Human T-cell Leukemia Virus Type I Tax Masks c-Myc Function through a cAMP-dependent Pathway*

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Human T-cell leukemia virus type I (HTLV-I) is the etiologic agent for adult T-cell leukemia (ATL) (1–3). HTLV-I encodes a 40-kDa phosphoprotein, Tax, which is essential for viral transcription (4–7). Tax has been proposed to be involved in molecular events leading to ATL (reviewed in Refs. 8–11). Numerous cellular findings, including the demonstration that Tax expression leads to immortalization of T-lymphocytes (12, 13) and transformation of rat fibroblasts (14, 15) ex vivo, are consistent with this proposition. In animals, transgenic mice that express Tax have constitutively activated T-cells (16), and targeted expression of Tax to T-lymphocytes results in development of large granular lymphocytic leukemias (17). How Tax effects these cellular changes is not well understood. It is, however, well demonstrated that this viral activator modulates a variety of cellular processes through signals transduced separately by CREB/ATF and NF-κB (reviewed in Ref. 18).

Transcription directed from the HTLV-I long terminal repeat and many cellular promoters (e.g. interleukin-2, interleukin-2Rα, transforming growth factor-β1, c-Fos, c-Jun, granulocyte-macrophage colony-stimulating factor, and epidermal growth factor receptor-1 among others (19–21)) is potently up-regulated by Tax. Three imperfectly repeated 21-base pair motifs, each containing a core 8-base pair cAMP-responsive element (22, 23), have been characterized as the cis-responsive long terminal repeat target for Tax regulation (24–28). Tax activates the viral long terminal repeat (and cellular promoters) by interacting with CREB/ATF (29, 30) bound at promoter-proximal cAMP-responsive elements (31–40). Tax also activates other promoters through alternative means that are less understood (41–44). Additionally, another function of Tax is reflected in its ability to induce the translocation of NF-κB from the cytoplasm into the nucleus (45–49), thereby modulating expression of a further class of genes including that encoding for interleukin-2Rα (50–54).

Recent evidence indicates that signaling pathways, previously thought to be discrete, are often intertwined (55–57). Homeostatically, it makes sense that each activating event in cells should be countered by a moderating reaction(s), although in the literature, descriptions of the former far exceed those of the latter. Thus, depending on context, activators should also frequently serve as repressors (reviewed in Ref. 58). In HTLV-I, many aspects of disease pathogenesis suggest the existence of tight checks on viral activation function. Most notable is the fact that <5% of all infected individuals ultimately develop ATL and usually not before a latency period greater than 20–30 years (59, 60). Hence, in vivo, disease development is slow and protracted. This contrasts sharply with the observed rapid and dramatic effects exerted by ectopic expression of HTLV-I proteins on cultured cells (discussed in Ref. 61). Such juxtaposition of findings suggests the existence of yet characterized biological controls on viral activation.

c-Myc is a 64-kDa nuclear phosphoprotein that regulates cell proliferation and differentiation (reviewed in Ref. 62). When dimerized with its heterologous partner Max, Myc is a sequence-specific DNA-binding protein (63–65) that has pleiotropic transcriptional activity (65, 66). Deregulated expression of c-Myc is linked to the development of many human cancers (reviewed in Ref. 62) and, in some context, leads to apoptosis (67, 68). Understandably, tight control of Myc expression, which has been described at transcriptional and post-transcriptional levels (reviewed in Ref. 62), is essential to normal cellular metabolism.

In searching for cellular targets of HTLV-I Tax, we unexpectedly observed a novel interaction with Myc. When cells were transfected to express Tax, the nuclear detection by a monoclonal antibody directed to an N-terminal epitope of Myc was masked. This effect did not affect steady-state stability/amount...
of Myc since detection of protein using other antibodies was unchanged. While Tax has been shown previously to cooperate with oncogenes (e.g. ras) (69) in transformation, its ability to antagonize also a second oncprotein through presumptive regulation at the level of local protein conformation is intriguing. We discuss the possible biological implications of this finding.

