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SFA-2, a Novel bZIP Transcription Factor Induced by Human T-Cell Leukemia Virus Type I, Is Highly Expressed in Mature Lymphocytes

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A novel cellular gene, SFA-2, was isolated by differential hybridization of a cDNA library, using probes obtained from an adult T-cell leukemia cell line in comparison with normal $CD4^+$ T cells and MOLT-4 cell line. The mRNA of the SFA-2 gene is approximately 0.9-kb in size and encodes a protein of 125 amino acids, containing a basic region-leucine zipper DNA-binding domain. The N-terminal region of SFA-2 is rich in serine and contains a consensus sequence for casein kinase II phosphorylation. The SFA-2 gene was strongly expressed in mature T and B lymphocytes, and was up-regulated after transformation by human T-cell leukemia virus type I. The SFA-2 did not homodimerize efficiently but formed heterodimer preferentially with c-Jun. The SFA-2/c-Jun heterodimer bound preferentially to the AP-1 and CRE sites.

Human T-cell leukemia virus type I (HTLV-I) is an exogenous human retrovirus closely linked with adult T-cell leukemia (ATL). HTLV-I has also been reported to be associated with myelopathy, alveolitis, arthropathy and uveitis which may result from immunologic alterations induced by HTLV-I infection (1–4). The HTLV-I genome encodes a 40-kDa protein, Tax, that functions as a transcriptional transactivator of the viral long terminal repeat and cellular genes. T cell proliferation and immunologic alterations observed during HTLV-I infection appear to be due to the effect of Tax on viral and cellular gene expression. Tax stimulates the expression of various cellular genes, including those for IL-2, IL-2 receptor α, IL-3, IL-4, GM-CSF, tumor necrosis factor β, TGF β, PTHrP, c-*fos*, c-*jun* and vimentin (5–12). However, the mechanism by which HTLV-I induces disease remains to be elucidated.

To examine the changes in $CD4^+$ T cells after HTLV-I transformation, we have performed differential hybridization of a cDNA library, using probes obtained from an ATL cell line in comparison with normal $CD4^+$ T cells and the MOLT-4 cell line. By differential screening of this library, a new bZIP transcription factor, SFA-2 (*SF*-HT-*a*ctivated gene-*2*), was isolated. In the present report, we describe the cloning and characterization of SFA-2, which was strongly expressed in mature lymphocytes and up-regulated by transformation with HTLV-I.

MATERIALS AND METHODS

Cells. Three human T lymphoblastic cell lines, MOLT-4, Jurkat and CEM, a human erythroleukemia cell line, K562, two human myelomonocytoid cell lines, HL-60 and U937, and three human carcinoma cell lines, PANC-1 (pancreas), SW1116 (colon adenocarcinoma) and A549 (lung), were obtained from the American Type Culture Collection (Rockville, MD). Two human pre B lymphoblastic cell lines, P30/OHK and NALM-6, and three human carcinoma cell lines, A172 (glioblastoma), Caki-1 (renal carcinoma) and Hep G2 (hepatocellular carcinoma), were obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan). An eosinophilic leukemia cell line, EoL-1, was obtained from Hayashibara Biochemical Laboratories (Okayama, Japan). JPX-9 cells (13) were kindly provided by Prof. K. Sugamura, Tohoku University, Sendai, Japan, HH-EB was a Epstein-Barr virus (EBV)-transformed B lymphoblastoid cell line. HTLV-I-transformed T-cell lines, MT-2 and MT-4, were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS; GIBCO, Grand

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Abbreviations: bZIP, basic region-leucine zipper; IL-2, interleukin-2; GM-CSF, granulocyte macrophage colony-stimulating factor; PTHrP, parathyroid hormone-related protein; PHA, phytohemagglutinin; TGF β, transforming growth factor β; MIP-1α, macrophage inflammatory protein-1α; CRE, cAMP response element.

