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Promoter Region of Interleukin-2 Gene Undergoes Chromatin Structure Changes and Confers Inducibility on Chloramphenicol Acetyltransferase Gene during Activation of T Cells

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The chromatin structure of the interleukin-2 (IL-2) gene was probed by DNase I treatment of isolated nuclei. The 5′ region of the IL-2 gene contains three regions of hypersensitivity to DNase I. When peripheral blood T cells or Jurkat T cells are stimulated with mitogens, IL-2 message is induced, and the promoter region of the IL-2 gene develops an additional hypersensitive site. This suggests that a DNA sequence close to the transcriptional start site is involved in the transduction of the extracellular signal. Such a conclusion is further supported by DNA transfection experiments. A short segment of DNA, which includes the region of induced hypersensitivity, confers inducibility on the linked chloramphenicol acetyltransferase gene in transiently transfected Jurkat cells. In addition, cells of nonhematopoietic origins exhibit a strikingly different chromatin pattern of IL-2, suggesting a role during differentiation for some of the hypersensitive sites.

Activation of T lymphocytes by mitogens or antigens leads to secretion of the growth factor interleukin-2 (IL-2) (14, 27, 30, 32, 34). This factor interacts with the IL-2 receptor on the surface of activated T cells, and such an interaction is required for progression of these cells through the cell cycle (3). T cells thus proliferate in part via an autocrine mechanism. Subsequent to the induction phase, IL-2 expression appears to be specifically down modulated (6). Consequently, the magnitude of the initial pulse of IL-2 synthesis determines to a large degree the magnitude of the proliferative stimulus. Several agents which interfere with IL-2 production, such as cyclosporin A (CsA) (7, 22) and glucocorticoids (12), have powerful immunosuppressive actions. These agents most likely prevent the expansion of clones of antigen-specific T lymphocytes. The effects of glucocorticoids are broad, whereas the dramatic immunosuppressive effects of CsA are based largely on the inhibition of IL-2 production and give some insight into the critical role of IL-2 regulation in governing the immune response.

The IL-2 gene product has been purified (28, 32), both cDNA and genomic clones for IL-2 have been identified, and the complete structure of the gene is known (11, 18, 19, 37). It has been established that the stimulation of IL-2 expression occurs at the transcriptional level (1, 23). Furthermore, the activation of the IL-2 gene after antigenic stimulation does not require new protein synthesis (6), indicating that the antigen receptor must communicate with the IL-2 gene without an intermediate newly synthesized gene product. At least one human cell line, Jurkat, serves as a model system for IL-2 activation. The Jurkat line can be stimulated to produce IL-2 by a combination of phytohemagglutinin (PHA) and phorbol myristate acetate (PMA) (14, 39–41). Either the OKT3 monoclonal antibody or the calcium ionophore A23187 can substitute for the PHA signal (39, 41). Normal peripheral blood T cells also require two signals for activation; namely, antigens (or mitogens) and macrophages (3, 26, 29, 38). Macrophages probably exert their effect through the IL-1 factor (5, 29). The mitogen PHA is thought to substitute for the signal normally provided by antigen, and PMA is probably a substitute for IL-1 or macrophages or both.

Despite such extensive knowledge about the structure and function of the IL-2 gene and the signals leading to its activation in both normal T cells as well as in model cell lines, very little is known about the intracellular events that result in transcriptional activation or the determinants for its tissue-specific expression. Therefore, we set out to study the regulation of the IL-2 gene at the DNA level. We approached this in two ways: first by probing the chromatin structure of IL-2 with DNase I in a variety of cell lines as well as in normal T cells before and after mitogenic stimulation, and second by DNA-mediated transfection experiments designed to locate the DNA segment(s) of the IL-2 gene that can confer inducibility on the linked chloramphenicol acetyltransferase (CAT) indicator gene (16).

We demonstrate that the IL-2 gene is associated with specific DNase I-hypersensitive sites located near its transcriptional start site. Such sites designate putative regulatory regions, because regulatory proteins bound to DNA are thought to perturb the regular chromatin structure such that DNA near them becomes hypersensitive to cutting by DNase I in the intact nuclei (8, 9, 43, 44). We show that several IL-2-nonproducing cells differ in their hypersensitive pattern from that in T cells. Furthermore, we show that stimulation of T cells leads to a dramatic change in the chromatin structure near the IL-2 promoter. This suggests that DNA sequence information near the promoter is involved with the transduction of the mitogenic signal received at the cell surface. The DNA transfection experiments reported here confirm this: a short DNA segment containing

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the IL-2 promoter confers inducibility on the linked CAT gene in transiently transfected Jurkat cells.

MATERIALS AND METHODS

Cells and cell lines. Human peripheral blood T cells were obtained from normal human donors by lymphopheresis on a Fenwall CS 3000 or an IBM COBE 2997 apparatus. Blood enriched for lymphocytes was subsequently purified over Ficoll-Hypaque gradients and nylon wool columns (see, for example, reference 10). The resulting cell preparation had consistently more than 90% T cells as judged by the presence of fluorescent OKT3 staining on the cell surface.

MCF-7 cells were kindly provided by L. Hennighausen; HeLa cells were kindly provided by J. Ostrove; Jurkat cells (14) were derived from those originally obtained from K. Smith and in addition were also kindly provided by K. Hardy; 8392 cells have been described previously (33); Raji cells were a gift from M. Trouco; HL60 cells were kindly provided by T. Breitman.

