Molecular Biology of the Insulin-Like Growth Factors

Relevance to Nervous System Function

Jessie English Hepler[1] and Pauline Kay Lund*[*,1]

[1]Curriculum in Neurobiology and [2]Departments of Physiology and Pediatrics, University of North Carolina at Chapel Hill, NC 27599

Contents

Index Entries: Molecular biology; insulin-like growth factors; nervous system; messenger RNA.

*Author to whom all correspondence and reprint requests should be addressed.

Molecular Neurobiology Ed.: N. Bazan

History

Discovery and Structural Characterization of the Insulin-Like Growth Factors (IGFs)

The discovery of the insulin-like growth factors arose from independent and parallel analyses of three different factors, each responsible for different biological activities:

1. The somatomedins, factors that mediate or influence the growth-promoting activities of growth hormone on cartilage (Salmon and Daughaday, 1957; Daughaday et al., 1972)
2. Nonsuppressible insulin-like activity (NSILA), factors that stimulate glucose uptake in adipose tissue even in the presence of antiinsulin antisera (Martin et al., 1958; Renold et al., 1960; Froesch et al., 1963) and
3. Multiplication stimulating activity (MSA), factors in serum (Pierson and Temin, 1972) or conditioned media (Dulak and Temin, 1973) that stimulate the proliferation of cells in culture.

Purification of somatomedins from human serum led to the identification of a neutral fraction termed somatomedin-A (Sm-A) (Hall, 1972; Uthne,1973) and a basic peptide termed somatomedin-C (Sm-C) (Van Wyk et al., 1974). An acidic fraction, originally termed somatomedin-B (Fryklund et al., 1976), later proved not to correspond to a somatomedin.

Analyses of NSILAs in human serum resulted in the first elucidation of the primary structures of two peptides that were termed human IGF-I (Rinderknecht and Humbel, 1978a) and human IGF-II (Rinderknecht and Humbel, 1978b). Human IGF-I and IGF-II show substantial structural similarity with each other and with proinsulin (Fig. 1). Somatomedin-C was subsequently shown to be identical in structure with IGF-I (Klapper et al., 1983), and somatomedin-A was shown to represent a mixture of IGF-II and IGF-I (Spencer et al., 1983).

Purification of MSAs in serum-free medium conditioned by the Buffalo rat liver cell line BRL-3A (Dulak and Temin, 1973) led to its structural characterization as the rat counterpart of human IGF-II (Marquardt et al., 1981; Nissley et al., 1983). The different terminologies ascribed to the IGFs as a result of their origins in diverse disciplines created some confusing nomenclature in the literature. Recently, workers in the field proposed that the IGF-I and IGF-II terminology be adopted (Daughaday et al., 1989).

Assays of IGF-I and IGF-II Biosynthesis

A number of different radioimmunoassays (RIAs) have been developed for the quantification of IGF-I and IGF-II. A comprehensive review of the status of IGF-I and IGF-II RIAs and of the immunoreactive forms detected in serum, body fluids, tissue extracts, cells in culture, and conditioned medium appeared in the literature recently (Daughaday and Rotwein, 1989) and will not be duplicated here. A number of problems in the analysis of IGF biosynthesis using immunological approaches are significant, however, in highlighting the utility of a molecular approach to the analyses of the IGFs in the nervous system. Success in development of high-titer, high-affinity antibodies that exclusively measure IGF-I or IGF-II and that are useful across mammalian species has been limited. This is especially so for IGF-II (Moses et al., 1980; Zapf et al., 1981; Enberg and Hall, 1984). In addition, high affinity binding proteins for the IGFs are present in serum and tissue extracts (Baxter and Martin, 1989). During RIA of the IGFs, therefore, a variety of preassay manipulations are necessary to remove interference from binding proteins. These can raise problems in comparing data obtained on samples subjected to different manipulations. In vivo, the plasma concentrations of the IGFs are higher relative to tissue concentrations, which creates difficulties in analysis of IGF synthesis in vivo using RIA (D'Ercole et al., 1984). The high plasma concentrations of the IGFs have been attributed to prolongation of the