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Island, NY). SF-HT, a leukemia cell line established from ATL patient, was maintained in RPMI 1640 medium with 10% FCS and 100 U/ml human recombinant IL-2 (Takeda Chemical Industries, Osaka, Japan).

Preparation of CD4$^+$ T cells and monocytes. Peripheral blood mononuclear cells were isolated from healthy individuals by Ficoll-Conray gradient centrifugation. Normal T and B cells were separated using the sheep erythrocyte-rosetting method. Human monocytes were prepared by using Nycodenz Monocyte (NYCOMED AS, Oslo, Norway) density centrifugation as described previously (14). CD4$^+$ T cells were enriched by treating with OKT8 (CD8) (Ortho Diagnostic Systems, Raritan, NJ), Leu11b (CD16) (Becton Dickinson, Mountain View, CA) and low-toxic rabbit C (Cedarlane, Hornby, Ontario, Canada), as described previously (15). The CD4$^+$ T cells, which had been cultured in RPMI 1640 medium with 10% FCS for 4 days after removal of PHA (GIBCO) and IL-2, were used for the experiment.

RNA isolation and Northern blot analysis. The total cellular RNA was extracted from the cells using the acid phenol-guanidinium isothiocyanate extraction method and Northern blot analysis was performed as described previously (16,17). The DNA probes used were a 0.9-kb SFA-2 cDNA and a 0.4-kb human β-actin cDNA (Wako Pure Chemical Industries, Osaka, Japan).

cDNA library and differential screening. Poly(A)$^+$ RNA from SF-HT cells was selected by oligo(dT) cellulose chromatography and then used to construct a cDNA library in the pcD-SRα expression vector system (18). Approximately 5×10^4 clones were screened by colony hybridization. For the subtractive probe preparation, single-stranded[^{32}P]-labeled cDNA probes were prepared from 10 μg of poly(A)$^+$ RNA isolated from SF-HT cells, the CD4$^+$ T cells or MOLT-4 cells with random DNA hexamers (Takara Shuzo, Kyoto, Japan), using the AMV reverse transcriptase system (BRL Life Technologies Inc., Gaithersburg, MD). Colonies that hybridized strongly with the probe from SF-HT cells were isolated for further analysis.

Protein–protein binding. The glutathione S-transferase (GST)-SFA-2 fusion protein was prepared using the GST gene fusion system (Pharmacia Biotech, Uppsala, Sweden). The sequence corresponding to amino acids 1-125 of SFA-2 was amplified with PCR primers. The PCR product was cloned into the *Sma*I site of the pGEX-4T vector (Pharmacia) and the resulting clones verified by direct sequencing. *E. coli* BL21 was transformed and the fusion protein was purified according to the manufacturer's instruction. The bZIP proteins were synthesized in rabbit reticulocyte lysates programmed with *in vitro*-transcribed mRNA in the presence of [^{35}S]-methionine according to the manufacturer's instruction (Stratagene, La Jolla, CA). Reticulocyte lysates containing bZIP proteins were mixed with GST-SFA-2 fusion protein, and the complexes were precipitated with glutathione-agarose beads as described (19). The precipitates were analyzed on a 12% polyacrylamide gel.

Gel mobility shift assays. The following double-stranded oligonucleotides were synthesized and used for gel mobility shift assays: AP-1 site, 5′-CCTAGTGATGACTCAGCCGGATCC-3′ and CRE site, 5′-CGATTGGCTGACGTCAGAGAGCTC-3′. After GST-SFA-2 fusion protein was dimerized with reticulocyte lysates containing c-Jun, gel mobility shift assays and competition analysis were performed using [^{32}P]-labelled or unlabelled oligonucleotides as described (19).

Nucleotide sequence accession number. The nucleotide sequence data of SFA-2 appears in the GSDB, DDBJ, EMBL and NCBI nucleotide sequence databases with the accession number D42106.

