Sp1 Family Proteins Recognize the U5 Repressive Element of the Long Terminal Repeat of Human T Cell Leukemia Virus Type I through Binding to the CACCC Core Motif

(Received for publication, September 12, 1995, and in revised form, February 8, 1996)

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We have identified several nuclear proteins binding to the U5 repressive element (USRE) at the U5 region of the human T cell leukemia virus type I (HTLV-I) long terminal repeat (LTR). In gel mobility shift assays with the USRE DNA probe, Jurkat T cell nuclear proteins generated five different complexes, named USRE binding protein complexes (USRP)-A1, -A2, -A3, -B, and -C. Only USRP-C was affected by pretreatment with an excess of poly(dI-dC) and was immunodepressed by anti-Ku/p80 antibodies, suggesting that USRP-C is a nonspecific complex involving Ku antigen. UV cross-linking showed at least six nuclear proteins involved in the other complexes, including USRP-A1, -A2, -A3, and -B. The sequence of the binding core element of these specific complexes, determined by competition assays and gel mobility shift assays using a series of the USRE mutants, is CACCC which is identical to that for the Sp1 transcription factor. LTR with a mutant USRE, which has no ability to bind with the nuclear proteins, showed stronger promoter activity than LTR with the wild USRE, suggesting that the specific interaction of these USRE-binding proteins might result in the US-mediated repression. USRP-A1 was supershifted by anti-Sp1 antibodies and USRP-A2 and -B were supershifted by anti-Sp3 antibodies, suggesting that Sp1 or Sp3 is involved in USRP-A1 or USRP-A2 and -B, respectively. Although the other nuclear proteins remain to be characterized, these findings suggest that USRE-binding proteins in USRP-A1, -A2, -A3, and -B are involved in HTLV-I gene repression.

**EXPERIMENTAL PROCEDURES**

**Cell Lines—**The human T cell line Jurkat (15) was cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (R10F medium).

**Crude Nuclear Extracts—**Nuclear extracts were prepared from Jurkat cells according to the method of Dignam et al. (22). Briefly, cells were washed with phosphate-buffered saline, suspended in 5 packed volumes of ice-cold lysing buffer A (10 mM HEPES, pH 8.0; 10 mM KC1; 15 mM MgCl2; 0.5 mM dithiorthreitol; 0.5 mM phenylmethylsulfonyl fluoride), kept on ice for 10 min, and centrifuged at 10,000 x g for 10 min. The cell pellet was resuspended in 2 volumes of ice-cold buffer A, homogenized 10–20 strokes with a Dounce homogenizer, and centrifuged at 10,000 x g for 10 min at 4°C. The nuclear pellet was resuspended in ice-cold extraction buffer 1 (200 mM HEPES, pH 8.0; 0.5 mM NaCl; 20% glycerol; 15 mM MgCl2; 0.2 mM EDTA; 0.5 mM dithiothreitol; 0.5 mM phenylmethylsulfonyl fluoride) at a ratio of 2.5 ml/106 cells, homogenized 10–20 strokes with a Dounce homogenizer, and kept on ice for 30 min. After centrifugation at 25,000 x g for 30 min at 4°C, the supernatant was dialyzed against dialysis buffer 2.

**Human T cell leukemia virus type I (HTLV-I)** is an etiological agent of adult T cell leukemia and HTLV-I-associated myelopathy or tropical spastic paraparesis (1–6). After infection into humans, however, the virus has a long latent period to induce such diseases. The mechanism of the viral latency has not yet been uncovered, although there are many studies on the regulation mechanisms of HTLV-I gene expression (7–19). The region was proposed to control virus basal gene expression (10), although this region represses HTLV-I gene transcription in the presence of the human cytomegalovirus IE2 protein (21). The US region of the U5-LTR was shown to contain a repressive element for the viral gene expression (16). Seki et al. (17) have shown that the region exerts its repressive effects at the post-transcriptional level. We recently reported that the US-mediated repression also occurred at a transcriptional level and that the USRE binding protein involved at least the autoantigen Ku protein complex p70/80 and an unknown protein p110 (15).

In this report, we further analyzed the binding complex with the USRE in detail, determined the sequence of the binding core motif, and identified some of the specific binding proteins to USRE, such as Sp1 and Sp3. Finally, we propose that the specific interaction of these binding proteins to USRE might result in the US-mediated repression.
(20 mM Tris-HCl, pH 8.0, 20% glycerol, 0.1 mM KCl, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride) for more than 8 h at 4°C. The dialyzed extract was cleared by centrifugation at 8,000 × g for 15 min, and the supernatant was used directly for further analysis or kept in aliquots at −80°C until use.

