P_{O_2} on K^+ currents in this experimental condition is shown in Fig. 3, which also illustrates the relative potency of test solutions with two different P_{O_2} values. A decrease of P_{O_2} from 150 to 110 mmHg caused a reduction of peak current amplitude of 19% (Fig. 3A), whereas decreasing P_{O_2} to 10 mmHg inhibited the current by about 32% (Fig. 3B). Recovery in both cases was almost complete. Peak K^+ current as a function of voltage in 150 and 10 mmHg P_{O_2} is shown in Fig. 3C. Although we did not study the K^+ current kinetics in detail, we observed that after recovery from extremely low P_{O_2}, the K^+ current had a faster activation time course than it did before exposure to hypoxia.

Our observations that type I cells have voltage-dependent Na^+, Ca^{2+}, and K^+ channels are of interest because type I cells, which are of neuroectodermal origin (*7*), have been thought to be nonexcitable (*5*), and this has influenced earlier models of the transduction mechanism in the carotid body. Thus, any explanation of transduction in this arterial chemoreceptor must now take into account the electrical properties of type I cells. The discovery in type I cells of a K^+ current sensitive to environmental P_{O_2} could be a direct result of the action of O_2 on K^+ channels, perhaps coupled to an O_2 sensor, or, as in other chemoreceptors (*8*), it may require an intracellular mediator. The fact that the inhibition of K^+ currents by low P_{O_2} was seen in dialyzed cells and appeared to be unaffected by intracellular Ca^{2+} and ATP favors the idea of a direct effect of molecular O_2 on the channels.

The ionic conductances of type I cells are well suited for a major contribution to stimulus-secretion coupling. The activation of Na^+ and Ca^{2+} channels causes the generation of action potentials and fast injection of Ca^{2+} into the cytosol, whereas the K^+ conductance sensitive to P_{O_2}, which has a relatively slow activation kinetic, may be critical for determining the firing frequency of the cells and thus may translate a decrease in P_{O_2} into the appropriate secretory response. We have recently observed that, as predicted by the voltage clamp recordings, type I cells fire action potentials repetitively after they are switched from voltage to current clamp mode and that hypoxia decreases only slightly the rate of repolarization of individual action potentials but it greatly increases the firing frequency of the cells (*9*).

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Selective Activation of Transcription by a Novel CCAAT Binding Factor

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A novel CCAAT binding factor (CBF) composed of two different subunits has been extensively purified from rat liver. Both subunits are needed for specific binding to DNA. Addition of this purified protein to nuclear extracts of NIH 3T3 fibroblasts stimulates transcription from several promoters including the α2(I) collagen, the α1(I) collagen, the Rous sarcoma virus long terminal repeat (RSV-LTR), and the adenovirus major late promoter. Point mutations in the CCAAT motif that show either no binding or a decreased binding of CBF likewise abolish or reduce activation of transcription by CBF. Activation of transcription requires, therefore, the specific binding of CBF to its recognition sites.

EUKARYOTIC GENES CONTAIN A COMplex array of cis-regulatory elements that mediate induced, repressed, or basal transcription rates (*1, 2*). One of these elements contains a conserved CCAAT sequence and is often present at about 80 to 120 bp upstream from the transcriptional start site. Studies with several promoters have indicated that the integrity of this CCAAT sequence is required for optimal promoter activity (*3, 4*). Several different DNA binding proteins can interact with CCAAT-containing promoter elements. Among these are a factor from murine erythroleukemia cells (*5*), CCAAT binding transcription factor/nuclear factor–1 (CTF/NF-1) from HeLa cells (*6*), CCAAT binding protein from rat liver cells (*4*), and nuclear factor–Y from B-lymphoma line M12 (*7*). We previously identified a factor that binds to a segment containing a CCAAT motif in the −80 region of the mouse α2(I) collagen promoter and CCAAT-containing sequences in a number of other promoters (*8, 9, 10*). This factor consists of two different protein components; both are required for DNA binding and are present in the DNA-protein complex (*10*). Point mutations in the −80 CCAAT motif of the α2(I) collagen promoter caused a decrease in promoter activity to 1/8 to 1/12 of normal, as assayed in DNA transfection of NIH 3T3 fibroblasts in culture. These same mutations also strongly inhibited interaction with a CCAAT binding factor (CBF) present in nuclear extracts of NIH 3T3 fibroblasts (*11*). CBF is distinguished from CTF/NF-1 by chromatographic properties and molecular size (*10*). In addition, direct binding and competition experiments indicate that this factor and CTF/NF-1 bind to different DNA segments (*9*). This suggests that sequence determinants outside the CCAAT motif help discriminate between these factors.

