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# cDNA CLONING OF IL-1α and IL-1β FROM mRNA OF U937 CELL LINE

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SUMMARY: Clones of cDNAs encoding growth inhibitory factors for human melanoma cell line A375 were isolated from cDNA library prepared by using mRNA derived from human histiocytic lymphoma cell line U937 induced with PMA and further stimulated with LPS. Cloning was achieved using Okayama-Berg cDNA expression vector system that permits expression of the inserted cDNA segments in mammalian cells. By assaying the transfected COS-1 cells supernatants and cell extracts, we isolated two distinct cDNA clones encoding growth inhibitory factors. It was determined by the nucleotide sequences of the inserts, the cDNAs corresponded to IL-1α and -1β. Our results indicate U937 cells can be induced to produce both interleukin-1s. 

Interleukin-1(IL-1) was first defined to be lymphocyte activating factor produced by monocytes-macrophages (1) and it has become evident that IL-1 activities are produced by numerous cell types (2) and IL-1 mediates a wide range of biological activities (2,3). IL-1 activities have been reported to be associated with proteins having isoelectronic points of about 5.0 and 7.0 (4-6). Recently March et al. reported the isolation of two distinct human IL-1 cDNAs(IL-1α and IL-1β) from a human macrophage cDNA library (7). Auron et al had reported the isolation of cDNA clone corresponding to the β form of IL-1 (8) and Furutani et al reported the cloning of cDNA corresponding to the α form of IL-1 (9). The α form has an isoelectric point of 5 (10), and the β form isofocuses at pI 7 (11). Although both IL-1s were produced by a population of macrophages in response to the same stimulant, it was not known whether a single cell was capable of producing both IL-1α and IL-1β simultaneously.

Abbreviations: IL-1, interleukin-1; GIF, growth inhibitory factor; poly(A)$^+$ RNA, polyadenylated RNA; cDNA, complementary DNA; bp, base pairs.

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On the other hand, human histiocytic lymphoma cell line U937 has been shown to differentiated into mature macrophage following induction with PMA (12). When U937 cells differentiated into macrophage were stimulated with LPS and MDP, we have found these cells produce growth inhibitory factors(GIF) for human melanoma cell line A375(13). To characterize GIF, we attempted to isolate cDNAs encoding GIF. In this report we show that two distinct cDNAs encoding GIF were isolated from stimulated U937 cells and these cDNAs are identical to human IL-1α and IL-1β cDNA,respectively.

## MATERIALS AND METHODS

Enzymes and chemicals: Restriction endonucleases were purchased from Takara Shuzo Co., Nippon Gene Co., and New England Biolabs. Avian myeloblastosis virus reverse transcriptase was obtained from Bio-Rad Laboratories, calf thymus terminal deoxynucleotidyl transferase, E.coli DNA ligase and RNase H from Pharmacia, E.coli DNA polymerase I from Boeringer Mannheim GmbH. All these enzymes were used according to the suppliers recommendations. [α-$^{32}$P]dCTP(3,000Ci/mmol and 400 Ci/mmol) were obtained from Amersham. Phorbol-12-myristate-13-acetate(PMA) was obtained from Pharmacia, Concanavalin A(ConA) from Sigma Chemical Co., E.coli lipopolysaccharide (LPS) from Difco Laboratories, Muramyl dipeptide (MDP) from Wako Pure Chemical Industries Ltd., oligo-dT cellulose from Collaborative Research Inc., RPMI-1640 medium and fetal calf serum from Gibco Laboratories, Eagle's minimum essential medium (Eagle's MEM) from Nissui Pharmaceutical Co., Ltd.

Cell lines and isolation of mRNA: The human histiocytic lymphoma cell line U937 (14) was cultured at $4\times10^5$ cells/ml in RPMI 1640 medium supplemented with 10% fetal calf serum. For differentiation of cells, cells were washed and resuspended in fresh RPMI 1640 medium supplemented with 10% calf fetal serum, 25ng/ml of PMA and 10μg/ml ConA. After incubation at 37°C for 3 days, cells were stimulated at 37°C in fresh RPMI 1640 medium supplemented with 10% calf fetal serum, 10μg/ml of LPS, 1μg/ml of MDP and 1ng/ml of PMA. After 18 hours, cells were harvested and total RNA was extracted by using the guanidinium-hot phenol method (15) and then the guanidinium-CsCl method (16), and poly(A)$^+$ RNA was selected by oligo(dT)-cellulose chromatography (17). The human melanoma cell line A375 (18) was maintained in Eagle's MEM supplemented with 10% fetal calf serum.

