Regulation of Expression of the Interleukin-2 Receptor on Hematopoietic Cells by Interleukin-3

MARIA C. BIRCHENALL-SPARKS, WILLIAM L. FARRAR, DONNA RENNICK, PATRICIA L. KILIAN, FRANCIS W. RUSCETTI*

Remarkable similarities in the intracellular and genetic events occur when lymphoid and hematopoietic cells are exposed to their specific growth factors. The interleukin-2 (IL-2) receptor, whose cell-surface expression is an absolute requirement for the growth and differentiation of lymphoid cells, was detected on various nonlymphoid hematopoietic cell types in this study. Cell lines consisting either of granulocyte-macrophage precursors or mast cells, which are dependent on interleukin-3 (IL-3) for their growth, expressed high levels of the IL-2 receptor on their surface. Analysis of the binding characteristics of these receptors with ^{125}I-labeled recombinant IL-2 revealed that only receptors with low affinity for IL-2 were present on these cells. Addition of purified recombinant IL-3 to these cell lines led to an increase in IL-2 receptor gene expression within 1 hour in isolated nuclei. This IL-3–induced increase in the number of IL-2 receptors on the cell surface is maximal within 24 hours. Addition of 10,000 units of IL-2 to these cells had no apparent effect on their growth or differentiation. The presence of the receptor with only low affinity for IL-2 on hematopoietic cells and the regulation by IL-3 suggest that this receptor is involved in some important metabolic event in hematopoiesis.

WHEN T LYMPHOCYTES ARE ACTIVATED after mitogen or antigen stimulation in vitro, they are induced to secrete a glycoprotein hormone, interleukin-2 (IL-2) (*1*), and to express specific membrane receptors for this molecule (*2*). The expression of these receptors on the cell surface after stimulation is transient, with peak expression for 3 to 7 days, then declining for 7 to 10 days to 5 to 15 percent of the maximum levels with a concomitant loss of proliferative capacity (*3*). Restimulation of these cells with either mitogen or antigen results in increased expression of high-affinity IL-2 receptors, which can then be bound by IL-2 to initiate further rounds of cell growth (*4*). Robb *et al.* (*5*) showed that there are two classes of IL-2 receptors with distinct affinities for the ligand on T cells. Both types of IL-2 receptors are also present on activated B cells (*6*).

