50. A. M. Campbell, *Jpn. J. Appl. Phys.* **26**, 2053 (1987); P. Kes, *Physica C* **153–155**, 1121 (1988); J. R. Clem, *ibid.*, p. 50.
51. F. Hellmann *et al.*, *J. Appl. Phys.* **63**, 447 (1988); E. A. Early, C. L. Seaman, K. N. Yang, M. B. Maple, *Am. J. Phys.* **56**, 617 (1988).
52. P. N. Peters, R. C. Sisk, E. W. Urban, C. Y. Huang, M. K. Wu, *Appl. Phys. Lett.* **52**, 2066 (1988); C. Y. Huang *et al.*, *Mod. Phys. Lett.* **B2**, 869 (1988); C. Politis and F. Stubhan, *Mod. Phys. Lett. B* **2**, 1119 (1988).
53. F. C. Moon, M. M. Yanoviak, R. Ware, *Appl. Phys. Lett.* **52**, 1534 (1988).
54. C. Giovannella *et al.*, *J. Appl. Phys.* **63**, 4173 (1988).
55. E. H. Brandt, *Appl. Phys. Lett.* **53**, 1554 (1988); *Am. J. Phys.*, in press.
56. ———, P. Esquinazi, H. Neckel, G. Weiss, *Phys. Rev. Lett.* **56**, 89 (1986); *J. Low Temp. Phys.* **63**, 187 (1986); P. Esquinazi, H. Neckel, G. Weiss, E. H. Brandt, *ibid.* **64**, 1 (1986); E. H. Brandt, *J. Phys. (Paris)* **48**, C8-31 (1987).
57. F. Shiota, K. Hara, T. Hirata, *Jpn. J. Appl. Phys.* **22**, 1439 (1983); *ibid.* **23**, L227 (1984).
58. G. S. La Rue, J. D. Phillips, W. M. Fairbank, *Phys. Rev. Lett.* **46**, 967 (1981); P. F. Smith *et al.*, *Phys. Lett. B* **153**, 188 (1985); *ibid.* **171**, 129 (1986).
59. E. Walker, P. Monnerat, M. Peter, *Physica C* **153–155**, 1425 (1988).
60. I thank my colleagues, in particular U. Essmann, for helpful discussions about this topic.

Contingent Genetic Regulatory Events in T Lymphocyte Activation

GERALD R. CRABTREE

Interaction of antigen in the proper histocompatibility context with the T lymphocyte antigen receptor leads to an orderly series of events resulting in morphologic change, proliferation, and the acquisition of immunologic function. In most T lymphocytes two signals are required to initiate this process, one supplied by the antigen receptor and the other by accessory cells or agents that activate protein kinase C. Recently, DNA sequences have been identified that act as response elements for one or the other of the two signals, but do not respond to both signals. The fact that these sequences lie within the control regions of the same genes suggests that signals originating from separate cell membrane receptors are integrated at the level of the responsive gene. The view is put forth that these signals initiate a contingent series of gene activations that bring about proliferation and impart immunologic function.

ALTHOUGH THE PRECURSORS FOR MOST T CELLS ARISE IN the bone marrow, only after migration to the thymus do they differentiate to express receptors capable of interacting with antigen (*1*). Thymic differentiation involves the rearrangement of genes encoding the antigen receptor (*2*) and the expression of a group of T cell surface proteins that divide mature T cells into those that suppress and those that enhance antibody production (*3*). After the rearrangement of antigen receptor genes, T cells are subject to selective mechanisms in the thymus that lead to the survival of cells able to respond to foreign antigen and the death of cells recognizing self-antigen (*4*). After these critical events, the T lymphocyte migrates through the peripheral blood to other tissues and remains quiescent until it comes into contact with its cognate antigen. A 7- to 10-day process then begins that results in cell division and immunologic functions such as cytotoxicity and the production of lymphokines that induce antibody production by B cells and control the growth of granulocyte and macrophage precursors. The initiation of this process requires a complex interaction of the antigen receptor with the combination of antigen and self-histocompatibility molecules on the surface of antigen-presenting cells. These complex requirements can in part be met by relatively simple stimuli such as calcium ionophores, plant lectins, and antibodies to the antigen receptor that are felt to mimic the effects of physiologic interactions with the T cell receptor. In addition, agents that activate protein kinase C (PKC), such as phorbol myristate acetate (PMA), are felt to mimic a requirement for accessory cells and their products. These aspects of T cell activation have been the subject of several recent reviews (*5*).

Along this pathway to immune function the T cell undergoes morphologic changes (blastogenesis) at about 12 hours, divides by 24 to 48 hours, and then differentiates as genes are sequentially activated for several days. More than 70 molecules are specifically regulated during this process (Table 1). These regulatory events begin within minutes of contact with antigen and continue for at least 10 days. By analogy to viral systems they can be roughly divided into immediate, early, and late genes, although sufficient information is not available to categorize every molecule listed. Table 1 presents data obtained primarily from human peripheral blood T cells; however, data are also included from murine nonmalignant T cell lines that may have differences in the degree or kinetics of induction of the molecules listed.

This complex sequence of gene activations leading to immunologic function can be approached by making analogies to several well-studied systems of differentiation. Generalities learned from these systems include the concept of commitment, after which differentiation progresses in a relatively autonomous way, and the concept of contingent regulatory events that provide temporal order to the process of morphologic and functional change. Commitment is used here to denote a programming event that may not be irreversible (*6*). Contingency simply indicates that a later event is dependent on an earlier event without implying a direct mechanism. These fundamental levels of control generally act by regulation of genes that commit a cell to a specific pathway of differentiation or allow it to proceed along a pathway. Recent developments have made it

The author is in the Department of Pathology, Stanford University Medical School, and Howard Hughes Medical Institute, Stanford, CA 94305.

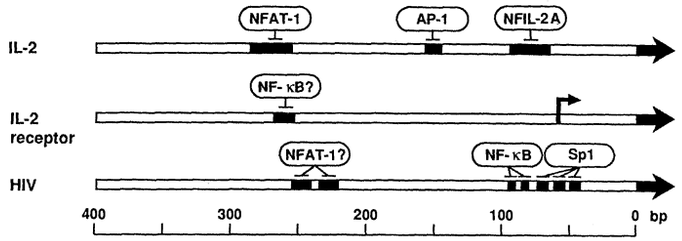

Fig. 1. Functional sequences and sites of DNA-protein interactions within the regulatory regions of the IL-2, IL-2 receptor genes, and the LTR of HIV. Sequences protected from DNase I digestion by nuclear extracts are shown as open boxes and identified as the binding site for the indicated nuclear factors. Numbers given at the bottom of the figure represent the position in base pairs relative to the initiation site of transcription. Sites that have not yet been clearly established are indicated with a question mark.

possible to search for the molecular basis of commitment and begin to identify the contingent regulatory events guiding T cell activation.

