Nucleic Acids Res. **11**, 1475 (1983).
27. M. P. Weekens, G. N. Buell, R. T. Schimke, *J. Biol. Chem.* **253**, 2483 (1978).
28. M. Mudryj, thesis, George Washington University, Washington, DC (1988).
29. T. A. Kunkel, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 488 (1985).
30. K. Harbers, M. Kuehn, H. Delius, R. Jaenisch, *ibid.* **81**, 1504 (1984).
31. A gift of R. Frunzio.
32. C. Gorman, L. Laimons, G. T. Merlino, P. Gruss, G. Khoury, B. Howard, in *Eukaryotic Gene Expression*, A. Kuman, Ed. (Plenum, New York, 1982), pp. 129–139.
33. S. L. Hu and J. L. Manley, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 820 (1981).

18 March 1988; accepted 13 June 1988

cDNA Expression Cloning of the IL-1 Receptor, a Member of the Immunoglobulin Superfamily

JOHN E. SIMS, CARL J. MARCH, DAVID COSMAN, MICHAEL B. WIDMER, H. ROBSON MACDONALD, CATHERINE J. MCMAHAN, CATHERINE E. GRUBIN, JANIS M. WIGNALL, JANA L. JACKSON, SUSAN M. CALL, DELLA FRIEND, ALAN R. ALPERT, STEVEN GILLIS, DAVID L. URDAL, STEVEN K. DOWER

Interleukin-1 alpha and -1 beta (IL-1α and IL-1β) are cytokines that participate in the regulation of immune responses, inflammatory reactions, and hematopoiesis. A direct expression strategy was used to clone the receptor for IL-1 from mouse T cells. The product of the cloned complementary DNA binds both IL-1α and IL-1β in a manner indistinguishable from that of the native T cell IL-1 receptor. The extracellular, IL-1 binding portion of the receptor is 319 amino acids in length and is composed of three immunoglobulin-like domains. The cytoplasmic portion of the receptor is 217 amino acids long.

INTERLEUKIN-1 ALPHA AND -1 BETA (IL-1α and IL-1β) have multiple biological activities in vivo and in vitro: stimulation of thymocyte proliferation ("lymphocyte activation"), accessory growth factor activity for certain T helper cells (*1*), stimulation of hematopoietic cell growth and differentiation (*2*), induction of acute phase protein synthesis, induction of prostaglandin and collagenase synthesis by fibroblasts and chondrocytes, and pyrogenicity (*3*). Human IL-1α and IL-1β show low amino acid identity (26%) (*4*), and yet both bind to the same cell-surface receptor (*5, 6*). This receptor is an 80-kD glycoprotein (*7*) with a broad tissue distribution (*8*), whose signal transduction mechanism is unknown. We isolated a cDNA clone of the IL-1 receptor, using a direct expression strategy, in order to study further its structure and mechanism of action.

As a source of mRNA encoding the IL-1 receptor, we used the cell line EL4 6.1 C10 (*9*), a subclone of the C57BL/6 mouse thymoma EL4. This cell line expresses about 10,000 surface IL-1 binding sites (*8, 10*), many more than most other cell lines or tissue types examined (*8*). Double-stranded cDNA was synthesized from EL4 6.1 C10 polyadenylated RNA (*11*), ligated into pDC201 (*12*), which is a vector designed to direct high-level expression of cloned cDNA molecules in mammalian cells, and used to transform *Escherichia coli*. Pools of 350 transformants were grown, and plasmid DNA was prepared and transfected into COS cells (*13*). After 3 days we screened for IL-1R cDNA clones by incubating the transfected cell monolayer with ^{125}I-labeled IL-1α. The plates were subjected to autoradiography; cells that bound elevated levels of IL-1 appeared as dark foci on a light gray background (Fig. 1B). The background (Fig. 1A) was caused by binding to the endogenous COS cell IL-1 receptors (approximately 500 per cell) (*14*).

One pool out of approximately 150,000 cDNA clones gave a positive signal (Fig. 1B) that was specific, and could be abolished by addition of a 100-fold excess of unlabeled IL-1α or IL-1β to the ^{125}I-labeled IL-1α during the binding incubation (*14*). Individual colonies from this pool were grown, and DNA from each was transfected into COS cells, until a single colony (clone 78) giving rise to IL-1 binding activity was identified (Fig. 1C).

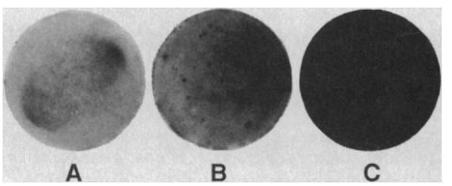


Fig. 1. Isolation of IL-1 receptor cDNA clones by direct expression screening (*23*). The autoradiographed plates of transfected COS cells had been probed with ^{125}I-labeled IL-1α to detect IL-1 receptor expression. (**A**) COS cells transfected with vector containing no insert, showing the background level of COS cell IL-1 receptor expression. (**B**) COS cells transfected with a positive pool of 350 individual cDNA clones, one of which encodes the IL-1 receptor. (**C**) COS cells transfected with pure clone 78 (IL-1 receptor) DNA.

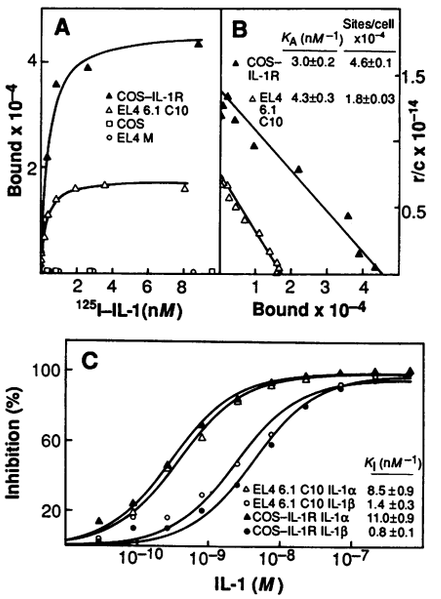


Fig. 2. Comparison of the IL-1 binding properties of natural and recombinant IL-1 receptors (*24*). (**A**) Direct binding of ^{125}I-labeled IL-1α to cells expressing native IL-1 receptors (EL4 6.1 C10) or recombinant receptors (COS–IL-1R). (**B**) Data from (A) replotted in the Scatchard coordinate system. (**C**) Inhibition of ^{125}I-labeled IL-1α binding by unlabeled IL-1α and IL-1β. c, concentration of IL-1 bound and added to the binding incubation (molar); r, molecules of IL-1 bound per cell; COS–IL-1R, COS cells transfected with clone 78 DNA. All parameter values are given ± the standard error.

