A viral long terminal repeat in the interleukin 2 gene of a cell line that constitutively produces interleukin 2

(transcriptional enhancers/viral integration/gibbon leukemia virus long terminal repeat/gene expression)

SU JANE CHEN*, NIKKI J. HOLBROOK†, KENNETH F. MITCHELL*, CAROL A. VALLONE*,
JUDITH S. GREENGARD‡, GERALD R. CRABTREE†‡, AND YUAN LIN*

*Central Research and Development Department, Glenolden Laboratory, E. I. du Pont de Nemours and Company, Glenolden, PA 19036; †Laboratory of Pathology, Building 10, Room 2N-113, National Cancer Institute, Bethesda, MD 20205; and ‡Department of Pathology, Room L224, Stanford University School of Medicine, Stanford, CA 94305

Communicated by Philip Leder, July 12, 1985

ABSTRACT The gibbon leukemia cell line MLA 144 differs from every other T-lymphocyte line in that it constitutively makes interleukin 2 (IL-2) (also called T-cell growth factor) without stimulation by antigen, lectin, or tumor promoters. Previous work in which glucocorticoids were used to inhibit IL-2 production has indicated that proliferation of this cell line is dependent upon endogenously produced IL-2. We have found that the MLA 144 cell line has a copy of the gibbon leukemia virus inserted into the 3' nontranslated region of the IL-2 gene. This integration event produces a composite mRNA made up of the protein coding sequences of the IL-2 gene transcript but incorporating the viral long terminal repeat (LTR) in the 3' nontranslated region of the mRNA. This composite mRNA transcript uses the polyadenylation signal in the viral 5' LTR and incorporates the viral transcriptional control regions. The integration event must involve only one allele of the IL-2 gene, since transcripts essentially identical to normal human IL-2 mRNA are also produced in cloned sublines of MLA 144. That the viral LTR contains a 94-base-pair repeat reminiscent of enhancer sequences in several viruses suggests that the integration of the viral LTR at the 3' end of the IL-2 gene is responsible for the constitutive production of IL-2 in the MLA 144 cell line.

MLA 144 (American Type Culture Collection no. TIB 201), a gibbon T-cell line originally established from a T-cell lymphoma, is a constitutive producer of interleukin 2 (IL-2) (1). Upon mitogen stimulation, the synthesis and secretion of IL-2 in these cells increases by 30- to 50-fold over the constitutive level (2). IL-2 purified from MLA 144 is similar to human IL-2 in both its biological activity and its molecular structure (3). Furthermore, after the cDNA and the gene encoding human IL-2 were cloned and sequenced in several laboratories (4–6), the sequence of the 5' flanking region of the human IL-2 gene was found to be somewhat homologous with the long terminal repeat (LTR) of the adult T-cell leukemia virus (7).

The MLA 144 cell line has been shown to harbor type C gibbon leukemia virus, San Francisco strain (GLV-SF) (8), a retrovirus associated with several types of hematopoietic neoplasms (9). Recent studies have indicated that the LTR of a retrovirus plays a regulatory role in the processes of viral transcription, integration, replication, and genome rearrangement (10, 11). Nucleotide sequence analysis of the LTR derived from a number of avian and mammalian retroviruses (12–16) has revealed the existence of consensus promoter sequences in the *U3* region located at similar distances from the RNA initiation site. Evidence is also mounting that the LTR can function to activate adjacent cellular genes follow-

ing the integration of proviral DNA 5' or 3' to a eukaryotic gene (17, 18). Short repeats of 50–100 nucleotides, shown to act as enhancers, have been found in a number of viruses (19). These enhancers increase transcriptional activity of adjacent genes in a manner that is independent of their position and orientation. Enhancers can also function beyond their own promoter elements to enhance gene activity from adjacent promoters (19).