We report here that the new heterodimeric CBF activates transcription from several promoters when the purified protein is added to NIH 3T3 nuclear extracts. The activation of transcription is specific since point mutations in the CCAAT motif which

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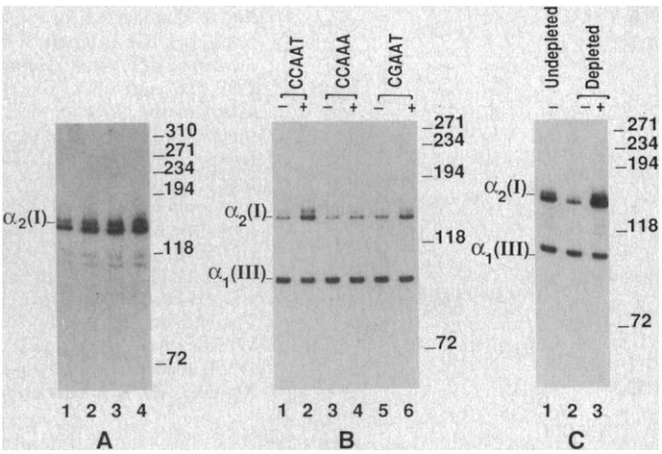

Fig. 1. In vitro transcription analysis of CBF activity. (**A**) Effect of CBF on transcription of the α2(I) collagen promoter. A supercoiled DNA template containing the mouse α2(I) collagen promoter, pR40 (300 ng), was transcribed with the use of NIH 3T3 nuclear extracts in presence of increasing amounts of purified CBF. (Lane 1) No CBF; (lane 2) 37 ng of CBF; (lane 3) 75 ng of CBF; and (lane 4) 150 ng of CBF. Plasmid pR40 (*25*) contains a segment of the mouse α2(I) collagen gene (−2000 to +54) fused to the bacterial chloramphenicol acetyltransferase (CAT) gene. Mouse NIH 3T3 fibroblasts grown in MEM (minimal essential medium) supplemented with 10% (v/v) calf serum were harvested at 80 to 100% confluency and nuclear extracts were prepared as described (*26*), except that leupeptin (10 μg/ml) and pepstatin (10 μg/ml) were included in all buffers. These extracts (final protein concentration in the reaction, 0.85 mg/ml) were added to 25-μl in vitro transcription reactions. The RNA synthesized in vitro was analyzed by primer extension (*27*). Primers were labeled to the same specific activity with T4 polynucleotide kinase and [γ-^{32}P]ATP. Quantitation was performed by scanning densitometry. All transcription experiments were repeated at least three times with two or more NIH 3T3 nuclear extract preparations and similar results were obtained. (**B**) Analysis of point mutations in the CCAAT motif. Supercoiled plasmid DNAs containing either the wild-type α2(I) collagen promoter or mutants in the CCAAT motif (300 ng) were mixed with 240 ng of p120, a supercoiled plasmid containing the α1(III) promoter, as an internal control. In plasmid p120 the sequence of the mouse α1(III) collagen gene between −80 and +16 is fused to the CAT gene (*28*). The mutant α2(I) collagen plasmids are identical to the wild-type plasmid except for the indicated mutation in the CCAAT motif. Mutations were introduced as described (*29*). The DNA templates were transcribed with NIH 3T3 nuclear extracts with or without CBF. (Lanes 1, 3, and 5) No CBF; (lanes 2, 4, and 6) 180 ng of CBF. (**C**) Transcription with CBF-depleted NIH 3T3 nuclear extracts. Supercoiled pR40 plasmid [α2(I) collagen] (300 ng) was mixed with supercoiled p120 plasmid [α1(III) collagen] (100 ng). The DNA templates were transcribed in undepleted extract (lane 1), in CBF-depleted extract (lane 2), and in CBF-depleted extract + 180 ng of CBF (lane 3).

inhibit binding of the protein also show a decreased activation of transcription.

Purified CBF from rat liver was used for in vitro transcription and DNA binding experiments. We purified this heterodimer by a factor of about 1200-fold from nuclear extracts by using the −80 CCAAT motif of the α2(I) collagen promoter as an affinity ligand (*10*). In the gel retardation assay the mobility of the retarded band is the same whether nuclear extracts or purified CBF is used. Furthermore, both with crude extracts and with purified rat liver CBF, DNA binding is dependent on two complementing factors.