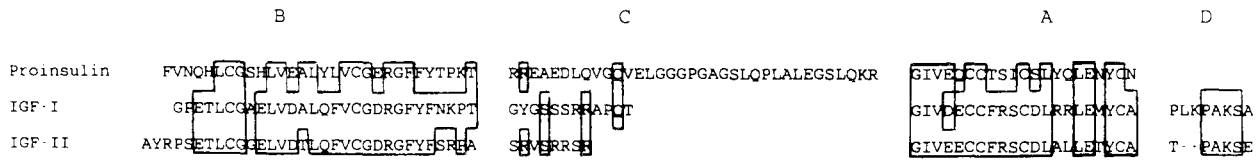

Fig. 1. Comparison of the amino acid sequences of human proinsulin, IGF-I, and IGF-II. The amino acids, in single letter code, are aligned to show regions of homology. Regions of homology are boxed. The peptide domains are identified by the letter above each group of amino adds.

plasma half-lives of the IGFs by their association with plasma-binding proteins, and to the limited storage and rapid secretion of IGFs in cells that synthesize them.

Because of the difficulties in analysis of the IGF immunoreactivity in tissue or cell extracts, most of our early information about regulation and sites of synthesis of the IGFs was based on plasma concentrations in vivo or IGF immunoreactivity in media from cultured tissue explants or cells. Liver was considered the primary site of IGF biosynthesis and the major, if not sole source of circulating IGFs in vivo. Increasing evidence pointed to nonhepatic synthesis of the IGFs and, therefore, the possibility of paracrine as well as endocrine actions of the IGFs (D'Ercole et al. 1984). These observations provided the impetus for development of sensitive methods to assay IGF synthesis in nonhepatic tissues, such as the nervous system.

Introduction

The advent of recombinant DNA technology provided an alternate strategy for analyses of IGF biosynthesis that could overcome the difficulties in analysis of IGF biosynthesis using immunological approaches. Complementary DNAs (cDNAs) encoding human insulin-like growth factors I and 11 (IGF-I and IGF-II) were isolated and sequenced in the early 1980s (Jansen et al., 1983; Bell et al., 1984; Dull et al., 1984). This and subsequent characterizations of IGF genes and cDNAs from other species have led to advances in our understanding of the molecular mechanisms that underlie IGF biosynthesis. The complexity of IGF-I and IGF-II gene and messenger RNA (mRNA) structure and expression is much greater than previously anticipated. Some basic structural information and information about the regulation and functional significance of a complex array of mRNAs and products that are derived from the IGF-I and IGF-II genes are still lacking. This review will describe in detail our current level of understanding of the pathway of IGF-I and IGF-II biosynthesis, and what is known about the regulation and sites of IGF synthesis in the nervous system and in liver, a major source of circulating IGFs for possible endocrine actions on the nervous system.

Cloning of IGF-I and IGF-II cDNAs also has led to widespread availability of recombinant IGF-I and IGF-II for biological testing and increased understanding of the biological actions of IGF-I and IGF-II in the nervous system. Much of the information about IGFs in the nervous system is based on studies in vitro, and the precise in vivo roles of the IGFs are still unclear. Available information about the biological actions of the IGFs in the nervous system will therefore be reviewed here with consideration to possible future directions that may define their precise roles in the nervous system. IGF receptors and binding proteins will be considered only briefly. For a more comprehensive appraisal of IGF receptors and bindings proteins, readers are referred to recent reviews (Baxter and Martin, 1989; Adamo et al., 1989a; Czech, 1989; Clemmons, 1989; Sara and Hall, 1990; Humbel, 1990).