In view of the unusual expression of the IL-2 gene in the MLA 144 cell line, we explored the organization and sequence of the IL-2 mRNA and IL-2 gene in MLA 144 cells and normal gibbon cells. The surprising finding that a viral LTR has been integrated at the 3' end of the IL-2 gene in the MLA 144 cell line suggests that constitutive production of IL-2 in the cell line may be related to this integration. The importance of constitutive IL-2 production in the proliferation of the MLA 144 cell line is suggested by Smith, who has used glucocorticoid inhibition of IL-2 production to demonstrate that the MLA 144 cell line proliferates via an autocrine mechanism involving IL-2 (20). Thus, integration of viral control regions in the IL-2 gene may be critical in regulating the growth of this malignant cell line.

MATERIALS AND METHODS

Isolation of DNA from the Blood of Gibbons. DNA was isolated by phenol extraction as described (21) from peripheral blood of 11 normal gibbons (Yerkes Regional Primate Center), 7 of which were unrelated.

Construction of a cDNA Library from MLA 144 mRNA and Screening for IL-2 cDNA. Double-stranded cDNA was synthesized from the poly(A)⁺ RNA of phytohemagglutinin-, phorbol 12-myristate 13-acetate-, LiCl-stimulated MLA 144 cells (2) by use of reverse transcriptase, RNase H, *Escherichia coli* DNA polymerase I, and *E. coli* DNA ligase (22). The double-stranded cDNA was then flush-ended with T4 DNA polymerase and ligated with *Eco*RI linkers at a ratio of 2:1. The ligated cDNA-linkers were digested with *Eco*RI restriction enzyme and loaded onto a 1-ml Sepharose CL-4B column for sizing. The large-size fractions [>500 base pairs (bp)] were ligated to *Eco*RI-cut and phosphatase-treated λ phage gt11 cloning vector and were packaged *in vitro* (23). A total of 3×10^5 recombinant phage were obtained. Of these 4×10^4 recombinant phage were screened for IL-2-containing clones with an affinity-purified IL-2 antibody probe. Four positive clones were selected. The same phage were also screened with human IL-2 [³²P]cDNA probes. In addition to these 4 clones, 19 more positive clones were selected.

Southern Blot Analysis. Genomic DNA digested with restriction enzymes was electrophoresed on a 0.8% agarose gel and transferred to nitrocellulose filter paper by the method of Southern (24). Nick-translated human IL-2 cDNA and GLV DNA (gift from Martin L. Scott, Stanford University, Cancer Biology Program) were used as probes for hybridizations. Hybridization and washing were performed as detailed in the figure legends.

Dot-Blot Analysis. mRNA from induced and uninduced MLA 144 cells was applied to nitrocellulose filter paper in serial dilutions, hybridized to nick-translated IL-2 cDNA, and washed under stringent conditions as described for Southern blots.

DNA Sequencing and Data Analysis. The cDNA insert from the λ gt11 clone was subcloned into the *Eco*RI site of M13 phage vector mp19, and the DNA sequence was determined by the dideoxy chain-termination method (25). The M13 universal primer and synthetic oligonucleotides were used as primers for the sequencing. Sequence data were compiled, and homologies between the DNAs were compared with the aid of the Wisconsin program (26).

Cloning of IL-2 Genomic Fragments. MLA 144 DNA was digested to completion with *Eco*RI, and fragments of 3.7, 4.5, and 6.0 kilobases (kb) were separated on an agarose gel, eluted, and cloned into λ gt11 as described for the cDNA above.

S1 Nuclease Analysis. A probe for analysis of transcripts from the IL-2 gene was produced by ligating the *Xba* I-*Stu* I fragment from the IL-2 cDNA to the *Stu* I-*Eco*RI fragment of the IL-2 gene. This probe was end-labeled at the *Xba* I site by using the Klenow fragment of *E. coli* DNA polymerase I and [³²P]dCTP. Approximately 1×10^5 cpm of the end-labeled 750-bp *Xba* I-*Eco*RI fragment (specific activity, 5×10^6 cpm/ μ g) was annealed at 47°C for 12 hr to 4 μ g of poly(A)-selected RNA from noninduced MLA-144 cells in 80% (vol/vol) formamide/400 mM NaCl/40 mM Pipes, pH 6.4/1 mM EDTA in sealed glass capillary tubes. The capillary tubes were opened, the hybrids were digested with 30 units of S1 nuclease (Sigma), and the resultant RNA-DNA duplexes were analyzed on 3.5% polyacrylamide nondenaturing gels as described (27).

