Elucidation of the mode of interaction in the UP1–telomerase RNA–telomeric DNA ternary complex which serves to recruit telomerase to telomeric DNA and to enhance the telomerase activity

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ABSTRACT

We found that UP1, a proteolytic product of heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1), both enhances and represses the telomerase activity. The formation of the UP1–telomerase RNA–telomeric DNA ternary complex was revealed by a gel retardation experiment. The interactions in the ternary and binary complexes were elucidated by NMR. UP1 has two nucleic acid-binding domains, BD1 and BD2. In the UP1–telomerase RNA binary complex, both BD1 and BD2 interact with telomerase RNA. Interestingly, when telomeric DNA was added to the binary complex, telomeric DNA bound to BD1 in place of telomerase RNA. Thus, BD1 basically binds to telomeric DNA, while BD2 mainly binds to telomerase RNA, which resulted in the formation of the ternary complex. Here, UP1 bridges telomerase and telomeric DNA. It is supposed that UP1/hnRNP A1 serves to recruit telomerase to telomeric DNA through the formation of the ternary complex. A model has been proposed for how hnRNP A1/UP1 contributes to enhancement of the telomerase activity through recruitment and unfolding of the quadruplex of telomeric DNA.

INTRODUCTION

Highly repetitive sequences called telomeres exist at the ends of eukaryotic chromosomes. Human telomeric DNA is 5- to 8-kb long and composed of repeats of the d(TTAGGG) sequence, with a 3′-single-stranded overhang of ~200 nt (1,2). Telomeres are associated with a specific set of proteins that serve to protect chromosome ends from fusion and degradation (3–5). In mammals, TRF2 binds to double-stranded telomeric DNA and protects the telomere ends (6). Another protein, POT1, is thought to modulate telomere elongation (7–9). The T-loop structure, in which the overhang loops back and invades the duplex telomeric DNA, can also protect chromosome ends (10).

The telomeres of most somatic cells become shorter on replication; whereas, those of germ cells and cancer cells maintain their lengths through elongation by telomerase, which is active in these cells (11). Telomerase is composed of reverse transcriptase (TERT) and telomerase RNA, which is used as a template for reverse transcription. It is unknown how telomerase is recruited to telomeres. Telomerase requires an accessible 3′-overhang for the elongation. DNA rich in guanosine residues tends to form a quadruplex with guanine-tetrad planes as core structures (12). Telomeric DNA is rich in guanosine residues and thus its quadruplex structure is supposed to play certain roles in the regulation of telomere length. In particular, the finding that telomerase activity is inhibited on formation of the quadruplex (13–15) has attracted attention in terms of the development of novel anticancer drugs.

Heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) is one of the most abundant nuclear proteins and involved in a variety of processes such as alternative splicing and mRNA transport (16–20). HnRNP A1 is composed of 320 residues. UP1 is a proteolytic product of hnRNP A1 and encompasses the N-terminal two-thirds

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of hnRNP A1, being composed of 196 residues. HnRNP A1 and UP1 possess two ribonucleoprotein (RNP)-type DNA/RNA-binding domains (BD1: K15-A89 and BD2: K106–A180). It has been shown that a deficiency of hnRNP A1 expression in a mouse erythroleukemic cell line is associated with short telomeres and that restoring hnRNP A1 expression increases the length of telomeres. Telomere elongation was also observed upon the introduction of exogenous UP1 (21). It was found that RNAi-induced reduction in hnRNP A1/A2 in HeLa cells was associated with a change in the distribution of the length of G-tails (22). Then, it was reported that hnRNP A1 stimulates telomerase activity. The association of hnRNP A1 with telomeres *in vivo* was also reported (23). A mechanism by which hnRNP A1/UP1 stimulates telomerase activity and modulates telomere length has been proposed. We demonstrated that UP1 can unfold the quadruplex structure of telomeric DNA into a single-stranded structure (24), which was later supported by another group (25). Here, UP1 may either directly unfold the quadruplex into the single-stranded structure or indirectly shift the equilibrium to the single-stranded structure through binding and stabilization of the single-stranded structure. We also revealed that DNA synthesis is arrested at the G-rich region of the synthetic template in a specific DNA polymerase stop assay due to the formation of the quadruplex and that UP1 can abrogate the arrest through unfolding of the quadruplex (24). Through the analogy, this finding led us to propose that hnRNP A1/UP1 facilitate telomerase activity through unfolding of the quadruplex of the overhang, which results in the provision of an accessible overhang (24). A similar role and mechanism in telomere maintenance were proposed for another related protein, hnRNP D, on the basis of its structure and ability to unfold the quadruplex (26). Later, a related idea was proposed by another group (23).

Another mechanism was also proposed. It was found that hnRNP A1 may interact simultaneously with telomerase RNA and telomeric DNA. It was suggested that hnRNP A1 may help recruit telomerase to the ends of chromosomes through the simultaneous interaction, resulting in the enhancement of the telomerase activity (27). Stimulated by this proposal, we started the present study. First, we found that UP1 both enhances and represses the synthesis of telomeric DNA, depending on the length of newly synthesized telomeric DNA. The formation of the UP1–telomerase RNA–telomeric DNA ternary complex was revealed by a gel retardation experiment. Then, the interactions in the binary and ternary complexes were elucidated for the first time at the residue level by NMR. On the basis of the elucidated interactions, a model accounting for the enhancement and repression of the telomerase activity by UP1 was constructed.

MATERIALS AND METHODS

Preparation of UP1

The expression plasmid pGEX-6P-2 was described previously (24). *Escherichia coli*, BL21(DE3), was transformed with this plasmid. The cells were grown in LB medium and GST-UP1 fusion protein was expressed. For the preparation of ^{13}C, ^{15}N-labelled UP1, the LB medium was replaced by M9 minimal medium containing $^{15}NH_4Cl$ and ^{13}C-glucose. GST-UP1 was purified with a glutathione Sepharose FF column (GE Healthcare, Buckinghamshire, England). Release of UP1 from GST-UP1 was performed with PreScission Protease (GE Healthcare). UP1 was further purified with a Q Sepharose FF column (GE Healthcare).

Assaying of the telomerase activity

A telomerase extract was prepared from HeLa cells in lysis buffer (11). The presence of either hnRNP A1 or hnRNP D was monitored by western blotting. Telomerase activity was examined by means of a modified TRAP method (28) with TeloChaser (Toyobo, Osaka, Japan). Products were run on a 10% polyacrylamide gel, stained with SYBER Green II, and detected with a FLA 5000 (Fuji Film, Tokyo, Japan). The intensities of bands were quantified with Image J.

