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Oxytricha telomere-binding protein: DNA-dependent dimerization of the α and β subunits

(chromosome structure/DNA–protein interactions/protein–protein interactions)

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ABSTRACT **A telomere-binding protein consisting of 56-kDa (α) and 41-kDa (β) subunits binds specifically to the single-stranded $T_4G_4T_4G_4$ sequence at the termini of macronuclear DNA molecules in *Oxytricha nova*. The recent availability of separate α and β polypeptides, expressed in *Escherichia coli*, allows investigation of the assembly of the telomeric complex ("telosome") from its individual components. By mixing wild-type subunits and electrophoretically distinct variants, we verify that the telosome contains one α and one β subunit. By using telomeric DNAs of two lengths, we find that there is one DNA molecule per telosome. The DNA–protein and subunit–subunit interactions were studied by glycerol gradient sedimentation and chemical cross-linking. The formation of α-DNA and β-DNA cross-links in the telomeric complex indicates that both subunits are in proximity to the DNA. When incubated together, both subunits exist predominantly as monomers in the absence of telomeric DNA. Upon binding to DNA, α and β subunits directly interact with each other to form a heterodimer. We suggest that this DNA-dependent dimerization may allow each subunit to carry out distinct functions as a monomer, in addition to its participation in chromosome capping as part of the heterodimer.**

Telomeres protect the natural ends of eukaryotic chromosomes from nuclease degradation and from end-to-end ligation (1–3). Telomere structures are also involved in chromosome organization and nuclear architecture (4–6). In addition, telomeric DNA is essential for the complete replication of chromosomes by serving as a substrate for telomerase (7). Molecular mechanisms of telomere function have begun to be elucidated in the past few years, especially in the ciliated protozoa (8, 9).

Each macronuclear chromosome of the hypotrichous ciliate *Oxytricha nova* has the same telomere sequence: 5′-$G_4T_4G_4T_4G_4T_4G_4T_4G_4$-3′; the last two T_4G_4 repeats protrude as single-stranded DNA (10). A protein consisting of 56-kDa (α) and 41-kDa (β) subunits binds specifically to the single-stranded extension of each macronuclear DNA terminus, forming a "telosome" (11–13). Both subunits remain tenaciously, yet noncovalently, bound to telomeric DNA and to each other under a wide range of conditions. Separation of the two subunits has only been achieved under conditions that inactivate telomere-binding protein (14).

Telomeric DNA of two or four repeats folds into intermolecular or intramolecular G-quartet structures, which are stabilized by Na^+ and K^+ ions (15–19). The fully folded G-quartet DNA is not recognized by the *Oxytricha* telomere-binding protein (20).

Genes encoding both subunits have been cloned and expressed in *Escherichia coli* (21, 22). Recombinant α subunit alone binds to DNA. The β subunit interacts with the α subunit to form a telomeric complex with the methylation footprint pattern indicative of telomeres *in vivo*. Expression of the recombinant α and β subunits separately in *E. coli* allows us to investigate the assembly of the telosome from its individual components.

This paper reports the study of subunit interactions by glycerol gradient sedimentation and chemical cross-linking. The α and β subunits exist predominantly as monomers in the absence of telomeric DNA. Upon binding to DNA, α and β subunits directly interact with each other to form a heterodimer. Thus, heterodimer formation is DNA dependent. In addition, α-DNA and β-DNA cross-links were detected in the telomeric complex in a glutaraldehyde cross-linking experiment. Therefore, both subunits are in proximity to DNA in the telomeric complex. By changing the charge properties of individual components of the complex, we demonstrate in mixing experiments that the telomeric complex contains one α subunit, one β subunit, and one DNA molecule.

MATERIALS AND METHODS

Oligonucleotides and Proteins. OXY2 (5′-TTTTGGGGTTTTGGGG-3′), OXY2T (5′-AAGACGACATCGCTCAGCCAGACATTTTGGGGTTTTGGGG-3′), OXY2LT (5′-AAGACGACATCGCTCAGCCAGACAGACGACATCGCTCAGCCAGATTTTGGGGTTTTGGGG-3′), and NSC (5′-AAGACGACATCGCTCAGCCAGACAGACGACATCGCTCAGC-3′) were made on an Applied Biosystems 380B DNA synthesizer. Protein expression and purification were done as described (22).