The Commitment to T Lymphocyte Activation

The commitment period for T cell activation has been defined with the use of concanavalin A (Con A), a plant lectin whose ability to activate T cells depends on the antigen receptor (*7, 8*). Cell-bound Con A can be removed by washing in the presence of α-methyl mannoside (*9*), which binds Con A and prevents binding to the T cell surface. The removal can be accurately followed by means of radiolabeled lectin. These studies indicated that only 2 hours of exposure are required to obtain maximal incorporation of [^{3}H]thymidine at 48 hours (*10*). In a second approach antibodies to the histocompatibility antigen, H-2K^b, were used to block activation of a murine H-2K^b–specific T cell clone. After as little as 1 hour the T cells are significantly refractory to the effects of the H-2K^b antibody and after 3 hours are fully refractory (*11*). In both of these studies, functions acquired late in T cell activation were not tested, and the commitment period may not be the same for all T cell subsets and all immunologic functions. Nevertheless, these studies indicate that essential events occur during the first 2 hours that are sufficient for DNA synthesis and morphological changes.

The membrane events initiated when antigen/major histocompatability complex (MHC) binds to the T cell antigen receptor are similar to those initiated by many hormones. Inositol phospholipids are hydrolyzed (*12*), intracellular stores of Ca^{2+} are mobilized (*13*), and membrane and cytoplasmic proteins are phosphorylated (*14*). The role of these events in conveying a signal to the nucleus is not clear, since recent reports indicate that interleukin-2 (IL-2) gene expression and proliferation can be initiated in the absence of phosphoinositide hydrolysis or an increase in intracellular calcium concentration ($[Ca^{2+}]_i$) (*15*). The immediate cell membrane and cytoplasmic events associated with triggering the antigen receptor are essentially complete within the first 5 minutes and yet 2 hours are required for commitment to T cell activation.

One possible way of interpreting the 2-hour requirement is that continuous contact with the antigen receptor–T3 complex is essential to maintain the concentration of a labile second messenger. Intracellular Ca^{2+} has been proposed to serve this function, since the $[Ca^{2+}]_i$ does not return to normal but rather intracellular levels fluctuate for more than an hour (*16–18*). Two hours of exposure to Con A are required for the activation of the IL-2 gene, corresponding closely to both the time during which alterations in $[Ca^{2+}]_i$ are maintained as well as the time during which commitment takes place (*19*). A T cell mutant with no sustained increase in $[Ca^{2+}]_i$ was defective in activation of the IL-2 gene (*16*), strengthening the correlation between a sustained increase in $[Ca^{2+}]_i$ and IL-2 gene activation. Other labile second messengers that might serve a role in commitment are transient phosphorylations noted after activation through the antigen receptor (*14, 20*), although a function for these events has not been discerned. One model is that spatially and temporally distributed Ca^{2+} fluxes between different cellular compartments produce sustained cellular activation (*18*). The view put forth here is that these second messengers are essential to activate critical genes that commit a T cell to activation.

If we assume that 2 hours of antigen stimulation are necessary to commit a cell to activation, then none of the genes listed in Table 1 activated before 2 hours are sufficient for commitment, but any of them might play a cooperative role. Nevertheless, genes activated at approximately 2 hours after stimulation could conceivably have this pivotal role. The recent identification of a single gene able to convert fibroblasts to muscle (*21*), as well as the necessary and sufficient role of the *lin 12* gene in determining cell type in *Caenorhabditis elegans* (*22*), indicates that it is not completely naïve to search for a gene with this type of function in T cell activation. Such an approach depends on the accuracy of precisely measuring the commitment period, defined here as the time interval during which lectin or antigen must be bound to lead to activation.

Several indirect lines of evidence indicate that the activation of IL-2 and its receptor might reflect a commitment event. (i) Since the same cell both produces IL-2 and responds to it (*23*), at least a subpopulation of T cells have an autocrine mode of proliferation; thus IL-2 and its receptor are required for at least the proliferative aspect of activation. (ii) Entry into the S phase of the cell cycle is dependent on the IL-2 concentration and the number of expressed IL-2 receptors per cell (*24*), implying that subsequent events are regulated by this step. (iii) The genes encoding both IL-2 and its receptor are expressed at about the time that T cells become committed to activation (*20, 25–27*). Presumably the mechanisms underlying commitment act on both genes. The additional information that these two genes are coactivated with the human immunodeficiency virus (HIV-1) (*28, 29*), are regulated by the *tat*-1 gene of the human T cell leukemia virus (HTLV-I) (*30, 31*), and may have a role in transformation lends additional interest to a search for the events controlling these two genes.

IL-2 Gene Activation and the Search for the Molecular Basis of Commitment

Most of the known regulatory influences over the IL-2 gene appear to be mediated by a transcriptional enhancer lying between −319 and −52 bp 5′ to the transcription initiation site of the gene (*30, 32–36*) (Fig. 1). The functional properties of the enhancer have been studied by testing its ability to direct transcription of indicator genes such as the chloramphenicol acetyltransferase (CAT) gene (*37*) or the firefly luciferase gene (*38*) coupled to a ubiquitously expressed promoter. When this three-part fusion gene is introduced into cell lines, it is expressed only after activation through the antigen receptor and only in T cells (*33*). In a more rigorous test of tissue specificity, the expression of a transgene in which the IL-2 enhancer–directed expression of the murine c-*myc* gene was restricted to activated T cells (*39*). T cells normally require both triggering of the antigen receptor and activation of PKC for IL-2 production and proliferation (*20, 40*). Likewise, the enhancer requires both of these signals for its function in transfected T cell lines (*33*). In a mutant cell line lacking the antigen receptor, neither the endogenous IL-2 gene nor the transfected IL-2 enhancer functions in response to lectins or antibodies to the antigen receptor (*32*).

However, the requirement for antigen receptor stimulation can be bypassed if the mutant cells are incubated with calcium ionophore and PMA, an activator of PKC (*33*). Furthermore, as with the endogenous IL-2 gene, the function of the transfected IL-2 enhancer can be completely inhibited with cyclosporin A (CsA) (*41*). Finally, a transfected IL-2–CAT construct containing the IL-2 enhancer that directs transcription through its own promoter can be activated by cotransfection of an HTLV-I tat-1–producing construct (*30, 31, 42, 43*). Thus, the IL-2 enhancer transmits all known physiologic and pathophysiologic regulatory information for the IL-2 gene.