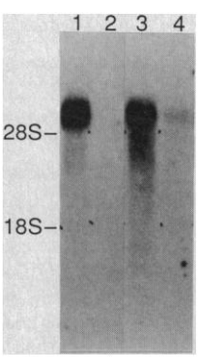
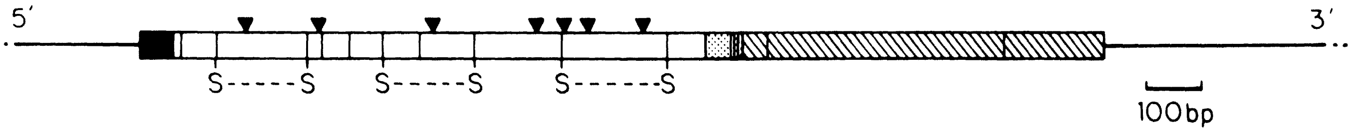


Fig. 3. RNA blot analysis of IL-1 receptor mRNA. EL4 6.1 C10 RNA (lane 1), EL4 M RNA (lane 2), 3T3 RNA (lane 3), L929 RNA (lane 4). Polyadenylated RNA (20 μg) from the indicated cell lines was loaded in each lane of a 1% formaldehyde-agarose gel (*25*). After electrophoresis, the RNA was blotted onto Hybond-N (Amersham) and the filter was hybridized at 63°C with the nick-translated insert from clone 78 (5×10^5 cpm/ml). The filter was washed at 63°C in 2× saline sodium citrate (SSC).

J. E. Sims, C. J. March, D. Cosman, M. B. Widmer, C. J. McMahan, C. E. Grubin, J. M. Wignall, J. L. Jackson, S. M. Call, D. Friend, A. R. Alpert, S. Gillis, D. L. Urdal, S. K. Dower, Immunex Corporation, 51 University Street, Seattle, WA 98101.
H. R. MacDonald, Ludwig Institute for Cancer Research, Lausanne Branch, 1066 Epalinges, Switzerland.

The DNA from clone 78, when transfected into COS cells, led to expression of IL-1 binding activity, which was virtually identical to that of EL4 6.1 C10 cells (Fig. 2). The affinity of ^{125}I-labeled IL-1α for each cell type was similar, and binding was completely inhibited by an excess of either unlabeled IL-1α or IL-1β (Fig. 2C). In addition, after surface iodination of the transfected COS cells, IL-1 receptor purified by affinity chromatography on IL-1α columns (*15*) comigrated on SDS-polyacrylamide gels with the