Both peripheral blood T cells (at 1×10^6 to 2×10^6 per ml) or Jurkat cells (at 4×10^5 to 8×10^5 per ml) were stimulated by using PHA-P (Burroughs Wellcome Co., Research Triangle Park, N.C.) at a concentration of 1 μg/ml and PMA at a concentration of 25 ng/ml for peripheral T cells and at 50 ng/ml for Jurkat cells, unless otherwise stated. Peripheral blood T-cell preparations can also be stimulated with PHA alone, presumably because there are sufficient macrophages present (no attempt was made to completely deplete the T cells of macrophages). However, this stimulation can usually be augmented by the addition of PMA.

DNase I hypersensitive analysis. The isolation of nuclei, digestion with DNase I, and subsequent purification of the DNA have been described extensively previously (33). Briefly, nuclei were isolated from cells by detergent lysis with Nonidet P-40, suspended at a concentration of between 2×10^7 and 5×10^7 nuclei per ml, and then digested with DNase I for 3 min at room temperature. The amounts of DNase I used are indicated in the figure legends.

Probes. The IL-2 probe used for DNase I hypersensitivity analysis is an *Xba*I-*Hin*dIII fragment derived from the p11 plasmid (19) (positions 4031 and 2482, respectively; see reference 18). The c-*myc* probe employed for hypersensitivity is a previously described *Xba*I-*Pvu*II fragment (33). The probe uscd to detect IL-2 message was derived from a full-length cDNA clone (19; N. J. Holbrook, unpublished data). The β-2 microglobulin cDNA-derived probe has been reported previously (36). Additional probes are described in the text.

Plasmid construction. A 632-base-pair (bp) fragment flanked by *Rsa*I restriction enzyme sites (−575 to +57) was excised from the *Eco*RI fragment spanning the IL-2 promoter fragment (subcloned as an *Eco*RI fragment in the plasmid p41 [19]). This fragment was subjected to brief BAL 31 digestion to remove the methionine initiator codon starting at position +54. After repair with Klenow polymerase, *Hin*dIII linker addition, and *Hin*dIII restriction, the fragments were electrophoresed on a low-melting-temperature agarose gel. Fragments which migrated close to the original size of 632 bp were excised and subsequently ligated into *Hin*dIII-cut pJYMOCAT. (pJYMOCAT was derived from pJYMCAT by deleting the *Hin*dIII insert containing the simian virus 40 early region promoter. pJYMCAT, in turn, was derived by ligation of the *Hin*dIII to *Eco*RI fragment of pJYMΔSph (25) containing the bulk of the pBR322 insert to the *Hin*dIII to *Eco*RI fragment of pSV2-CAT [16].) To select subclones with short deletions and to determine the extent of the deletions, various BAL 31 mutants were end labeled after *Hin*dIII digestion and then further digested with *Hin*fI and electrophoresed on 8 M urea–6% polyacrylamide sequencing gels. Two plasmids whose inserts had very short deletions on either end and which specifically had 2 and 10 bp deleted from the end carrying the initiator codon of the IL-2 gene were selected for further study (pIL-2-CAT 19 and pIL-2-CAT 1, respectively; see also Fig. 5). An additional plasmid was selected, pREV-IL-2-CAT, in which the IL-2 insert is transcriptionally opposite to the CAT gene and in which approximately 10 bp were removed from both ends of the insert.

Transfections and CAT assays. Cells were transfected by a DEAE-dextran procedure. Samples of 6×10^6 cells (taken from cells grown to a density of about 3×10^5/ml) were gently suspended in 0.25 mg of DEAE-dextran (Sigma Chemical Co., St. Louis, Mo.) per ml–0.1 mM chloroquine diphosphate (Sigma)–10 μg of DNA per ml in RPMI medium at 22°C. The cell samples were incubated at 37°C for 1.5 h, and then the cells were washed. Subsequently, the cells were incubated for 5 h in RPMI with 10% fetal bovine serum and then for another 36 h with or without 20 μg of PHA-P (Difco Laboratories, Detroit, Mich.) per ml and 10 ng of PMA (Chemicals for Cancer Research) per ml. The cells were harvested thereafter, and CAT assays were performed as described previously (15, 16).

RESULTS

Chromatin structure of IL-2 gene in normal human peripheral blood T cells before and after activation with mitogens. Peripheral blood T cells were isolated from the mononuclear cell fraction of blood obtained from normal human donors (see Materials and Methods). The cells were purified by Ficoll-Hypaque gradient centrifugation, and the nylon wool-nonadherent cell fraction was isolated. Consistently more than 90% of such cells stained with the monoclonal antibody OKT3 (data not shown). Cells isolated in this manner were used directly for DNase I hypersensitive studies. We prepared nuclei and digested them with a range of DNase I concentrations (see Materials and Methods). Subsequently, we isolated DNA from these nuclei, digested it with the restriction enzyme *Xba*I, electrophoresed it on 0.9 or 1% agarose gels, transferred it to nitrocellulose, and hybridized it to a ^{32}P-labeled probe (35). We visualized the hypersensitive sites as subbands by the indirect end-labeling technique (42). The probe shown in Fig. 1B labels the original genomic DNA restriction fragment in addition to the subbands created by the partial cutting of DNase I at the hypersensitive sites. These subbands become more visible with increasing amounts of DNase I as seen in Fig. 1A for the resting T lymphocytes. Three main regions of hypersensitivity, marked I, II, and IV, are apparent, and their positions are indicated in Figure 1B.

