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Interferon regulates c-*myc* gene expression in Daudi cells at the post-transcriptional level

(blot hybridization/cell growth inhibition/*in vitro* nuclear transcription)

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ABSTRACT **c-*myc* gene mRNA is reduced by >75% in the human lymphoblastoid cell line Daudi when growth is inhibited by treatment with human interferon β (IFN-β). In the present communication, we describe the effect of IFN-β treatment on transcription of the c-*myc* gene and on the steady-state level of c-*myc* mRNA in the cytoplasm of Daudi cells. The results show that, although the rate of c-*myc* transcription is not significantly different in nuclei isolated either from untreated cells or from those treated with IFN-β for 3 or 24 hr, the level of c-*myc* mRNA in the cytoplasm is reduced by 60% within 3 hr of IFN-β treatment. These results suggest that IFN-β regulates the c-*myc* mRNA at a post-transcriptional level. These results are in contrast to the regulation of two IFN-β-induced genes that under identical conditions are regulated in these cells at the transcriptional level. We have also detected induction of the (2′-5′)oligoadenylate synthetase (2-5A synthetase) gene in IFN-β-treated Daudi cells. Since certain c-*myc* transcripts have the capacity to form double-stranded RNA regions, we propose that one mechanism by which c-*myc* could be regulated post-transcriptionally in IFN-β-treated cells is by activating, through its own double-strandedness, the 2-5A synthetase/RNase L endonuclease system, which would cause selective degradation of the c-*myc* RNA.**

Several lines of evidence suggest that expression of the c-*myc* gene may be related to the regulation of cellular proliferation and the process of neoplastic transformation. Kelly *et al.* (1) have reported a striking increase in c-*myc* RNA in normal fibroblasts and lymphocytes during the transition from the quiescent to the proliferating state induced by either growth or mitogenic factors. Campisi *et al.* (2) have confirmed these results on the cell cycle dependency of c-*myc* expression in normal cells and, in addition, have shown that c-*myc* becomes constitutively expressed in chemically transformed cells. Deregulated expression of c-*myc* has also been observed in avian B-cell lymphomas (3), human Burkitt lymphomas and murine plasmacytomas (reviewed in ref. 4), and isolates of various tumor types (5). Neoplastic transformation has been achieved by co-transfection of rat embryo fibroblasts with c-*myc* and c-*ras* genes (6). On the other hand, reduced c-*myc* RNA expression was observed in F9 teratocarcinoma cells whose growth was inhibited when induced to differentiate (2). In addition, when cells of the human promyelocytic leukemia line HL-60 are induced to differentiate by a vitamin D metabolite and cease to proliferate, c-*myc* expression is greatly reduced (7).

The interferons (IFNs) are secreted proteins that exhibit multiple biological activities, including inhibition of cell growth (8) and reversion of the transformed phenotype (9). The molecular mechanisms by which IFN mediates these phenomena have not been elucidated in detail. One approach is to determine the effect of IFN on the expression of genes with oncogenic potential. IFN treatment has been shown to reduce the synthesis of $pp60^{src}$ and expression of the transformation phenotype in Rous sarcoma virus-transformed rat cells (10), to reduce the amount of c-Ha-*ras* mRNA and c-Ha-*ras*-specified protein in RS485 mouse cells (9), and to reduce *src* and Ha-*ras* mRNAs in human RT4 cells (11). In light of the data implicating c-*myc* in cell growth and neoplasia, we have investigated the effect of IFN-β on the expression of c-*myc* in human Daudi cells, a line of lymphoblastoid cells derived from an African Burkitt lymphoma (12), whose growth can be inhibited by low concentrations of IFN (13, 14). Our experiments showed a >75% reduction in c-*myc* mRNA in Daudi cells whose growth had been inhibited by IFN-β (15). This reduction appeared to be selective because the level of actin mRNA was not diminished and neither *in vitro* translation of mRNA extracted from IFN-β-treated cells nor *in vivo* synthesis of cellular proteins in IFN-β-treated cells was quantitatively affected. However, the above observations were made after 24 hr of exposure to IFN, thus weakening any mechanistic implications of the reduced amounts of c-*myc* mRNA. It was therefore of interest to determine whether the IFN-induced reduction in c-*myc* mRNA precedes detectable inhibition of Daudi cell growth and whether c-*myc* expression is regulated at the transcriptional or the post-transcriptional level. We report here (*i*) that there is a 60% reduction in c-*myc* mRNA in Daudi cells as early as 3 hr after the addition of IFN-β and (*ii*) that IFN-β does not change the rate of c-*myc* gene transcription.