A



B

```
          TGGGTCGTCTGACTAGAAGTGAGCTGTCTGTCATTCTTGTGCACGCCAGCCCAGTAATCATTTGGAGGCAAAGCAAACTGTAAGTAATGCTGTCCTGGGCTGACT   -177

TGAGGAGGCAGTTTTCGTTTTAACAGCCAGTGTTTATTTGCTCAGCAAACGTTGTCTCGGGGAGAAATGTCGCTGGATGTCATCAGAGTTCCCAGTGCCCCGAACCGTGAACAACACAA   -58

Met Glu Asn Met Lys Val Leu Leu Gly Leu Ile Cys Leu Met Val Pro Leu Leu Ser↓Leu Glu Ile Asp Val Cys Thr Glu Tyr Pro Asn   11
ATG GAG AAT ATG AAA GTG CTA CTG GGG CTC ATT TGT CTC ATG GTG CCT CTG CTG TCG CTG GAG ATT GAC GTA TGT ACA GAA TAT CCA AAT   33

Gln Ile Val Leu Phe Leu Ser Val Asn Glu Ile Asp Ile Arg Lys [Cys] Pro Leu Thr Pro Asn Lys Met His Gly Asp Thr Ile Ile Trp   41
CAG ATC GTT TTG TTT TTA TCT GTA AAT GAA ATT GAT ATT CGC AAG [TGT] CCT CTT ACT CCA AAT AAA ATG CAC GGC GAC ACC ATA ATT TGG   123

Tyr Lys Asn Asp Ser Lys Thr Pro Ile Ser Ala Asp Arg Asp Ser Arg Ile His Gln Gln Asn Glu His Leu Trp Phe Val Pro Ala Lys   71
TAC AAG AAT GAC AGC AAG ACC CCC ATA TCA GCG GAC CGG GAC TCC AGG ATT CAT CAG CAG AAT GAA CAT CTT TGG TTT GTA CCT GCC AAG   213

Val Glu Asp Ser Gly Tyr Tyr Tyr [Cys] Ile Val Arg Asn Ser Thr Tyr Cys Leu Lys Thr Lys Val Thr Val Thr Val Leu Glu Asn Asp   101
GTG GAG GAC TCA GGA TAT TAC TAT [TGT] ATA GTA AGA AAC TCA ACT TAC TGC CTC AAA ACT AAA GTA ACC GTA ACT GTG TTA GAG AAT GAC   303

Pro Gly Leu Cys Tyr Ser Thr Gln Ala Thr Phe Pro Gln Arg Leu His Ile Ala Gly Asp Gly Ser Leu Val [Cys] Pro Tyr Val Ser Tyr   131
CCT GGC TTG TGT TAC AGC ACA CAG GCC ACC TTC CCA CAG CGG CTC CAC ATT GCC GGG GAT GGA AGT CTT GTG [TGC] CCT TAT GTG AGT TAT   393

Phe Lys Asp Glu Asn Asn Glu Leu Pro Glu Val Gln Trp Tyr Lys Asn Cys Lys Pro Leu Leu Leu Asp Asn Val Ser Phe Phe Gly Val   161
TTT AAA GAT GAA AAT AAT GAG TTA CCC GAG GTC CAG TGG TAT AAG AAC TGT AAA CCT CTG CTT CTT GAC AAC GTG AGC TTC TTC GGA GTA   483

Lys Asp Lys Leu Leu Val Arg Asn Val Ala Glu Glu His Arg Gly Asp Tyr Ile [Cys] Arg Met Ser Tyr Thr Phe Arg Gly Lys Gln Tyr   191
AAA GAT AAA CTG TTG GTG AGG AAT GTG GCT GAA GAG CAC AGA GGG GAC TAT ATA [TGC] CGT ATG TCC TAT ACG TTC CGG GGG AAG CAA TAT   573

Pro Val Thr Arg Val Ile Gln Phe Ile Thr Ile Asp Glu Asn Lys Arg Asp Arg Pro Val Ile Leu Ser Pro Arg Asn Glu Thr Ile Glu   221
CCG GTC ACA CGA GTA ATA CAA TTT ATC ACA ATA GAT GAA AAC AAG AGG GAC AGA CCT GTT ATC CTG AGC CCT CGG AAT GAG ACG ATC GAA   663

Ala Asp Pro Gly Ser Met Ile Gln Leu Ile [Cys] Asn Val Thr Gly Gln Phe Ser Asp Leu Val Tyr Trp Lys Trp Asn Gly Ser Glu Ile   251
GCT GAC CCA GGA TCA ATG ATA CAA CTG ATC [TGC] AAC GTC ACG GGC CAG TTC TCA GAC CTT GTC TAC TGG AAG TGG AAT GGA TCA GAA ATT   753

Glu Trp Asn Asp Pro Phe Leu Ala Glu Asp Tyr Gln Phe Val Glu His Pro Ser Thr Lys Arg Lys Tyr Thr Leu Ile Thr Thr Leu Asn   281
GAA TGG AAT GAT CCA TTT CTA GCT GAA GAC TAT CAA TTT GTG GAA CAT CCT TCA ACC AAA AGA AAA TAC ACA CTC ATT ACA ACA CTT AAC   843

Ile Ser Glu Val Lys Ser Gln Phe Tyr Arg Tyr Pro Phe Ile [Cys] Val Val Lys Asn Thr Asn Ile Phe Glu Ser Ala His Val Gln Leu   311
ATT TCA GAA GTT AAA AGC CAG TTT TAT CGC TAT CCG TTT ATC [TGT] GTT GTT AAG AAC ACA AAT ATT TTT GAG TCG GCG CAT GTG CAG TTA   933

Ile Tyr Pro Val Pro Asp Phe Lys Asn Tyr Leu Ile Gly Gly Phe Ile Ile Leu Thr Ala Thr Ile Val Cys Cys Val Cys Ile Tyr Lys   341
ATA TAC CCA GTC CCT GAC TTC AAG AAT TAC CTC ATC GGG GGC TTT ATC ATC CTC ACG GCT ACA ATT GTA TGC TGT GTG TGC ATC TAT AAA   1023

Val Phe Lys Val Asp Ile Val Leu Trp Tyr Arg Asp Ser Cys Ser Gly Phe Leu Pro Ser Lys Ala Ser Asp Gly Lys Thr Tyr Asp Ala   371
GTC TTC AAG GTT GAC ATA GTG CTT TGG TAC AGG GAC TCC TGC TCT GGT TTT CTT CCT TCA AAA GCT TCA GAT GGA AAG ACA TAC GAT GCC   1113

Tyr Ile Leu Tyr Pro Lys Thr Leu Gly Glu Gly Ser Phe Ser Asp Leu Asp Thr Phe Val Phe Lys Leu Leu Pro Glu Val Leu Glu Gly   401
TAT ATT CTT TAT CCC AAG ACC CTG GGA GAG GGG TCC TTC TCA GAC TTA GAT ACT TTT GTT TTT AAA CTG TTG CCT GAG GTC TTG GAG GGA   1203

Gln Phe Gly Tyr Lys Leu Phe Ile Tyr Gly Arg Asp Asp Tyr Val Gly Glu Asp Thr Ile Glu Val Thr Asn Glu Asn Val Lys Lys Ser   431
CAG TTT GGA TAC AAG CTG TTC ATT TAT GGA AGG GAT GAC TAT GTT GGA GAA GAT ACC ATC GAG GTT ACT AAT GAA AAT GTA AAG AAA AGC   1293

Arg Arg Leu Ile Ile Ile Leu Val Arg Asp Met Gly Gly Phe Ser Trp Leu Gly Gln Ser Ser Glu Glu Gln Ile Ala Ile Tyr Asn Ala   461
AGG AGG CTG ATT ATC ATT CTA GTG AGA GAT ATG GGA GGC TTC AGC TGG CTG GGC CAG TCA TCT GAA GAG CAA ATA GCC ATA TAC AAT GCT   1383

Leu Ile Gln Glu Gly Ile Lys Ile Val Leu Leu Glu Leu Glu Lys Ile Gln Asp Tyr Glu Lys Met Pro Asp Ser Ile Gln Phe Ile Lys   491
CTC ATC CAG GAA GGA ATT AAA ATC GTC CTG CTT GAG TTG GAG AAA ATC CAA GAC TAT GAG AAA ATG CCA GAT TCT ATT CAG TTC ATT AAG   1473

Gln Lys His Gly Val Ile Cys Trp Ser Gly Asp Phe Gln Glu Arg Pro Gln Ser Ala Lys Thr Arg Phe Trp Lys Asn Leu Arg Tyr Gln   521
CAG AAA CAC GGA GTC ATT TGC TGG TCA GGA GAC TTT CAA GAA AGA CCA CAG TCT GCA AAG ACC AGG TTC TGG AAA AAC TTA AGA TAC CAG   1563

Met Pro Ala Gln Arg Arg Ser Pro Leu Ser Lys His Arg Leu Leu Thr Leu Asp Pro Val Arg Asp Thr Lys Glu Lys Leu Pro Ala Ala   551
ATG CCA GCC CAA CGG AGA TCA CCA TTG TCT AAA CAC CGC TTA CTA ACC CTG GAT CCT GTG CGG GAC ACT AAG GAG AAA CTG CCG GCA GCA   1653

Thr His Leu Pro Leu Gly End                                                                                               557
ACA CAC TTA CCA CTC GGC TAG CATGGCAAAAGTGGGCAGGCCAAGAACTTCGGAATATCTCCCATCATAAGAGGCTGCAGCTGGGCTGTGCCTCCCAGTAAAACAGTCACGA   1765

ACCAAACCTGTGCAGTCCCTTGTTCCAGATCACCTGGAACTGGATTGGGAAGAGAACAGGACTTGGTGGCCAGGACCGCTCAGAGAGCCATGGTTGCTCAGGGATGCTGCTCCGGGATG   1884

CTTGACTAACAGTCGAGGCAGTGAACTGGGTGTAGAAAGCGTCAGGAAATGGCCACATGTGTGGATGGTTTAATTAGATTCTGTGGAGTCTCACAGTGGGATTGTGGCTGTCTGAGGAC   2003

ACTTTGGGGGGTCGCTGTCCAAGAAGTGGCTCCCCAAAGTATAAGTGCGGGTGAGGTTTACTGATACCCCAC                                                   2075
```