In that CBF was purified with an oligonucleotide containing the −80 CCAAT motif of the mouse α2(I) collagen promoter as DNA-affinity ligand, we first examined the transcriptional stimulatory activity of this factor with the α2(I) collagen promoter as template. Increased concentrations of purified CBF added to the transcription reaction caused increased transcription from the α2(I) collagen promoter (Fig. 1A). The maximum increase in this experiment was about fourfold. To determine the specificity of this activation, two point mutations in the CCAAT motif of the α2(I) collagen promoter were examined. In one the T in the CCAAT motif was mutated to an A, and in the other the second C was mutated to a G. Since CBF is unable to bind to the mouse α1(III) collagen promoter (*8*) (Fig. 2) and also does not stimulate transcription from this promoter (Figs. 1, B and C, and 3), this template was used as an internal control in the subsequent transcription reactions. Whereas CBF-stimulated transcription from the wild-type α2(I) collagen promoter template was four times greater than normal, there was no increase with the CCAAA mutant (Fig. 1B). With the CGAAT mutant as template, the increase (2.5 times greater) of transcription by CBF was less pronounced than with the wild-type template.

The binding affinities of these templates for CBF were also compared. DNA binding was assayed by gel retardation (*12, 13*) and deoxyribonuclease (DNase) I footprinting (*14*). In DNase I footprints, the area of protection of the α2(I) collagen promoter lies approximately between −100 and −73 (Fig. 2B). The DNA with the CCAAA mutation exhibits no detectable binding of CBF in either a gel retardation assay or a DNase I footprint assay (Fig. 2, A and B). In DNA with the CGAAT mutation, binding of CBF was detectable in both assays but greatly decreased. A direct correlation exists, therefore, between the capacity of CBF to stimulate the transcriptional activity of these templates and its ability to bind to them.

NIH 3T3 nuclear extracts were depleted of CBF by mixing the extracts with the DNA affinity resin that was used to purify CBF. A gel retardation assay showed that 90 percent of the CBF DNA binding activity in the extracts was removed by this procedure. Transcription of the α2(I) collagen promoter template was decreased in depleted extracts, whereas transcription of the α1(III) promoter was unchanged (Fig. 1C). Addition of purified CBF to the depleted extract increased the transcriptional activity of the α2(I) collagen DNA template, whereas transcription from the α1(III) collagen promoter was unchanged.

The transcriptional stimulation by CBF was also tested with other promoters. CBF increases transcription of the α1(I) collagen promoter about threefold (Fig. 3). CBF binds to the α1(I) collagen promoter (Fig. 2), which contains two CCAAT sequences (between −96 and −100, and between −122 and −126). Only the sequence surrounding the proximal CCAAT sequence (−96 to −100) is protected by CBF in DNase I footprints (Fig. 2B), suggesting a difference in affinity. When the proximal CCAAT segment is deleted, no binding is detected to the distal CCAAT sequence as assayed by gel retardation. Although there is little sequence similarity surrounding the CCAAT motif in the CBF binding sites of the α1(I) and α2(I) collagen promoters, the affinity of CBF for these sites is similar. The promoter of RSV-LTR is also stimulated about 2.5-fold by CBF (Fig. 3). DNase I footprint analysis showed that CBF binds to two different sites in the RSV-LTR promoter (*10*). We do not know yet whether both sites are needed for the activation of transcription. In other experiments the RSV-LTR promoter was stimulated at least fivefold. CBF also increases transcription of adenovirus major late promoter about twofold (Fig. 3). CBF does not detectably activate transcription of the herpes simplex virus thymidine kinase promoter. The affinity of CBF for this promoter is much less than for the α2(I) collagen promoter (*10*).

CBF-stimulated transcription from the α2(I) collagen promoter is more pronounced compared to that from the other promoters (compare Fig. 1C and Fig. 3). Perhaps the activity of this promoter is more dependent on CBF than are other promoters, which would be more dependent on other factors. For instance, the activity of

