MOLECULAR AND CELLULAR BIOLOGY, Aug. 1989, p. 3438–3446
0270-7306/89/083438-09$02.00/0

Vol. 9, No. 8

Isolation and Characterization of Glucocorticoid- and Cyclic AMP-Induced Genes in T Lymphocytes

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Received 27 February 1989/Accepted 9 May 1989

Glucocorticoids and cyclic AMP exert dramatic effects on the proliferation and viability of murine T lymphocytes through unknown mechanisms. To identify gene products which might be involved in glucocorticoid-induced responses in lymphoid cells, we constructed a λcDNA library prepared from murine thymoma WEHI-7TG cells treated for 5 h with glucocorticoids and forskolin. The library was screened with a subtracted cDNA probe enriched for sequences induced by the two drugs, and cDNA clones representing 11 different inducible genes were isolated. The pattern of expression in BALB/c mouse tissues was examined for each cDNA clone. We have identified two clones that hybridized to mRNAs detected exclusively in the thymus. Other clones were identified that demonstrated tissue-specific gene expression in heart, brain, brain and thymus, or lymphoid tissue (spleen and thymus). The kinetics of induction by dexamethasone and forskolin were examined for each gene. The majority of the cDNA clones hybridized to mRNAs that were regulated by glucocorticoids and forskolin, two were regulated only by glucocorticoids, and three hybridized to mRNAs that required both drugs for induction. Inhibition of protein synthesis by cycloheximide resulted in the induction of all mRNAs that were inducible by glucocorticoids. Preliminary sequence analysis of four of the 11 cDNAs suggests that two cDNAs represent previously undescribed genes while two others correspond to the mouse VL30 retrovirus-like element and the mouse homolog of chondroitin sulfate proteoglycan core protein.

Glucocorticoids induce cytolysis in susceptible lymphocyte populations, including immature thymocytes and certain leukemias and lymphomas. Steroid-induced cell death is thought to occur through the activation of an endogenous suicide process. Glucocorticoid treatment of murine lymphocytes results in severe alterations in cellular metabolism which include an inhibition of glucose transport (50), increased RNA (14) and protein (44) degradation, a rise in intracellular calcium levels (37), and decreased incorporation of thymidine into DNA (9). Glucocorticoid-induced lymphocytolysis is preceded by cell cycle arrest (28) and extreme morphological changes. These include widespread chromatin condensation, which is associated with extensive DNA fragmentation in both human and murine lymphocytes (22, 58). The DNA cleavage appears to result from the activation of a preexisting calcium-dependent endonuclease (17, 59). Glucocorticoid-induced DNA fragmentation and cell death are prevented when RNA and protein syntheses are inhibited (17). In addition, there is genetic evidence in mouse and human cells for the existence of a locus involved in cell lysis (25, 26, 63). Therefore, glucocorticoids may induce the synthesis of a specific protein(s) involved directly or indirectly in lysis of lymphocytes. No such protein has yet been identified, and the mechanism of glucocorticoid-induced cytolysis remains obscure.

In addition to glucocorticoids, several other agents, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (46) and cyclic AMP (cAMP) (20), are toxic to lymphoid cells. Agents that increase intracellular levels of cAMP cause cell cycle arrest in G1 (16) and produce the same pattern of DNA fragmentation as do glucocorticoids (58). A functional interaction between glucocorticoids and cAMP was observed in WEHI-7 lymphoma cells, as cAMP increased the glucocorticoid binding capacity of the steroid receptor (27). cAMP is also known to regulate the expression of several genes at the level of transcription (5, 15, 49). Moreover, cAMP and glucocorticoids can act synergistically to induce gene expression (21, 32, 53, 62). The possibility thus exists that both glucocorticoids and cAMP regulate the expression of genes involved in the lytic process.

Glucocorticoids and cAMP are known to regulate the levels of several proteins in lymphocytes. For example, glucocorticoids induce glucocortin in rat thymus (18), glutamine synthetase in the CEM-C7 leukemic cell line (29), interleukin-1 receptor in human B lymphocytes (2), and insulin receptor in the human IM-9 lymphocyte cell line (47). In contrast, expression of the proto-oncogenes c-myc, c-myb, and c-Ki-ras is inhibited by glucocorticoids in the T lymphoma S49 cell line (23) as is expression of thymidine kinase, thymidylate synthetase, and ornithine decarboxylase in P1798 lymphosarcoma cells (4). cAMP also produces a decrease in ornithine decarboxylase activity in S49 cells (36). Recently, several cDNA clones representing mRNAs repressed by glucocorticoids in rat thymus were isolated (33); however, the products of these genes have yet to be identified. Although the levels of the above-mentioned proteins are regulated by glucocorticoids or cAMP, there is no evidence to suggest that they are involved in cytolysis.

As a first step towards identifying the gene products responsible for the effects of glucocorticoids in T lymphocytes, we utilized a subtractive hybridization strategy to isolate glucocorticoid- and cAMP-inducible genes from the glucocorticoid-sensitive murine thymoma cell line, WEHI-7TG (8, 31). The isolation and characterization of such genes will provide useful information towards understanding the cytolytic process as well as other hormone-regulated responses in T cells. Eleven cDNA clones have been isolated that correspond to different mRNAs that are induced by treatment with glucocorticoids and cAMP. The expression of several of these mRNAs is lymphoid specific.

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MATERIALS AND METHODS

Cell culture and nucleic acid isolation. WEHI-7TG is a thioguanine-resistant derivative of the glucocorticoid-sensitive BALB/c murine thymoma line WEHI-7 (8, 31). CXG56D3 is a variant of WEHI-7TG that was selected for resistance to cAMP and dexamethasone (dex^r $cAMP^r$) by Don Gruol (The Salk Institute) by a two-step selection procedure described previously (27). All cells were grown in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.). For induction of glucocorticoid-regulated sequences, WEHI-7TG cells were treated with triamcinolone acetonide (TA; 1 μM) or dexamethasone (1 μM) for 5 h. TA and dexamethasone are both synthetic glucocorticoids with high affinities for the hormone receptor. In some experiments, forskolin (an agent which increases intracellular levels of cAMP) was added to a concentration of 12 μM and cycloheximide was added at 10 μg/ml. Total cellular RNA was extracted from cells and BALB/c mouse tissues according to Chirgwin et al. (11) or alternatively by an acid guanidinium thiocyanate-phenol-chloroform procedure (12). Poly(A)$^+$ RNA was selected by affinity chromatography on oligo(dT) cellulose (45).

cDNA library construction. Poly(A)$^+$ RNA was isolated from WEHI-7TG cells treated for 5 h with 1 μM TA and 12 μM forskolin. Double-stranded cDNA was made from 5.0 μg of poly(A)$^+$ RNA by using the Amersham cDNA synthesis kit (Amersham Corp., Arlington Heights, Ill.). The cDNA was methylated prior to ligation with *Eco*RI linkers to protect internal *Eco*RI sites. After removal of excess linkers, a portion of the cDNA was inserted into the λZAP vector (Stratagene, La Jolla, Calif.) and resulted in a library of 5×10^5 recombinants. The unamplified library was plated at 50,000 plaques per 150-mm dish in 0.7% top agarose supplemented with 30% glycerol (40), and lifts were done with Hybond-N (Amersham). The plates were stored at −70°C.