Fig. 4. Sequence of the murine IL-1 receptor cDNA clone (*11*). (**A**) Schematic diagram of the sequence features. Untranslated regions are lines; the IL-1 receptor coding region is a box. The signal peptide is filled in, the extracellular portion is open, the transmembrane region is stippled, and the cytoplasmic segment is cross-hatched. Inverted triangles above the box show potential *N*-glycosylation sites. Vertical lines indicate cysteine residues, and the proposed Ig-like disulfide bonds are indicated. (**B**) The sequence of the cDNA insert of the mouse IL-1 receptor clone 78. Amino acids are numbered from the NH_2-terminal amino acid of the mature protein; nucleotides are numbered from the first base of the codon for this amino acid. The arrow marks the site of cleavage of the signal peptide. The 26–amino acid sequence derived from the mature protein by NH_2-terminal sequencing is underlined with a thick bar. The transmembrane region is thinly underlined. Inverted triangles mark potential sites for *N*-linked glycosylation, and the cysteines believed to be involved in Ig-like intradomain disulfide bonding are boxed.

receptor from EL4 6.1 C10 cells, at 80 kD.

At saturating DNA concentrations, the transfected COS cell monolayer expressed, on average, 45,000 sites per cell. Since the parental COS cells expressed only about 500 sites per cell, more than 98% of all IL-1 binding sites in the transfected population were attributable to the expressed product of the cDNA clone. However, flow cytometry with fluorescein-conjugated IL-1α revealed that only 4.2% of the cells stained brightly (*14*); therefore, those few COS cells that were transfected and expressed the receptor contained on average 1.1×10^6 IL-1 binding sites, suggesting that the protein encoded by clone 78 can, by itself, bind IL-1α. Alternatively, if another polypeptide is also required for IL-1 binding, the transfected COS cells must provide this hypothetical second protein in great abundance.

Clone 78 contained a cDNA insert of roughly 2.35 kbp, which was used as a probe in RNA blot analysis. It hybridized to an EL4 6.1 C10 RNA that had an electrophoretic mobility slightly less than that of 28*S* ribosomal RNA (Fig. 3, lane 1), and which was undetectable in an IL-1 receptor–negative variant of EL4, EL4 M (Fig. 3, lane 2). The probe hybridized to a similarly sized RNA in the mouse fibroblast cell lines 3T3 and L929 (Fig. 3, lanes 3 and 4). The 3T3 cells, which have approximately ten times more surface IL-1 receptors per cell than do L929 cells (*8*), also contained more of the hybridizing RNA (Fig. 3). The correlation of receptor expression with abundance of hybridizing RNA is further evidence that clone 78 is derived from IL-1 receptor mRNA. As the size of the mRNA is at least 5 kb, clone 78 contains only a portion of it.

The 2.35-kb insert of clone 78 was subcloned and sequenced by standard techniques. The sequence had a single open reading frame of 576 amino acids (Fig. 4). To confirm that this reading frame encodes the IL-1 receptor, we purified the receptor protein to homogeneity from detergent extracts of EL4 6.1 C10 cells, and obtained the sequence of the NH_2-terminal 26 amino acids. This corresponded exactly to part of the amino acid sequence deduced from the open reading frame of the cDNA clone (Leu^1 through Lys^{26}; Fig. 4).

Upstream of the NH_2-terminal leucine of the mature receptor is a presumed signal peptide, and potential initiating methionine residues at positions −19 and −16. The sequences surrounding each methionine do not correspond well to the consensus sequence (GCCGCCRCCATGG) for good translation initiation sites (*16*), making it difficult to predict which one would actually be used. For simplicity, we assume translation initiates at the distal residue (Met^{-19}). An in-frame stop codon lies three codons upstream of Met^{-19}. The amino acid sequence surrounding Leu^1 is typical of signal peptide cleavage sites (*16*).