Glycerol Gradient Sedimentation. Recombinant telomere-binding protein (2 or 4 μM) was incubated with or without 2 μM OXY2 in 200 μl of binding buffer (20 mM Hepes, pH 7.5/1 mM EDTA/20 mM NaCl/10 mM dithiothreitol) for 60 min at room temperature. Samples were then loaded onto 5-ml 3–15% (vol/vol) linear glycerol gradients in the binding buffer with 0.3 ml of 50% glycerol at the bottom of the gradients. Centrifugation was done in a SW55 rotor for 15 hr at 237,000 × *g* and 4°C. Fractions of 400 μl were manually collected from the top of the centrifuge tubes and assayed by SDS/10% PAGE. Silver staining was performed according to the manufacturer's instructions (Pierce).

Construction of α_s, β_s, and Fusion Proteins. α_s (αC74S) and β_s (βC41S) were constructed by site-directed mutagenesis (23); they are mutants in which the single cysteine residue in each subunit was mutated to a serine. The VP16/α fusion protein gene was constructed by PCR recombination. DNA encoding the herpes simplex virus VP16 activation domain (amino acids 413–490) was amplified from plasmid pJL2 (24). DNA encoding the first 16 amino acids of phage T7 gene *10* protein on plasmid p56A (22) was fused to the 5′ end of the VP16/α coding sequence by subcloning to increase protein

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Abbreviations: DST, disuccinimidyl tartarate; DSP, dithiobis(succinimidyl propionate); DMS, dimethyl sulfate.

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expression. His-Gly was introduced at the fusion junction between ϕ10 and VP16 during plasmid construction. The βC237/VP16 gene encoding amino acids 1–237 of β followed by amino acids 413–490 of VP16 was constructed by PCR amplification and subcloning. βC237 was chosen because of the availability of its *Bst*XI restriction endonuclease site.

Chemical Cross-Linking. Telomere-binding protein (α_s and β_s, at 700 nM or 2 μM each) was incubated with or without 700 nM OXY2 or OXY2T in 15 μl of 25 mM Hepes, pH 7.5/1 mM EDTA/300 mM NaCl for 60 min at room temperature. Then disuccinimidyl tartarate (DST), dithiobis(succinimidyl propionate) (DSP), or glutaraldehyde was added to a final concentration of 50 μg/ml, 5 μg/ml, or 0.001%, respectively. Cross-linking was allowed to proceed at room temperature for 30 min (DSP) or 60 min (DST and glutaraldehyde). Reactions were quenched by adding 6 μl of 4× SDS/PAGE sample loading buffer (0.25 M Tris·HCl, pH 6.8/1.3% SDS/30% glycerol/0.005% bromophenol blue) and analyzed by SDS/7% PAGE. Silver staining was performed according to the manufacturer's instructions (Pierce). Reversal of DST and DSP cross-linking was done by incubating gel slices at room temperature in 30 mM sodium periodate for 20–60 min or in 5% 2-mercaptoethanol for 5–15 min, respectively.

RESULTS

α/β Dimerization is DNA Dependent. Purified recombinant telomere-binding polypeptides were incubated with or without telomeric DNA and sedimented through a 3–15% glycerol gradient. Fractions of the gradient were analyzed by SDS/PAGE.

By itself, the α subunit (56 kDa) sediments slower than bovine serum albumin (67 kDa), but slightly faster than chicken egg albumin (45 kDa) (Fig. 1*A*). The β subunit (41 kDa) sediments slower than chicken egg albumin (Fig. 1*B*). Therefore, each subunit sediments according to its own molecular mass. When incubated with OXY2 (two repeats of T_4G_4 DNA), the α subunit sediments toward higher molecular mass fractions (Fig. 1*C*). Since the α·DNA complex has a half-life much less than 1 min (25), even a rather unstable interaction can be detected under these assay conditions. When incubated together, the majority of the α subunit and the majority of the β subunit retain their own characteristic sedimentation rates and peak at different fractions. Only a very small amount of each subunit cosediments with the other subunit toward higher molecular mass fractions (Fig. 1*D*). Thus, both subunits exist predominantly as monomers even at this high concentration (2 μM for each subunit). However, the α and β subunits cosediment toward higher molecular mass fractions in the presence of oligonucleotide OXY2 (Fig. 1*E*). When the β subunit is in excess, free β subunit is separated from the bound β (Fig. 1*F*). Hence, α, β, and DNA form a stable complex.

