Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 68–72, January 1985
Biochemistry

Use of a cDNA expression vector for isolation of mouse interleukin 2 cDNA clones: Expression of T-cell growth-factor activity after transfection of monkey cells

(helper T cells/lymphokine/gene cloning/transient expression/DNA sequence analysis)

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Communicated by Arthur Kornberg, September 4, 1984

ABSTRACT **A cDNA sequence coding for mouse interleukin 2 (IL-2) has been cloned from a cDNA library prepared from mRNA derived from a concanavalin A-activated mouse T-cell clone. The library was constructed by using the pcD vector system, which permits the expression of cDNA inserts in mammalian cells. Screening of the library was performed by transfecting COS-7 monkey cells with pools of cDNA clones in order to express the products encoded by full-length cDNA inserts. By assaying the supernatant fluid, IL-2 cDNA clones that express T-cell growth-factor (TCGF) activity were identified. The DNA sequence codes for a polypeptide of 169 amino acid residues including a putative signal peptide. The mouse IL-2 amino acid sequence deduced from the nucleotide sequence of its cDNA shares extensive homology with the human IL-2 amino acid sequence reported previously. These results demonstrate that identification of full-length cDNA clones for many lymphokines may be achieved entirely on the basis of detection of the functional polypeptides in mammalian cells.**

T lymphocytes may regulate the growth and differentiation of certain lymphopoietic and hematopoietic cells through the action of secreted protein factors. Helper T cells are capable of producing a variety of these factors after activation by either antigen or lectin. One such factor is interleukin 2 (IL-2), originally described as a lymphokine that enhanced thymocyte mitogenesis and maintained *in vitro* growth of T-cell lines (1–4). Lymphokines exhibiting these activities have been characterized in several species, including human (5), mouse (6), rat (7), and chicken (8). Mouse and human IL-2 have similar biological activities; both mouse and human IL-2 stimulate growth of mouse T-cell lines, whereas only human IL-2 stimulates human T-cell lines (9). They differ in apparent molecular weight (M_r 30,000 for mouse vs. M_r 15,000 for human) under nondenaturing conditions and isoelectric point (pI = 4.3–4.9 for mouse vs. pI = 6.5–7.8 for human) (6, 10). Although the human IL-2 gene has been cloned and characterized at both the cDNA (11) and genomic (12) levels, no clones containing the mouse IL-2 gene have been reported previously.

Recently, we have begun to clone and express a variety of cDNAs that encode T-cell-specific gene products in order to resolve the multiple factors that exist in the supernatants from activated helper T-cell clones (13). Using a pcD cDNA library established with mRNA from a mouse T-cell line, we have adopted a screening procedure employing transfection of plasmid DNAs into mammalian cells followed by assaying the transfected cell supernatants for T-cell growth-factor (TCGF) activity. In this manner we identified IL-2 cDNA clones based entirely on the synthesis of a functional product in mammalian cells. This approach requires no prior information on the structure of the polypeptide and involves only a specific assay for its biological activity.

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MATERIALS AND METHODS

Cell Lines. The T-cell line LB2-1 is an antigen-specific C57BL/6 mouse T-cell line specific for a chicken erythrocyte alloantigen. In response to antigen and syngeneic spleen cells, or Con A, LB2-1 produces large amounts of IL-2, interleukin 3 (IL-3), and γ-interferon. Details of the derivation and properties of this cell line will be published elsewhere.

The HT-2 mouse T-cell line (14) was obtained from S. Strober and grown in complete growth medium. The MC/9 mouse mast cell line (15) was obtained from G. Nabel.

TCGF Assay. The colorimetric proliferation assay was performed as described (16). Briefly, serial 1:2 or 1:3 dilutions of growth factor were made in 50 μl of RPMI 1640 medium and 10% fetal calf serum in Falcon flat-bottomed 96-well trays. HT-2 cells (2000 cells in 50 μl) were then added. After incubation at 37°C for 20 hr, 0.01 ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution [5 mg/ml in phosphate-buffered saline (P_i/NaCl)] was added to each well and incubation was continued for another 4 hr. Acid-isopropanol (0.04 M HCl in isopropanol) was added in 0.115 ml to each well and thoroughly mixed. Optical density was measured at 570 nm. Units of TCGF were calculated by determining the dilution of factor required to give 50% of the maximal stimulation, and 1 unit is defined as the amount of TCGF required to give 50% of the maximal signal using 2000 HT-2 cells in a volume of 0.1 ml.