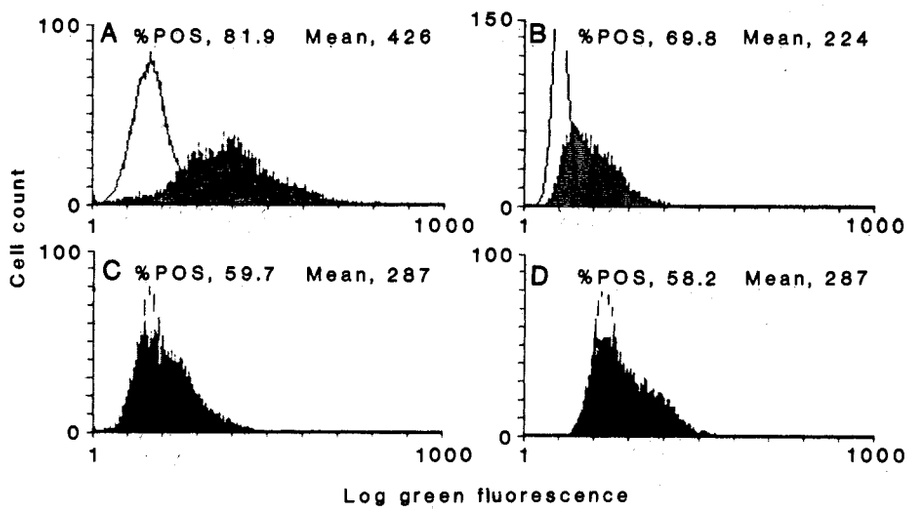

Fig. 1. Expression of 7D4 antigen on various cell lines. Solid lines represent the fluorescence profile of 7D4 antigen distribution. Broken lines show levels of nonspecific staining after incubation in excess unlabeled 7D4. Shaded area shows background with avidin–fluorescein isocyanate (FITC) alone. (A) CT-6, a cytotoxic T-cell line from C57BL/6 (*25*); (B) FDC-P1, an IL-3–dependent granulocyte-macrophage progenitor cell line (*7*); and (C) DX-1 and (D) DX-2 (two newly derived mast cell lines with the phenotype Thy-1$^-$, Lyt-1$^-$, Lyt-2$^-$, Ly-5.1$^+$, I-A^{d+}, I-E^{d+}, FcγR$^+$, FcϵR$^+$, Mac 1$^-$) were independently established from bone marrow cultures of C57BL/6 (DX-1) or BALB/c (DX-2) as described (*26*) and cultured in RPMI 1640 containing 5% fetal calf serum (FCS), 5×10^{-5}*M* β-mercaptoethanol (β-ME), and a source of IL-3. Cytofluorometric analyses for 7D4 expression were performed as follows. Cells were washed three times in RPMI 1640 and incubated with saturating amounts of 7D4 or a control monoclonal antibody for 1 hour at 4°C. After two washings, cells were incubated in FITC-conjugated goat antibody to rat IgM (Cappel, Cochranville, PA). After two more washings cells were analyzed on an Ortho Cytofluorograph System 50 with an argon ion laser at 488 nm. Live cells were gated. Information was stored on an Ortho 2150 computer system. Fluorescence intensity was compared by using the mean channel number for different histograms. The percentage of cells expressing the 7D4 antigen (%POS) was calculated against a background of nonspecific labeling with normal IgM (1% to 3%).

IL-3 has the ability to promote the growth of diverse myeloid cells, particularly their progenitor cells (*7, 8*). Recently, some of these IL-3–dependent cells have been shown to respond to another growth factor, granulocyte-macrophage colony-stimulating factor (GM-CSF); this suggested that different growth factor receptors can be expressed simultaneously on the same myeloid cell (*9*).

In the course of studies on the intracellular consequences of growth factor stimulation of lymphocytes and hematopoietic cells, we were struck by the similarities between the responses of IL-2–dependent cells to IL-2 and the responses of IL-3–dependent cells to IL-3 (*10*). In both cases, translocation of protein kinase C to the cell membrane, increases in intracellular calcium ($[Ca^{2+}]_i$) flux, and the rapid transcription of certain cellular oncogenes seem to characterize the response to these specific growth factors (*10*). Since IL-2 stimulation of IL-2–dependent T cells results in increased transcription of the IL-2 receptor gene (*11*), we investigated the possibility that the regulation of this gene is a general physiological response of hematopoietic cells to their growth factors. FDC-P1, a murine cell line consisting of granulocyte and macrophage progenitor cells (*7*) and two newly derived murine mast cell lines, DX-1 and DX-2, were tested for the presence of IL-2 receptors.

A rat monoclonal antibody, 7D4, to the murine IL-2 receptor (*12*) allowed flow cytofluorimetric analysis of several murine cell types. More than 60 percent of all three nonlymphoid cells express the 7D4 antigen in asynchronous cultures (Fig. 1A). However, the mean fluorescent intensity of the 7D4 antigen binding on FDC-P1, DX-1, and DX-2 was 50 percent of the mean seen on the IL-2–dependent cytotoxic T-cell clone CT-6 (Fig. 1A), indicating that the density of receptors per cell was less than on T cells.

M. C. Birchenall-Sparks, W. L. Farrar, F. W. Ruscetti, Laboratory of Molecular Immunoregulation, Biological Response Modifiers Program, Division of Cancer Treatment, National Cancer Institute–Frederick Cancer Research Facility, Frederick, MD 21701.
D. Rennick, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304.
P. L. Kilian, Department of Immunopharmacology, Hoffmann–La Roche, Nutley, NJ 07107.

*To whom correspondence should be addressed.