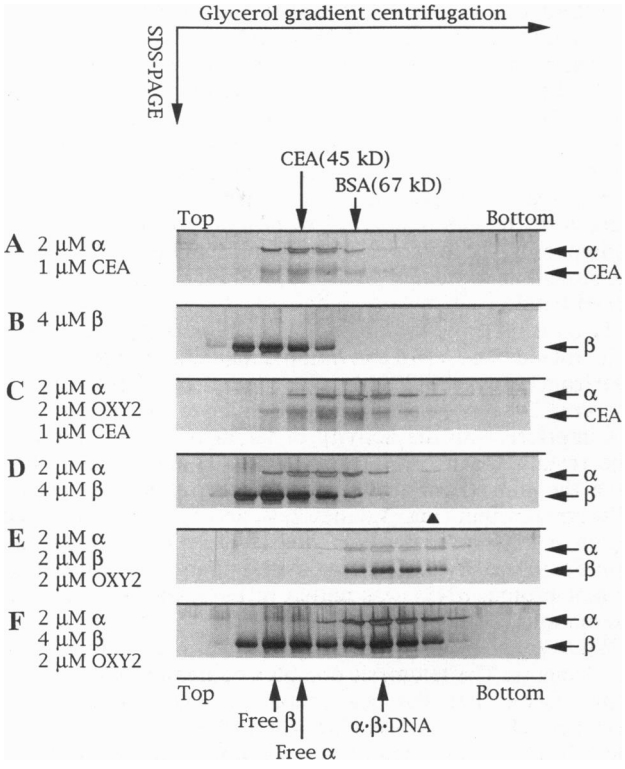

FIG. 1. Glycerol gradient sedimentation of the telomere-binding protein and DNA–protein complexes relative to marker proteins. Fractions were analyzed by SDS/10% PAGE, and proteins were visualized by silver staining. CEA, chicken egg albumin; BSA, bovine serum albumin. The arrowhead in *D* indicates the expected position of the $\alpha\cdot\beta$ heterodimer in the absence of DNA.

The α and β subunits could contact each other in the complex. Alternatively, both subunits could bind to the DNA without any direct contact between the subunits. To distinguish these possibilities, chemical cross-linking experiments were performed. A complication arose because the α and β subunits each contain one cysteine residue, and small amounts of disulfide-linked hetero- and homodimers formed unless dithiothreitol was present. Since dithiothreitol reacts with the protein cross-linking reagents used in this study, the cysteine residue in each subunit was mutated to serine to eliminate the background cross-linking by disulfide bonds. The resulting mutants αC74S (α_s) and βC41S (β_s), which were fully active in telomeric complex formation as assayed by dimethyl sulfate (DMS) methylation footprinting (data not shown), were analyzed for subunit interactions.

DST, DSP, and glutaraldehyde were used as cross-linking reagents. All three react with primary amino groups (26–28). α_s alone is not cross-linked as a homodimer either in the absence or in the presence of OXY2 (Fig. 2 *A–C*, lanes 2 and 3). The 60-kDa† band in lane 3 of Fig. 2*C* represents a product cross-linked between α_s and the DNA, since it shifts to the 65-kDa position and becomes radioactive when labeled OXY2T replaces OXY2 (Fig. 2*C*, lane 4; autoradiograph not shown). (OXY2T has two repeats of T_4G_4 at its 3′ end and a 24-nucleotide nontelomeric tail sequence at its 5′ end.) The α_s-OXY2T cross-linking is more efficient than the α_s-OXY2 cross-linking (Fig. 2*C*, lanes 3 and 4). β_s alone is not cross-linked as a homodimer either in the absence or in the presence of OXY2 (Fig. 2 *A* and *B*, lanes 5 and 6; Fig. 2*C*, lanes 6 and 7). However, it is cross-linked to labeled OXY2T by glutaraldehyde to give a 57-kDa radioactive band (Fig. 2*C*, lane 8).

When the two subunits (each at 700 nM) were mixed together in the absence of telomeric DNA, no cross-linked product was detected (Fig. 2 *A* and *B*, lanes 8; Fig. 2*C*, lane 10). In the presence of OXY2, two cross-linked bands (95 kDa and 120 kDa) were detected (Fig. 2 *A* and *B*, lane 9; Fig. 2*C*, lane 11). The substitution of OXY2T for OXY2 increased the cross-linking efficiency of these two bands (Fig. 2*C*, lane 12). (In the presence of OXY2T, the cross-linking efficiencies were 2–4% for the 95-kDa band and 6–12% for the 120-kDa band, determined by comparing the band intensities with those of a series of dilutions of telomere-binding protein as standards.) These two bands were not radioactive when labeled DNA was used (data not shown), and their mobilities were independent of the length of the DNA (Fig. 2*C*, lanes 11 and 12). Thus we conclude that they result from protein-protein cross-links. This conclusion is further supported by

†Throughout this work, molecular masses assigned to cross-linked complexes on SDS gels are apparent molecular masses based on polypeptide standards.