Gel retardation experiments

Cy5-labelled 15-mer telomerase RNA, r(UGAGAAGGGC GUAGG), was incubated with various amounts of UP1 at 4°C for 30 min in a 20 μl solution comprising 10 mM Tris (pH 7.5), 50 mM NaCl, 1 mM EDTA, 5% glycerol and 2 μg BSA. Competitor tRNA was also added in some case. For the examination of the formation of the ternary complex, 12-mer telomeric DNA, d(GTCTTAGGGCAG), was added to the solution of the UP1:15-mer telomerase RNA binary complex. DNA possessing one telomeric repeat in the middle of a non-telomeric sequence was used, because DNA with multiple telomeric repeats sometimes could form the artificial aggregate caused by intra- and intermolecular interactions through multiple interactive sites. The mixtures were run on a 10% polyacryl-amide gel, with detection with a FLA2000 (Fuji Film).

NMR spectroscopy

UP1 was dissolved in a solution comprising 20 mM Na-phosphate buffer (pH 6.5), either 0 or 150 mM NaCl, 2.7 mM ^2H-DTT, 1 mM protease inhibitors, Pefabloc SC(AEBSF) (Roche Applied Science, Mannheim, Germany) and 5% ^2H_2O. The UP1 concentrations were 0.5–1.0 mM for the structural analysis and 0.2 mM for the titration. NMR spectra were recorded with Bruker (Rheinstetten, Germany) DRX600 and DRX800 spectrometers equipped with a cryoprobe. The following NMR spectra were obtained to assign resonances: HSQC, ^{15}N-edited NOESY-HSQC, ^{13}C-edited NOESY-HSQC, HNCA, HN(CO)CA, HNCO, HN(CA)CO, HNCACB, CBCA (CO)NH, C(CO)NH and H(CCO)NH. For the titration experiments as to the formation of binary complexes, a concentrated solution of either 15-mer telomerase RNA or 12-mer telomeric DNA was added step by step to the UP1 solution. For the titration experiment as to the formation of the ternary complex, a concentrated solution of 12-mer telomeric DNA was added step by step to the solution of UP1–telomerase RNA binary complex. An equimolar amount of telomeric DNA to telomerase RNA was added at the end of the titration. Spectra were

processed and analyzed with XWIN-NMR (Bruker), NMRPipe (29), Capp/Pipp/Stapp (30), Sparky (31) and Kujira (32). Combined chemical shift perturbation as to H^N and N was defined and calculated as

$$[(\Delta\delta_{NH}{}^*600)^2 + (\Delta\delta_N{}^*60{}^*2)^2]^{1/2}$$

where $\Delta\delta_{HN}$ and $\Delta\delta_N$ are the chemical shift perturbations for H^N and N resonances, respectively.

RESULTS

Effect of UP1 on the telomerase activity

The effect of UP1 on the telomerase activity was examined. Before the examination, it was revealed on western blotting that hnRNP A1 and hnRNP D were almost completely depleted in the course of the preparation of the telomerase extract from HeLa cells, although the HeLa cells originally contained these proteins (data not shown). It is supposed that endogenous hnRNP A1 and hnRNP D bound to endogenous DNA/RNA and were co-precipitated during the preparation, resulting in the depletion. Then, Figure 1 shows the effect of UP1 on the telomerase activity of the extract prepared from HeLa cells, as monitored with a modified TRAP method. The intensities of all bands were summed up for each lane in Figure 1. The total intensity in each lane was calibrated with the intensity of the band of the internal control. The calibrated total intensities in individual lanes were compared. The relative intensities were 1.0, 1.7, 1.3 and 1.0 for the UP1 concentrations of 0 µM, 1 µM, 2.5 µM and 5 µM, respectively. Thus, UP1 increases the total activity of telomerase. The primer used in a modified TRAP method contains three d(GGGG) sequences and one d(GG) sequence (28), and so potentially may form a quadruplex. When telomeric DNA repeats are added to the primer by telomerase, the formation of the quadruplex is more likely. It is supposed that the enhancement of the activity is brought about by UP1 through recruitment of telomerase to telomeric DNA and also through unfolding of the quadruplex of telomeric DNA, as discussed later. The enhancement became the maximum with the UP1 concentration of ~1 µM and was counteracted with higher UP1 concentrations. UP1 is denatured and removed from a system before a PCR step in the procedure with a TeloChaser. Therefore, it is certain that the affect of UP1 on the TRAP assay is at the level of extension by telomerase and not PCR amplification.

It should be noted that the intensities of bands corresponding to relatively short DNA increase on the addition of UP1, while those corresponding to relatively long DNA decrease. For example, when the intensities with 1 µM UP1 (lane 3) are compared with those with no UP1 (lane 2), an increase is observed for bands corresponding to less than ~160 bp, while a decrease is observed for those corresponding to more than ~160 bp.

Enhancement of the telomerase activity by hnRNP A1/UP1 was reported by another group (23). They did not report that the enhancement becomes maximum at a certain hnRNP A1/UP1 concentration and that it is
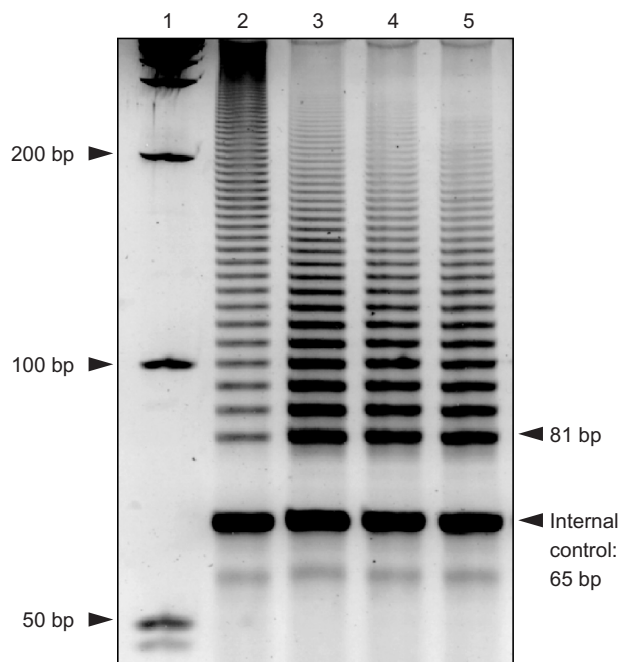

Figure 1. The effect of UP1 on the telomerase activity, as monitored by the TRAP method. The elongation of telomeric DNA was carried out with a telomerase extract prepared from HeLa cells with increasing amounts of UP1. Lane: 1, markers; 2–5 µM, 0 µM, 1 µM, 2.5 µM and 5 µM UP1.

counteracted with higher hnRNP A1/UP1 concentrations. Thus, our results are consistent with their report and provide a more comprehensive view as to the effect of hnRNP A1/UP1 on the telomerase activity. In fact, it was reported by other group that binding of hnRNP A1/UP1 to single-stranded telomeric repeats inhibits telomerase extension (33). The apparent discrepancy may be rationalized to some extent by taking into account the hnRNP A1/UP1 concentrations applied to each experiment.