Sucrose Gradient Sedimentation—One ml of the JURKAT nuclear extract was applied on 4 ml of a 5–30% sucrose gradient bed and centrifuged at 100,000 × g for 18 h at 4°C using SW55 rotor (Beckman; LB-80 × 60 m). Each fraction from the bottom puncture of the centrifuge tube was recovered step wise in 500-μl amounts and analyzed.

Oligonucleotides for Probes and Competitors—The oligonucleotides were synthesized using a DNA synthesizer (Cyclone Plus DNA Synthesizer, Applied Biosystems, Foster City, CA). The sequence of the DNA is indicated in the Figs. 5 and 6.

Gel Mobility Shift Assay—The crude nuclear extracts or partially purified fractions were incubated with 32P-end-labeled DNA probes in binding buffer 1 (20 mM Tris-HCl, pH 7.4, 50 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 5% glycerol) before preincubation with or without poly(dI-dC)poly(dI-dC) (designated poly(dI-dC) DNA; Pharmacia, Uppsala, Sweden) for 30 min at 25°C. Aliquots of the reaction mixtures were loaded onto a 5% polyacrylamide gel, followed by electrophoresis for 90 min at 150 V in the electrophoresis buffer (TAE; 40 mM Tris acetate buffer, 1 mM EDTA) as described elsewhere (15). The mobility-retarded DNA bands were visualized by autoradiography with x-ray film.

For immunodepression assays, 1 μl of the appropriate antibody was simultaneously added into the binding reaction mixtures. After incubation for 15 min at 25°C, the reaction mixtures were further incubated with protein G and A agarose (Oncogene Science, Manhasset, NY) for 30 min; thereafter, the supernatants of these reaction mixtures were processed as described above. For supershift assays, 1 μl of the appropriate antibody was added to the binding reaction mixture 20 min prior to the loading of the gel.

Anti-p40tax monoclonal antibodies (L-4) were kindly provided by Y. Tanaka (23). Anti-Sp1 antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-Sp3 antibodies were kindly provided by G. Hagen (24).

For UV cross-linking, the binding reaction mixtures were irradiated at 254 nm for 30 min at 25°C at a distance of 3 cm and then loaded onto a 5% polyacrylamide gel. From the gel slice including the target band, proteins were eluted in SDS-PAGE loading buffer, heated at 90°C for 5 min, and loaded onto an SDS-10% polyacrylamide gel. After electrophoresis, the gel was dried on Whatman 3MM paper and exposed on the x-ray film.

Transfection and Chloramphenicol Acetyltransferase (CAT) and Luciferase Assays—Three luciferase (luc) expression plasmids derived from the HTLV-I LTR promoter with the U5RE (wild type), or the U5RE DNA probe. In this study, however, at least five bands were synthesized using a DNA synthesizer (Cyclone Plus DNA Synthesizer, Applied Biosystems, Foster City, CA). The sequence of the DNA is indicated in the Figs. 5 and 6.

Gel Mobility Shift Assay—The crude nuclear extracts or partially purified fractions were incubated with 32P-end-labeled DNA probes in binding buffer 1 (20 mM Tris-HCl, pH 7.4, 50 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 5% glycerol) before preincubation with or without poly(dI-dC)poly(dI-dC) (designated poly(dI-dC) DNA; Pharmacia, Uppsala, Sweden) for 30 min at 25°C. Aliquots of the reaction mixtures were loaded onto a 5% polyacrylamide gel, followed by electrophoresis for 90 min at 150 V in the electrophoresis buffer (TAE; 40 mM Tris acetate buffer, 1 mM EDTA) as described elsewhere (15). The mobility-retarded DNA bands were visualized by autoradiography with x-ray film.

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Transfection and Chloramphenicol Acetyltransferase (CAT) and Luciferase Assays—Three luciferase (luc) expression plasmids derived from the HTLV-I LTR promoter with the USRE (wild type), or the USRE M13 or M17 (mutant type; see Fig. 6) were constructed as described previously (15); pBLTR-M13-luc, or pBLTR-M17-luc, in which the luc gene was under the control of the U3-R-U5 (−321 to +316) region of the wild type LTR or the mutant type LTR which is introduced as a single point mutation (C to G or A) at nucleotide +275 or +279 of the USRE region as shown in Fig. 7, respectively.