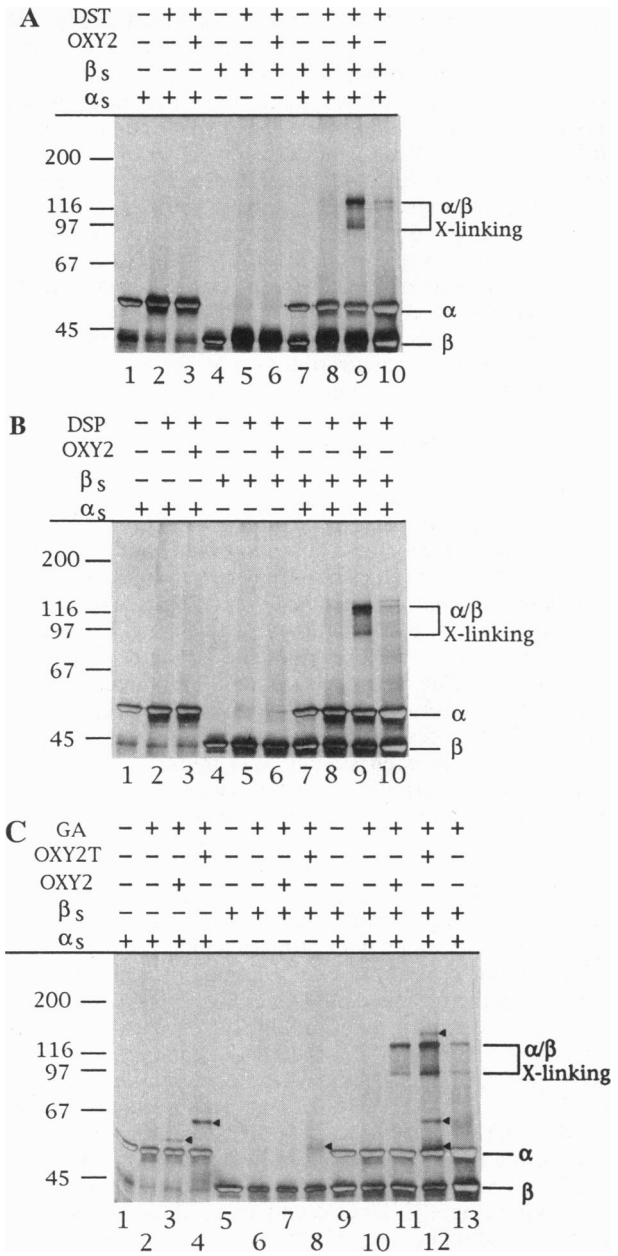

FIG. 2. Cross-linking of the telomere-binding protein and DNA–protein complexes by DST (*A*), DSP (*B*), and glutaraldehyde (GA) (*C*). Cross-linked products were analyzed by SDS/7% PAGE and visualized by silver staining. The DNA and protein concentrations were 700 nM except lane 10 of *A* and *B* and lane 13 of *C*, where the protein concentration was 2 μM. Arrowheads in *C* point to the bands that were radioactive when labeled DNA was used. These radioactive bands represent cross-linked DNA–protein complexes. X-linking, cross-linking. Molecular size markers (in kDa) are given at left.

the observation that one or both of these two bands were detected at a higher protein concentration (2 μM α_s plus 2 μM β_s), though less efficiently, in the absence of telomeric DNA (Fig. 2 *A* and *B*, lane 10; Fig. 2*C*, lane 13). Reversal of DST and DSP cross-linking in gel slices (see *Materials and Methods*) and electrophoresis into a second dimension showed that each of the two bands contains both α and β subunits (data not shown). Thus, we conclude that the interactions between the α and β subunits are DNA facilitated. In the presence of telomeric DNA, these two subunits directly contact each other.

In addition to the two protein-protein cross-links, three radioactive cross-linked products (57 kDa, 65 kDa, and 140 kDa) were detected in the presence of labeled OXY2T (Fig. 2*C*, lane 12). All three bands disappeared when digested with proteinase K or extracted with phenol (data not shown). Thus, they represent DNA-protein cross-links rather than DNA-DNA cross-links. The 57-kDa radioactive band just above the uncross-linked α_s comigrates with the cross-linked β_s·OXY2T complex (Fig. 2*C*, lanes 8 and 12). The 65-kDa band comigrates with the cross-linked α_s·OXY2T complex (Fig. 2*C*, lanes 4 and 12). Therefore, these two bands appear to represent β_s-OXY2T and α_s-OXY2T cross-links. On the basis of its migration rate and cross-linking efficiency, the 140-kDa band might represent a doubly cross-linked product. The α_s subunit in the presence of the β_s subunit also cross-links very weakly to OXY2 as a 60 kDa band, which can be detected in the presence of labeled OXY2 (data not shown).