Isolation of mRNA from Con A-Stimulated LB2-1 Cells. Total cellular RNA was extracted by using the guanidinium thiocyanate method (17) and the poly(A)$^+$ RNA was selected by oligo(dT)-cellulose chromatography. The yield of poly(A)$^+$ mRNA from each time point was about 0.01 mg from about 0.3 mg of total RNA from 1.55×10^8 cells.

Construction of cDNA Library. The cDNA library was constructed by using the pcDV1 vector-primer and the pL1 linker fragment according to the procedure of Okayama and Berg (18) as described (13). The cyclized vector-cDNA preparation was used to transform competent *Escherichia coli* MC1061 (19) cells by using calcium chloride (20). About 6 μg of poly(A)$^+$ RNA from Con A-stimulated LB2-1 cells yielded 10^5 independent transformants. Sublibraries based on the size of the cDNA insert, corresponding to cDNA insert sizes of 0–1, 1–2, 2–3, 3–4, 4–5, and 5 kilobases (kb) were prepared from a total cDNA library as described (13,

Abbreviations: IL-2, interleukin 2; IL-3, interleukin 3; kb, kilobase(s); MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; PMA, phorbol 12-myristate 13-acetate; TCGF, T-cell growth factor.

14). A collection of 10^4 independent clones was picked at random from the sublibrary enriched for cDNA inserts of 1–2 kb and propagated individually in wells of microtiter dishes.

DNA Transfection of Monkey Cells. COS-7 cells were transfected with plasmid DNA by using DEAE-dextran as described (13). After a 4-hr incubation at 37°C, the cells were washed and then incubated with Dulbecco's modified Eagle's medium (DME medium) containing 150 μM chloroquine for 3 hr. This was then replaced with DME medium containing 4% fetal calf serum. Seventy-two hours later the medium was collected and assayed for TCGF activity.

DNA Sequence Analysis. The nucleotide sequence of the cDNA inserts of IL-2 cDNA was determined by using the M13 dideoxy chain-termination method (21) and a modified procedure of Maxam and Gilbert (22, 23).

Other Procedures. Human IL-2 cDNA clones were isolated from a cDNA library established in a pcD vector by using mRNA prepared from a Con A-activated human helper T-cell clone (obtained from G. Nabel) and will be described elsewhere. Other procedures were as described (13).

RESULTS

Induction of IL-2 Synthesis and Evaluation of mRNA. Based on previous experiments showing that the synthesis of IL-2 occurs mainly during the first 10–12 hr following Con A stimulation, we prepared mRNA at 0, 6, 8, and 11 hr after induction and evaluated the content of active IL-2 mRNA by translation in oocytes. The results shown in Table 1 indicate that mRNA for IL-2 was undetectable in uninduced cells, whereas the mRNA collected at 6, 8, and 11 hr showed high activity. These results show that production of TCGF mRNA is inducible by Con A. Aliquots of the three induced mRNA samples were then pooled for the construction of a cDNA library in a pcD vector.

Screening the cDNA Library by DNA Transfection of Monkey Cells. Based on the predicted sizes of the polypeptide and mRNA, a sublibrary enriched for cDNA inserts 1- to 2-kb long was screened for TCGF activity. Bacterial clones were pooled into groups of 48 clones, and plasmid DNA was isolated from 58 such pools. Each pool of plasmids was transfected into COS-7 cells by using DEAE-dextran. After 72 hr, the supernatants from the transfected cells were assayed for TCGF activity (Table 2). Four pools (groups 6, 33, 40, and 56) yielded TCGF activity. Group 33 was subdivided into 8 pools, each containing 6 of the original pools. Only 1 of these pools (group c) was positive in the transfection assay. Each of the plasmids in group c was transfected individually into COS-7 cells. Only 1 clone, designated MT-18, was active in producing TCGF activity.

Table 1. Production of TCGF activity by LB2-1

Con A	LB2-1 supernatant, units per 10^6 cells	Oocyte supernatant, units per 10^6 cell equivalents
Without	<10	<0.5
With		
At 6 hr	10,377	293
At 8 hr	16,496	52
At 11 hr	14,604	112

LB2-1 cells were harvested at 6, 8, and 11 hr after the addition of Con A. Uninduced cells were grown identically, except omitting Con A. Poly(A)$^+$ RNA was prepared from each cell pellet and then injected into *Xenopus* oocytes. The supernatants from stimulated and unstimulated cells and the oocyte supernatants were assayed for TCGF activity on the HT-2 cell line.