Molecular Biology of the IGFs

IGF-I Precursors Predicted from Sequencing of cDNAs

A similar IGF-I precursor has been described in human, cow, pig, sheep, rat, mouse, chicken, xenopus, and salmon based on sequencing of cDNAs (Jansen, et al., 1983; Honegger and Humbel, 1986; Rotwein et al., 1986; Tavaklol et al., 1988; Francis et al., 1989: Casella et al., 1987; Roberts et al., 1987a; Murphy et al., 1987a; Bell et al., 1986; Kajimoto and Rotwein, 1989, 1990; Cao et al., 1989). In each of these species, the 70 amino acid IGF-I sequence (B,C,A, and D domains) is flanked by precursor sequences at both the amino terminus (putative signal peptide) and carboxyl terminus (E domain or trailer peptide) (Fig. 2). The structure of the IGF-I precursor is highly conserved across species. The highest degree of sequence conservation is within the sequence for mature IGF-I (Fig. 2). There is also a high degree of sequence conservation of the carboxyl-terminal E domains (Fig. 2). IGF-I cDNAs encoding two distinct E domain types, termed IA and IB, have been demonstrated in human, rat, and mouse, and appear to derive from alternate splicing of the primary IGF-I gene transcript (Fig. 2; *see below;* Rotwein et al.,1986; Shimatsu and Rotwein 1987; Bell et al., 1986). The characterized salmon E domain shows structural similarity to both IA- and IB-type E domains of mammals (Fig. 2).

At present, the biological relevance of the E domains on IGF-I precursors is not clear, but their conservation during evolution from lower vertebrates to mammals provides support for a biological role. To date, the pathway of post-translational processing and secretion of IGF-I precursors has not been analyzed in detail. Human fibroblasts have, however, been shown to secrete an IGF-I precursor with an amino acid composition similar to that predicted for IGF-IB (Clemmons and Shaw, 1986; Clemmons, 1989). Antisera developed to recognize the IA-type E domain detect the pro-IGF-IA peptide in plasma (Powell et al., 1987). Based on these observations, it is tempting to speculate that the E domains could be important in IGF-I action at a point downstream from secretion, possibly in targeting of IGF-I to its sites of action. Support for this contention derives from observations in rat that IGF-I mRNAs encoding IB-type E domains are expressed primarily in liver (Hoyt et al., 1988, Lowe et al., 1988), which appears to be a primary source of circulating IGF-I. In contrast, rat IGF-I mRNAs encoding IA-type E domains are expressed not only in liver, but also in nonhepatic tissues, including brain. Thus, IA-type IGF-I precursors may give rise to IGF-I that acts in a local, paracrine manner. Further analysis of biosynthesis, processing, secretion, and actions of different IGF-I precursor types will clearly be required to define the roles of the precursor E domain peptides.

The amino terminal precursor sequences of IGF-I precursors also show some homology across species, although to a much lesser extent than other regions of the precursor (Fig. 2). The hydrophobicity of the amino terminal precursor peptides indicates that they function as signal peptides. The precise location of the initiator methionine has yet to be clearly defined in any species. The human IGF-I cDNA sequence predicts potential initiator methionines at -48, -25, and -22 relative to the IGF-I sequence. The methionine at -48 is conserved across human, rat, chicken, and xenopus (Fig. 2). In vitro translation studies using in vitro synthesized RNAs, transcribed from human, chicken, and xenopus IGF-I cDNAs, indicate that the -48 methionine can function as an initiator and that the 48 amino acid amino terminal precursor is cotranslationally removed by microsomal membranes (Rotwein et al., 1987; Kajimoto and Rotwein 1989,1990). Together these data support the notion that the 48 amino acid amino terminal precursor is a signal peptide, even though it is rather long compared with other signal peptides. Methionines at -25 and -22 are also con-

