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Activation of Latent Epstein-Barr Virus Genomes: Selective Stimulation of Synthesis of Chromosomal Proteins by a Tumor Promoter

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The tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA) is a potent inducer of Epstein-Barr virus (EBV) gene expression. The optimal conditions for maximum activation of latent EBV genomes by TPA were determined. Although TPA is able to induce replication of EBV genomes in P3HR-1 cells in all phases of growth, the greatest increase in viral genome copies per cell (15-fold above the control level) occurred in nonproliferating cells as opposed to cells growing exponentially (6-fold above the control level). The synthesis of chromosomal proteins in nonproliferating cells under the conditions that induce maximum activation of latent virus genomes by TPA was studied. Selective stimulation in chromosomal protein synthesis accompanied the increase in EBV genomes in P3HR-1 cells despite an overall reduction in total cellular protein synthesis. Comparison of the chromosomal proteins from TPA-induced P3HR-1 cells and from superinfected Raji cells revealed comigrating chromosomal polypeptides of 145K, 140K, 135K, 110K, 85K, and 55K that are presumably EBV associated. The selective stimulation of synthesis of these chromosomal proteins in TPA-treated P3HR-1 cells was closely associated with the activation of latent EBV genomes.

Human lymphoblastoid cell lines carrying latent Epstein-Barr virus (EBV) genomes vary widely in the expression of virus antigens and in the production of infectious virus particles. In nonproducer lines, cells do not spontaneously enter the viral cycle; they carry multiple copies of the EBV genome (21) and express nuclear antigen (22), but no other known viral antigens have been detected.

An abortive viral cycle can be induced in some nonproducer lines by the halogenated pyrimidines (6), by *n*-butyrate (18), and by tumor promoters (15–17, 24). In virus producer lines, a small proportion of the cells are continuously activated to enter the lytic cycle (8). Virus activation in producer cells (P3HR-1) after treatment with 12-O-tetradecanoyl-phorbol-13-acetate (TPA) results in a dramatic increase in EBV DNA replication (15–17) paralleling the appearance of viral antigen synthesis (15), whereas comparable treatment of nonproducer cells (Raji) results in synthesis of only early antigen (15, 24).

EBV, like other herpesviruses, may cause a latent or productive infection; in addition, latent virus may be reactivated. The molecular and cellular control mechanisms for these processes are almost totally unknown. The present studies use TPA induction as a model for studying molecular mechanisms of EBV latency and reactivation. We have shown (14) that activation of latent EBV DNA replication in a Burkitt somatic cell hybrid line (D98/HR-1) by iododeoxyuridine is associated with the selective stimulation of synthesis of several chromatin-bound proteins. Polypeptides that are in some way involved in DNA replication or control of gene expression are likely to be chromatin associated. We establish here optimized conditions for TPA induction that provide maximum activation of latent EBV genomes and allow selective labeling of virus-associated polypeptides with at the same time marked suppression of cellular protein synthesis. Under these circumstances we have been able to identify directly without immunoprecipitation several EBV-associated chromosomal proteins that appear to be linked to the activation of latent EBV genomes.

MATERIALS AND METHODS

Chemicals. [^{35}S]methionine (755 Ci/mmol) was obtained from Amersham Corp., Arlington Heights, Ill. TPA (lot 48C-0039) was from Sigma Chemical Co., St. Louis, Mo.

Cell cultures. The virus producer (P3HR-1) cells were grown in tissue culture flasks in RPMI-1640

medium as described elsewhere (15). A steady-state mode of growth was established as follows. Cells were seeded at a density of 4×10^5 to 6×10^5 cells per ml and were counted daily until they reached a density of 2×10^6 cells per ml. During this interval the cells are growing in a strict exponential fashion. Density-inhibited cultures were obtained by growing the cells for 6 days without additional medium. During this period, the cells enter a quiescent stationary phase of growth. Cell viability was determined by the trypan blue exclusion method.

Assay of viral DNA replication and antigen synthesis in TPA-treated cells. For analysis of viral antigen synthesis, both exponentially growing and density-inhibited cultures were harvested by centrifugation and incubated in fresh medium containing TPA (30 ng/ml) (15). Samples of cells were taken daily and assayed for viral antigen induction by an indirect immunofluorescence method (7). For determination of virus genome replication, samples of P3HR-1 cells at different growth phases were taken and incubated in fresh medium containing TPA for 7 days. At the end of TPA induction DNA was isolated, and EBV genome copies per cell were determined as described elsewhere (15, 16).

