stimulated by the preovulatory gonadotropin surge suggests that the half-lives of the inhibin mRNAs are likely to be very short. This is consistent with inhibin production being tightly regulated in response to transient conditions during the estrous cycle, and suggests that the major site of control of inhibin biosynthesis is at the level of transcription. Our results indicate that one component of this control is likely to be the gonadotropin surges. Further elucidation of the molecular mechanisms by which gonadotropins and other hormonal and developmental cues modulate inhibin biosynthesis promises to enhance our understanding of the control of the mammalian reproductive system.

REFERENCES AND NOTES

1. A. N. Hirshfield, *Biol. Reprod.* **34**, 229 (1987); J. S. Richards *et al.*, *Recent Prog. Horm. Res.* **43**, 231 (1987); A. J. W. Hsueh, E. Y. Adashi, P. B. C. Jones, T. H. Welsh, *Endocr. Rev.* **5**, 76 (1984); S. Chappel, *ibid.* **4**, 179 (1983); A. Amsterdam and S. Rotmensch, *ibid.* **8**, 309 (1987).
2. A. V. Schally *et al.*, *Science* **173**, 1036 (1971).
3. R. T. Savoy-Moore and N. B. Schwartz, in *Reproductive Physiology*, R. O. Greep, Ed. (University Park Press, Baltimore, 1980), vol. 3, pp. 203–248.
4. F. H. Bronson and C. P. Channing, *Endocrinology* **103**, 1894 (1978); S. C. Chappel, J. A. Holt, H. G. Spies, *Proc. Soc. Exp. Biol. Med.* **163**, 310 (1980); K. F. Miller, J. A. Wesson, O. J. Ginther, *Biol. Reprod.* **21**, 867 (1979); R. L. Stouffer, J. L. Coensgen, G. S. di Zegera, G. D. Hodgen, in *Dynamics of Ovarian Function*, N. B. Schwartz and M. Hunzicker-Dunn, Eds. (Raven, New York, 1981), pp. 185–190; C. P. Channing, K. Tanabe, H. D. Guthrie, S. K. Batta, D. J. Hoover, in *Intragonadal Regulation of Reproduction*, P. Franchimont and C. P. Channing, Eds. (Academic Press, New York, 1981), pp. 365–376.
5. M. L. Marder, C. P. Channing, N. B. Schwartz, *Endocrinology* **101**, 1639 (1977).
6. J. C. Hoffmann, J. R. Lorenzen, T. Weil, N. B. Schwartz, *ibid.* **105**, 200 (1979); N. B. Schwartz and C. P. Channing, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5721 (1977); R. Welschen, J. Dullaart, F. H. de Jong, *Biol. Reprod.* **18**, 421 (1978).
7. J. Rivier, J. Spiess, R. McClintock, J. Vaughan, W. Vale, *Biochem. Biophys. Res. Commun.* **133**, 120 (1985); D. M. Robertson *et al.*, *ibid.* **126**, 220 (1985); K. Miyamoto *et al.*, *ibid.* **129**, 396 (1985); N. Ling *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7217 (1985).
8. W. Vale *et al.*, *Nature (London)* **321**, 776 (1986); N. Ling *et al.*, *ibid.*, p. 779.
9. T. K. Woodruff, H. Meunier, P. B. C. Jones, A. J. W. Hsueh, K. E. Mayo, *Mol. Endocrinol.* **1**, 561 (1987).
10. F. S. Esch *et al.*, *ibid.*, p. 338; A. J. Mason *et al.*, *Nature (London)* **318**, 659 (1985); R. G. Forage *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 3091 (1986); K. E. Mayo *et al.*, *ibid.*, p. 5849.