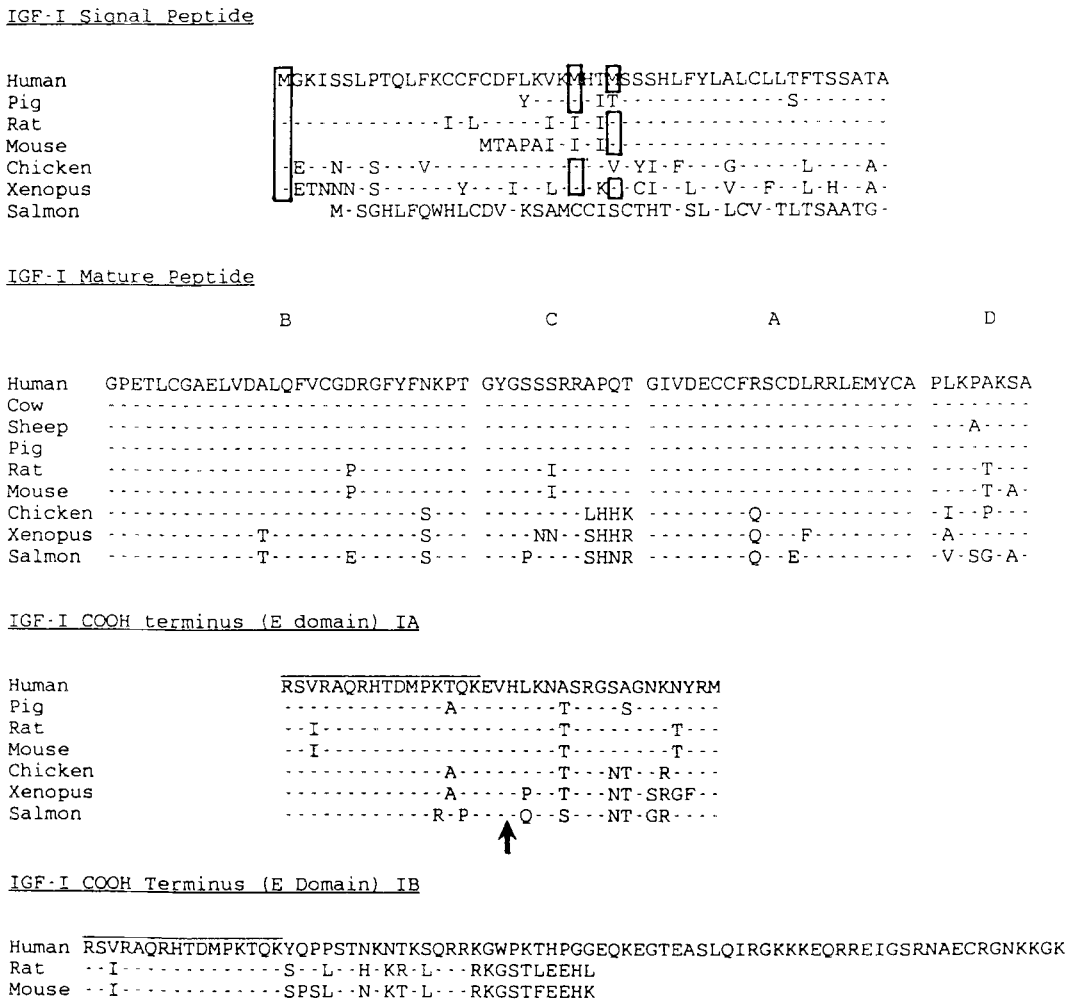

Fig. 2. Comparison of the amino acid sequences of IGF-I precursors in several species. Dashes indicate identical amino acids compared with the human IGF-I precursor. Methionine residues in the signal peptides that are conserved across species and represent potential intitiators are boxed. The first 16 amino acids shown for the COOH terminal E domain IA and IB (overlined) are residues common to IA and IB E domains. An arrow underneath the salmon IA E domain indicates the point where 27 amino acids were deleted to maximize homology with the IA E domains of other species. (Note that there is a minor degree of homology of this omitted 27 residue sequence with mammalian IB E domains) (not shown).