MATERIALS AND METHODS

Plasmid Constructs—Tax-expressing constructs have been described previously (19, 70). Wild-type Myc and Myc deletion constructs are described elsewhere (71). Myc\textsuperscript{D45–175} was constructed by digestion of wild-type Myc cDNA with Sst\textsubscript{I} and religation to form the described in-frame deletion. This fragment was transferred to a glutathione S-transferase fusion vector for subsequent expression. Myc\textsuperscript{45–130} was constructed by polymerase chain reaction amplification of the appropriate fragment and subsequent placement into the same glutathione S-transferase fusion vector.

Cell Culture and Transfection—HeLa and Rat1a cells and Rat1a derivatives were grown in Dulbecco's modified Eagle's medium containing 2\,mM L-glutamine, 10\% fetal calf serum, and 100 units/ml penicillin/streptomycin. The S49.1 derivative (B1R) was grown in RPMI 1640 medium containing 2\,mM L-glutamine, 10\% horse serum, and 100 units/ml penicillin/streptomycin. Jurkat cells were propagated in RPMI 1640 medium containing 2\,mM L-glutamine, 10\% fetal calf serum, and 100 units/ml penicillin/streptomycin. For immunofluorescence, HeLa cells were seeded at 1 \times 10^5 cells/well onto coverslips in 100-mm\textsuperscript{2} culture dishes. Calcium phosphate-mediated transfections were performed 24 h later as described previously (72). Cells were washed twice and then fed with 5 ml of medium 16 h after introduction of calcium phosphate precipitates. Cells were fixed 24 h later for microscopy.

Transfection of suspension cells was achieved using Lipofectin (Life Technologies, Inc.) according to manufacturer's suggestions. Following transfection, the cells were attached to polylysine-coated glass coverslips and processed for microscopy as described for adherent cells.

Chloramphenicol Acetyltransferase Assays—HeLa cells were seeded at 5 \times 10^5 cells/well onto 6-well tissue culture plates. Calcium phosphate-mediated transfections were performed as described above. Extracts were made by freeze-thawing and were assayed for chlorampheni-
icol acetyltransferase activity (73). Enzymatic activity was quantitated on a Fujii phosphoimager after resolution of acetylated forms by thin-layer chromatography. All assays were performed within the linear range of enzymatic activity.

Immunoblot Analysis—Whole cell extracts were made by dissolving cell pellets in 23 Laemmli buffer (74) and boiling for 10 min. Extracts were clarified by centrifugation and then resolved on a 10% SDS-polyacrylamide gel. Following separation, the proteins were transferred to polyvinylidene difluoride membrane. Nonspecific sites were blocked by preincubation in BLOTTO (75). Primary antibody was reacted with the filters overnight at 4°C in BLOTTO. The filters were then washed in 1% casein in PBS and incubated with an alkaline phosphatase-conjugated second antibody for 1 h at room temperature. Excess antibody was removed with extensive washes in 1% casein in PBS and visualized by chemiluminescence according to the manufacturer's protocols (WesternLite, Tropix Inc.).

Confocal Microscopy—Cells were seeded onto coverslips and transfected as described above. The adherent cells were fixed with fresh 4% paraformaldehyde (pH 7.0) for 10 min at room temperature. Fixed cells were permeabilized with a 2-min wash in 100% MeOH at room temperature followed by a wash in 4% bovine serum albumin dissolved in PBS. All subsequent steps were performed in bovine serum albumin/PBS. Appropriately diluted primary antibody was incubated with coverslips overnight at 4°C. Excess antibody was removed with four washes in bovine serum albumin/PBS. Species-specific second antibody conjugated to fluorescein isothiocyanate or tetramethylrhodamine B isothiocyanate was then reacted with the coverslips for 1 h at room temperature with four subsequent washes in bovine serum albumin/PBS. The final samples were mounted onto slides and visualized using a Zeiss Axiophot confocal microscope.