The sequences within the IL-2 enhancer mediating these functions were defined more precisely by comparing the activity of internal deletion mutants and clustered base-pair mutants with the wild-type enhancer, after transfection into the Jurkat T cell line (*32, 36*). This approach identified four regions, deletions of which impair the ability of the enhancer to activate a linked promoter in response to signals from the antigen receptor. A more rigorous and positive test of function is to investigate the ability of multimers of an individual site to activate a linked promoter. When this test was applied to the sequences identified from the deletion analysis, two of the four sequences, −285 to −255 and −93 to −63, will activate a

Table 1. T lymphocyte activation molecules. The categories are based on an analogy to viral gene expression. Immediate events are independent of protein synthesis; early events require protein synthesis (data not always available) but precede cell division, and late events occur after cell division. Time refers to the earliest time that the molecule can be detected or that an increase could be detected. A/NA refers to the relative levels in activated versus nonactivated cells at peak induction. In some cases these data were not available since only fluorescent intensity was measured. Act-2, gene expressed in activated human T cells encoding a 99–amino acid protein that is potentially secreted; bcl-2, gene located at the chromosomal breakpoint of several human lymphomas and leukemias expressed on activated human peripheral blood lymphocytes (pbls); TCA 3, murine gene encoding a protein of 69 amino acids that is probably secreted; pBK791 and pBK642 are murine cDNAs obtained from cytotoxic T cell lines (CTLs); ODC, ornithine decarboxylase; SAM, *S*-adenosylmethionine; TGF, transforming growth factor; IL-3 to IL-6, interleukin-3 to interleukin-6; Ea1, Ea2, and Ea3 are early activation antigens on human and murine pbls; rpt-1, regulatory protein of T lymphocytes encoding a nuclear protein that potentially suppresses the IL-2R gene and the HIV-1–LTR; 4F2, cell surface protein of activated pbls; p28, 28-kD antigen present on human peripheral T cells; SECT, gene of human T cells encoding a protein with homology to serine esterase; T305, antigen on human peripheral T cells; L-35 and L-36 are antigens on mitogen-stimulated T cells; MLR3 is an antigen present on cells stimulated in a mixed-lymphocyte reaction; TLiSA1, T lineage–specific antigen on murine CTL clones; GM-CSF, granulocyte-macrophage colony-stimulating factor; T10, antigen on human T cells; MALA-1, murine-activated lymphocyte antigen-1; HIVEN86A, 86-kD nuclear protein that is probably identical to NF-κB and binds the enhancer of HIV-1; Ta_1, 105-kD antigen of human T4 and T8 cells; Tp 103, similar to Ta_1; CTLA-1 to CTLA-4, CCPI and CCPII, HF, and the Granzymes are cytotoxic T cell–associated sequences present in the granules of T cells felt to have a role in cell killing—identities among this group of proteins are not yet clear [compare, for example, (*110*) with (*111*)];HLA-DR, major histocompatibility antigen, class II; RANTES and 519 are genes expressed in CTLs and activated human T cells but not malignant cells; GP-26, 26-kD glycoprotein on human pbls; VLA-1 to VLA-5 are heterodimeric proteins made up of a common β chain and a unique α chain with sequence similarity to each other and to a variety of adhesive proteins including the vitronectin receptor-platelet GPIIb/IIIa family and the position-specific (PS) antigen involved in *Drosophila* embryogenesis; Act 1, activated T cell antigen on human peripheral T cells; LDA-1, late differentiation antigen associated with helper inducer function of human T cells.

Name	Time	Location*	Ratio (A/NA)	Reference
	Immediate			
c-*fos*†	15 min	N	>100	(*67*)
Act-2†	15	S	>10	(*77*)
NFAT-1	20	N	~50	(*27*)
c-*myc*†	30	N	20	(*67, 68*)
NF-κB†	30	N	>10	(*48, 50*)
	Early			
γ-Interferon†	30 min	S	>100	(*20*)
IL-2†	45	S	>1000	(*20, 25, 27*)
bcl-2†	<1 hour	C?	20	(*78*)
TCA 3†	<1	S?	>100	(*79*)
pBK 791†	1	?	>10	(*80*)
pBK 642†	1	?	>50	(*80*)
ODC†	1	C	10	(*81*)
c-*abl*†	1	N	~10	(*67*)
SAM decarboxylase†	1	C	5	(*82*)
Insulin receptor†	1	CM	3	(*67*)
HIV-1†	<2		>10	(*28, 29, 46–49*)
TGF-β†	<2	S	>10	(*83*)
Actin†	2	C	3	(*81*)
IL-2 receptor†	2	S	>50	(*26, 67, 84*)
IL-3†	1–2	S	>100	(*80, 85, 86*)
Lymphotoxin†	1–3	S	>100	(*87*)
Ea1	3	CM	>10	(*88*)
Ea2	3	CM	>10	(*88*)
Ea3	3	CM	>10	(*88*)
Proenkephalin†	2–4	S	>20	(*80*)
rpt-1†	<4	N	1/5	(*89*)
4F2†	4	CM	?	(*90*)
p28	4	CM	?	(*91*)
Cyclin†	4–6	C	>10	(*92*)
SECT†	<6	C	370	(*93*)
IL-4†	<6	S	>100	(*94*)
IL-5†	<6	S	>100	(*95*)
IL-6†	<6	S	>100	(*96*)
	Early			
c-*myb*†	16	N	100	(*67, 72, 97*)
Transferrin†	6–8	S	?	(*98*)
T-305†	8	CM	?	(*99*)
L-35	12	CM	?	(*100*)
L-36	12	CM	?	(*100*)
Transferrin receptor†	14	CM	5	(*67, 101*)
MLR3	18	CM	?	(*102*)
TLiSA1†	18–24	CM	?	(*90*)
GM-CSF†	<20	S	?	(*85, 103*)
Galactosyltransferase†	21	CM	12	(*104*)
T10	24	CM	?	(*105*)
MALA-1	24	CM	10	(*106*)
Neuraminadase†	24–48	C	5	(*107*)
Histone H3†	24–48	N	>10	(*67*)
HIVEN86A	<48	N	10	(*66*)
	Late			
Ta_1	2 days	CM	?	(*108*)
Tp 103	2	CM	?	(*109*)
CCPI,† CTLA-1,† Granzyme B†	2	?	>100	(*110, 111, 112*)
CTLA-4†	2–3	CM	>100	(*111*)
HLA-DR†	3–5	CM	10	(*113*)
RANTES†	3–5	S	10	(*114*)
GP-26	3–5	CM	>10	(*115*)
VLA-4	4	CM	>100	(*116*)
HF,† CTLA-3,† Granzyme A†	<5	?	>100	(*110, 118*)
CTLA-2†	<5	?	>100	(*111, 112*)
CCPII†	<5	?	>100	(*119*)
519†	5	N	>50	(*120*)
Act 1	5–6	CM	>100	(*121*)
LDA-1	9	CM	?	(*117*)
VLA-1†	7–14	CM	>100	(*122*)
VLA-2	7–14	CM	?	(*122*)
VLA-3, VLA-5	7–14	CM	?	(*123*)

*Location refers to the location of the molecule within the cell: S, secreted; CM, cell membrane; C, cytoplasmic; N, nuclear. †Indicates genes that have been cloned.

linked promoter in response to signals from the antigen receptor (*32*).