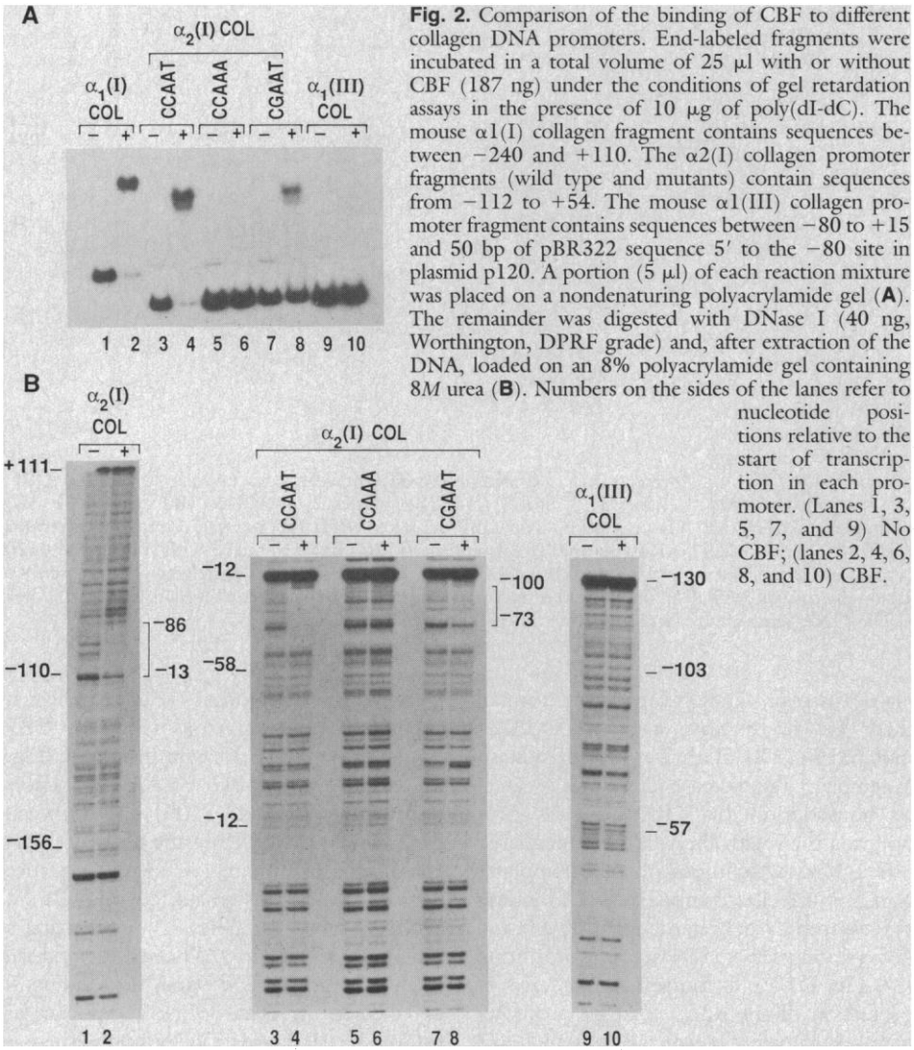

Fig. 2. Comparison of the binding of CBF to different collagen DNA promoters. End-labeled fragments were incubated in a total volume of 25 μl with or without CBF (187 ng) under the conditions of gel retardation assays in the presence of 10 μg of poly(dI-dC). The mouse α1(I) collagen fragment contains sequences between −240 and +110. The α2(I) collagen promoter fragments (wild type and mutants) contain sequences from −112 to +54. The mouse α1(III) collagen promoter fragment contains sequences between −80 to +15 and 50 bp of pBR322 sequence 5′ to the −80 site in plasmid p120. A portion (5 μl) of each reaction mixture was placed on a nondenaturing polyacrylamide gel (**A**). The remainder was digested with DNase I (40 ng, Worthington, DPRF grade) and, after extraction of the DNA, loaded on an 8% polyacrylamide gel containing 8*M* urea (**B**). Numbers on the sides of the lanes refer to nucleotide positions relative to the start of transcription in each promoter. (Lanes 1, 3, 5, 7, and 9) No CBF; (lanes 2, 4, 6, 8, and 10) CBF.

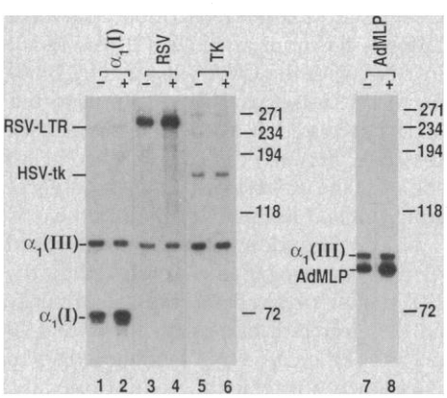

Fig. 3. Effect of CBF on transcription of other promoters. Reactions (25 μl) contained p120, the plasmid containing the α1(III) collagen promoter (100 ng), except for the reaction in lanes 7 and 8, which contained 210 ng of this plasmid. In addition, reactions contained the following DNAs. (Lanes 1 and 2) 30 ng of p13-3 [α1(I) collagen promoter (*30*)]; (lanes 3 and 4) 300 ng of pRSV-CAT (RSV-LTR) (*31*); (lanes 5 and 6) 300 ng of pTK-CAT [HSV-thymidine kinase (*32*)]; and (lanes 7 and 8) 75 ng of pG91 [adenovirus major late promoter (*33*)]. Reactions in lanes 1 to 6 were performed with CBF-depleted NIH 3T3 nuclear extracts. Reactions in lanes 7 and 8 were performed with undepleted extract. (Lanes 1, 3, 5, and 7) No CBF; (lanes 2, 4, 6, and 8) 180 ng of CBF.