We also analyzed the hypersensitive pattern in T lymphocytes activated with PHA and PMA in vitro (see Materials and Methods) for 6.5 and 11 h. Figure 1A shows those patterns obtained at optimal DNase I concentrations. In addition to the hypersensitive sites seen for resting T cells, stimulated cells have one additional site marked III, located near the IL-2 promoter (Fig. 1B). Stimulation with PHA alone also shows this new hypersensitive site, if only slightly weaker (data not shown). We further established that these cells stimulated with PHA and PMA produce IL-2 message as judged by Northern blot analysis with an IL-2 cDNA

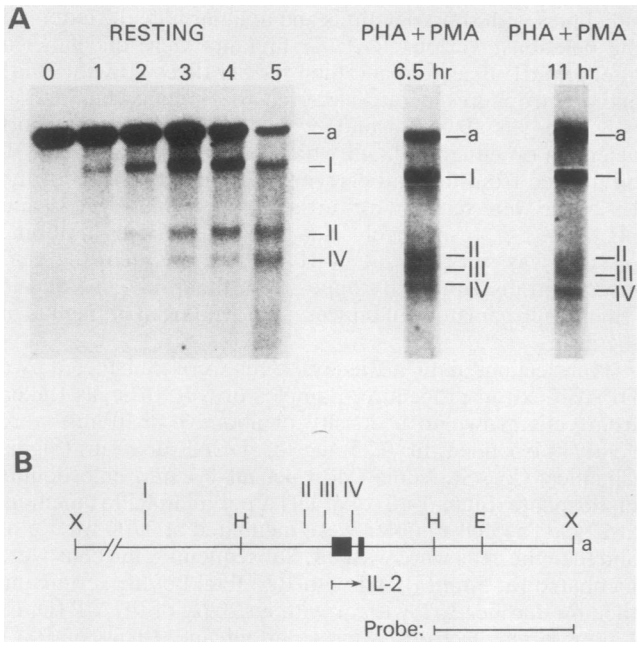

FIG. 1. Chromatin structure of the IL-2 gene in peripheral blood T cells. (A) Resting peripheral blood T cells or T cells stimulated for either 6.5 or 11 h with PHA and PMA (see Materials and Methods) were subjected to DNase I digestion (see Materials and Methods). In the resting pattern, increasing amounts of DNase I were used (left to right): 0, no DNase I; 1, 2 U of DNase I per ml of suspended nuclei; 2, 8 U/ml; 3, 12 U/ml; 4, 16 U/ml; 5, 24 U/ml. In the case of the stimulated cells, only an optimal DNase I concentration pattern is shown, which was obtained at 24 U of DNase I per ml of suspended nuclei. a marks the position of the genomic *Xba*I fragment to which the probe hybridizes. I, II, and IV and in the case of the stimulated cells also III mark the position of the subbands created by the cutting of DNase I at the hypersensitive sites. (B) Position of the hypersensitive sites relative to the first two exons of the IL-2 gene (shown by black boxes). The location of the probe is shown, as well as the start point of transcription (arrow) and the genomic fragment to which the probe hybridizes (a). X, *Xba*I; H, *Hin*dIII; E, *Eco*RI; only some of restriction enzyme sites are indicated.

probe, while no message was visible in resting T cells (Fig. 2). IL-2 message can already be detected after 2 h of stimulation in this experiment (although the exact onset may vary between blood donors [see references 21 and 23]). Message levels continue to rise for at least 24 h, possibly in part because the cells are turned on asynchronously.

Chromatin structure of IL-2 gene in Jurkat cell line before and after activation with mitogens. The Jurkat cell line is a model system in which to study the activation of the IL-2 gene. IL-2 can be induced with PHA plus PMA (or any signals which will substitute for one or the other), whereas either one of these agents alone essentially does not induce or does so only weakly (39–41). We performed DNase I-hypersensitive site analyses on normal Jurkat cells, on Jurkat cells stimulated for either 2.5 or 4 h with PHA and PMA, and on cells stimulated for 10 h with either PMA alone or PHA and PMA. Jurkat cells are rapidly inducible for IL-2 with substantial amounts of IL-2 message apparent after 2.5 h of stimulation. High message levels for IL-2 were observed for well over 8.5 h (Fig. 2B), but decreased appreciably by 22 h, possibly owing to posttranscriptional mechanisms (6). PHA and PMA together (at 2.5, 4, or 10 h) induced the same new hypersensitive site III which was evident in activated normal human peripheral blood T cells (Fig. 3A and B). In fact, the whole pattern of hypersensitivity in Jurkat cells was similar to that of normal T cells. Hypersensitive site I appeared somewhat weaker in Jurkat cells. In addition, induction of the Jurkat cells decreased the intensity of hypersensitive sites II and IV slightly when compared with the pattern in induced normal T cells. Visualization of such a decrease may be a direct consequence of the homogeneous Jurkat cell population which is strongly inducible, as opposed to the heterogeneous population of T-cell subclasses that are probably asynchronously turned on. Such an interpretation is supported by the slow increase in IL-2 message production up to at least 24 h of stimulation in peripheral blood T cells (Fig. 2), as opposed to the sharp rise and fall of message in Jurkat cells. Nonetheless, aside from these minor differences, the patterns of hypersensitivity confirm each other and clearly suggest that a segment of DNA located around hypersensitive site III plays a major role during the