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 (19) with poly(A)$^+$ RNA from the stimulated U937 cells.

Screening of cDNA library by transfection: Bacterial clones in the cDNA library were pooled into groups of approximately 70 clones, and plasmid DNA was isolated from 400 such pools. Each pool of plasmids was transfected into COS-1 monkey cells (20) by using DEAE-dextran as described (21). After 72 hours, the supernatants and cell extracts prepared with freezing and thawing were assayed for GIF activities as described below. Positive pools yielding GIF activities were subdivided into 24 pools, each containing 10 clones of the original pools and the process was repeated. Each of the plasmids in positive subpools was transfected individually into COS-1 cells.

DNA sequence analysis: The nucleotide sequences were determined by the dideoxy chain-termination method (22) with M13 phage templates (23) or plasmid pUC 19 DNA (24).

Northern blot analysis: 15μg of poly(A)$^+$ RNA was fractionated through 1.0% agarose gel containing formaldehyde (25) and transferred to nitrocellulose filters (26). The blots were hybridized with the radiolabeled cDNA probes by nick translation(27).

GIF assay: The growth inhibitory factors were assayed for cytostatic or cytolytic activity for human melanoma A375 cells as described(28). Briefly A375 cells were added into 96 wells microtiter trays ($2x10^3$ cells/well) in Eagle's MEM supplemented with 10% fetal calf serum containing the test sample. After the incubation at 37°C for 4 days, viable cells were measured by neutral-red dye-uptake method.

One GIF unit per ml represents the reciprocal of the dilution of GIF causing 50% cytostasis or cytolysis for 4 days cultures.

## RESULTS AND DISCUSSION

In order to isolate cDNA encoding GIF, the cDNA library was constructed with mRNA from stimulated U937 cells by using a pcDV-1 vector-primer and a pL1 linker fragment (19). Bacterial clones in the cDNA library were divided into 400 groups, each group containing approximately 70 clones for the primary screening. By the transfection of each group of plasmid DNA into the COS-1 monkey cells, we obtained eleven groups producing GIF activities. As described in MATERIALS AND METHODS, individual cDNA clones of GIF were finally isolated from positive each group.

It was shown that ten cDNAs have the same restriction map, but one cDNA, pcD-GIF-207, has the distinct map. pcD-GIF-16 as the representative cDNA of ten cDNAs and pcD-GIF-207 have the distinct restriction map each other as shown in Fig.1, but both cDNAs produce GIF in COS-1 monkey cells as shown in Table 1. The cDNA inserts of both clones were characterized by sequence analysis (Fig.2 and 3). The nucleotide sequence of cDNA insert of pcD-GIF-16 was in complete agreement of IL-1β cDNA reported by March et al.(7) except the silent substitution of the third codon of the 105th amino acid codon. Plasmid pcD-GIF-16 has a cytosine instead of a thymine for the third nucleotide. The nucleotide sequence of cDNA insert of pcD-GIF-207 was also in complete agreement of IL-1α cDNA reported by them except the location of polyadenylation site which was located at 278 bp upstream than in case of their IL-1α cDNA. In case of pcD-GIF-207,