These functional sequences within the IL-2 enhancer presumably activate transcription by binding regulatory proteins. The functional sequences between −285 to −255 and −93 to −63 within the IL-2 enhancer are protected from deoxyribonuclease (DNase) I digestion by proteins in fractionated nuclear extracts from activated T lymphocytes (*32*). Competition studies with oligonucleotides from the protected regions indicate that separate factors with quite distinct characteristics bind to these two sites (*32*). The nuclear factor, NFIL-2A, binds to the most proximal regulatory site identified in the IL-2 enhancer between −93 and −63 (Fig. 1). This factor is present in nuclear extracts of both activated and resting T cells (*32*). Nevertheless, an oligonucleotide representing the sequence protected from DNase I digestion can activate a linked promoter in response to signals from the T cell antigen receptor. However, in contrast to the intact enhancer or the endogenous IL-2 gene, PMA makes no contribution to the function of the oligonucleotide (*32*). For this reason, this sequence has been referred to as an antigen receptor response element or ARRE-1. Despite the constitutive presence of the protein that binds to the −63 to −93 site, this region becomes hypersensitive to DNase I in whole nuclei from activated T cells but not other cell types (*34*). Additional evidence that this site is functional in the regulation of the IL-2 gene is that the NFIL-2A binding site can make an unrelated promoter suppressible by CsA (*41*). Interestingly, the NFIL-2A protein may have a negative function in resting T cells. Mutations at the contact guanosines of the NFIL-2A binding site presumably disrupt protein binding and lead to constitutive activity of the promoter when the SV-40 enhancer is placed upstream of a truncated IL-2 enhancer (*44*). Together these results suggest that the NFIL-2A protein both suppresses expression of the IL-2 promoter in nonactivated T cells and activates it after cells are stimulated.

Another antigen receptor response element (ARRE-2) is present between −285 and −255 of the IL-2 enhancer (Fig. 1). This sequence forms a DNA-protein complex first designated NFIL-2E (*32*). Later the factor was renamed nuclear factor of activated T cells (NFAT-1) (*27*) because of its selective expression in activated T cells. In the human Jurkat T cell line the following evidence indicates that activation of the IL-2 gene is dependent on the prior activation of NFAT-1: (i) Deletion of the NFAT-1 binding site significantly impairs activity of the IL-2 enhancer (*32*). (ii) The binding site fused to an unrelated promoter will make the promoter T cell–specific and responsive to signals initiated at the T cell antigen receptor (*27, 32*). (iii) Cyclosporin A blocks both the activation of the IL-2 gene and the ability of the ARRE-2 to activate a linked promoter in response to signals from the antigen receptor (*41*). (iv) NFAT-1 binding activity is present in nuclear extracts of activated T cells in amounts about 50 times those of other cell types (*27*). (v) In the human Jurkat T cell line the appearance of NFAT-1 precedes the earliest detectable appearance of IL-2 mRNA by about 10 minutes (*27*). (vi) Studies in which anisomycin (*45*), a rapidly acting inhibitor of protein synthesis, was added at progressively later times after stimulation with PMA and phytohemagglutinin (PHA) indicate that the activator of the IL-2 gene is synthesized in Jurkat T cells about 20 min after T cell activation (*27*); this time corresponds to the appearance of NFAT-1 after stimulation (*27*). Together, these results indirectly indicate that the activation of the IL-2 gene is contingent on the formation of the NFAT-1 complex (Fig. 2). The formation of the NFAT-1 complex is illustrated as requiring transcription of a new gene, since binding activity is dependent on RNA synthesis (*27*). A single NFAT-1 binding site does not activate transcription from an unrelated promoter but requires either reiteration or another functional site (*32*). Thus the 10- to 20-min delay between the appearance of NFAT-1 and the first detection of IL-2 mRNA may reflect the assembly of some form of functional complex. The observation that deletion of either the NFAT-1 or the NFIL-2A site abolishes most of the activity of the IL-2 enhancer suggests that the two sites cooperate for full activity; however, each protein binds independently (*32*). To date, the data that indicate that IL-2 gene activation is dependent on the expression of NFAT-1 come only from studies with the Jurkat T cell line and need to be confirmed in subpopulations of normal T cells.

Although the relation of NFAT-1 to the commitment event is unclear, recent evidence suggests that it may have a role in activating transcription of HIV-1 (*27*). The latent virus is activated to produce RNA and initiate replication in T cells stimulated with PMA and PHA; an event that may be involved in the breakdown of latent HIV-1 infection (*46, 47*). Transcription from the viral long terminal repeat (LTR) is activated between 30 and 120 min after T cell stimulation (*28, 29*), and recent studies indicate that the transcriptional enhancer within the LTR responds to PMA (*46–49*). Within the enhancer there are two binding sites for NF-κB (*50*) (Fig. 1), a nuclear factor that binds to the immunoglobulin κ chain enhancer and regulates transcription of this gene in response to physiologic stimuli and also to PMA (*48*). Deletion of these sites significantly impairs the activity of the enhancer. However, additional sequences within the LTR that are responsive to PHA-stimulated T cell activation lie upstream of the enhancer between −278 and −176 relative to the start of transcription (*29*). The sequences within this functional region between −216 and −254 of the HIV-1–LTR are protected from DNase I digestion after incubation with nuclear extracts of activated but not nonactivated cells. The protection of this region is effectively inhibited by competition with the NFAT-1 binding site, but not other oligonucleotides (*27*). In addition, as with the NFAT-1 binding site in the IL-2 enhancer, nuclear extracts of cells stimulated in the presence of protein synthesis inhibitors do not protect the −216 to −254 sequences of the HIV-1–LTR. Finally, the formation of the NFAT-1 complex is inhibited by competition with the HIV-1–LTR region extending from −342 to −154 (*27*). These results indicate that either NFAT-1 or a protein with related biological and DNA-binding characteristics is capable of interacting with the PHA-responsive region of the HIV-1–LTR (Fig. 1).

The studies of NFIL-2A and NFAT-1 do not explain why PMA is required for activation of the intact enhancer as well as the IL-2 gene, since the binding sites for these proteins do not activate transcription in response to PMA alone or in conjunction with a signal from the antigen receptor. However, an AP-1 binding site is present in the IL-2 gene at position −240 (*51, 52*) (Fig. 1). AP-1 is the cellular homolog of the v-*jun* oncogene (*53*), and its binding site will make a normally unresponsive promoter responsive to agents that activate PKC (*51, 54*). Deletion of the AP-1 site from the IL-2 enhancer reduces the response of the enhancer to PMA stimulation in the EL-4 murine T cell line (*52*). A functional AP-1 site in the IL-2 gene suggests that the control region of the gene is a mosaic of elements that respond to signals emanating from the antigen receptor and to signals initiated with the activation of PKC. Thus, signals initiated through two cell membrane receptors may be integrated at the level of the responsive gene. A tentative model for the activation of the IL-2 gene depicting the relative roles of AP-1, NFIL-2A, and NFAT-1 is shown (Fig. 2). Deletion analysis indicates that the NFIL-2A site contributes most to the function of the enhancer (*32*).