The mature protein has one extended stretch of uncharged amino acids, from Asp^{320} to Tyr^{340}. This presumed transmembrane region contains three cysteine residues that could be involved in either lipid derivatization or covalent binding to other proteins. NH_2-terminal to this segment is a stretch of 319 amino acids that contains all seven of the potential *N*-linked glycosylation sites; thus, this portion of the molecule may lie exterior to the cell and be responsible for IL-1 binding. The IL-1 receptor is glycosylated on asparagine residues (*15*), and the removal of the carbohydrate with *N*-glycanase decreases the size of the receptor from 80 kD to 62 kD (*15*), close to the 64,598 daltons predicted from the cDNA sequence. The COOH-terminal, presumably cytoplas-

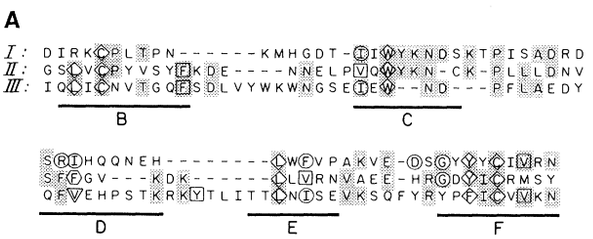


Fig. 5. Relation of the NH_2-terminal portion of the IL-1 receptor to the Ig superfamily. (**A**) Alignment of the three domains of the IL-1 receptor. Residues present in more than one domain are stippled. The geometric symbols enclose residues conserved in all members of the Ig superfamily (◇), V domains (○), C1 domains (□), V and C1 domains (▽), V and C2 domains (○). Stretches of amino acids involved in β-strand formation are underlined and labeled B to F; see (*17*) for full discussion. (**B**) Scores generated by the computer program ALIGN (*18*) when the three IL-1 receptor domains are compared with one another and with other members of the Ig superfamily. Scores above 3.00 are boxed, and are statistically significant. The Ig superfamily sequences were from GenBank or from the sources in (*17*), except for hc-fms, mPDGFR, hCEA, and hCD3γ (*26*). Nomenclature is as given in (*17*). The ALIGN program was run using the parameters given in (*17*). For the IL-1 receptor, the search strings used were: domain I, amino acids 23–80; domain II, amino acids 122–180; domain III, amino acids 228–296. Definitions: human, h; murine, m; rat, r; rabbit, rb; carcinoembryonic antigen, CEA; α1B-glycoprotein, α1B-GP; Fc receptor, FcR; cluster designation, CD; immunoglobulin, Ig; constant region, C; variable region, V; T cell antigen receptor, TcR; β2-microglobulin, β2-MG; poly immunoglobulin receptor, Poly IgR; major glycoprotein of peripheral myelin, P_0 MP; and leukocyte common antigen, LCA.

B

	Murine IL-1 Receptor Domains		
	I	II	III
mIL-1R (I)			
mIL-1R (II)	[3.7]		
mIL-1R (III)	1.3	[4.2]	
C2 Domains			
hc-fms (I,II,III,IV)	1.0,1.3 [3.1] 1.1	2.2,2.6,2.6,0.0	1.4,0.8 [3.1,4.1]
mPDGFR (I,III)	1.6,2.6	[3.5,3.3]	[3.5] 2.8
cN-CAM (IV)	[3.2]	3.0	[4.1]
rMAG (III,IV)	1.7,1.6	[3.0,3.6]	2.4,0.0
hCEA (II,III,IV,V)	2.5,2.1 [3.3] 2.2	2.3 [3.6] 2.8 [3.1]	[3.0] 2.3 [3.4] 2.1
hαlB-GP (III)	1.9	2.1	[3.6]
mFcR (I,II)	2.9 [3.3]	[3.8] 2.3	2.5,1.4
rCD2 (II)	0.5	[3.0]	1.1
hCD3γ	1.2	[0.3]	2.9
hCD3ε	[6.6]	[3.0]	2.7
C1 Domains			
hIg C lambda	2.1	2.7	[3.9]
hIg C kappa	2.3	2.9	[3.6]
hIg C heavy (I,III)	2.2,0.9	[4.1,4.9]	[3.9] 2.9
hTcR beta	1.2	[3.7]	[5.7]
mTcR gamma	2.6	1.8	2.2
hβ2-MG	1.7	2.3	0.2
hMHC I alpha 3	2.0	2.4	[3.6]
hMHC II beta 2	[3.7]	[3.7]	[3.2]
hCD1 alpha 3	2.4	1.1	[4.4]
V Domains			
mIg V lambda	2.8	2.9	2.0
hIg V heavy	[3.5]	2.6	[3.9]
mTcR V alpha	[3.8]	2.8	2.3
hTcR V beta	[4.7]	1.8	2.5
hCD4 (I)	[3.2]	2.9	[4.0]
rCD8 (chain I)	[3.8]	[3.6]	[3.2]
rCD8 (chain II)	[5.0]	2.2	[5.2]
rbPoly IgR (III)	2.3	[4.8]	1.4
rMRC OX-2 (I)	2.9	2.1	[3.1]
rP_0 MP	2.1	[3.0]	1.7
rThy-1	2.1	2.4	1.8
hc-fms (V)	1.2	[3.8]	2.2
ΔS-S Domains			
rCD2 (I)	2.6	2.1	2.9
mPDGFR (IV)	[4.5]	0.9	1.1
hCEA (I)	1.0	1.0	2.3
Negatives			
hIL-2R (p55)	a	a	1.2
LCA	a	1.5	a
hCD5	a	0.9	0.5

mic, part of the protein contains 217 amino acids.

The extracellular portion of the IL-1 receptor is organized into three domains, similar to those of members of the immunoglobulin (Ig) gene superfamily (Fig. 5A). Immunoglobulin-like domains typically possess only minimal amino acid similarity but share a common three-dimensional structure consisting of two β sheets held together by a disulfide bond (*17*). The cysteine residues involved in formation of this disulfide bond, as well as a few other critical residues, are highly conserved and occur in the same relative position in almost all members of the family (*17*). Members of the Ig superfamily include not only Ig constant and variable regions but also a number of other cell surface molecules. In particular, certain other hormone receptors, such as those for platelet-derived growth factor and colony-stimulating factor 1, also share Ig homology (*17*).