served across a number of species and represent candidate initiators (Fig. 2). Precise assignment of initiator methionines for IGF-I precursors, however, awaits analyses in whole cell systems with full-length IGF-I mRNAs. Precise assignment of the initiator methionines may have significance for a number of reasons. Little is known about co- and posttranslational processing of IGF-I precursors. In mouse and rat, there are "variant" IGF-I mRNAs that differ in 5' sequence and possibly in the sequence of amino terminal precursor peptides *(see below)*. Since these variant mRNAs are expressed in a cell and tissue specific fashion (Hoyt et al. 1988, Lowe et al., 1987), the amino terminal precursor peptide may play a role in IGF-I precursor processing or secretion. Recent findings that CUG codons can function as initiators when basic fibroblast growth factor (bFGF) mRNAs are translated in whole cell systems (Prats et al., 1989) also raise

the possibility that growth factors may utilize unusual biosynthetic mechanisms.

IGF-I Gene Structure and mRNA Complexity

Rat IGF-I Genes and mRNAs

Despite much progress, our information about rat IGF-I gene structure, mRNA precursor processing, and therefore, gene regulation is still incomplete. Six exons spanning more than 80 kb of genomic DNA have been mapped for the rat IGF-I gene (Fig. 3; Shimatsu and Rotwein, 1987; Bucci et al., 1989; Hoyt et al., manuscript in preparation). Signal peptide, IGF-I, and a portion of E domain coding sequence common to all rat IGF-I mRNAs are represented on two exons (exons 2 and 3; Fig. 3). Alternate inclusion or exclusion of exon 4, a 52-base mini-exon, leads to the formation of IGF-I mRNAs encoding the distinct IA- and IB-type E domains (Figs. 3 and 4) Rotwein 1986; Roberts et al., 1987a; Shimatsu and Rotwein, 1987; Bell et al., 1986). The longest and most 3' exon (exon 5) specifies 3'UT common to all rat IGF-I mRNA types. This exon contains multiple polyadenylation signals that result in rat IGF-I mRNAs that differ in size (Fig. 3; Hoyt et al., manuscript in preparation).

In liver, the most abundant source of IGF-I, IGF-I mRNAs exist as two predominant forms of estimated size, 7.5–7.0 kb and 1.2–0.9 kb, as well as minor forms intermediate in size (Lund et al., 1986; Lowe et al., 1987, 1988; Hoyt et al., 1988; Fig. 5). In contrast, in brain and a number of other nonhepatic tissues, the 7.5–7.0 mRNA predominates with low or barely detectable levels of the smaller forms (Fig. 5). The 7.5–7.0 kb IGF-I mRNAs differ in size from the 1.2–0.9 kb forms because of unusually long 3'UTs of greater than 6 kb (Lund et al., 1989) derived from use of an alternate polyadenylation site within exon 5 (Hoyt et al., manuscript in preparation). Recent studies revealed that the 7.5–7.0 kb mRNAs have shorter half-lives than the smaller forms in vitro and in vivo (Hepler et al., 1990). Furthermore, the long 3'UT of these large mRNAs contains structural features implicated in mRNA destabilization such as sequences with the potential to form secondary structure as well as multiple AU rich sequences (Hoyt et al., manuscript in preparation). These findings suggest posttranscriptional regulation of IGF-I mRNA synthesis at the level of mRNA stability, and this may be especially relevant for regulation of IGF-I synthesis in brain and nonhepatic tissues where the large IGF-I mRNAs predominate (Lund et al., 1986).

The structure of the 5' end of the rat IGF-I gene and mRNAs and the number of 5' genomic exons are still uncertain. IGF-I mRNA types expressed in liver, brain, and most, if not all, nonhepatic tissues contain 5' sequences (5'UT and possibly portions of signal peptide coding sequence) specified by the most 5' of the rat IGF-I exons characterized to date (exon 1; Figs. 3 and 4; Shimatsu and Rotwein, 1987). IGF-I mRNAs containing exon 1 sequences have been variously termed Class C (Lowe et al., 1987; Roberts et al., 1987b) and type 1 (Hoyt et al., 1988; Fig. 4). At present, the 5' extent of these mRNAs has not been conclusively mapped within the rat IGF-I gene, and it is not known whether their 5' sequences are represented in entirety within exon 1 or are specified by more 5' exons. The latter possibility is supported by the absence of consensus sequences associated with promoters and transcription start sites, such as a TATA box and a CAAT box in proximity to known exon 1 sequences. This is not unprecedented, however, since the IGF-II gene (de Pagter-Holthuizen et al., 1987) contains a promoter that lacks such consensus sequences.