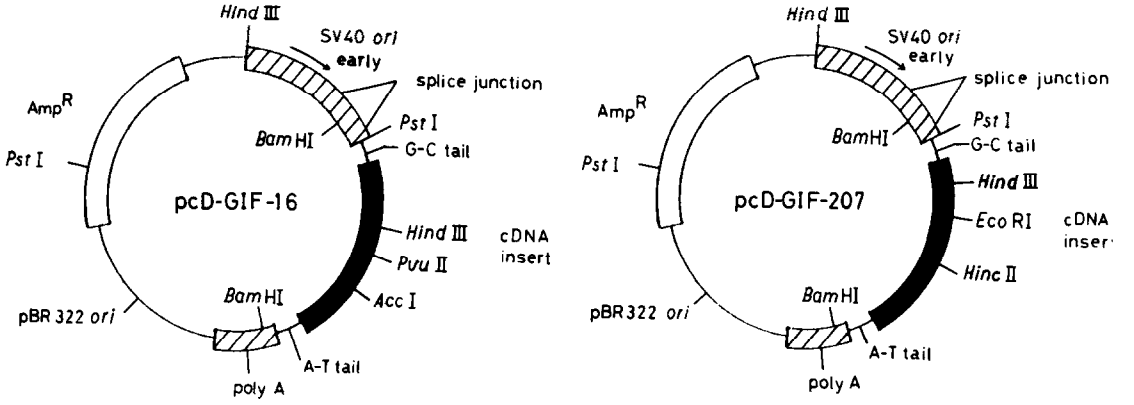


Fig. 1. Map of plasmid pcD-GIF-16 and pcD-GIF-207. The cDNA insert is shaded in black. The hatched boxes represent SV40 DNA segments. The direction of transcription from the SV40 early promoter is indicated by the arrow. The locations of the splice doner and acceptor sites and the polyadenylation signal are shown. The box and line show pBR322 region including the β-lactamese gene ($Amp^R$) and the origin of replication.

the nucleotide sequence AATAAA at 18 bp upstream of polyadenylation site may be recognized as polyadenylation signal.

The results presented here indicate that GIFs, that are inhibitors of growth in the human melanoma A375 cells, are identical to human IL-1α and β. Recently Lachman et al. have shown that human recombinant IL-1β exhibits a growth inhibitory function against A375 cells (29). Our data suggest that both forms of IL-1 directly inhibit the growth of some tumor cells. We have shown that both forms of IL-1 have growth inhibitory activity against a limited number of malignant cell lines (30).

Although IL-1α and IL-1β mRNA are induced in human peripheral blood monocyte population by the same stimulus (LPS), it is not known whether

Table I. Transient expression of GIF in monkey cells

| Clone | Cell extract (U/ml) | Culture medium (U/ml) |
|---|---|---|
| Mock | 0 | 0 |
| pcD-GIF-16 | 120.7 | 48.3 |
| pcD-GIF-207 | 196.9 | 62.6 |