Comparison of the three IL-1 receptor domains with one another using the ALIGN program (*18*) shows that domains 1 and 2 are related (alignment score = 3.7), as are domains 2 and 3 (alignment score 4.2); but that domains 1 and 3 are less similar (alignment score 1.3). Alignment scores of >3.00 are considered significant (*18*). Comparison of each domain with selected members of the Ig superfamily, however, reveals significant matches by all three domains (Fig. 5B). The closest relationships, as percentage amino acid identity between the IL-1 receptor and other members of the Ig superfamily, are domain 1 with rat CD8 chain II (31.6%), domain 2 with human Ig Cγ1 (31.6%), and domain 3 with human T cell receptor Cβ (33.3%).

How the cytoplasmic domain functions in signal transduction is unknown. Computer searches of the August 1987 edition of GenBank, the April 1987 edition of the European Molecular Biology Laboratory databases, and the June 1987 edition of the National Biomedical Research Foundation protein database have not revealed significant similarity to any currently available sequences. No sequences typical (*19*) either of protein tyrosine kinases or of protein tyrosine kinase acceptor sites are present. The sequence Lys-Lys-Ser-Arg-Arg (amino acids 429 to 433) resembles a protein kinase C acceptor site (*19*); phosphorylation at this position may regulate either the activity or the cell surface expression of the IL-1 receptor.

The cDNA clone we have isolated encodes a polypeptide that binds both forms of IL-1 in a manner quantitatively indistinguishable from that of the receptor on EL4 6.1 C10 cells. This suggests that no other protein is required for IL-1 binding. It is not known whether the binding activity is a property of the monomer or of multimeric forms of the receptor.

A second class of higher affinity ($K_a \sim 10^{11} M^{-1}$) IL-1 receptors has been reported (*10, 20*). We have not found such receptors on our subclone of EL4 6.1 C10 cells, although we can detect them in concanavalin A–stimulated primary murine spleen cells (*14*). The nature and relevance of these higher affinity receptors is unknown. However, since there is only one class of IL-1 receptor present in our EL4 6.1 C10 cell line, and the binding of IL-1 to these receptors is sufficient to induce the EL4 cells to secrete T cell growth factor activity (*21*), the molecule we have cloned must be the functional IL-1 receptor (or, alternatively, the IL-1 binding subunit of a hypothetical functional IL-1 receptor complex). The IL-1 receptor described here, unlike the p55 subunit of the IL-2 receptor, has a cytoplasmic domain large enough in principle to possess some IL-1–regulated enzymatic activity. Whether the cloned IL-1 receptor molecule is functional in signal transduction can now be addressed by appropriate transfection experiments.

IL-1 receptors on B cell lines and fibroblasts differ in size or binding properties, or both, from those on EL4 cells (*5, 20, 22*). Further comparison of these receptors to the T cell IL-1 receptor may provide insight into the relation between the structure and function of this molecule.

