1 and UP1 proteins (GST-A1 and GST-UP1) can bind directly and specifically to short single-stranded oligonucleotides carrying 2–4 contiguous vertebrate TTAGGG telomeric repeats (29). As monitored by gel shift assays, A1 and UP1 did not bind to a 22-nucleotide yeast telomeric sequence (data not shown). Because mammalian chromosomes end with G-rich single-stranded overhangs, we tested the influence of non telomeric sequences flanking the telomeric repeats. Binding assays using UP1 and oligonucleotides carrying non telomeric sequences upstream or downstream of the telomeric cassette indicated a slight preference.
for telomeric repeats located at the 3'-end (Fig. 1A, compare lanes 1–3 with lanes 9–11). Likewise, UP1 bound slightly more efficiently to oligonucleotides carrying a double-stranded portion at the 5'-end rather than at the 3'-end (Fig. 1A, compare lanes 5–7 with lanes 13–15). Binding assays performed in the presence of an excess of unlabeled competitor oligomers confirmed that UP1 interacts slightly better with an oligomer that contains telomeric sequences at the 3' terminus (Fig. 1B).

Because the length of single-stranded G-rich overhangs in human and mouse telomeres ranges from 50 to 150 nucleotides (4–6), we examined the binding of A1/UP1 to a more natural substrate (TS10) containing 10 contiguous telomeric TTAGGG repeats and, hence, nine complete A1 binding sites (TAGGGT). A mobility shift assay performed with GST-UP1 revealed the assembly of three complexes with TS10 (Fig. 2, lanes 1–6). A similar result was obtained with GST-A1, and no complex was formed when GST-A1 or GST-UP1 was incubated with a control oligonucleotide of similar length (data not shown). The profile of complex formation on TS10 was different when a preparation of UP1 lacking the GST moiety was used (Fig. 2, lanes 8–10). In this case, a small complex appeared at a low concentration of UP1, several complexes of intermediate mobility appeared as the concentration of UP1 was increased, and a single highly retarded complex was detected at the highest concentration of UP1. Thus, although the presence of a bulky GST domain does not dramatically affect the affinity of UP1 to a single-stranded telomeric extension, it influences the architecture of the complex.

**RRM1 Is Sufficient for Strong and Specific Binding to Telomeric Sequences**—To determine whether the binding of UP1 to telomeric sequences requires both nucleic acid binding domains, we tested the binding activity of UP1 molecules deprived of either RRM2 or part of RRM1 (Fig. 3, A and B). The UP1 derivative lacking the C-terminal RRM2 domain (UP1Δ2) was almost as efficient as the complete UP1 protein at binding to TS10 (Fig. 3C). The binding of UP1Δ1 remained specific, since short oligonucleotides carrying mutated telomeric repeats were bound less efficiently by UP1Δ2 (Fig. 3D). A UP1 derivative lacking most of RRM1 (UP1Δ1) was considerably less efficient than UP1 and UP1Δ2 at binding to TS10 (Fig. 3C). These results indicate that the N-terminal portion of UP1 that contains RRM1 is sufficient for stable and specific binding. Since stable UP1 binding requires a minimum of two TAGGGT units (29), each unit may be bound by the RRM1 domain of one UP1 molecule.