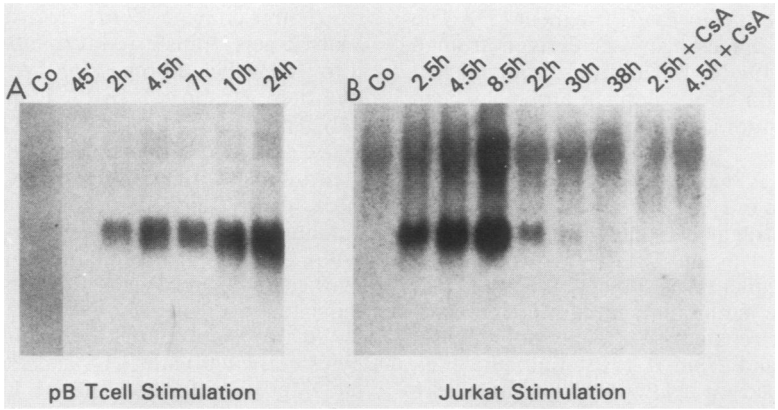

FIG. 2. IL-2 message induction in peripheral blood T cells and Jurkat cells. Peripheral blood (pB) T cells and Jurkat cells were stimulated with PHA and PMA for the times indicated and as described in Materials and Methods. Total RNA was extracted by the guanidine isothiocyantate extraction method (4) and blotted as described previously (24). The probe used was a full-length IL-2 cDNA (see Materials and Methods). For the Jurkat experiment, approximately similar amounts of total RNA were loaded in each lane, and for the peripheral blood T cells, the RNA from an equal number of cells was loaded in each lane (except for the 7-h pB T-cell lane, which had slightly less RNA), and this was further confirmed by rehybridizing these blots with a cDNA probe of β-2 microglobulin, which showed constant message levels (see Materials and Methods; data not shown). In the case of the Jurkat cells, a 2.5- and 4.5-h stimulation were done also in the presence of CsA at 1 μg/ml (see text), which prevented IL-2 message induction.

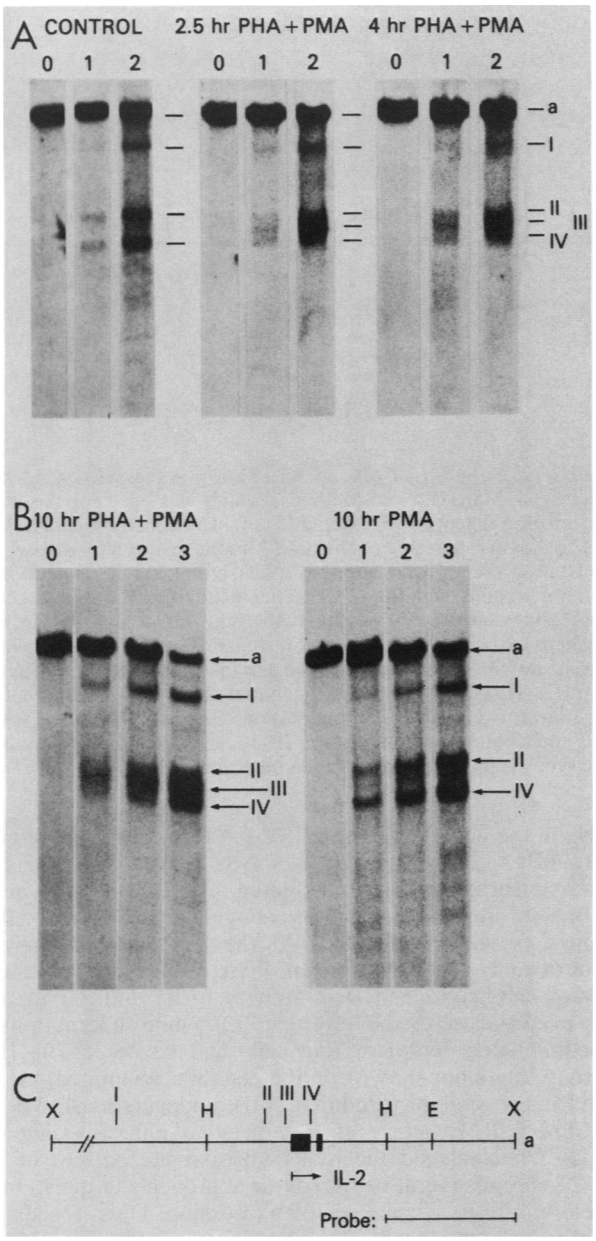

FIG. 3. Chromatin structure of IL-2 gene in Jurkat cells. (A) Hypersensitive sites for unstimulated cells (control) and for 2.5- and 4-h PHA- and PMA-stimulated Jurkat cells; (B) pattern of hypersensitivity obtained after 10 h of stimulation with PHA and PMA or PMA alone (see Materials and Methods); (C) location of the hypersensitive sites and the probe used. For further description see the legend to Fig. 1. DNase I concentrations: control and 2.5-h PHA + PMA, 0 = no DNase I, 1 = 12 U/ml, 2 = 32 U/ml; 4-h PHA + PMA, 1 = 16 U/ml, 2 = 32 U/ml; 10-h PHA + PMA and 10-h PMA, 1 = 16 U/ml, 2 = 24 U/ml, 3 = 32 U/ml.

induction process. This is further supported by the experiment shown in Fig. 3B, in which only PMA was administered. PMA alone is a poor activating agent for IL-2 production, and this is reflected in the barely detectable appearance of hypersensitive site III in cells so treated (as seen only in long exposures such as that in Fig. 3B). A similar result was obtained with PHA alone, an only marginally better inducer than PMA alone (data not shown). Furthermore, the addition of CsA at a concentration of 1 μg/ml completely prevented the formation of hypersensitive site III when added just 30 min before the addition of PHA and PMA (data not shown); under these conditions, no IL-2 message was observed (Fig. 2).