MATERIALS AND METHODS

Cells and Cell Fractionation. Human Daudi cells were grown in RPMI medium (GIBCO) supplemented with 10% fetal bovine serum (GIBCO) and gentamicin (GIBCO) at 37°C in 5% CO_2/95% air. Human IFN-β was produced and purified to homogeneity as described (16). Cells were washed twice with phosphate-buffered saline, then homogenized at 4°C in a metal Dounce homogenizer in 10 mM Tris·HCl, pH 7.4/10 mM NaCl/5 mM $MgCl_2$/1 mM dithiothreitol. Nuclei were removed by centrifugation of the homogenate at 800 × *g* for 2 min and the nuclear pellet was used for *in vitro* transcription experiments. The supernatant was centrifuged at 7000 × *g* for 10 min and the resulting supernatant was used for preparation of cytoplasmic RNA.

Transcription in Isolated Nuclei. The incorporation of [α-^{32}P]UTP into RNA in isolated nuclei was carried out as described by Hofer *et al.* (17). For each experimental point, nuclei from 10^8 cells were washed once with reaction buffer [20 mM Tris·HCl, pH 7.9/140 mM KCl/10 mM $MgCl_2$/2 mM dithiothreitol/20% (vol/vol) glycerol/1 mM ATP/CTP/GTP/5 μM UTP] and then suspended in 200 μl of reaction buffer containing 250 μCi of [α-^{32}P]UTP (3000 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear). The nuclei were incubat-

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Abbreviations: IFN, interferon; kb, kilobase(s); 2-5A synthetase, (2′-5′)oligoadenylate synthetase.

ed for 10 min at 30°C and the [^{32}P]RNA was extracted as described (18). After phenol extraction, the [^{32}P]RNA was prepared for hybridization by precipitation with trichloroacetic acid followed by limited base hydrolysis (19).

RNA Isolation. Total cellular RNA was isolated by a modified guanidinium isothiocyanate/cesium chloride method (15, 20). Cytoplasms were made 0.1% in lithium dodecyl sulfate, extracted three times with phenol/chloroform and once with chloroform, and ethanol precipitated. Poly(A)$^+$ RNA was purified by oligo(dT)-cellulose chromatography (21).

Hybridization. Dot-blot hybridization of [^{32}P]RNA from isolated nuclei to excess DNA was carried out according to the procedure of Hofer *et al.* (17) with the following modification: before being loaded on nitrocellulose paper, plasmid DNAs were linearized with *Eco*RI and denatured by heating at 55°C for 15 min in 50% formamide/2.5 mM Na_4EDTA/2.0 M formaldehyde/40 mM triethanolamine·HCl, pH 7.5 (22). Five micrograms of plasmid DNA was loaded per individual dot. Filters were prehybridized for 6–8 hr and hybridized with the labeled RNA at 5×10^6 cpm per blot for 42–45 hr under recommended conditions. To decrease background radioactivity, the blots were treated during washing with RNase T1 at 10 units/ml and bovine pancreatic RNase at 0.3 unit/ml. RNA blot hybridization and ^{32}P labeling of probes by either nick-translation or end labeling were carried out as described (15).