**A1 and UP1 Can Protect Telomeric Sequences from Nuclease Digestion**—We next examined whether UP1 binding could protect telomeric sequences against nucleolytic activities. Incubation of naked TS10 with DNase I yielded a profile indicative of preferential cleavage after each G in every repeat (Fig. 4A, lane 7; B, lane 6; and C, lane 9). The sites of cleavage were confirmed by comparison with a profile obtained by DMS treatment, which generates cuts at all G nucleotides (data not shown). At the lowest concentrations of GST-A1, DNase I cleavage occurred preferentially at the T of each repeat (Fig. 4A, lanes 1–3). Complete protection of TS10 was observed at higher concentrations of GST-A1 (lanes 4–6). An assay performed with GST-UP1 and a higher level of DNase I yielded similar results (Fig. 4B). Likewise, a preparation of UP1 lacking the GST moiety offered a level of protection that was equivalent to the level obtained with GST derivatives (Fig. 4C, lanes 1–8). In contrast, incubation of GST-A1 or UP1 with an oligonucleotide (C6) not bound by these proteins offered little or no protection against DNase I (Fig. 4A, lanes 8–14; C, lanes 10–18). Thus, low concentrations of A1 or UP1 changed the cleavage profile obtained with DNase I, consistent with the notion that TAGGGT is the unit bound by A1 and UP1. At high concentrations of A1 or UP1, complete protection was observed. The binding of A1 and UP1 to single-stranded telomeric sequences
can therefore promote the assembly of a complex that is highly resistant to endonuclease attack. The derivative UP1Δ1, which binds weakly to TS10, did not offer significant protection against DNase I (Fig. 4A, lanes 8–10). Although UP1Δ2 binds to TS10 nearly as efficiently as UP1, it was less efficient at protecting TS10 against DNase I (Fig. 4A, lanes 5–7). Thus, strong binding is not sufficient to confer full protection, suggesting that portions of UP1 help prevent access of the endonuclease to DNA.

Protection assays were also performed with the E. coli 3′ exonuclease I, which carries out nucleotide removal on naked TS10 or C6 to yield a ladder of products (Fig. 4E, lanes 3 and 6). It is unclear why no products shorter than 35 nucleotides are detected with TS10. The highest concentration of UP1 decreased the level of TS10 Exo I products by 2-fold while providing no protection to the C6 oligonucleotide (lanes 2 and 5, respectively). A similar experiment performed with Bal31 exonuclease showed that the addition of increasing amounts of
UP1 protects oligonucleotide TS10 but not C6 against Bal31 activity (data not shown). The efficiency of UP1 binding to the oligonucleotides correlated well with the level of protection from Bal31 (Fig. 4F). Our results clearly indicate that UP1 binding to a long telomeric substrate provides protection against the activity of endo- and exonucleases.

**A1 and UP1 Inhibit Telomerase-mediated Extension**—We next investigated whether A1 or UP1 could affect the ability of telomerase to extend telomeric DNA in vitro. Recombinant UP1 lacking the GST moiety was prebound to TS10, and the complex was incubated in a HeLa S100 extract. In this conventional telomerase assay, the extension products are monitored directly following fractionation in a denaturing gel. In the absence of UP1, telomerase added multiple repeats to TS10, indicative of high processivity (Fig. 5A, lane 1). No activity was detected when the S100 extract was treated with RNase A (lane 2). Preincubation with increasing amounts of UP1 led to a gradual reduction in telomerase activity (lanes 3–5) such that few products were made at concentrations of UP1 that shifted TS10 into low mobility complexes (Fig. 5B, lanes 1–4). A similar result was obtained with GST-A1 (Fig. 5A, lanes 6–8). Inhibition appeared A1/UP1 protein-specific, since preincubation with the single-stranded DNA-binding gp32 was less efficient at inhibiting telomerase (Fig. 5A, lanes 9–11) despite the fact that gp32 assembled TS10 into low mobility complexes as efficiently as UP1 (Fig. 5B, lanes 5–7).

Inhibition of telomerase activity by UP1 was less apparent when a short substrate was used. The appearance of the shorter extension products derived from oligonucleotide TS was not compromised by large amounts of UP1 (Fig. 5C, lane 2). However, UP1 promoted a small but reproducible decrease in the appearance of the largest (+20) extension product (lane 3). While oligonucleotide TS is a common substrate in telomerase (TRAP) assays (35), it does not contain a complete telomeric repeat and is not bound by UP1 (not shown). Because the largest TS extension product carries three complete A1 binding sites (TAGGGT), the product carrying two sites is the first substrate sensitive to the addition of UP1. This is consistent with our observation that at least two TAGGGT units are required for stable UP1 binding (29). We also tested a derivative carrying one complete telomeric repeat (TS1). While TS1 can be extended in a S100 extract, the addition of UP1 compromised the appearance of a shorter (+14) extension product (Fig. 5C, lane 4), again consistent with the notion that at least two TAGGGT units are required for UP1 binding.