RESULTS AND DISCUSSION

The cDNA clones of RNA from an ATL cell line, SF-HT, were differentially screened and found to show greater hybridization with SF-HT than with normal CD4$^+$ T cell and MOLT-4 cDNA probes. Thirty-two cDNA clones differentially hybridized to the SF-HT cDNA probe on the first screening of about 5×10^4 clones. Rescreening and additional Northern blot analysis yielded eight HTLV-I-induced cellular genes. Searches of the GenBank and EMBL databases using the sequences of these eight clones identified MIP-1α, TGF β, c-*jun*, vimentin and 4 unknown genes. Of these, one clone, designated SFA-2, was analyzed further.

The expression of the SFA-2 gene in various hematopoietic and non-hematopoietic cell lines was evaluated by hybridizing SFA-2 or β-actin probes with blots of lymphocyte or cell line RNAs (Fig. 1A, 1B and 1C). The mRNA of the SFA-2 gene was found to be approximately 0.9-kb in size. Expression of SFA-2 was increased more than 3-fold in the ATL cell line, SF-HT, and in two HTLV-I-transformed T cell lines (MT-2 and MT-4), compared with the level seen in CD4$^+$ T cells. SFA-2 RNA was either absent or present at very low levels in all hematopoietic cells except lymphoid cells (monocytes, U937, HL-60, EoL-1 and K562) and in various non-hematopoietic cell lines: A172, Caki-1, PANC-1, Hep G2, SW1116 and A549. As shown in Fig. 1C, the SFA-2 gene was significantly expressed in mature lymphoid cells, HTLV-I- or EBV-transformed cell lines, T lymphocytes and B lymphocytes, while none of the immature T- or pre B-lymphoblastic cell lines (MOLT-4, Jurkat, CEM, P30/OHK and NALM-6) expressed detectable levels of SFA-2 mRNA.

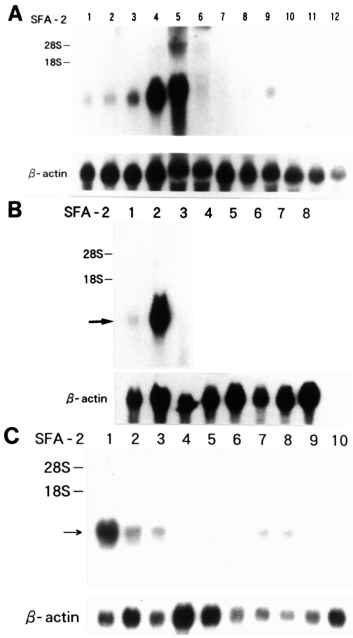

FIG. 1. A. Expression of the SFA-2 gene in hematopoietic cells. Total RNA (10 μg) was size-fractionated on formaldehyde agarose gels, transferred to charged nylon membranes, and hybridized with SFA-2 or β-actin cDNA probes. RNA samples are as follow: lane 1, normal T cells; lane 2, normal $CD4^+$ T cells; lane 3, SF-HT; lane 4, MT-2; lane 5, MT-4; lane 6, MOLT-4; lane 7, Monocytes; lane 8, U937; lane 9, normal B cells; lane 10, EoL-1, lane 11, HL-60; lane 12, K562. B. Expression of the SFA-2 gene in non-hematopoietic cell lines. Lane 1, normal T cells; lane 2, MT-2; lane 3, A172 (glioblastoma); lane 4, Caki-1 (renal carcinoma); lane 5, PANC-1 (pancreatic carcinoma); lane 6, Hep G2 (hepatocellular carcinoma); lane 7, SW1116 (colon adenocarcinoma); lane 8, A549 (lung cancer). C. Expression of the SFA-2 gene in lymphoid cell lines. Lane 1, SF-HT; lane 2, normal T cells; lane 3, normal $CD4^+$ T cells; lane 4, MOLT-4; lane 5, Jurkat; lane 6, CEM; lane 7, normal B cells; lane 8, HH-EB; lane 9, P30/OHK; lane 10, NALM-6.