RESULTS

Tax Masks Detection of a c-Myc Epitope—The modulatory effects of Tax on various cellular genes have been extrapolated largely from chimeric reporter experiments and from cell-free biochemical assays. Direct characterization of Tax effects within intact cells has generally been difficult. To address this, we sought to visualize in situ interactions between Tax and endogenous transcription factors using confocal microscopy. We transfected cells with a Tax-expressing vector. Subsequently, the transfected cells were double-stained with anti-Tax rabbit serum (19) and a collection of mouse monoclonal antibodies specific for selected cellular factors. Some of these
factors colocalized with Tax; and from one series of study, an unexpected interaction between Tax and c-Myc was observed. When Tax-expressing HeLa cells (Fig. 1A, arrow 1) were visualized using anti-Myc monoclonal antibody C-8, no Myc fluorescence was detected in the nucleus (Fig. 1B, arrow 1). In the same field, HeLa cells that did not express Tax stained brightly for c-Myc (Fig. 1B, arrow 2). This finding was reproduced in all other fields, and quantitation of fluorescent signals showed that Tax-expressing cells (Fig. 1C, trace 1) measured less than one-tenth the staining intensity of that seen in Tax-nonexpressing counterparts (Fig. 1C, trace 2).

The epitope in c-Myc recognized by the C-8 monoclonal antibody was unknown. To check whether the disappearance of Myc fluorescence was due to a reduction in steady-state protein or was a result of epitope masking, two other Myc-specific antibodies were tested. Anti-Myc C-33 is a monoclonal antibody directed to an epitope different from C-8, although this reactivity has also not been mapped (Fig. 1E). Anti-Myc 9E10 recognizes amino acids 408–439 (Fig. 1G). When a population of HeLa cells transfected with a Tax vector was costained with anti-Tax and either C-33 or 9E10, clear images of c-Myc appeared in the nucleus, regardless of whether the cell expressed Tax protein or not (Fig. 1, compare arrowed cells in D and E and those in F and G). These findings contrasted with those obtained using the C-8 monoclonal antibody and suggested that Tax expression did not perturb steady-state Myc levels, but instead masked detection of one antigenic epitope.

To better understand this masking, we mapped the epitope recognized by the C-8 monoclonal antibody. Wild-type and 10 mutant proteins that contained selected amino acid deletions were expressed in Escherichia coli (Fig. 2A). This panel of 11 Myc polypeptides was probed with C-8 in immunoblot assays. The results revealed that MycD1–48 and MycD106–143 were reactive to C-8, while MycD48–175 was not (Fig. 2A). Further analysis revealed that an expressed peptide containing amino acids 45–130 was recognized by C-8 (Fig. 2B, lane 8), deductively narrowing the epitope to amino acids 45–105. Of interest, we note that amino acids 45–105 are wholly contained within the previously characterized transcriptional activation domain of Myc (reviewed in Ref. 76).

Tax Represses Myc-induced Anchorage-independent Cell Growth—Tax interference with the nuclear detection of the N-terminal Myc activation domain suggested functional significance. Possibly, some aspect of Myc function is perturbed in the presence of Tax. Because singular overexpression of Myc confers anchorage-independent growth to Rat1a cells (77), we selected for Rat1a cells that ectopically overexpress Myc and Tax and assessed their growth phenotype. Starting with parental Rat1a cells (R1aH), using hygromycin selection, cells that express Myc (RM8), Myc + Tax (RM8X), or Myc + an inactive Tax mutant (RM8XG320 (19)) were established (Fig. 3). Tax protein in cells was verified by both immunofluorescence and Western blotting. In all cases, Rat1a cells that ectopically overexpressed Myc or Myc + Tax were able to form colonies on soft agar, whereas Rat1a cells that ectopically overexpressed Myc + an inactive Tax mutant were unable to form colonies on soft agar (Fig. 3).
cence (Fig. 3A) and immunoblotting (Fig. 3B). RM8X and RM8XG320 cells were found to express Tax, while R1aH and RM8 did not (Fig. 3A and B). For Myc expression, similar amounts (after normalizing for background protein intensities) were detected for RM8 (Fig. 3D, lane 2), RM8X (lanes 3 and 4), and RM8XG320 (lanes 5 and 6). The lower level of endogenous Myc was not visualized clearly in R1aH cells (Fig. 3D, lane 1) by this assay. Overall, the immunoblots are consistent with the complementary immunofluorescent images shown in Fig. 1, confirming that Tax does not affect steady-state Myc levels.