cDNA probe preparation and library screening. Single-stranded [^{32}P]cDNA was made by using poly(A)$^+$ RNA isolated from WEHI-7TG cells treated with TA and forskolin for 5 h. With the Amersham cDNA synthesis kit, the first-strand synthesis reaction was adjusted to contain 0.5 mM each of dATP, dGTP, and dTTP, 0.1 mM dCTP, 50 μg of actinomycin D per ml, and 200 μCi of [α-^{32}P]dCTP (3,889 Ci/mmol). After first-strand synthesis, the template RNA was removed by incubation in 0.2 N NaOH at 70°C for 20 min. The reaction was neutralized with HCl, and the cDNA was ethanol precipitated in the presence of 2.5 M ammonium acetate. The specific activity of the cDNA was approximately 1×10^8 to 2×10^8 cpm/μg. The [^{32}P]cDNA was hybridized to a 40-fold mass excess of uninduced CXG56D3 poly(A)$^+$ RNA to a R_0t of 1,000 (mol/liter) × s in 0.5 M sodium phosphate buffer (pH 7), 0.1% sodium dodecyl sulfate (SDS), and 0.001 M EDTA. Unhybridized cDNA was collected by hydroxylapatite chromatography (Bio-Rad, Richmond, Calif.) in 0.12 M phosphate buffer–0.1% SDS at 60°C. After two cycles of hybridization, the remaining single-stranded cDNA was used to screen the WEHI-7TG cDNA library. Filters were hybridized at 42°C in a solution of 50% formamide, 5× SSPE (1× SSPE is 0.18 M NaCl, 0.01 M $NaHPO_4$, 0.001 M EDTA), 0.5% SDS, 5% (wt/vol) dextran sulfate, 7× Denhardt solution (1× Denhardt solution is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), and 200 μg of denatured salmon sperm DNA per ml. Filters were washed in 0.3× SSPE–0.1% SDS at 65°C and exposed to Kodak XAR-5 film at −70°C. Positive plaques were rescreened at low density with the subtracted probe or by using duplicate lifts hybridized to unsubtracted [^{32}P]cDNA probes prepared from poly(A)$^+$ RNA of induced WEHI-7TG cells or untreated CXG56D3 cells.

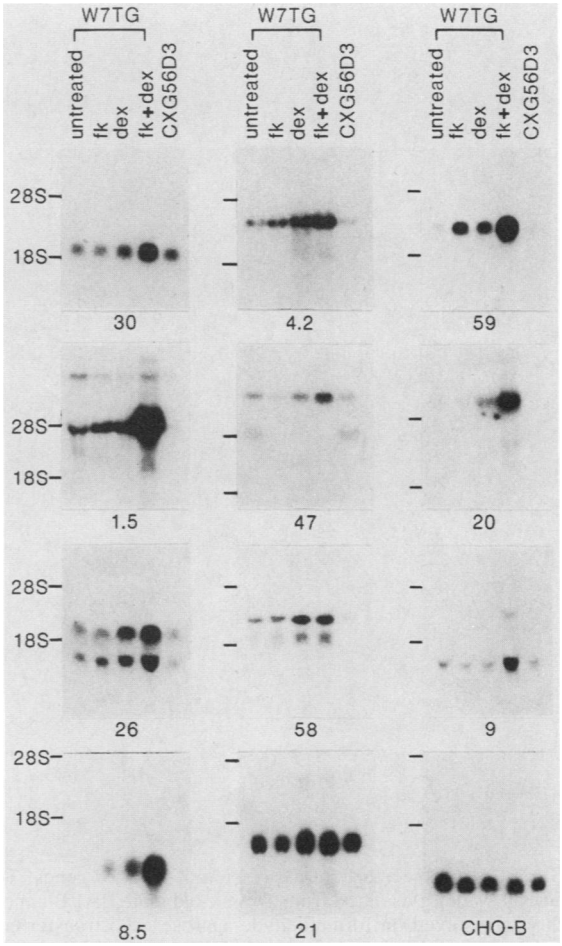

FIG. 1. Northern blot analysis of induced mRNAs. Total cellular RNA (20 μg) from untreated WEHI-7TG cells, WEHI-7TG cells treated for 5 h with 12 μM forskolin (fk), 1 μM dexamethasone (dex), or a combination of the two drugs (fk + dex), and untreated CXG56D3 cells was fractionated on formaldehyde-agarose gels and transferred to Hybond-N. Labeled cDNA inserts from each clone were used as probes. As a control, a cDNA corresponding to CHO-B (30), a constitutively expressed, unregulated mRNA, was used as a probe. Migration of the 18S (2-kb) and 28S (5-kb) ribosomal RNAs is indicated.

Northern blot analysis. Total cellular RNA was fractionated in 1% agarose gels containing 2.2 M formaldehyde and was transferred to Hybond-N. The pBluescriptSK(−) plasmid containing the cDNA inserts was excised from the λZAP vector by an in vivo excision protocol as recommended by the supplier (Stratagene). cDNA inserts were labeled to 1×10^9 to 2×10^9 cpm/μg with the Multiprime DNA labeling kit from Amersham. The filters were hybridized at 42°C in a solution of 50% formamide, 5× SSPE, 8× Denhardt solution, 0.5% SDS, and 400 μg of denatured salmon sperm DNA per ml (Fig. 1) or in 50% formamide at 42°C as described by Church and Gilbert (13) (Fig. 2 and 3). The latter procedure produced a higher signal but otherwise identical results. Filters were washed in 0.3× SSPE–0.1% SDS at 65°C and exposed to Kodak XAR-5 film at −70°C.

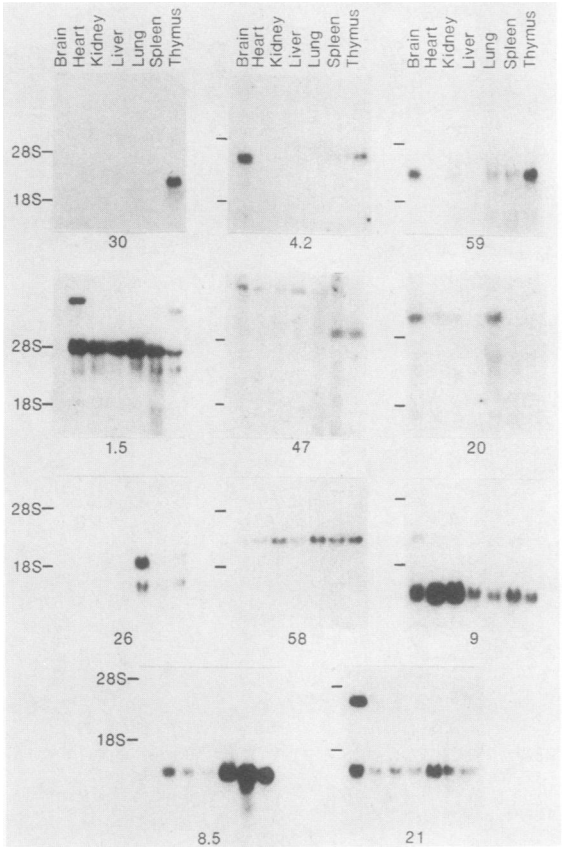

FIG. 2. Tissue specificity of expression of induced genes. Total cellular RNA (20 μg) isolated from 5-week-old male BALB/c mouse tissues was resolved on formaldehyde-agarose gels, transferred to Hybond-N, and hybridized to labeled cDNA probes. Migration of the 18S (2-kb) and 28S (5-kb) ribosomal RNAs is indicated.