Roberts et al. (1987b) described a rat IGF-I cDNA that contained 5' sequences distinct from those in exon 1 (Figs. 3 and 4). Corresponding mRNAs have been variously termed the Class B (Lowe et al., 1987) or the type 2 IGF-I mRNAs (Hoyt et al., 1988), and are expressed primarily if not exclusively in liver and only postnatally (Hoyt et al., 1988; Lowe et al., 1987). Tissue and development specific mechanisms therefore

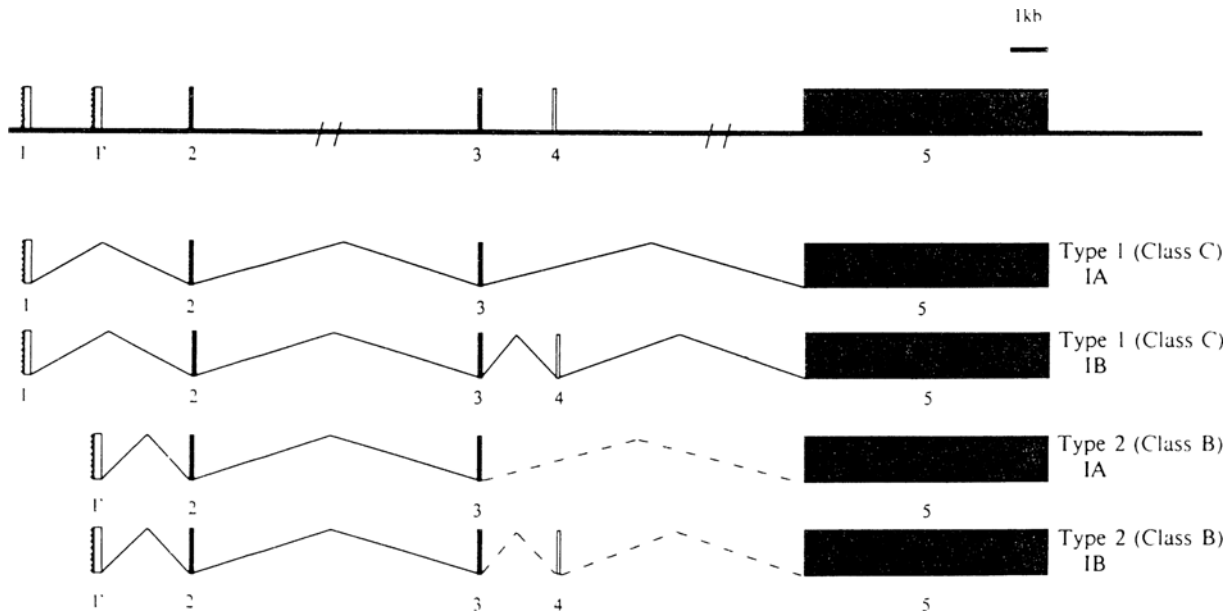

Fig. 3. Diagram of the rat IGF-I gene (top). Black boxes indicate the exons common to all characterized human IGF-I cDNAs. Open boxes indicate exons that are alternately spliced. Jagged edges indicate that the complete exon has not been mapped. Middle and bottom panels illustrate the alternate splicing of exons 1 and 1' resulting in the type 1 (Class C) and type 2 (Class B) 5' sequences, respectively. The inclusion or exclusion of exon 4 results in the IA and IB-type E domain coding sequences. The dashed lines in the bottom two panels reflect uncertainty about whether both E domain types can be linked to the type 2 (Class B) 5' sequences. (//) indicates introns that are not drawn to scale because of their large size. Not shown are multiple polyadenylation sites found in exon 5 (Shimatsu and Rotwein, 1987; Hoyt et al., manuscript in preparation). Note that the exon for Class A 5' sequences is not shown because it has not yet been mapped in the IGF-I gene.