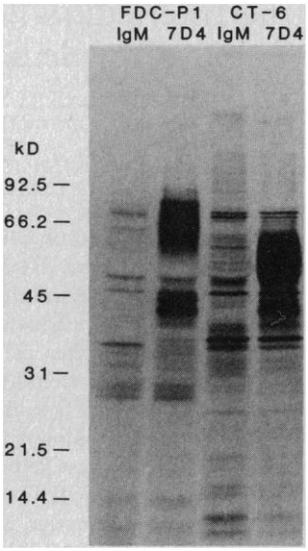

Fig. 2. 7D4-immunoprecipitated membrane components from different cell lines. Electrophoretic patterns were obtained after immunoprecipitation of lysates obtained from the IL-2– and IL-3–dependent cell lines CT-6 and FDC-P1, respectively, with an IgM control or the 7D4 antibody. Cultured CT-6 lymphocytes and FDC-P1 myeloid cells were washed free of growth medium and resuspended in 2 ml of methionine-free RPMI 1640 (Gibco) and equilibrated at 37°C for 30 minutes. The cultures were then incubated for 4 hours in the presence of 0.5 mCi of [^{35}S]methionine (New England Nuclear). After several washings with ice-cold RPMI 1640 containing methionine, the cells were lysed in 10-mm tris-HCl (*p*H 7.5) buffer containing 1% Triton X-100. After centrifugation at 100,000*g* for 1 hour at 4°C, the supernatants were cleared and subjected to immunoprecipitation with 7D4, a rat monoclonal antibody to mouse IL-2 receptor or a control IgM monoclonal antibody as described earlier (*12*). After protein A–immunoabsorbents were washed extensively with TTO buffer [100 m*M* tris-HCl, 0.5% Tween 20, and ovalbumin (1 mg/ml)], bound antigens were eluted by boiling for 5 minutes in 2% SDS-TTO sample buffer and resolved on an SDS–polyacrylamide gel (7.5% to 15%) according to the method of Laemmli (*27*). After being strained with Coomassie brilliant blue R250, the gel was treated with En-Hance (New England Nuclear) and dried. Fluorography was performed at −80°C.

The 7D4 antigens immunoprecipitated from the myeloid or T-cell lines were then compared by analysis on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 2). Immunoprecipitation of endogenously labeled IL-2 receptor from CT-6 cells showed two principal species on SDS-PAGE analysis. A low molecular weight form at M_r 36,000 to 38,000 and a broad band of higher molecular weight forms at M_r 58,000 to 60,000. These molecular weights are in agreement with those obtained earlier with immunoprecipitates from either murine or human T cells, for which [^{35}S]methionine-labeled antibody to the IL-2 receptor was used (*13, 14*). The lower molecular weight form has been suggested to be primarily an immature receptor modified by post-translational processing by *N*- and *O*-linked glycosylation to yield the higher molecular weight species, with diffuse electrophoretic properties imparted by variable glycosylations (*13*). Immunoprecipitation of the IL-2 receptor from FDC-P1 cells revealed a low molecular weight form at M_r 36,000 to 38,000, similar to that seen in T cells. However, the broad band of higher molecular weight forms was seen at M_r 62,000 to 70,000, which is 8,000 to 10,000 higher than that observed in murine T cells. All these forms are specifically recognized by the 7D4 antibody and not by a nonspecific antibody of the same isotype (Fig. 1B). Therefore, both T cells and promyeloid cells exhibited similar putative intracellular immature forms of IL-2 receptors identified by the 7D4 monoclonal immunoglobulin M (IgM). However, pronounced differences were seen with the higher molecular weight species in these cell lines. This heterogeneity in the immunoprecipitates is most likely due to differences in glycosylation (*13, 14*), but it requires more detailed biochemical analysis to determine its relation to binding affinity for IL-2 on cells of different lineages.

The affinity for IL-2 of the IL-2 receptors present on the cell surface of various cell lines was then examined in binding experiments with ^{125}I-labeled IL-2. The IL-3–dependent cell lines FDC-P1 (myeloid) and DX-1 (mast cell) expressed low-affinity receptors but not high-affinity receptors for IL-2 on their cell surfaces (Fig. 3 and Table 1). Dissociation constants (K_d) of the receptors were $1.4 \times 10^{-8}M$ and $3.3 \times 10^{-8}M$ for DX-1 and FDC-P1, respectively, and receptor numbers were 1.2×10^4 and 1.8×10^4 per cell, respectively. In contrast, the IL-2–dependent T-cell clone CT-6 expressed both high- and low-affinity receptors on its cell surface (Fig. 2 and Table 1). No IL-2 receptors were detected on the mouse myeloma cell line NS-1.