These findings showed that the SFA-2 gene was specifically expressed in mature lymphocytes. To investigate whether the SFA-2 gene is transactivated by Tax, we examined the kinetics of SFA-2 gene expression in JPX-9 cells, a stable transformant of Jurkat with the plasmid pMAXRHneo-1 containing the metallothionein promoter-driven Tax gene. Tax is undetectable in JPX-9 cell mRNA without stimulation, but can be induced by addition of $CdCl_2$ to the culture medium. As shown in Fig. 2, induction of the SFA-2 gene was observed after expression of Tax. In contrast, SFA-2

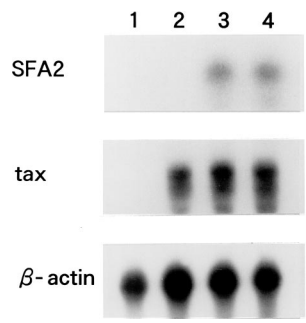

FIG. 2. Kinetics of SFA-2 gene expression by Tax in JPX-9 cells. Northern blot analysis was performed with SFA-2, tax or β-actin probes. The JPX-9 cells were cultured in RPMI 1640 medium supplemented with 10% FCS and 10 μM $CdCl_2$ and harvested at 0, 12, 24, and 48 h after the addition of $CdCl_2$ (lane 1, 2, 3 and 4, respectively).

expression was not induced by the addition of $CdCl_2$ to parental Jurkat cells (data not shown). This indicated that SFA-2 was transactivated by Tax.

The complete nucleotide and deduced amino acid sequences of the SFA-2 gene are shown in Fig. 3. The SFA-2 gene shown in Fig. 3 is nearly full-length, since it is close to the expected size. The 3′ untranslated region contains a potential polyadenylation signal, AATAAA. Translation probably starts from the ATG sequence at nucleotides 160 to 162, since an in-frame stop codon, TAG, occurs at nucleotides 49 to 51. The SFA-2 gene has a 375-nucleotide open reading frame and encodes a protein consisting of 125 amino acids, with a calculated molecular weight of 13,700. SFA-2 has a linked basic DNA-binding domain and a leucine zipper structure (20). The N-terminal region (residues 1 to 26) is rich in serine and contains a consensus sequence for casein kinase II phosphorylation, SDSSDS, which may play a role in the response to transduced signals or transcriptional activation. A number of bZIP proteins have been cloned and have been divided into the following families: AP-1 (Jun/Fos), CREB/ATF, C/EBP and Maf (21). Comparing the bZIP do-

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   1  GGA CGC AGG GGT CAG AGG TGG CTA CAG GGC AGG CAG AGG AGG CAC CTG      48
      *
  49  TAG GGG GTG GTG GGC TGG TGG CCC AGG AGA AGT CAG GAA GGG AGC CCA      96

  97  GCT GGT GAC AAG AGA GCC CAG AGG TGC CTG GGG CTG AGT GTG AGA GCC     144

   1                      M   P   H   S   S   D   S   S   D   S   S      11
 145  CGG AAG ATT TCA GCC ATG CCT CAC AGC TCC GAC AGC AGT GAC TCC AGC     192

  12   F   S   R   S   P   P   P   G   K   Q   D   S   S   D   D   V      27
 193  TTC AGC CGC TCT CCT CCC CCT GGC AAA CAG GAC TCA TCT GAT GAT GTG     240

  28   R   R   V   Q   R   R   E   K   N   R   I   A   A   Q   K   S      43
 241  AGA AGA GTT CAG AGG AGG GAG AAA AAT CGT ATT GCC GCC CAG AAG AGC     288

  44   R   Q   R   Q   T   Q   K   A   D   T   L   H   L   E   S   E      59
 289  CGA CAG AGG CAG ACA CAG AAG GCC GAC ACC CTG CAC CTG GAG AGC GAA     336