11. N. B. Schwartz and W. L. Talley, *Biol. Reprod.* **17**, 820 (1978); L. V. De Paulo, D. Shandler, P. Wise, C. Barrclough, C. P. Channing, *Endocrinology* **105**, 647 (1979).
12. D. C. Hoak and N. B. Schwartz, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4953 (1980).
13. R. T. Savoy-Moore, N. B. Schwartz, J. A. Duncan, J. C. Marshall, *Science* **209**, 942 (1980).
14. N. B. Schwartz, in *Intraovarian Control Mechanisms*, C. P. Channing and S. J. Segal, Eds. (Plenum, New York, 1982), pp. 15–36.
15. Sprague-Dawley CD rats 60 days old (Charles River Laboratories) were housed in temperature-controlled quarters on a light:dark cycle at a ratio of 14 to 10 with lights on at 0500 hours. Water and laboratory chow were continuously available. Cycle stage was initially assessed by daily inspection of vaginal cytology. Only those animals displaying two consecutive 4-day estrous cycles were used. We confirmed the cycle stage by several criteria, including uterine and oviduct ballooning, uterine intraluminal water weight, histological detection of ova in oviducts, and serum hormone concentrations. Four animals were killed at each selected time point during the 4-day estrous cycle. All animals were killed within 30 seconds of handling to minimize stress effects on serum hormone levels. One ovary and a lobe of liver were removed and immediately frozen on dry ice. The oviduct from the second ovary was promptly observed under a dissecting microscope for ballooning or for ova and was fixed for histological analysis.
16. S. R. Davis *et al.*, *Biochem. Biophys. Res. Commun.* **138**, 1191 (1986).
17. P. Cuevas *et al.*, *ibid.* **142**, 23 (1987).
18. T. K. Woodruff, J. D'Agostino, N. B. Schwartz, K. E. Mayo, in preparation.
19. C. Rivier, J. Rivier, W. Vale, *Science* **234**, 205 (1986); T. A. Bicsak *et al.*, *Endocrinology* **119**, 2711 (1986).
20. Y. Hasegawa *et al.*, in *Inhibin-Non-Steroidal Regulation of Follicle Stimulating Hormone Secretion*, H. G. Burger, D. M. deKretser, J. K. Findlay, M. Igarashi, Eds. (Raven, New York, 1987), pp. 119–133.
21. K. Zinn, D. DiMaio, T. Maniatis, *Cell* **34**, 367 (1983); D. A. Melton *et al.*, *Nucleic Acids Res.* **12**, 7035 (1984).
22. P. Chomczynski and N. Sacchi, *Anal. Biochem.* **162**, 156 (1987).
23. Trunk blood was collected and allowed to clot overnight at 4°C. After centrifugation, serum was divided into aliquots and frozen at −20°C. LH was measured in duplicate by the ovine-ovine radioimmunoassay with NIH-LH-S16 used as the standard and GDN15 antiserum to LH. FSH was determined in duplicate by the rat-rat system with FSH-RP1 as the standard (from NIH). The assay coefficient of variation for low and high serum pools was 6 and 5% for LH, and 5 and 3% for FSH.
24. A. Haase, M. Brahic, L. Stowring, H. Blum, *Methods Virol.* 7, 189 (1984).