Because of the limits in the sensitivity of the binding assay, less than 200 high-affinity receptors for IL-2 cannot be reliably detected on any cell line (*2*). Thus, the effect of recombinant IL-2 on the proliferative response of IL-3–dependent cell lines was determined. An IL-2–dependent T-cell clone was stimulated to near maximal growth at a concentration of 1 to 2 units (100 to 200 p*M*) of recombinant IL-2, indicating that binding to high-affinity receptors is correlated with growth stimulation (Table 1) (*3*). In contrast, IL-3 had no effect on growth of CT-6 (Table 1). Neither FDC-P1 nor DX-1 cells were stimulated by IL-2 at concentrations up to 10,000 unit/ml (1 μ*M*). Thus, as judged from the calculated K_d values in Table 1, the equilibrium constants for IL-2 binding sites on non-T cells were 14 to 33 n*M*. Consequently, 1 μ*M* should saturate these low-affinity binding sites. In addition, IL-2 did not induce proliferation in these IL-3–dependent cells in the presence of limiting doses of IL-3, 12-*O*-tetradecanoyl phorbol-13-acetate, or Ca^{2+} ionophore A23187. Thus, it seems likely that IL-2 binding to the IL-2 receptors expressed on IL-3–dependent cells is not sufficient to transmit a growth signal because they are predominantly, if not entirely, receptors with low affinity for IL-2.

Cell-surface expression of IL-2 receptors on these IL-3–dependent cells was augmented after IL-3 was added to quiescent FDC-P1 and DX-1 cells (Table 1). IL-2 increases IL-2 receptor expression on T cells (*15*) by increasing transcription of the IL-2 receptor gene (*11*). The possibility that IL-3 can similarly regulate transcription of the IL-2 receptor gene in FDC-P1 was explored

Table 1. Numbers and affinity values of IL-2 receptors and growth effect of IL-2 and IL-3 for various cell lines. Receptor numbers and K_d values were determined by Scatchard analysis as described in the legend to Fig. 1. Determination of growth effect of IL-2 or IL-3 (*23*) on cells was done as follows. Cells were incubated at 37°C, free of growth factors in order to obtain quiescent cultures. Then, 100,000 cells were placed in 0.2 ml of RPMI 1640 containing 5% FCS and $5 \times 10^{-5}M$ β-ME with the indicated amounts of recombinant IL-2 and IL-3 (*24*). After incubation at 37°C for 20 hours, the cells were labeled with [^{3}H]thymidine for 4 hours and harvested. IL-2 receptors were determined on quiescent cells and after 24 hours of growth factor stimulation (values ± standard error for three assays). Abbreviations: T, T cells; G, granulocyte progenitors; M, mast cells; ND, not done.

Cell type	Cell line	Ligand added (unit/ml)	High-affinity sites per cell ($\times 10^4$)	Receptors K_d (n*M*)	Low-affinity sites per cell ($\times 10^4$)	Receptors K_d (n*M*)	[^{3}H]TdR (counts/min) ($\times 10^3$)
T	CT-6		5.8 ± 1.5	0.13 ± 0.07	17 ± 5.2	28 ± 10	0.7
G	FDC-P1		<200		1.2 ± 0.4	32 ± 16	0.8
M	DX-1		<200		2.1 ± 0.7	14 ± 9	0.7
T	CT-6	IL-2 (1)	ND	ND	ND	ND	103
G	FDC-P1	IL-2 (10,000)	<200		1.0 ± 0.4	33 ± 15	0.8
M	DX-1	IL-2 (10,000)	<200		2.1 ± 0.7	14 ± 9	1.4
T	CT-6	IL-3 (500)	4.9 ± 1.3	0.15 ± 0.07	18 ± 4.5	27 ± 8	0.5
G	FDC-P1	IL-3 (50)	<200		2.5 ± 0.8	33 ± 15	42
M	DX-1	IL-3 (50)	<200		4.1 ± 1.1	14 ± 9	27

by trying to detect increased IL-2 receptor transcripts in isolated nuclei through hybridization with complementary DNA (cDNA) probes specific for the IL-2 receptor (*16*). Increased IL-2 receptor transcripts were observed 1 hour after IL-3 stimulation of quiescent FDC-P1 cells (Fig. 4). Increased transcription of c-*myc* and ribosomal RNA–specific transcripts was also seen, suggesting that this increase in expression of the IL-2–receptor gene is part of the quiescent cell's physiological response to growth factor stimulation. This represents specific gene induction since albumin and c-*ras* transcripts were not increased after IL-3 stimulation. Increased cytoplasmic messenger RNA (mRNA) specific for the IL-2 receptor was seen by Northern blot transfer of RNA at 4 hours after IL-3 stimulation and led to a doubling of the receptor number in 24 hours. The predominant mRNA species was 4.7 kilobases (kb) (*16*) in both CT-6 and FDC-P1 cells, although all IL-2 receptor mRNA's were seen in both cell types.