Hybrid Selection for the Detection of IL-2 mRNA Containing a Viral Insert. To detect the presence of the IL-2 mRNA containing the viral insert, the LTR portion of the GLV was hybridized to the mRNA isolated from the unstimulated MLA 144 cells. mRNA hybridized to the LTR was injected into the oocytes to determine the presence of IL-2 activity (28). The hybridization and elution conditions were identical to those described by Parnes *et al.* (29). IL-2 activity was determined by using an IL-2-dependent T-cell line, OH-1, and the activity was expressed as the amount of [³H]thymidine incorporated (1).

RESULTS

Southern Blot Analysis of DNA of Human and Gibbon Cells.

Fig. 1 shows the Southern blot analysis of *Eco*RI fragments of DNA from normal human peripheral blood lymphocytes, a gibbon B-cell line, and two gibbon T-cell lines (6G1 and MLA 144) with IL-2 cDNA used as probe. Except for MLA 144, a single band at 3.7 kb was observed. Even though two *Eco*RI fragments encompass the IL-2 gene, their sizes are too similar to be distinguished by the agarose gel (6). In contrast, MLA 144 DNA showed three distinct size fragments—3.7, 4.5, and 6.0 kb—that hybridized to IL-2 cDNA. The middle band was consistently less intense than the other two bands. Additionally, DNA samples from peripheral blood of 11 normal gibbons (at least 7 of which were unrelated) were similarly analyzed. A single band at 3.7 kb was observed (data not shown). Thus, it appears that the three-band

FIG. 1. Southern blot-hybridization analysis of human and gibbon chromosomal DNAs. Chromosomal DNA was prepared from human peripheral blood lymphocytes (lane a), gibbon normal B-cell line GB737 (lane b), and two gibbon T-cell lymphoma lines, MLA 144 (lane c) and 6G1 (lane d). Each DNA (10 μ g) was digested with *Eco*RI, electrophoresed on a 0.8% agarose gel, and blotted to a nitrocellulose filter. Human IL-2 [³²P]cDNA was used as a probe. The filter was hybridized in 40% (vol/vol) formamide containing 4 $\times$ NaCl/Cit (1 $\times$ = 0.15 M NaCl/0.015 M sodium citrate, pH 7), 0.8 $\times$ Denhardt's solution (1 $\times$ = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin), 10% dextran sulfate, and 20 μ g of salmon sperm DNA per ml at 42°C overnight and was washed for 15 min in 2 $\times$ NaCl/Cit containing 0.1% NaDodSO₄ at room temperature and then for 30 min in 0.1 $\times$ NaCl/Cit containing 0.1% NaDodSO₄ at 50°C for 30 min. Sizes are shown in kb.

restriction pattern for the IL-2 gene is unique to MLA 144 cells.

MLA 144 cells were established originally from polyclonal lymphoma cells. Thus, to ensure that the difference in restriction patterns does not originate from a mixed population of cells, single-cell clones were prepared by plating MLA 144 cells to less than one cell per well. The *Eco*RI fragments of DNA extracted from 10 of these clones all show the same three-band pattern as in lane c of Fig. 1. Thus, it is clear that the extra bands observed in the Southern blot do not arise from a mixed population of cells but instead are derived from the genome of homogeneous cells.

The IL-2 Genes of MLA Cells Are Inducible with Mitogen. Dot-blot analysis using human IL-2 cDNA as a probe indicated that the level of IL-2 mRNA was much higher in the mitogen-induced MLA 144 cells than in the uninduced cells (Fig. 2). This result agrees with those determined by the biological activity assay (2).