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Alternative Mechanisms for Activation of Human Immunodeficiency Virus Enhancer in T Cells

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The expression of human immunodeficiency virus (HIV) after T cell activation is regulated by NF-κB, an inducible DNA-binding protein that stimulates transcription. Proteins encoded by a variety of DNA viruses are also able to activate expression from the HIV enhancer. To determine how this activation occurs, specific genes from herpes simplex virus type 1 and adenovirus that activate HIV in T lymphoma cells have been identified. The cis-acting regulatory sequences in the HIV enhancer that mediate their effect have also been characterized. The relevant genes are those for ICP0—an immediate-early product of herpes simplex virus type 1—and the form of E1A encoded by the 13*S* messenger RNA of adenovirus. Activation of HIV by adenovirus E1A was found to depend on the TATA box, whereas herpesvirus ICP0 did not work through a single defined cis-acting element. These findings suggest multiple pathways that can be used to bypass normal cellular activation of HIV, and they raise the possibility that infection by herpes simplex virus or adenovirus may directly contribute to the activation of HIV in acquired immunodeficiency syndrome by mechanisms independent of antigenic stimulation in T cells.

EXPRESSION OF HUMAN IMMUNODEFICIENCY virus (HIV) increases after activation of inducer T cells by phorbol esters and lectins (*1, 2*). This stimulation is mediated by NF-κB (*3*), a factor that regulates transcription and binds to the twice-repeated 11-bp κB motif in the HIV enhancer (Fig. 1). This 11-bp motif is also found in the immunoglobulin light chain enhancer (*4*). Mutations of nucleotides within these sites that eliminate the binding of NF-κB also abolish the increase in HIV gene expression seen in activated T cells (*3*). DNA from primate viruses induced HIV expression when cotransfected into fibroblasts with a plasmid containing the HIV enhancer linked to the chloramphenicol acetyltransferase (CAT) gene (*5–7*), but the

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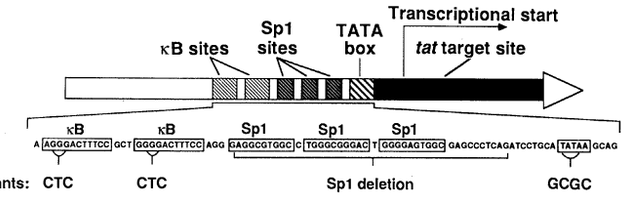

Fig. 1. Schematic representation of HIV-CAT plasmid and mutant plasmids. Mutant plasmids contained either alterations in both κB sites as shown (mutant κB), deletion of all Sp1 sites (mutant Sp1), alteration of TATA box (mutant TATA), or alteration of both the κB and TATA sites (mutant κB plus TATA) as shown. The κB sites were mutated as described (*3*). The TATA box was altered, and Sp1 sites were deleted as shown by using the gap heteroduplex method for site-directed mutagenesis (*3*).

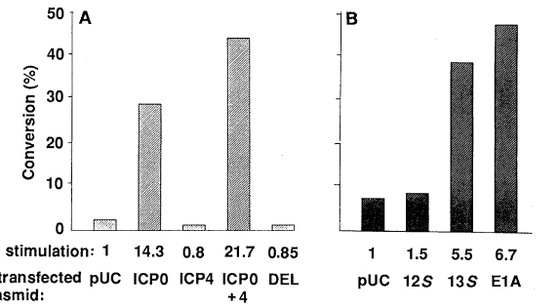

Fig. 2. Effect of DNA virus genes on HIV-CAT expression. (**A**) Effect of herpes simplex virus immediate-early products. The HIV-CAT plasmid (5 μg) was cotransfected into Jurkat *tat*-III cells (10^7) by using DEAE-dextran (*17*) with 5 μg of pUC13, or plasmid containing herpes simplex virus ICP0 (pSHZ), ICP4 (pEK4), ICP0 in combination with ICP4 (pSG1), or a deletion (DEL) mutant of ICP0 (pSHZ-d11). Conversion of chloramphenicol to its acetylated forms was determined (*3*), and relative stimulations by the cotransfected plasmids are indicated. The plasmid pSHZ contains a 4.7-kb Sac I to Hpa I insert encoding the intact ICP0 gene (*43*) cloned into pUC-19. A deletion derivative, pSHZ-d11, was made by deleting a 2.1-kb Xho I to Sal I fragment from pSHZ, removing a large segment of the ICP0 coding region (*36*). The plasmid pEK4, encoding ICP4, contains a previously described 8.5-kb Eco RI to Kpn I insert (*36*) cloned into pUC19. The plasmid pSG1 (*44*) contains an 18-kb insert that encodes both ICP0 and ICP4. (**B**) Effect of adenovirus E1A, and 12*S* and 13*S* mRNA products of E1A on HIV-CAT expression. HIV-CAT (5 μg) was cotransfected into Jurkat *tat*-III cells (10^7) by using DEAE-dextran (*17*) with 5 μg of pUC13, a plasmid containing the 12*S* subunit of E1A (DOL-12*S*), a plasmid with the 13*S* subunit of E1A (DOL-13*S*), or the adenovirus DNA fragment H3G (*20*) containing the entire E1A gene. DOL-12*S* and DOL-13*S* were derived by ligation of E1A complementary DNAs, as described (*45*), into the Bam HI cloning site of DOL. For data in Figs. 2 to 4, no less than two replicate plasmids were analyzed for each group presented and independent transfections were performed in duplicate or triplicate. Values reported are accurate to a SD ≤10%.