Recently, it was reported that murine L cells genetically transfected with a cloned human IL-2 receptor gene express receptors for IL-2, which appear to be entirely of low affinity (*17*). Our results show that lineages of normal cells exist that, without molecular manipulation, express IL-2 receptors with low affinity. It has been shown that transfection of the human cDNA for the IL-2 receptor into a mouse T-cell line results in a functionally active human type receptor that binds IL-2 with high affinity (*18*). This suggests that lymphoid cells expressing the IL-2 receptor need accessory proteins or molecules for binding IL-2 with high affinity. Thus, since there is only one copy of the IL-2 receptor gene present in the genome (*16*) and the same mRNA species are seen in both hematopoietic cells and lymphoid cells, it is clear that the IL-2 receptor gene as defined by molecular biology is under regulatory control in hematopoietic cells despite the absence of a high-affinity interaction between the receptor and its hormone.

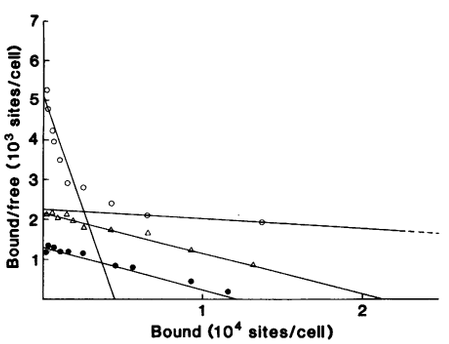

Fig. 3. Scatchard analysis of IL-2 binding to IL-2– and IL-3–dependent cell lines. The following cell lines were analyzed for ^{125}I-labeled IL-2 binding by Scatchard analysis: CT-6, (○); FDC-P1, (●); and DX-1, (△). For accurate measurement of ligand binding, especially on cells growing in IL-2, any receptor-bound IL-2 was dissociated by a 60-second exposure to RPMI 1640 plus 25 m*M* sodium acetate (*p*H 4.0) and washed three times with cold (4°C) RPMI 1640 containing 10% FCS. For equlibrium binding analysis, serial dilutions of ^{125}I-labeled IL-2 were incubated with 1×10^5 to 10×10^5 cells (200 μl) for 20 minutes at 37°C. To quantitate binding to a receptor with low affinity, nanomolar amounts of IL-2 were used (*5*). Cell-bound and free radioactivity were determined after separation by centrifugation (13,000*g* for 90 seconds) through a mixture of 84% silicone oil and 16% paraffin oil as described earlier (*2*). The number of binding sites per cell was determined by Scatchard analysis after subtraction of nonsaturable binding in the presence of a 150-fold excess of unlabeled IL-2. The number of units per milliliter for both labeled and unlabeled IL-2 was determined in a standard IL-2 proliferation assay in which CTLL-2 (*23*) was used with serial dilutions of IL-2 and then analyzed by probit analysis. Protein determinations were performed by the Lowry method. CT-6 (○) intercepted the *x*-axis at 1.7×10^5 low-affinity binding sites per cell.

However, it is surprising that the predominant IL-2 receptor expressed on hematopoietic cells is a receptor with low affinity for IL-2. These IL-3–dependent cell lines are not affected by the addition of large amounts of IL-2, suggesting that low-affinity IL-2 receptors are not able to stimulate a growth response when bound by IL-2. Indeed, recent experiments have shown that the binding of IL-2 to cell lines possessing only receptors with low affinity for IL-2 fails to transmit several transmembrane biochemical signals associated with binding to the high-affinity receptor (*19*). However, it is possible that IL-2 triggering of the low-affinity receptor stimulates an unrecognized differentiation event.

The finding of binding sites with multiple affinities for a ligand is not unique to the IL-2 receptor. The receptors for several polypeptide growth factors such as nerve growth factor, epidermal growth factor, platelet-derived growth factor, and interferon bind with two different affinities (*20*). The affinities of some of these receptors could be interconverted with lectins or monoclonal antibodies (*21*), indicating that variations in ligand-receptor affinities could be due to conformational changes of the protein. Alternatively, the extensive post-translational glycosylation of the IL-2 receptor observed in T cells (*13, 14*) and hematopoietic cells could account for the differences in receptor affinities. The fact that the IL-2 receptor on hematopoietic cells exists solely as a low-affinity receptor and that post-translational modifications made the receptor appreciably larger than on T cells make these observations different from those reported previously for other receptors.