It was not reported either that the effect of hnRNP A1/UP1 depends on the length of synthesized telomeric DNA, because the synthesis of relatively short telomeric DNA was examined previously (23). The interpretation of our findings will be discussed later.

Formation of the UP1–telomerase RNA–telomeric DNA ternary complex revealed by a gel retardation experiment

It was shown that UP1 specifically binds to telomerase RNA (27). Analysis with a deletion mutant of telomerase RNA indicated that binding of UP1 is detected to the 5′-terminal 71 nt of telomerase RNA, while binding is not detected to the 5′-terminal 56 nt of telomerase RNA (27). This suggests that residues 56–71 of telomerase RNA may be the binding site for UP1. We examined if UP1 binds to 15-mer telomerase RNA, r(UGAGAAGGGCGUAGG), corresponding to residues 56–71 of telomerase RNA through a gel retardation experiment. Figure 2A shows that UP1 binds to the 15-mer. The binding is rather strong, the dissociation constant being estimated to be between 5×10^{-8} M and 5×10^{-7} M from Figure 2A. This is the first demonstration that the short fragment of telomerase RNA is bound by UP1.
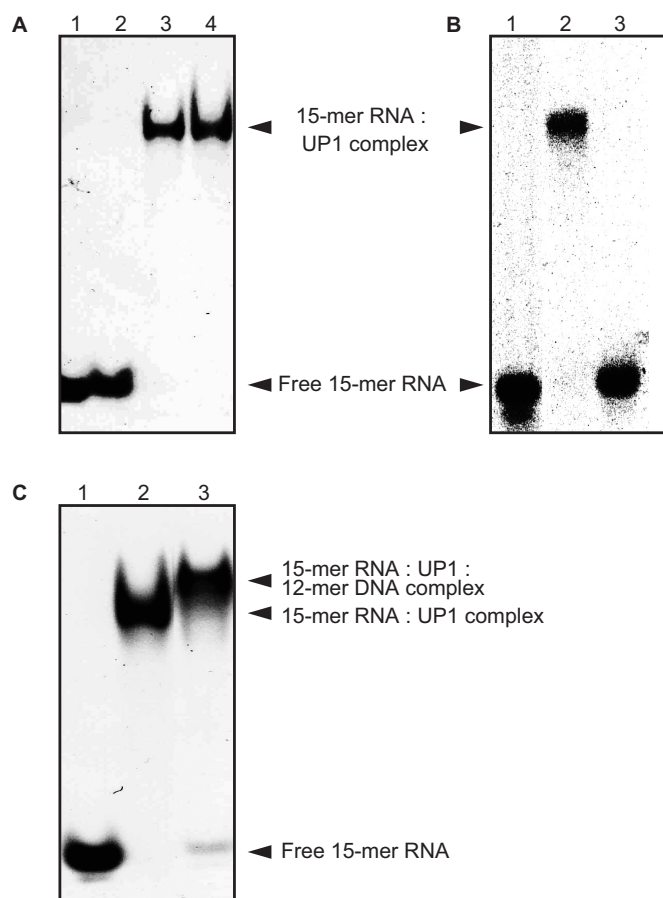
Figure 2. The formation of the binary and ternary complexes, as revealed by gel retardation experiments. (**A**) The binding of UP1 to 15-mer telomerase RNA. Cy5-labelled 15-mer telomerase RNA was incubated with increasing amounts of UP1, $0\,\mu$M, $0.05\,\mu$M, $0.5\,\mu$M and $1\,\mu$M for lanes 1–4. The mixtures were run on a polyacrylamide gel. (**B**) The binding of UP1 to 15-mer telomerase RNA in the presence of competitor tRNA. Cy5-labelled 15-mer telomerase RNA and UP1 ($5\,\mu$M) were incubated in the presence of an excess amount of competitor tRNA. The weight ratios of tRNA/15-mer were 200 and 2000 for lanes 2 and 3, respectively. The 15-mer alone was applied for lane 1. (**C**) The formation of the UP1–telomerase RNA–telomeric DNA ternary complex. Cy5-labelled 15-mer telomerase RNA was incubated alone (lane 1) or with UP1 ($5\,\mu$M) (lane 2). Then, 12-mer telomeric DNA ($5\,\mu$M) was added to the solution of the UP1: 15-mer telomerase RNA binary complex (lane 3).

The dissociation constant of the UP1:full-length telomerase RNA complex was shown to be between $2.5 \times 10^{-7}\,$M and $5 \times 10^{-7}\,$M (26). Thus, the affinity of UP1 to 15-mer RNA is comparable to or even higher than that to the full-length RNA (27). It was shown that the binding of UP1 to full-length telomerase RNA is specific (27). Figure 2B shows that the addition of a 200-fold excess of tRNA as a competitor does not disrupt the binding of UP1 to the 15-mer (lane 2). This implies that the binding of UP1 to the 15-mer may also be specific like that to full-length telomerase RNA, which is expected from rather strong affinity of UP1 to the 15-mer.

When 12-mer DNA containing a telomeric DNA sequence, d(GTCTTAGGGCGA), was added to the UP1:15-mer telomerase RNA binary complex, a super-shift of the band which may correspond to the formation

of the ternary complex was observed in the gel retardation experiment (lane 3 in Figure 2C). The results of western blotting confirmed that the super-shifted band still contains UP1 (data not shown). Therefore, the super shift strongly suggests the formation of the UP1:15-mer telomerase RNA:12-mer telomeric DNA ternary complex. The formation of the ternary complex was suggested on the basis of the results of affinity chromatography, in which longer telomerase RNA and DNA possessing multiple telomeric repeats were used (27). Our results on gel retardation are consistent with this idea.