DNA virus transactivators and their sites of action in HIV were unclear.

Herpes simplex virus type 1 encodes several gene products that activate transcription of other genes. One set of herpesvirus transactivators is synthesized immediately after infection (immediate-early gene products). These include the known transactivating proteins ICP0 (ICP, infected cell protein), which is 110 kD in size as determined by SDS–polyacrylamide gel electrophoresis, and ICP4, which is 175 kD (*8*). ICP4 is required for later gene transcription essential to viral replication, and both gene products can stimulate viral gene expression in transfected cells.

Similar to herpesvirus, adenovirus encodes polypeptides expressed early after infection that are required for virus replication. The E1A gene, located at the left end of the adenovirus genome, codes for three overlapping messenger RNAs (mRNAs) that share a common 5′ end but are differentially spliced (*9*). Two of these transcripts, the 13*S* and the 12*S* forms of E1A, are the first products expressed after adenovirus infection, and they encode polypeptides of 288 and 242 amino acids, respectively (*10*). They differ only by the presence of an additional 46 amino acids in the 13*S* form (*11*). The phosphoprotein encoded by the 13*S* transcript regulates mRNA levels of other early viral and cellular genes, whereas the 12*S* form stimulates the growth of adenovirus in quiescent cells (*12–16*). The relative contributions of these proteins to viral replication and cellular transformation are unknown.

To determine which transactivators from herpes simplex virus and adenovirus were responsible for activation of HIV, we cotransfected known transactivator genes from these viruses with an HIV-CAT plasmid. To determine the sites of action, we examined expression by using HIV-CAT plasmids containing mutations in the κB, Sp-1, or TATA sites (Fig. 1).

Plasmid DNAs that encoded known viral transactivators from either herpes simplex virus type 1 or adenovirus type 5 were cotransfected (*17*) with an HIV-CAT plasmid [pURIII-CAT (*18*)] into Jurkat *tat*-III cells (*19*). The HIV-CAT plasmid contained the *tat*-III target region, thus allowing us to increase the sensitivity of the system by using Jurkat cells that stably expressed the *tat*-III product. To validate the use of *tat*-III in this way, we first studied a plasmid in which the *tat*-III target region was deleted. This deletion decreased basal activity 15-fold, but cotransfection with ICP0 or E1A increased CAT activity 16- and 8-fold, respectively, indicating that the stimulatory activity of these proteins is independent of the *tat*-III gene. Cotransfection of Jurkat *tat*-III cells with HIV-CAT and plasmids containing ICP0 or ICP0 plus ICP4 resulted in a 14- to 22-fold activation of HIV-CAT expression (Fig. 2A). Neither the ICP4 gene alone nor pUC13, which resembled the vector for the viral genes, caused activation. Similarly, cotransfection of the E1A gene (*20*) increased HIV-CAT expression 5- or 6-fold in Jurkat *tat*-III cells, and the 13*S* but not the 12*S* product mediated this effect (Fig. 2B).

When Jurkat *tat*-III cells were transfected with a mutant HIV-CAT plasmid, which no longer binds NF-κB (Fig. 1), little or no increase in CAT activity was found in activated T cells compared to resting cells (Fig. 3). This result had been described with Jurkat cells that do not express *tat*-III (*3*). When either ICP0 or E1A genes were cotransfected with these mutant plasmids, however, a 15- to 20-fold stimulation of CAT activity was evident (Fig. 4A). Thus, these genes function as stimulators by a mechanism apparently independent of the NF-κB binding site.

To localize other sites critical to the activity of ICP0 or E1A, we used plasmids either deleted of their Sp1 sites, mutated in the TATA site, or mutated in both κB and TATA. The plasmid with deleted Sp1 sites had a somewhat reduced basal expression but was stimulated 5- to 20-fold by the viral activator genes (Fig. 4B). Mutation of the TATA also reduced activity (Fig. 4C) and abolished the ability of E1A to stimulate, although it had little effect on ICP0 stimulation (Fig. 4C). For example, ICP0 could stimulate even when both the κB and TATA sites were altered (Fig. 4C) and was active when the entire enhancer (U3 region) except for the TATA box and 8 bp of adjacent upstream sequence was removed. Thus E1A appears to depend on the TATA site, but ICP0's action is independent of any single defined site and may not require a cis-active element at all.