However, the physiological role of these receptors with low affinity for IL-2 in hematopoiesis is not clear. Recent evidence suggests most of the receptors for hematopoietic growth factors are linked in a hierarchical manner that allows binding of certain factors to their receptors to down-regulate and activate different receptors without specific ligand binding (for example, IL-3 can activate the GM-CSF receptor) (*22*). It is possible that the receptor with low affinity for IL-2 is part of this linkage of hematopoietic growth factors and has some important metabolic function on hematopoietic cells that is unrelated to its ability to bind IL-2. It is also possible that this receptor binds another ligand with high affinity or that IL-2 binding triggers an as yet unrecognized event. In summary, we have shown that the IL-2 receptor gene is regulated by IL-3 in IL-3–

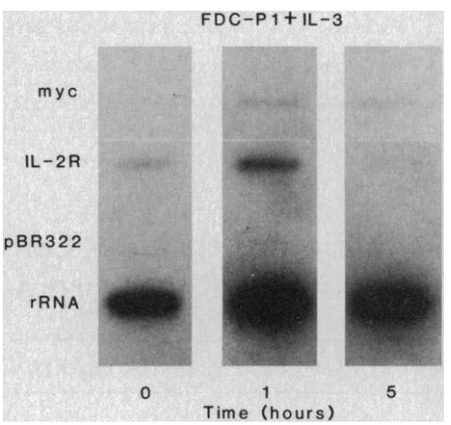

Fig. 4. Analysis of IL-2 receptor transcripts by nuclear transcription runoff assay. The FDC-P1 cell line was incubated for 2 hours in the absence of IL-3. Then, recombinant purified IL-3 at 100 units per 10^6 cells was added to the cultures (this was designated 0 time). The nuclear transcriptional runoff was then performed at various times. In this assay, RNA transcripts which are already initiated are faithfully elongated giving an accurate measure of transcriptional level at time of lysis (*28*). Briefly, 10^8 cells after various treatments and time intervals were washed three times with ice-cold phosphate-buffered saline and lysed in NP-40 lysis buffer [10 m*M* tris (*p*H 7.4), 10 m*M* NaCl, 3 m*M* $MgCl_2$, 0.5% (v/v) NP-40], incubated on ice for 5 minutes, and centrifuged at 500 rev/min for 5 minutes. The nuclei were washed in lysis buffer and resuspended in 100 μl of reaction mixture [10 m*M* tris-HCl (*p*H 8.0); 5 m*M* $MgCl_2$; 300 m*M* KCl; 0.5 m*M* each of ATP, CTP, and GTP, and 200 μCi of [α-^{32}P]UTP (3000 Ci/mm, Amersham)]; and incubated at 30°C for 15 minutes. The ^{32}P-labeled RNA was then isolated and precipitated with ethanol as described elsewhere (*28*). This RNA solution was hybridized to linearized plasmid DNA immobilized on nitrocellulose for 36 hours at 65°C as described (*28*). The filters were then washed in 2× standard saline citrate (SSC) [1× SSC is 0.15*M* NaCl, 0.0125*M* sodium citrate (*p*H 7)] for 2 hours at 65°C and incubated at 37°C with ribonuclease A (10 μg/ml), washed, air-dried, and exposed to Kodak x-ray film. Plasmids containing human IL-2 receptor were hybridized under conditions for mouse IL-2 receptor mRNA (*16*).

dependent cell lines in the same fashion that it is regulated by IL-2 in IL-2–dependent T-cell lines. Thus, in both T cells and hematopoietic cells, IL-2 receptor expression is a component of very similar cellular responses to specific growth factor stimulation. These responses also include protein kinase C translocation, $[Ca^{2+}]_i$ flux, and increased expression of c-*fos*, c-*myc*, and c-*myb*. Our results suggest that the IL-2 receptor is important in regulating the growth of hematopoietic cells.