  60   D   L   E   K   Q   N   A   A   L   R   K   E   I   K   Q   L      75
 337  GAC CTG GAG AAA CAG AAC GCG GCT CTA CGC AAG GAG ATC AAG CAG CTC     384

  76   T   E   E   L   K   Y   F   T   S   V   L   N   S   H   E   P      91
 385  ACA GAG GAA CTG AAG TAC TTC ACG TCG GTG CTG AAC AGC CAC GAG CCC     432

  92   L   C   S   V   L   A   A   S   T   P   S   P   P   E   V   V     107
 433  CTG TGC TCG GTG CTG GCC GCC AGC ACG CCC TCG CCC CCC GAG GTG GTG     480

 108   Y   S   A   H   A   F   H   Q   P   H   V   S   S   P   R   F     123
 481  TAC AGC GCC CAC GCA TTC CAC CAA CCT CAT GTC AGC TCC CCG CGC TTC     528

 124   Q   P   *                                                       125
 529  CAG CCC TGA GCT TCC GAT GCG GGG AGA GCA GAG CCT CGG GAG GGG CAC     576

 577  ACA GAC TGT GGC AGA GCT GCG CCC ATC CCG CAG AGG CCC CTG TCC ACC     624

 625  TGG AGA CCC GGA GAC AGA GGC CTG GAC AAG GAG TGA ACA CGG GAA CTG     672

 673  TCA CGA CTG GAA GGG CGT GAG GCC TCC CAG CAG TGC CGC AGC GTT TCG     720

 721  AGG GGC GTG TGC TGG ACC CCA CCA CTG TGG GTT GCA GGC CCA ATG CAG     768

 769  AAG AGT ATT AAG AAA GAT GCT CAA GTC CCA TGG CAC AGA GCA AGG CGG     816

 817  GCA GGG AAC GGT TAT TTT TCT AAA TAA ATG CTT AAA AAA AAA AAA AAA     864

 865  AAA A                                                           868
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FIG. 3. Nucleotide and deduced amino acid sequences of the SFA-2 cDNA clone. The predicted amino acid sequence is shown above the nucleotide sequence and the amino acid numbers start at the initiation methionine. The basic DNA-binding domain is overlined. The leucine residues in the leucine zipper domain are circled. In-frame stop codons are indicated by asterisks. A potential polyadenylation signal, AATAAA, is boxed. The sequence appears in the GSDB/DDBJ/EMBL/NCBI nucleotide sequence databases with the accession number D42106.

mains (residues 28 to 82) of these members, SFA-2 appears to be most closely related to ATF-3 (23 of 55 residues), while the next closest relation is Fra-1 of the AP-1 family (20 of 55 residues), and the C/EBP and Maf families appear more distantly related (data not shown). No sequence similarity was detected between SFA-2 and any member of the bZIP family outside the bZIP domain.

Homo- or heterodimerization occur between some pairs of bZIP factors (21). We examined the ability of binding of SFA-2 to the five representative bZIP proteins, ATF-3, CREB, c-Fos, c-Jun and NF-IL6. As shown in Fig. 4, the SFA-2 did not homodimerize efficiently. However, the SFA-2 formed heterodimerization preferentially with c-Jun but not with the other 4 bZIP proteins, ATF-3, CREB, c-Fos and NF-IL6. We next analyzed the DNA binding activities of the SFA-2 complexes by gel mobility shift assays using the oligonucleotides containing the AP-1 (TGACTCA) or CRE (TGACGTCA) sites (Fig. 5). The SFA-2/c-Jun heterodimer bound strongly to the AP-1 and CRE sites, while the bindings of SFA-2 alone or c-Jun homodimer were undetectable or much weaker, respectively. The AP-1 site is remarkably similar to the CRE site, and the AP-1 family is closely related to the CREB/ATF family (19). Fos and Jun proteins can also associate with the CRE site, and Jun/ATF heterodimers can bind with either the AP-1 or CRE sites (22, 23).