Table 2. DNA transfection assay for TCGF activity from pools of plasmid DNA

	DNA	Units/ml
First screening	1–5	each <10
	6	144
	7–32	each <10
	33	128
	34–39	each <10
	40	128
	41–55	each <10
	56	140
Second screening*	a and b	each <10
	c	1669
	d–h	each <10
Third screening†	1 and 2	each <10
	3 (MT-18)	9020
	4–6	each <10
Fourth screening‡	MT-1	5851
	MT-2 to MT-5	each <10
	MT-7	<10
	MT-17	<10
	MT-19	<10
	MT-20	6800
	MT-21 to MT-23	each <10
	MT-26	<10
	MT-28	8200
	MT-29	<10
Mock-infected COS-7		<10
EL4 supernatant		107,126
Human COS-IL-2		51,100

Independent bacterial colonies (10^4) picked from a sublibrary of the total cDNA library that was enriched for 1- to 2-kb cDNA inserts were propagated into 96-well microtiter dishes. For the first screening, 58 pools of plasmid DNA each containing 48 random cDNA clones were prepared. Each plasmid DNA (18 μg) was transfected into COS-7 cells and the supernatant from the transfected cells was assayed for TCGF activity. The second and third screenings were performed essentially as above except that each transfection was performed with pools of 6 cDNA clones and a single cDNA clone, respectively. For the fourth screening, the same 58 pools of the sublibrary were screened by colony hybridization using a nick-translated *Hin*dIII–*Pst* I fragment of the MT-18 cDNA clone. Fifteen cDNA clones were isolated. Plasmid DNA of each cDNA clone was transfected into COS-7 cells. Positive clones MT-1, MT-20, and MT-28 that express TCGF activity were isolated from groups 6, 40, and 56 in the first screening, respectively. Mock-infected COS-7 cells were treated identically but DNA was omitted. The supernatants of COS-7 cells transfected with human IL-2 cDNA clone (COS-IL-2) and PMA-stimulated EL4 cells were used as a control.

*Groups of 6 clones from group 33.

†Single clones from group c.

‡Single clones hybridized with cDNA insert of clone MT-18.

Isolation of Full-Length Mouse cDNA Clones That Express TCGF Activity. The cDNA insert isolated from the MT-18 clone was labeled with ^{32}P and used to screen the same set of cDNA clones by colony hybridization. Fifteen positive clones were identified and individually transfected into COS-7 cells. Of these clones, only 3, contained in pools 6, 40, and 56, produced high levels of TCGF activity (Table 2). Thus, it appears that these cDNA inserts contain sufficient information to direct the synthesis of a functional TCGF polypeptide and each of these clones accounts for the activity of these pools in the initial random screening. The restriction analysis showed that these clones share essentially the same structure. These active clones, MT-1, MT-20, and MT-28, along

with MT-18, were designated as mouse TCGF cDNA clones.

Hybridization with Human IL-2 cDNA Probe. The extent of homology between our mouse TCGF cDNA clones and the human IL-2 cDNA originally isolated by Taniguchi *et al.* (11) was examined. A [^{32}P]cDNA probe derived from a human IL-2 cDNA clone (isolated from a human helper T-cell cDNA library) was hybridized with a filter containing 48 colonies from group 33. After washing the filter under low-stringency conditions (50°C), only clone MT-18 hybridized with the human IL-2 cDNA probe, indicating that the mouse MT-18 cDNA clone that expresses TCGF activity shares regions of homology with the human IL-2 cDNA (Fig. 1). Hybridization was not detected after washing the filter at 68°C (data not shown), suggesting that the homology is not perfect.

Blotting Analysis of LB2-1 mRNA with Mouse IL-2 cDNA Probe. Clone MT-18 plasmid DNA was labeled with ^{32}P by nick-translation and used as a probe with an RNA blot of Con A-activated and unactivated LB2-1 mRNA. As shown in Fig. 2, cDNA of clone MT-18 hybridizes to a single mRNA species about 0.9 kb long that is strongly inducible by Con A. No hybridization was detected with unactivated mRNA. The same cDNA probe also hybridizes with a single mRNA species of similar size isolated from the T-cell thymoma cell line EL4 induced by phorbol 12-myristate 13-acetate (PMA) (data not shown).