REFERENCES AND NOTES

1. D. A. Morgan, F. W. Ruscetti, R. Gallo, *Science* **193**, 1007 (1976); F. W. Ruscetti, D. A. Morgan, R. C. Gallo, *J. Immunol.* **119**, 131 (1977).
2. R. J. Robb, A. Munck, K. A. Smith, *J. Exp. Med.* **154**, 1455 (1981); W. J. Leonard *et al.*, *Nature (London)* **300**, 267 (1982).
3. D. A. Cantrell and K. A. Smith, *J. Exp. Med.* **158**, 1895 (1983).
4. J. M. Depper, W. J. Leonard, M. Kronke, T. A. Waldemann, W. C. Greene, *J. Immunol.* **133**, 3054 (1984).
5. R. J. Robb, W. C. Greene, C. M. Rusk, *J. Exp. Med.* **160**, 1126 (1984).
6. S. J. Korsmeyer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4522 (1983); M. Tsudo, T. Uchiyama, H. Uchino, *J. Exp. Med.* **160**, 612 (1984); R. H. Zubler *et al.*, *ibid.*, p. 1170; J. W. Lowenthal, R. H. Zubler, M. Nabholz, H. R. MacDonald, *Nature (London)* **315**, 669 (1985).
7. T. M. Dexter, J. Garland, D. Scott, E. Scolnick, D. Metcalf, *J. Exp. Med.* **152**, 1036 (1980).
8. J. N. Ihle *et al.*, *J. Immunol.* **131**, 282 (1983); J. W. Schrader and G. J. V. Nossal, *Immunol. Rev.* **53**, 61 (1980); G. Nabel, S. J. Galli, A. M. Dvorak, H. F. Dvorak, H. Cantor, *Nature (London)* **291**, 332 (1981).
9. A. J. Hapel, H. S. Warren, D. A. Hume, *Blood* **64**, 786 (1984).
10. W. L. Farrar and W. B. Anderson, *Nature (London)* **315**, 233 (1985); W. L. Farrar, T. P. Thomas, W. B. Anderson, *ibid.*, p. 236; J. L. Rossio, W. L. Farrar, F. W. Ruscetti, *Lymphokine Res.*, in press; M. Birchenall-Sparks, W. L. Farrar, F. W. Ruscetti, in preparation.
11. J. Depper *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4230 (1985); T. Malek and J. Ashwell, *J. Exp. Med.* **161**, 1575 (1985).
12. T. R. Malek, R. J. Robb, E. M. Shevach, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5694 (1983).
13. J. M. Depper *et al.*, *J. Immunol.* **133**, 1691 (1984); J. M. Yodoi *et al.*, *J. Exp. Med.* **161**, 924 (1985).
14. W. Leonard *et al.*, *J. Biol. Chem.* **260**, 1872 (1985).
15. K. A. Smith and D. A. Cantrell, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 864 (1985); K. Welte *et al.*, *J. Exp. Med.* **160**, 1390 (1984); A. Reeves *et al.*, *J. Biol. Resp. Mod.* **4**, 31 (1985).
16. W. Leonard *et al.*, *Nature (London)* **311**, 626 (1984); T. Nikaido *et al.*, *ibid.*, p. 631; J. Miller *et al.*, *J. Immunol.* **134**, 4212, (1985); W. Leonard *et al.*, *Science* **230**, 633 (1985).
17. W. C. Greene *et al.*, *J. Exp. Med.* **162**, 363 (1985).
18. S. Kondo *et al.*, *Nature (London)* **320**, 75 (1986).
19. W. L. Farrar *et al.*, in preparation.
20. S. E. Buxser, D. J. Kelleher, L. Watson, P. Puma, G. L. Johnson, *J. Biol. Chem.* **258**, 3741 (1983); T. Kawamoto, J. D. Sato, A. Le, A. J. Polikoff, G. H. Sato, J. Mendelsohn, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1337 (1983); L. T. Williams, P. M. Tremble, M. F. Lavin, M. E. Sunday, *J. Biol. Chem.* **259**, 5287 (1984); G. E. Hannigan, D. R. Gewert, B. Williams, *ibid.*, p. 9456.
21. P. M. Grob and M. A. Bothwell, *J. Biol. Chem.* **258**, 14136 (1983); J. A. Fernandez-Pol, D. J. Kios, P. D. Hamilton, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **43**, 1980 (1984).
22. F. Walker, N. Nicola, D. Metcalf, A. Burgess, *Cell* **43**, 269 (1985).
23. S. Gillis *et al.*, *J. Immunol.* **120**, 2027 (1978).
24. Recombinant human IL-2 was generously provided by Biogen Inc. (Geneva, Switzerland), and recombinant mouse IL-3 was generously provided by DNAX (Palo Alto, CA).
25. J. J. Farrar *et al.*, *Immunol. Rev.* **63**, 129 (1982).
26. G. Nabel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1157 (1981).
27. U. Laemmli, *Nature (London)* **227**, 680 (1970).
28. M. Gourdine, M. Peretz, H. Weintraub, *Mol. Cell. Biol.* **1**, 281 (1981); M. Greenberg and E. Ziff, *Nature (London)* **311**, 433 (1984).