Protein synthesis in P3HR-1 cells. Samples of cells at different growth phases were taken and labeled with [^{35}S]methionine (10 μCi/ml) for 18 h. Acid-precipitable radioactivity was determined (14).

Incorporation of [^{35}S]methionine into total cellular and chromosomal proteins. Stationary-phase P3HR-1 cells (6-day-old culture) were incubated in fresh medium with or without TPA, and protein synthesis was determined daily by pulse-labeling as described above. Incorporation of [^{35}S]methionine into total cellular and chromosomal proteins was determined as previously reported (14).

Electrophoretic analysis of chromosomal protein synthesized in TPA-induced cells. The [^{35}S]methionine-labeled chromosomal proteins were analyzed on slab gels by the method of Laemmli (12). The proteins were treated with sample buffer, and samples of 50 μl containing 60 μg of protein with various amounts of radioactivity were electrophoresed at 25 mA per slab for 8 h. Radiolabeled proteins in the gels were detected by fluorography (2).

RESULTS

Protein synthesis in P3HR-1 cells in different phases of growth. Formerly we were unable to detect virus-induced polypeptides associated with viral antigen synthesis and EBV genome replication in exponentially growing cells exposed to TPA, probably because of the masking effect of high rates of cellular protein synthesis in exponentially growing cells. Thus, we decided to attempt to radiolabel the virus-associated polypeptides induced by TPA when cellular protein synthesis is suppressed. As the first step, we determined the time point when synthesis of cellular proteins is at its minimal rate.

P3HR-1 cells were subcultured at 3-day intervals. The synthesis of total cellular proteins was determined daily for 9 days after subculture. The rate of protein synthesis paralleled the rate of cell multiplication (17) and reached a peak on day 3 (Fig. 1). As the cells reached a stationary phase of growth (after day 4) there was a rapid decrease in protein synthesis, which leveled off at a low rate after 6 days in culture. The rate of total cellular protein synthesis of 6-day-old cultures (stationary-phase cells) was only about 40% of that of the exponentially growing cells (3-day culture). The viability of 6-day-old cultures was maintained at 90%. Therefore, 6-day-old cultures were used for the subsequent TPA induction experiments.

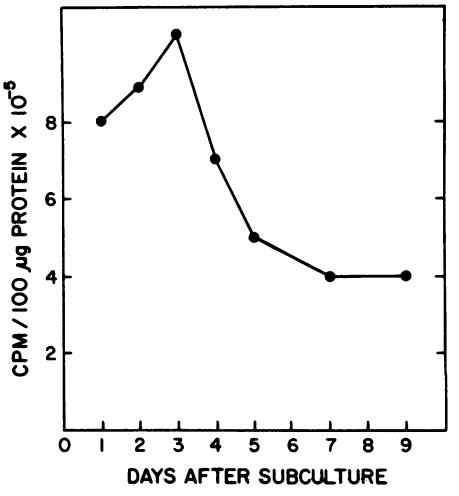

FIG. 1. Protein synthesis in P3HR-1 cells at different growth phases. Samples of cells were pulse-labeled with [^{35}S]methionine for 18 h at daily intervals after subculture, and acid-precipitable counts per minute per 100 μg of protein were determined.

Effect of growth phase on the induction of viral antigen synthesis by TPA. We then determined whether cells in the stationary phase (analogous to the G_0 stage) and actively cycling cells are equally susceptible to TPA induction. The P3HR-1 cell line is a spontaneous producer; under our experimental conditions the spontaneous expression of early antigen (EA) and viral capsid antigen (VCA) fluctuated around 5 to 10% (data not shown). The kinetics of EA and VCA synthesis in P3HR-1 cells in response to TPA induction was indistinguishable between stationary-phase and exponentially growing cells (Fig. 2). However, the intensity of immunofluorescent staining in stationary-phase cells appeared to be stronger than in exponentially growing cells. By 5 days approximately 95% of cells became EA and VCA positive in both cases. These results indicate that stationary-phase cells may be more responsive to TPA induction, but the limitation of the immunofluorescence assay did not permit precise quantitation.