Individual GIF cDNA plasmids were transfected into COS-1 cells using DEAE-dextran. Cells and culture medium were harvested 72 hours after the transfection. Mock-infected COS cells were treated with plasmid pcDV-1 (15) identically.

```
                                                                    ACCAACC
TCTTCGAGGCACAAGGCACAACAGGCTGCTCTGGGATTCTCTTCAGCCAATCTTCATTGCTCAAGTGTCTGAAGCAGCC
                              -50
 1                                                                          20
Met Ala Glu Val Pro Glu Leu Ala Ser Glu Met Met Ala Tyr Tyr Ser Gly Asn Glu Asp
ATG GCA GAA GTA CCT GAG CTC GCC AGT GAA ATG ATG GCT TAT TAC AGT GGC AAT GAG GAT
1                                                                       50
                                                                           40
Asp Leu Phe Phe Glu Ala Asp Gly Pro Lys Gln Met Lys Cys Ser Phe Gln Asp Leu Asp
GAC TTG TTC TTT GAA GCT GAT GGC CCT AAA CAG ATG AAG TGC TCC TTC CAG GAC CTG GAC
                                                 100
                                                                           60
Leu Cys Pro Leu Asp Gly Gly Ile Gln Leu Arg Ile Ser Asp His His Tyr Ser Lys Gly
CTC TGC CCT CTG GAT GGC GGC ATC CAG CTA CGA ATC TCC GAC CAC CAC TAC AGC AAG GGC
                                       150
                                                                           80
Phe Arg Gln Ala Ala Ser Val Val Val Ala Met Asp Lys Leu Arg Lys Met Leu Val Pro
TTC AGG CAG GCC GCG TCA GTT GTT GTG GCC ATG GAC AAG CTG AGG AAG ATG CTG GTT CCC
                          200
                                                                          100
Cys Pro Gln Thr Phe Gln Glu Asn Asp Leu Ser Thr Phe Phe Pro Phe Ile Phe Glu Glu
TGC CCA CAG ACC TTC CAG GAG AAT GAC CTG AGC ACC TTC TTT CCC TTC ATC TTT GAA GAA
          250                                                              300
                                                                          120
Glu Pro Ile Phe Phe Asp Thr Trp Asp Asn Glu Ala Tyr Val His Asp Ala Pro Val Arg
GAA CCT ATC TTC TTC GAC ACA TGG GAT AAC GAG GCT TAT GTG CAC GAT GCA CCT GTA CGA
                                                      350
                                                                          140
Ser Leu Asn Cys Thr Leu Arg Asp Ser Gln Gln Lys Ser Leu Val Met Ser Gly Pro Tyr
TCA CTG AAC TGC ACG CTC CGG GAC TCA CAG CAA AAA AGC TTG GTG ATG TCT GGT CCA TAT
                                                 400
                                                                          160
Glu Leu Lys Ala Leu His Leu Gln Gly Gln Asp Met Glu Gln Gln Val Val Phe Ser Met
GAA CTG AAA GCT CTC CAC CTC CAG GGA CAG GAT ATG GAG CAA CAA GTG GTG TTC TCC ATG
                                       450
                                                                          180
Ser Phe Val Gln Gly Glu Glu Ser Asn Asp Lys Ile Pro Val Ala Leu Gly Leu Lys Glu
TCC TTT GTA CAA GGA GAA GAA AGT AAT GAC AAA ATA CCT GTG GCC TTG GGC CTC AAG GAA
                          500
                                                                          200
Lys Asn Leu Tyr Leu Ser Cys Val Leu Lys Asp Asp Lys Pro Thr Leu Gln Leu Glu Ser
AAG AAT CTG TAC CTG TCC TGC GTG TTG AAA GAT GAT AAG CCC ACT CTA CAG CTG GAG AGT
          550                                                              600
                                                                          220
Val Asp Pro Lys Asn Tyr Pro Lys Lys Lys Met Glu Lys Arg Phe Val Phe Asn Lys Ile
GTA GAT CCC AAA AAT TAC CCA AAG AAG AAG ATG GAA AAG CGA TTT GTC TTC AAC AAG ATA
                                                      650
                                                                          240
Glu Ile Asn Asn Lys Leu Glu Phe Glu Ser Ala Gln Phe Pro Asn Trp Tyr Ile Ser Thr
GAA ATC AAT AAC AAG CTG GAA TTT GAG TCT GCC CAG TTC CCC AAC TGG TAC ATC AGC ACC
                                                 700
                                                                          260
Ser Gln Ala Glu Asn Met Pro Val Phe Leu Gly Gly Thr Lys Gly Gly Gln Asp Ile Thr
TCT CAA GCA GAA AAC ATG CCC GTC TTC CTG GGA GGG ACC AAA GGC GGC CAG GAT ATA ACT
                                       750
                                  269
Asp Phe Thr Met Gln Phe Val Ser Ser
GAC TTC ACC ATG CAA TTT GTG TCT TCC TAA AGAGAGCTGTACCCAGAGAGTCCTGTGCTGAATGTGGAC
                          800

TCAATCCCTAGGGCTGGCAGAAAGGGAACAGAAAGGTTTTTGAGTACGGCTATAGCCTGGACTTTCCTGTTGTCTACAC
850                                                 900

CAATGCCCAACTGCCTGCCTTAGGGTAGTGCTAAGAGGATCTCCTGTCCATCAGCCAGGACAGTCAGCTCTCTCCTTTC
                    950                                                    1000

AGGGCCAATCCCCAGCCCTTTTGTTGAGCCAGGCCTCTCTCACCTCTCCTACTCACTTAAAGCCCGCCTGACAGAAACC
                                        1050

ACGGCCACATTTGGTTCTAAGAAACCCTCTGTCATTCGCTCCCACATTCTGATGAGCAACCGCTTCCCTATTTATTTAT
          1100                                                  1150

TTATTTGTTTGTTTGTTTTATTCATTGGTCTAATTTATTCAAAGGGGGCAAGAAGTAGCAGTGTCTGTAAAAGAGCCTA
                                  1200

GTTTTTAATAGCTATGGAATCAATTCAATTTGGACTGGTGTGCTCTCTTTAAATCAAGTCCTTTAATTAAGACTGAAAA
  1250                                                  1300

TATATAAGCTCAGATTATTTAAATGGGAATATTTATAAATGAGCAAATATCATACTGTTCAATGGTTCTGAAATAAACT
                          1350                                          1400

TCTCTGAAGAAAAAAAAAAAAAAAAAAAA.....
```