To confirm that UP1 binding to the telomerase substrate was responsible for the inhibition, we preincubated UP1 with a molar excess of an oligonucleotide bound by UP1 (TS8A) or an oligonucleotide not bound by UP1 (MS2). TS8A and MS2 are not efficiently extended by telomerase (data not shown). Preincubating UP1 with TS8A stimulated extension of TS10 (Fig. 5D, lanes 3 and 4), while preincubation with MS2 had little effect (lanes 5 and 6). Overall, these results show that inhibition of telomerase activity by UP1 is specific to substrates carrying high affinity A1 binding sites.

The inhibitory activity of UP1 derivatives lacking either RRM1 or RRM2 was also tested. Although UP1A2 binds to TS10 as efficiently as the complete UP1 protein, it did not inhibit telomerase extension of TS10 (Fig. 5E). UP1Δ1, which does not bind efficiently to TS10, also did not affect telomerase extension (data not shown).

**UP1 Inhibits the Activity of Other DNA Polymerases**—To determine whether UP1 could affect the activity of other DNA polymerases, we first tested the activity of calf thymus termi-
**Fig. 6. UP1 represses TdT activity.** UP1, GST-UP1Δ2, GST-UP1Δ1, and gp32 were prebound to TS10 (20 nM) followed by the addition of TdT. The concentrations of proteins used are indicated in μM above each lane.

**Fig. 7. UP1 inhibits rNTP-dependent lagging strand synthesis.** A, the lagging strand synthesis assay was performed with TS10 (0.5 μM) in the absence of dGTPs and rGTPs. rNTPs are UTP, rCTP, and rATP. The band identified as X (also seen in B and C) is of unknown origin but was always seen when TS10 was incubated in the absence of rNTPs. B, UP1 does not inhibit rNTP-independent lagging strand synthesis. In addition to TS10 (0.5 μM), the mixture contained an oligonucleotide complementary to TS10 (C5, 1 μM) and dNTPs (dCTP, dATP, and dTTP). C, rescue of rNTP-dependent lagging strand synthesis by oligonucleotides. In the assay shown in lanes 1–7, the amount of TS10 was increased while keeping the concentration of UP1 constant. In the assay shown in lanes 8–14, oligonucleotides Tel2 and Ne4 were co-incubated with TS10 in the HeLa extract without exogenous UP1. Tel2, but not Ne4, is bound by UP1 in gel shift assays (not shown). The assay depicted in lanes 15–20 monitors the effect of co-incubating oligonucleotide Tel2 in a HeLa nuclear extract (HeLa), a HeLa S100 extract (S100), and a nuclear extract prepared from mouse erythroblastic CB3 cells that are severely deficient in hnRNPA1 protein (CB3; Ref. 29). Endogenous levels of A1 are high in the HeLa nuclear extract and low in the S100 extract.

Intracellular deoxynucleotidyltransferase (TdT), which like telomerase carries out extension on a 3′-end. Unlabeled TS10 oligomer was incubated with TdT in the presence of UP1 and radiolabeled TTP. Nucleotide addition was monitored on a denaturing polyacrylamide gel. As shown in Fig. 6, UP1 strongly inhibited extension of TS10 (lanes 2–4). UP1Δ2 also efficiently repressed TdT activity (lanes 5–7), while UP1Δ1 had little effect (lanes 8–10). The inhibitory effect of UP1 was specific, since an oligonucleotide lacking an A1 binding site (TS oligo) was efficiently extended by TdT in the presence of a high concentration of UP1 (data not shown). Notably, the addition of gp32 also prevented extension of TS10 by TdT (lanes 11–14). Thus, although UP1Δ2 and gp32 were poor inhibitors of telomerase, both could prevent TdT access to the substrate. These results suggest that the mechanism by which UP1 inhibits telomerase is different from the mechanism that inhibits TdT.