Nucleotide Sequence of the Mouse IL-2 cDNA. Fig. 3 shows the structure of mouse pcD-IL-2 and the restriction endonuclease cleavage map of one of the longest cDNA inserts that produces biologically active IL-2 (clone MT-1 in Table 2). The DNA sequence of the cDNA insert for clone MT-1 was determined and is shown in Fig. 4. The cDNA insert contains a single open reading frame. The first ATG is found 49–51 nucleotides from the 5′ end and is followed by 169 codons before the termination triplet TAA at nucleotide positions 555–558. The NH_2-terminal segment of the predicted IL-2 amino acid sequence is hydrophobic as would be expected for a signal peptide.

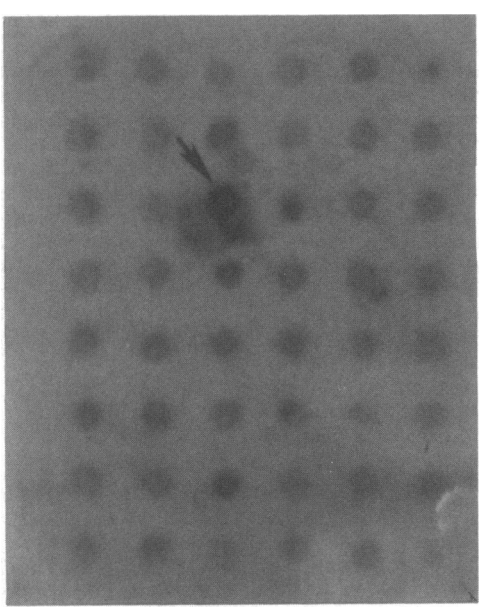

FIG. 1. Colony hybridization of mouse cDNA clones with human IL-2 cDNA probe. Individual clones from a subpool (group 33) of 48 cDNA clones were grown in wells of a 96-well microtiter dish. Colonies were replicated onto duplicate nitrocellulose filters where the cDNA was immobilized. The ^{32}P-labeled human IL-2 cDNA probe was prepared by nick-translation using a 540-base-pair *Pst* I–*Stu* I fragment from the cDNA insert (11). Hybridization was performed at 42°C in 1.08 M NaCl/60 mM sodium phosphate, pH 7.4/6 mM EDTA/10% formamide. The filter was washed extensively at 50°C in 0.36 M NaCl/20 mM sodium phosphate, pH 7.4/2 mM EDTA. Clone MT-18 is indicated by an arrow.

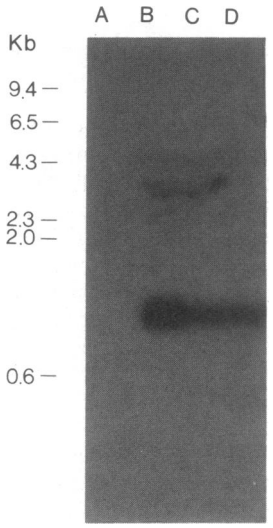

FIG. 2. RNA blot analysis of Con A-activated and unactivated LB2-1 mRNA. Poly(A)$^+$ mRNA (1 μg) isolated from unactivated or Con A-activated LB2-1 cells was denatured, treated with formaldehyde, and separated on a 1% agarose/formaldehyde gel. The RNA was transferred from the gel to nitrocellulose, and the filter was hybridized with nick-translated mouse IL-2 cDNA clone MT-18. Lanes: A, unactivated LB2-1; B, C, and D, LB2-1 mRNA prepared 6, 8, and 11 hr, respectively, after Con A-activation. Arrows indicate the locations of size markers.