DNA Probes. Plasmid pKW3 was provided by W. Hayward and contains the *Pst* I segment from the second exon of human c-*myc* (23). Two plasmids, pIF-IND-1 and pIF-IND-2, were isolated from a cDNA library of IFN-β-treated human diploid fibroblasts (24). The pIF-IND-1 clone hybridizes to a 2.9-kilobase (kb) mRNA from IFN-β-treated cells. Clone pIF-IND-2 hybridizes under the same conditions to a 1.9-kb mRNA. The insert of pIF-IND-2 is complementary to the 3′ end of the mRNA coding for a 56-kDa protein that is inducible in IFN-α- and IFN-β-treated cells (25). Also, this plasmid hybrid-selects from IFN-β-treated cells a mRNA that on *in vitro* translation produces a 56-kDa protein (unpublished observation).

A 20-nucleotide-long probe for (2′-5′)oligoadenylate synthetase (2-5A synthetase) was prepared by R. Arentzen (Central Research and Development Department). The probe sequence 5′ G-G-G-A-C-C-C-A-T-C-C-C-A-A-T-T-C-T-T-A 3′ is complementary to the sequence of a 2-5A synthetase cDNA between nucleotides 234 and 253 derived from published sequence data of Merlin *et al.* (25).

Quantitation of Autoradiograms. Films from DNA dot blots and RNA hybridizations after different exposures were quantitated as reported (15) and as described in the legend of Fig. 2.

RESULTS

The reduced concentration of c-*myc* mRNA in IFN-β-treated Daudi cells that we observed (15) could be due to either reduced synthesis or increased degradation. To distinguish between transcriptional and post-transcriptional control, we have compared the rate of c-*myc* gene transcription in control and IFN-β-treated cells by using an *in vitro* nuclear transcription assay in combination with a dot-blot procedure (17, 19). In this approach, isolated nuclei are incubated for 10 min in a reaction mixture containing [α-^{32}P]UTP. Labeled nascent RNA is prepared and hybridized to specific DNAs bound to nitrocellulose paper. Reaction conditions of the assay support elongation of initiated RNA transcripts but do not permit reinitiation (17, 19). Therefore, the amount of [^{32}P]RNA that hybridizes to a specific DNA reflects the number of RNA chains initiated prior to cell harvesting and can serve as an estimate of *in vivo* transcription rate.

In preliminary experiments, we determined that the level of c-*myc* mRNA was reduced in Daudi cells treated with IFN-β for 3 hr and even further in cells treated for 24 hr (data not shown). Based on this observation, [^{32}P]RNA prepared from nuclei of control cells and cells treated with IFN-β for 3 or 24 hr was used for hybridization. The result of the dot-blot analysis is shown in Fig. 1*A*. It is evident that the extent of hybridization to c-*myc* DNA (dots 2) is not significantly different among the three types of [^{32}P]RNA. To exclude the possibility that the c-*myc* DNA on the blot is limiting and the assay cannot register a decrease in c-*myc* transcripts reliably, we also increased the amount of [^{32}P]RNA used for hybridization by 50%. Under those conditions, the amount of c-*myc*-specific hybridization showed a concurrent 50% increase, indicating that the c-*myc* DNA on the blot was in excess (data not shown). These results indicate that IFN-β treatment of Daudi cells for 3 or 24 hr does not significantly change the rate of c-*myc* RNA synthesis from that in control cells. A blot analysis of the c-*myc* mRNA isolated from the cytoplasmic fractions of the cells whose nuclei were used for the *in vitro* nuclear transcription experiment shown in Fig. 1*A* is presented in Fig. 1*B*. A significant reduction in c-*myc* mRNA is evident in cells treated for 3 hr with IFN-β. Fig. 2 is a histogram of the data in Fig. 1 *A* and *B* after quantitation by densitometer measurement. The data in Fig. 2*A* show that the rate of synthesis of c-*myc* RNA does not change after exposure to IFN-β for 3 or 24 hr while the data in Fig. 2*B* show a 60% decrease in cytoplasmic c-*myc* mRNA after only 3 hr of IFN-β exposure. These data indicate that IFN-β reduces the level of c-*myc* mRNA in Daudi cells by a post-transcriptional mechanism and not by reducing its rate of synthesis. There is no effect of IFN-β on DNA synthesis in Daudi cells, as measured by [^{3}H]thymidine incorporation, up