Whether herpesvirus or adenovirus can actually potentiate HIV production in an infected individual is not known, but it is evident in this model system that genes of the two viruses stimulate HIV-mediated CAT production by disparate mechanisms, both unrelated to the potentiation of CAT

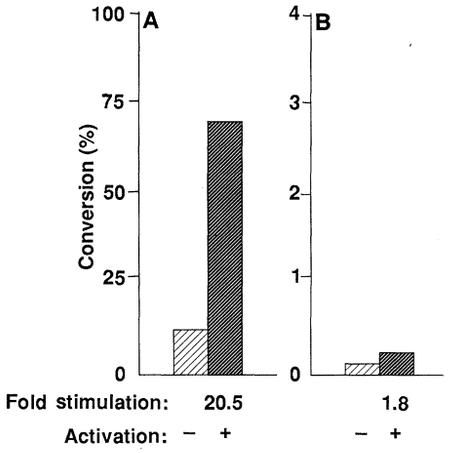

Fig. 3. Expression of HIV-CAT (**A**) and HIV-CAT κB mutant (**B**) activity after activation of Jurkat *tat*-III cells. To determine expression of HIV-CAT in activated cells, Jurkat *tat*-III cells were transfected with HIV-CAT or mutant κB plasmids and activated with phorbol myristate acetate and phytohemagglutinin as described (*3*). The percentage conversion of chloramphenicol to its acetylated form was determined (*3*), and the fold induction for HIV-CAT or mutant HIV-CAT plasmids was calculated.

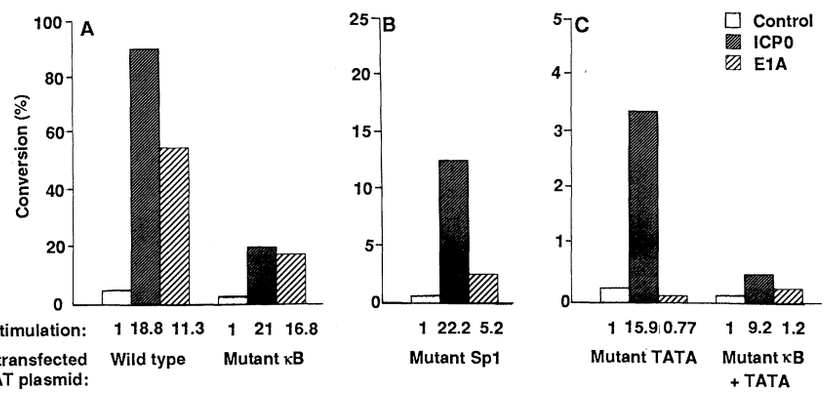

Fig. 4. Stimulation of HIV-CAT variants in the presence of ICP0 or adenovirus E1A. Plasmids encoding ICP0 or E1A (5 μg) were cotransfected with mutant plasmids (5 μg) by using DEAE dextran (*17*) into Jurkat *tat*-III cells. To determine control levels of activity, HIV-CAT was cotransfected with the ICP0-deletion mutant (Fig. 2). Similar levels of activity were found when cotransfection with pUC13 was performed.

synthesis during activation of T cells. Both the ICP0 and E1A products are known to affect the activity of other genes, although there is little evidence that either transactivator interacts directly with DNA. However, E1A can inhibit transcriptional activity under some conditions, as in simian virus 40 (SV40), adenovirus, polyoma, or immunoglobulin enhancer–dependent systems (*21–24*). Type 12 adenovirus E1A can also decrease expression of class I major histocompatibility molecules (*25–27*). In other instances, E1A can activate transcription, as with several early adenovirus genes (*11, 12, 14, 15*), and with certain cellular genes, such as β-tubulin or heat shock (*28, 29*), or transfected preproinsulin or β-globin genes (*30–32*). In the case of adenovirus E1A activating E1B, the enhanced expression also depends on the TATA box and appears to alter the rate but not the site of transcriptional initiation (*33*). We do not yet know whether E1A affects the rate or the site of initiation in the activation of HIV since we cannot detect signals by S1 nuclease analysis using RNA expressed in cells transfected with the TATA box mutant. Regardless of which mechanism is operative, however, E1A stimulation appears independent of the κB regulatory site.