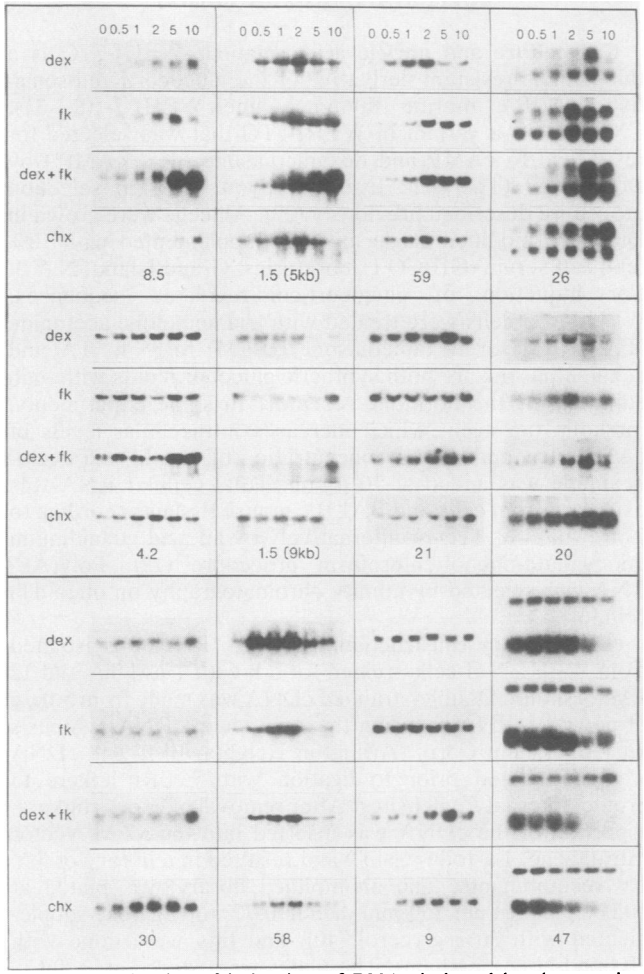

FIG. 3. Kinetics of induction of RNAs induced by dexamethasone, forskolin, and cycloheximide. Total cellular RNA (20 μg per lane) isolated from untreated WEHI-7TG cells or WEHI-7TG cells treated for the indicated times (hours shown at top) with 1 μM dexamethasone (dex), 12 μM forskolin (fk), a combination of dexamethasone and forskolin (dex + fk), or 10 μg of cycloheximide (chx) per ml was fractionated on formaldehyde-agarose gels, transferred to Hybond-N, and hybridized to each cDNA clone. The results for the major mRNA species hybridizing to each clone are shown.

Northern (RNA) blots were quantified with an LKB Ultroscan XL densitometer.

Sequence analysis. Computer searches of GenBank were done through Bionet with the FASTA program of Pearson and Lipman (54). Partial nucleotide sequences were compared with release 59 of GenBank.

RESULTS

Preparation and screening of a cDNA library from WEHI-7TG cells. We were interested in isolating genes induced by glucocorticoids and cAMP in T lymphocytes. Therefore, a λZAP cDNA library was prepared by using poly(A)$^+$ RNA from WEHI-7TG cells treated for 5 h with 1 μM TA and 12 μM forskolin. A library of approximately 5×10^5 independent recombinant phage was obtained and plated without amplification. ^{32}P-labeled single-stranded cDNA was prepared from the induced WEHI-7TG mRNA and was submitted to subtractive hybridization against mRNA extracted from the CXG56D3 (dex^r cAMP^r) variant. These cells were chosen as a source of uninduced RNA because the responses to both glucocorticoids and forskolin are blocked and therefore mRNAs inducible by hormone or cAMP should be underrepresented. The single-stranded cDNA remaining after two cycles of subtraction was used as a probe to screen the WEHI-7TG cDNA library. Approximately 1,000 plaques per 5×10^5 gave a positive signal. Representative plaques (100) with signals of various intensities were selected for a secondary screen and were hybridized at low density with the subtracted probe. Alternatively, positive plaques were rescreened by differential hybridization in which duplicate lifts were hybridized to cDNA probes prepared from induced WEHI-7TG or untreated CXG56D3 poly(A)$^+$ RNA. Plaques giving hybridization signals of different intensity with the two probes were selected for further study.

Characterization of cDNA clones and corresponding mRNAs. Northern blots were used to verify that the selected cDNA clones represented inducible genes. cDNA inserts were subcloned into pBluescriptSK by using an in vivo excision protocol (Stratagene), digested with *Eco*RI, and gel purified. Radioactive probe prepared from each insert was hybridized to Northern blots of total cellular RNA extracted from untreated CXG56D3 cells, untreated WEHI-7TG cells, and WEHI-7TG cells treated for 5 h with either dexamethasone, forskolin, or both drugs in combination. Of the cDNA clones, 42 represented induced sequences while the remain-

TABLE 1. Summary of cDNA clones

Clone	Size of cDNA insert (bp)[a]	No. of isolates[b]	Size (kb) of[c]: Major mRNA	Minor mRNA
30	400	1	2.4	
4.2	900	13	3.7	
59	1,550	1	3.0	
1.5	2,200	15	5.0	3.5
			9.0	
			11.0	
47	1,450	1	9.0	
			6.0	
20	1,250 + 1,850	1	6.5	
26	700	1	2.3	
			1.5	
58	1,100	2	3.1	2.3
9	700 + 1,350	1	1.4	3.0
8.5	800	5	1.2	
21	200 + 400	1	1.6	3.7

[a] Sizes of DNA fragments generated by *Eco*RI digestion of cDNA clones.
[b] Determined as the number of times the sequence was isolated from the 100 selected cDNAs.
[c] Sizes of mRNAs are based on migration of 18S and 28S ribosomal RNAs.

der gave signals of equal intensity in induced and untreated RNA samples. The latter clones most likely represent sequences that were incompletely removed during the subtractive hybridization. Several cDNAs hybridized to mRNAs of the same size with similar patterns of dexamethasone and forskolin induction. We tested the inserts for sequence similarity by cross-hybridization to identify cDNA clones corresponding to the same mRNA. In this manner, we identified cDNA clones representing 11 different glucocorticoid- and cAMP-regulated genes. The mRNA sizes ranged between 1.2 and 11 kilobases (kb), with several of these cDNA clones hybridizing to more than one mRNA species (Table 1; Fig. 1). Individual clones representing the 11 inducible genes were selected for further characterization.

Northern blots of RNA from untreated CXG56D3 or WEHI-7TG cells or from WEHI-7TG cells treated with either dexamethasone, forskolin, or both drugs in combination were probed with each of the 11 cDNA inserts and are shown in Fig. 1. In addition, hybridization to a probe for a constitutively expressed gene, *CHO-B*(30), is included as a control to check that the same amount of RNA has been loaded in each lane. The mRNAs corresponding to five of the cDNA clones (1.5 [5 kb], 4.2, 8.5, 26, and 59) were induced by both dexamethasone and forskolin after 5 h of treatment. Induction of the 1.4- and 3.0-kb mRNAs for clone 9, the 11-kb mRNA for clone 1.5, and the 9-kb mRNA for clone 47 depended on the addition of both dexamethasone and forskolin, as no increase in expression was seen when the cells were treated with either drug alone. Clones 20, 21, 30, and 58 hybridized to mRNAs that were induced only by dexamethasone at 5 h. Although the mRNAs corresponding to clones 20 and 30 showed no induction with forskolin alone, when forskolin was combined with dexamethasone an increased response was observed. In two cases (clones 1.5 and 47), treatment of WEHI-7TG cells with dexamethasone or forskolin alone reduced the levels of a 9- or 6-kb mRNA, respectively. Exposure of the cells to a combination of the two drugs, however, caused no change in the accumulation of the 9-kb mRNA of clone 1.5, while the 6-kb mRNA of clone 47 remained reduced.