The mixture of 1 μg of pBLTR-Wt-luc, pBLTR-M13-luc, or pBLTR-M17-luc expression plasmids and 2 μg of the internal control CAT expression plasmid pRSV-CAT (15) was transfected into JURKAT cells using a Lipofectin KS reagent (Life Technologies, Inc.) in serum-free culture medium, followed by culturing for 16 h, and was further cultured for 48 h in RPMI medium. Thereafter, the cells were harvested, and whole cell lysates in 100 μl of reporter lysis buffer (Promega, Madison, WI) were prepared. The lysates were cleared by centrifugation at 15,000 × g for 10 min. The supernatants were recovered and assayed for CAT and luciferase activities. CAT activity was measured by chemiluminescence using a luminometer (Lumat model LB9501; Berthold, Wildbad, Ger.

1. 2 3 4 5 6 7 8 9 10

**Fig. 1.** Demonstration of five different complexes in gel mobility shift assays of JURKAT nuclear proteins with the USRE DNA probe. Gel mobility shift assays were performed using 1 ng of the 32P-labeled USRE DNA as a probe after preincubation of the nuclear extracts (2 μg) with (lanes 1–5) or without (lanes 6–10) the poly(dI-dC) DNA (1 μg). Five major bands are indicated as U5RP-A1, -A2, -A3, -B, and -C. For competition gel shift assays, reactions were performed in the absence (lanes 1 and 6) or in the presence of a 10- or 100-fold (lanes 2, 4, 7, 9, and 3, 5, 8, and 10, respectively) molar excess of the USRE DNA (lanes 2, 3, 7, and 8) or the nonspecific DNA (lanes 4, 5, 9, and 10) respectively, as a competitor.

Anti-Ku p80 Monodonal Antibody Preparation—Anti-Ku/p80 monodonal antibodies were generated by injecting mice with bacterially expressed Ku80 p80 proteins. Brieﬂy, the CDNA fragment comprising the coding region for the Ku80 protein (26) was ligated with a pGEX-XZT expression vector (Pharmacia) to obtain the plasmid capable of expressing a Ku80 protein as a fusion protein with glutathione S-transferase (GST-Ku/p80). The recombinant GST-Ku/p80 protein expressed in E. coli was puriﬁed using a prepacked glutathione-Sepharose 4B column (Pharmacia) and loaded onto a preparative polyacrylamide gel (Bio-Rad model 491). Fractions containing GST-Ku/p80 were collected and used as immunogens in mice. We screened a hybrid-myceloma cell line from which the culture supernatant speciﬁcally reacts with both the recombinant Ku80 protein and the natural Ku80 protein in HeLa cells (ATCC no. CCL2) by Western blotting. The supernatant from this clone was used as anti-Ku/p80 monodonal antibodies.

RESULTS

Detection of Five Distinct Binding Complexes to the USRE—We previously described that one major shift band was detectable in gel mobility shift assays with the 32P-labeled USRE DNA probe. In this study, however, at least five bands were distinguishable when assayed under the conditions with modifications as described under "Experimental Procedures." The slowest three mobility complexes appear to be very closely retarded bands, which addressed USRP-A1, -A2, and -A3. Here, we name Group A inclusive of these three bands. The other two separated bands are designated as USRP-B and -C (Fig. 1). The complex formation of these bands appeared to be very fragile, because the intensity of these bands decreased when the nuclear extract proteins were used after freezing and thawing (data not shown). Thus, the JURKAT nuclear extracts without treatment by freezing and thawing were used in this study. As shown in Fig. 1, lanes 1 and 6, the band intensity of USRP-C was shown to decrease after preincubation of the nuclear extracts with the poly(dI-dC) DNA for 10 min on ice; however, the intensity of the others, USRP-A1, -A2, -A3, and -B, was not affected. Competition gel shift assays revealed that the bands,
Group A and USRP-B, were clearly competed with the USRE DNA but not with the nonspecific DNA (Fig. 1). In addition, these complexes were competed with similar efficiencies by the USRE competitor. These results suggested the presence of specific binding proteins in USRP-A1, -A2, -A3, and -B, but not in USRP-C. To characterize these complexes further, Jurkat nuclear extracts were subjected to sucrose gradient sedimentation, and each fraction was analyzed by gel mobility shift assays (Fig. 2). The highest peak of the complexes of Group A and USRE-B was detected in the same fraction (fraction 3; Fig. 2, lane 4); whereas that of the other USRP-C was in another fraction (fraction 4; Fig. 2, lane 5), suggesting that DNA-protein complexes contained in Group A and USRP-B are distinct from those in USRP-C. Our previous studies have shown that Ku or Ku-related proteins are involved in the USRE-binding protein complex (15). Thus, to clarify which complex involves Ku, we performed gel mobility immunodepression assays using monoclonal antibody raised against Ku/p80 antigen as described under “Experimental Procedures.” The anti-p80/Ku antibody appeared to depress DNA binding only in USRP-C; whereas neither the anti-p40tax monoclonal antibody nor a negative control culture supernatant (NC, lane 4) were tested. Lanes 1 and 2, no antibody. USRP-A1, -A2, -A3, -B, and -C are indicated by A1, A2, A3, B, and C, respectively, at the left. An immunodepressed band in lane 5 is indicated by an arrowhead at the right.