FIG. 2. Inducible and constitutive levels of IL-2 expression in MLA 144; quantitation of IL-2 mRNA in both uninduced and induced MLA 144. A representative dot-blot of poly(A)⁺ RNA from MLA 144 cells stimulated (row a) and not stimulated (row b) by phorbol 12-myristate 13-acetate/phytohemagglutinin/LiCl. Serial dilutions (1:1) of RNA samples were applied to each dot. The highest concentration, 5 μ g, was loaded onto the first dot on the left. Human IL-2 [³²P]cDNA was used as hybridization probe.

Nucleotide Sequence Analysis. Three IL-2 cDNA inserts—425 bp (clone CM1), 694 bp (clone CM11), and 927 bp (clone CM9) in length—and a portion of the 3' genomic *EcoRI* fragment were sequenced. Clone CM1 and CM11 are identical in sequence; however, clone CM9 contains a different sequence at the 3' end as compared to the other two clones. The sequences of clones CM11 and CM9 are compared to the human IL-2 cDNA (4) in Fig. 3, which shows two types of cDNA that share the same coding region with the exception of a silent base change at position 264 from C to T. Both clones also share a 118-base homology beyond the termination codon in the 3' noncoding region, with the exception of a deletion of G-T-A-A in clone CM9. Downstream from this segment of 118 bases, the sequence of CM9 and the genomic DNA is entirely different from that of human IL-2. Clone CM11 continues to show homology to the human IL-2 cDNA. There are only four differences between the 3' noncoding regions of human and gibbon IL-2 cDNA. Clone CM9 shares the same homology with both human IL-2 and gibbon clone CM11 until position 624, 118 bp beyond the termination codon. At this point, there is a sequence of 487 bases, ending in a poly(A) tail, that is not found in CM11. When this segment of 487 bases was compared to the GLV LTR (15, 16), >75% homology was found within the 487 bases. Within this

sequence a "TATA" box, a "CAT" box, and a poly(A) addition signal are found. In addition, there are two 94-bp direct repeating sequences that are separated by four bases. This repeat represents the major point of divergence from the GLV sequences previously reported (15, 16), which contain only a single copy of this 94-bp repeat. Within the repeats there is an enhancer-like sequence T-G-T-G-G-T. Even though there is no direct repeat in the LTR of GLV in strain San Francisco, a 50-base direct repeat was found in strain SEATO of GLV (16). Fig. 4 gives a diagrammatic representation of the sequence homology between the human and gibbon IL-2 cDNA as well as the sequence homology between CM9 and the LTR of GLV.

Southern Blot Analysis of MLA 144 DNA with 5' and 3' Human IL-2 and GLV Probes. To determine which of the three *EcoRI* bands of MLA 144 DNA are homologous to the *EcoRI* fragments encompassing the normal human IL-2 gene, the following experiment was done. MLA 144 DNA restricted with *EcoRI* was blotted and hybridized with either p15 (5' probe) or pX-R (3' probe) human IL-2 genomic DNA probes. The p15 plasmid (see ref. 6) contains the 2100-bp *HindIII* fragment in the 5' *EcoRI* segment of the gene, including about 1000 bp 5' to the gene, exons 1 and 2, and part of the second intron. pX-R is an *Xba* I-*EcoRI* fragment encompassing

FIG. 3. Comparison of nucleotide sequences of human IL-2 cDNA, two gibbon IL-2 cDNAs, and the LTR of GLV-SF. Translation of the protein coding region is shown on the top of the sequences. Rows: a, human IL-2 cDNA sequence by Taniguchi *et al.* (4); b, normal gibbon IL-2 cDNA sequence (clone CM11); c, gibbon IL-2 cDNA (clone CM9), which contains the LTR sequence of GLV-SF at the 3' untranslated region; d, LTR of GLV-SF by Trainor *et al.* (16). An alignment of the sequences maximizing the number of correspondences with the minimum introduction of gaps was done by the Wisconsin computer program. The 94-bp direct repeat is underlined. The major structural features, TATA box, CAT box, polyadenylation signal, and U3/R, and R/U5 borders in the LTR of GLV-SF, are indicated.