Biological Activity of IL-2 Expressed in Monkey Cells. Both human and mouse COS-IL-2 induce the proliferation of the mouse T-cell line HT-2 (Table 2). In addition, we evaluated the ability of mouse and human COS-IL-2 to support the proliferation of the LB2-1 cell line (source of mRNA for the cloned IL-2 cDNA). Recently stimulated, rapidly growing LB2-1 cells were washed free of endogenous factors and used as target cells in the TCGF assay. Fig. 5 shows that LB2-1 is factor-dependent for proliferation and that this requirement can be met by spleen conditioned medium, mouse COS-IL-2, or by human COS-IL-2. Maximal proliferation could be obtained with all three growth factor preparations. If equal units of mouse or human COS-IL-2 (as defined on HT-2 cells) are added to IL-2-dependent phytohemagglutinin-stimulated human peripheral blood cells, the response to human COS-IL-2 is approximately five times as strong as to mouse COS-IL-2 (data not shown). Mouse COS-IL-3 had no activity on dividing LB2-1 cells (Fig. 5).

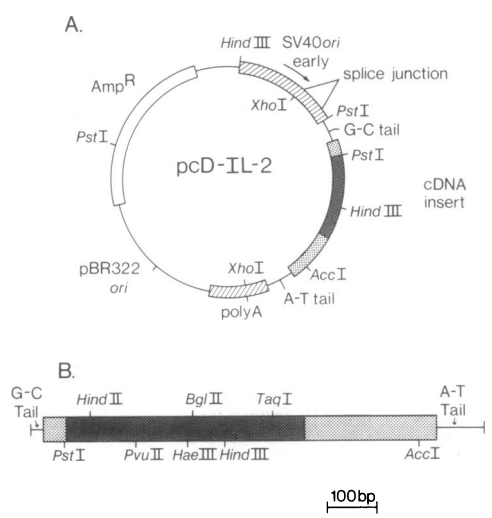

FIG. 3. (*A*) Diagram of pcD-IL-2, the plasmid carrying the nearly full-length mouse IL-2 cDNA insert. The cDNA insert [816 base pairs (bp)] is heavily shaded. The direction of transcription from simian virus 40 (SV40) early promoter is indicated by the arrow. The locations of the splice donor and acceptor sites and the polyadenylylation signal are shown (18). (*B*) Restriction endonuclease cleavage map of the mouse IL-2 cDNA insert. The IL-2 coding region is heavily shaded and the noncoding regions are lightly shaded.

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       10          20          30          40                          63
TATCACCCTT GCTAATCACT CCTCACAGTG ACCTCAAGTC CTGCAGGC ATG TAC AGC ATG CAG
                                                      MET Tyr Ser MET Gln

                  78                  93                  108
CTC GCA TCC TGT GTC ACA TTG ACA CTT GTG CTC CTT GTC AAC AGC GCA CCC ACT
Leu Ala Ser Cys Val Thr Leu Thr Leu Val Leu Leu Val Asn Ser Ala Pro Thr
                                                          ↑
    123                 138                 153                 168
TCA AGC TCC ACT TCA AGC TCT ACA GCG GAA GCA CAG CAG CAG CAG CAG CAG CAG
Ser Ser Ser Thr Ser Ser Ser Thr Ala Glu Ala Gln Gln Gln Gln Gln Gln Gln

            183                 198                 213
CAG CAG CAG CAG CAG CAC CTG GAG CAG CTG TTG ATG GAC CTA CAG GAG CTC CTG
Gln Gln Gln Gln Gln His Leu Glu Gln Leu Leu MET Asp Leu Gln Glu Leu Leu

228                 243                 258                 273
AGC AGG ATG GAG AAT TAC AGG AAC CTG AAA CTC CCC AGG ATG CTC ACC TTC AAA
Ser Arg MET Glu Asn Tyr Arg Asn Leu Lys Leu Pro Arg MET Leu Thr Phe Lys

        288                 303                 318                 333
TTT TAC TTG CCC AAG CAG GCC ACA GAA TTG AAA GAT CTT CAG TGC CTA GAA GAT
Phe Tyr Leu Pro Lys Gln Ala Thr Glu Leu Lys Asp Leu Gln Cys Leu Glu Asp

                348                 363                 378
GAA CTT GGA CCT CTG CGG CAT GTT CTG GAT TTG ACT CAA AGC AAA AGC TTT CAA
Glu Leu Gly Pro Leu Arg His Val Leu Asp Leu Thr Gln Ser Lys Ser Phe Gln

    393                 408                 423                 438
TTG GAA GAT GCT GAG AAT TTC ATC AGC AAT ATC AGA GTA ACT GTT GTA AAA CTA
Leu Glu Asp Ala Glu Asn Phe Ile Ser Asn Ile Arg Val Thr Val Val Lys Leu