The four established cell lines were assayed also for anchorage-independent growth (Fig. 3C). When plated into soft agar, control R1aH cells (Myc⁺/Tax⁻) failed to form significant colonies (Fig. 3C). RM8 (Myc⁺/Tax⁻), consistent with previous findings (77), produced many large foci (Fig. 3C). RM8XG320 (Myc⁺/inactive Tax (19)) resembled RM8 in also producing many large colonies. However, RM8X (Myc⁺/Tax⁺) (Fig. 3C), which expressed both Myc and Tax, showed much reduced cell masses in agar. One interpretation of these results is that Tax, perhaps as a consequence of epitope masking, repressed the induction by Myc of anchorage independence in cells. We caution that our findings do not eliminate the possibility that Tax has other more general effects on the cells, thereby reducing proliferative capacity.

Tax Affects Transcriptional Activation by Myc—Myc activates transcription from responsive promoters. A contribution of the transcriptional activity of Myc to cellular transformation has been suggested (reviewed in Ref. 76). That the N-terminal activation domain of Myc (66, 71, 78, 79) is perhaps conformationally altered in cells that express HTLV-I Tax prompted us to examine what effect, if any, Tax has on Myc-modulated transcription. To explore this issue, we introduced into CV-1

![Diagram](http://www.jbc.org)
Tax Masks c-Myc Function

Fig. 6. Effect of PKA modulation on masking of Myc. HeLa cells were exposed to forskolin for 4 h, immediately fixed, and stained. Myc masking was confirmed by comparing changes in the intensity of C-8-specific fluorescence relative to fluorescence from polyclonal pan-Myc. In untreated cells (A and B), equal intensities were observed with the two antibodies. In the presence of forskolin, C-8-specific immunostaining was reduced compared with pan-Myc-specific immunostaining (compare C and D). In a converse assay, HeLa cells were seeded onto coverslips and transfected with a Tax expression vector followed by simultaneous visualization for both Tax and Myc, whether activation of protein kinase A (PKA) signaling, in the absence of Tax, perturbs the Myc C-8 epitope. We observed that mock-treated cells stained well with either C-8 (Fig. 6A) or pan-reactive polyclonal (Fig. 6B) serum in double simultaneous stainings. In contrast, cells incubated for 4 h with forskolin were not stained with C-8 (Fig. 6C), while the polyclonal antiserum readily visualized nuclear fluorescence of Myc in the identical cells (Fig. 6D).

As a complementary assay, we reasoned that if cAMP-PKA stimulation through either Tax or forskolin effects masking, then inhibitors of PKA activation should blunt this effect. To test this, we examined the ability of Tax to mask the C-8 epitope in cells treated with kinase inhibitor H-9. HeLa cells seeded onto coverslips were transfected with Tax-expressing plasmid and then incubated with H-9 for 12 h. Cells were fixed vector (TaxD). Chloramphenicol acetyltransferase activities were determined 48 h later. As expected, we observed that MLPCAT was activated significantly by RSVmyc (Fig. 4, top). Interestingly, this stimulation was abolished when Tax was coexpressed (Fig. 4, top). In contrast, coexpression of a plasmid expressing an inactive Tax mutant (TaxD) did not affect Myc activation of MLPCAT, controlling for functional specificity. As additional controls, we replicated the transfections using instead plasmid reporter MLPDMCAT (Fig. 4, bottom) that contained mutated Myc-responsive elements. In this instance, promoter expression was not influenced by coexpression of Myc, Tax, or Myc + Tax. These findings demonstrate that intact Myc-binding sites are necessary for activation and that functional Tax protein repressed this activation.