Activation of the IL-2 receptor (IL-2R) gene is also essential for committment to cell division and immunologic function (*55*). Present data indicate that high affinity binding of IL-2 to its receptor involves a trimolecular interaction among IL-2 and the two

cell membrane–bound polypeptide chains that constitute the complete receptor (*56*). A 55-kD chain has been cloned (*57*) and is found primarily on activated T cells but is also present on certain other cell types (*26, 58*). Unlike the IL-2 gene, which is strictly dependent on signals from the antigen receptor, the 55-kD chain is expressed after activation with tumor necrosis factor (*59*), IL-1 (*60*), IL-5 (*61*), or simply with PMA (*26*). However, in murine T cell clones, a signal from the antigen receptor is sufficient to activate expression of the 55-kD chain without a requirement for accessory cell stimuli (*62*). A 70-kD chain is expressed on a variety of cell types including nonactivated T cells (*56, 63*). Although the sequences responsible for the PMA-induced activation of the IL-2 receptor gene have been localized 5′ of the transcription initiation site (*30, 43*), there is some disagreement about the exact sequences required (*30, 31, 43, 64, 65*). Recently a binding site for the PMA-responsive transcription factor NF-κB or a protein with very similar characteristics, HIVEN86A (*66*), was localized between −255 and −268 of the transcription initiation site (*65*). Furthermore, this site was found to activate a nonregulated promoter in response to PMA-activation of T cells transfected with a fusion gene made up of several of the binding sites coupled to the thymidine kinase promoter. The proposed binding site for NF-κB in the IL-2 receptor was also found to compete for binding of authentic NF-κB to the site that had been previously identified in the HIV-1 enhancer (*48, 65*). Thus multiple transcription factors responding to PMA-induced signals (AP-1 and NF-κB) and to signals emanating from the antigen receptor (NFAT-1 and NFIL-2A) appear to be involved in the early regulatory events of T cell activation (Figs. 1 and 2).

The Role of Cellular Oncogenes in T Cell Activation

At least four oncogenes and one tyrosine kinase homologous to c-*src* are regulated during T cell activation (Table 1). Since they are expressed at quite different times they might be expected to have different roles in this process. An important question is which later events, if any, are contingent upon the expression of these genes.

Early studies showed that c-*myc* gene rearrangements were associated with both T and B cell lymphomas and that activation of the c-*myc* gene followed PHA stimulation by about 30 min (*67, 68*). This activation appeared to be a primary response since it could not be blocked by cycloheximide (*68*). The association of c-*myc* activation with proliferation was supported by studies with antisense oligonucleotides to inhibit the activation of the c-*myc* gene (*69*). These oligonucleotides were added to the cell culture media and were taken up by the T cells where they persisted for several hours. Cells given short antisense oligonucleotides complementary to the c-*myc* RNA had delayed entry into S phase, but cells given a noncomplementary oligonucleotide did not. Morphologic blast transformation was not affected. Thus the response was biologically specific; c-*myc* was not essential for the morphologic changes associated with T cell activation but was required for DNA synthesis. Immunologic functions related to T cell activation were not studied in these experiments. The actual function of c-*myc* is unknown but these studies imply that it may contribute to entry into S phase during T cell activation.

Recent developments have suggested an intriguing role of c-*fos* in T cell activation. As in most cells, transient (about 1 hour) expression of c-*fos* is rapidly induced after PMA stimulation (*67*). This activation appears to be a direct response to stimulation, since accumulation of cytoplasmic mRNA cannot be blocked with cycloheximide. The rapidity of its induction (10 to 15 min) also suggests a direct mechanism. A role for c-Fos in the activation of the IL-2 gene is suggested by the finding that c-Fos will associate with the transcriptional factor AP-1 (*70*) and that these complexes can stimulate transcription (*64*). The IL-2 enhancer contains a binding site for AP-1 (*51*) (Fig. 1), and this site probably contributes the requirement for PMA, since PMA is known to activate AP-1 by a posttranscriptional mechanism (*51, 54, 71*). Because the c-*fos* gene is also induced by PMA, the complex of activated AP-1 and the induced c-Fos could have a role in activating the IL-2 gene.

The activation of at least two early genes have been found to be contingent on prior expression of IL-2. Interaction of IL-2 with its receptor is required for expression of the mRNA for c-*myb* about 6 hours after PHA stimulation of T cells during mid-G_1 (*67, 72*). The induction of c-*myb* mRNA precedes DNA synthesis and possibly is required for entry into S phase. The activation of the transferrin receptor gene also appears to be a consequence of IL-2 interacting with its receptor, since it can be blocked with antibodies to either IL-2 or its receptor (*73*). These studies, together with the ones mentioned above, suggest that at least two levels of contingent events might be operating in T cell activation: early events, such as IL-2 expression, that are dependent on induction of NFAT-1 and later gene activations such as c-*myb*, that are dependent on IL-2 interacting with its receptor.

Events Providing Temporal Order to T Cell Activation

A central issue for understanding differentiation is the nature of the mechanisms providing temporal order. The complexity of this problem in T cell activation is illustrated by Table 1. Over 70 genes are activated at times ranging from 15 min to 14 days. In several lower eukaryotes, evidence suggests that a cascade of gene activations might provide temporal order to pathways of differentiation. Such cascades have been clearly demonstrated only in prokaryotes

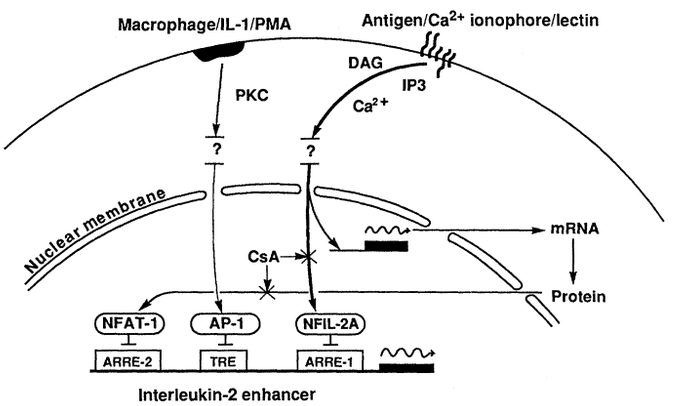

Fig. 2. A tentative model of the pathways involved in the activation of the IL-2 gene in the Jurkat human T cell line. The relative contribution of each pathway to the activation of the IL-2 gene is indicated by the thickness of the arrow and is estimated from the effects of deletion of the response element from the intact enhancer in transfection studies. The gene activated to produce the NFAT-1 complex may not encode the DNA binding activity but could only contribute to the formation of the complex. CsA indicates the proposed site of action of cyclosporin A. The effects of CsA are shown as acting after the protein is produced, since it does not interfere with binding activity, but only with the ability of multimers of the binding site to activate a linked promoter in transfected Jurkat cells stimulated with PHA and PMA (*41*). DAG (diacylglycerol), Ca^{2+}, IP3 (inositol 1,4,5-trisphosphate), and PKC are postulated to be second messengers in transmitting signals initiated at the antigen receptor. TRE is the TPA or PMA response element defined by Serfling *et al.* (*52*).

