Transcriptional activation of homologous viral long terminal repeats by the human immunodeficiency virus type 1 or the human T-cell leukemia virus type I tat proteins occurs in the absence of *de novo* protein synthesis

KUAN-TEH JEANG^{*†}, PETER R. SHANK[‡], AND AJIT KUMAR[§]

^{*}Laboratory of Molecular Virology, National Cancer Institute, Bethesda, MD 20892; [‡]Division of Biology and Medicine, Brown University, Providence, RI 02912; and [§]Department of Biochemistry, George Washington University, Washington, DC 20037

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ABSTRACT The genomes of human retroviruses [human immunodeficiency virus type 1 (HIV-1) and human T-cell leukemia virus (HTLV-I)] encode positive trans-activator proteins, named tat. In the presence of tat, the transcriptional activity of the homologous HIV-1 or HTLV-I long terminal repeat (LTR) promoter is markedly increased. We have constructed mammalian cell lines that contain stably integrated copies of a HIV-1 or a HTLV-I LTR–chloramphenicol acetyltransferase (CAT) gene. When presynthesized HIV-1 or HTLV-I tat proteins were separately introduced into these cells in the presence of cycloheximide, we found a strong increase in the steady-state expression of the homologous viral LTR. Nuclear “run-on” assays verified that this tat-mediated enhancement, occurring in the absence of *de novo* cellular protein synthesis, was due to increased transcriptional initiation at the LTR promoter. We conclude that one aspect of transcriptional trans-activation of viral LTR by the HIV-1 and HTLV-I tat proteins does not require the production of new cellular proteins.

Human immunodeficiency virus type 1 (HIV-1) and human T-cell leukemia virus type I (HTLV-I) are, respectively, the etiological agents for acquired immunodeficiency syndrome (AIDS; refs. 1 and 2) and one form of adult T-cell leukemia (3, 4). The genomes of these two human retroviruses contain *gag*, *pol*, and *env* genes typical of the known animal retroviruses. In addition, HIV-1 and HTLV-I encode a viral trans-activator protein, tat, that is located in the 3' portion of the viral genomes (for reviews, see refs. 5 and 6). It has been demonstrated that the expression of genes placed downstream of the HIV-1 or HTLV-I long terminal repeats (LTRs) is substantially increased in the presence of the homologous tat protein (7–16).

The HIV-1 and HTLV-I *tat* genes share no significant nucleotide sequence homology with each other, although both polypeptides are encoded by doubly spliced mRNAs (7, 8, 17, 18). The HIV-1 tat protein varies in size from 86 amino acids (HXB2) to 101 amino acids (SF2), depending upon the particular viral isolate (19). However, functional ability to trans-activate the HIV-1 LTR has been shown to be entirely contained within the first 58 N-terminal amino acids of the protein (20). The counterpart HTLV-I trans-activator is a 40-kDa phosphoprotein, commonly designated as p40^x (21–23). As yet no concise definition of the minimal protein domain necessary for p40^x-mediated trans-activation is available.

The precise mechanism(s) of human retrovirus tat-mediated regulation of viral LTR remains unclear. For HTLV-I p40^x, the experimental data favor a transcriptional

role (13–16), possibly through the mediation of cellular factors (24, 25). For HIV-1 tat, there are suggestions that it can act at both a transcriptional (9–11, 26–28) and posttranscriptional (9, 29, 30) level.

There is currently no clear experimental evidence for a direct interaction between either HIV-1 or HTLV-I tat proteins with nucleic acids. Indeed, a number of studies have reported on the binding of cellular proteins to tat-responsive sequences in both the HIV-1 and HTLV-I LTRs (24, 25, 31–33). Hence, one viable model for tat-mediated trans-activation suggests the induction/recruitment of cellular transcription factors.

We have produced functional HIV-1 and HTLV-I trans-activator proteins in abundance by using a baculovirus expression vector (34, 35). In this study, we report on the individual introduction through cell–cell fusion of both presynthesized tat proteins into mammalian cells containing integrated forms of a HIV-1 or HTLV-I LTR–chloramphenicol acetyltransferase (CAT) gene. The induction of the viral LTRs by the introduced tat polypeptides was assayed for in the presence and absence of protein synthesis inhibitor. Analysis of steady-state and nuclear “run-on” transcriptions showed that neither HIV-1 tat nor HTLV-I p40^x requires the *de novo* synthesis of cellular proteins in the transcriptional activation of homologous LTR. We propose that one mechanism of trans-activation by HIV-1/HTLV-I tat involves the interaction of the viral protein with preexisting cellular factors.