Resonance assignments and the deduced secondary structure of UP1

The polypeptide produced on the cleavage of GST-UP1 with PreScission Protease is composed of the UP1 (M1-R196) protein and an N-terminal linker peptide (GPLGSPGIRCSV), totally 208 residues. Sequential assignments of the main chain H^N, N, C^α, C^β and C' resonances were made for the peptide of 208 residues in the same way as reported for a related protein, hnRNP D (26,34,35). Resonance assignments were accomplished for ca. 90% of the polypeptide. The polypeptide corresponding to the N-terminal half of UP1 was analyzed and the assignments were reported (36). It was confirmed that our assignments for the whole UP1 are consistent with those reported for the N-terminal half.

The secondary structure of UP1 was deduced on the basis of the resonance assignments with the chemical shift index method (37). It was confirmed that the deduced secondary structure in solution is basically consistent with the structure found in a crystal (38–40).

Interactions in the UP1–telomerase RNA and UP1–telomeric DNA binary complexes revealed by NMR

The chemical shift perturbations of the ^{1}H-^{15}N HSQC correlation peaks of UP1 on the addition of 15-mer telomerase RNA were observed until a 1:1 molar ratio (data not shown). No further chemical shift perturbation was observed when further 15-mer was added. The increase in the line width of the correlation peak was moderate. These results indicate that one telomerase RNA molecule binds to one UP1 molecule. The chemical shift perturbations of the UP1 correlation peaks on the addition of telomerase RNA are mapped on the UP1 monomer structure of the dimer observed in a crystal (38–40). Most chemical shift perturbations are located on the two β-sheets side, and a few chemical shift perturbations on the back side (Figure 3A), which indicates that the two β-sheets side is the binding surface. The binding on the β-sheet side was observed for other RNP-type binding domains, including those of a related protein, hnRNP D (26,34,35).

It was suggested that BD2 of hnRNP A1/UP1 is responsible for binding to telomerase RNA (26). However, the chemical shift perturbations were observed not only for BD2 but also for BD1 of UP1 (Figure 3A). This indicates that both BDs bind to telomerase RNA to form the UP1–telomerase RNA binary complex under the conditions used for NMR experiments (Figure 3C).
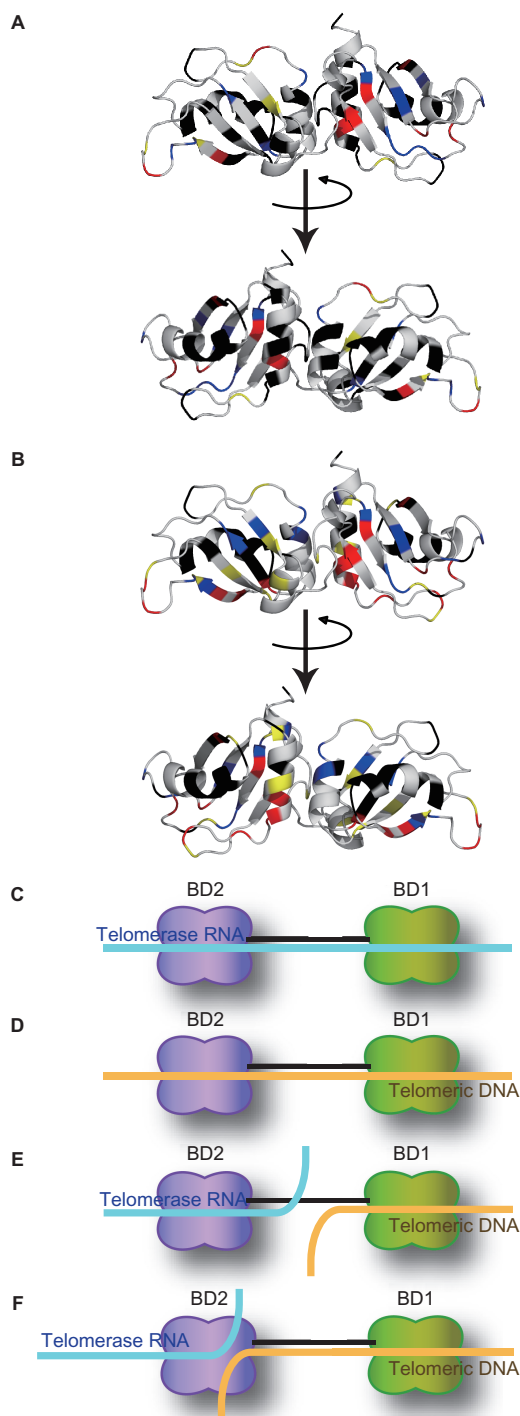
Figure 3. Summary of the interactions and the schematic representations of the binary and ternary complexes. Mapping of chemical shift perturbations upon binding of 15-mer telomerase RNA (**A**) and 12-mer telomeric DNA (**B**), respectively. The crystal structure of UP1 (PDB ID: 2up1) is used for mapping. The residues with combined chemical shift perturbations as to H^N and N of >75 Hz, 75–45 Hz and 45–0 Hz are colored red, yellow and black, respectively, and those whose ^{1}H-^{15}N correlation peak disappeared are colored blue. The residues whose chemical shift perturbations were not unambiguously determined due to overlapping of peaks are colored gray. Schematic representations of the UP1–telomerase RNA binary complex (**C**), the UP1–telomeric DNA binary complex (**D**), the UP1–telomerase RNA–telomeric DNA ternary complex (**E**) and the more precise UP1–telomerase RNA–telomeric DNA ternary complex (**F**).

The chemical shift perturbations mainly on the two β-sheets side were also observed when 12-mer telomeric DNA was added to the UP1 solution (Figure 3B). It was suggested that BD1 of hnRNP A1/UP1 is responsible for binding to telomeric DNA, although the possibility of the binding of BD2 to telomeric DNA was preserved (27). This time, the chemical shift perturbations were observed for both BDs of UP1, indicating that both BDs bind to telomeric DNA to form the UP1–telomeric DNA binary complex under the present conditions (Figure 3D).

The crystal structure of UP1 bound to the single-stranded telomeric repeat DNA was reported (40). In crystal, dimeric UP1 bound to two oligodeoxyribonucleotides. Judging from the narrow line widths of ^{1}H-^{15}N HSQC correlation peaks of either UP1–telomerase RNA or UP1–telomeric DNA binary complexes, it is supposed that UP1 is monomeric in both the complexes.