and yeasts, although there is compelling evidence with some of the DNA tumor viruses that early genes may directly activate late genes. During T cell activation IL-2 gene transcription is probably contingent on the prior activation of a gene contributing to NFAT-1 binding activity with later gene activations contingent on IL-2 interacting with its receptor. If contingent gene activations are in use to order developmental processes, a problem is raised. Most primary gene activations in eukaryotes require only about 15 to 20 min from the time of the initial stimulus to the time that mRNA appears, indicating that if one regulatory gene were to simply activate the next regulatory gene plus a group of functional genes, there would be hundreds if not thousands of such contingent steps in a complex process of differentiation lasting many days. One mechanism by which genes such as the very late activation antigens (VLA-1 and 2) could be activated many days after initial contact with antigen is suggested by the presence of very large genes. For example, the muscular dystrophy gene is 2000 kbp (*74*). DNA polymerase would require 33 hours to transcribe this gene beginning at the promoter at the calculated rate of 15 bp/s (*75*). These figures suggest that the transit time of polymerase could be used to order gene activations in a complex cascade of gene activations. The presence of a large gene in a contingent series could cause a delay of more than a day in the appearance of an activity associated with its protein. Since most of the length of large genes is made up of intervening sequences, which evolve rapidly, the repetitive DNA in introns could contribute to the temporal phasing of gene expression during a developmental pathway. Perhaps the most intriguing implication of this notion is that heterochronic mutants, having differences in timing of developmental pathways (*76*), would arise as a result of variations in intron size. Intron size is one of the most rapidly evolving aspects of genomic structure. Since abundant evidence indicates that species differ from one another not in their structural or functional genes but in the timing and degree of expression of their genes (*75*), such rapidly generated heterochronic mutants could play a role in speciation.

The events providing temporal order to T cell activation will almost certainly involve mechanisms that cannot be anticipated at the present time. Analogies to well-studied simple systems point to the importance of understanding at least three aspects of T cell activation. First, careful determinations of when pure T cell types are committed to a variety of late functions will be essential to decipher the role of the early activation genes in the initiation of T cell activation. A knowledge of these initiation events is particularly important for understanding how drugs such as CsA and glucocorticoids are able to block most of the late events in T cell activation, presumably by affecting early events. Furthermore, since activation of the latent LTR of the HIV-1 virus may be under the same control mechanisms as those involved in the initiation of T cell activation (*28, 29, 46, 47, 49*), an understanding of these early events may lead to insights into the breakdown of latency. Second, in order to establish causal relations between cell membrane events and the activation of genes in the nucleus, it will be essential to understand how the proteins binding to the antigen receptor response elements are activated to initiate transcription of early genes. Knowing the biochemical events involved in the activation of these proteins will provide an understanding of the pathways transmitting signals from the antigen receptor to genes responsible for T cell proliferation and the acquisition of immune function. A third significant direction is the establishment of contingent relations among the known gene activations to determine how the process is temporally ordered and how late functions such as cytotoxicity are controlled. To this end, mutations in the immediate events of T cell activation have already been selected (*7, 16*) and new insights will emerge as these approached are extended to the selection of defects in later events.