Purified UP1 was reported to stimulate the activity of DNA polymerase α when poly(dA/dT) or a template DNA partially degraded with E. coli exonuclease III is used as substrate (36, 37). To determine whether UP1 displays a similar activity on a telomeric substrate, we used an in vitro assay to monitor the synthesis of the mammalian telomere lagging strand. Using TS10 as DNA template, DNA polymerase α/primase was shown to catalyze synthesis of the complementary strand, a process that required both dNTPs and rNTPs (38). In agreement with this report, we observed that incubation of unlabeled TS10 in the HeLa nuclear extract leads to the synthesis of products shorter than 60 nucleotides (Fig. 7A, lane 3). Synthesis required rNTPs and dNTPs, but omission of GTP and/or rGTP did not affect the appearance of labeled products (lane 2, and data not shown). The rNTP dependence of the assay is indicative of DNA polymerase α/primase activity. When the complementary oligonucleotide C5 was preincubated with TS10, lagging strand synthesis occurred in the absence of rNTPs, suggesting DNA polymerase δ and/or ε activity (Fig. 7B, lane 2). To confirm that the rNTP-dependent activity was mediated by DNA polymerase α/primase, we tested the inhibitory effect of a monoclonal antibody specific for DNA polymerase α/primase (38). The SJK 132-20 antibody completely inhibited rNTP-dependent DNA synthesis but did not affect rNTP-independent DNA synthesis (data not shown).

Notably, the addition of increasing amounts of GST-UP1 inhibited rNTP-dependent synthesis in the HeLa nuclear extract (Fig. 7A, lanes 5–8) but did not affect rNTP-independent synthesis (Fig. 7B, lanes 4–7). Inhibition was also seen with UP1, GST-A1, and commercial preparations of SSB and gp32 proteins (data not shown).

It is unlikely that our protein preparations contain a non-specific inhibitor of DNA polymerase α/primase activity, since increasing the amounts of TS10 in mixtures containing UP1 stimulated lagging strand synthesis (Fig. 7C, lanes 5–7). Consistent with this observation, the addition of a shorter telomeric oligonucleotide (Tel2) to a HeLa mixture containing TS10 but not recombinant UP1 also stimulated rNTP-depend-
ent lagging strand synthesis (Fig. 7C, compare lane 12 with lane 13). In contrast, the addition of a control oligonucleotide lacking telomeric sequences did not stimulate lagging strand synthesis (lane 14). These results suggest that Tel2 can specifically suppress the inhibition caused by nuclear factor that binds to TS10. Given that recombinant A1 protein also inhibits lagging strand synthesis, endogenous A1 proteins are probably repressing lagging strand activity in the HeLa extract. This conclusion is supported by the following experiment in which rNTP-dependent activity was monitored in a HeLa nuclear extract, a HeLa postnuclear S100 extract, which contains residual levels of hnRNP A1, and a nuclear extract prepared from a mouse cell line (CB3), which is severely deficient in hnRNP A1 (29, 32, 33). Compared with the strong stimulation obtained when Tel2 is added to the HeLa nuclear extract (Fig. 7C, compare lane 15 with lane 18), Tel2 only had a modest stimulatory effect when added to the HeLa S100 (compare lane 17 with lane 20) and no effect when added to the CB3 extract (compare lane 16 with lane 19). These results indicate that endogenous A1 proteins repress lagging strand synthesis in the HeLa nuclear extract, and that sequestration of A1 by Tel2 considerably improves rNTP-dependent synthesis on TS10.

Inhibition of lagging strand synthesis could also be obtained with UP1Δ2 but not with UP1Δ1 (data not shown). Thus, the mechanism by which UP1 inhibits lagging strand synthesis and TdT activity may be similar but distinct from the mechanism by which UP1 prevents telomerase extension.

**DISCUSSION**

We have shown previously that hnRNP A1 and its shortened derivative UP1 can promote telomere elongation in mammalian cells (29). The ability of A1 and UP1 to bind specifically to single-stranded telomeric repeats suggests that A1 may interact with telomeric extensions at the ends of mammalian chromosomes. In addition, recombinant UP1 but not recombinant A1 protein can recover telomerase activity from a cell extract (29). Because a proteolytic activity that produces UP1 from A1 has been reported, this conversion may represent an important step in the production of a factor that can interact with telomerase. Alternatively, post-translational modifications occurring at the C-terminal glycine-rich domain of A1 may modulate the interaction with telomerase. These possibilities are currently being investigated. In this study, we have addressed the effect of recombinant A1 and UP1 on a variety of processes that are relevant to telomere biogenesis. We find that the binding of A1 or UP1 protects a single-stranded telomeric substrate against the activity of endo- and exonucleases. Moreover, recombinant A1 and UP1 proteins prevent telomerase extension and telomere replication by DNA polymerase α/primase in vitro.