Interactions in the UP1–telomerase RNA–telomeric DNA ternary complex revealed by NMR

When 12-mer telomeric DNA was added to a solution of the UP1–telomerase RNA binary complex, interesting changes in the chemical shift perturbations were observed. The position of an HSQC correlation peak of a certain residue for the UP1–telomerase RNA binary complex (green) is generally different from that for the UP1–telomeric DNA binary complex (black), as shown in Figure 4. For most residues of BD1, when an equimolar amount of telomeric DNA to telomerase RNA was added to the UP1–telomerase RNA binary complex, the correlation peak originally observed at the position for the UP1–telomerase RNA binary complex appeared at the position for the UP1–telomeric DNA binary complex (Figure 4A–C). This indicates that a portion of telomerase RNA originally bound to BD1 dissociated and that telomeric DNA bound to BD1 in place of telomerase RNA (Figure 3E). Although a weak correlation peak remains at the position for the UP1–telomerase RNA binary complex for some residues of BD1, a dominant stronger correlation peak nonetheless appears at the position for the UP1–telomeric DNA binary complex (Figure 4D and E). This once again reveals that BD1 is basically bound by telomeric DNA in the ternary complex.

hnRNP A2 also has two DNA/RNA-binding domains, BD1 and BD2. DNA/RNA binding of hnRNP A2 was characterized, and the possibility was raised that telomeric DNA may compete with telomerase RNA for binding to BD1 of hnRNP A2 (41). This is actually what we experimentally found for BD1 of hnRNP A1. Thus, hnRNP A1 and hnRNP A2 may share some features on DNA/RNA binding, although they exhibit differences in other aspects (41).

For many residues of BD2, in contrast, a correlation peak remains at the position for the UP1–telomerase RNA binary complex even after the addition of telomeric DNA (Figure 4F and G). This indicates that the portion of telomerase RNA originally bound to BD2 remains there (Figure 3E). In summary, analysis of chemical shift perturbations revealed the formation of the UP1–telomerase RNA–telomeric DNA ternary complex in which BD1
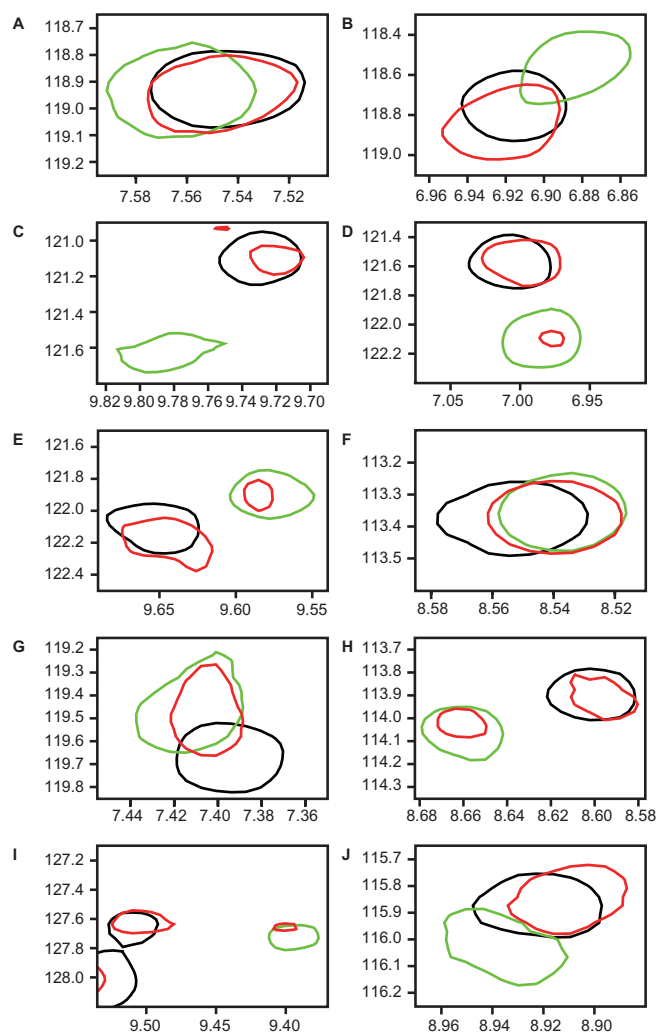
Figure 4. The formation of the ternary complex, as revealed by NMR chemical shift perturbations. Comparison of the position of a ^{1}H-^{15}N correlation peak for the UP1–telomerase RNA–telomeric DNA ternary complex (red) with those for either the UP1–telomerase RNA binary complex (green) or the UP1–telomeric DNA binary complex (black). The ternary complex was made by the titration of telomeric DNA to the UP1–telomerase RNA binary complex. An equimolar amount of telomeric DNA to telomerase RNA was added to the binary complex at the end of the titration. (**A–E**): D42, V68, K87, A74 and H77 of BD1, respectively; (**F–J**): H119, I162, F150, F153 and I107 of BD2, respectively.

and BD2 bind to telomeric DNA and telomerase RNA, respectively (Figure 3E).

It was noticed that for some residues of BD2, a dominant correlation peak appears at the position for the UP1–telomeric DNA binary complex, although a weak correlation peak remains at the position for the UP1–telomerase RNA binary complex (Figure 4H and I). There is even a case that a correlation peak of a residue of BD2 appears at the position for the UP1–telomeric DNA binary complex, exclusively (Figure 4J). These results imply that a portion of telomerase RNA originally bound to BD2 also dissociated to some extent and that telomeric DNA bound to BD2 in place of telomerase RNA in the ternary complex (Figure 3F).