Although ICP0 appears to activate many cellular and viral genes, its mechanism of action is not understood. This 110-kD immediate-early polypeptide of herpes simplex virus can enhance expression of immediate-early, early, and late herpes simplex promoters (*34–39*), as well as the SV40 early promoter (*40*). These promoters do not appear to share a common cis-acting regulatory sequence, suggesting that ICP0 may not act through a unique cis element. Although ICP0 can increase the levels of mRNA expressed from these genes (*37, 41*), it has not been proved that this effect is transcriptional. It remains possible that ICP0 affects gene expression at a post-transcriptional level, such as RNA transport or stability. Mosca *et al.* have also shown that ICP0 activates the HIV enhancer (*42*). They observed no stimulation when 5′ deletions extended through the region containing κB and Sp1 sites, possibly because of the use of deletion rather than point mutation of the enhancer, because their transfections were performed in non-T cells, or because of lower sensitivity in the CAT assay. The use of *tat*-III–infected Jurkat cells in our system allows measurement of signals that would otherwise be undetectable.

It is evident that HIV RNA accumulation is sensitive to various influences. The effects described here are distinct from activation by the *tat*-III gene product. Furthermore, the κB-dependent effect of T cell activation is quite separate from the effects of ICP0 and E1A, suggesting that these latter agents might activate HIV in the absence of immune stimulation in an infected T cell. These suggestions, generated from examination of cell culture systems and transiently transfected plasmids, must be tested in more clinical settings to determine whether they apply to the progression of HIV-induced disease.

REFERENCES AND NOTES

1. D. Zagury *et al.*, *Science* **231**, 850 (1986).
2. S. Harada *et al.*, *Virology* **154**, 249 (1986).
3. G. Nabel and D. Baltimore, *Nature (London)* **326**, 711 (1987).
4. R. Sen and D. Baltimore, *Cell* **46**, 705 (1986).
5. H. E. Gendelman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9759 (1986).
6. J. D. Mosca *et al.*, *Nature (London)* **325**, 67 (1987).
7. R. F. Rando, P. E. Pellett, P. A. Luciw, C. A. Bohan, A. Srinivasan, *Oncogenes* **1**, 13 (1987).
8. Reviewed by G. S. Hayward, in *Cancer Cells*, vol. 4, *DNA Tumor Viruses: Control of Gene Expression and Replication*, T. Grodzicker, M. Botchan, P. A. Sharp, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1986), pp. 59–77.
9. Reviewed by M. S. Horwitz, in *Virology*, B. Fields, Ed. (Raven Press, New York, 1985), pp. 433–476.
10. M. Perricaudet, G. Akusjärvi, A. Virtanen, U. Pettersson, *Nature (London)* **281**, 694 (1979); H. J. van Ormandt, J. Maat, R. Dijkema, *Gene* **12**, 63 (1980).
11. R. P. Ricciardi *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6121 (1981).
12. A. J. Berk *et al.*, *Cell* **17**, 935 (1979).
13. L. R. Carlock and N. C. Jones, *J. Virol.* **40**, 657 (1981).
14. N. Jones and T. Shenk, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3665 (1979).
15. C. Montell, E. F. Fisher, M. H. Caruthers, A. J. Berk, *Nature (London)* **295**, 380 (1982).
16. J. Nevins, *Cell* **29**, 913 (1982).
17. C. Queen and D. Baltimore, *ibid.* **33**, 741 (1983).
18. C. A. Rosen, J. Sodroski, W. A. Haseltine, *ibid.* **41**, 813 (1985).
19. W. C. Greene *et al.*, *Science* **232**, 877 (1986).
20. A. Fire *et al.*, *J. Virol.* **40**, 703 (1981).
21. E. Borrelli, R. Hen, P. Chambon, *Nature (London)* **312**, 608 (1984).
22. A. Velcich and E. Ziff, *Cell* **40**, 705 (1985).
23. D. H. Smith, M. Kegler, E. B. Ziff, *Mol. Cell. Biol.* **5**, 2684 (1985).
24. R. Hen *et al.*, *Science* **230**, 1391 (1985).
25. R. Bernards *et al.*, *Nature (London)* **305**, 776 (1983).
26. K. B. Eager *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5525 (1985).
27. R. Vasavada, K. B. Eager, G. Barbanti-Brodano, A. Caputo, R. P. Ricciardi, *ibid.* **83**, 5257 (1986).
28. R. Stein and E. B. Ziff, *Mol. Cell. Biol.* **4**, 2792 (1984).
29. H.-T. Kao and J. R. Nevins, *ibid.* **3**, 2058 (1983).
30. C. Svensson and G. Akusjärvi, *EMBO J.* **3**, 789 (1984).
31. R. B. Gaynor, D. Hillman, A. J. Berk, *Proc. Natl.*