            453                 468                 483
AAG GGC TCT GAC AAC ACA TTT GAG TGC CAA TTC GAT GAT GAG TCA GCA ACT GTG
Lys Gly Ser Asp Asn Thr Phe Glu Cys Gln Phe Asp Asp Glu Ser Ala Thr Val

498                 513                 528                 543
GTG GAC TTT CTG AGG AGA TGG ATA GCC TTC TGT CAA AGC ATC ATC TCA ACA AGC
Val Asp Phe Leu Arg Arg Trp Ile Ala Phe Cys Gln Ser Ile Ile Ser Thr Ser

                    570         580         590         600         610
CCT CAA TAACT ATGTACCTCC TGCTTACAAC ACATAAGGCT CTCTATTTAT TTAAATATTT
Pro Gln

       620         630         640         650         660         670         680
AACTTTAATT TATTTTTGGA TGTATTGTTT ACTATCTTTT GTAACTACTA GTCTTCAGAT GATAAATATG

       690         700         710         720         730         740         750
GATCTTTAAA GATTCTTTTT GTAAGCCCCA AGGGCTCAAA AATGTTTTAA ACTATTTATC TGAAATTATT

       760         770         780         790         800         810         820
TATTATATTG AATTGTTAAA TATCATGTGT AGGTAGACTC ATTAATAAAA GTATTTAGAT GATTCAAATA

TAAAA
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FIG. 4. Nucleotide sequence and predicted amino acid sequence of the mouse IL-2 cDNA insert. The nucleotide sequence begins with position 1 at the first nucleotide following the oligo(dG) segment. The amino acid sequence begins with the first in-phase ATG codon for the single long open reading frame. The arrow indicates the putative cleavage site of the leader sequence.

DISCUSSION

We describe here the isolation of cDNA clones that encode the mouse IL-2. Important aspects of the cloning protocol include (*i*) the use of a reliable and sensitive bioassay for TCGF activity, (*ii*) the use of a helper T-cell line, LB2-1, as an enriched source of biologically active TCGF mRNA, and (*iii*) the construction of a cDNA library in a mammalian expression vector. Throughout this work, a rapid and sensitive colorimetric assay for T-cell proliferation (16) was employed. The high IL-2 production and low mRNA content of the T-cell line LB2-1 led us to expect that mRNA from this T-cell line would be a relatively enriched source of IL-2 mRNA; this was confirmed by translation in *Xenopus laevis* oocytes.

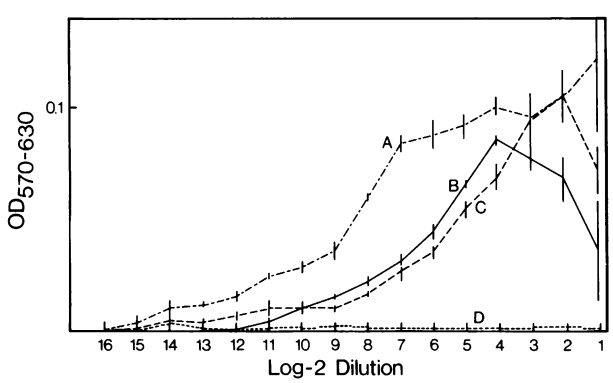

FIG. 5. Response of LB2-1 to COS-IL-2. LB2-1 cells were stimulated with antigen (0.02%) in the presence of irradiated [2500 rad (1 rad = 0.01 gray)] syngeneic spleen cells (2×10^6 per ml). After 24 hr, the cells were washed to remove IL-2 synthesized in response to stimulation, and the washed cells were used as target cells at 2.5×10^3 cells per well in a TCGF assay. Growth factor samples were human-COS-IL-2 (A), partially purified conditioned medium (B), mouse-COS-IL-2 (C), and mouse-COS-IL-3 (D). Incubation was for 3 days; the plates were developed and the viable cells were determined by the standard MTT assay.

A clone library in the pcD expression vector provided an opportunity to identify complete cDNA clones by direct expression in mammalian cells. In this work, complete mouse IL-2 cDNA clones were directly identified by transfecting COS-7 cells with randomly picked cDNA clones and measuring the TCGF activity secreted into the cell supernatant. Our results indicate that the identification of full-length cDNA clones of many lymphokines or hormones may be achieved solely on the basis of detection of a functional polypeptide in mammalian cells.