Tax activates transcription of viral and cellular genes through distinct enhancers - namely, the 21-bp enhancer of HTLV-I (24), the CArG box of *c-fos* (7), and NF-κB-binding site of IL-2, IL-2 receptor α, and vimentin genes (11, 25, 26). Tax does not bind directly to these DNA elements. However, Tax has been reported to bind to enhancer-binding proteins and thus binds to enhancer DNA indirectly; these DNA-binding proteins are CREB and CREM for the 21-bp enhancer (27,28), serum response factor for the CArG box (29, 30), and NF-κB1 p50 for the NF-κB-binding site (30). Tax binding to these enhancer-binding proteins plays a critical role in transcriptional activation (28,30). In addition to the binding to these DNA-binding proteins in the nucleus, Tax has been also shown to bind to NF-κB1 p105, a precursor of NF-κB1 p50, IκB-α and -γ in the cytoplasm, resulting in nuclear translocation of Rel/NF-κB proteins (31,32,33), whereas the binding of Tax to NF-κB2 p100 did not induce efficient dissociation of the cytoplasmic complexes p100/c-Rel or p100/RelA, and thus did not induce nuclear translocation of c-Rel or RelA (34,35). These findings suggest that Tax can directly or indirectly alter the activity of a variety of different transcription factors to increase the level of gene expression. In addition, Tax can also stimulate the DNA binding properties of a variety of bZIP proteins at their cognate binding sites (36,37). Therefore, SFA-2/c-Jun heterodimer may modulate the expression of some Tax-activating cellular genes,

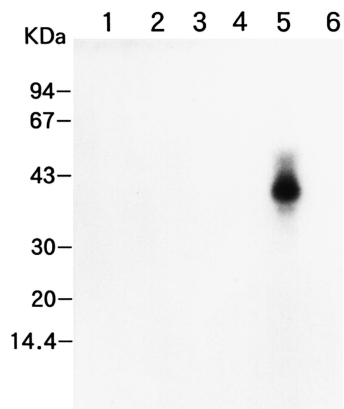

FIG. 4. Binding of SFA-2 to the bZIP proteins. The six [^{35}S]-labelled bZIP proteins, SFA-2 (lane 1), ATF-3 (lane 2), CREB (lane 3), c-Fos (lane 4), c-Jun (lane 5) and NF-IL6 (lane 6), were translated *in vitro* with rabbit reticulocyte lysate and bound to GST-SFA-2 fusion protein. After precipitation with glutathione-agarose beads, the products were electrophoresed.

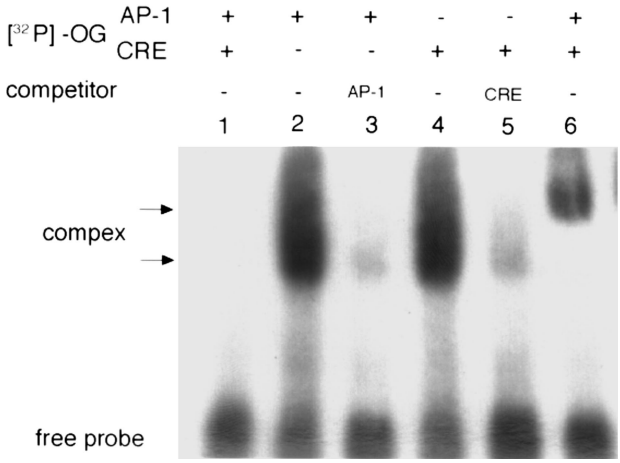

FIG. 5. Gel mobility shift analysis of SFA-2 alone, SFA-2/c-Jun heterodimer and c-Jun homodimer with the oligonucleotides containing the AP-1 or CRE sites. GST-SFA-2 alone (lane 1), the mixture of GST-SFA-2 and reticulocyte lysate containing c-Jun (lane 2–5) and *in vitro* translated c-Jun homodimer (lane 6) were assayed for the ability to bind to the [^{32}P]-labelled oligonucleotides in the absence (lane 1, 2, 4 and 6) and the presence (lane 3 and 5) of the unlabelled competitors. The bindings of c-Jun homodimer or SFA-2/c-Jun heterodimer to the oligonucleotides are shown by upper or lower arrows, respectively.

