The murine leukemia inhibitory factor receptor α-chain (mLIFR) exists in a membrane-bound and a soluble form. The two major classes of mRNA transcript correspond to either the soluble or membrane-bound form of the mLIFR. In this study we have identified a complex and heterogeneous pattern of expression of mRNA transcripts for this receptor in normal mouse tissues and cell lines. In order to understand the molecular basis of these transcripts, genomic clones encompassing the region of divergence from the soluble to the membrane-bound form of the receptor were isolated. cDNAs encoding the membrane-bound form of the mLIFR were generated by an alternative splicing event where an exon that is specific to the soluble mLIFR was skipped. The membrane-bound form of the mLIFR was heterogeneously polyadenylated with at least five different sites of polyadenylation. The mRNA transcript encoding the soluble form of the mLIFR contained a region highly homologous to a murine B2 repetitive element, thus providing a possible explanation for the genesis of this transcript. The different forms of the mLIFR were analyzed in a wide range of mouse tissues in pseudopregnant mice and in mice at various stages of pregnancy. Only liver, placenta, and uterus showed an increase in the levels of mLIF mRNA expression during pregnancy, indicating an important role for the LIFR in this process. However, somewhat surprisingly, there was no detectable difference in mLIF mRNA levels or levels of soluble protein in leukemia inhibitory factor nullizygous mice when compared with normal mice.

Leukemia inhibitory factor (LIF) is a multifunctional cytokine that was initially purified and cloned on the basis of its ability to induce differentiation and suppress donogenicity in the mouse leukemic cell line M1 (1). The pleiotropic actions of LIF include its ability to maintain embryonic stem cells in their pluripotent state, stimulate acute phase synthesis by hepatocytes, inhibit adipogenesis, regulate nerve differentiation, stimulate the function of osteoblasts and the proliferation of DA-1 hemopoietic cells, and potentiate the action of interleukin-3 on megakaryocyte precursors (reviewed in Ref. 2).

The high affinity LIF receptor complex is composed of a specific LIF-binding component, the α-chain, and an affinity converter, gp130 (3, 4). Several other cytokines including ciliary neurotrophic factor (5) and oncostatin-M (6, 4) require the LIFR α-chain to transduce a biological signal. The recently characterized cytokine cardiotrophin-1 has also been shown to bind to the LIFR α-chain with low affinity (7, 8). The high affinity receptor complexes of these cytokines as well as those for interleukin (IL)-6 (9) and IL-11 (10, 11) also contain gp130, and this sharing of receptor components may account for the overlapping actions and functional redundancy of this group of cytokines.

The LIF receptor α-chain (LIFR) and gp130 are members of the hemopoietin family of receptors (12, 13). The LIFR contains its extracellular region two hemopoietin domains that are separated by an immunoglobulin-like module. In addition, three fibronectin type III (FNIII) domains are located immediately N-terminal to the transmembrane domain. cDNA clones that encode receptors corresponding to a membrane-bound form of the LIFR were isolated from a human placental cDNA library (3). Further cDNA clones, that have been isolated from murine liver cDNA libraries, encode stop codons just after the second FNIII domain and are thus predicted to encode soluble receptors (3, 14). A naturally occurring LIF-binding protein is present in high levels in normal mouse serum and was shown to be a soluble form of the mLIFR (15, 16). The LIF-binding protein is able to bind mLIF with similar characteristics to the membrane-bound form of the receptor, but unlike the soluble form of the IL-6 receptor (17) it acts as an inhibitor of LIF activity rather than as an agonist (18). The physiological function of this form of the mLIFR has not yet been determined, but it may serve to sequester excess LIF in the circulation.