The positions of the hypersensitive sites in Jurkat cells were also confirmed by *Eco*RI digestion of the DNA after isolation from the DNase I-treated nuclei. The resultant Southern blots were then hybridized with an appropriate probe (*Cla*I [position 1951]-*Eco*RI [position 3013] [18]) (data not shown).

Homologies surrounding regions of hypersensitivity. Regions of hypersensitivity are potential binding sites for regulatory proteins. Although we know nothing about such putative sequences or proteins for the IL-2 gene, hypersensitive sites II and III are surrounded by sequence homologies to HTLV-I and to gamma-interferon (11, 18). The HTLV-I virus (human T-cell leukemia virus I) is expressed in T cells and as such should contain sequence information allowing it to be recognized by transcriptional factors in T lymphocytes. The gamma-interferon gene is expressed in T cells and furthermore is induced in a manner analogous to that of IL-2 (41). Therefore, shared transacting proteins might be involved in the regulation of all these promoters, and thus sequence homologies between HTLV-I, IL-2, and gamma-interferon may be significant. In addition, the promoter region of the IL-2 receptor gene, again a gene which is induced in activated T cells, appears to share a short nucleotide sequence with the IL-2 gene (20). Most, but not all (18), of these described homologies are near hypersensitive sites II and III. The hypersensitive sites as well as these homologous regions and their origins are indicated in Fig. 4. Hypersensitive site I lies in a region further upstream which has not been sequenced.

DNA-mediated transient transfection of a CAT construct driven by an IL-2 promoter-containing fragment. To define the sequences involved in the activation of the IL-2 gene, we constructed plasmids to be used in DNA transfection experiments, in which fragments extending from about 570 bp upstream to about 50 bp downstream of the IL-2 transcriptional start site were inserted 5′ to the CAT gene (see Materials and Methods and Fig. 5). Two of these plasmids, pIL-2-CAT 1 and pIL-2-CAT 19, have IL-2 inserts in the same orientation as the CAT gene, and another, pREV-IL-2-CAT, has an IL-2 insert in the opposite orientation. These CAT constructs were transfected into either the Jurkat cell line or the Raji cell line. Raji cells are Epstein-Barr virus-positive Burkitt lymphoma B cells and were used as control cells in these experiments. A CAT construct driven by the Rous sarcoma virus long terminal repeat (pRSV-CAT) served as a parallel control template, since it is expressed constitutively in a variety of cells (15). The cells were transfected by a DEAE-dextran method and subsequently either stimulated with PHA and PMA for 36 h or left unstimulated before extraction to determine CAT activity (see Materials and Methods).

In the Jurkat cell line transfected with pRSV-CAT, stimulation with PHA and PMA resulted in only a slight increase in CAT activity from an already high constitutive level (Fig. 6A, lanes 1 and 2). Expression of pIL-2-CAT1 and -19 constructs, on the other hand, could not be detected in untreated Jurkat cells, while strong expression occurred in PHA- and PMA-stimulated cells (Fig. 6A, lanes 6 and 5; data shown only for pIL-2-CAT1). Moreover, the pREV-IL-2-CAT construct (negative control) was not expressed in the induced or noninduced state (Fig. 6A, lanes 3 and 4). In contrast to the Jurkat cells, expression of the pIL-2-CAT construct was not detectable in PHA- and PMA-stimulated Raji cells (which are not inducible for IL-2 expression),