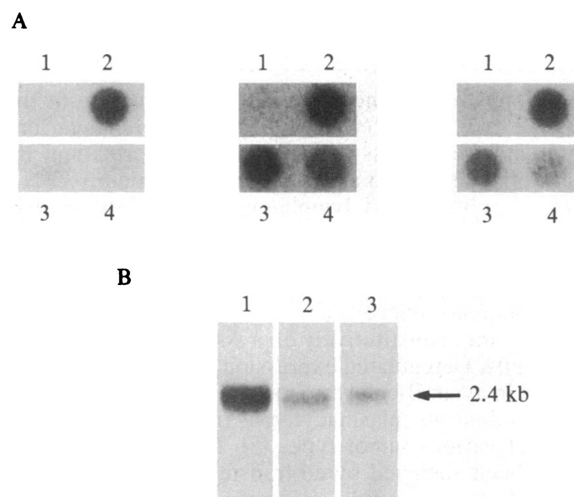

FIG. 1. Hybridization analyses of RNA from control and IFN-β-treated Daudi cells. Daudi cells were either not treated (control) or treated with IFN-β at 50 units/ml for 3 or 24 hr. The cells were disrupted by Dounce homogenization and the nuclear and cytoplasmic fractions were isolated by differential centrifugation. (*A*) Dot-blot hybridization. Isolated nuclei were incubated for 10 min with [α-^{32}P]UTP, and RNA was isolated and hybridized to plasmids spotted on nitrocellulose filters. (*Left*) Control. (*Middle*) IFN-β treatment for 3 hr. (*Right*) IFN-β treatment for 24 hr. Dots: 1, pBR322; 2, pKW3; 3, pIF-IND-2; 4, pIF-IND-1. (*B*) Blot hybridization. Poly(A)$^+$ RNA was isolated from the cytoplasmic fraction by phenol/chloroform extractions and chromatography on oligo(dT)-cellulose. Ten micrograms of each RNA was separated on 1.4% agarose gel containing formaldehyde. The RNA was transferred to nitrocellulose paper and the blot was hybridized with ^{32}P-labeled pKW3 probe. Lanes: 1, RNA from control cells; 2, RNA from cells treated with IFN-β for 3 hr; 3, RNA from cells treated with IFN-β for 24 hr.

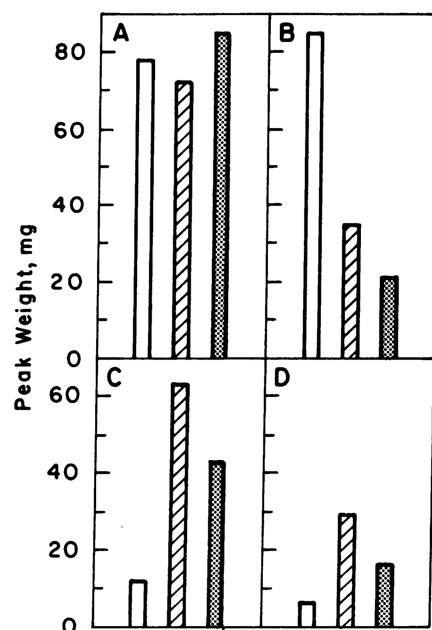

FIG. 2. Densitometric analysis of the experiments shown in Fig. 1. Films from the dot-blot and blot-hybridization experiments were scanned on an MK III microdensitometer (Joyce–Loebl). Individual peaks from each tracing were cut out and weighed, and the weights (in mg) were plotted. (*A*) Dot blots with the c-*myc* probe. (*B*) Blot hybridization with the c-*myc* probe. (*C*) Dot blots with the pIF-IND-2 probe. (*D*) Dot blots with the pIF-IND-1 probe. Open bars, RNA from control cells; hatched bars, RNA from cells treated with IFN-β for 3 hr; stippled bars, RNA from cells treated with IFN-β for 24 hr. Duplicate filters were scanned for the dot-blot experiments and averages of these determinations are shown.