MATERIALS AND METHODS

Cells and Viruses. Infection and propagation of insect SF9 cells were conducted as described (36). The recombinant baculovirus containing the HIV-1 *tat* encoding exon 1 (vActat) and the recombinant baculovirus containing the HTLV-I p40^x cDNA (vAcPx) have been characterized in detail (34, 35). Wild-type baculovirus was provided by M. Summers (Texas A & M University). CV-1 cell lines containing one integrated copy of the pBENNCAT (HIV-1 LTR–CAT; ref. 37) or the pU3RCAT (HTLV-I LTR–CAT; ref. 12) genes were derived by using G418 coselection. The cBENNCAT and cU3RCAT cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% (vol/vol) fetal bovine serum.

RNA Analysis. Total cellular RNA was isolated by the procedure of Chirgwin *et al.* (38). A single-stranded DNA probe was generated from a M13–CAT construction (39) for use in steady-state quantitation of CAT mRNA by S1 nucle-

ase protection assay. Conditions for S1 nuclease analysis have been published (16). Synthesis of radiolabeled run-on RNAs in isolated nuclei was performed as described by Treisman and Maniatis (40).

Polyethylene Glycol (PEG)-Mediated Cell-Cell Fusion. PEG-mediated fusion of baculovirus-infected SF9 cells with mammalian fibroblasts was performed by using established conditions (35). Briefly, a suspension of SF9 cells infected with the appropriate strain of baculovirus for 36 hr was mixed with an equal number of trypsinized mammalian fibroblasts. The mixture of cells was pelleted in a conical centrifuge tube at 2000 rpm for 5 min by using a table-top centrifuge and a Sorvall RT 6000 rotor. The mixed cell pellet was then gently resuspended into 2 ml of a 100% (wt/vol) PEG 1000 (Carbowax, Fisher) solution made with serum-free DMEM buffered with 100 mM Hepes and titrated to pH 7.0 with NaOH. The cells after standing for 1 min at room temperature were pelleted, washed once with serum-free DMEM, and then seeded into culture flasks containing the appropriate medium. Usually 10^6 cells were used for each CAT assay and a minimum of 10^7 cells were used for each RNA analysis. We generally found by light microscopy that at least one-quarter of the seeded mammalian fibroblasts appeared to be fused to SF9 cells.

RESULTS

Presynthesized tat Proteins Activated the Homologous, But Not the Heterologous, LTR in a Cell-Cell Fusion Assay. One

approach in studying the mechanism(s) of the HIV-1 and HTLV-I tat proteins is to generate sufficient quantities of biologically active polypeptides for experimental analysis. Toward that end we have constructed eukaryotic recombinant baculoviruses that contain the functional HIV-1 *tat* coding for exon 1 (vActat) or the HTLV-I p40^x cDNA (vAcPx). In Fig. 1A we show that, in infected SF9 cells, vActat synthesized an additional 13-kDa polypeptide (tat; Fig. 1A, lane 1) not found in mock-infected cells. Similarly, vAcPx-infected SF9 cells contained an additional 40-kDa protein (p40^x; Fig. 1A, lane 3). The identities of both polypeptides were verified by immunoprecipitation with specific antiserum directed against the respective HIV-1 or HTLV-I tat protein (Fig. 1A).

To assess the biological usefulness of the baculovirus-synthesized tat proteins, a functional trans-activation assay was optimized for both proteins by using CV-1-derived cell lines containing either an integrated copy of a HIV LTR-CAT (cBENNCAT; ref. 37) or a HTLV-I LTR-CAT (cU3RCAT; ref. 12) gene. The baculovirus-produced tat proteins were delivered into the mammalian cell lines by PEG-mediated cell-cell fusion. CAT enzyme activity was assayed 18 hr after fusing infected SF9 cells with cBENNCAT or cU3RCAT cells. We found that the tat proteins markedly increased the acetylase activity in the homologous LTR-CAT cells (Fig. 1B, lanes 3 and 6). No significant cross-activation of the heterologous viral LTR was seen for either of the tat proteins (Fig. 1B, lanes 1 and 4). Nor did we find significant response by cBENNCAT or cU3RCAT cells