The gene for the murine LIF receptor (mLIFR) has been mapped to the proximal region of chromosome 15 and is located within a cluster of cytokine receptor lod that includes growth hormone receptor, interleukin-7 receptor, and prolactin receptor (19). Targeted disruption of the low affinity mLIFR results in a wide range of defects including abnormal placentation, severe osteopenia, various neural defects, and metabolic disorders of the liver (20). mLIFR-deficient animals die during or shortly after birth. Mice in which the LIF gene has been dis-
ruptured have also been described (21, 22), and although these mutant mice display retarded postnatal growth they are generally viable. Interestingly, blastocysts from LIF null females are viable but are only able to implant if transferred to heterozygous or wild type pseudopregnant females, indicating a requirement for LIF in the implantation process. An important role for LIF during pregnancy is also implied by studies of normal mice. We have also investigated the levels of the soluble and membrane-bound forms of the mouse LIFR. Examination of normal mice revealed that the soluble and membrane-bound forms of the mouse LIFR (15, 16) increase dramatically during gestation. The maximum serum levels of soluble mLIFR occur several days after the transient burst of LIF expression on day 4 of pregnancy (23).

In this study we have characterized the molecular basis for the soluble and membrane-bound forms of the mouse LIFR. Given the role of LIF and its receptor in pregnancy we have examined the mRNA levels of the soluble and membrane-bound forms of the mLIFR in a variety of tissues during gestation in normal mice. We have also investigated the levels of mLIFR mRNA expression in mice in which the LIF gene has been ablated.

MATERIALS AND METHODS

Library Screening—Initially, cDNA clones encoding the mouse LIF receptor were isolated by screening a 14-day mouse fetal liver cDNA Unizap library with a radiolabeled full-length cDNA clone encoding the human LIFR (24). Mouse cDNA clones were detected following moderate stringency washing conditions (2X SSC, 0.1% SDS at 65°C). Additional cDNA clones were isolated by screening a commercial (Stratagene) adult mouse liver cDNA λ ZAP library using radioactive probes derived either from cDNA clones or subcloned restriction fragments from genomic clones. All DNA probes used in this study were labeled to a specific activity of 3 × 10^6 dpm/μg using (α-32P)dATP (Bresatec) and a Gigaprim (Bresatec) random hexamer DNA labeling kit.

In order to isolate the mLIF gene a mouse 129SV genomic library in λ FIXII (Stratagene) was screened under high stringency conditions (0.2X SSC, 0.1% SDS at 65°C) with radiolabeled probes (a BglII-HindIII fragment from the extracellular domain that encompassed fibronectin III domains I and II plus a BglII-BstEII fragment from the cytoplasmic domain) derived from DNA clones. Positive plaques were purified, and phage DNA was prepared using standard methods (25). After restriction mapping, and hybridization using exon-specific oligonucleotide primers, genomic insert fragments were digested with appropriate restriction enzymes and subcloned into the pBluescript SK II vector.

DNA Sequence Analysis—Sequence analysis of cDNAs and subcloned restriction fragments of genomic clones was carried out by the dideoxy nucleotide chain termination method (26) using either a PRISM Ready Reaction DyeDeoxy Terminator Cycle sequencing kit on an Applied Biosystems 373 DNA sequencer or a T7 DNA polymerase-based Ready Reaction DyeDeoxy Terminator Cycle sequencing kit on an Applied Biosystems 373 DNA sequencer or a T7 DNA polymerase-based Pharmacia Biotech Inc. kit. DNA sequences were analyzed using the program Autoassembler or programs from the GCG sequence analysis software package.

Southern Blot Analysis of Genomic DNA—DNA was isolated from either W9.5 embryonic stem cells (27) or the brain of a normal female C57BL/6 × C57BL/10 mouse using standard procedures (25). The DNA (10 μg) was digested with restriction enzymes according to the manufacturer’s instructions, electrophoresed in 0.8% agarose gels, and blotted onto GeneScreen Plus membranes using 0.4 M NaOH as the transfer medium (28). The blots were probed with a radiolabeled polymerase chain reaction-generated probe spanning nucleotides 2169–3389 of the published mLIFR sequence (14). Hybridization was carried out overnight at 65°C in a solution containing 10% dextran sulfate, 1% SDS, and 1 M NaCl, and then filters were washed at 65°C using high stringency conditions in 0.2X SSC, 0.1% SDS.