Identification of the Binding Core Motif(s) of USRP-A1, -A2, -A3, and -B—To identify the binding core motif of Group A and USRP-B, competition analysis was performed in gel mobility shift assays using a series of mutant USRE competitor DNAs, M1–M5 (Fig. 5A). Group A and USRP-B complexes binding to the wild USRE probe were competed with M1, M3, and M5 as well as with the wild USRE but not with M2 nor M4 (Fig. 5A). From these results summarized in Fig. 5B, we suspected that a binding core motif of Group A and USRP-B was involved in the TTCCACCC sequence. To further analyze the Group A and USRP-B binding core motif in detail, we performed a competition study with another series of mutant USRE competitor DNAs, M11–M21 (Fig. 6B). Group A and USRP-B complexes binding to the wild USRE probe were competed with M11, M13, M15, M16, and M17 respectively, at the left. An immunodepressed band in lane 5 is indicated by an arrowhead at the right.

3. The USRP-C complex involves Ku antigen. For immunodepression assays, the appropriate antibody was simultaneously added into the binding reaction mixture involving Jurkat nuclear proteins with (lane 1) or without the poly(dI·dC) DNA pretreatment (lanes 2–5) and the 32P-labeled USRE DNA probe. Monoclonal antibodies to Ku/p80 antigen (lane 5) and to p40tax protein (lane 3), and negative control culture supernatant (NC, lane 4) were tested. Lanes 1 and 2, no antibody. USRP-A1, -A2, -A3, -B, and -C are indicated by A1, A2, A3, B, and C, respectively, at the left. An immunodepressed band in lane 5 is indicated by an arrowhead at the right.

4. Analysis of the proteins in each complexes by UV cross-linking. UV cross-linking assays were performed using 2 ng of the 32P-labeled USRE DNA as a probe with the nuclear extracts (4 μg). Elution samples of two gel slices, including Group A (USRP-A1, -A2, and -A3) (lanes 1 and 3) and USRP-B (lanes 2 and 4) bands, respectively, were analyzed on 10% SDS-PAGE. After electrophoresis, the gel was dried on Whatman paper and exposed on the x-ray film for short (lanes 3 and 4) or long periods (lanes 1 and 2). The molecular masses (kDa) of the markers ([3H]-labeled methylated protein mixture, high molecular mass range; Amersham Corp.) are indicated to the right.

DNAs, M11–M21 (Fig. 6C) in gel mobility shift assays. M13, M15, M16, and M17 had no effect on the USRE binding (Fig. 6A; lanes 6, 8, 9, and 10, respectively). M19 and M20 (lanes 2 and 3, respectively) were less competitive than was wild USRE. Furthermore, gel mobility shift assays were performed using the 32P-labeled DNAs from M11 to M21 as a probe (Fig. 6B). Compared with the wild USRE probe, neither M16 nor M17 was detectable the Group A and USRP-B complexes. The complexes were detectable with the other mutants, and the intensity of these bands with M11, M12, M14, M18, M19, and M20 were relatively comparable to that with the wild USRE.
The intensity of the bands with M13 and M15 became weaker. These results are summarized in Fig. 6, indicating that the CACCC sequence is the core binding motif of both of the Group A and U5RP-B. A single point mutation (A to T at the 276 nucleotide of the U5RE region) maintained the binding activity with the U5RE binding proteins (Fig. 6), suggesting that the C(A/T)CCC sequence is the consensus core binding motif.