**Interaction of A1/UP1 with Telomeric Repeats**—A UP1 derivative lacking RRM2 binds to a telomeric oligonucleotide with an affinity and specificity that are nearly equivalent to the complete UP1 protein. In contrast, deleting part of RRM1 severely compromises binding. Thus, while the RRM1 domain is essential for binding, the RRM2 domain appears dispensable. This conclusion contrasts with a previous observation indicating that both RRM1 and RRM2 are required to recover molecules carrying RNA versions of telomeric repeats from a pool of random sequence (39). Because these experiments were performed with derivatives carrying the C-terminal glycine-rich domain, it is possible that this domain affects the binding properties of the preceding RRM. The deletion of RRM2 in A1 would position the glycine-rich domain next to RRM1 and could alter its binding specificity. Because the glycine-rich domain is absent from UP1, the deletion of RRM2 would maintain the binding specificity of RMM1 in our assays. Thus, while cooperativity between RRMs has been assumed to be a general property of proteins with multiple RRMs, our results clearly show that RRM2 is not required for strong and specific binding to telomeric oligonucleotides. Because at least two TAGGGT repeats are required for the assembly of a stable complex with A1 or UP1, our results suggest that a minimal complex involves two A1 or two UP1 molecules, each protein using only RRM1 to bind to one repeat unit. The recent x-ray structure of UP1 bound to 12 nucleotides of telomeric DNA indicates that UP1 binds as a dimer to two strands of telomeric DNA, the RRM1 and RRM2 of one UP1 monomer binding to separate strands (40). Whereas each telomeric repeat is contacted by only one RRM, RRM1 and the adjacent linker region make more contacts with the bases of a telomeric repeat than RRM2 (40), suggesting that the N-terminal half of UP1 plays a dominant role in the affinity and/or specificity of binding. In contrast to the work of Ding et al. (40), our results suggest that UP1 does not simultaneously interact with different strands, since the incubation of UP1 with two small telomeric oligonucleotides of different lengths does not lead to the assembly of complexes containing both oligonucleotides.2

2 F. Dallaire and B. Chabot, unpublished results.
A Role for A1/UP1 in the Maintenance of a Telomeric 3' Overhang—Maintaining the integrity of telomeric 3' overhangs is paramount to telomere function. In mammals, this conclusion is supported by recent studies performed with TRF2, a protein that binds to double-stranded telomeric repeats. Overexpression of a dominant negative variant of TRF2 promotes the loss of 3' overhangs, an event associated with chromosome fusions and apoptosis (41, 42). Consistent with the properties expected for a protein that binds to 3' overhangs, A1/UP1 displays specific binding to mammalian telomeric repeats and prefers binding to telomeric sequences that are located at the 3' terminus of an oligonucleotide. While specific binding is also a property of the telomere single-stranded binding proteins of *Oxytricha* and *Euplotes*, the yeast Cdc13p/Est4p and Est1p proteins can interact, albeit with reduced affinity, to vertebrate and ciliate telomeric sequences (9, 19, 20, 43). The proteins can interact, albeit with reduced affinity, to vertebrate telomeric partial filling by DNA polymerase in replication and the activity of specific nucleases (Fig. 8), the must gain access to a 3' overhang may remain free of A1/UP1 for a long period of time to allow extension by telomerase and partial filling by DNA polymerase α/primase (Fig. 8). The subsequent binding of A1/UP1 to newly made telomeric tails would help maintain long 3' overhangs throughout the rest of the cell cycle.

In conclusion, our study indicates that the binding of A1/UP1 to single-stranded telomeric sequences provides protection against nucleolytic activities. A1/UP1 binding also renders the 3'-end of a telomeric substrate inaccessible to a variety of polymerases. We can now begin to envision how the protective and replication-modulating activities associated with the binding of A1/UP1 contribute to telomere biogenesis in mammalian cells.

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