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RESULTS

Expression of Multiple mLIFR Chain Isoforms in Normal Mouse Tissues—Northern analysis of poly(A)^+ RNA from a range of mouse tissues, using a cDNA probe derived from the extracellular region of the mLIFR, revealed a complex and heterogeneous pattern of expression of mRNA transcripts (Fig. 1A and B). Three major transcripts with approximate molecular sizes of 3, 5, and 10 kb were detected in the tissues analyzed. In addition to these three major transcripts, several minor transcripts in liver and placenta were observed. In the liver, two additional transcripts of 2.2 and 1.8 kb were apparent. A further band that was specific to liver had a molecular size of approximately 10.2 kb (seen in Fig. 1A in 16-day fetal liver and more clearly for adult liver in Fig. 4A). In the placenta there were multiple additional transcripts including those at approximately 2, 4.5, 7, and 8.5 kb. Expression of the three major transcripts was preferentially expressed in various tissues. Testis, eye, skeletal muscle, ovary, uterus, thymus, brain, and fat expressed predominantly the 10-kb form. The 3-kb form was more predominant in liver and pancreas. In other tissues the 10- and 3-kb forms were present in approximately equal ratios. Expression of the mLIFR could not be detected in small intestine.
F9, PC13, NIH3T3-L1, and embryonal stem cells. Peritoneal macrophages from mice carrying a DNA insert encoding granulocyte-macrophage colony-stimulating factor as a transgene also expressed mLIFR transcripts. mLIFR transcripts were not detected in FD-CP1 and WEHI 3BD cells. Embryonal stem cells showed a similar expression pattern of transcripts to placenta, although the 5-kb band was absent. All three major forms of transcript were present in M1 cells.

Expression of the mLIFR in fetal tissues was analyzed (Fig. 1D). In contrast to adult liver, in fetal liver the 3-kb band was expressed in lower amounts relative to the 10-kb band. A similar pattern was observed in liver samples from embryonic days 15 and 19 and newborn mice.

Identification of cDNAs Encoding mLIFR α-Chain—In order to obtain cDNA clones encoding the mLIFR a mouse liver library was screened with a human LIF receptor cDNA (24). In addition to many clones encoding soluble mLIFR transcripts, one incomplete clone (mLIFR52.4A) encoding the cytoplasmic domain, the transmembrane domain, and part of the third FNIII domain of the membrane-bound mLIFR was isolated.

This partial clone contained 3′-untranslated sequence that extended 526 bp further than that previously published (14) and contained a poly(A) tail. A canonical poly(A) signal was not present. However a motif, GATAAA, that may function weakly as a poly(A) signal (31) was present upstream of the poly(A) tail. The 3′-untranslated region of this membrane-bound mLIFR mRNA transcript was compared with other known transcripts by searching the GenBank™ data base. Interestingly, it contained a domain with a high degree of homology to the corresponding region in the hLIFR cDNA sequence (3) (Fig. 3A). There was no homology to the 3′-untranslated region of the soluble mLIFR transcript. A 30-bp region on the DNA minus strand in the 3′-untranslated region of this membrane-bound mLIFR transcript was homologous to the negative regulatory region of the mouse proliferating cell nuclear antigen (PCNA) gene (32) (Fig. 3B).

The nucleotide sequence of clones encoding soluble mLIFRs obtained in this study corresponded with the sequence that has been previously reported (3, 14). However, when the sequence of the 3′-untranslated region of the soluble receptor clones was...
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Fig. 2. Nucleotide sequence of 3'-untranslated region of the membrane-bound mLIFR cDNA clone mLIFR52.4A. DNA sequence that has been previously described in Ref. 14 is shown in lowercase, and additional DNA sequence is shown in uppercase. The translational termination codon is indicated in boldface type. The putative poly(A) signal is underlined. The numbers shown on the left relate to the nucleotide sequence reported in Ref. 14.