FIG. 4. Schematic diagram depicting the regions of sequence homology between two gibbon IL-2 cDNAs (clones CM11 and CM9), human IL-2 cDNA (TIL2), and the LTR of GLV-SF. , Protein encoding region; , 99% homology between the sequences; , 75% homology between the sequences; , 94-bp direct repeat.

the exons 3 and 4 and flanking sequence 3' to the gene.

Fig. 5 *Right* shows the Southern blot results. The 5' probe (lane a) recognized the 3.7-kb band and the 4.5-kb fragment. In contrast, the 3' probe (lane b) recognized the 3.7-kb and 6.0-kb fragments. Hybridization with the human IL-2 cDNA (with homologies to both the 3' and 5' *Eco*RI fragments) gave the expected three-band pattern (lane c). These data clearly assign the 6.0-kb and 4.5-kb fragments to the 3' and 5' ends of the gene, respectively. Southern blots of MLA 144 cellular DNA digested with *Eco*RI were hybridized with a probe containing the entire GLV viral DNA. Numerous bands indicated viral insertion at multiple sites in the genome (Fig. 5 *Left*). Similar blots with normal human DNA and normal gibbon DNA showed no homology to GLV (not shown).

Expression of LTR-Containing IL-2 mRNA in Unstimulated Cells. Table 1 gives the results of experiments in which mRNA hybrid-selected by the GLV LTR was translated into proteins after injection into oocytes. mRNA selected by the GLV LTR produced 298 ± 17 units of IL-2 as opposed to only 24 ± 4 units of IL-2 produced by mRNA selected by pBR322. Since the mRNA used in these experiments was from noninduced MLA 144, the results indicate that the allele of the IL-2 gene containing the GLV insert is transcribed without induction by phytohemagglutinin and phorbol 12-myristate 13-acetate.

FIG. 5. Southern blot of MLA 144 DNA with IL-2 and GLV probes. (*Right*) MLA 144 DNA was digested with *Eco*RI and, after electrophoresis, was transferred to nitrocellulose and hybridized with radiolabeled DNA corresponding to either the 5' *Eco*RI fragment (lane a) or 3' *Eco*RI fragment (lane b) of the normal human gene or to the complete DNA (lane c). (*Left*) Blots prepared from *Eco*RI-digested MLA 144 DNA were hybridized with the complete viral genome of the San Francisco isolate of GLV cloned in λ phage. Both lanes represent the same DNA and are essentially duplicates. Sizes are shown in kb.

S1 Nuclease Analysis of IL-2 RNA Transcripts. The 750-bp *Xba* I-*Eco*RI fragment made up of the *Xba* I-*Stu* I cDNA sequences and the *Stu* I-*Eco*RI genomic sequences was hybridized to noninduced MLA 144 poly(A)-selected mRNA. Hybrids digested with S1 nuclease revealed a protected fragment of 350 bp, corresponding to the distance from the *Xba* I site to the viral insertion site, but no protected fragment of 500 bp, corresponding to the distance from the *Xba* I site to the normal polyadenylation site. When the end-labeled *Xba* I-*Eco*RI fragment was hybridized to liver cell mRNA, neither the 350-bp or the 500-bp fragment was protected (data not shown).