Fig. 2. Nucleotide sequence and deduced amino acid sequence for cDNA insert of clone pcD-GIF-16. Numbering of nucleotides is negative for the 5' non-coding region and positive from the presumed initiator methionine codon. The amino acid sequence begins at the initiator methionine. The polyadenylation signal, AATAAA, is underlined.

```
                                                                    TCAAG
 1                                                                      20
Met Ala Lys Val Pro Asp Met Phe Glu Asp Leu Lys Asn Cys Tyr Ser Glu Asn Glu Glu
ATG GCC AAA GTT CCA GAC ATG TTT GAA GAC CTG AAG AAC TGT TAC AGT GAA AAT GAA GAA
1                                                          50
                                                                       40
Asp Ser Ser Ser Ile Asp His Leu Ser Leu Asn Gln Lys Ser Phe Tyr His Val Ser Tyr
GAC AGT TCC TCC ATT GAT CAT CTG TCT CTG AAT CAG AAA TCC TTC TAT CAT GTA AGC TAT
                                                  100
                                                                       60
Gly Pro Leu His Glu Gly Cys Met Asp Gln Ser Val Ser Leu Ser Ile Ser Glu Thr Ser
GGC CCA CTC CAT GAA GGC TGC ATG GAT CAA TCT GTG TCT CTG AGT ATC TCT GAA ACC TCT
                                        150
                                                                       80
Lys Thr Ser Lys Leu Thr Phe Lys Glu Ser Met Val Val Val Ala Thr Asn Gly Lys Val
AAA ACA TCC AAG CTT ACC TTC AAG GAG AGC ATG GTG GTA GTA GCA ACC AAC GGG AAG GTT
                              200
                                                                      100
Leu Lys Lys Arg Arg Leu Ser Leu Ser Gln Ser Ile Thr Asp Asp Asp Leu Glu Ala Ile
CTG AAG AAG AGA CGG TTG AGT TTA AGC CAA TCC ATC ACT GAT GAT GAC CTG GAG GCC ATC
          250                                                          300
                                                                      120
Ala Asn Asp Ser Glu Glu Glu Ile Ile Lys Pro Arg Ser Ala Pro Phe Ser Phe Leu Ser
GCC AAT GAC TCA GAG GAA GAA ATC ATC AAG CCT AGG TCA GCA CCT TTT AGC TTC CTG AGC
                                                  350
                                                                      140
Asn Val Lys Tyr Asn Phe Met Arg Ile Ile Lys Tyr Glu Phe Ile Leu Asn Asp Ala Leu
AAT GTG AAA TAC AAC TTT ATG AGG ATC ATC AAA TAC GAA TTC ATC CTG AAT GAC GCC CTC
                                                400
                                                                      160
Asn Gln Ser Ile Ile Arg Ala Asn Asp Gln Tyr Leu Thr Ala Ala Ala Leu His Asn Leu
AAT CAA AGT ATA ATT CGA GCC AAT GAT CAG TAC CTC ACG GCT GCT GCA TTA CAT AAT CTG
                                        450
                                                                      180
Asp Glu Ala Val Lys Phe Asp Met Gly Ala Tyr Lys Ser Ser Lys Asp Asp Ala Lys Ile
GAT GAA GCA GTG AAA TTT GAC ATG GGT GCT TAT AAG TCA TCA AAG GAT GAT GCT AAA ATT
                              500
                                                                      200
Thr Val Ile Leu Arg Ile Ser Lys Thr Gln Leu Tyr Val Thr Ala Gln Asp Glu Asp Gln
ACC GTG ATT CTA AGA ATC TCA AAA ACT CAA TTG TAT GTG ACT GCC CAA GAT GAA GAC CAA
          550                                                          600
                                                                      220
Pro Val Leu Leu Lys Glu Met Pro Glu Ile Pro Lys Thr Ile Thr Gly Ser Glu Thr Asn
CCA GTG CTG CTG AAG GAG ATG CCT GAG ATA CCC AAA ACC ATC ACA GGT AGT GAG ACC AAC
                                                  650
                                                                      240
Leu Leu Phe Phe Trp Glu Thr His Gly Thr Lys Asn Tyr Phe Thr Ser Val Ala His Pro