Formation of the 65-kDa α_s·OXY2T cross-linked product in the presence or in the absence of the β_s subunit was not blocked by competition by addition of a 25-fold excess of a nontelomeric oligonucleotide (NSC at 17.5 μM) and is therefore specific. Although the β_s-OXY2T cross-link (57-kDa band) in the β_s·DNA complex was blocked by competition by 17.5 μM NSC, the same cross-link in the telomeric complex was resistant to competition (data not shown).

α and β Subunits and Telomeric DNA Form a 1:1:1 Complex. As described above, cross-linking gave two α/β products with apparent molecular masses of 95 and 120 kDa. It seemed possible that the band of lower mobility was a doubly cross-linked product and might therefore represent a protein trimer. To test this possibility, the stoichiometry of the telomeric complex was determined in mixing experiments.

Native gel electrophoresis was used to physically separate the telomeric complex. The α subunit alone binds to telomeric DNA, giving a smear during the native gel electrophoresis. The β subunit supershifts the α·DNA complex into the well (data not shown). Deletion of the C-terminal highly basic one-third of the β subunit makes the telomeric complex slowly migrate into the gel as a single band. The resulting mutant protein, βC232, contains the N-terminal 232 amino acids of the β subunit and is fully active in the formation of the telomeric complex as assayed by methylation footprinting (25). We reasoned that if the negative charge of the telomeric DNA or of each subunit were increased, the telomeric complex should migrate faster in the native gel. If complexes were formed with a mixture of normal and fast-migrating components, the different complexes could be separated and the stoichiometry could be determined by counting the number of bands.

Two fusion proteins and one deletion mutant protein were constructed. The VP16 activation domain (amino acids 413–490) from herpes simplex virus type 1 contains 18 net negative charges (29). Fusion of this acidic domain to each subunit did not interfere with the activity of telomere-binding protein. The resulting chimeric proteins VP16/α and βC237/VP16 gave the same DMS methylation footprint pattern as the wild-type protein (Fig. 3, lanes 2, 4, and 5). The N-terminal region of the α subunit is highly basic. Deletion of the first 33 amino acids removes eight net positive charges. The resulting mutant protein αN34 was active in DNA-binding and α/β interactions (Fig. 3, lane 3) (25).

Wild-type α, αN34, and VP16/α were used in mixing experiments. The telomeric complex containing VP16/α migrated faster than the one containing wild-type α during native gel electrophoresis (Fig. 4*A*, lanes 1 and 3). When α and VP16/α were mixed together and then added to βC232 and OXY2T, only two well-separated bands were detected, and no band with intermediate mobility was observed (Fig. 4*A*, lane 2). Mixing experiments performed with α and αN34 gave the same result (Fig. 4*A*, lanes 4–6). If there were two α subunits per telomeric complex, one additional band with intermediate mobility and twice the intensity should have

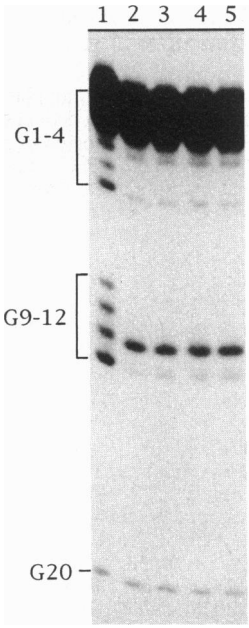

FIG. 3. DMS methylation footprint assay for binding of αN34, VP16/α, and βC237/VP16. The 5′ labeled BOXY2T (a biotinylated version of OXY2T; ref. 25) was incubated with *E. coli* lysates containing recombinant telomere-binding protein and its variants. Streptavidin paramagnetic beads were added for 30 min, followed by treatment with 0.1% DMS for 10 min at room temperature. Methylation was stopped, the streptavidin beads were collected and washed, and DNA was cleaved with pyrrolidine. Products were analyzed by 12% polyacrylamide/8 M urea gel electrophoresis (25). G20 is a guanine residue in the tail sequence, which serves as an internal control for loading differences. Lanes: 1, control lysate of *E. coli* transformed with expression vector; 2, α plus β; 3, αN34 plus β; 4, VP16/α plus β; 5, α plus βC237/VP16.

been detected. The lack of an intermediate band leads us to conclude that there is only one α subunit per telomeric complex.