DISCUSSION

The regulation of IL-2 gene expression in MLA 144 is different from that in normal lymphocytes and that of many established malignant gibbon or human T-cell lines whose IL-2 genes are not active unless they are stimulated with mitogen or antigen (30). The culture medium of unstimulated MLA 144 cells has been used as a source of IL-2 for the maintenance of cultured T-cells before purified IL-2 was available. Recently, it was demonstrated that, although expression of IL-2 is constitutive, the level of IL-2 produced from MLA cells increases ≈ 30 -fold after mitogen stimulation (2). Southern blot analysis indicates that there is additional sequence inserted into the MLA 144 IL-2 gene as compared to the normal gibbon gene. This difference in restriction pattern is not related to individual polymorphisms because DNA from 10 normal gibbons did not display the unusual pattern observed in MLA 144. The discovery of a viral insert at the 3' end of one of the IL-2 alleles gives an explanation of the 6.0-kb fragment observed on Southern blots of *Eco*RI-digested MLA 144 cells. In preliminary experiments, the subcloned 4.5-kb fragment at the 5' end of the rearranged IL-2 gene hybridized to the GLV LTR, suggesting that this fragment also contains GLV sequences. Thus, it is possible that two integration events have occurred adjacent to the IL-2 gene (data not shown).

The fact that the GLV LTR can be used to hybrid-select biologically active IL-2 mRNA from noninduced MLA-144 cells (Table 1) indicates that the allele containing the GLV insert is active in noninduced cells. Furthermore, S1 nuclease

Table 1. [3 H]Thymidine incorporation by the IL-2-dependent T-cell line OH-1

mRNA*	[3 H]Thymidine, cpm $\pm$ SEM ($n = 4$)
Total mRNA of MLA 144	554 $\pm$ 9
LTR-hybridized mRNA†	298 $\pm$ 17
Control‡	24 $\pm$ 4

*Oocytes were injected with total mRNA of MLA 144 or hybrid-selected mRNA.

†pBR322 plasmid DNA with LTR insert was used for hybridization. The mRNA bound to the filter paper was eluted and injected into oocytes for IL-2 assay.

‡For control, pBR322 DNA at the same concentration was used for hybridization.

analysis of IL-2 mRNA transcripts from noninduced cells indicates that few if any IL-2 transcripts arise from the normal allele in noninduced cells. These observations strongly suggest a role for viral insertion in the constitutive activation of the IL-2 gene.

The presence of a proviral insert has been demonstrated to have a significant effect in modulating gene expression in several systems. In an avian leukosis virus-induced bursal lymphoma, the avian leukosis virus DNA has been shown to enhance the expression of c-myc RNA when it was placed in the same transcriptional orientation either upstream (5') or downstream (3') to the c-myc gene locus (18). Our data for the MLA 144 IL-2 genomic nucleotide sequences indicates that the GLV provirus integrated into the 3' untranslated region of the IL-2 gene, so that the IL-2 transcripts proceed through *U3* and terminate in the *R* region of the virus LTR. Enhancer elements have been identified within the *U3* region of a number of retroviral LTRs (19, 31, 32). In Moloney murine sarcoma virus, the 73- to 72-bp repeats within the LTR were shown to enhance the level of gene expression at both 5' and 3' locations and in either orientation relative to the indicator gene (32). Also, the prototype enhancer, a 72-bp tandem repeat of simian virus 40 DNA was shown to be responsible for the early gene activity. Deletion of this element reduced transcriptional activity by at least 100-fold (33, 34). The presence of a viral 94-bp direct repeat in one of the alleles of the IL-2 gene of MLA 144 cells may play an important role in the constitutive expression of the IL-2 gene in this cell line.

We thank Ellen Doran for the synthesis of oligonucleotides used in this study, Gail Sandlin for cell culture, Martin Scott for the GLV probes, and Drs. Harvey Rabin and Darrel Stafford for continuous encouragement and support. These studies were supported in part by Public Health Service Grant CA 39612 to G.R.C. This paper is contribution no. 3769 from the Central Research and Development Department, Glenolden Laboratory, E. I. du Pont de Nemours and Company, Glenolden, PA 19036.