DISCUSSION

We demonstrated that UP1 enhances the synthesis of relatively short telomeric DNA by telomerase. We revealed the formation of the UP1–telomerase RNA–telomeric DNA ternary complex through a gel retardation experiment, which is consistent with the previous suggestion derived from the experiment involving affinity chromatography (27). NMR chemical shift perturbation analysis provided detailed views of the interactions in the ternary complex, together with those in the UP1–telomerase RNA and UP1–telomeric DNA binary complexes. On the basis of the obtained information, we propose a model of how UP1 enhances the telomerase activity. First, UP1 captures telomerase through the interaction of both the BDs with telomerase RNA (Figure 5A), as revealed by NMR chemical shift perturbation analysis (Figure 3A and C). It is supposed that the presence of TERT bound to telomerase RNA does not interfere with the UP1–telomerase RNA interaction. In fact, it was shown that a column carrying GST-UP1 can specifically recover telomerase activity from a cell extract (21). At least 20% of total telomerase activity was present in the UP1-bound fraction (21). Then, telomerase-bound UP1 effectively searches for telomeric DNA with its affinity to telomeric DNA, particularly with the affinity of BD1 to telomeric DNA. When telomerase-bound UP1 encounters telomeric DNA, the BDs of UP1, particularly BD1, unfold the quadruplex structure of telomeric DNA into a single-stranded structure. Then, the UP1–telomerase–telomeric DNA ternary complex is formed through the interaction of BD1 with telomeric DNA (Figure 5B), as NMR indicated that telomeric DNA bound to BD1 in place of telomerase RNA (Figures 3E, F and 4). UP1 bridges telomerase and telomeric DNA through simultaneous BD1–telomeric DNA and BD2–telomerase RNA interactions. In this way, UP1 serves to recruit telomerase to telomeric DNA. Thus, telomerase is brought to telomeric DNA where it should function. Finally, telomerase RNA associates with telomeric DNA using the complementarity to telomeric DNA, which is present at the edge of the template region of telomerase RNA, and TERT synthesizes new telomeric DNA, UP1 being released meanwhile (Figure 5C). In this model, the enhancement of the telomerase activity is brought about through the recruitment of telomerase to telomeric DNA and the unfolding of the quadruplex structure of telomeric DNA into a single-stranded structure by UP1.

It was proposed that POT1 protein may disrupt quadruplex structures in telomeric DNA and thereby allow proper elongation by telomerase (42). So, hnRNP A1/UP1 and POT1 may share the similar role in telomere maintenance, at least in terms of unfolding. Therefore, the unfolding may be performed by POT1, in place of hnRNP A1/UP1, in some cases.

When the UP1 concentration was raised to 2.5 μM, the beginning of the decrease in the total telomerase activity was observed (Figure 1). Occupation of the 3′-overhang of telomeric DNA by an excess amount of UP1 and resultant interference with binding of telomerase to telomeric DNA may account for this phenomenon (Figure 5D).
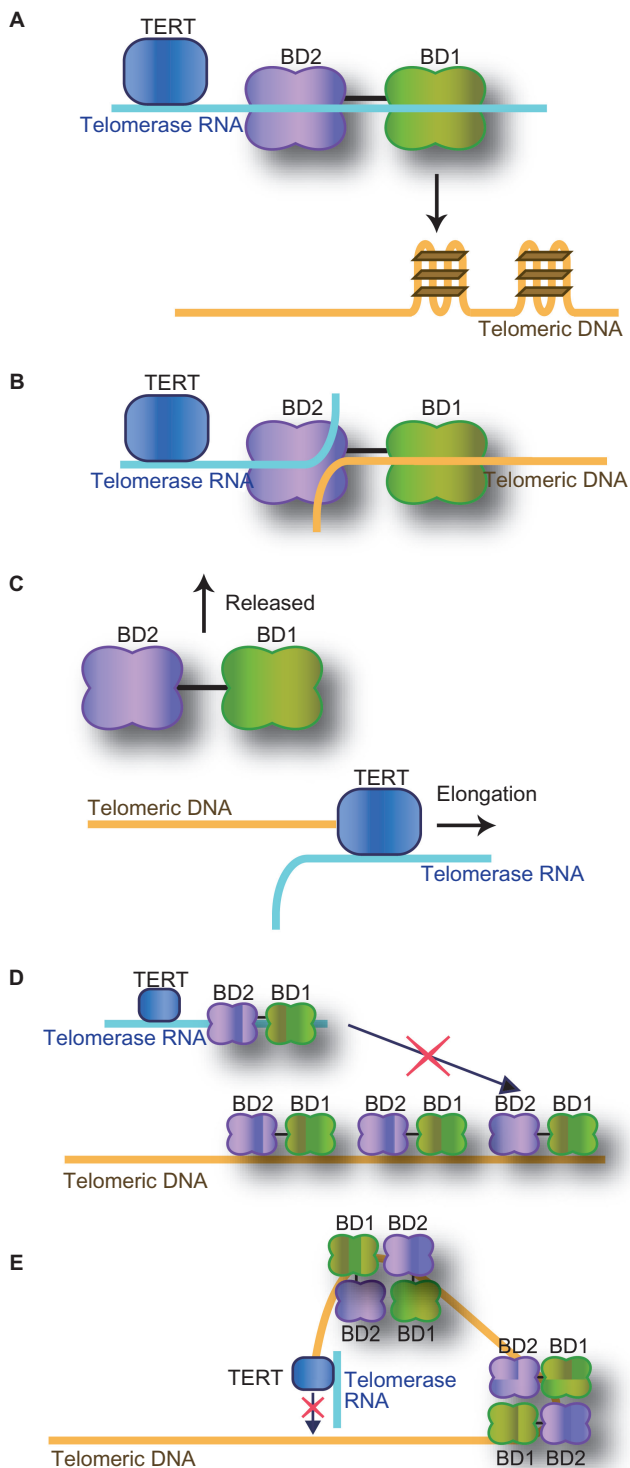
Figure 5. A model for how hnRNP A1/UP1 both enhances and represses the telomerase activity. A model for enhancement of the telomerase activity through the recruitment of telomerase to telomeric DNA by UP1 composed of BD1 and BD2 (**A–C**). (A) UP1 captures telomerase through binding of both BD1 and BD2 to telomerase RNA. Telomerase-bound UP1 searches for telomeric DNA with the affinity of BDs, particularly that of BD1, to telomeric DNA. (B) When telomerase-bound UP1 encounters telomeric DNA, the BDs of UP1, particularly BD1, unfold the quadruplex structure of telomeric DNA into a single-stranded form. Then, UP1 bridges telomerase and telomeric DNA through simultaneous BD1–telomeric DNA and BD2–telomerase RNA interactions. (C) Telomerase brought to telomeric DNA forms the telomerase RNA–telomeric DNA complex through their complementarity and begins to elongate the telomeric DNA. UP1 is released meanwhile. (**D**) An excess amount of UP1 may occupy the 3′-overhang of telomeric DNA and thus interfere with access of telomerase-bound UP1 to this region, which may result in the reduction of the telomerase activity. (**E**) When newly synthesized telomeric DNA becomes long, a certain higher order structure such as a loop structure may be formed with the assistance of UP1. The formed structure may sterically have an inhibitory effect on further elongation of telomeric DNA by telomerase. This may account for the reduction of the synthesis of longer telomeric DNA in the presence of UP1.