since SFA-2 and *c-jun* are also transactivated by Tax. A high level of C/EBPα expression has been reported to occur specifically in highly differentiated cells such as hepatocytes and adipocytes (38). The differentiation of mouse preadipocytes into adipocytes is characterized by the induction of C/EBPα expression (38). C/EBPα regulates several genes expressed in differentiated cells, including the serum albumin (39), stearoyl coenzyme A desaturase (40), α_1-antitrypsin (41), and insulin-like growth factor II genes (42). SFA-2 may also play a role in differentiation or the maintenance of the mature state in lymphocytes. Further studies on the interactions between SFA-2 and other cellular genes are now in progress using SFA-2-expressing transformants.

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REFERENCES

1. Gessain, A., Barin, F., Vernant, J. C., Gout, O., Maurs, L., Calender, A., and De-The, G. (1985) *Lancet* **ii,** 407–409.
2. Osame, M., Usuku, K., Izumo, S., Ijichi, N., Amatani, H., Igata, A., Matsumoto, M., and Tara, M. (1986) *Lancet* **i,** 1031–1032.
3. Nishioka, N., Maruyama, I., Sato, K., Kitajima, I., Nakajima, Y., and Osame, M. (1989) *Lancet* **i,** 441.
4. Mochizuki, M., Yamaguchi, K., Takatsuki, K., Watanabe, T., Mori, S., and Tajima, K. (1992) *Lancet* **339,** 1110.
5. Inoue, J. K., Seiki, M., Taniguchi, T., Tsuru, S., and Yoshida, M. (1986) *EMBO J.* **5,** 2883–2888.
6. Siekevitz, M., Feinberg, M. B., Holbrook, N., Wong-Staal, F., and Greene, W. C. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5389–5393.
7. Fujii, M., Sassone-Corsi, P., and Verma, I. M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8526–8530.
8. Miyatake, S., Seiki, M., Yoshida, M., and Arai, K. (1988) *Mol. Cell. Biol.* **8,** 5581–5587.
9. Tschachler, E., Robert-Guroff, M., Gallo, R. C., and Reitz, M. S. (1989) *Blood* **73,** 194–201.
10. Kim, S. J., Kehrl, J. H., Burton, J., Tendler, C. L., Jeang, K. T., Danielpour, D., Thevenin, C., Kim, K. Y., Sporn, M. B., and Roberts, A. B. (1990) *J. Exp. Med.* **172,** 121–129.
11. Lilienbaum, A., Duc Dodon, M., Alexandre, C., Gazzolo, L., and Paulin, D. (1990) *J. Virol.* **64,** 256–263.