**Comparison with GenBank Database**

In order to determine the molecular origin of the various forms of the mLIFR we analyzed the genomic structure of this region of the mLIFR locus. Exon-intron boundaries of this section of the mLIFR gene were established by sequence analysis and are shown in Table I. All the exon-intron boundaries conformed to the consensus splice donor and acceptor sequences including the GT/AG motif (34). Intron sizes were estimated by a combination of sequencing and polymerase chain reaction using exon-specific oligonucleotides. The genomic structure of this region of the mLIFR locus, and the predicted splicing pattern is shown in Fig. 5B.

**Isolation of Phage Clones Containing the Murine LIF-R α-Chain Gene**

In order to determine the molecular origin of the various forms of the mLIFR we analyzed the genomic segment of DNA spanning the region around the point of divergence between the soluble and the membrane-bound forms of the mLIFR. We screened a mouse 129SV genomic library using a BglII-HindIII fragment from the extracellular domain (comprising fibronectin III domains 1 and 2) and a BglII-BstII fragment (cytoplasmic domain). Several positive clones were isolated and initially characterized using oligonucleotide probes specific to various regions of the mLIFR. Three clones, AmlIFR10.5, AmlIFR17.1, and AmlIFR18.4, encompassed the region of interest (Fig. 5A). The restriction map of these clones was determined using BamHI, EcoRI, and HindIII (Fig. 5A). Genomic DNA derived from a C57BL/6 × C57BL/10 mouse was digested with the same restriction enzymes and analyzed with a polymerase chain reaction-generated probe that extended from nucleotide 2169 to 3389 of the published mLIFR sequence (14). The map derived from the genomic clones agreed with the restriction pattern in the genomic Southern blot except that a 600-bp HindIII fragment represented in the genomic phage clones was not observed (data not shown). This difference was attributed to an interstrain polymorphism. Several genomic restriction fragments that spanned the relevant regions were subcloned into pBlueScript SKII to allow more detailed analysis of the intron-exon organization of this section of the mLIFR locus. Exon-intron boundaries of this region of the mLIFR gene were established by sequence analysis and are shown in Table I. All the exon-intron boundaries conformed to the consensus splice donor and acceptor sequences including the GT/AG motif (34). Intron sizes were estimated by a combination of sequencing and polymerase chain reaction using exon-specific oligonucleotides. The genomic structure of this region of the mLIFR locus, and the predicted splicing pattern is shown in Fig. 5B.

**Description of Gene Structure from the Soluble and Membrane-bound Divergence Point to the Cytoplasmic Domain of the mLIFR**

In order to generate a transcript that encodes a soluble mouse LIF receptor an alternative splicing event was required. The soluble receptor transcript uses a specific exon so that the nucleotide sequence diverges at nucleotide position