CTC CTC TTC TTC TGG GAA ACT CAC GGC ACT AAG AAC TAT TTC ACA TCA GTT GCC CAT CCA
                                                700
                                                                      260
Asn Leu Phe Ile Ala Thr Lys Gln Asp Tyr Trp Val Cys Leu Ala Gly Gly Pro Pro Ser
AAC TTG TTT ATT GCC ACA AAG CAA GAC TAC TGG GTG TGC TTG GCA GGG GGG CCA CCC TCT
                                        750
                                                  271
Ile Thr Asp Phe Gln Ile Leu Glu Asn Gln Ala
ATC ACT GAC TTT CAG ATA CTG GAA AAC CAG GCG TAG GTCTGGAGTCTCACTTGTCTCACTTGTGCAG
                              800

TGTTGACAGTTCATATGTACCATGTACATGAAGAAGCTAAATCCTTTACTGTTAGTCATTTGCTGAGCATGTACTGAGC
850                                                          900

CTTGTAATTCTAAATGAATGTTTACACTCTTTGTAAGAGTGGAACCAACACTAACATATAATGTTGTTATTTAAAGAAC
                          950                                                1000

ACCCTATATTTTGCATAGTACCAATCATTTTAATTATTATTCTTCATAACAATTTTAGGAGGACCAGAGCTACTGACTA
                                              1050

TGGCTACCAAAAAGACTCTACCCATATTACAGATGGGCAAATTAAGGCATAAGAAAACTAAGAAATATGCACAATAGCA
               1100                                                1150

GTTGAAACAAGAAGCCACAGACCTAGGATTTCATGATTTCATTTCAACTGTTTGCCTTCTGCTTTTAAGTTGCTGATGA
                                    1200

ACTCTTAATCAAATAGCATAAGTTTCTGGGACCTCAGTTTTATCATTTTCAAAATGGAGGGAATAATACCTAAGCCTTC
    1250                                                1300

CTGCCGCAACAGTTTTTTATGCTAATCAGGGAGGTCATTTTGGTAAAATACTTCTCGAAGCCGAGCCTCAAGATGAAGG
                               1350                                          1400

CAAAGCACGAAATGTTATTTTTTAATTATTATTTATATATGTATTTATAAATATATTTAAGATAATTATAATATACTAT
                                                  1450

ATTTATGGGAACCCCTTCATCCTCTGAGTGTGACCAGGCATCCTCCACAATAGCAGACAGTGTTTTCTGGGATAAGTAA
                    1500                                                1550

GTTTGATTTCATTAATACAGGGCATTTTGGTCCAAGTTGTGCTTATCCCATAGCCAGGAAACTCTGCATTCTAGTACTT
                                        1600

GGGAGACCTGTAATCATATAATAAATGTACATTAATTACCTTGAAAAAAAAAAAAAAAAAAAA.....
          1650
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Fig. 3. Nucleotide sequence and deduced amino acid sequence for cDNA insert of clone pcD-GIF-207. The numbering and the polyadenylation signal are those explained in the legend to Fig.2.

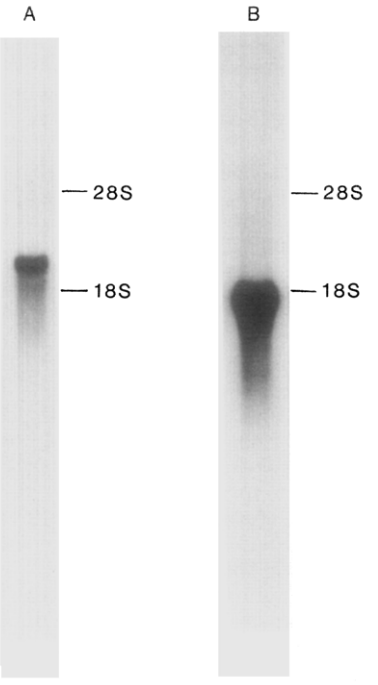


Fig. 4. Northern blot analysis of mRNA from stimulated U937 cells. Poly(A)+ RNA was subjected to blotting analysis by using the nick-translated PstI-EcoRI cDNA fragment of pcD-GIF-207 (A) and PstI-PvuII cDNA fragment of pcD-GIF-16 (B), as probes.

they are produced by the same cell type (7). We analysed the mRNA for IL-1α and β in stimulated U937 cells by Northern blot analysis (Fig.4). The predominant RNA sizes were about 1800 nucleotides for IL-1β and about 2200 nucleotides for IL-1α. The level of IL-1β mRNA seemed to be higher than that of IL-1α mRNA in stimulated U937 cells. We have been able to isolate both forms of IL-1s cDNA from mRNA of stimulated U937 cells. The ratio of IL-1β cDNA and IL-1α cDNA clones was 15 to 1 in our cDNA library.

Recently we found that the expression of IL-1β mRNA, but not IL-1α mRNA, was induced with PMA, and the induction of IL-1α mRNA was achieved by stimulating the differentiated U937 cells with LPS (Nishida. T., et al, unpublished data).

These results suggest that the same cells, the stimulated U937 cells, are capable of producing both forms of IL-1 with the same stimulus and the expression of two IL-1 mRNAs are independently regulated.

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