U5RE-binding Proteins Involved in the Repression of the LTR-directed Expression—As described above, the mutant M17 had no binding ability with the U5RE-binding proteins, but the mutant M13 had weak binding ability. To test whether the U5 region with the M17 or M13 mutation exerts its repressive effect on the LTR-directed expression, three luciferase (luc) expression plasmids derived from the HTLV-I LTR promoter with the wild type, the M13, or M17 mutant type within the U5RE region were constructed as described under “Experimental Procedures” and designated pBLTR-Wt-luc, pBLTR-M13-luc, or pBLTR-M17-luc, respectively (Fig. 7A). The luciferase activities of these three reporter genes were measured and compared in at least four independent experiments. Fig. 7B shows that the activities of pBLTR-M17-luc appeared to be approximately twice those of the wild pBLTR-Wt-luc or of pBLTR-M13-luc. Therefore, a single point mutation (C to A at the 279 nucleotide of the U5RE region) diminishes not only the binding activity with the U5RE binding proteins but also the repressive effect on the LTR-directed expression. On the other hand, another single point mutation (C to G at the 275 nt) decreases the binding activity but still retains the repressive effect. Thus, we argued that the protein components in U5RP-A1, -A2, -A3, and -B play an important role in the repression of HTLV-I gene expression.
Sp1 and Sp3 Binding to HTLV-I U5 Repressive Element

FIG. 7. Involvement of USRE-binding proteins in the repression on the LTR-directed expression. A, schematic diagram of three luciferase (luc) expression plasmids, pBLTR-Wt-luc, pBLTR-M13-luc, and pBLTR-M17-luc, in which the luc gene was under the control of the U3-R-U5 (−325 to +316) region of the wild type LTR and the mutant type LTRs which are introduced as a single point mutation (C to G or A) at nucleotide +275 or +279 of the USRE region, respectively. B, significant differences in the promoter activity of pBLTR-M13-luc and pBLTR-M17-luc, compared with that of pBLTR-Wt-luc. The mixture of 1 μg of each expression plasmids and 2 μg of the internal control CAT expression plasmid pRSV-CAT was transfected into Jurkat cells, followed by culturing for 16 h, and was further cultured for 48 h in medium containing 10% fetal bovine serum. Thereafter, the supernatants of the cell lysates were recovered and assayed for CAT and luciferase activities.

FIG. 8. Involvement of Sp1 family proteins in USRP-A and -B. Gel mobility shift assays were performed with the 32P-labeled USRE DNA as a probe using the Jurkat nuclear extracts (NE) with the poly(dI-dC) DNA pretreatment. Lane 1, no nuclear extract. Five major bands are indicated by A1, A2, A3, B, and C at the left. For supershift assays, anti-Sp1 antibodies (lane 3), anti-Sp3 antibodies (lane 4), or anti-Sp1 and anti-Sp3 antibodies (lane 5) were added to the binding reaction mixture 20 min prior to the loading of the gel. Lanes 1 and 2, no antibody addition. In the presence of anti-Sp1 antibodies, USRP-A1 was supershifted. In the presence of anti-Sp3 antibodies, USRP-A2 and -B were supershifted. Supershifted bands are indicated by an arrowhead at the right.

DISCUSSION

Here, we described five different binding complexes to USRE, namely USRP-A1, -A2, -A3, -B, and -C. At first, we named Group A inclusive of USRP-A1, -A2, and -A3 because they are hardly distinguishable without supershift assays using anti-Sp1 and -Sp3 antibodies. These complexes, except for USRP-C, were found to specifically bind to USRE. The immunodepression assays revealed that only USRP-C reacted to the anti-p80Ku antibody, suggesting that the Ku antigen is involved in USRP-C. In contrast to this finding, we previously reported that Ku antigen is involved in USRP-B and its binding to USRE is specific (15). This discrepancy may be explained by the difference in these two experimental conditions; previously, we simultaneously analyzed for binding specificity of the other specific complexes to USRE involving the nonspecific complexes. Here, we conclude that nonspecific binding of Ku antigen to USRE occurs. The nonspecific binding affinity of USRP-C to USRE might be due to the possibility that Ku antigen also binds to the termini of DNA fragments. Some groups have shown that Ku antigen has binding activity at the double-stranded DNA end (29–32).