FIG. 1. Activation of homologous, but not heterologous, viral LTR by HIV-1 and HTLV-I (p40^x) tat proteins synthesized by using baculovirus vectors. (A) Synthesis of HIV-1 and HTLV-I (p40^x) tat proteins by using recombinant baculoviruses. Lanes: 1, immunoprecipitation of tat from vActat (HIV-1 *tat* recombinant baculovirus)-infected SF9 cells by using anti-HIV tat serum; 2, immunoprecipitation of tat from mock-infected SF9 cells; 3, immunoprecipitation of tat from vAcPx (HTLV-I *tat* recombinant baculovirus)-infected SF9 cells by using anti-HTLV-I tat serum; 4, immunoprecipitation of tat from mock-infected SF9 cells; M, ¹⁴C-labeled molecular weight markers. Cells were labeled with [³⁵S]cysteine. tat and p40^x designate the respective HIV-1 and HTLV-I proteins. (B) CAT activities in cells from the fusion of baculovirus-infected SF9 cells to cell lines containing a HIV-1 LTR-CAT (cBENNCAT) or HTLV-I LTR-CAT (cU3RCAT) gene. Lanes: 1, fusion of vAcPx-infected SF9 cells to cBENNCAT; 2, fusion of wild-type baculovirus-infected SF9 cells to cBENNCAT; 3, fusion of vActat-infected SF9 cells to cBENNCAT; 4, fusion of vActat-infected SF9 cells to cU3RCAT; 5, fusion of wild-type baculovirus-infected SF9 cells to cU3RCAT; 6, fusion of vAcPx-infected SF9 cells to cU3RCAT. CAT activities were assayed by using TLC on reaction mixtures containing extracts prepared 18 hr after cell-cell fusion.

to fusion with SF9 cells infected with wild-type baculovirus (Fig. 1B, lanes 2 and 5).

Transcriptional Activation of Viral LTRs Occurs in the Absence of Cellular Protein Synthesis. The cointroduction into cells of plasmids directing the synthesis of either the HIV-1 or the HTLV-I tat proteins with a homologous viral LTR-reporter gene has been useful in answering some questions about tat-mediated trans-activation. This approach, however, does not permit one to distinguish between the induction of new cellular gene products versus the interaction with preexisting cellular factors by HIV-1/HTLV-I tat proteins in the trans-activation mechanism. We introduced presynthesized functional tat proteins into cells treated with a protein synthesis inhibitor to study this aspect of trans-activation.

We inhibited cellular protein synthesis in parallel samples of cBENNCAT and cU3RCAT cells with cycloheximide (final concentration, 50 μ g/ml) starting 30 min before fusion with infected SF9 cells. After PEG-induced cell-cell fusion, cycloheximide-treated cells were maintained in the presence

of inhibitor. Untreated cells were fed with fresh DMEM medium. Total cellular RNAs were isolated 18 hr later (38), and CAT-specific transcription was quantitated by using a 32 P-labeled single-stranded DNA probe (39). We found that the basal transcriptional activity of the HIV-1 (cBENNCAT; Fig. 2A, lane 1) and HTLV-I (cU3RCAT; Fig. 2A, lane 5) LTRs was extremely low after fusion with mock-infected SF9 cells. However, treatment with cycloheximide for 18 hr increased the steady-state CAT mRNA levels in cBENNCAT and cU3RCAT cells by 13-fold (Fig. 2A, lane 2) to 18-fold (Fig. 2A, lane 6). Introduction of appropriate tat proteins into the same cells similarly increased LTR-mediated expression by 8-fold (Fig. 2A, lane 3) to 10-fold (Fig. 2A, lane 7). When tat protein was delivered into indicator cells in the presence of cycloheximide, an additive effect was seen (Fig. 2A, lanes 4 and 8). Densitometric tracings indicated that HIV-1 tat protein with cycloheximide increased CAT transcripts in cBENNCAT cells by 23-fold (compare Fig. 2A, lanes 1 and 4) and that HTLV-I p40^x with