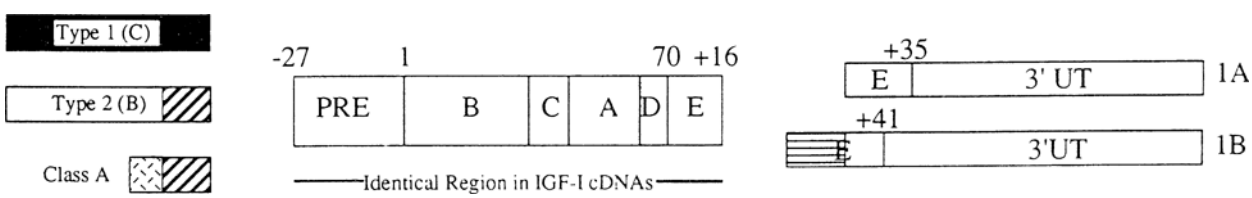

Fig. 4. Schematic of rat IGF-I mRNAs. The middle bar (Pre, B, C, A, D, E) represents an identical sequence reported in all rat IGF-I cDNAs. Bars labeled Type 1 (C), Type 2 (B), and Class A represent alternate 5' sequences. The diagonal striped bar indicates the identical region of Class A and Type 2 (B) cDNAs. The horizontal striped bar represents the 52 base sequence included in IGF-IB type cDNAs that leads to a shift in translation reading frame and stop codon compared with IGF-IA type cDNAs. Bars 3' to the E domain sequence labeled 3'UT illustrates the identical 3'UT sequences in both IA and IB type cDNAs that derive from exon 5.

appear to regulate the expression of the type 2/ Class B mRNAs. Genomic sequences corresponding to these type 2 (Class B) 5' sequences have recently been mapped (exon 1' Fig. 3; Bucci et al., 1989), but the 5' boundary of this exon has not been defined.

A rat IGF-I cDNA with third 5' sequence type (termed Class A) was described by Roberts et al. (1987b). This cDNA contains a short amount of sequence that is identical with the type 2 or Class B sequence and then diverges (Fig. 4). The divergent sequence is an invert repeat of a portion of the IGF-I 3'UT (Roberts et al., 1987b). This feature can arise as an artifact during reverse transcription of mRNA into cDNA. Since the point of divergence of the Class A cDNA from the type

Fig. 5. Autoradiograms of northern blots of IGF mRNAs in fetal and adult rat liver and brain. *Upper panel,* 10 μg of Poly A+ RNA from fetal (F) or adult (A) rat liver hybridized with a rat IGF-I cDNA (*right*) and a human IGF-II cDNA (*left*). *Bottom panel,* 20 μg of Poly A+ RNAs from fetal (F) and adult (A) rat brain RNA hybridized with a rat IGF-I cDNA (*right*) and a human IGF-II cDNA (*left*).

2/Class B mRNA is not a consensus intron/exon, boundary the possibility that the Class A cDNA is a cloning artifact must be considered. On the other hand, using RNase protection assays, complete protection of a Class A antisense RNA probe is observed, and differential regulation of this mRNA subtype has been reported (Lowe et al., 1987). Understanding the significance of the Class A cDNAs and mRNAs awaits confirmation of genomic coding sequences, and transcriptional and posttranscriptional mechanisms that account for them.

Until the 5' ends of the various IGF-I mRNA subtypes are defined, it will not be clear whether there are as yet unknown exons at the 5' end of the IGF-I gene, and whether the different IGF-I mRNA types derive from use of different promoters and transcription start sites, or use of a single transcription start site and differential exon splicing. Complete characterization of the 5' end of the rat IGF-I gene also is necessary before promoters and regulatory elements that control IGF-I synthesis can be defined.