12. Watanabe, T., Yamaguchi, K., Takatsu, K., Osame, M., and Yoshida, M. (1990) *J. Exp. Med.* **172,** 759–765.
13. Miura, S., Ohtani, K., Numata, N., Niki, M., Ohbo, K., Ina, Y., Gojobori, T., Tanaka, Y., Tozawa, H., Nakamura, M., and Sugamura, K. (1991) *Mol. Cell. Biol.* **11,** 1313–1325.
14. Hasegawa, H., Satake, Y., and Kobayashi, Y. (1990) *Microbiol. Immunol.* **34,** 459–466.
15. Hasegawa, H., Utsunomiya, Y., Yasukawa, M., Yanagisawa, K., and Fujita, S. (1994) *J. Virol.* **68,** 5326–5329.
16. Hasegawa, H., Sakai, A., and Sugino, A. (1989) *Yeast* **5,** 509–524.
17. Hasegawa, H., Yoshida, M., Shiosaka, T., Fujita, S., and Kobayashi, Y. (1992) *Virology* **191,** 158–165.
18. Takebe, Y., Seiki, M., Fujisawa, J., Hoy, P., Yokota, K., Arai, K., Yoshida, M., and Arai, N. (1988) *Mol. Cell. Biol.* **8,** 466–472.
19. Hai, T., and Curran, T. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 3720–3724.
20. Landschulz, W. H., Johnson, P. F., and McKnight, S. L. (1988) *Science* **240,** 1759–1764.
21. Hurst, H. C. (1995) *Protein Profile* **2,** 101–168.
22. Hurst, H. C., Masson, N., Jones, N. C., and Lee, K. A. W. (1990) *Mol. Cell. Biol.* **10,** 6192–6203.
23. Ryseck, R.-P., and Bravo, R. (1991) *Oncogene* **6,** 533–542.
24. Fujisawa, J., Seiki, M., Sato, M., and Yoshida, M. (1986) *EMBO J.* **5,** 713–718.
25. Cross, S. L., Feinberg, M. B., Wolf, J. B., Wong-Staal, F., and Leonard, W. J. (1987) *Cell* **49,** 47–56.
26. Leung, K., and Nabel, G. J. (1988) *Nature* **333,** 776–778.
27. Zhao, L., and Giam, C. Z. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 7070–7074.
28. Suzuki, T., Fujisawa, J., Toita, M., and Yoshida, M. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 610–614.
29. Fujii, M., Tsuchiya, H., Chujoh, T., Akizawa, T., and Seiki, M. (1992) *Genes Dev.* **6,** 2066–2076.
30. Suzuki, T., Hirai, H., Fujisawa, J., Fujita, T., and Yoshida, M. (1993) *Oncogene* **8,** 2391–2397.
31. Watanabe, M., Muramatsu, M., Hirai, H., Suzuki, T., Fujisawa, J., Yoshida, M., Arai, K., and Arai, N. (1993) *Oncogene* **8,** 2949–2958.
32. Hirai, H., Suzuki, T., Fujisawa, J., Inoue, J., and Yoshida, M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 3584–3588.
33. Suzuki, T., Hirai, H., Murakami, T., and Yoshida, M. (1995) *Oncogene* **10,** 1199–1207.
34. Beraud, C., Sun, S., Ganchi, P., Ballard, D. W., and Greene, W. C. (1994) *Mol. Cell. Biol.* **14,** 1374–1382.
35. Murakami, T., Hirai, H., Suzuki, T., Fujisawa, J., and Yoshida, M. (1995) *Virology* **206,** 1066–1074.
36. Wagner, S., and Green, M. R. (1993) *Science* **262,** 395–399.
37. Baranger, A. M., Palmer, C. R., Hamm, M. K., Giebler, H. A., Brauweiler, A., Nyborg, J. K., and Schepartz, A. (1995) *Nature* **376,** 606–608.
38. Umek, R. M., Friedman, A. D., and McKnight, S. L. (1991) *Science* **251,** 288–292.
39. Friedman, A. D., Landschulz, W. H., and McKnight, S. L. (1989) *Genes Dev.* **3,** 1314–1322.
40. Christy, R. J., Yang, V. W., Ntambi, J. M., Geiman, D. E., Landschulz, W. H., Friedman, A. D., Nakabeppu, Y., Kelly, T. J., and Lane, M. D. (1989) *Genes Dev.* **3,** 1323–1335.
41. Costa, R. H., Grayson, D. R., Xanthopoulos, K. G., and Darnell, J. E. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3840–3844.
42. van Dijk, M. A., Rondenburg, J. T., Holtzhuizen, P., and Sussenbach, J. S. (1992) *Nucleic Acids Res.* **20,** 3099–3104.