Tissue-specific expression of the induced mRNAs. Another important aspect in the characterization of the selected cDNA clones is their pattern of expression in murine tissues. Northern blots were prepared with total RNA isolated from BALB/c mouse tissues and were hybridized to the cDNA insert for each clone (Fig. 2). Expression of the genes corresponding to several of the clones showed specificity for lymphoid tissues. Clone 30 hybridized strongly to a 2.4-kb mRNA in the thymus but showed no detectable hybridization to mRNA from any of the other tissues tested. This pattern of expression was also observed for the 9-kb mRNA hybridizing to clone 1.5. The gene corresponding to clone 4.2 was expressed in the brain and the thymus with a very low level of hybridization to mRNA from the spleen. The gene corresponding to clone 59 was expressed in several tissues but was most abundant in the brain and thymus. Clone 47 hybridized to mRNAs of two different sizes. The smaller message (6 kb) appeared to be lymphoid specific as it was detected only in spleen and thymus. The larger mRNA (9 kb) was detected in all of the tissues examined.

The expression of several genes demonstrated tissue specificity in nonlymphoid tissues. The 3.7-kb mRNA of clone 21 was detected at very high levels in the brain relative to the other tissues. In contrast, the 1.6-kb mRNA of clone 21 was detected at various degrees in all tissues tested. Similarly, an 11-kb mRNA corresponding to clone 1.5 was detected exclusively and substantially in the heart, while the 5-kb mRNA was detected in all tissues except the brain. The genes corresponding to clones 8.5, 9, 20, 26, and 58 were expressed at various degrees in most of the tissues examined. However, the mRNA corresponding to clone 20 was expressed predominantly in brain and lung, whereas expression of the mRNAs for clone 26 were elevated in the lung.

Time course of induction with glucocorticoids and forskolin. The preceding analysis of the mRNA response to dexamethasone and forskolin (Fig. 1) was performed at 5 h of treatment. It is possible that the level of induction of some of the mRNAs, in particular those that showed no response to dexamethasone or forskolin alone, may change at earlier or later times. WEHI-7TG cells were treated with dexamethasone, forskolin, or both drugs for periods of up to 10 h to determine the time course of appearance and decay of the mRNAs. Total RNA was isolated at various times and analyzed by Northern hybridization to labeled insert from each cDNA clone. Figure 3 shows the kinetics of induction by dexamethasone (see Fig. 4 for quantification). The mRNAs for seven of the 11 clones (1.5 [5 kb], 4.2, 20, 26, 30, 58, and 59) were increased (onefold or more) within 30 min to 1 h after the addition of dexamethasone. Of these clones, 1.5 (5 kb), 58, and 59 reached maximal level of induction by 2 h and then decreased. The mRNAs hybridizing to clones 20 and 26 reached peak levels at 5 h, while the mRNAs of clones 30 and 4.2 remained at elevated levels after 10 h of treatment. A second type of response was observed for the mRNAs of clones 8.5 and 21 which were not significantly induced (i.e., not more than onefold) until 2 and 5 h, respectively. An increase in mRNA levels was not detectable by 10 h for clones 9 and 47 (9 kb). Two clones hybridized to mRNAs that were decreased in abundance by dexamethasone. The levels of the 9-kb mRNA hybridizing to clone 1.5 and the 6-kb mRNA hybridizing to clone 47 were decreased by treatment with dexamethasone at 5 and 10 h, respectively.

The kinetics of induction by forskolin for each of the mRNAs is also shown in Fig. 3. The mRNA levels of clones 1.5 (5 kb), 20, 58, and 59 were all increased after 30 min to 1 h and reached peak levels at 2 h, except 59 which peaked between 2 and 5 h. The levels of induction as well as the time

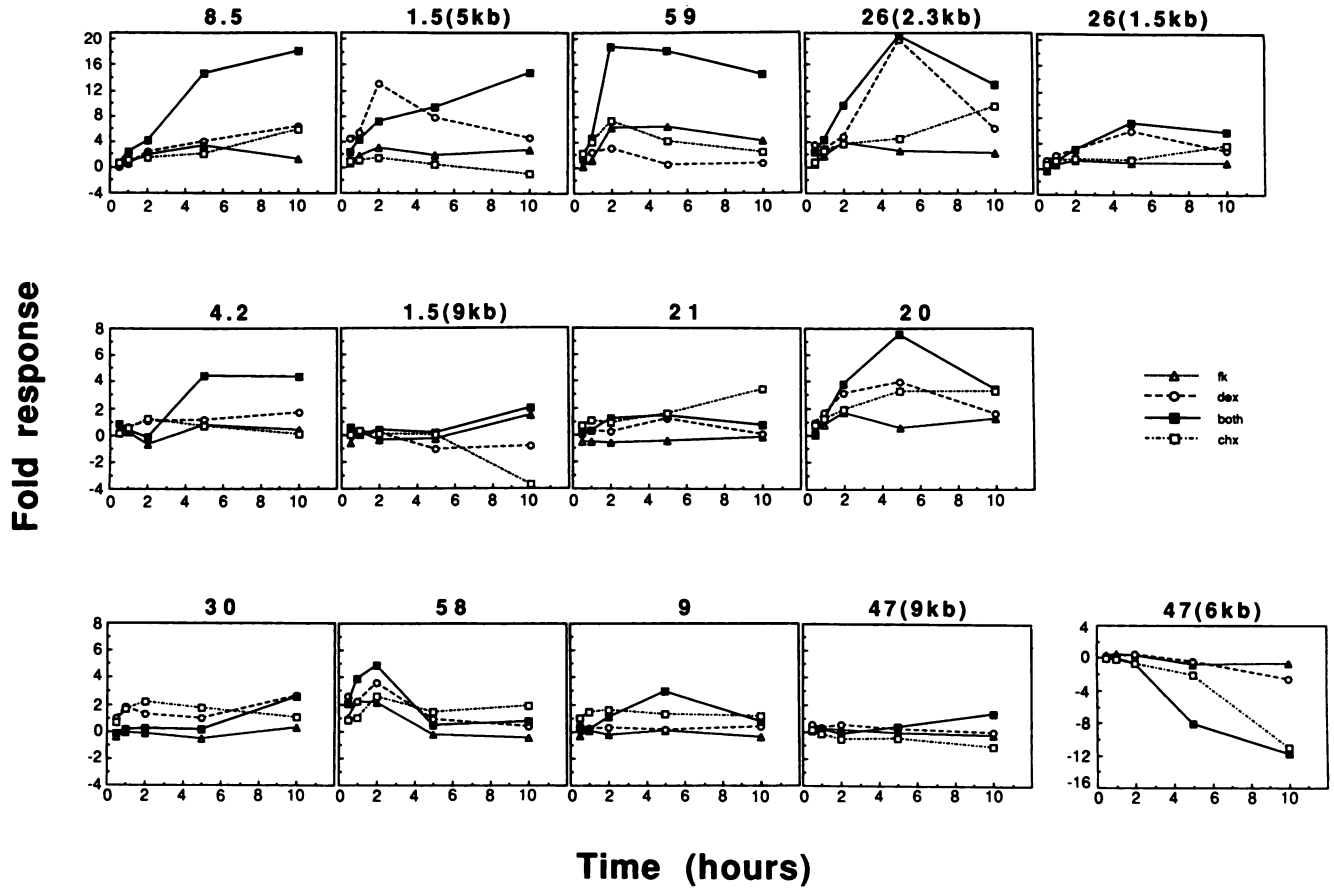

FIG. 4. Quantification of Northern blots from time course experiments. Autoradiographs (from preflashed film) of the Northern blots shown in Fig. 3 were scanned by laser densitometry. Appropriate film exposures that were determined to be in the linear range of the densitometer (0.1 to 2.0 absorbance units) were used for quantification. The expression of each mRNA in response to treatment with cycloheximide (chx), dexamethasone (dex), forskolin (fk), or a combination of dexamethasone and forskolin (both) is plotted relative to untreated RNA levels. The fold response relative to untreated (time zero) levels was calculated according to the following equation: (value at time n − value at time zero)/value at time zero. In all graphs, the time (h) axis remains unchanged. The fold response axis varies between each row and for clone 47 (6 kb).