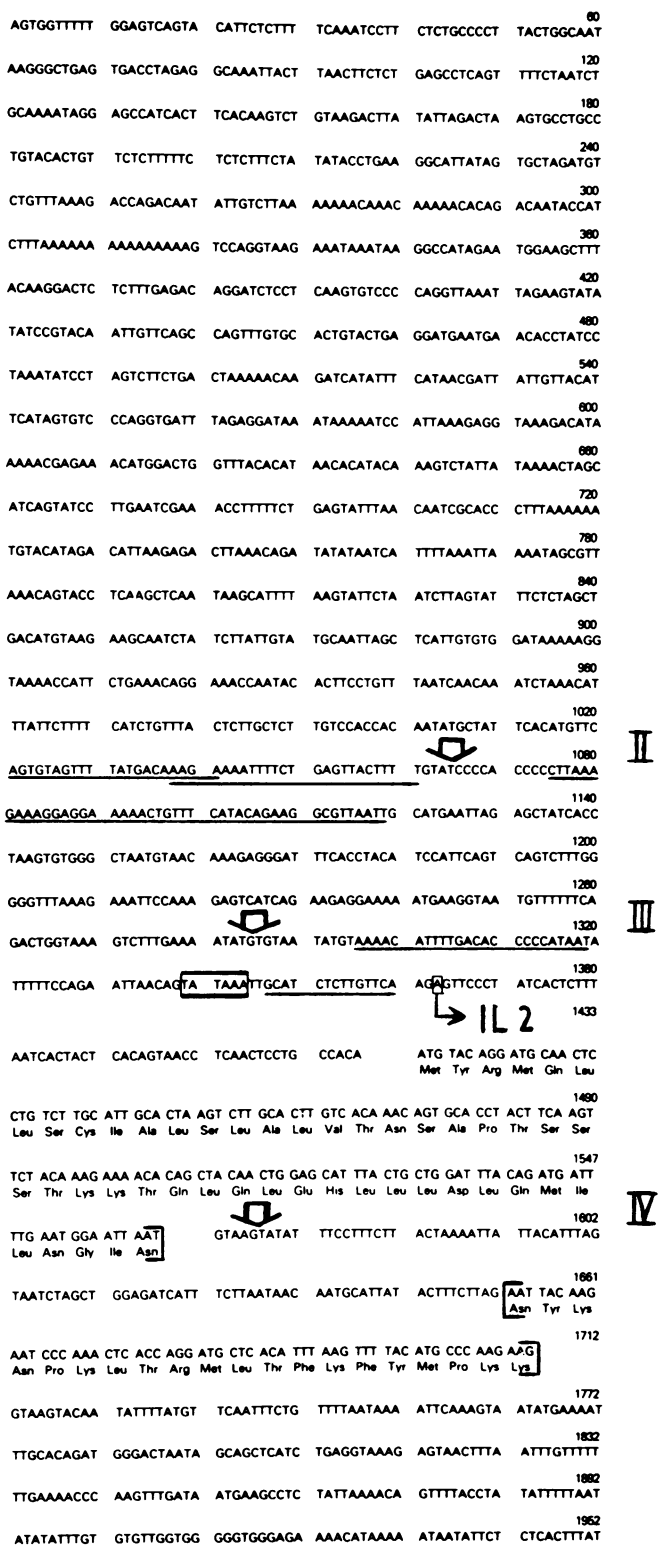

FIG. 4. Location of IL-2 hypersensitive sites and sequence homologies. The approximate positions of the hypersensitive sites II, III, and IV are marked relative to the sequence of the 5′ end of the IL-2 gene (18). (The center of the hypersensitive bands was measured. Measurements of independent experiments varied by about ± 25 bp). Also shown are the TATA motif (boxed) and the transcriptional start site (arrow), in addition to the first two exons of the IL-2 gene. Hypersensitive site I is located upstream of the sequence shown. Various homologies are underlined: position 1021

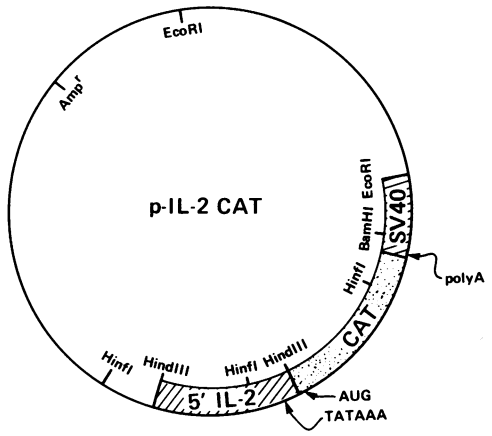

FIG. 5. Structure of pIL-2-CAT. Shown is the pIL-2-CAT construct (see Materials and Methods) which was used for the transfection experiments (see text and Fig. 6). The construct contains the IL-2 promoter inclusive of the TATA motif (marked by arrow) and the transcriptional start site (but exclusive of the IL-2 methionine initiator codon) and the CAT gene including its initiator codon (marked by arrow). 3′ of the CAT gene lies a simian virus 40 segment harboring the early-region simian virus 40 polyadenylation signal, and the remaining sequence derives from pBR. The structure of pREV-IL-2-CAT is the same as that of pIL-2-CAT except that the IL-2 insert is in the reverse orientation. Only the *Hin*fI sites within and immediately proximal to the IL-2 insert are shown; they were used for mapping analysis of the constructs.

despite the activity of the pRSV-CAT vector in these cells (Fig. 6B).

To ensure that the transfected pIL-2-CAT was being expressed in parallel with the endogeneous IL-2 gene, IL-2 bioassays were performed. At the time of cell harvest, supernatants from cultures of PHA- and PMA-stimulated Jurkat cells contained IL-2 activity of 65 units or greater (assayed as described in reference 13), while supernatants of nonstimulated Jurkat or Raji cells had no detectable IL-2 activity (data not shown). In the cell lines examined, pIL-2-CAT expression mirrored that of the endogenous IL-2 gene.

Chromatin structure of IL-2 gene in nonexpressing cell types. We analyzed the hypersensitive-site pattern of the IL-2 gene in several cell types to approach the question of the origin of its tissue-specific expression. Only T cells are known to produce IL-2 when stimulated appropriately. Therefore, in an initial screening, we determined the IL-2 chromatin structure in two hematopoietic cell lines as well as in two cell lines of epithelial origin. The HL60 promyelocytic cells exhibit the same pattern of hypersensitivity for IL-2 as that in uninduced T cells (Fig. 7B), except that hypersensitive site II appears to be reduced in intensity relative to sites

to position 1041 (inclusive) has a 17 of 21 nucleotide match with a sequence in the HTLV-I genome, at about the same distance upstream of the promoter of that virus (18); 1038 to 1061 has a 20 of 24 match with an intronic gamma-interferon sequence, which is also associated with a hypersensitive site (17); 1075 to 1119 has a 33 of 45 match with a gamma-interferon sequence just upstream of the promoter of that gene (11); 1296 to 1319 has a 15 of 24 match with a gamma-interferon sequence just upstream of the promoter of that gene (11); 1347 to 1360 has a 12 of 14 match with an HTLV-I sequence which is located just downstram of the TATA motif of that virus (18); 1274 to 1284 has a 9 of 11 match with an IL-2 receptor sequence, which is located at about the same distance upstream of the TATA motif of that gene (20) (not underlined in the figure).