An analogous mixing experiment performed with βC232 and βC237/VP16 leads us to conclude that there is only one β subunit per telomeric complex (Fig. 4*B*, lanes 1–3).

Two different DNA probes, OXY2T and OXY2LT, were used in mixing experiments. Both probes contain two telomeric repeats (T_4G_4) at the 3′ ends, but OXY2LT has a longer 5′ nontelomeric tail. As predicted, the telomeric complex containing OXY2LT migrates faster than the one containing OXY2T during native gel electrophoresis (Fig. 4*C*, lanes 1, 3, 4, and 6). When OXY2T and OXY2LT were mixed together and added to α and βC232 or to αN34 and βC232, only two well-separated bands were detected in each case (Fig. 4*C*, lanes 2 and 5). We conclude that there is only one DNA molecule per telomeric complex. Therefore, the telomeric complex contains one α subunit, one β subunit, and one DNA molecule.

DISCUSSION

We have shown that both subunits of the telomere-binding protein exist predominantly as monomers in the absence of telomeric DNA, although a small amount of heterodimer appears to form at a high protein concentration (each subunit at 2 μM). Upon binding to DNA, the α and β subunits form a heterodimer, as evidenced by their cross-linking with three reagents of different lengths. Further evidence that α and β interact on the DNA is presented elsewhere, where we showed that the α and β subunits bind to the telomeric DNA cooperatively and that the α subunit has separable DNA-binding and dimerization domains (25). Therefore, we conclude that heterodimer formation is DNA facilitated and, at all but the highest protein concentrations, DNA dependent. DNA-dependent protein–protein interaction has been observed in other DNA-binding proteins. For example, homodimer formation between the DNA-binding domains of two glucocorticoid receptor proteins and between two GAL4 DNA-binding domains is DNA dependent (30–33).

There are two possible explanations for DNA-dependent dimerization of α and β. The interactions between the two subunits may not be strong enough to form a stable dimer without DNA holding the subunits together. Alternatively, DNA binding may cause a conformation change for one or both of the subunits, thereby exposing the dimerization interface(s). In addition, binding of one subunit may change the DNA conformation, promoting binding of the other subunit. We are not able to differentiate these possibilities.

In addition to the α-β cross-links, we also detect cross-linking between α and DNA by glutaraldehyde, consistent with previous observation that the α subunit by itself binds to telomeric DNA (22). Under the higher salt condition used here, only the 3′ telomeric repeat sequence is bound by the α subunit in the absence of β (ref. 25; cf. ref. 22). The α subunit cross-links to OXY2T better than to OXY2, both in the absence and in the presence of the β subunit. Due to the cooperative binding of the α and β subunits to telomeric DNA (25), any α-DNA complex should be converted to the α-β-DNA complex in the presence of both subunits with the protein concentration used here. Thus, it is likely that the α-DNA cross-link formed in the presence of the β subunit results from the α-β-DNA complex. We conclude that the α subunit contacts the DNA in both complexes.

The β subunit can be cross-linked to OXY2T but not to OXY2, both in the absence and in the presence of α. In addition, the β-OXY2T cross-link in the telosome cannot be blocked by competition by nonspecific DNA. We conclude that the β subunit makes direct DNA contacts in the telomeric complex. Consistent with our data, it has been shown previously that the β subunit by itself is cross-linked to OXY2T by UV radiation (B. Hicke, M. Willis, T. Koch, and T.R.C., unpublished results).

Protein-protein cross-linking of the telomeric complex gave two products (95 kDa and 120 kDa), each containing both subunits. Cross-linking of *Oxytricha* telomere-binding protein bound to macronuclear chromosomes at a comparable DSP concentration revealed only one cross-linked band at