course of decay for these mRNAs differed (Fig. 4). Other clones did not show induction by forskolin until later times. The genes corresponding to clones 26 and 8.5 were not significantly induced until 2 h and peaked at 2 and 5 h, respectively. The mRNA hybridizing to clone 4.2 decreased at 2 h but increased (onefold) by 5 h. This decrease was observed in two different experiments. The genes corresponding to clones 9 and 47 (9 kb) were not induced by dexamethasone and also showed no increase with forskolin alone. The genes corresponding to clones 21 and 30 were not affected by forskolin, although dexamethasone induced these mRNAs by 30 min. The 6-kb mRNA hybridizing to clone 47 was decreased by forskolin at 5 h as was seen with dexamethasone. The 9-kb mRNA of clone 1.5 was also decreased by forskolin at 5 h but returned to untreated levels at 10 h.

Therefore, the kinetics of induction by dexamethasone and forskolin were not the same for all clones. This implies different mechanisms of gene activation and, thus, the potential for additive or synergistic effects when the two drugs are used in combination. The time course of appearance for the inducible mRNAs in cells treated with both dexamethasone and forskolin is shown in Fig. 3. Seven of the 11 clones (1.5 [5 kb], 4.2, 8.5, 20, 26, 58, 59) hybridized to mRNAs that were induced by 30 min to 1 h. The mRNAs hybridizing to clones 9 and 21 did not increase until 2 h of treatment. The mRNAs corresponding to three clones (1.5 [9 kb], 30, and 47 [9 kb]) were induced at 10 h. This time is longer than what was observed in our initial screen of clone 47 (Fig. 1), where induction of the 9-kb mRNA was observed at 5 h of treatment. A similar discrepancy was observed with clone 30, for which induction by dexamethasone and forskolin (Fig. 3) was observed only after 10 h of treatment, whereas the initial screen (Fig. 1) detected induction at 5 h. This variation may be attributed to differences in the handling of the cells necessary for the large-scale RNA isolation in the time course experiments. The times of maximum induction for all clones varied from 2 h to >10 h. The mRNA hybridizing to clone 4.2 reproducibly decreased to untreated levels at 2 h, followed by reinduction at 5 h.

The response to dexamethasone and forskolin used in combination can be compared with the response observed with each drug used individually. The mRNA levels at the time of peak induction with both drugs were additive in relation to the levels seen with each drug alone for clones 21, 26, 30, and 58 (Fig. 3 and 4). In contrast, clones 1.5 (5 kb), 4.2, 8.5, 20, and 59 were synergistically induced by a combination of dexamethasone and forskolin. Two of the

cDNA clones, 9 and 47 (9 kb), hybridized to mRNAs that required the presence of both dexamethasone and forskolin for induction. The 6-kb mRNA hybridizing to clone 47 that was decreased by dexamethasone and forskolin alone was also decreased when both drugs were combined.

Effect of cycloheximide. The inhibition of protein synthesis has been shown to induce the expression of genes responsive to growth factors and serum (3, 41). To assess the effect of cycloheximide on the expression of the dexamethasone- and forskolin-regulated mRNAs isolated in this study, WEHI-7TG cells were treated for various times with cycloheximide and Northern blots of total RNA were hybridized to radiolabeled insert from each clone. The results are shown in Fig. 3 (quantification shown in Fig. 4). The majority of the mRNAs (clones 1.5 [5 kb], 4.2, 8.5, 9, 20, 21, 26, 30, 58, and 59) were induced by treatment with cycloheximide. However, the mRNAs hybridizing to clones 1.5 (9 kb) and 47 were repressed by cycloheximide after 5 to 10 h of treatment.

The effect of cycloheximide on the mRNA responses to dexamethasone and forskolin for each clone was also studied (data not shown). For the majority of mRNAs, an analysis of the effect of cycloheximide on the hormone response was inconclusive because of the large induction by cycloheximide alone relative to the induction observed with hormone. However, cycloheximide partially blocked the mRNA induction by a combination of dexamethasone and forskolin for clone 4.2 but did not block the induction by dexamethasone for clones 1.5 (9 kb), 4.2, 8.5, 20, and 59 or by cAMP for clones 1.5, 8.5, and 9.

Identification of cDNA clones. Preliminary sequence data have been obtained for several of the cDNA clones to determine if they represent new sequences or correspond to previously described genes. Partial nucleotide sequences for clones 1.5, 8.5, 30, and 59 compiled from the original cDNA clones and additional overlapping cDNAs from the λZAP library were used to search GenBank. For clone 30, approximately 900 nucleotides (corresponding to approximately one-third of the detected mRNA) adjacent to the poly(A) tail were compared with sequences in the data base and no significant similarity was observed. Likewise, searches of GenBank with sequences from clone 59 (approximately 500 nucleotides at the 3′ end of the mRNA and approximately 800 nucleotides further upstream) revealed no significant similarity. Therefore, clones 30 and 59 may correspond to unique, undescribed genes.

Sequence analysis of clones 1.5 and 8.5 resulted in the tentative identification of these cDNAs. A comparison of approximately 300 nucleotides near the 3′ end of clone 1.5 with the sequences in GenBank detected a 94% identity to the mouse retroelement VL30 (1). The mouse VL30 elements are a retroviruslike gene family with 100 to 200 copies dispersed throughout the genome (38, 39). The VL30 elements are 5 to 6 kb in length and contain long terminal repeats similar to the long terminal repeats of retroviruses (10, 34, 35). The cDNA clone we have isolated appears to be the same as NVL-3, a VL30 element whose sequence was recently reported (1). Computer searches with partial sequences of clone 8.5 consisting of approximately 300 nucleotides adjacent to the 3′ end or approximately 300 nucleotides located further upstream found these segments to be 65 or 85% identical to the rat chondroitin sulfate proteoglycan core protein (7), respectively. This suggests that clone 8.5 corresponds to the mouse homolog of this gene.

DISCUSSION

Immature T cells in the murine thymus are susceptible to the lytic effects of glucocorticoids, and during T-cell maturation this sensitivity is lost (56). The molecular basis of the glucocorticoid sensitivity and the acquisition of resistance is unknown. The effects of glucocorticoids are often exerted at the level of transcription, yet very little is known about the genes regulated by glucocorticoids in T lymphocytes. As a first step towards understanding the effects of glucocorticoids on T-cell viability, we utilized a subtractive hybridization procedure to isolate glucocorticoid- and cAMP-regulated genes. In this study, we have isolated 11 cDNA clones from a murine T lymphoma cell line that correspond to genes that are induced by glucocorticoids, forskolin, or a combination of the two drugs. Clone 30 is of special interest as it corresponds to a gene that appears to be expressed exclusively in the thymus and is regulated by glucocorticoids. The expression of this gene in the thymus and not in the spleen suggests it may be expressed in early T cells and not in peripheral T lymphocytes. As such, it would be of great interest as a marker for studying T cell differentiation. The possibility remains that the gene is expressed in thymus epithelium; however, this is unlikely since the WEHI-7TG cell line from which the clone was isolated is of lymphoid origin. Preliminary sequence analysis suggests that clone 30 represents a previously undescribed gene. Further work is necessary to determine the identity of this new gene.