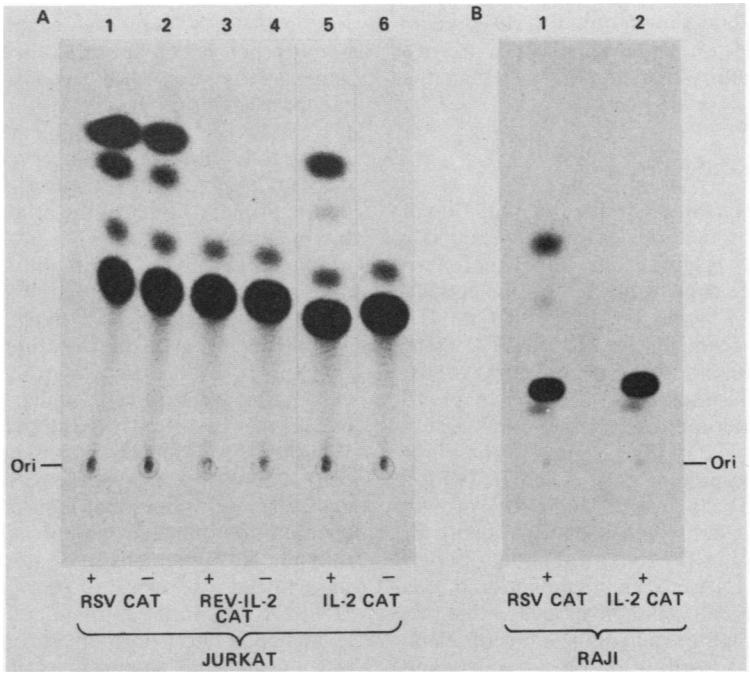

FIG. 6. 5′-flanking region of the IL-2 gene confers inducibility onto the CAT gene . (A) Expression of CAT recombinant plasmids in Jurkat cells. Jurkat cells (6×10^6) were transfected with 20 μg of pRSV-CAT (lanes 1 and 2), pREV-IL-2-CAT (lanes 3 and 4), and pIL-2-CAT1 (lanes 5 and 6) (see Materials and Methods for transfection conditions and extraction). Ori refers to the origin at which the samples were spotted onto the thin-layer chromatography plates, and + indicates stimulation with PHA and PMA before extraction for analysis of CAT activity, whereas − indicates no stimulation. Percent conversions from [^{14}C] chloramphenicol to 1- or 3-acetylated chloramphenicol (e.g., upper two spots in lane 1) were as follows: lane 1, 38%; lane 2, 20%; lane 3, 0%; lane 4, 0%; lane 5, from 0.6 to 4.2% in five experiments for IL-2-CAT1 and -19; lane 6, 0%. (B) Expression of CAT recombinant plasmids in Raji cells. Conditions were as in panel A except that pIL-2-CAT19 was transfected (see Materials and Methods); however, this plasmid is expressed in a quantitatively similar manner to pIL-2-CAT1 in Jurkat cells (data not shown). Percent conversions: lane 1, 9.1%; lane 2, 0%.

I and IV. A similar result was obtained with 8392, an Epstein-Barr virus-transformed B-cell line (data not shown). Thus, it is possible that these cells have a slightly different composition of nuclear factors interacting with the IL-2 gene, resulting in slightly decreased hypersensitivity at site II. On the other hand, two cell lines of nonhematopoietic origin, namely, the human breast carcinoma cell line MCF-7 and the HeLa cell line derived from a human cervical carcinoma, showed essentially no hypersensitive sites (Fig. 7A, data shown only for HeLa cells). To control for proper DNase I digestion, we also probed these latter DNA samples for c-*myc* hypersensitivity, which yielded the expected pat-