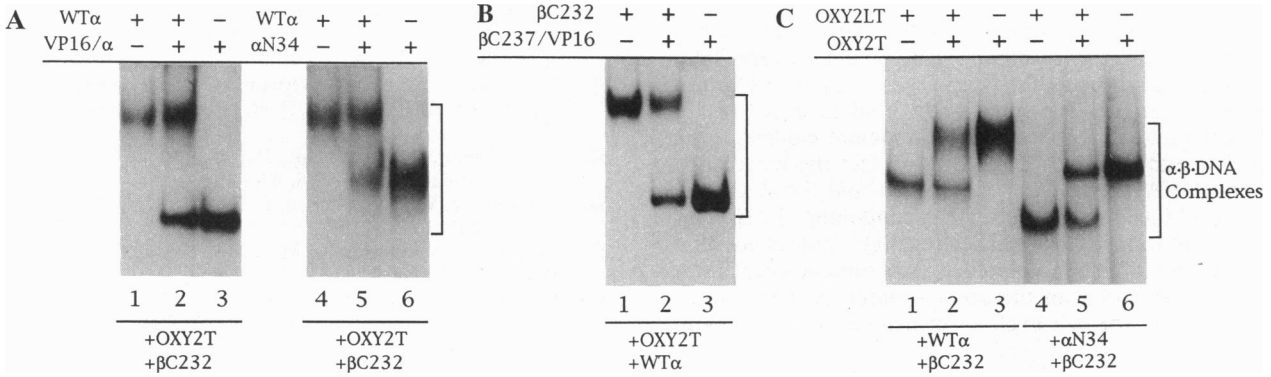

FIG. 4. There is one α subunit (*A*), one β subunit (*B*), and one DNA molecule (*C*) per telomeric complex. The composition of the telomeric complex was determined by native gel electrophoresis in mixing experiments (see text). *E. coli* lysates containing telomere-binding protein and its variants were incubated with 5′ labeled OXY2T and/or OXY2LT and then subjected to native 8% PAGE at 4°C (25). WT, wild type.

≈120 kDa (14), which suggests a subtle difference between the *Oxytricha* protein and the recombinant protein.

The apparent molecular mass of the lower band (95 kDa) is the sum of the molecular masses of the α (56 kDa) and β (41 kDa) subunits. Thus, this band can be attributed to a cross-linked heterodimer. Two possible explanations were considered for the upper band (120 kDa). It might represent an $\alpha \cdot \beta$ heterodimer cross-linked at a position different from that of the lower band, which would require either two populations of telomeric complexes or multiple contact sites between the two subunits within one complex. Alternatively, the upper band might represent a doubly cross-linked protein trimer. The distribution of the two cross-linked products favors the upper band, even at low concentrations of the cross-linking reagents and at short cross-linking time (data not shown), which is contradictory to the double cross-link hypothesis unless two single cross-links form cooperatively. To further test the protein trimer possibility, we determined the stoichiometry of the telomeric complex.

The stoichiometry of the telomeric complex was studied previously by protein concentration titration (22). It was concluded that the telomeric complex contains a 1:1 molar ratio of heterodimer to DNA. Titration experiments require accurate determination of protein concentration, which is method dependent, and assumption of protein activity, which is difficult to measure independently. Thus, a 2-fold inaccuracy in stoichiometry is difficult to exclude. In addition, the titration experiment gives the molar ratio of protein to DNA but not the physical composition of the complex since it does not physically determine the size of the complex. Thus, the previous conclusion of a 1:1:1 complex of α subunit, β subunit, and DNA required the assumption of one DNA molecule per complex (22). To study the physical composition of the telomeric complex, we performed mixing experiments with DNA and protein subunits of different electrophoretic mobility (e.g., refs. 34–36).

Increasing the negative charge of the telomeric complex makes the complex migrate faster in a native gel. We were able to fuse the highly acidic VP16 activation domain from herpes simplex virus to each subunit while maintaining their activities in telomere binding. By using these chimeric proteins and different DNA probes, we demonstrated that the telomeric complex consists of one α subunit, one β subunit, and one DNA molecule. An active deletion mutant (αN34) of the α subunit gave the same result. Given the 1:1:1 stoichiometry of the telomeric complex, the 120-kDa band detected in the chemical cross-linking experiment is unlikely to be a protein trimer. Because native gel electrophoresis resolved only a single complex, it is likely that the appearance of two bands (95 and 120 kDa) on denaturing SDS gels represents different positions of cross-linking between the α and β subunits.

Why is heterodimer formation DNA dependent? It has been shown that the α subunit can bind to every T_4G_4 telomere repeat along a $(T_4G_4)_n$ multimer (22). Therefore, it is possible that the α subunit might bind to every newly synthesized telomere repeat during telomere elongation by telomerase and thereby temporarily protect the lengthened telomeric DNA. Recently, we observed that the β subunit can promote the association of DNA containing $(T_4G_4)_2$ into dimers and tetramers (unpublished results). Thus, in addition to forming the telomeric complex, each subunit might have functions independent of the other subunit, perhaps at different phases of the cell cycle or of macronuclear development. Preformed heterodimer would prevent each subunit from carrying out its own distinctive functions *in vivo*.