Other cDNA clones isolated in this study demonstrated tissue-specific patterns of gene expression for lymphoid or nonlymphoid tissues. Clone 47 hybridized to a 6-kb mRNA in the spleen and thymus and may correspond to a gene expressed specifically in lymphoid cells. Clones 4.2 and 59 hybridized to mRNAs detected at high levels in the thymus and brain. This pattern of expression is similar to that of several members of the immunoglobulin supergene family (e.g., Ox-2 and Thy-1 antigens) which are found in cells of the mammalian nervous system as well as on thymocytes (for a review, see reference 24). However, on the basis of our estimation of the mRNA size and preliminary sequence data for clone 59, the genes we have isolated do not appear to be the Ox-2 or Thy-1 genes. Clone 1.5 hybridized to a mRNA (11 kb) detected only in the heart as well as to a 9-kb mRNA found only in the thymus, and clone 21 hybridized to a mRNA (3.7 kb) detected only in the brain. Further study of the regulation of these genes may provide information relevant to the control of tissue-specific gene expression. Finally, it should be noted that the 6.5-kb mRNA hybridizing to clone 20 was not measurably detected in the thymus, although the clone was isolated from the immature T cell line WEHI-7TG. The expression of this gene, while strongly induced by dexamethasone, was very low in untreated WEHI-7TG cells and in murine tissues. It is possible that the abundance of this mRNA is low in untreated thymocytes or is expressed only in a subset of thymocytes and therefore is undetectable in total thymus mRNA.

The expression of many hormone-regulated genes is under multifactor control (for a review, see reference 60). The genes corresponding to the cDNA clones we have isolated are regulated by glucocorticoids and cAMP. The molecular mechanisms of glucocorticoid and cAMP regulation have been studied in a variety of systems. Glucocorticoids can increase mRNA levels through effects on mRNA stabilization (21, 57), RNA processing (19), and by activating transcription as a result of hormone-receptor binding to the target DNA (for reviews, see references 6 and 61). cAMP

also induces mRNA transcription via binding of a transcription factor to the cAMP-responsive sequence element (48). The mRNAs for five of the cDNA clones isolated in this study (clones 1.5 [5 kb], 4.2, 8.5, 20, and 59) were synergistically induced by dexamethasone and forskolin, while the effects appeared additive for four others (clones 21, 26, 30, and 58). Future experiments aimed at measuring mRNA half-life and rates of transcription initiation will enable us to determine the mechanisms of glucocorticoid and cAMP induction of these mRNAs. The genes isolated in this study that required both drugs for induction (clones 1.5 [11 kb], 9, and 47) will be useful in studying the functional interactions between these two signaling systems.

Treatment of WEHI-7TG cells with cycloheximide alone resulted in the induction of all the mRNAs with the exception of the 1.5 (9-kb) and 47 (6-kb) mRNAs, which were both decreased in the presence of cycloheximide. Induction of the mRNAs by cycloheximide suggests that protein synthesis may be required for RNA destabilization or to inhibit transcription. A combination of these mechanisms is responsible for the cycloheximide induction of the immediate early genes which are induced by growth factor stimulation (3, 41). A common mechanism may exist for repressing transcription or for controlling mRNA stability of the genes we have isolated, as all of the glucocorticoid-induced mRNAs identified are induced by cycloheximide.

The induction of the majority of mRNAs corresponding to the selected cDNA clones involved an early response to dexamethasone and forskolin with an increased level of mRNA detected at 30 min to 1 h. The response for a few clones was slower, requiring 2 to 5 h for induction, suggesting that, in these cases, the response is an indirect effect of the hormone and may be dependent on protein synthesis. To examine this further, we analyzed the effect of cycloheximide on the mRNA responses to dexamethasone and forskolin for all of the clones (data not shown). Cycloheximide partially blocked the mRNA induction by a combination of dexamethasone and forskolin observed for clone 4.2. It is possible that an intermediate protein is involved in the regulation of this mRNA as was suggested for other glucocorticoid-responsive genes (51–53). In other cases, cycloheximide did not block the induction by dexamethasone (clones 1.5 [9 kb], 4.2, 8.5, 20, and 59) or cAMP (clones 1.5, 8.5, and 9), suggesting that the response does not require protein synthesis. For the remainder of the mRNAs, the induction with hormone was small and the mRNAs were induced by cycloheximide alone; thus, it was not possible to unambiguously assess the effect of cycloheximide on the hormone response.

Several of the cDNA clones hybridized to more than one mRNA species that was not coordinately regulated. A possible explanation for the multiple mRNAs detected by a single clone is that concatemerized, unrelated cDNAs were inserted into the vector during the library construction. This explanation is unlikely for two reasons. First, it is doubtful that two cDNAs corresponding to genes regulated by glucocorticoids would be inserted into the same vector. Second, in the cases of clones which contained more than one *Eco*RI fragment, each fragment hybridized to the same mRNAs on Northern blots (data not shown). Thus, the multiple mRNAs may represent genes that are structurally related but under the control of separate promoters, or they may be the result of alternative splicing of a common precursor RNA. Differential gene regulation by dexamethasone has been observed for calcitonin/α-CGRP and β-CGRP, two closely related members of a neuroendocrine gene family (55). In rat medullary thyroid carcinoma cells, dexamethasone increased the transcription rate of calcitonin/α-CGRP, while the level of β-CGRP mRNA did not change. In our study, the multiple mRNAs hybridizing to clones 1.5 and 47 were the most striking examples of noncoordinate regulation, as one mRNA was repressed in the WEHI-7TG cells after treatment with forskolin or dexamethasone and other mRNAs were induced by the same treatment. Negative regulation by glucocorticoids is not well understood (for a review, see reference 6). Further study of the noncoordinate regulation of the multiple mRNAs corresponding to clones 1.5 and 47 should provide useful information in this area.

Preliminary sequence analysis of four of the 11 clones isolated in this study indicates that at least two of the cDNAs (clones 30 and 59) represent new, undescribed genes regulated by glucocorticoids and cAMP in T lymphocytes. Two other clones (1.5 and 8.5) correspond to known genes. Clone 1.5 encodes a mouse VL30 element. The major transcription product of the mouse VL30 elements is a 30S (approximately 5-kb) poly(A)$^+$ RNA (39). In addition to a 5-kb mRNA, we observed a highly expressed mRNA of approximately 11 kb in the heart as well as a 9-kb mRNA in the thymus in Northern blots hybridized to clone 1.5. Further experiments will determine the nature of these previously undetected transcripts. Clone 8.5 appears to encode a mouse chondroitin sulfate proteoglycan, a molecule known to be synthesized and secreted by human and murine T lymphocytes (42). Stimulated T lymphocytes secrete a B-cell-stimulatory factor that copurifies with chondroitin sulfate proteoglycan, and recent evidence suggests that this activity may actually be chondroitin sulfate proteoglycan (43). To our knowledge, the synergistic regulation of chondroitin sulfate proteoglycan mRNA by dexamethasone and cAMP in T lymphocytes has not been previously observed. Sequence analysis of all clones isolated in this study is in progress and will provide information about the structure and function of these genes.