- **Fig. 3.** Nucleotide sequence comparison of the 3'-untranslated region of the membrane-bound mLIFR cDNA 52.4A with the corresponding region of the membrane-bound mLIFR cDNA. The numbers on the left of each DNA sequence refer to the nucleotide sequence for the mLIFR presented in Fig. 2 and for the mLIFR sequence described in Ref. 3 as indicated. Identities are indicated by dashed lines. B, nucleotide sequence comparison of a 30-bp section on the minus strand of the 3'-untranslated region of the membrane-bound mLIFR with the mouse PCNA gene negative regulatory region. The numbers on the left of each DNA sequence refer to the nucleotide sequences of mLIFR cDNA clone 52.4A as indicated. Identities are indicated by dashed lines.
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When a Northern blot containing poly(A) confirmed this as "intronic" sequence from genomic DNA. A comparison of the region immediately downstream of the translational termination codon sequence appeared to read through the poly(A) signal soluble transcripts. This clone, however, contained additional because of a stop codon at the same position observed in the 5' region. A further 526 bp, 474 bp, and 1.4 kb, respectively, 3' of the sequence described in Ref. 14. Thus three sites of polyadenylation were identified within this region of genomic DNA. All of the sequence constituting the 3'-untranslated region of the membrane-bound mLIFR transcript was not extended beyond the HindIII fragment mLIFR319 but that additional sites of polyadenylation remained to be defined. To identify these cDNA sequences corresponding to this region of genomic DNA we screened a mouse liver cDNA library with genomic HindIII fragment mLIFR319, (Fig. 6A), a signal corresponding to the 10-kb mLIFR transcript was detected. This was evident on poly(A)+ mRNA from brain and salivary gland (Fig. 7). There was a significant amount of smearing in the liver and placenta lanes, which is possibly due to the presence of repetitive sequences. When a Northern blot was probed with genomic HindIII restriction fragments mLIFR316 and mLIFR318 (Fig. 6A), no signal was detected (data not shown). This suggested that mLIFR transcripts did not extend beyond the HindIII fragment mLIFR319 but that additional sites of polyadenylation remained to be defined. To identify these cDNA sequences corresponding to this region of genomic DNA we screened a mouse liver cDNA library with genomic HindIII fragment mLIFR319. Of the large number of hybridizing clones, six were analyzed by sequencing and then aligned using the GCG DNA sequence analysis programs Pileup and Wordsearch. Two of the six clones (mLIFR329 and mLIFR330, Fig. 6B), were polyadenylated. Sequence analysis of cDNA clone mLIFR329 revealed a canonical poly(A) signal. Sequence comparisons with genomic DNA showed that this poly(A) signal was located approximately 160 bp upstream of the second HindIII site (Fig. 6A) identified in genomic DNA (Fig. 6B). cDNA clone mLIFR330 was also positioned by sequence comparison with genomic DNA, and its 5' end was located approximately 145 bp downstream of the second genomic BamHI site (Fig. 6A). Thus these cDNA clones utilized polyadenylation signals up to 6 kb downstream of the clones described above. Additional cDNA clones overlapped these two regions (Fig. 6B). Because we have not identified full-length, complete cDNA transcripts it was not possible to definitively assign the different 3'-untranslated re-

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**Fig. 4. Northern analysis of mLIFR transcripts.** The locations of RNA size markers (kb) are shown on the right in each panel. A, Northern blot of poly(A)+ RNA (3 µg/lane) from mouse placenta and liver hybridized with a cDNA probe to the extracellular region of the mLIFR. B, Northern blot of poly(A)+ RNA (3 µg/lane) from mouse placenta and liver hybridized with a cDNA probe specific to the cytoplasmic domain of the membrane-bound mLIFR. C, Northern blot of poly(A)+ RNA (3 µg/lane) from mouse placenta and liver hybridized with a cDNA probe encoding the 3'-untranslated region of the membrane-bound mLIFR. D, Northern blot of poly(A)+ RNA (3 µg/lane) from various mouse tissues and cell lines hybridized with a cDNA probe containing the B2 element in the 3'-untranslated region of the soluble mLIFR.
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Expression of the mLIFR during Gestation—It has been previously reported that the levels of mLBP undergo a dramatic increase during pregnancy (15, 16). We therefore undertook a detailed study of the different forms of the mLIFR in a variety of tissues in pseudopregnant mice and in mice at various stages of pregnancy. Female C57BL/6 × C57BL/10 mice at 6–8 weeks of age and varying stages of pregnancy were sacrificed. Serum was analyzed by a competition radioimmunoassay for levels of soluble mLIFR. Several tissues, including uterus, brain, thymus, salivary gland, heart, lung, muscle, spleen, kidney, liver, and placenta were tested for expression of mLIFR transcripts using a mLIFR fragment that detects all mLIFR transcripts. The blots were also probed with glyceraldehyde-3-phosphate dehydrogenase (to normalize the different samples for RNA loading), analyzed using a PhosphorImager, and quantified using Imagequant version 3.0 software. The only tissues to demonstrate an increase in mLIFR transcripts during pregnancy were liver, placenta, and uterus. Fig. 8A shows the expression of the 3- and 10-kb bands in the liver during various stages of pregnancy. The two highest molecular weight isoforms of the membrane-bound mLIFR were analyzed as a single band of approximately 10 kb. The 3-kb band (encoding the soluble receptor) increased sharply around day 8 of pregnancy, peaked at day 12, and dropped to relatively low levels at day 21. This pattern correlated with the changing levels of soluble mLIFR that were detected in the serum (Fig. 8B). An approximately 20-fold increase in the level of the 3-kb transcript and the levels of soluble mLIFR was observed between normal and day 12 pregnant mice. The levels of the 10-kb transcript were maximal around days 8–12 of pregnancy and then gradually
that were polyadenylated are followed by restriction map in and are further described under "Results." Their positions relative to the genomic map can be obtained by visual alignment with the italics B map.