REFERENCES AND NOTES

1. E. A. Kurt-Jones, S. Hamberg, J. Ohara, W. E. Paul, A. K. Abbas, *J. Exp. Med.* **166**, 1774 (1987).
2. D. Y. Mochizuki, J. R. Eisenman, P. J. Conlon, A. D. Larsen, R. J. Tushinski, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5267 (1987); M. A. S. Moore and D. J. Warren, *ibid.*, p. 7134.
3. J. J. Oppenheim, E. J. Kovacs, K. Matsushima, S. K. Durum, *Immunol. Today* **7**, 45 (1986); C. A. Dinarello, *Rev. Infect. Dis.* **6**, 51 (1984); S. B. Mizel, *Immunol. Rev.* **63**, 51 (1982).
4. C. J. March *et al.*, *Nature* **315**, 641 (1985).
5. S. K. Dower *et al.*, *ibid.* **324**, 266 (1986).
6. P. L. Kilian *et al.*, *J. Immunol.* **136**, 4509 (1986).
7. S. K. Dower *et al.*, *J. Exp. Med.* **162**, 501 (1985).
8. S. K. Dower and D. L. Urdal, *Immunol. Today* **8**, 46 (1987).
9. H. R. MacDonald, R. K. Lees, C. Bron, *J. Immunol.* **135**, 3944 (1985).
10. J. W. Lowenthal and H. R. MacDonald, *J. Exp. Med.* **164**, 1060 (1986).
11. Double-stranded, blunt-ended cDNA was synthesized from EL4 6.1 C10 polyadenylated RNA as described [U. Gubler and B. J. Hoffman, *Gene* **25**, 263 (1983); *cDNA Synthesis System*, Amersham International (1985); J. Sims, unpublished data] with random hexanucleotide primers (Pharmacia). The blunt-ended cDNA was fractionated by size on an agarose gel, and material larger than 1600 bp was eluted, ligated into Sma I–cleaved, dephosphorylated pDC201, and transformed into competent DH5α *E. coli* cells [D. Hanahan, *J. Mol. Biol.* **166**, 557 (1983)]. The transformed cells were plated on ampicillin, pooled in groups of 350 and grown in L broth for DNA extraction [H. C. Birnboim and J. Doly, *Nucleic Acids Res.* 7, 1513 (1979)]. Clone 78 was identified as described in Fig. 1 and its restriction map was determined by standard techniques. DNA sequencing [F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977); M. D. Biggin, T. J. Gibson, G. F. Hong, *ibid.* **80**, 3963 (1983)] was done on selected restriction fragments subcloned into M13mp18 and mp19 vectors [J. Norrander, T. Kempe, J. Messing, *Gene* **26**, 101 (1983)]. The entire clone was sequenced on both strands and across all internal restriction sites used in subcloning. Protein sequencing was performed as described (*4*).
12. The plasmid pDC201 was assembled from (i) the SV40 origin of replication, enhancer, and early and late promoters; (ii) the adenovirus-2 major late promoter and tripartite leader; (iii) SV40 polyadenylation and transcription termination signals; (iv) adenovirus-2 virus-associated RNA genes (VAI and VAII); and (v) pMSLV (*27*). The multiple cloning site contained sites for Kpn I, Sma I, and Bgl II. Details of the construction of pDC201 are available on request.
13. Y. Gluzman, *Cell* **23**, 175 (1981).
14. S. K. Dower, unpublished data.
15. D. L. Urdal, S. M. Call, J. L. Jackson, S. K. Dower, *J. Biol. Chem.* **263**, 2870 (1988); C. Bron and H. R. MacDonald, *FEBS Lett.* **219**, 365 (1987).
16. M. Kozak, *Nucleic Acids Res.* **15**, 8125 (1987); G. von Heijne, *J. Mol. Biol.* **184**, 99 (1985).
17. A. F. Williams, *Immunol. Today* **8**, 298 (1987).
18. M. O. Dayhoff, W. C. Barker, L. T. Hunt, *Methods Enzymol.* **91**, 524 (1983).
19. T. Hunter, *Cell* **50**, 823 (1987); C. House, R. E. H. Wettenhall, B. E. Kemp, *J. Biol. Chem.* **262**, 772 (1987).
20. T. A. Bird and J. Saklatvala, *Nature* **324**, 263 (1986); J. Chin, P. M. Cameron, J. A. Schmidt, *J. Exp. Med.* **165**, 70 (1987).
21. B. Mosley, S. K. Dower, S. Gillis, D. Cosman, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4572 (1987).
22. K. Matsushima, T. Akahoshi, M. Yamada, Y. Furutani, J. J. Oppenheim, *J. Immunol.* **136**, 4496 (1986); T. A. Bird, A. J. H. Gearing, J. Saklatvala, *FEBS Lett.* **225**, 21 (1987); R. Horuk, J. J. Huang, M. Covington, R. C. Newton, *J. Biol. Chem.* **262**, 16275 (1987).
23. COS cells were grown and transfected as described (*27*) with the DNA from a 1.5-ml culture of plasmid-containing *E. coli*. After 72 hours culture supernatants were discarded, and dishes were washed once with phosphate-buffered saline (*p*H 7.4) (PBS) containing 1% bovine serum albumin (BSA) and 0.1% sodium azide (binding buffer). To each dish was added 3 ml of binding buffer containing ^{125}I-labeled recombinant IL-1α (3×10^{-10} *M*) (*5*). After 2 hours at 8°C, the dishes were washed once with binding buffer, twice with PBS, and then fixed by incubation for 30 min at room temperature with 2.5% glutaraldehyde in PBS. The fixed preparations were stained with methylene blue to visualize the cell monolayer (to ensure an even distribution of cells), washed, and air-dried. The vertical edges of the culture dishes were removed and the bound ^{125}I-labeled IL-1α was visualized by autoradiography (Kodak XR-OMAT film for 72 hours at −70°C).
24. COS cells were transfected with vector pDC201 containing the murine IL-1 receptor cDNA insert (clone 78) or with control vector containing no insert as described in Fig. 1. After 72 hours of culture, cells were washed once with 10 ml of PBS, and then treated for 20 min at 37°C with an EDTA solution (0.005*M* in PBS, *p*H 7.4), and harvested. EL4 6.1 C10 cells and EL4 M cells were grown and harvested as described (*15*). For binding assays, COS cells were resuspended at 1.7×10^6 cell/ml with EL4 M (1.5×10^7 cell/ml) cells as carriers. EL4 M and EL4 6.1 C10 were resuspended at 1.5×10^7 cell/ml. All cell suspensions were made and binding assays done in RPMI 1640, 10% BSA, 0.1% sodium azide, 20 m*M* Hepes, *p*H 7.4. Binding incubations with ^{125}I-labeled IL-1α or ^{125}I-labeled IL-1α and unlabeled IL-1α or IL-1β were done as described (*5*). Data were analyzed and theoretical curves calculated and plotted with RS/1 (Bolt, Beranek and Newman) as described (*7*). In the competition experiment the free ^{125}I-labeled IL-1α concentration was 7.7×10^{-10}*M*. On the transfected COS cells the maximal binding was $2.98 \pm 0.3 \times 10^4$ molecules per cell (no inhibition) and the back-

ground (measured in the presence of $6 \times 10^{-7} M$ unlabeled IL-1α) was 921 ± 60 molecules per cell (100% inhibition). On the EL4 6.1 C10 cells maximal binding was $1.33 \pm 0.02 \times 10^4$ molecules per cell and background (see above) was 47 ± 2 molecules per cell.
25. D. A. Goldberg, *Proc. Natl. Acad. Sci. U.S.A.* 77, 5794 (1980).
26. L. Coussens *et al.*, *Nature* **320**, 277 (1986); Y. Yarden *et al.*, *ibid.* **323**, 226 (1986); S. Oikawa, H. Nakazato, G. Kosaki, *Biochem. Biophys. Res. Commun.* **142**, 511 (1987); G. W. Krissansen, M. J. Owen, W. Verbi, M. J. Crumpton, *EMBO J.* **5**, 1799 (1986).
27. D. Cosman *et al.*, *Nature* **312**, 768 (1984).
28. We thank A. Akelis, K. Barker, V. Batler, K. Biggs, D. Crockett, T. Davis, S. Gimpel, J. Hesselberg, J. Slack, and T. VandenBos for technical help, D. Cerretti for computer assistance, B. Gallis for erudition on protein kinases, and N. Divers and L. Troup for preparation of the manuscript. Supported by Immunology Ventures, a joint venture of Immunex and Eastman Kodak, and by a scholarship from the Leukemia Society of America to M.B.W.

8 March 1988; accepted 10 June 1988

Intervacuole Exchange in the Yeast Zygote: A New Pathway in Organelle Communication

LOIS S. WEISMAN AND WILLIAM WICKNER*

A new pathway of vesicle traffic between organelles has been identified. The vacuoles (lysosomes) of *Saccharomyces cerevisiae* zygotes rapidly exchange their contents at a specific point in the cell cycle. With the use of fluorescence microscopy, "tracks" were observed that connect the original parental vacuoles to the newly forming bud vacuoles. These observations suggest that vacuole-derived vesicles rapidly move along the tracks in both directions, equilibrating vacuole contents. This rapid vesicle movement may be responsible for vacuole formation in newly developing cells.