Acad. Sci. U.S.A. **81**, 1193 (1984).
32. M. Green, R. Treisman, T. Maniatis, *Cell* **35**, 137 (1983).
33. L. Wu, D. S. E. Rosser, M. C. Schmidt, A. Berk, *Nature (London)* **326**, 512 (1987).
34. R. D. Everett, *EMBO J.* **3**, 3135 (1984).
35. P. O'Hare and G. S. Hayward, *J. Virol.* **53**, 751 (1985).
36. M. P. Quinlan and D. M. Knipe, *Mol. Cell. Biol.* **5**, 957 (1985).
37. I. H. Gelman and S. Silverstein, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5265 (1985).
38. P. O'Hare and G. S. Hayward, *J. Virol.* **56**, 723 (1985).
39. P. Mavromara-Nazos *et al.*, *Virology* **149**, 152 (1986).
40. P. O'Hare, J. D. Mosca, G. S. Hayward, in *Cancer Cells*, vol. 4, *DNA Tumor Viruses: Control of Gene Expression and Replication*, T. Grodzicker, M. Botchan, P. A. Sharp, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1986), pp. 175–188.
41. L. Su and D. M. Knipe, *J. Virol.* **61**, 615 (1987).
42. J. D. Mosca *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 7408 (1987).
43. L. J. Perry, F. J. Rixon, R. D. Everett, M. C. Frame, D. J. McGeoch, *J. Gen. Virol.* **67**, 2365 (1986).
44. A. L. Goldin *et al.*, *J. Virol.* **38**, 50 (1981).
45. B. Roberts *et al.*, *ibid.* **56**, 404 (1985).
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Regulation of Gene Expression by Interferons: Control of H-2 Promoter Responses

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The magnitude of the response to interferons and the requirement for individual elements in the promoter of the H-2D^d gene were shown to be cell-specific and dependent on the type of interferon used. Three DNA sequences in the promoter were found to bind murine nuclear factors. Two of these sequences are in functionally defined enhancer regions and also bind to the transcription factor AP-1. The third sequence is part of the region involved in interferon regulation and is homologous to the enhancer element of the interferon β gene. A model for interferon regulation of H-2 promoters is discussed.

INTERFERONS (IFNs) AFFECT SUCH cellular processes as anti-viral responses, cell growth, differentiation, and gene regulation. Type I IFNs (α and β) and type II IFN (γ) are synthesized by different cells, and it is likely that they use different mechanisms to elicit their different cellular responses (*1–3*).

Major histocompatibility (H-2) class I genes are regulated by type I and type II IFNs (*4*). The murine H-2 class I proteins are highly polymorphic, 45-kD polypeptides that function during antigen presentation as cell-surface recognition molecules for cytotoxic T lymphocytes (*5*) and are expressed on most somatic tissues at tissue-specific levels (*6*). Early embryonic tissues do not express H-2 antigens, although expression can be induced by IFNs (*7*). Adult brain cells respond to IFN γ with a 30-fold increase in the expression of H-2 antigens; heart and kidney cells with a 13- to 17-fold increase; and the tissues that have the highest basal levels of H-2 antigens, like the spleen and lymph nodes, show the least change in expression (*8*).