29. We thank J. Mikovits and C. Mantel for their excellent technical assistance and J. Oppenheim, L. Varesio, H. Young, and D. Longo for helpful discussions concerning the manuscript. Plasmids were kindly provided by L. Varesio (rRNA), R. Dalla-Favera (c-*myc*), and W. Greene (IL-2 receptor).

15 November 1985; accepted 6 May 1986

Induction of the c-*fos* Oncogene by Thyrotropic Hormone in Rat Thyroid Cells in Culture

GIULIA COLLETTA, ANNA MARIA CIRAFICI, GIANCARLO VECCHIO

Rat thyroid cells in culture, rendered quiescent by hormone deprivation, can be stimulated to undergo DNA synthesis in the absence of serum by the addition of purified thyrotropin. The primary effect in response to thyrotropin action in thyroid cells is the induction of the c-*fos* oncogene, followed by c-*myc* expression. This suggests that thyrotropin acts as a competence growth factor.

SEVERAL PROTO-ONCOGENES HAVE been directly implicated in the control of cellular proliferation. Expression of oncogenes coding for nuclear proteins, such as c-*fos* and c-*myc*, is enhanced by stimulation by growth factors and may transduce signals that affect the cellular response to exogenous stimuli.

Moreover, three distinct proto-oncogenes (c-*sis*, c-*erb*B, and c-*fms*) seem to control critical events in the transduction of extracellular growth signals into the cell. Sequence comparisons suggest that c-*sis* codes for platelet-derived growth factor (PDGF) (*1, 2*) and that the c-*erb*B product is partially homologous to the epidermal growth factor (EGF) receptor (*3*). Finally, the mouse c-*fms* product, which is found in high concentrations in macrophages, is closely related to the colony-stimulating factor–1 receptor (*4*).

The c-*myc* oncogene is induced in resting cells of both hematopoietic and fibroblast origin, in response to mitogens and growth factors, and in regenerating liver tissue after partial hepatectomy (*5–7*). The transcription of c-*fos* is also greatly increased within minutes of the administration of purified growth factors to quiescent rat fibroblasts. This is followed by the induction of c-*myc* messenger RNA (mRNA) and the subsequent rapid disappearance of almost all c-*fos* mRNA (*8–10*). This suggests that c-*fos* induction is one of the earliest effects on gene expression triggered by growth factors.

We focused our analysis on the effect of a purified hormone, thyrotropin (TSH), on c-*myc* and c-*fos* expression in epithelial thyroid cells, which are the physiological target for the hormone in vivo. The rat thyroid cell line (FRT-L-5 cell clone) has the following differentiated functions: (i) TSH dependence for growth, (ii) thyroglobulin synthesis and secretion in the culture medium, and (iii) ability to take up iodide. FRT-L-5 cells grow in the presence of calf serum and require a mixture of six hormones (6H), which includes TSH (10 mU/ml), somatostatin (10 μg/ml), insulin (10 μg/ml), glycyl-L-hystidyl-L-lysine acetate (10 μg/ml), transferrin (5 μg/ml), and hydrocortisone ($10^{-8}M$). Under these conditions, the cells have a doubling time of 32 hours and display the fully differentiated phenotype that is typical of the original tissue (*11*). The hormone mixture, and in particular the TSH hormone, is an absolute requirement for FRT-L-5 cell growth. Without the 6H mixture, thyroid cells remain attached to the plate, develop morphological alterations, and do not divide (*12*).

In this system, depriving rat thyroid cells of the hormone mixture can make them quiescent even in the presence of 5% calf serum; only background levels of incorporated [^{3}H]thymidine were observed. However, to exclude any interference by serum proteins in the action of growth factors, we carried out experiments on synchronized thyroid cells in serum-free medium. Treatment of quiescent FRT-L-5 cells with 6H resulted in a 20- to 30-fold increase in the incorporation of [^{3}H]thymidine into trichloroacetic acid (TCA)–precipitable material that reached a peak 19 hours after stimulation (Fig. 1). These data demonstrate a synchronous progression of rat thy-

Dipartimento di Biologia e Patologia Cellulare e Molecolare, Centro di Endocrinologia ed Oncologia Sperimentale del Consiglio Nazionale delle Ricerche, II Facoltà di Medicina e Chirurgia, Via Sergio Pansini 5, Naples, Italy.