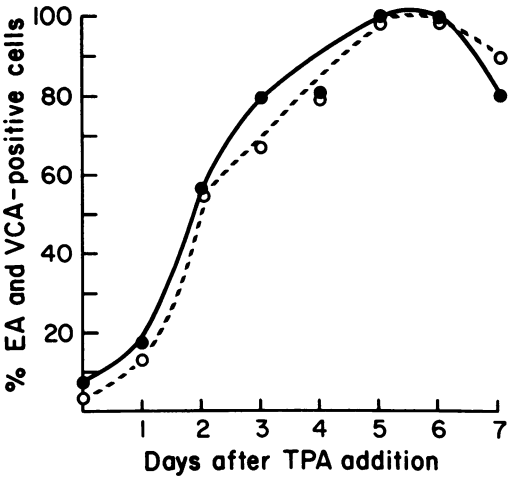

FIG. 2. Effect of growth phase on the induction of EA/VCA in TPA-treated P3HR-1 cells. Both exponentially growing and stationary cells were harvested and incubated in fresh TPA medium. Samples of cells were taken daily and assayed for the percentage of viral antigen-positive cells. Symbols: (○) exponentially growing cells exposed to TPA; (●) stationary-phase cells exposed to TPA.

Effect of growth phase on the induction of EBV genomes by TPA. To determine the effect of different growth phases on TPA induction in P3HR-1 cells, we employed a more sensitive quantitative method, cRNA-DNA hybridization. Figure 3 illustrates the effect of growth phase on the magnitude of induction of EBV genomes by TPA. As the time of TPA addition (indicated by arrows) was progressively postponed, there was an increasing inductive effect. Although TPA was able to induce EBV genome replication at all phases of growth, the most dramatic increase occurred on day 6 (approximately 15-fold above the control level) and remained at this level thereafter when cells reached the stationary phase, as opposed to only a 6-fold increase in exponentially growing cells. The EBV genome copies per cell in nontreated cultures fluctuated around 250 copies (Fig. 3).

Protein synthesis in stationary-phase P3HR-1 cells exposed to TPA. The results in Fig. 3 clearly indicate that stationary-phase cells were approximately two to three times more responsive to TPA induction. To study the kinetics of protein synthesis, stationary-phase P3HR-1 cells (6-day-old cultures) were incubated in fresh medium with or without TPA, and protein synthesis was determined daily. The incorporation of [^{35}S]methionine into total cellular proteins in nontreated cells increased sharply within 1 day after subculture, reaching a peak on day 2, and then decreased very rapidly after day 3 (Fig. 4A). The synthesis of total cellular proteins in TPA-treated cells also increased within 1 day, reaching a peak on day 2, and then decreased very rapidly after day 4 (Fig. 4A). However, the magnitude of synthesis in TPA-treated cells remained 30 to 50% lower than in nontreated cells.

The incorporation of [^{35}S]methionine into chromosomal proteins was determined in samples of cells from the above experiments (Fig. 4A). Chromatin was isolated, and chromosomal proteins were dissociated and separated from DNA as detailed earlier (13, 14). Figure 4B shows that the synthesis of chromosomal proteins in the nontreated cells followed kinetics similar to those of the synthesis of total cellular proteins. However, synthesis leveled off after day 3 in culture. Synthesis of chromosomal proteins in TPA-treated cells increased very rapidly on day 1 with a slight lag on day 2. In contrast to the rapid decrease in total cellular protein synthesis after day 3 (Fig. 4A), the synthesis of chromosomal proteins kept increasing and reached a peak on day 5 (Fig. 4B). These results indicate that the synthesis of chromosomal proteins was selectively stimulated in TPA-treated cells.

Electrophoretic analysis of chromosomal proteins synthesized in stationary-phase P3HR-1 cells exposed to TPA. To characterize the selectively