- Rabin, H., Hopkins, R. F., III, Ruscetti, R. W., Neubauer, R. H., Brown, R. L. & Kawakami, T. G. (1981) *J. Immunol.* **127**, 1852-1856.
- Lin, Y. & Robb, R. J. (1985) *Lymphokine Res.* **4**, 1-4.
- Henderson, L. E., Hewetson, J. F., Hopkins, R. F., III, Sowder, R. C., Neubauer, R. H. & Rabin, H. (1983) *J. Immunol.* **131**, 810-814.
- Taniguchi, T., Matsui, H., Fujita, T., Takaoka, C., Kashima, N., Yoshimoto, R. & Hamuro, J. (1983) *Nature (London)* **302**, 305-310.
- Fujita, T., Takaoka, C., Matsui, H. & Taniguchi, T. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 7437-7441.
- Holbrook, N. J., Smith, K. A., Fornace, A. J., Jr., Comeau, C. M., Wiskocil, R. L. & Crabtree, G. R. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1634-1638.
- Holbrook, N. J., Lieber, M. & Crabtree, G. R. (1984) *Nucleic Acids Res.* **12**, 5005-5012.
- Kawakami, T. G., Huff, S. D., Buckley, P. M., Dungworth, D. L., Snyder, S. P. & Gilden, R. V. (1972) *Nature (London)* **235**, 170-171.
- Snyder, S. P., Dungworth, D. L., Kawakami, T. G., Caloway, E. & Lau, D. (1973) *J. Natl. Cancer Inst.* **51**, 89-94.
- Varmus, H. E. (1982) *Science* **216**, 812-820.
- Temin, H. M. (1982) *Cell* **28**, 3-5.
- Van Beveren, C., Rands, E., Chattopadhyay, S. K., Lowy, D. R. & Verma, I. M. (1982) *J. Virol.* **41**, 542-556.
- Ju, G. & Skalka, A. M. (1980) *Cell* **22**, 379-386.
- Chen, H. R. & Barker, W. C. (1984) *Nucleic Acids Res.* **12**, 1767-1778.
- Scott, M. L., McKereghan, K., Kaplan, H. S. & Fry, K. E. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4213-4217.
- Trainor, C. D., Scott, M. L., Josephs, S. F., Fry, K. E. & Reitz, M. S., Jr. (1984) *Virology* **137**, 201-205.
- Hayward, W. S., Neel, B. G. & Astrin, S. M. (1981) *Nature (London)* **290**, 475-480.
- Payne, G. S., Bishop, J. M. & Varmus, H. E. (1982) *Nature (London)* **295**, 209-213.
- Khoury, G. & Gruss, P. (1983) *Cell* **33**, 313-314.
- Smith, K. A. (1982) *Immunobiology* **161**, 157-173.
- Crabtree, G. R., Comeau, C. M., Fowlkes, D. M., Fornace, A. J., Malley, J. D. & Kant, J. K. (1985) *J. Mol. Biol.*, in press.
- Gubler, U. & Hoffman, B. J. (1983) *Gene* **25**, 263-269.
- Young, R. A. & Davis, R. W. (1983) *Science* **222**, 778-782.
- Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503-507.
- Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463-5467.
- Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12**, 387-395.
- Crabtree, G. R. & Kant, J. A. (1982) *Cell* **31**, 159-166.
- Lin, Y., Stadler, B. M. & Rabin, H. (1982) *J. Biol. Chem.* **257**, 1587-1590.
- Parnes, J. R., Velan, B., Felsenfeld, A., Ramanathan, L., Ferrini, U., Appella, E. & Deiman, J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2253-2257.
- Gillis, S. & Watson, J. (1980) *J. Exp. Med.* **152**, 1709-1719.
- Laimins, L. A., Khoury, G., Gorman, C., Howard, B. & Gruss, P. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6453-6457.
- Laimins, L. A., Gruss, P., Pozzatti, R. & Khoury, G. (1984) *J. Virol.* **49**, 183-189.
- Benoist, C. & Chambon, P. (1981) *Nature (London)* **290**, 304-310.
- Gruss, P., Dhar, R. & Khoury, G. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 943-947.