The binding core element of USRP-A1, -A2, -A3, and -B was determined by competition assays and gel shift mobility assays using a series of mutant USRE competitor DNAs (Figs. 5 and 6). The results showed that USRP-A1, -A2, -A3, and -B recognize the CACCC sequence as a core motif with identical affinities. Thereafter, a single point mutation (C to A at the 279 position of the USRE region) diminished not only the binding activity with the USRE binding proteins but also the repressive effect on the LTR-directed expression. In addition to the previous report, in which we have observed a 2-5-fold increase in basal promoter activity when the USRE domain was deleted (15), this evidence strongly suggested that the protein components in USRP-A1, -A2, -A3, and -B play an important role in the repression of HTLV-I gene expression.

The CACCC sequence is well known as the motif bound to some transcription factors such as Sp1 and Kruppel type zinc-finger proteins (28, 33). Group A and USRP-B were then subjected to supershift assays with anti-Sp1 and anti-Sp3 antibod-
ies. The results indicated that USRP-A1, corresponding to the slowest band, was found to include Sp1 or Sp1-related protein. A molecular mass of Sp1 is known to be 100 – 110 kDa (24), which is consistent with that of the slowest band in the UV cross-linking assay as described above, and a 110-kDa protein was previously identified in the USRE-binding complexes as described (15). Cloning and functional analysis of three other major proteins of about 64, 72, and 76 kDa in Group A will be required to establish their role. Moreover, USRP-A2 and -B were retarded by gel shift assays using anti-Sp3 antibodies, indicating the possible involvement of Sp3 or Sp3-related protein in both of the USRP-A2 and -B complexes. A recent report (24) described that the anti-Sp3 antiserum, which we also used in this study, specifically recognized 97-, 60-, and 58-kDa proteins in a nuclear extract from HeLa cells. The 95-kDa protein shown in our UV cross-linking assay seems to correspond to the largest 97-kDa protein in that immunoblot, suggesting that it is Sp3. The intensity of the band of the 52-kDa protein observed to be the strongest. In supershift assays, anti-Sp3 antibodies completely supershifted the USRP-B complex band. These findings suggest that this 52-kDa protein associates with Sp3 binding through the USRE DNA. Further characterization of this 52-kDa protein remains to be determined. The 95-kDa protein was also contained in Group A, and anti-Sp3 antibodies supershifted the USRP-A2 complex band, suggesting that Sp3 is in USRP-A2.

As in our results, a similar pattern of DNA specificity was observed using both the GC box (GGGGCGGC) and the GT motif (GAGTGTGGC) as a probe (24, 34). We additionally found the third complex, namely USRP-A3, specifically binding to the CACCC motif, but these antibodies did not affect the binding of USRP-A3. Thus, it is remained to be determined what proteins were involved in USRP-A3. We speculate the possible involvement of the other CACCC binding protein family, such as a Sp1 family, Sp2 or Sp4. It is unlikely, however, that Sp4 is involved, because Sp4 is known to be a brain-specific protein (27, 28, 33).

Our demonstration that Sp1 family proteins are involved in USRE-binding will aid in examining the mechanism of the LTR US-mediated repression. Originally identified as a cellular transcription factor required for SV40 gene expression, Sp1 plays a role in the down-regulation of HTLV-I gene expression (47). The Sp1 consensus binding sequence, CACCC, can confer equal responsiveness to RB. The retinoblastoma protein is directly or indirectly involved in Sp1 binding. These findings provide evidence for a functional link between retinoblastoma and Sp1. Based on these ideas, there is a possibility that association of Sp1 with some cofactors might result in the US-mediated repression. Furthermore, an unidentified binding protein(s) to USRE, which is involved in the USRP-A3 complex, might be involved in the repression.

Taken together, we propose that the USRE plays an important role in the down-regulation of HTLV-I gene expression. Moreover, in viral latency, some contribution that HTLV-I gene expression is down-regulated at the transcriptional levels by the USRE-binding proteins, such as Sp1, Sp3, and others, might be suggested.

Acknowledgments—We thank Dr. Gustav Hagen for kindly providing anti-Sp3 antibodies and Dr. Yutetsu Tanaka for kindly providing antitax immunological antibodies. We are also thank Drs. Yorio Hinuma, Masakazu Hatanaka, and Osamu Yoshiie for helpful discussions.

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Sp1 Family Proteins Recognize the U5 Repressive Element of the Long Terminal Repeat of Human T Cell Leukemia Virus Type I through Binding to the CACCC Core Motif

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J. Biol. Chem. 1996, 271:12944-12950.
doi: 10.1074/jbc.271.22.12944

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