FIG. 2. Increase in steady-state expression of the HIV-1 and HTLV-I LTRs does not require *de novo* protein synthesis. (A) S1 nuclease analyses of CAT mRNAs synthesized in cBENNCAT and cU3RCAT cells after cell-cell fusion with SF9 cells infected with vActat or vAcPx. (Upper) Lanes: 1, cBENNCAT fused with mock-infected SF9 cells; 2, cBENNCAT fused with mock-infected SF9 cells and maintained in cycloheximide (50 μ g/ml); 3, cBENNCAT fused with vActat-infected SF9 cells; 4, cBENNCAT fused with vActat-infected SF9 cells and maintained in cycloheximide (50 μ g/ml); 5, cU3RCAT fused with SF9 cells; 6, cU3RCAT fused with SF9 cells and maintained in cycloheximide (50 μ g/ml); 7, cU3RCAT fused with vAcPx-infected SF9 cells; 8, cU3RCAT fused with vAcPx-infected SF9 cells and maintained in cycloheximide (50 μ g/ml). The relative ratios of CAT mRNA protected by probe in lanes 1–4 are 1:13:8:23 and in lanes 5–8 are 1:18:10:30. (Lower) Normalization controls showing the endogenous actin mRNA in each of the samples used for S1 analysis. M, molecular size markers in kDa; probe, intact size of the single-stranded DNA probe; 256, expected band size of protection for CAT mRNA. (B) CAT activities for each of the respective fusions examined by S1 analysis in A. Note that the activation of CAT mRNA seen in the presence of cycloheximide in A (lanes 2, 4, 6, and 8) is not seen at the CAT enzyme level. This reflects the efficacy of protein synthesis inhibition.

cycloheximide increased CAT mRNAs in cU3RCAT cells by 30-fold (compare Fig. 2A, lanes 5 and 8).

The amount of RNA used in the S1 nuclease analyses was internally controlled by hybridizing each sample with an actin-specific probe (Fig. 2A Lower) and by ethidium staining of the 28S and 18S rRNAs (data not shown). An additional control was for the efficacy of protein synthesis inhibition. Fig. 2B shows the counterpart CAT enzyme assays of the S1-treated RNA samples presented in Fig. 2A. As a measure of the tightness of translational inhibition, substantial increases in the CAT mRNA levels in the presence of cycloheximide treatment (Fig. 2A; lanes 2, 4, 6, and 8) were not reflected by enhanced enzyme activities (Fig. 2B; lanes 2, 4, 6, and 8).

tat-Activation of Viral LTR Occurs at the Level of Transcriptional Initiation. The separate and additive effects of cycloheximide and tat on the steady-state expression of the LTR-linked CAT gene suggest the existence of different pathways of activation. We examined whether HIV-1 or HTLV-I tat trans-activation is mediated at the level of transcriptional initiation and/or transcript stabilization. As in our previous analysis, cBENNCAT or cU3RCAT cells were separately fused to SF9 cells infected with vActat or vAcPx. In this case, isolated nuclei were prepared 18 hr after fusion and preinitiated transcriptional complexes were permitted to extend *in vitro* in the presence of [³²P]UTP. The radiolabeled run-on transcripts were purified and used as probe against nitrocellulose filters containing slots of λ phage, actin, or CAT DNAs. The λ and the actin DNAs represent negative and positive controls, respectively, for hybridization. Specific signals annealing to the CAT template would be a measure of the amount of preinitiated transcriptional complexes within the HIV-1 and HTLV-I LTRs.

We found that the baculovirus-synthesized HIV-1 and HTLV-I tat proteins substantially increased the number of transcriptional initiations in the homologous LTRs (Fig. 3, vActat and vAcPx). This effect was seen despite the presence of cycloheximide (Fig. 3, vActat-cyclo and vAcPx-cyclo) and hence suggests that *de novo* cellular protein synthesis is not required in tat-mediated transcriptional activation. Parallel fusions of mock-infected SF9 cells with or without the presence of cycloheximide demonstrated insignificant hybridizations to the CAT template. These results are consistent with the elevation of steady-state CAT mRNA in the

presence of cycloheximide (Fig. 2A) occurring from a post-transcriptional (e.g., mRNA stabilization) rather than transcriptional initiation mechanism.

DISCUSSION

There is increasing evidence that many viral trans-activator proteins exert their effects in concert with host cell factors. For example, various investigators have shown that the simian virus 40 tumor antigen, the adenovirus E1a gene product, and the herpes-virus immediate-early proteins are each capable of activating genes that do not conserve a common target sequence (for review, see ref. 41). These findings suggest that these viral proteins and perhaps others as well may each be capable of interacting with more than one host cell protein in the trans-activation of the various target sequences.

The HIV-1/HTLV-I tat proteins may similarly activate the homologous viral LTR by using cellular factors. This would be compatible with reports on the binding of cellular proteins to regulatory sequences within the HIV-1 (31–33) and the HTLV-I (24, 25) LTRs. It is additionally known that the HTLV-I p40^x protein can trans-activate the cellular promoter-regulatory sequences of genes such as interleukin 2 (42) or the interleukin-2 receptor (42, 43). Since neither of these responsive sequences contain a single obviously conserved target with each other or with the HTLV-I LTR, it is unlikely that the trans-activation effect is mediated by direct binding of p40^x to DNA. Indeed, as yet, no significant nucleic acid-binding properties have been shown for either HIV-1 tat or HTLV-I p40^x.