sequencing of cDNA clones. The relative positions and identification numbers of subcloned genomic fragments are indicated stages of pregnancy (Fig. 8 also analyzed in the mRNA from placentae of mice at varying pseudopregnant mice. Abledifference in mLIFR transcript levels between normal and this transcript was approximately 10-fold. There was no detect-

descended by day 21 (Fig. 8A), and the increase in the levels of this transcript was approximately 10-fold. There was no detect-
able difference in mLIFR transcript levels between normal and pseudopregnant mice.

The levels of the 3-4, 5-, 7-, 8-, and 10-kb bands were also analyzed in the mRNA from placentae of mice at varying stages of pregnancy (Fig. 8C). The placental sample taken at day 8 of pregnancy included the embryos. Nonpregnant uterus is shown for comparison. There was an increase of 6-9-fold in the levels of all the mLIFR isoforms (from day 8 of pregnancy) until day 15 of pregnancy, after which the levels of all the isoforms declined. There was an increase in LIFR mRNA in uterine tissue (Fig. 8D), although it is possible that this was partly due to contamination with placental tissue.

LIFR Expression in LIF Nullizygous Mice—Because a potential role of the soluble mLIFR in mouse serum may be to act as an inhibitor of excess systemic LIF, we were interested to know what the levels of soluble mLIFR would be when no LIF was present. Mice (both male and female) that were nullizygous for the LIF gene were analyzed for expression of the various forms of the mLIFR. The sera from these mice were analyzed by a competition radioimmunounassay for soluble mLIFR (Fig. 9A). There appeared to be a wide range in the levels of soluble mLIFR. The serum levels in the LIF +/- mice seemed to be slightly higher than those of LIF +/- mice and the normal LIF +/- littermate controls. A Northern blot containing a range of tissues derived from a LIF +/- mouse, probed with a cDNA probe encoding the mLIFR, showed that there was no difference in the pattern of levels of mLIFR expression compared with a similar blot derived from a normal littermate (data not shown). The levels of the 3- and 10-kb mLIFR transcripts in the livers of several LIF +/- mice, LIF +/- mice and LIF +/- littermates were analyzed using a Phosphorlimer as described previously (Fig. 9B). Again there appeared to be a range in the levels of mLIFR transcripts, but there was no difference or if anything an increase in levels in LIF +/- mice compared with LIF +/- mice and LIF +/- littermates. The slight elevation in levels of soluble mLIFR in the serum of the LIF nullizygous mice may be explained by the possibility that in normal LIF +/- mice soluble LIFR may be complexed to endogenous

Table I

| Intronic size (kb) | Acceptor sequence | Domain (exon) | Exon size (bp) | Amino acid at 5' boundary | Phase | Donor sequence |
|-------------------|------------------|---------------|---------------|--------------------------|-------|---------------|
| 0.248             | -atccctacag      | Second half FNI1I2 (FNI1I2b) | 102 | Asp | 1 | AA CTG G gtaagtttatt-- |
| 0.972             | -gtgcttcag       | Soluble specific (SS) | 278 | Glu | 1 | AG CCA C gtagaaat-- |
| 1.16              | -tttcctgtag      | First half of FNI1I3 (FNI1I3b) | 167 | Ala | 1 | AA AAC T gtagttcgg-- |
| 1.16              | -tttcctgtag      | Second half of FNI1I3 (FNI1I3b) | 162 | His | 1 | AA AAC T gtagttcgg-- |
| 1.53              | -ttatctcag       | Transmembrane (TM) | 94 | Ser | 1 | A GAA TCG gtagtttgc-- |
| 1.53              | -ttatctcag       | Cytoplasmic box 1 (CB1) | 76 | Trp | 2 | TGC GAG gtaacctgc-- |
| 3.3               | -ttatctcag       | Cytoplasmic | 625 | Gly | 0 | |