The cDNA clones we have isolated represent an important step in understanding the effects of glucocorticoids in T lymphocytes. To date, few T-cell-specific, glucocorticoid-regulated genes have been identified. Further analysis of the genes corresponding to the cDNA clones isolated in this study will increase our understanding of the mechanism of action of glucocorticoids, perhaps to include the molecular mechanisms of glucocorticoid-induced cytolysis and acquired glucocorticoid resistance during T-cell maturation. In addition, identification of genes expressed in specific populations of thymocytes will be useful for the study of T-cell differentiation.

ACKNOWLEDGMENTS

We thank Don Gruol for providing CXG56D3 cells, Lois Chandler, Glen Evans, and John Moffett for critical reading, and Gloria Swart for assistance in preparation of the manuscript.

This work was supported by Public Health Service grant CA36146 from the National Institutes of Health and by a grant from the Elsa U. Pardee Foundation (S.B.). Computer searches of GenBank were done through Bionet (Public Health Service grant P41RR01685). M.T.H. was supported by Public Health Service training grant 5T32CA09254 to Melvin Cohn.

LITERATURE CITED

1. **Adams, S. E., P. D. Rathjen, C. A. Stanway, S. M. Fulton, M. H. Malim, W. Wilson, J. Ogden, L. King, S. M. Kingsman, and A. J. Kingsman.** 1988. Complete nucleotide sequence of a mouse VL30 retro-element. Mol. Cell. Biol. **8**:2989–2998.
2. **Akahoshi, T., J. J. Oppenheim, and K. Matsushima.** 1988. Induction of high-affinity interleukin 1 receptor on human pe-

ripheral blood lymphocytes by glucocorticoid hormones. J. Exp. Med. **167**:924–936.
3. **Almendral, J. M., D. Sommer, H. MacDonald-Bravo, J. Burckhardt, J. Perera, and R. Bravo.** 1988. Complexity of the early genetic response to growth factors in mouse fibroblasts. Mol. Cell. Biol. **8**:2140–2148.
4. **Barbour, K. W., S. H. Berger, F. G. Berger, and E. A. Thompson, Jr.** 1988. Glucocorticoid regulation of the genes encoding thymidine kinase, thymidylate synthetase, and ornithine decarboxylase in P1798 cells. Mol. Endocrinol. **2**:78–84.
5. **Beale, E. G., J. L. Hartley, and D. K. Granner.** 1982. N6,O2'-Dibutryl cyclic AMP and glucose regulate the amount of messenger RNA coding for hepatic phosphoenolpyruvate carboxykinase (GTP). J. Biol. Chem. **257**:2022–2028.
6. **Beato, M.** 1989. Gene regulation by steroid hormones. Cell **56**:335–344.
7. **Bourdon, M. A., M. Shiga, and E. Ruoslahti.** 1985. Identification from cDNA of the precursor form of chondroitin sulfate proteoglycan core protein. J. Biol. Chem. **261**:12534–12537.
8. **Bourgeois, S., and R. F. Newby.** 1977. Diploid and haploid states of the glucocorticoid receptor gene of mouse lymphoid cell lines. Cell **11**:423–430.
9. **Bradshaw, Jr., H. D., and W. V. Vedeckis.** 1983. Glucocorticoid effects on thymidine incorporation into the DNA of S49 lymphoma cells. J. Steroid Biochem. **18**:691–698.
10. **Carter, A. T., J. D. Norton, and R. J. Avery.** 1983. A novel approach to cloning transcriptionally active retrovirus-like genetic elements from mouse cells. Nucleic Acids Res. **11**:6243–6254.
11. **Chirgwin, J. M., A. E. Przybla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**:5295–5299.
12. **Chomczynski, P., and N. Sacchi.** 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162**:156–159.
13. **Church, G. M., and W. Gilbert.** 1984. Genomic sequencing. Proc. Natl. Acad. Sci. USA **81**:1991–1995.
14. **Cidlowski, J. A.** 1982. Glucocorticoids stimulate ribonucleic acid degradation in isolated rat thymic lymphocytes *in vitro*. Endocrinology **111**:184–190.
15. **Cimbala, M. A., W. H. Lamers, K. Nelson, J. E. Monahan, H. Yoo-Warren, and R. W. Hanson.** 1982. Rapid changes in the concentration of phosphoenolypyruvate carboxykinase mRNA in rat liver and kidney. J. Biol. Chem. **257**:7629–7636.
16. **Coffino, P. J., W. Gray, and G. M. Tomkins.** 1975. Cyclic AMP, a nonessential regulator of the cell cycle. Proc. Natl. Acad. Sci. USA **72**:878–882.
17. **Cohen, J. J., and R. C. Duke.** 1984. Glucocorticoid activation of a calcium-dependent endonuclease in thymocyte nuclei leads to cell death. J. Immunol. **132**:38–42.
18. **Colbert, R. A., and D. A. Young.** 1986. Glucocorticoid-mediated induction of glucocortin: a rapid primary response common to major target tissues. Proc. Natl. Acad. Sci. USA **83**:72–76.
19. **Cote, G. J., and R. F. Gagel.** 1986. Dexamethasone differentially affects the levels of calcitonin and calcitonin gene-related peptide mRNAs expressed in a human medullary thyroid carcinoma cell line. J. Biol. Chem. **261**:15524–15528.
20. **Daniel, V., G. Litwack, and G. M. Tomkins.** 1973. Induction of cytolysis of cultured lymphoma cells by adenosine 3':5'-cyclic monophosphate and the isolation of resistant variants. Proc. Natl. Acad. Sci. USA **70**:76–79.
21. **Dean, D. C., R. F. Newby, and S. Bourgeois.** 1988. Regulation of fibronectin biosynthesis by dexamethasone, transforming growth factor β, and cAMP in human cell lines. J. Cell Biol. **106**:2159–2170.
22. **Distelhorst, C. W.** 1988. Glucocorticosteroids induce DNA fragmentation in human lymphoid leukemia cells. Blood **72**: 1305–1309.
23. **Eastman-Reks, S. B., and W. V. Vedeckis.** 1986. Glucocorticoid inhibition of c-myc, c-myb, and c-ki-ras expression in a mouse lymphoma cell line. Cancer Res. **46**:2457–2462.
24. **Evans, G. A.** 1987. Genes and gene families related to immunoglobulin genes, p. 225–257. *In* J. Patrick and S. Heinemann (ed.), Molecular neurobiology: recombinant DNA approaches Plenum Publishing Corp., New York.
25. **Gasson, J. C., and S. Bourgeois.** 1983. A new determinant of glucocorticoid sensitivity. J. Cell Biol. **96**:409–415.
26. **Gasson, J. C., T. Ryden, and S. Bourgeois.** 1983. Role of *de novo* DNA methylation in the glucocorticoid resistance of a T-lymphoid cell line. Nature (London) **302**:621–623.
27. **Gruol, D. J., N. F. Campbell, and S. Bourgeois.** 1986. Cyclic AMP-dependent protein kinase promotes glucocorticoid receptor function. J. Biol. Chem. **261**:4909–4914.
28. **Harmon, J. M., M. R. Norman, B. J. Fowles, and E. B. Thompson.** 1979. Dexamethasone induces irreversible G1 arrest and death of a human lymphoid cell line. J. Cell Physiol. **98**:267–278.
29. **Harmon, J. M., and E. B. Thompson.** 1982. Glutamine synthetase induction by glucocorticoids in the glucocorticoid-sensitive human leukemic cell line CEM-C7. J. Cell. Physiol. **110**:155–160.
30. **Harpold, M. M., R. M. Evans, M. Salditt-Georgieff, and J. E. Darnell.** 1979. Production of mRNA in Chinese hamster cells: relationship of the rate of synthesis to the cytoplasmic concentration of nine specific mRNA sequences. Cell **17**:1025–1035.
31. **Harris, A. W., A. D. Bankhurst, S. Mason, and N. L. Warner.** 1973. Differentiated functions expressed by cultured mouse lymphoma cells. J. Immunol. **110**:431–438.
32. **Higuchi, H., H.-Y. T. Yang, and S. L. Sabol.** 1988. Rat neuropeptide Y precursor gene expression. J. Biol. Chem. **263**: 6288–6295.
33. **Hoshikawa, Y., M. Izawa, and S. Ichii.** 1988. Cloning of glucocorticoid-responsive mRNA in rat thymus. Endocrinol. Jpn. **35**:429–437.
34. **Itin, A., and E. Keshet.** 1986. Diverse long terminal repeats are associated with murine retrovirus-like (VL30) elements. Mol. Cell. Biol. **6**:1276–1282.
35. **Kaghad, M., L. Maillet, and P. Brulet.** 1985. Retroviral characteristics of the long terminal repeat of murine E. Tn sequences. EMBO J. **4**:2911–2915.
36. **Kaiser, N., H. R. Bourne, P. A. Insel, and P. Coffino.** 1979. Regulation of phosphodiesterase and ornithine decarboxylase by cAMP is cell cycle independent. J. Cell Physiol. **101**:369–374.
37. **Kaiser, N., and I. S. Edelman.** 1977. Calcium dependence of glucocorticoid-induced lymphocytolysis. Proc. Natl. Acad. Sci. USA **74**:638–642.
38. **Keshet, E., and A. Itin.** 1982. Patterns of genomic distribution and sequence heterogeneity of a murine "retrovirus-like" multigene family. J. Virol. **43**:50–58.
39. **Keshet, E., Y. Shaul, J. Kaminchik, and H. Aviv.** 1980. Heterogeneity of "virus-like" genes encoding retrovirus-associated 30S RNA and their organisation within the mouse genome. Cell **20**:431–439.
40. **Klinman, D. M., and D. I. Cohen.** 1987. Preserving primary cDNA libraries. Anal. Biochem. **161**:85–88.
41. **Lau, L. F., and D. Nathans.** 1987. Expression of a set of growth-related immediate early genes in BALB/c 3T3 cells: coordinate regulation with c-fos or c-myc. Proc. Natl. Acad. Sci. USA **84**:1182–1186.
42. **Levitt, D., and P.-L. Ho.** 1983. Induction of chondroitin sulfate proteoglycan synthesis and secretion in lymphocytes and monocytes. J. Cell Biol. **97**:351–358.
43. **Levitt, D., and L. Olmstead.** 1987. B-cell stimulation by T-cell-secreted proteoglycan. Cell. Immunol. **110**:425–430.
44. **MacDonald, R. G., T. P. Martin, and J. A. Cidlowski.** 1980. Glucocorticoids stimulate protein degradation in lymphocytes: a possible mechanism of steroid-induced cell death. Endocrinology **107**:1512–1524.
45. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, p. 197. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
46. **McConkey, D. J., P. Hartzell, S. K. Duddy, H. Hakansson, and S. Orrenius.** 1988. 2,3,7,8-Tetrachlorodibenzo-p-dioxin kills immature thymocytes by Ca^{+}-mediated endonuclease activation. Science **242**:256–259.