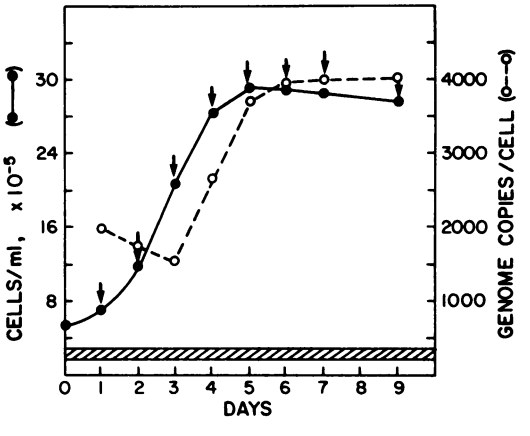

FIG. 3. Effect of growth phase on the induction of EBV genome replication in TPA-treated P3HR-1 cells. Exponentially growing cells were seeded at a density of 6×10^5 cells per ml. At different phases of growth (indicated by arrows), samples of viable cells were harvested and incubated in fresh medium containing TPA for 7 days. At the end of TPA induction, DNA was isolated, and EBV genome copies per cell were determined by cRNA-DNA hybridization. Symbols: (●) growth curve of P3HR-1 cells in culture; (○) EBV genome copies per cell; the hatched horizontal bar represents the range of EBV genome copies per cell in nontreated cells during experimental period.

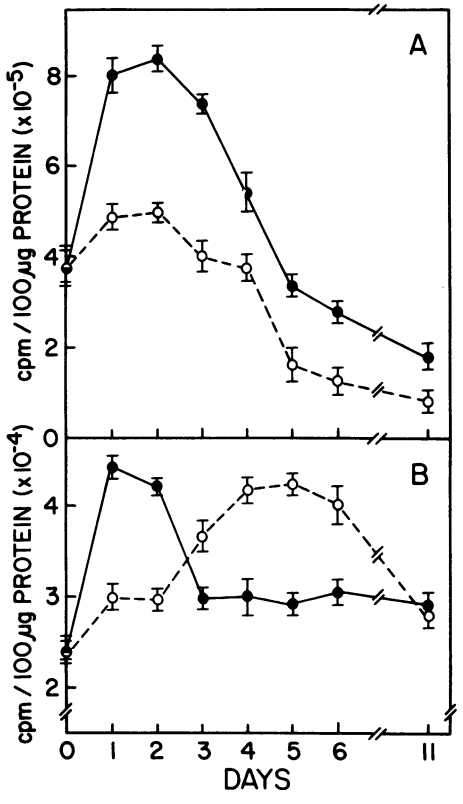

FIG. 4. Protein synthesis in stationary-phase P3HR-1 cells exposed to TPA. Stationary-phase P3HR-1 cells (6-day-old culture) were incubated in fresh medium with or without TPA, and protein synthesis was determined daily as in Fig. 1. A, Total cellular protein; B, chromosomal protein synthesis. Symbols: (○) TPA-treated cells; (●) nontreated cells; bars indicate standard errors.

synthesized chromosomal proteins, stationary-phase P3HR-1 cells (6-day-old cultures) were pelleted and suspended in fresh medium with or without TPA. Samples of cells were taken on days 0, 3, 4, and 5 and were labeled for 18 h with [^{35}S]methionine. The labeled chromosomal proteins were isolated and analyzed on SDS-polyacrylamide gels. Figure 5 shows the results of a fluorogram made by exposing an electropherogram of ^{35}S-labeled chromosomal proteins. There was a significant difference in protein banding patterns before (gel 0) and after (gels 3 to 5) TPA induction. At least six distinct chromosomal proteins of 145K, 140K, 135K, 110K, 85K, and 55K were selectively induced into synthesis in TPA-treated cells. The quantities of these six newly synthesized chromosomal proteins increased in cells with the time of TPA exposure, and their appearance paralleled the increase in EBV genome numbers (15). These six chromosomal proteins were either synthesized at very low levels or undetectable before TPA treatment (gel 0). In addition, the chromosomal protein around 50K was greatly enhanced and formed a diffuse band after TPA exposure. The synthesis of the bulk of cellular proteins was inhibited under these induction conditions.