*Including soluble exon.
LIF and hence the levels measured by a competitive assay are underestimated, whereas this would not be the case in LIF nullizygous mice.

**DISCUSSION**

The low affinity LIF receptor participates in a number of cytokine receptor signaling systems, including those of LIF, ciliary neurotrophic factor, oncostatin-M, and cardiotoxin-1, which are involved in processes regulating the growth and development of neurons, hemopoietic precursors, hepatocytes, adipocytes, myoblasts, cardiac myocytes, and osteoblasts (36). Membrane-bound receptors for LIF have been found on a wide range of mouse cells (37), and a protein present at relatively high levels in mouse serum was shown to be a truncated form of the LIFR (15). cDNA clones encoding a predicted membrane-bound form and also potentially encoding a soluble form of the mLIFR have now been isolated. In this study we show that there are specific transcripts for both the predicted soluble and membrane-bound mLIFRs, and that they are both expressed in a wide range of mouse tissues and several mouse cell lines. The two transcript forms were differentially expressed in the tissues examined. Furthermore, the expression pattern of membrane-bound mLIFR transcripts was found to be highly complex and variable between different tissues. The presence of LIFR transcripts in liver, brain, bone marrow, muscle, placentas, fat, kidney, and spleen is in agreement with the LIF responsiveness of these tissues (2). LIFR transcripts in tissues such as lung, intestine, and pancreas may indicate further roles for LIFR or an alternative LIFR-associated cytokine involved in the growth and differentiation of these tissues. The LIFR has been shown to play a critical role in many important physiological processes since targeted disruption of the LIF receptor gene in mutant animals resulted in defects in bone formation, placentation, metabolic disorders in the liver, reduced numbers of astrocytes in the brain, and failure to survive beyond the day of birth (20).

Transcripts encoding the membrane-bound mLIFR were heterogeneous, and their expression pattern was complex. In this study, we have shown that the heterogeneity can be accounted for, at least in part, by differences in the lengths of the 3'-...
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Expression of mLIFR and detection of soluble mLIFR protein in LIF nullizygous mice. Detection of mLIFR mRNA expression in mice that were genotypically LIF–/–, +/–, or +/+ was carried out as described in the legend to Fig. 8. A, levels of soluble LIFR protein in the serum of male (M) or female (F) mice that were genotypically LIF–/–, +/–, or +/+ Data represent the mean ± S.D. of at least two experiments. B, quantitative analysis of Northern blot expression of mLIFR transcripts (10 and 3 kb) in the livers from male (M) or female (F) mice that were genotypically LIF–/–, +/–, or +/+ The relative order of the samples analyzed is identical to that described in panel A of this figure.

The fibronectin (FN) type III domain is a 90-amino acid residue unit that was originally noted in fibronectin, where it is repeated 15 times (39). Domains homologous to this repeat have now been shown to be a feature of many other proteins with protein-protein interaction functions including contactin, N-CAM, and fasciculin (40). Members of the hemopoietin family of receptors including the receptors for growth hormone, prolactin, IL-5, and LIF were shown to contain at least one copy of an FNIII domain in their extracellular regions. In receptors such as that for growth hormone the FNIII domain contains residues that constitute the binding site for its cognate ligand. Receptors such as those for G-CSF, gp130, and LIF contain additional FNIII domains that are C-terminal to the “binding” domains (41). FNIII domains are typically flanked by phase 1 introns, which are defined as falling between the first and second base of a codon (42). This phasing arrangement has also been conserved in the FNIII exons of this section of the mLIFR.