Negative Signaling through a CREB/ATF Pathway Correlates with Masking of Myc—Tax activates gene expression pleiotropically using at least two major signal transduction pathways defined by either NF-κB or CREB/ATF (reviewed in Refs. 8 and 10). Previously, we (19) and others (80) have delineated separate domains within Tax that specify interaction with either NF-κB or CREB/ATF. That analysis prompted the generation of a series of single amino acid Tax mutants such that different mutants were restricted to unimodal activation of either CREB/ATF or NF-κB. We tested six of these mutants (Fig. 5A) in Myc masking assays to characterize better underlying mechanism(s). Tax mutants C23 (Fig. 5B, panels C and D), N43 (panels E and F), and A113 (panels G and H) have been defined previously to activate only CREB/ATF. In contrast, TaxS29 (Fig. 5B, panels I and J), TaxN52 (panels K and L), and TaxN84 (panels M and N) interact with NF-κB, but not CREB/ATF (19). When these versions of Tax were introduced into cells followed by simultaneous visualization for both Tax and Myc, we observed two types of results. All three CREB/ATF-active Tax proteins masked Myc (Fig. 5B, panels C–H), while none of the NF-κB-active Tax polypeptides influenced Myc detection (panels I–N). In other experiments, a direct protein-protein complex between Tax and c-Myc was not found (not in glutathione S-transferase protein chromatography, communoprecipitations, or yeast two-hybrid assays; data not shown). Furthermore, the NF-κB and CREB/ATF mutations distributed to discrete regions in Tax (Fig. 5A), making it, although not impossible, unlikely that any three-point mutations would define one domain for protein-protein contact. Collectively, these findings favor a mechanism linked to induction of CREB/ATF signaling rather than one of direct stoichiometric protein-protein shielding for the masking phenomenon.

If Tax represses Myc detection through activation of cAMP-dependent signaling, then one might expect that an independent means of cAMP stimulation would produce a similar picture. To investigate this possibility, we treated HeLa cells with the adenylyl cyclase activator forskolin and investigated whether activation of protein kinase A (PKA) signaling, in the absence of Tax, perturbs the Myc C-8 epitope. We observed that mock-treated cells stained well with either C-8 (Fig. 6A) or pan-reactive polyclonal (Fig. 6B) serum in double simultaneous stainings. In contrast, cells incubated for 4 h with forskolin were not stained with C-8 (Fig. 6C), while the polyclonal antiserum readily visualized nuclear fluorescence of Myc in the identical cells (Fig. 6D).

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and stained with antiserum. In the absence of H-9, Tax-expressing cells (Fig. 6E) showed reduced C-8 fluorescence (Fig. 6F). Tax-expressing cells treated with with H-9 (Fig. 6G) recovered the normal intensity of C-8 as compared with Tax-nonexpressing cells (Fig. 6H), confirming that protein kinase inhibitors can blunt masking of Myc.

We further studied cells that are genetically defective in the cAMP-PKA axis. The murine B1R cell line is a PKA-defective clonederived from the S49.1 lymphoma (81). These cells fail to respond to cAMP as the result of an absence of cAMP-binding protein (82). If cAMP-PKA signaling is required by Tax to mask Myc, then one should observe staining of Myc by C-8 despite expression of Tax in B1R cells. We, in fact, saw brightly stained Myc protein (Fig. 7E, arrowhead) in B1R cells that expressed Tax (Fig. 7D, arrowhead). In comparison, a suspension T-cell line (Jurkat) not defective in cAMP-PKA signaling, when similarly stained with C-8 (Fig. 7B), showed reduced Myc fluorescence in a cell that expressed Tax (Fig. 7A). Thus, the results from B1R (Fig. 7), considered collectively with those from Tax mutants (Fig. 5) and from forskolin- and protein kinase inhibitor-treated cells (Fig. 6), underscore a linkage between the cAMP-PKA signaling pathway and Tax masking of the Myc C-8 epitope.

**DISCUSSION**

Tax functions are complex. Examples of activation of the HTLV-I long terminal repeat through CREB/ATF and activation of the promoters for human immunodeficiency virus type 1, interleukin-2Rα, and granulocyte-macrophage colony-stimulating factor (among others) through NF-κB illustrate intrinsic interplay between divergent signaling pathways (reviewed in Refs. 8 and 10). In understanding the role of Tax in HTLV-I lymphoproliferation, one faces the issue of a long latency between virus infection and development of ATL. Indeed, only a small minority of infected individuals (~5%) progress to ATL, invariably more than 20–30 years after initial exposure (9, 59, 60). Hence, in vivo, Tax is only mildly oncogenic, and its transforming properties are arguably incidental to its pleiotropic effects on cellular genes.