To correlate these TPA-induced chromosomal

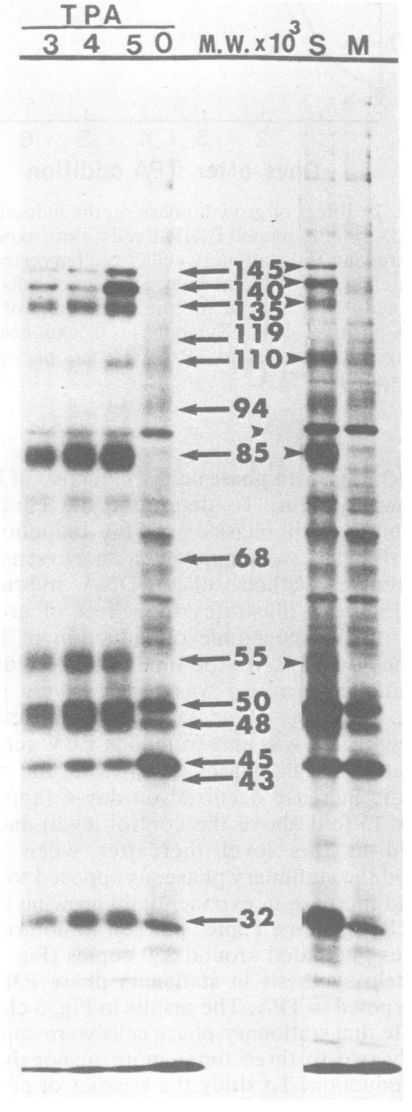

FIG. 5. Time course of appearance of TPA-induced chromosomal proteins synthesized in stationary P3HR-1 cells. Cells were pulse-labeled with [^{35}S]methionine, and chromosomal proteins were analyzed with 7.5% gel as described in the text. TPA, TPA-treated P3HR-1 cells; the numerical numbers, 0, 3, 4, and 5 are days of TPA treatments; S and M, superinfected and mock-infected Raji cells, respectively.

proteins with EBV-associated polypeptides, chromosomal proteins isolated from superinfected and mock-infected Raji cells were analyzed in adjacent slots of the same gel (designated as S and M). It appears that the major polypeptides 145K, 140K, 135K, 110K, 85K, and 55K (indicated by arrowheads), consistently detected and identified previously as probable EBV-specific polypeptides (4), are chromatin-bound proteins.

The new chromosomal proteins synthesized in TPA-treated P3HR-1 cells could result from artifactual conditions created by TPA. To test this possibility, an EBV genome-negative cell line, BJAB, was similarly exposed to TPA, and chromosomal proteins were assayed by the same method. Identical chromosomal protein banding patterns were observed before and after TPA treatment; they did not exhibit the same polypeptides found in TPA-treated P3HR-1 cells (data not shown).

DISCUSSION

We have developed here an optimized system which provides maximum induction by TPA of EBV DNA replication and allows selective labeling of polypeptides, probably chiefly virus associated, under the conditions in which general cellular protein synthesis is suppressed. The system involves using stationary-phase cells (6-day-old cultures) which are arrested at the G_1 to G_0 phase of the cell cycle. Upon exposure of these growth-arrested cells to fresh medium containing TPA, viral functions are greatly activated, whereas the cellular functions are presumably inhibited. Under these circumstances, virus-associated chromosomal proteins are selectively labeled with radiolabeled amino acids and can be detected directly by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

We show here that the magnitude of activation of latent EBV genome by TPA depends upon the physiological state of the cells when they are exposed to the drug. As the time of TPA addition was progressively postponed, there was an increasing inductive effect. Although TPA was able to induce EBV genome replication in all phases of growth, the most dramatic increase (15-fold above the control level) occurred in quiescent stationary-phase cells (a state analogous to G_0) as opposed to cells that were growing exponentially (6-fold above the control level). We have also found that treatment of stationary-phase P3HR-1 cells with TPA results in approximately a threefold further increase in biologically active virus (determined by superinfection of Raji cells) as compared with virus from exponentially growing cells exposed to TPA (unpublished data). These findings are in contrast to the induction by halogenated pyrimidines in which the S phase is a critical period for activation of latent EBV genomes (6). The mechanisms underlying the enhancement of activation of latent EBV genomes in nonproliferative cells are not understood. However, these results suggest that the increase in inductive effect is linked to intracellular events that vary as the cells pass through an actively cycling to a resting phase. Decreased proliferation of these cells is normally accompanied by a stepdown in cellular protein (Fig. 1) and DNA synthesis (unpublished data). Recently we reported (17) that TPA is a potent antiproliferative agent for the lymphoblastoid cells carrying EBV genomes; the TPA-treated cells were arrested at the G_1 to G_0 phase of the cell cycle. When cell mechanisms are inhibited, the endogenous viral functions may take over and proceed from latency into a replicative cycle. The possible explanation for this control mechanism is substantiated by the finding that the K_m of EBV DNA polymerase for dTTP is sixfold less than that of host DNA polymerase (3), indicating that virus DNA polymerase can utilize substrates more efficiently to replicate DNA under stringent nonproliferative physiological conditions.