In this study we have explored one aspect of the mechanism(s) by which HIV-1 tat or HTLV-I p40^x transcriptionally activate their respective LTRs. We have introduced, by using a PEG fusion technique, presynthesized tat proteins into mammalian cells that contain an integrated copy of a responsive LTR–CAT gene. Measurement of cellular CAT mRNA levels after fusion demonstrated that both tat proteins substantially elevated the steady-state transcriptional activity of the LTRs (Fig. 2A). This activation was shown to be mediated at the level of transcriptional initiation and was independent of cellular protein synthesis (Fig. 3). However, it is not clear whether the enhanced transcriptional initiation can totally account for the increase in steady-state mRNA.

FIG. 3. Nuclear run-on analyses of cell-cell fusions. cBENNCAT or cU3RCAT cells were fused to mock-infected SF9 cells or SF9 cells infected with vActat or vAcPx viruses. Cells after fusion were maintained in the presence or absence of cycloheximide. Nuclei were isolated from fused cells 18 hr after fusion and run-on RNAs were completed in the presence of [³²P]UTP. Total nuclear RNAs were purified and hybridized to nitrocellulose filters containing slots of immobilized phage λ , actin, or CAT DNAs. λ was used as a negative hybridization control. Actin was used to normalize the CAT hybridization signals.

We also found that inhibition of protein synthesis by cycloheximide alone was capable of increasing the steady-state level of LTR-driven CAT mRNA (Fig. 2A). This effect was not reflected at the nuclear run-on level and probably represents transcript stabilization.

Our work was not designed to address the possible post-transcriptional mechanism(s) of the HIV-1 or the HTLV-I tat proteins. When considering the activation of transcription within the viral LTRs, we believe that neither tat protein needs *de novo* synthesis of cellular gene products. Mechanistically, this suggests that trans-activation is facilitated either by a direct interaction of the viral proteins with nucleic acid sequences or by an indirect interaction mediated through preexisting cellular transcription factors. For the various reasons presented above, we favor the latter hypothesis. If such is the situation, it may mean that the human retrovirus tat proteins can work in a fashion analogous to that reported for the adenovirus E1a protein (i.e., posttranslational modification of cellular factors; ref. 44).