Another thing is that the synthesis of relatively long-telomeric DNA was repressed by UP1 (Figure 1). When the elongated telomeric DNA becomes longer, several distant parts of the elongated DNA may form a certain structure such as a loop structure with the assistance of UP1 (Figure 5E). It was found in the crystal structure that two telomeric DNA strands are brought close to each other through the interactions with a UP1 dimer (40). This kind of interaction could result in the formation of the loop structure. The formed structure may sterically inhibit further proceeding of telomerase, resulting in a decrease in the synthesis. Reduction of the synthesis of long-telomeric DNA caused by UP1 may be rationalized in this way. Alternative interpretation may also be possible. Binding of UP1 to single-stranded telomeric DNA might enhance the dissociation of telomerase from the telomeric DNA, which would result in the production of shorter telomeric DNA.

It may be the case that the enhancement of the synthesis of relatively short DNA is sufficient for hnRNP A1/UP1 to contribute to maintenance of the telomere *in vivo*. Alternatively, a not yet identified cellular factor, which was depleted during the course of the preparation of the telomerase extract as hnRNP A1 and hnRNP D were, may facilitate further enhancement by hnRNP A1/UP1 *in vivo*. For example, a factor that promotes the dissociation of hnRNP A1/UP1 from the telomeric DNA might be present in cells to guarantee the enhancement of the synthesis of relatively long DNA.

We found for the first time that UP1 has the ability to unfold the quadruplex structure of telomeric DNA into a single-stranded structure (24). On the basis of this finding, we proposed that UP1 may serve to enhance the telomerase activity through unfolding of the quadruplex structure of telomeric DNA and resultant provision of the accessible overhang (24). We also proposed a similar effect for a related protein, hnRNP D (26). We assume that both unfolding and recruitment by hnRNP A1/UP1 contribute to the enhancement of the telomerase activity and the maintenance of the proper telomeric DNA length. Thus, hnRNP A1/UP1, together with hnRNP D, may be promising targets for controlling the activity of telomerase which is linked to several cancers.

It must be a next target to determine the structures of the UP1–telomerase RNA and UP1–telomeric DNA binary complexes and the ternary complex. The effort is in progress to improve the relatively poor solubility and stability of these complexes.