Intuitively, it is reasonable that Tax cooperates with cytoplasmic oncogenes such as ras (69) in transformation. It was unexpected that Tax might antagonize the anchorage-independent cell growth and transcription functions of Myc. While the short half-lived Myc protein has been shown to be regulated at discrete stages in transcriptional initiation, transcriptional elongation, and post-transcriptional processing (reviewed in Ref. 62), regulation at the level of protein conformation has not, heretofore, been proposed. The region in Myc perturbed by Tax (amino acids 45–106) is contained within a highly conserved transformation/activation domain (66, 71, 78, 79, 83–85). While we do not understand fully the mechanistic details responsible for the Tax-Myc interaction, our experimental evidence supports the likelihood that the N-terminal portion of Myc is conformationally altered by Tax, without additional effects on overall protein stability. A conformational disruption of this protein domain could reasonably account for an abrogation of Myc's transactivation and transformation properties. p53 and Rb are two examples of regulatory proteins targeted for modulation by viral oncogenes (reviewed in Ref. 86). While there are many biological instances in which mechanisms such as phosphorylation and protein degradation are used to control...
function, one of the increasingly common regulatory themes is exemplified by formation of protein-protein complexes, such as those between p53/Rb and viral oncoproteins. Indeed, in one setting, Myc function is known to be regulated by direct binding to Rb (84, 87). In comparison, a common mechanism of Tax action appears to be the facilitation of protein-protein dimerization. This has been illustrated well by effects of Tax on bZIP action (33–35, 88, 89), and this latter ability could, in part, explain its coexistence with host cells. In this regard, the fact that some of the non-intuitive observations with Tax are generally relevant and similarly operative in other biological paradigms.