Identification of relevant cDNA clones based on the functional expression of the gene offers advantages over hybrid selection procedures. Identification of cDNA clones by hybrid selection relies on nucleotide sequence homology between mRNA and cDNA inserts. We found that some of the functionally inactive cDNA clones that hybridized with the mouse IL-2 cDNA probe (MT-3, MT-4, MT-5, MT-7, MT-17, MT-19, MT-21, MT-22, MT-23, and MT-29 in Table 2) have different restriction maps from that of the functional mouse IL-2 cDNA (data not shown). These inactive clones also hybridized with the human IL-2 cDNA probe (data not

FIG. 6. Comparison of mouse and human IL-2 amino acid sequences. Two sequences deduced from the nucleotide sequences of their cDNA clones were aligned to maximize the homology between the two IL-2 peptides by eye. Identical residues are shown with boxes. The possible processing site is indicated by the arrows.

shown). Thus, it is possible to isolate irrelevant clones on the basis of partial sequence homology within the cDNA inserts. Furthermore, our cloning protocol is particularly useful in cases in which the large amounts of mRNA needed for hybrid selection are not available.

The homologies between the mouse IL-2 cDNA and the human IL-2 cDNA, first detected by hybridization, were confirmed by nucleotide sequence comparison. Overall, there is about 70% homology between the two IL-2 cDNA sequences (24). In particular, the regions covered by nucleotide positions 2–128, 232–356, and 419–682 share extensive homology with the corresponding regions of human IL-2 cDNA. However, the trinucleotide sequence CAG, which is repeated 12 times within the mouse IL-2 cDNA coding region, is not present in the human IL-2 cDNA.

The mouse IL-2 cDNA contains a single open reading frame consisting of 169 codons corresponding to a protein with a calculated M_r of ≈19,000, whereas the human IL-2 cDNA contains 153 codons (11). The homology between the two IL-2 coding regions is even more evident if the deduced amino acid sequences are compared (Fig. 6). Ninety-four of the predicted 169 amino acid residues of mouse IL-2 are conserved in human IL-2. Downstream of the putative initiation codon in the two IL-2s is a region rich in hydrophobic amino acids. Beginning with an asparagine codon at position 19 there are 9 contiguous amino acids, which are identical in human IL-2. This sequence contains an alanine residue, which is the NH_2-terminal amino acid deduced for mature human IL-2 (11, 25). It is likely, therefore, that the mature form of the secreted mouse IL-2 begins with an alanine residue and the preceding 20 amino acids constitute the putative leader sequence that is removed by proteolytic processing. If this is true, mature mouse IL-2 would consist of 149 amino acid residues and would have a M_r of ≈16,000. Twelve glutamine residues encoded by the CAG block share no homology with human IL-2. This CAG block was found in all active clones (MT-1, MT-18, MT-20, and MT-28) and truncated IL-2 clones (MT-2 and MT-26).

Mouse IL-2 contains 20 acidic (aspartic acid and glutamic acid) and 14 basic (arginine and lysine) amino acids, whereas human IL-2 has equal numbers of acidic and basic residues in its mature form (11). The excess of acidic amino acids accounts for the more acidic isoelectric point (pI = 4.3–4.9) of mouse IL-2 (6, 10). There are no potential N-glycosylation sites (Asn-X-$\frac{\text{Ser}}{\text{Thr}}$) (26); however, modification by sialylation or O-glycosylation cannot be excluded. It has been reported that the apparent M_r of mouse IL-2 is 30,000, whereas the mature IL-2 predicted from our nucleotide sequence is M_r 16,000. This discrepancy suggests that mouse IL-2 consists of a dimer, as suggested also by molecular weight determination of $NaDodSO_4$-denatured IL-2 (27).

We thank Debra Lancaster for isolating the LB2-1 cell line, Ellen Jacob for DNA transfections, Nancy Larson for screening the library, Elizabeth Baheri for microinjections, Patricia Meyerson and Gloria Yang for bioassays, and Lynn DeOgny for DNA sequencing analysis. We thank Gordon Freeman for advice on DNA transfection in the presence of chloroquine and Hiroto Okayama, Gerard Zurawski, and Albert Zlotnik for helpful discussions. We also thank Paul Berg and Charles Yanofsky for critical reading of the manuscript.

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