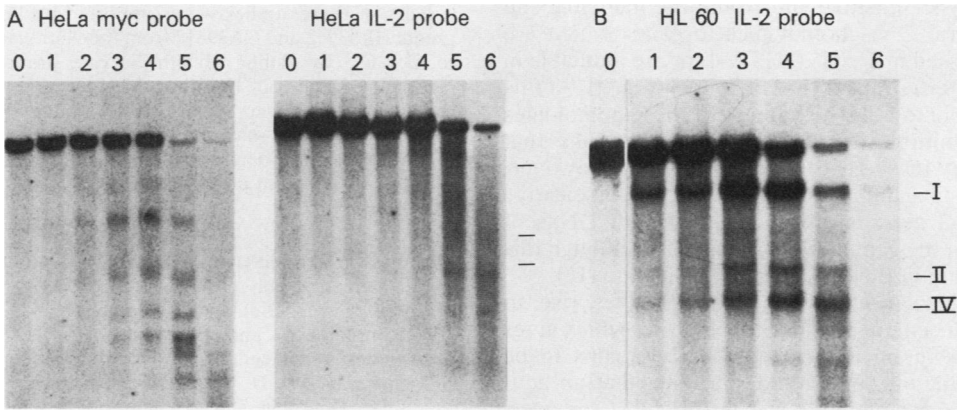

FIG. 7. Chromatin structure of IL-2 gene in non-T cells. (A) No true hypersensitive sites for the IL-2 gene were seen in HeLa cells (*Xba*I digest and IL-2 probe as described in the legends to Fig. 1 and 3) even after overexposing the film. Some regions of very weak hypersensitivity are visible in the lanes which were highly digested with DNase I, but even these regions do not coincide with the hypersensitive sites seen strongly in T cells at much lower DNase I concentrations. The positions were hypersensitive sites I, II, and IV occur in T cells are marked by lines next to lane 6. The same HeLa digest shows a normal hypersensitive pattern when probed with a c-*myc* probe (see Materials and Methods and reference 33). See the legend to Fig. 1 for further description. DNase I concentrations were as follows: 0, no DNase I; 1, 8 U/ml; 2, 16 U/ml; 3, 32 U/ml; 4, 48 U/ml; 5, 96 U/ml; 6, 128 U/ml. (B) The IL-2 hypersensitive pattern in HL60 cells (same restriction digest and same probe as in panel A). The hypersensitive sites I, II, and IV are marked. DNase I concentrations were as follows: 0, no DNase I; 1, 2 U/ml; 2, 8 U/ml; 3, 16 U/ml; 4, 32 U/ml; 5, 48 U/ml; 6, 64 U/ml.

tern for this gene (Fig. 7A, data shown only for HeLa cells) (33). Consequently, both HeLa and MCF-7 cells have a completely different chromatin structure of IL-2 than the hematopoietic cells tested.

DISCUSSION

Data presented in this paper establish that the induction of IL-2 expression in T cells is mediated via DNA sequence elements that lie near the promoter of this gene. Two complementary experimental approaches led to this conclusion. First, a new DNase I hypersensitive site (site III) appears promptly just upstream of the IL-2 TATA motif after stimulation of peripheral blood T cells and Jurkat cells, which are inducible for IL-2 expression. As shown previously by nuclear run-on experiments, this induction occurs at the transcriptional level (1, 23). The change in the chromatin structure becomes apparent only when activation conditions are used that lead to the expression of this growth factor; stimulation of Jurkat cells with a partial signal, for example, PMA alone, does not precipitate this new hypersensitive site. Additionally, CsA blocks the induction of this new site in stimulated Jurkat cells, establishing also that this drug acts before the chromatin rearrangement of IL-2. Although these data no not constitute proof of a cause and effect relationship between changed hypersensitivity and transcriptional activation, they do provide an intriguing correlation between these two phenomena. Furthermore, these data do not address the potential involvement of elements additional to hypersensitive site III in the induction process.

The DNA transfection experiments described here represent the second experimental approach. These experiments reveal that a fragment about 600 bp in length, including the transcription start site of IL-2 and the segments around hypersensitive sites II and III, is apparently sufficient to confer inducibility onto a CAT gene during a transient transfection assay in Jurkat cells. This suggests that all the information necessary for induction is contained within these approximately 600 bp and provides further support for the conclusion reached from the chromatin analysis.

The DNA segment just upstream of the IL-2 promoter, which contains hypersensitive site II and the inducible site III, harbors several DNA homologies to genes which are specifically expressed in T cells (HTLV-I) or are inducible in T cells (gamma-interferon and IL-2 receptor) (Fig. 4). At this time it is impossible to tell which if any of these homologies are indeed recognition sites for regulatory proteins that might be shared by these genes. It is noteworthy, however, that a homology to gamma-interferon near hypersensitive site II of the IL-2 gene is also associated with DNase I hypersensitivity in the gamma-interferon gene, although this sequence is intronic in the gamma-interferon gene (17).

The exact molecular architecture which gives rise to hypersensitivity in general and more specifically which gives rise to the change upon induction of IL-2 remains to be elucidated. Nonetheless, one could speculate that an activated transcription factor binds just upstream of (or overlapping) the TATA box after T-cell stimulation. For example, steroid activation of the mouse mammary tumor virus promoter correlates with hypersensitivity next to a hormone-receptor DNA-binding site (44). In the case of IL-2, it is equally possible that a transcription-associated complex located near site III is modified or that a protein normally shielding this region from DNase I is in fact removed to allow transcription. Less likely is that the observed change in chromatin structure is a consequence of transcriptional activity per se, as several other transcriptionally inducible genes such as c-*fos*, heat shock genes, c-*myc*, and the IL-2 receptor gene do not display such a new hypersensitive site (31, 42; U. Siebenlist, unpublished observations). It is worth noting that although the observed chromatin change may be directly involved in the transcriptional activation, it may not be the primary target of the stimulation signal received from the membrane.

By comparing the chromatin structure of the IL-2 gene in nonproducing cells with that in T cells, one might be able to pinpoint sequences most likely responsible for creating a T-cell-specific gene during differentiation. We report here that the IL-2 gene in two other hematopoietic cells has a chromatin structure very similar to that in uninduced T cells, except that hypersensitive site II is somewhat less apparent. Whether this difference is sufficient to prevent the induction of IL-2 and thus of hypersensitive site III or whether these cells also lack additional factors is undetermined. The pattern in two nonhematopoietic cells is dramatically different, though. No hypersensitive sites are present in IL-2, even though the expressed c-*myc* gene carries the expected DNase I sites in these same cells. Thus, the IL-2 gene may be in a completely different chromatin arrangement, possibly existing in a heterochromatic form in these cells. By the same reasoning then, the hypersensitive sites associated with the IL-2 gene in hematopoietic cells may be markers of activation events during development and differentiation. In addition, T cells only might elaborate nuclear factors that lead to increased sensitivity near site II and that allow formation of site III.

The work presented here provides the basis for a more detailed dissection of the molecular events which lead to the activation of the IL-2 gene in T cells and ultimately to T-lymphocyte proliferation.

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