REFERENCES AND NOTES

1. W. Born, J. Yague, E. Palmer, J. Kappler, P. Marrack, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 2925 (1985); H. R. Snodgrass, Z. Dembic, M. Steinmetz, H. von Boehmer, *Nature* **315**, 232 (1985); H. R. Snodgrass, P. Kisielow, M. Kiefer, M. Steinmetz, H. von Boehmer, *ibid.* **313**, 592 (1985); I. S. Trowbridge, J. Lesley, J. Trotter, R. Hyman, *ibid.* **315**, 666 (1985).
2. M. M. Davis, *Annu. Rev. Immunol.* **3**, 537 (1985); S. M. Hedrick, D. I. Cohen, E. A. Nielsen, M. M. Davis, *Nature* **308**, 149 (1984).
3. E. L. Reinherz and S. F. Schlossman, *Cell* **19**, 821 (1980).
4. J. W. Kappler, U. Staerz, J. White, P. C. Marrack, *Nature* **332**, 35 (1988); J. W. Kappler, N. Roehm, P. Marrack, *Cell* **49**, 273 (1987).
5. M. M. Davis and P. J. Bjorkman, *Nature* **334**, 395 (1988); R. H. Schwartz, *Ann. N.Y. Acad. Sci.* **505**, 1 (1987); E. R. Unanue and P. M. Allen, *Science* **236**, 551 (1987); H. Clevers, B. Alarcon, T. Wileman, C. Terhorst, *Annu. Rev. Immunol.* **6**, 629 (1988).
6. C. P. Chiu and H. M. Blau, *Cell* **37**, 879 (1984); H. M. Blau *et al.*, *Science* **230**, 758 (1985).
7. A. Weiss and J. Stobo, *J. Exp. Med.* **160**, 1284 (1984).
8. A.-M. Schmitt-Verhulst *et al.*, *Nature* **325**, 628 (1987); J. M. Kanellopoulos, S. De Petris, G. Leca, M. J. Crumpton, *Eur. J. Immunol.* **15**, 479 (1985).
9. A. Novogrodsky and E. Katchalski, *Biochim. Biophys. Acta* **228**, 579 (1971).
10. S. M. Milner, *Nature* **268**, 441 (1977).
11. J. W. Lowenthal, C. Tougne, R. MacDonald, K. A. Smith, M. Nabholz, *J. Immunol.* **134**, 931 (1985).
12. J. J. O'Shea, J. B. Harford, R. D. Klausner, *ibid.* **137**, 971 (1986); H. Hasegawa-Sasaki and T. Sasaki, *Biochim. Biophys. Acta* **754**, 305 (1983); M. V. Taylor *et al.*, *Nature* **312**, 462 (1984).
13. J. B. Imboden and J. D. Stobo, *J. Exp. Med.* **161**, 446 (1985); J. B. Imboden, A. Weiss, J. D. Stobo, *J. Immunol.* **134**, 663 (1985); S. Treves *et al.*, *J. Exp. Med.* **166**, 33 (1987); M. H. Freedman, M. C. Raff, B. Gomperts, *Nature* **255**, 378 (1975); R. Y. Tsien, T. Rozzan, T. Rink, *ibid.* **295**, 68 (1982).
14. R. B. Acres, P. J. Conlon, D. Y. Mochizuki, B. Gallis, *J. Immunol.* **139**, 2268 (1988); L. E. Samelson, M. D. Patel, A. M. Weissman, J. B. Harford, R. D. Klausner, *Cell* **46**, 1083 (1986); J. J. O'Shea *et al.*, *J. Immunol.* **139**, 3463 (1987).
15. J. J. Sussman *et al.*, *Nature* **334**, 625 (1988); W. Holter, G. F. Fischer, O. Majdic, H. Stockinger, W. Knapp, *J. Exp. Med.* **163**, 654 (1986).
16. M. A. Goldsmith and A. Weiss, *Science* **240**, 1029 (1988).
17. I. Kojima, K. Kojima, H. Rasmussen, *J. Biol. Chem.* **260**, 9177 (1985).
18. D. L. Alkon and H. Rasmussen, *Science* **239**, 998 (1988).
19. A. Weiss, R. Shields, M. Newton, B. Manger, J. Imboden, *J. Immunol.* **138**, 2169 (1987).
20. R. Wiskocil, A. Weiss, J. Imboden, R. Kamin-Lewis, J. Stobo, *ibid.* **134**, 1599 (1985).
21. R. L. Davis, H. Weintraub, A. B. Lassar, *Cell* **51**, 987 (1987).
22. P. W. Sternberg and H. R. Horvitz, *Annu. Rev. Genet.* **18**, 489 (1984); E. L. Ferguson, P. W. Sternberg, H. R. Horvitz, *Nature* **326**, 259 (1987); P. W. Sternberg and H. R. Horvitz, *Cell* **44**, 761 (1986).
23. S. C. Meuer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1509 (1984).
24. D. A. Cantrell and K. A. Smith, *Science* **224**, 1312 (1984).
25. S. Gillis and J. Watson, *J. Exp. Med.* **152**, 1709 (1980).
26. W. J. Leonard, M. Kronke, N. J. Peffer, J. M. Depper, W. C. Greene, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 6281 (1985).
27. J. P. Shaw *et al.*, *Science* **241**, 202 (1988).
28. S. E. Tong-Starksen, P. A. Luciw, B. M. Peterlin, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 6845 (1987).
29. M. Siekevitz *et al.*, *Science* **238**, 1575 (1987).
30. M. Maruyama *et al.*, *Cell* **48**, 343 (1987).
31. M. Siekevitz, M. B. Feinberg, N. Holbrook, F. Wong-Staal, W. C. Greene, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5389 (1987).
32. D. B. Durand *et al.*, *Mol. Cell. Biol.* **8**, 1715 (1988).
33. D. B. Durand, M. R. Bush, J. G. Morgan, A. Weiss, G. R. Crabtree, *J. Exp. Med.* **165**, 395 (1987).
34. U. Siebenlist *et al.*, *Mol. Cell. Biol.* **6**, 3042 (1986).
35. T. Fujita, H. Shibuya, T. Ohashi, K. Yamanishi, T. Taniguchi, *Cell* **46**, 401 (1986).
36. T. M. Williams *et al.*, *J. Immunol.* **141**, 662 (1988).
37. C. M. Gorman, L. F. Moffat, B. H. Howard, *Mol. Cell. Biol.* **2**, 1044 (1982).
38. J. R. de Wet, K. V. Wood, M. DeLuca, D. R. Helinski, S. Subramani, *ibid.* **7**, 725 (1987).
39. L. Lacy, J. P. Shaw, F. Bachmann, J. Greengard, G. R. Crabtree, unpublished observations. Three lines of transgenic mice were prepared by injection of embryos with a DNA construct of murine c-*myc* that contained 590 bp of the human IL-2 enhancer/promoter region, and 49 bp of the c-*myc* untranslated region. Transcripts from 12 different tissues, including activated and non-activated lymphocytes, were assayed for proper initiation by the ribonuclease protection method.
40. D. Rosenstreich and S. Mizel, *J. Immunol.* **123**, 1749 (1979); A. Weiss, R. L. Wiskocil, J. D. Stobo, *ibid.* **133**, 123 (1984).
41. D. B. Durand, J. P. Shaw, E. A. Emmel, G. R. Crabtree, in preparation.
42. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
43. S. L. Cross *et al.*, *Cell* **49**, 47 (1987).
44. G. J. Nabel, C. Gorka, D. Baltimore, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2934 (1988).
45. P. A. Grollman, *J. Biol. Chem.* **242**, 3226 (1967).
46. D. Zagury *et al.*, *Science* **231**, 850 (1986).
47. S. Harada, Y. Koyanagi, H. Nakashima, N. Kobayashi, N. Yamamoto, *Virology* **154**, 249 (1986).