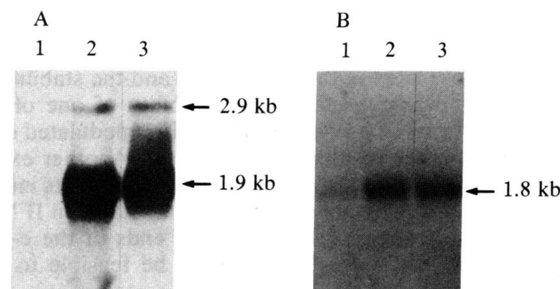

FIG. 3. Blot-hybridization analysis of RNA from Daudi cells. (*A*) The blot of cytoplasmic poly(A)$^+$ RNA from control and IFN-β-treated cells used in the experiment shown in Fig. 1*B* was stripped of the c-*myc* probe by boiling for 5 min in H_2O (15) and then hybridized with a 1:1 mixture of ^{32}P-labeled pIF-IND-1 and pIF-IND-2. (*B*) The blot of total poly(A)$^+$ RNA from Daudi cells was hybridized with the ^{32}P-labeled synthetic probe for 2-5A synthetase. Lanes: 1, RNA from control cells; 2, RNA from cells treated with IFN-β for 3 hr; 3, RNA from cells treated with IFN-β for 24 hr.

to 18 hr of IFN-β exposure (unpublished observation). Thus, the reduction in the level of cytoplasmic c-*myc* mRNA precedes any measurable effect of IFN-β on DNA synthesis. This suggests that the IFN-induced reduction in c-*myc* mRNA is not simply a consequence of the cessation of cell growth.

In contrast to the above results, the rates of synthesis of the RNAs from IFN-induced genes pIF-IND-1 and pIF-IND-2 are dramatically increased after 3 hr of IFN-β treatment (Fig. 1*A*, dots 3 and 4). Quantitatively, the rates of synthesis of the RNAs from these two genes increased 500–600% (Fig. 2 *C* and *D*). We have also determined the effect of IFN-β on the cytoplasmic levels of the mRNAs for pIF-IND-1 and pIF-IND-2 in Daudi cells by RNA blot analysis. In addition, we have used a synthetic DNA probe to determine the cytoplasmic level of the mRNA for the IFN-induced enzyme 2-5A synthetase. As shown in Fig. 3, the mRNAs for pIF-IND-1 (2.9 kb), pIF-IND-2 (1.9 kb), and 2-5A synthetase (1.8 kb) reach maximum concentrations after 3 hr of IFN-β exposure. These data are in good agreement with those shown in Fig. 2 since maximum RNA synthesis from genes pIF-IND-1 and pIF-IND-2 occurs with only 3 hr exposure to IFN-β.

DISCUSSION

The object of this research was to determine the kinetics and level at which IFN-β regulates the steady-state level of c-*myc* mRNA in Daudi cells. Our results show that, although the steady-state level of c-*myc* mRNA is reduced by 60% in IFN-β-treated cells within 3 hr, the rate of c-*myc* gene transcription is virtually unaffected even after 24 hr of IFN-β exposure.

The reduction in c-*myc* mRNA is detectable several hours before the IFN-β-treated Daudi cells cease to proliferate. At the low level of IFN-β (50 units/ml) used in our study, the proliferation is not reduced until after 18–20 hr of IFN-β treatment. This is in agreement with findings of others who observed that a marked reduction in c-*myc* mRNA precedes cessation of cell proliferation by a number of hours (2, 7). Taken together, these results suggest that the reduction in c-*myc* mRNA cannot simply be a consequence of the cessation of cell growth. Furthermore, they raise the question of whether the reduction in c-*myc* expression plays a role in the mechanism of growth inhibition. The experiments of Kelly *et al.* (1) show that c-*myc* expression is induced during the competence process in fibroblasts on their exposure to platelet-derived growth factor. Those authors proposed that c-*myc* expression may be a part of the competence process necessary for the entry of cells into S phase. It is tempting to infer from these observations that the reduction in c-*myc* expression brought about by external agents such as the IFNs may disrupt the process of competence and therefore inhibit optimum growth.