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1. Muller, H. J. (1938) *The Collecting Net-Woods Hole* **13**, 181–198.
2. McClintock, B. (1941) *Genetics* **26**, 234–282.
3. McClintock, B. (1942) *Proc. Natl. Acad. Sci. USA* **28**, 458–463.
4. Agard, D. A. & Sedat, J. W. (1983) *Nature (London)* **302**, 676–681.
5. Rawlins, D. J., Highett, M. I. & Shaw, P. J. (1991) *Chromosoma* **100**, 424–431.
6. de Lange, T. (1992) *EMBO J.* **11**, 717–724.
7. Blackburn, E. H. (1992) *Annu. Rev. Biochem.* **61**, 113–129.
8. Zakian, V. A. (1989) *Annu. Rev. Genet.* **23**, 579–604.
9. Blackburn, E. H. (1991) *Nature (London)* **350**, 569–573.
10. Klobutcher, L. A., Swanton, M. T., Donini, P. & Prescott, D. M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3015–3019.
11. Gottschling, D. E. & Cech, T. R. (1984) *Cell* **38**, 501–510.
12. Gottschling, D. E. & Zakian, V. A. (1986) *Cell* **47**, 195–205.
13. Price, C. M. & Cech, T. R. (1987) *Genes Dev.* **1**, 783–793.
14. Price, C. M. & Cech, T. R. (1989) *Biochemistry* **28**, 769–774.
15. Williamson, J. R., Raghuraman, M. K. & Cech, T. R. (1989) *Cell* **59**, 871–880.
16. Sundquist, W. I. & Klug, A. (1989) *Nature (London)* **342**, 825–829.
17. Sen, D. & Gilbert, W. (1990) *Nature (London)* **344**, 410–414.
18. Kang, C. H., Zhang, X., Ratliff, R., Moyzis, R. & Rich, R. (1991) *Nature (London)* **356**, 126–131.
19. Smith, F. & Feigon, J. (1992) *Nature (London)* **356**, 164–168.
20. Raghuraman, M. K. & Cech, T. R. (1990) *Nucleic Acids Res.* **18**, 4543–4552.
21. Hicke, B. J., Celander, D. W., MacDonald, G. H., Price, C. M. & Cech, T. R. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1481–1485.
22. Gray, J. T., Celander, D. W., Price, C. M. & Cech, T. R. (1991) *Cell* **67**, 807–814.
23. Kunkel, T. A., Roberts, J. D. & Zakour, R. A. (1987) *Methods Enzymol.* **154**, 367–382.
24. Sadowski, I., Ma, J., Triezenberg, S. & Ptashne, M. (1988) *Nature (London)* **335**, 563–564.
25. Fang, G., Gray, J. T. & Cech, T. R. (1993) *Genes Dev.* **7**, 870–882.
26. Lomant, A. J. & Fairbanks, G. (1976) *J. Mol. Biol.* **104**, 243–261.
27. Peters, K. & Richards, F. M. (1977) *Annu. Rev. Biochem.* **46**, 523–551.
28. Smith, R. J., Capaldi, R. A., Muchmore, D. & Dahlquist, F. (1978) *Biochemistry* **17**, 3719–3723.
29. Triezenberg, S. J., Kingsbury, R. & McKnight, S. L. (1988) *Genes Dev.* **2**, 718–729.
30. Hard, T., Kellenbach, E., Boelens, R., Maler, B. A., Dahlman, K., Freedman, L. P., Carlstedt-Duke, J., Yamamoto, K. R., Gustafsson, J. A. & Kaptein, R. (1990) *Science* **249**, 157–160.
31. Schwabe, J. W. R., Neuhaus, D. & Rhodes, D. (1990) *Nature (London)* **348**, 458–461.
32. Luisi, B. F., Xu, W. X., Otwinowski, Z., Freedman, L. P., Yamamoto, K. R. & Sigler, P. B. (1991) *Nature (London)* **352**, 497–505.
33. Marmorstein, R., Carey, M., Ptashne, M. & Harrison, S. C. (1992) *Nature (London)* **356**, 408–414.
34. Landschulz, W. H., Johnson, P. F. & McKnight, S. L. (1989) *Science* **243**, 1681–1688.
35. Gregor, P. D., Sawadogo, M. & Roeder, R. G. (1990) *Genes Dev.* **4**, 1730–1740.
36. Nye, J. A., Petersen, J. M., Gunther, C. V., Jonsen, M. D. & Graves, B. J. (1992) *Genes Dev.* **6**, 975–990.