48. G. Nabel and D. Baltimore, *Nature* **326**, 711 (1987).
49. J. D. Kaufman *et al.*, *Mol. Cell. Biol.* 7, 3759 (1987).
50. R. Sen and D. Baltimore, *Cell* **47**, 921 (1986).
51. P. Angel *et al.*, *ibid.* **49**, 729 (1987).
52. E. Serfling *et al.*, *EMBO J.*, in press.
53. K. Ryder, L. F. Lau, D. Nathans, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1487 (1988); K. D. Harshman, W. S. Moye-Rowley, C. S. Parker, *Cell* **53**, 321 (1988); P. Angel *et al.*, *Nature* **332**, 166 (1988); T. J. Bos, D. Bohmann, H. Tsuchie, R. Tjian, P. K. Vogt, *Cell* **52**, 705 (1988); D. Bohmann *et al.*, *Science* **238**, 1386 (1987).
54. W. Lee, P. Mitchell, R. Tjian, *Cell* **49**, 741 (1987).
55. K. A. Smith, *Science* **240**, 1169 (1988).
56. M. Sharon, R. D. Klausner, B. R. Cullen, R. Chizzonite, W. J. Leonard, *Science* **234**, 859 (1986).
57. W. J. Leonard *et al.*, *ibid.* **230**, 633 (1985); T. Nikaido *et al.*, *Nature* **311**, 632 (1984); D. Cosman *et al.*, *ibid.* **312**, 768 (1984).
58. M. C. Mingari *et al.*, *Nature,* **312**, 641 (1984).
59. J. C. Lee, A. Trueneh, M. F. Smith, Jr., K. Y. Tsang, *J. Immunol.* **139**, 1935 (1987).
60. G. Pletinck, W. Declercq, J. Tavernier, M. Nabholz, W. Fiers, *Eur. J. Immunol.* **17**, 1835 (1987).
61. M. S. Loughnan, K. Takatsu, N. Harada, G. J. Nossal, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5399 (1987).
62. M. K. Jenkins, D. M. Pardoll, J. Mizuguchi, T. M. Chused, R. H. Schwartz, *ibid.*, p. 5409.
63. M. Nishi, Y. Ishida, T. Honjo, *Nature* **331**, 267 (1988).
64. R. Chiu *et al.*, *Cell* **54**, 541 (1988).
65. E. Böhnlein *et al.*, *ibid.* **53**, 827 (1988).
66. B. R. Franza, S. F. Josephs, M. Z. Gilman, W. Ryan, B. Clarkson, *Nature* **330**, 391 (1987).
67. J. C. Reed, J. C. Alpers, P. C. Nowell, R. G. Hoover, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 3982 (1986).
68. K. Kelly, B. H. Cochran, C. D. Stiles, P. Leder, *Cell* **35**, 603 (1983).
69. R. Heikkila *et al.*, *Nature* **328**, 445 (1987).
70. F. J. Rauscher III, L. C. Sambucetti, T. Curran, R. J. Distel, B. M. Spiegelman, *Cell* **52**, 471 (1988); F. J. Rauscher III *et al.*, *Science* **240**, 1010 (1988); P. Sassone-Corsi, W. W. Lamph, M. Kamps, I. M. Verma, *Cell* **54**, 553 (1988).
71. R. Chiu, M. Imagawa, R. J. Imbra, J. R. Bockoven, M. Karin, *Nature* **329**, 648 (1987).
72. J. B. Stern and K. A. Smith, *Science* **233**, 203 (1986).
73. L. M. Neckers and J. Cossman, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3494 (1983).
74. M. Koenig *et al.*, *Cell* **50**, 509 (1987).
75. E. H. Davidson, *Gene Activity in Early Development* (Academic Press, New York, ed. 2, 1976), p. 363.
76. G. R. De Beer, *Embryos and Ancestors* (Clarendon, Oxford, ed. 3, 1958).
77. M. A. Lipis, N. T. Clang, W. J. Leonard, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
78. J. C. Reed, Y. Tsujimoto, J. D. Alpers, C. M. Croce, P. C. Nowell, *Science* **236**, 1295 (1987).
79. P. R. Burd *et al.*, *J. Immunol.* **139**, 3126 (1987).
80. B. S. Kwon *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2896 (1987).
81. M. W. White, T. Kameji, A. E. Pegg, D. R. Morris, *Eur. J. Biochem.* **170**, 87 (1987); M. W. White, A. K. Oberhauser, C. A. Kuepfer, D. R. Morris, *Mol. Cell. Biol.* 7, 3004 (1987).
82. M. Mach, M. W. White, M. Neubauer, J. L. Degen, D. R. Morris, *J. Biol. Chem.* **261**, 11697 (1986).
83. J. H. Kehrl *et al.*, *J. Exp. Med.* **163**, 1037 (1986).
84. M. Kronke, W. J. Leonard, J. M. Depper, W. C. Greene, *ibid.* **161**, 1593 (1985); T. Uchiyama, S. Broder, T. A. Waldmann, *J. Immunol.* **126**, 1393 (1981).
85. A. Kelso and T. Owens, *J. Immunol.* **140**, 1159 (1988).
86. S. C. Clark and R. Kamen, *Science* **236**, 1229 (1987).
87. P. W. Gray *et al.*, *Nature* **312**, 721 (1984).
88. W. Newman, V. A. Fanning, P. E. Rao, E. F. Westberg, E. Patten, *J. Immunol.* **137**, 3702 (1986).
89. P. Patarca *et al.*, *Proc. Natl. Sci. U.S.A.* **85**, 2733 (1988).
90. B. F. Haynes *et al.*, *J. Immunol.* **126**, 1409 (1981); G. F. Burns, T. Triglia, J. A. Werkmeister, C. G. Begley, A. W. Boyd, *J. Exp. Med.* **161**, 1063 (1985); E. Quackenbush *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 6526 (1987); Gottesdiener *et al.*, *Mol. Cell Biol.* **8**, 3809 (1988).
91. M. C. Udey, M. D. Jendrisak, C. D. Parker, *J. Immunol.* **128**, 1870 (1982).
92. P. Kurki, M. Lotz, K. Ogata, E. M. Tan, *ibid.* **138**, 4114 (1987).
93. A. Caputo *et al.*, *J. Biol. Chem.* **263**, 6363 (1988).
94. F. Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2061 (1986); Y. Norma *et al.*, *Nature* **319**, 640 (1986).
95. T. Kinashi *et al.*, *Nature* **324**, 7 (1986).
96. T. Hirano *et al.*, *ibid.*, p. 73.
97. G. Torelli, L. Selleri, A. Donelli, S. Ferrari, *Mol. Cell. Biol.* **5**, 2874 (1985).
98. J. B. Lum, A. J. Infante, D. M. Makker, F. Yang, B. H. Bowman, *J. Clin. Invest.* 77, 841 (1986).
99. R. I. Fox *et al.*, *J. Immunol.* **131**, 762 (1983).
100. V. C. Maino and M. E. Janszen, *ibid.* **137**, 3093 (1986).
101. I. S. Trowbridge and M. B. Omary, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3039 (1981).
102. M. E. Cosulich, A. Rubartelli, A. Risso, F. Cozzolino, A. Bargellesi, *ibid.* **84**, 4205 (1987).
103. G. G. Wong *et al.*, *Science* **228**, 810 (1983).
104. J. T. Lamont, J. L. Perotto, M. M. Weiser, K. J. Isselbacher, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3726 (1974).
105. C. Terhorst *et al.*, *Cell* **23**, 771 (1981).
106. F. Takei, *J. Immunol.* **133**, 345 (1984).
107. N. F. Landolfi, J. Leone, J. E. Womack, R. G. Cook, *Immunogenetics* **22**, 159 (1985).
108. D. A. Fox *et al.*, *J. Immunol.* **133**, 1250 (1984).
109. B. Fleisher, D. J. Schendel, D. Von Steldern, *Eur. J. Immunol.* **16**, 741 (1986).
110. D. Masson and J. Tschopp, *Cell* **49**, 679 (1987).
111. J. F. Brunet, F. Denizot, M. F. Luciani, M. Roux-Dosseto, *Nature* **328**, 267 (1987).
112. J. F. Brunet, F. Denizot, M. Suzan, W. Haas, *J. Immunol.* **138**, 4102 (1987); J. Brunet *et al.*, *Nature* **322**, 268 (1986).
113. S. M. Fu *et al.*, *J. Exp. Med.* **148**, 1423 (1978).
114. T. J. Schall *et al.*, *J. Immunol.* **141**, 1018 (1988).
115. T. Hercend, L. M. Nadler, J. M. Pesando, E. L. Reinherz, S. F. Schlossman, J. Ritz, *Cell. Immunol.* **64**, 192 (1981).
116. M. E. Hemler *et al.*, *J. Biol. Chem.* **262**, 11478 (1978).
117. N. Suciu-Foca *et al.*, *Nature* **318**, 465 (1985).
118. H. K. Gershenfeld and I. L. Weissman, *Science* **232**, 854 (1986).
119. C. G. Lobe, B. B. Finlay, W. Paranchych, V. H. Paetkau, R. C. Bleackley, *ibid.*, p. 858.
120. J. Jongstra *et al.*, *J. Exp. Med.* **165**, 601 (1987).
121. A. I. Lavarovits *et al.*, *J. Immunol.* **133**, 1857 (1984).
122. M. E. Hemler, J. G. Jacobson, J. L. Strominger, *J. Biol. Chem.* **260**, 15246 (1985).
123. M. E. Hemler, C. Huang, L. Schwarz, *ibid.* **262**, 3300 (1987); X. Takeda, J. L. Strominger, M. E. Hemler, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3239 (1987).
124. I wish to thank M. Karin and E. Serfling for sharing unpublished information and A. Weiss, H. Blau, A. Krensky and C. Edwards for thoughtful comments on the manuscript. This work was supported by grants CA 39612 from the National Cancer Institute and HL 33942 from the National Heart, Lung, and Blood Institute of the NIH.