To assess the effect of IFN-β on the rate of c-*myc* gene transcription, we have used an established *in vitro* transcription assay that has been used successfully to study regulation of cellular genes by IFN-β (24). The sensitivity of our assay system can be considered satisfactory, because it detected the response of two cellular genes that have been shown previously to be transcriptionally regulated. Kinetically, the response of these two genes of IFN-β in Daudi cells reported here is similar to their response in normal human fibroblasts, involving both a dramatic increase in the rate of transcription after 3 hr of IFN-β exposure and a decrease to low levels by 24 hr (24). However, within the detection limits of our assay, the rate of transcription of the c-*myc* gene is not significantly different in nuclei isolated from either control or IFN-β-treated cells over the span of a 24-hr exposure. On the basis of these results we surmise that the reduction in the steady-state level of c-*myc* mRNA caused by IFN-β cannot be due to a decreased rate of c-*myc* gene transcription and must be accounted for by some post-transcriptional event(s).

It is not possible to infer from the present data at which post-transcriptional level the c-*myc* transcripts are regulated. However, from published results on the structure of c-*myc* transcripts we have noted several features that may play a role in their stability. Two potential polyadenylylation sequences are present in the c-*myc* gene (26). The results in the literature indicate that the choice of polyadenylylation signal may modulate gene expression. The maturation of adenovirus transcripts (27, 28) and the growth-dependent expression of transfected dihydrofolate reductase genes (29) have been

shown to be dependent on the choice of polyadenylylation signal. While no detailed study is available on the utilization of either of these sites in the c-*myc* gene and the stability of the resulting mRNA, preferential instability of one of the possible two forms of c-*myc* mRNA in IFN-modulated cells may account for the results presented here. Another explanation may be that one of the two possible transcripts initiated on the c-*myc* dual promoter (26) is more stable in IFN-β-treated cells. By mapping the 5′ and 3′ ends of the c-*myc* mRNAs in IFN-treated cells, it should be feasible to test these possibilities.

One of the postulated mechanisms by which IFN can regulate gene expression at the post-transcriptional level is by the 2-5A synthetase/RNase L system (reviewed in ref. 30). The role of this system in the antiproliferative activity of IFN was considered (31) but there has been no demonstration of a specific cellular double-stranded RNA to activate the 2-5A synthetase. However, Battey *et al.* (26) and Saito *et al.* (32) have reported that c-*myc* mRNA has the capacity to form double-stranded RNA structures either within the first exon or between the first and the second exons. Saito *et al.* have proposed that such a structure may regulate c-*myc* expression at the translational level because the AUG codon would be sequestered in the secondary structure and not readily accessible for ribosome binding. Since, in Daudi cells, the 2-5A synthetase mRNA is induced at the time the steady-state level of c-*myc* mRNA is reduced, we suggest that this system may play a role in the post-transcriptional regulation of c-*myc* mRNA.

The role of expression of the c-*myc* gene in the regulation of cell growth remains to be proven. However, the use of agents such as the IFNs should provide data that will help elucidate the molecular mechanisms that regulate cell proliferation.

Note Added in Proof. Since communication of this manuscript, Mechti *et al.* (33) have also reported that the c-*myc* mRNA is regulated at a post-transcriptional level in IFN-treated Daudi cells.