47. **McDonald, A. R., and I. D. Goldfine.** 1988. Glucocorticoid regulation of insulin receptor gene transcription in IM-9 cultured lymphocytes. J. Clin. Invest. **81**:499–504.
48. **Montminy, M. R., and L. M. Bilezikjian.** 1987. Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. Nature (London) **328**:175–178.
49. **Montminy, M. R., K. A. Sevarino, J. A. Wagner, G. Mandel, and R. H. Goodman.** 1986. Identification of a cyclic-AMP-responsive element within the rat somatostatin gene. Proc. Natl. Acad. Sci. USA **83**:6682–6686.
50. **Munck, A.** 1968. Metabolic site and time course of cortisol action on glucose uptake, lactic acid output, and glucose-6-phosphate levels of rat thymus in vitro. J. Biol. Chem. **243**:1039–1042.
51. **Nakamura, T., S. Niimi, K. Nawa, C. Noda, A. Ichihara, Y. Takagi, M. Anai, and Y. Sakaki.** 1987. Multihormonal regulation of transcription of the tryptophan 2,3-dioxygenase gene in primary cultures of adult rat hepatocytes with special reference to the presence of a transcriptional protein mediating the action of glucocorticoids. J. Biol. Chem. **262**:727–733.
52. **Nawa, K., T. Nakamura, A. Kumatori, C. Noda, and A. Ichihara.** 1986. Glucocorticoid-dependent expression of the albumin gene in adult rat hepatocytes. J. Biol. Chem. **261**:16883–16888.
53. **Nebes, V. L., and S. M. Morris, Jr.** 1988. Regulation of messenger ribonucleic acid levels for five urea cycle enzymes in cultured rat hepatocytes. Requirements for cyclic adenosine monophosphate, glucocorticoids, and ongoing protein synthesis. Mol. Endocrinol. **2**:444–451.
54. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85**:2444–2448.
55. **Russo, A. F., C. Nelson, B. A. Roos, and M. G. Rosenfeld.** 1988. Differential regulation of the coexpressed calcitonin/α-CGRP and β-CGRP neuroendocrine genes. J. Biol. Chem. **263**:5–8.
56. **Scollay, R., and K. Shortman.** 1985. Thymocyte subpopulations: an experimental review, including flow cytometric cross-correlations between the major murine thymocyte markers. Thymus **5**:245–295.
57. **Shapiro, D. J., J. E. Blume, and D. A. Nielsen.** 1987. Regulation of messenger RNA stability in eucaryotic cells. Bioessays **6**:221–226.
58. **Vedeckis, W. V., and H. D. Bradshaw, Jr.** 1983. DNA fragmentation in S49 lymphoma cells killed with glucocorticoids and other agents. Mol. Cell. Endocrinol. **30**:215–227.
59. **Wyllie, A. H.** 1980. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature (London) **284**:555–556.
60. **Wynshaw-Boris, A., J. M. Short, and R. W. Hanson.** 1987. Regulation of gene transcription by multiple hormones: organization of regulatory elements, p. 59–87. *In* W. E. Cohn and K. Moldave (ed.), Progress in nucleic acid research and molecular biology, vol. 34. Academic Press, Inc., San Diego, Calif.
61. **Yamamoto, K.** 1985. Steroid receptor regulated transcription of specific genes and gene networks. Annu. Rev. Genet. **19**: 209–252.
62. **Yoshikawa, K., and S. L. Sabol.** 1986. Glucocorticoids and cyclic AMP synergistically regulate the abundance of preproenkephalin messenger RNA in neuroblastoma-glioma hybrid cells. Biochem. Biophys. Res. Commun. **139**:1–10.
63. **Yuh, Y., and E. B. Thompson.** 1987. Complementation between glucocorticoid receptor and lymphocytolysis in somatic cell hybrids of two glucocorticoid-resistant human leukemic clonal cell lines. Somatic Cell Mol. Genet. **13**:33–46.