Increases in chromosomal protein synthesis (Fig. 4B) accompany an overall reduction in total cellular protein synthesis (Fig. 4A). This evident dichotomy between the incorporation of [^{35}S]methionine into total cellular proteins and into chromosomal proteins after TPA induction indicates a selective induction of synthesis of chromatin-bound proteins and rules out the possibility that these changes were simply due to an increased uptake of amino acids in TPA-treated cells. A selective increase in chromosomal protein synthesis has been reported previously in iododeoxyuridine-induced Burkitt somatic hybrid cells containing latent EBV genomes (14).

Analysis of chromosomal proteins synthesized in TPA-induced P3HR-1 cells revealed that six distinct polypeptides of 145K, 140K, 135K, 110K, 85K, and 55K were selectively induced; the kinetics of their appearance paralleled the increase in EBV genome copies per cell (15). The synthesis of these new chromosomal proteins is EBV associated rather than a result of artifacts created by TPA, because (i) these polypeptides are not synthesized in an EBV-genome-negative cell line (BJAB) similarly exposed to TPA, and (ii) the same polypeptides appears to be found in superinfected Raji cells (Fig. 5). It should be noted that three EBV-associated chromosomal proteins (95K, 110K, and 130K) in the same molecular weight range were detected in iododeoxyuridine-induced Burkitt somatic hybrid cells (D98/HR-1) (14). Whether these iododeoxyuridine-induced chromosomal proteins in D98/HR-1 cells represent a subset of TPA-in-

duced chromosomal proteins in P3HR-1 cells remains to be determined.

In previous reports with TPA induction exponentially growing cells were used (9, 15, 16, 24). Since cellular protein synthesis continues in exponentially growing cells throughout induction, immunoprecipitation was needed to identify virus-specific proteins (19, 20, 23). The patterns of precipitated polypeptides varied with sera of different sources and titer (1, 11; our unpublished data). The advantage of TPA induction with nonproliferative cells is the marked reduction in cellular protein synthesis which makes possible the direct detection of TPA-induced chromosomal proteins associated with the activation of latent EBV genomes. We emphasize that these induced proteins merely accompany activation; we do not ascribe a cause or effect relation with either transcription or replication in the present evidence.

Recently several attempts were made to identify the EA complex (5, 10, 11, 20). These reports involved using mixtures of EA/VCA-positive antisera to immunoprecipitate the presumptive early polypeptides associated with viral infection or chemical induction. Since immunoreagents monospecific for EA are not available, the results reported by these authors varied. EA synthesis in nonproliferating Raji cells activated by TPA increased 300-fold (from 0.1% to 30% EA-positive cells), in contrast to a 100-fold increase (from 0.1% to 10%) in exponentially growing cells (unpublished data). The optimized conditions described in this report should offer an alternative approach to characterize and compare the components of the EA complex with those of previous reports.

These results indicate that TPA induction can provide as efficient a system as superinfected Raji cells for radiolabeling EBV-associated proteins. The results, taken together, indicate that TPA-induced chromosomal proteins in P3HR-1 cells may well be inherently associated with the activation of latent viral genomes. Studies are in progress to define these EBV-associated chromosomal proteins further in relation to their possible linkage to regulation of expression of latent EBV genomes.