REFERENCES

1. Makarov,V.L., Hirose,Y. and Langmore,L.P. (1997) Long G tails at both ends of human chromosomes suggest a C strand degradation mechanism for telomere shortening. *Cell*, **88**, 657–666.
2. McElligott,R. and Wellinger,R.J. (1997) The terminal DNA structure of mammalian chromosomes. *EMBO J.*, **16**, 3705–3714.
3. Blackburn,E.H. (2000) Telomere states and cell fates. *Nature*, **408**, 53–56.
4. van Steensel,B., Smogorzewska,A. and de Lange,T. (1998) TRF2 protects human telomeres from end-to-end fusions. *Cell*, **92**, 401–413.
5. Hackett,J.A., Feldser,D.M. and Greider,C.W. (2001) Telomere dysfunction increases mutation rate and genomic instability. *Cell*, **106**, 275–286.
6. Smogorzewska,A. and de Lange,T. (2004) Regulation of telomerase by telomeric proteins. *Annu. Rev. Biochem.*, **73**, 177–208.
7. Baumann,P. and Cech,T.R. (2001) Pot1, the putative telomere end-binding protein in fission yeast and humans. *Science*, **292**, 1171–1175.
8. Colgin,L.M., Baran,K., Baumann,P., Cech,T.R. and Reddel,R. (2003) Human POT1 facilitates telomere elongation by telomerase. *Curr. Biol.*, **13**, 942–946.
9. Loayza,D. and de Lange,T. (2003) POT1 as a terminal transducer of TRF1 telomere length control. *Nature*, **423**, 1013–1018.
10. Griffith,J. D., Comeau,L., Rosenfield,S., Stansel,R. M., Bianchi,A., Moss,H. and de Lange,T. (1999) Mammalian telomeres end in a large duplex loop. *Cell*, **97**, 503–514.
11. Kim,N.W., Piatyszek,M.A., Prowse,K.R., Harley,C.B., West,M.D., Ho,P.L., Coviello,G.M., Wright,W.E., Weinrich,S.L. and Shay,J.W. (1994) Specific association of human telomerase activity with immortal cells and cancer. *Science*, **266**, 2011–2015.
12. Phan,A.T., Kuryavyi,V. and Patel,D.J. (2006) DNA architecture: from G to Z. *Curr. Opin. Struct. Biol.*, **16**, 288–298.
13. Zahler,A.M., Williamson,J.R., Cech,T.R. and Prescott,D.M. (1991) Inhibition of telomerase by G-quartet DNA structures. *Nature*, **350**, 718–720.
14. Zaug,A.J., Podell,E.R. and Cech,T.R. (2005) Human POT1 disrupts telomeric G-quadruplexes allowing telomerase extension in vitro. *Proc. Natl Acad. Sci. USA*, **102**, 10864–10869.
15. Oganesian,L., Moon,I.K., Bryan,T.M. and Jarstfer,M.B. (2006) Extension of G-quadruplex DNA by ciliate telomerase. *EMBO J.*, **25**, 1148–1159.
16. Mayeda,A. and Krainer,A.R. (1992) Regulation of alternative pre-mRNA splicing by hnRNP A1 and splicing factor SF2. *Cell*, **68**, 365–375.
17. Mayeda,A., Munroe,S.H., Caceres,J.F. and Krainer,A.R. (1994) Function of conserved domains of hnRNP A1 and other hnRNP A/B proteins. *EMBO J.*, **13**, 5483–5495.
18. Yang,X., Bani,M.R., Lu,S.J., Rowan,S., Ben-David,Y. and Chabot,B. (1994) The A1 and A1B proteins of heterogeneous nuclear ribonucleoparticles modulate 5′ splice site selection *in vivo*. *Proc. Natl Acad. Sci. USA*, **91**, 924–6928.
19. Blanchette,M. and Chabot,B. (1999) Modulation of exon skipping by high-affinity hnRNP A1-binding sites and by intron elements that repress splice site utilization. *EMBO J.*, **18**, 1939–1952.
20. Izaurralde,E., Jarmolowski,A., Beisel,C., Mattaj,I.W., Dreyfuss,G. and Fischer,U. (1997) A role for the M9 transport signal of hnRNP A1 in mRNA nuclear export. *J. Cell Biol.*, **137**, 27–35.
21. LaBranche,H., Dupuis,S., Ben-David,Y., Bani,M.R., Wellinger,R.J. and Chabot,B. (1998) Telomere elongation by hnRNP A1 and a derivative that interacts with telomeric repeats and telomerase. *Nat. Genet.*, **19**, 199–202.
22. Patry,C., Bouchard,L., Labrecque,P., Gendron,D., Lemieux,B., Toutant,J., Lapointe,E., Wellinger,R. and Chabot,B. (2003) Small interfering RNA-mediated reduction in heterogeneous nuclear ribonucleoparticle A1/A2 proteins induces apoptosis in human cancer cells but not in normal mortal cell lines. *Cancer Res.*, **63**, 7679–7688.
23. Zhang,Q.S., Manche,L., Xu,R.M. and Krainer,A.R. (2006) hnRNP A1 associates with telomere ends and stimulates telomerase activity. *RNA*, **12**, 1116–1128.
24. Fukuda,H., Katahira,M., Tsuchiya,N., Enokizono,Y., Sugimura,T., Nagao,M. and Nakagama,H. (2002) Unfolding of quadruplex structure in the G-rich strand of the minisatellite repeat by the binding protein UP1. *Proc. Natl Acad. Sci. USA*, **99**, 12685–12690.
25. Myers,J.C., Moore,S.A. and Shamoo,Y. (2003) Structure-based incorporation of 6-methyl-8-(2-deoxy-beta-ribofuranosyl)isox-anthopteridine into the human telomeric repeat DNA as a probe for UP1 binding and destabilization of G-tetrad structures. *J. Biol. Chem.*, **278**, 42300–42306.
26. Enokizono,Y., Konishi,Y., Nagata,K., Ouhashi,K., Uesugi,S., Ishikawa,F. and Katahira,M. (2005) Structure of hnRNP D complexed with single-stranded telomere DNA and unfolding of the quadruplex by heterogeneous nuclear ribonucleoprotein D. *J. Biol. Chem.*, **280**, 18862–18870.
27. Fiset,S. and Chabot,B. (2001) hnRNP A1 may interact simultaneously with telomeric DNA and the human telomerase RNA *in vitro*. *Nucleic Acids Res.*, **29**, 2268–2275.
28. Tatematsu,K., Nakayama,J., Danbara,M., Shionoya,S., Sato,H., Omine,M. and Ishikawa,F. (1996) A novel quantitative 'stretch PCR assay' that detects a dramatic increase in telomerase activity during the progression of myeloid leukemias. *Oncogene*, **13**, 2265–2274.
29. Delaglio,F., Grzesiek,S., Vuister,G.W., Zhu,G., Pfeifer,J. and Bax,A. (1995) NMRPipe: a multidimensional spectral processing system based on UNIX pipes. *J. Biomol. NMR*, **6**, 277–293.
30. Garrett,D.S., Powers,R., Gronenborn,A.M. and Clore,G.M. (1991) A common sense approach to peak picking in two-, three-, and four-dimensional spectra using automatic computer analysis of contour diagrams. *J. Magn. Reson.*, **95**, 214–220.
31. Goddard,T.D. and Kneller,D.G. (2006) *SPARKY 3*, University of California, San Francisco.
32. Kobayashi,N., Iwahara,J., Koshiba,S., Tomizawa,T., Tochio,N., Guntert,P., Kigawa,T. and Yokoyama,S. (2007) KUJIRA, a package of integrated modules for systematic and interactive analysis of NMR data directed to high-throughput NMR structure studies. *J. Biomol. NMR*, **39**, 31–52.
33. Dellaire,F., Dupuis,S., Fiset,S. and Chabot,B. (2000) Heterogenous nuclear ribonucleoprotein A1 and UP1 protect mammalian telomeric repeats and modulate telomere replication *in vitro*. *J. Biol. Chem.*, **275**, 14509–14516.
34. Nagata,T., Kurihara,Y., Matsuda,G., Saeki,J., Kohno,T., Yanagida,Y., Ishikawa,F., Uesugi,S. and Katahira,M. (1999) Structure and interactions with RNA of the N-terminal UUAG-specific RNA-binding domain of hnRNP D0. *J. Mol. Biol.*, **287**, 221–237.
35. Katahira,M., Miyanoiri,Y., Enokizono,Y., Matsuda,G., Nagata,T., Ishikawa,F. and Uesugi,S. (2001) Structure of the C-terminal RNA-binding domain of hnRNP D0 (AUF1), its interactions with RNA and DNA, and change in backbone dynamics upon complex formation with RNA. *J. Mol. Biol.*, **311**, 973–988.
36. Garrett,D.S., Lodi,P.J., Shamoo,Y., Williams,K.R., Clore,G.M. and Gronenborn,A.M. (1994) Determination of the secondary structure and folding topology of an RNA binding domain of mammalian hnRNP A1 protein using three-dimensional hetero-nuclear magnetic resonance. *Biochemistry*, **33**, 2852–2858.

37. Wishart,D.S., Sykes,B.D. and Richards,F.M. (1992) The chemical shift index: a fast and simple method for the assignment of protein secondary structure through NMR spectroscopy. *Biochemistry*, **18**, 1647–1651.

38. Shamoo,Y., Krueger,U., Rice,L.M., Williams,K.R. and Steitz,T.A. (1997) Crystal structure of the RNA binding domains of human hnRNP A1 at 1.75 Å resolution. *Nat. Struct. Biol.*, **4**, 215–222.

39. Xu,R.M., Jokhan,L., Cheng,X., Mayeda,A. and Krainer,A. (1997) Crystal structure of human UP1, the domain of hnRNP A1 that contains two RNA-recognition motifs. *Structure*, **5**, 559–570.

40. Ding,J., Hayashi,M.K., Zhang,Y., Manche,L., Krainer,A.R. and Xu,R.M. (1999) Crystal structure of the two-RRM domain of hnRNP A1 (UP1) complexed with single-stranded telomeric DNA. *Genes Dev.*, **13**, 1102–1115.

41. Moran-Jones,K., Wayman,L., Kennedy,D.D., Reddel,R.R., Sara,S., Snee,M.J. and Smith,R. (2005) hnRNP A2, a potential ssDNA/RNA molecular adapter at the telomere. *Nucleic Acids Res.*, **33**, 486–496.

42. Zaug,A.J., Podell,E.R. and Cech,T.R. (2005) Human POT1 disrupts telomeric G-quadruplexes allowing telomerase extension *in vivo*. *Proc. Natl Acad. Sci. USA*, **102**, 10864–10869.