The FNIII domains in the region of the mLIFR gene described in this study are encoded by two exons. The cytoplasmic domain of the LIFR has been shown to contain two functional motifs, box 1 and box 2, which are involved in signal transduction (35). These two motifs are conserved among cytokine receptors including those for granulocyte-macrophage colony-stimulating factor (43), G-CSF, and gp130 (44). Box 1 is generally encoded by a single exon located immediately downstream of the transmembrane domain and has a phase 2 intron on its 5’ side and a phase 0 intron on its 3’ side. This arrangement has been conserved in the FNIII exons of this section of the mLIFR. This conservation of intron-exon arrangement supports the notion that the domains in this group of receptors have a common evolutionary origin (43).

LIF is known to have a critical function in the implantation process, as mice nullizygous for the LIF gene are viable but female mice are infertile due to failure of implantation (21). The levels of transcripts encoding the soluble mLIFR in the liver have been previously shown to increase during pregnancy, and they correlate with increasing levels of soluble LIFR present in the serum (14–16). The results from this study confirm these findings and also show that the levels of mRNA transcripts for the membrane-bound mLIFR increase in the liver during pregnancy. Several other tissues were examined for differences in mLIFR mRNA expression during pregnancy, but the only tissues to show an increase were liver, placenta, and uterus.

A large number of soluble receptors for cytokines and growth factors have been described. Although the level of soluble mLIFR in normal mouse serum is 1–2 μg/ml and increases 20–25-fold during pregnancy, the physiological levels of most of

untranslated region of the transcripts. The function of these transcripts is unclear, however, as they are all predicted to give rise to identical proteins. In order to determine the molecular basis governing the expression of the soluble and membrane-bound forms of the mLIFR, we have analyzed the corresponding genomic regions and have shown that the different forms of the mLIFR are derived from the same genetic locus by differential splicing. The membrane-bound form of the receptor is generated by an alternative splicing event and skips an exon that contains sequences specific to the soluble receptor. The 3’-untranslated region of the soluble mLIFR transcript contained part of a mouse B2 repetitive element (33), which provided the polyadenylation signal. There was no similar element present in the immediate 3’-untranslated region of membrane-bound mLIFR cDNAs. There are many examples of B2 elements in the vicinity of expressed genes, and there is evidence that the presence of these elements and similar elements near a polymerase II-transcribed gene can affect its levels of expression (38). The presence of a B2-like element in the mouse but not the hLIFR gene provides an explanation both for the mechanism and high levels of the soluble LIFR transcript in the mouse and the failure to detect these transcripts in the human (3). The 3’-untranslated region of the membrane-bound mLIFR mRNA transcripts was compared with other known transcripts by searching the GenBankTM data base. Interestingly, in the region just after the translational termination codon there was a high degree of homology with the hLIFR cDNA. This may point to an important regulatory role for this region of DNA in the transcription of membrane-bound forms of the LIFR, as it was not homologous to the 3’-untranslated region of the soluble mLIFR. A 30-bp region on the DNA minus strand in the 3’-untranslated region of a membrane-bound mLIFR transcript described in this study was homologous to the negative regulatory region of the mouse PCNA gene (32). The significance of this homology, however, is unclear.
these receptors is generally low (45). Some soluble forms of receptors e.g. the IL-6 receptor (17) are able, when complexed to their respective ligands, to act as agonists on responsive cells, while soluble mLIFR seems to act as an antagonist to LIF function. The transient burst of LIF expression during the implantation process precedes the rise in soluble LIFR levels. We therefore reasoned that the elevated levels of soluble LIFR might be a physiological response to elevated levels of LIF. The maintenance of higher levels of soluble LIFR mRNA transcript and protein in mutant mice lacking a LIF gene indicates that the LIFR is not regulated directly in response to the amount of LIF present in the serum or other tissues. Rather, it appears that the soluble mLIFR is constitutively expressed even in LIF nullizygous cells and is regulated at the transcriptional level by additional signaling processes associated with pregnancy.

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