IFN regulation of class I gene expression involves at least two mechanisms, one dependent on sequences upstream and the other on sequences downstream of the H-2 promoter region (*9–12*). IFN induction utilizing the upstream sequences accounts for less than a twofold enhancement of class I gene expression, corresponding to less than 40% of the entire IFN response in L cells (*10*). It is dependent on the presence of a 37-bp region, designated the IRS (IFN response sequence) (*11*) that lies between nucleotides 159 and 122 upstream of the transcription initiation site of the H-2D^d gene (*10*). Induction of class I expression by type I IFNs in L cells requires the concomitant presence of a second sequence located just upstream of the IRS (*10, 11*); this sequence is not required in NIH 3T3 cells (*12*). These conclusions were based on cells that express high endogenous levels of H-2 antigens that are only moderately inducible (2.5- to 8-fold) by IFNs. We have assessed the relative activities of the upstream regulatory sequences in cells in which H-2 antigen expression is highly inducible by IFNs.

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Table 1. Induction of CAT constructs in different cell types by murine type I and type II IFNs. The enhancement factor is a ratio of specific CAT activity (CAT activity per protein concentration) in cells treated with IFN to that in untreated cells. It is independent of the cotransfection efficiency in the individual pools of cells transformed with a mixture of *neo* gene and CAT constructs in all cell lines used. Ten cloned cell lines from a BL5 transfected cell pool were analyzed as controls; all were positive for CAT expression. The basal levels of CAT gene expression and their ability to respond to IFNs varied moderately between different cloned transfectants. Clone data confirmed results observed on pooled transfectants. Levels of class I antigens on different cell lines were measured with different antibodies by radioimmunoassay (*32*), and compared on a per cell basis with the levels of the class I antigen expression on spleen cells of the same haplotype. L cells expressed 5- to 10-fold higher levels than H-2^k C3H spleen cells, BLK SV cells expressed 3- to 6-fold more than H-2^b C57BL/6 spleen cells, MPC 11 cells expressed 1.5- to 6-fold higher levels than H-2^{d}Balb/c spleen cells, and BL5 cells expressed lower levels of H-2^b antigens than C57BL/6 spleen cells (ranging from 0.25-fold to comparable levels of expression).

Construct	Enhancement factor							
	BL5 + IFN γ		BL5 + IFN (α + β)		BLK SV + IFN γ		BLK SV + IFN (α + β)	
	Range	$\bar{x}$ ± SD (*n*)	Range	$\bar{x}$ ± SD (*n*)	Range	$\bar{x}$ ± SD (*n*)	Range	$\bar{x}$ ± SD (*n*)
L^{d}CAT	3.0– 5.2	4.5 ± 0.7 (3)	2.5– 6.6	4.5 ± 1.1 (4)	5.3– 9.9	8.0 ± 0.9 (5)	4.7–8.0	6.6 ± 0.8 (4)
D^{d}CAT	9.7–10.4	10.1 ± 0.2 (3)	9.9–16.7	12.3 ± 1.5 (4)	5.5–11.8	7.9 ± 0.9 (6)	3.8–8.3	5.8 ± 1.0 (4)
Δ-385CAT	5.2– 6.1	5.5 ± 0.2 (3)	4.9– 6.2	5.6 ± 0.4 (3)				
Δ-236CAT	1.9– 4.9	2.9 ± 1.0 (3)	3.4– 5.6	4.5 ± 0.7 (3)				
Δ-159CAT	2.0– 6.4	4.1 ± 0.5 (9)	4.9– 6.3	5.8 ± 0.2 (6)	1.9– 7.0	3.2 ± 0.6 (8)	0.7–1.4	1.1 ± 0.1 (7)
Δ-122CAT*	0.7– 1.1	1.0 ± 0.1 (3)	0.7– 1.2	1.0 ± 0.1 (4)	0.8– 1.6	1.1 ± 0.1 (5)	1.1–1.2	1.2 ± 0.1 (4)

*Endogenous levels of H-2 antigens in Δ-122CAT transfectants were inducible by IFNs to the same degree as in the untransfected cells. The ability of these molecules to respond to IFN served as an internal control for the noninducible phenotype of Δ-122CAT gene.