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LITERATURE CITED

1. **Bodemer, W. W., W. Summers, and J. C. Niederman.** 1980. Detection of virus-specific antigen in EB-(P3HR-1) virus-superinfected Raji cells by immunoprecipitation. Virology **103**:340–349.
2. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium labeled proteins and nucleic acid in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
3. **Datta, A. K., B. M. Colby, J. E. Shaw, and J. S. Pagano.** 1980. Acyclovir inhibition of Epstein-Barr virus replication. Proc. Natl. Acad. Sci. U.S.A. **77**:5163–6166.
4. **Feighny, R. J., M. P. Farrell, and J. S. Pagano.** 1980. Polypeptides synthesis and phosphorylation in Epstein-Barr virus infected cells. J. Virol. **34**:455–463.
5. **Feighny, R. J., B. E. Henry, and J. S. Pagano.** 1981. Epstein-Barr virus polypeptides: identification of early proteins and their synthesis and glycosylation. J. Virol. **39**:651–655.
6. **Hampar, B., J. G. Derge, L. M. Martos, M. A. Tagamets, S. Y. Chang, and M. Chakrabarty** 1973. Identification of critical period during the S phase for activation of the Epstein-Barr virus by 5-iododeoxyuridine. Nature (London) New Biol. **244**:214–217.
7. **Henle, G., and W. Henle.** 1966. Immunofluorescence in cells derived from Burkitt's lymphoma. J. Bacteriol. **91**:1248–1256.
8. **Hinuma, Y., M. Konn, J. Yamaguchi, D. J. Wudarski, J. R. Blakeslee, and J. T. Grace.** 1967. Immunofluorescence and herpes-type virus particles in the P3HR-1 Burkitt lymphoma cell lines. J. Virol. **1**:1045–1051.
9. **Hudewentz, J., G. W. Bornkamm, and H. zur Hausen.** 1980. Effect of the diterpene ester TPA on Epstein-Barr virus antigen and DNA synthesis in producer and nonproducer cell lines. Virology **100**:175–178.
10. **Kallin, B., J. Luka, and G. Klein.** 1979. Immunochemical characterization of Epstein-Barr virus associated early and late antigens in *n*-butyrate-treated P3HR-1 cells. J. Virol. **32**:710–716.
11. **Kawanishi, M., K. Sugawara, and Y. Ito.** 1981. Epstein-Barr virus-induced polypeptides: a comparative study with superinfected Raji, IUdR-treated and N-butyrate-treated P3HR-1 cells. Virology **109**:72–81.
12. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
13. **Lin, J. C., C. Nicolini, and R. Baserga.** 1974. A comparative study of some properties of chromatin from normal diploid and SV-40 transformed human fibroblasts. Biochemistry **13**:4127–4133.
14. **Lin, J. C., and J. S. Pagano.** 1980. Synthesis of chromosomal proteins and Epstein-Barr virus DNA in activated Burkitt somatic cell hybrids. Virology **106**:50–58.
15. **Lin, J. C., J. E. Shaw, M. C. Smith, and J. S. Pagano.** 1979. Effect of 12-O-tetradecanoyl-phorbol-13-acetate on the replication of Epstein-Barr virus. I. Characterization of viral DNA. Viroloy **99**:183–187.
16. **Lin, J. C., M. C. Smith, and J. S. Pagano.** 1981. Induction of replication of Epstein-Barr virus DNA by 12-O-tetradecanoyl-phorbol-13-acetate. II. Inhibition by retinoic acid and 9-(2-hydroxyethoxymethyl)guanine. Virology **111**:294–298.
17. **Lin, J. C., M. C. Smith, and J. S. Pagano.** 1982. Effects of 12-O-tetra-decanoyl-phorbol-13-acetate on cell proliferation and proliferation and Epstein-Barr virus DNA replication. Virology **117**:186–194.
18. **Luka, J., B. Kallin, and G. Klein.** 1979. Induction of Epstein-Barr virus cycle in latently infected cells by n-butyrate. Virology **94**:228–231.
19. **Mueller-Lantzsch, N., B. Georg, N. Yamamoto, and H. zur Hausen.** 1980. Epstein-Barr virus proteins. III. Analysis of polypeptides from P3HR-1-EBV-superinfect NC37 cells by immunoprecipitation. Virology **102**:231–233.
20. **Mueller-Lantzsch, N., N. Yamamoto, and H. zur Hausen.** 1979. Analysis of early and late Epstein-Barr virus associated polypeptides by immunoprecipitation. Virology **97**:378–387.
21. **Nonoyama, M., and J. S. Pagano.** 1971. Detection of Epstein-Barr viral genome in nonproductive cells. Nature (London) New Biol. **233**:103–104.

22. **Reedman, B. M., and G. Klein.** 1973. Cellular localization of an Epstein-Barr virus-associated complement fixing antigen in producer and nonproducer lymphoblastoid cell lines. Int. J. Cancer 11:499–520.
23. **Roubal, J., B. Kallin, J. Luka, and G. Klein.** 1981. Early DNA-binding polypeptides of Epstein-Barr virus. Virology 113:285–292.
24. **zur Hausen, H., F. J. O'Neill, and J. K. Freeze.** 1978. Persisting oncogenic herpes virus induced by the tumor promoter TPA. Nature (London) 272:275–373.