CELL DIVISION REQUIRES THAT ALL cellular constituents be replicated and segregated faithfully. Whereas mitosis ensures the equal partitioning of chromosomes between two daughter cells, little is known about how other cellular components are segregated. Previous observations of yeast vacuoles strongly suggest that their distribution between mother and daughter cells does not occur by a random process (*1*). *Saccharomyces cerevisiae* grows by an asymmetric process of budding. The bud receives a small vacuole when it is approximately 1% of the mother cell volume (*1*). Although obvious growth is seen only in the bud vacuole and there is no synchronous fragmentation of the maternal vacuole, about 50% of the bud's vacuole contents are derived directly from the mother cell vacuole (*1, 2*). To account for this pattern of inheritance, we proposed that vacuole formation entails continual vesicular traffic between the mother and bud vacuoles. We now report that parental vacuoles within a zygote mix their contents without fusing, presumably by a vesicle-mediated process. This "communication" between parental vacuoles may be related to the process of vacuole formation.

The two parental vacuoles in a zygote can be distinguished by mating an *ade2* strain with an *ADE* strain. Vacuoles of *ade2* yeast accumulate a fluorescent dye when the cells are deprived of adenine. Synthesis of the dye is suppressed by adenine, and the previously accumulated dye is stable (*1, 3*). When yeast zygotes first form, the endogenous *ade2* fluorophore remains with the original parental vacuole (Fig. 1, a and b). The fluorophore appears in the *ADE* parental vacuoles sometime after bud formation (Fig. 1, c to f). Often vesicles can be seen along a "path" from each major vacuole into the bud (Fig. 1c). Although the vacuole contents mix, the original parental vacuoles never fuse (Fig. 1, e and f).

The transfer of vacuole contents occurs at a specific point in zygote development. Zygotes were scored both for bud size and for dye transfer (Fig. 2). At 2.9 hours after mating, most of the zygotes had no bud or a small bud. None of the zygotes scored had dye present in the *ADE* parental vacuoles. In contrast, many of the zygotes scored at time points ranging from 3.4 to 4.8 hours after mating had mixed the contents of the parental vacuoles. In all of the zygotes with no bud and most of the zygotes with a small bud, the *ade2* fluorophore appeared exclusively in the *ade2* parental vacuole. In contrast, both parental vacuoles are labeled in almost all of the large-budded zygotes. In zygotes with medium-sized buds, about 50% have label in both parental vacuoles. From these observations we conclude that transfer of fluorescent dye to the unmarked parental vacuole generally occurs when the bud is medium-sized. To more precisely determine the size of the bud at transfer, we observed and photographed individual zygotes until fluorescence appeared in the *ADE* parental vacuole. During observations of nine such zygotes, we measured the size of the bud at the time that transfer occurred. The volume of the bud at transfer was 9 ± 3% (SD) of the zygote (two fused parental cells plus the bud). Buds eventually grow to a maximum volume of 31 ± 4% (SD) of the zygote. Thus, transfer occurs when the bud is about one-third of its final volume. In addition, transfer occurs in a

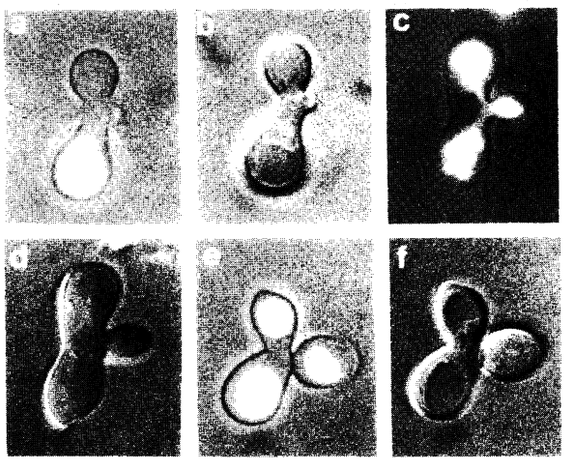


Fig. 1. Zygotes observed after mating *ade2* yeast with *ADE* yeast. The zygotes in this figure are oriented such that the two parental lobes are positioned on the vertical axis and the bud emerges on the horizontal axis. DBY1398 [*MATa ade2 ura3*] were grown in yeast extract–peptone dextrose (YEPD) (*21*) for 3 days until the vacuoles were highly fluorescent. One milliliter of culture was diluted into 25 ml of YEPD with adenine (160 μg/ml) to a final concentration of 2×10^7 cells per milliliter. The culture was aerated at 30°C for 2 hours, then mixed with an equal volume of a culture of X2180-1B and [*MATα*] (Yeast Genetics Stock Center) at the same cell concentration. Cells were incubated with slow shaking at 30°C. Under these conditions, zygotes were observed in 2.5 to 3 hours. Glass slides were treated with concanavalin A (0.5 mg/ml) before application of cells (*5*). Fluorescence of the cells was observed as described (*1*). In addition, the cells were illuminated with visible light so that the cell outlines could be seen. (**a**, **c**, and **e**) Fluorescence micrographs. (**b**, **d**, and **f**) The corresponding Nomarski photographs. (a) In zygotes with no bud, and most zygotes with a small bud, the endogenous *ade2* fluorescence is confined to the original *ade2* parental vacuole. (c) In approximately one-half of the zygotes with a medium-sized bud, fluorescence is seen in both parental vacuoles and in the bud. In addition, vesicles or fluorescent trails appear to connect these areas. (e) When the bud is large, most zygotes contain fluorescence in both parental vacuoles as well as in the bud and the fluorescent trails are no longer visible.

Molecular Biology Institute and Department of Biological Chemistry, University of California, Los Angeles, CA 90024.

*To whom correspondence should be addressed.