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- Barre-Sinoussi, F., Chermann, J. C., Ray, F., Nugeyre, M. T., Chamaret, S., Gruest, J., Dauget, C., Axler-Blin, C., Vozinet-Brun, F., Rouzioux, C., Rozenbaum, W. & Montagnier, L. (1983) *Science* **220**, 868–871.
- Broder, S. & Gallo, R. C. (1984) *N. Engl. J. Med.* **311**, 1292–1297.
- Hinuma, Y., Nagata, K., Misoka, M., Nakai, M., Matsumoto, T., Kiroshita, K., Shirakawa, S. & Miyoshi, I. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6476–6480.
- Kalyanaraman, V. S., Sarngadharan, M. G., Nakao, Y., Ito, Y., Aoki, T. & Gallo, R. C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1653–1657.
- Gallo, R. C. (1986) *Sci. Am.* **255**, 88–98.
- Rabson, A. B. & Martin, M. A. (1985) *Cell* **40**, 477–480.
- Sodroski, J., Rosen, C., Wong-Staal, F., Salahuddin, S. Z., Popoyic, M., Arya, S., Gallo, R. C. & Haseltine, W. A. (1985) *Science* **227**, 171–173.
- Arya, S. K., Guo, C., Josephs, S. F. & Wong-Staal, F. (1985) *Science* **229**, 69–73.
- Cullen, B. R. (1986) *Cell* **46**, 973–982.
- Peterlin, B. M., Luciw, P. A., Barr, P. J. & Walker, M. D. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9734–9738.
- Muesing, M. A., Smith, D. H. & Capon, D. J. (1987) *Cell* **48**, 691–701.
- Sodroski, J. G., Rosen, C. A. & Haseltine, W. A. (1984) *Science* **225**, 381–385.
- Felber, B. K., Paskalis, H., Kleinman-Ewing, C., Wong-Staal, F. & Pavlakis, G. N. (1985) *Science* **229**, 675–679.
- Fujisawa, J., Seiki, M., Sato, M. & Yoshida, M. (1986) *EMBO J.* **5**, 713–718.
- Seiki, M., Hattori, S. & Yoshida, M. (1986) *EMBO J.* **5**, 561–565.
- Brady, J., Jeang, K.-T., Duvall, J. & Khoury, G. (1987) *J. Virol.* **61**, 2175–2181.
- Seiki, M., Hikikoshi, A., Taniguchi, T. & Yoshida, M. (1985) *Science* **228**, 1532–1534.
- Wachsman, W., Golde, D. W., Temple, P. A., Orr, E. C., Clark, S. C. & Chen, I. S. Y. (1985) *Science* **228**, 1534–1537.
- Myers, G., Josephs, S. F., Rabson, A. B., Smith, T. F. & Wong-Staal, F. (1988) in *Human Retroviruses and AIDS: A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences* (Los Alamos Natl. Lab., Los Alamos, NM).
- Seigel, L. J., Ratner, L., Josephs, S. F., Derse, D., Feinberg, M. B., Reyes, G. R., O'Brien, S. J. & Wong-Staal, F. (1986) *Virology* **148**, 226–231.
- Lee, T. H., Coligan, J. E., Sodroski, J. G., Haseltine, W. A., Salahuddin, S. Z., Wong-Staal, F., Gallo, R. C. & Essex, M. (1984) *Science* **226**, 57–61.
- Slamon, D. J., Shimotohno, K., Cline, M. J., Golde, D. & Chen, I. S. Y. (1984) *Science* **226**, 61–65.
- Jeang, K.-T., Brady, J., Radonovich, M., Duvall, J. & Khoury, G. (1988) *UCLA Symp. Mol. Cell. Biol. New Ser.* **67**, 181–187.
- Altman, R., Herrich, D., Garcia, J. A. & Gaynor, R. B. (1988) *J. Virol.* **62**, 1339–1346.
- Nyborg, J. K., Dynan, W. S., Chen, I. S. Y. & Wachsman, W. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6364–6368.
- Hauber, J., Perkins, A., Heimer, E. P. & Cullen, B. R. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6364–6368.
- Kao, S.-Y., Calman, A. F., Luciw, P. A. & Peterlin, B. M. (1987) *Nature (London)* **330**, 489–493.
- Rice, A. P. & Mathews, M. B. (1988) *Nature (London)* **332**, 551–553.
- Rosen, C. A., Sodroski, J. G., Goh, W. C., Dayton, A. I., Lippke, J. & Haseltine, W. A. (1986) *Nature (London)* **319**, 555–559.
- Feinberg, M. B., Jarrett, R. F., Aldovini, A., Gallo, R. C. & Wong-Staal, F. (1986) *Cell* **46**, 807–817.
- Jones, K. A., Kadonaga, J. T., Luciw, P. A. & Tjian, R. (1986) *Science* **232**, 755–759.
- Garcia, J. A., Wu, F. K., Mitsuyasu, R. & Gaynor, R. B. (1987) *EMBO J.* **6**, 3761–3770.
- Franza, B. R., Josephs, S. F., Gilman, M. Z., Ryan, W. & Clarkson, B. (1988) *Nature (London)* **330**, 391–395.
- Jeang, K.-T., Giam, C.-Z., Nerenberg, M. & Khoury, G. (1987) *J. Virol.* **61**, 708–713.
- Jeang, K.-T., Shank, P. R., Rabson, A. B. & Kumar, A. (1988) *J. Virol.* **62**, 3874–3878.
- Summers, M. D. & Smith, G. E. (1987) *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures* (Texas Agriculture Exp. Station, College Station, TX) Bull. 1555.
- Gendelman, H. E., Phelps, W., Feigenbaum, L., Ostrove, J. M., Adachi, A., Howley, P. M., Khoury, G., Ginsberg, H. S. & Martin, M. A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9759–9763.
- Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
- Loeken, M. R., Khoury, G. & Brady, J. (1986) *Mol. Cell. Biol.* **6**, 2020–2026.
- Treisman, R. & Maniatis, T. (1985) *Nature (London)* **315**, 72–74.
- Kingston, R. E., Baldwin, A. S. & Sharp, P. A. (1985) *Cell* **41**, 3–5.
- Inoue, J.-I., Seiki, M., Taniguchi, T., Tsumu, S. & Yoshida, M. (1986) *EMBO J.* **5**, 2883–2888.
- Cross, S. L., Feinberg, M. B., Wolf, J. B., Holbrook, N. J., Wong-Staal, F. & Leonard, W. (1987) *Cell* **49**, 47–56.
- Reichel, R., Kovcsdi, I. & Nevin, J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 387–390.