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REFERENCES

1. POIEZ, B. J., Ruscotti, F. W., Gazdar, A. F., Bunn, P. A., Minna, J. D., and Gallo, R. C. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 7415–7419
2. Yoshida, M., Miyoshi, J., and Hinuma, Y. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 2031–2035
3. Robert-Guroff, M., Nakao, Y., Notake, K., Ito, Y., Sliski, A., and Gallo, R. C. (1982) Science 215, 975–978
4. Sodroski, J. G., Rosen, C. A., and Haseltine, W. A. (1984) Science 225, 381–385
5. Carr, A. J., Rosenblatt, J. D., Wachsmann, W., Shah, N. P., and Chen, I. S. (1985) Nature 318, 571–574
6. Felber, B. K., Paskalis, H., Kleinman-Ewing, C., Wong-Staal, F., and Pavlakis, G. N. (1985) Science 220, 675–679
7. Fujisawa, J., Seki, M., Miyokawa, T., and Yoshida, M. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 2277–2281
8. Smith, M. R., and Greene, W. C. (1991) Clin. Invest. 87, 761–766
9. Feur, G., and Chen, I. S. (1992) Biochim. Biophys. Acta 1114, 223–233
10. Sodroski, J. (1992) Biochim. Biophys. Acta 1114, 19–29
11. Yoshida, M. (1993) Trends Microbiol. 1, 131–135
12. Grassmann, R., Dengler, C., Muller-Fleckenstein, I., Fleckenstein, B., McGuire, K., Dokhelar, M. C., Sodroski, J. G., and Haseltine, W. A. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 3351–3355
13. Grassmann, R., Berchicki, S., Radant, I., Alt, F., Fleckenstein, B., Sodroski, J. G., Haseltine, W. A., and Ramstedt, U. (1992) J. Virol. 66, 4570–4575
14. Tanaka, A., Takahashi, C., Yamada, S., Nosaka, T., Maki, M., and Hatanaka, M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1071–1075
15. Smith, M. R., and Greene, W. C. (1991) J. Clin. Invest. 87, 1038–1042
16. Kelly, K., Davis, P., Mitsuya, H., Irving, S., Wright, J., Grassmann, R., Fleckenstein, B., Wanyo, Y., Greene, W., and Siebenlist, U. (1992) Oncogene 7, 1463–1470
17. Grossman, W. J., Kimata, J. T., Wong, F. H., Zutter, M., Ley, T. J., and Ratner, L. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1057–1061
18. Frank, A. A., and Nyborg, J. K. (1995) J. Biol. Chem. 270, 17–29
19. Semmes, O. J., and Jang, K.-T. (1992) J. Virol. 66, 7183–7192, and references cited therein
20. Kim, S. J., Kehrl, J. H., Burton, J., Tendler, C. L., Jang, K.-T., Danielpour, D., Thevaraj, C., Kim, K. Y., Sohn, M. S., and Roberts, A. B. (1990) J. Exp. Med. 172, 121–129, and references cited therein
21. Sakamoto, K. M., Nimer, S. D., Rosenblatt, J. D., and Gasson, J. C. (1992) Oncogene 7, 2235–2240, and references cited therein
22. Montminy, M. R., Sevarino, K. A., Wagner, J. A., Mandel, G., and Goodman, R. H. (1988) Proc. Natl. Acad. Sci. U. S. A. 83, 6682–6686
23. Tschida, T., Fink, J. S., Mandel, G., and Goodman, R. H. (1987) J. Biol. Chem. 262, 8743–8747
24. Jang, K.-T., Boris, I., Brady, J., Radonovich, M., and Khoury, G. (1988) J. Virol. 62, 4499–4505
25. Giam, C. Z., and Xu, Y. L. (1989) J. Biol. Chem. 264, 15236–15241
26. Tan, T. H., Jia, R., and Roeder, R. G. (1989) J. Virol. 63, 3761–3768
27. Kadison, P., Poteat, H. T., Klein, K. M., and Fallar, D. V. (1990) J. Virol. 64, 2141–2148
28. Poteat, H. T., Chen, F. Y., Kadison, P., Sodroski, J. G., and Haseltine, W. A. (1990) J. Virol. 64, 1264–1270
29. Minnich, M. R., and Bilezikjian, L. M. (1987) Nature 328, 175–178
30. Lin, Y. S., and Green, M. R. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 3396–3400
31. Adam, E., Kerkdijk, P. M., Manneperde, M., Kettmann, R., Burny, A., Droogmans, L., and Willems, L. (1994) J. Virol. 68, 5845–5853
32. Willems, L., Kettmann, R., Chen, G., Portetelle, D., Burny, A., and Derse, D. (1992) J. Virol. 66, 765–772
33. Adya, N., and Giam, C. Z. (1995) J. Virol. 69, 1834–1841
34. Yun, J. M., Paulussen, E. J., Seeler, J. S., and Gaynor, R. B. (1995) J. Virol. 69, 3420–3432
35. Goren, I., Iba, O., Jang, K.-T., and Moelling, K. (1995) J. Virol. 70, 5806–5811
36. Armstrong, A. P., Franklin, A. A., Uittenbogaard, M. N., Giebler, H. A., and Nyborg, J. K. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 7303–7307
37. Franklin, A. A., Kubak, M. F., Uittenbogaard, M. N., Brauweiler, A., Utaisincharoen, P., Matthews, M. A., Dynan, W. S., Hoeffler, J. P., and Roberts, A. B. (1993) J. Virol. 67, 21225–21231
38. Wagner, S., and Green, M. R. (1993) Science 262, 395–399
39. Baranger, A. M., Palmer, C. R., Hamm, K. M., Giebler, H. A., Brauweiler, A., Nyborg, J. K., and Schepert, A. Z. (1995) Nature 376, 606–608
40. Peller, G., Wagner, S., and Green, M. R. (1995) Nature 376, 602–605
41. Fujii, M., Sassone-Corsi, P., and Verma, I. M. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 8526–8530
42. Nakata, K., Ohtani, K., Nakamura, M., and Sugamura, K. (1989) J. Virol. 63, 3761–3764
43. Alexandre, C., Charnay, P., and Verrier, B. (1991) Oncogene 6, 1851–1857
44. Fujii, M., Tsujiaya, H., Chuhaj, T., Akizawa, T., and Seki, M. (1992) Gene Dev. 6, 2066–2076
45. Ballard, D. W., Bohnlein, E., Lowenthal, J. W., Wanyo, Y., Franzan, B. R., and

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3 D. Y. J. in and K.-T. J. eang, manuscript in preparation.