the major late promoter of adenovirus is strongly stimulated by another factor (USF), which binds more proximally to the start site of transcription in this promoter (*15, 16*). Alternatively, it is possible that the location of the binding site of CBF in the α2(I) collagen promoter provides more favorable interactions with other transcriptional components.

A few transcriptional factors have been shown to consist of two different domains, a DNA binding domain and a domain that interacts with other transcriptional components (*17–20*). One of the subunits of CBF could correspond to the binding domain of these proteins, and the second could provide the interactions with other transcriptional components, as well as control binding of the first subunit to DNA. This model is also illustrated by the chicken ovalbumin upstream promoter (COUP) and S300 factors isolated from HeLa cells (*21, 22*). However, in this system the COUP factor binds the ovalbumin promoter by itself, whereas the S300 factor is needed for transcription and was shown to stabilize the COUP-DNA complex. In contrast, CBF requires both factors for DNA binding and subsequent transcriptional activation. Alternatively, both subunits of CBF could cooperatively bind to DNA, provide interactions with other components of the transcriptional machinery, and be targets for regulatory modifications. Regardless of which model is valid, the finding that the CBF transcription factor consists of two different subunits broadens the regulatory potential of such a factor.

After this report was submitted, a similar heterodimeric CCAAT binding factor from HeLa cells which binds to the major late promoter of adenovirus and a few other promoters was described (*23*). Interestingly, the subunits of a yeast CCAAT binding factor are functionally interchangeable with the subunits of this HeLa cell factor (*24*).

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

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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).

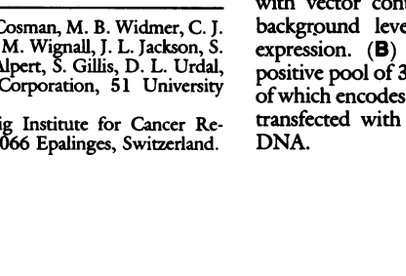

Fig. 1. Isolation of IL-1 receptor cDNA clones by direct expression screening (*23*). The autoradiographed plates of transfected COS cells had been probed with ^{125}I-labeled IL-1α to detect IL-1 receptor expression. (**A**) COS cells transfected with vector containing no insert, showing the background 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.

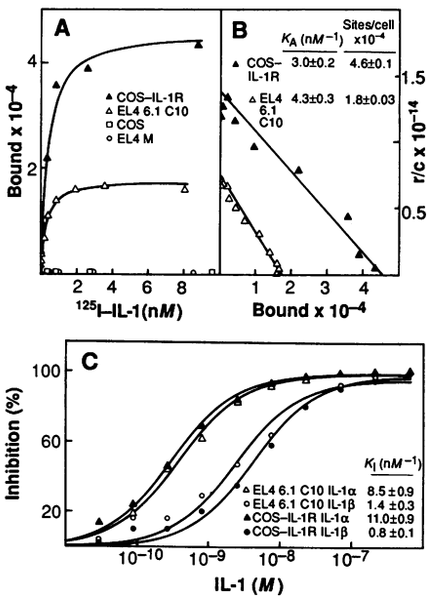

Fig. 2. Comparison of the IL-1 binding properties of natural and recombinant IL-1 receptors (*24*). (**A**) Direct binding of ^{125}I-labeled IL-1α to cells expressing native IL-1 receptors (EL4 6.1 C10) or recombinant receptors (COS–IL-1R). (**B**) Data from (A) replotted in the Scatchard coordinate 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.

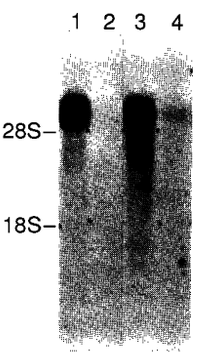

Fig. 3. RNA blot analysis of IL-1 receptor mRNA. EL4 6.1 C10 RNA (lane 1), EL4 M RNA (lane 2), 3T3 RNA (lane 3), L929 RNA (lane 4). Polyadenylated RNA (20 μg) from the indicated cell lines was loaded in each lane of a 1% formaldehyde-agarose gel (*25*). After electrophoresis, the RNA was blotted onto 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).

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