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1. Kelly, K., Cochran, B. H., Stiles, C. D. & Leder, P. (1983) *Cell* **35**, 603–610.
2. Campisi, J., Gray, H. E., Pardee, A. B., Dean, M. & Sonenshein, G. E. (1984) *Cell* **36**, 241–247.
3. Hayward, W. S., Neel, B. G. & Astrin, S. M. (1981) *Nature (London)* **290**, 475–480.
4. Leder, P., Battey, J., Lenoir, G., Moulding C., Murphy, W., Potter, H., Stewart, T. & Taub, R. (1983) *Science* **222**, 765–771.
5. Eva, A., Robbins, K. C., Anderson, P. R., Srinivasan, A., Tronick, S. R., Reddy, E. P., Nelson, W. E., Galen, W. T., Lautenberger, J. A., Papas, T. S., Westin, E. H., Wong-Stahl, F., Gallo, R. C. & Aaronson, S. A. (1982) *Nature (London)* **295**, 116–119.
6. Land, H., Parada, L. F. & Weinberg, R. A. (1983) *Nature (London)* **304**, 596–602.
7. Reitsma, P. H., Rothberg, P. G., Astrin, S. M., Trial, J., Bar-Shavit, Z., Hall, A., Teitelbaum, S. L. & Kahn, A. J. (1983) *Nature (London)* **306**, 492–494.
8. Paucker, K., Cantell, K. & Henle, W. (1962) *Virology* **17**, 324–338.
9. Samid, D., Cheng, E. H. & Friedman, R. M. (1984) *Biochem. Biophys. Res. Commun.* **119**, 21–28.
10. Lin, S. L., Garber, E. A., Wang, E., Caliguiri, L. A., Schellekens, H., Goldberg, A. R. & Tamm, I. (1983) *Mol. Cell. Biol.* **3**, 1656–1664.
11. Soslau, G., Bogucki, A. R., Gillespie, D. & Hubbell, H. R. (1984) *Biochem. Biophys. Res. Commun.* **119**, 941–948.
12. Klein, E., Klein, G., Nadkarni, J. S., Nadkarni, J. J., Wigzell, H. & Clifford, P. (1967) *Cancer Res.* **28**, 1300–1310.
13. Adams, A., Strander, H. & Cantell, K. (1975) *J. Gen. Virol.* **28**, 207–217.
14. Hilfenhaus, J., Damm, H., Karges, H. E. & Mantley, K. F. (1976) *Arch. Virol.* **51**, 87–97.
15. Jonak, G. J. & Knight, E., Jr. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1747–1750.
16. Knight, E., Jr., & Fahey, D. (1981) *J. Biol. Chem.* **256**, 3609–3611.
17. Hofer, E., Hofer-Warbinek, R. & Darnell, J. E., Jr. (1982) *Cell* **29**, 887–893.
18. Soeiro, R. & Darnell, J. E. (1969) *J. Mol. Biol.* **44**, 551–562.
19. Groudine, M., Peretz, M. & Weintraub, H. (1981) *Mol. Cell. Biol.* **1**, 281–288.
20. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
21. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
22. Gurney, T., Jr., Sorenson, D. K., Gurney, E. J. & Wills, N. M. (1982) *Anal. Biochem.* **125**, 80–90.
23. Wiman, K. G., Clarkson, B., Hayday, A. C., Saito, H., Tonegawa, S. & Hayward, W. S. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6798–6802.
24. Larner, A. C., Jonak, G., Cheng, Y.-S. E., Korant, B., Knight, E. & Darnell, J. E., Jr. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6733–6737.
25. Merlin, G., Chebath, J., Benech, P., Metz, R. & Revel, M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4904–4908.
26. Battey, J., Moulding, C., Taub, R., Murphy, W., Stewart, T., Potter, H., Lenoir, G. & Leder, P. (1983) *Cell* **34**, 779–787.
27. Nevins, J. R. & Darnell, J. E. (1978) *Cell* **15**, 1477–1493.
28. Shaw, A. & Ziff, E. (1980) *Cell* **22**, 905–916.
29. Kaufman, R. J. & Sharp, P. A. (1983) *Mol. Cell. Biol.* **3**, 1598–1608.
30. Lengyel, P. (1982) *Annu. Rev. Biochem.* **51**, 251–282.
31. Kimchi, A., Shure, H. & Revel, M. (1979) *Nature (London)* **282**, 849–851.
32. Saito, H., Hayday, A. C., Wiman, K., Hayward, W. S. & Tonegawa, S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 7476–7480.
33. Mechti, N., Blanchard, T. M., Piechaczyk, M., Dani, C., Jeanteur, P. H. & Lebleu, B. (1984) *Biol. Cell* **52**, 106a.