Human IGF-I Gene and mRNAs

Compared with rat, much less is known about the human IGF-I gene and mRNAs (Rotwein et al., 1986). The human IGF-I gene spans more than 90 kb and includes at least five exons (Fig. 6). As in the rat, exons 2 and 3 encode the signal peptide, IGF-I, and a part of the E domain. IGF-I cDNAs and mRNAs encoding two different E domains (1A and 1B) exist in humans as in rodents. In humans, the two mRNA types arise from alternate splicing of two entirely different 3' exons, exon 4 or exon 5, rather than inclusion or exclusion of a mini-exon as in rat (Fig. 6; Rotwein, 1986). The relative expression of the human IGF-I mRNAs with IA- versus IB-type coding sequence has not been extensively studied in different tissues.

Currently, it is not known whether humans express IGF-I mRNAs with different 5' sequences. Only one 5' sequence type has been found in characterized human cDNAs (Jansen et al., 1983; Rotwein, 1986).

Neither the 5' nor 3' ends of the human IGF-I genes and mRNAs have been completely characterized. The largest human IGF-I mRNA is 8.0 kb. Characterized human IGF genomic exons

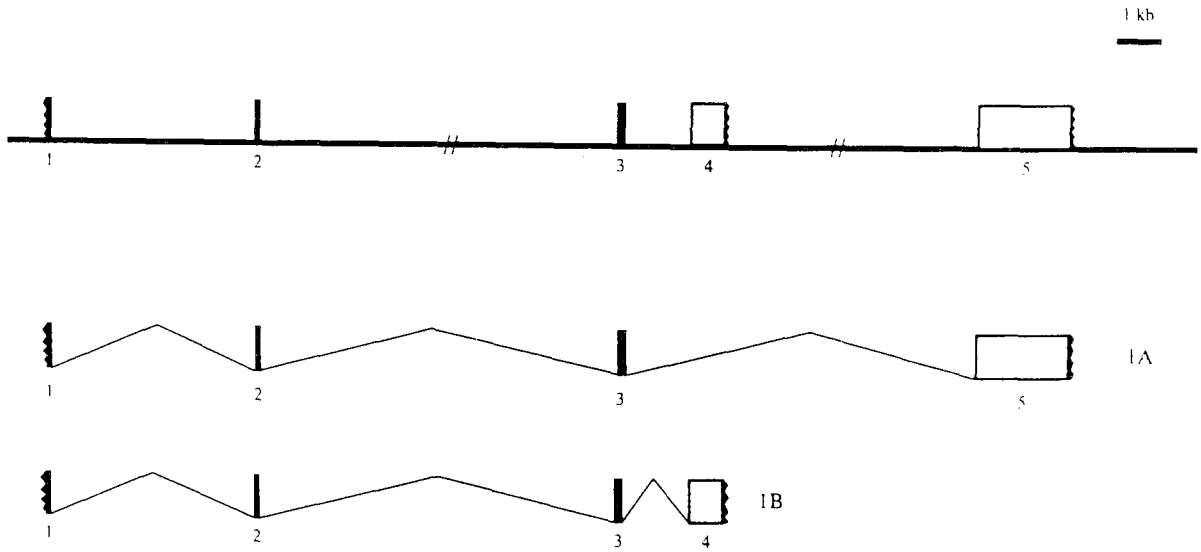

Fig. 6. Schematic of the human IGF-I gene (top). Black boxes indicate the exons common to all characterized IGF-I cDNAs. Open boxes indicate exons that are alternately spliced. Jagged edges indicate that the complete exon has not been mapped. (11) indicates introns that are not drawn to scale because of their large size. Middle and bottom panels illustrate that alternate splicing of either exon 5 or exon 4 results in an IGF-I mRNAs With IA or IB-type E domain coding sequences, respectively.

account for less than 2 kb of this sequence. By analogy with rat, it seems likely that a long 3'exon specifying the 3'UT accounts for most of the unknown sequence within the human IGF-I gene.

Human IGF-I mRNAs range in size from 8.0–0.7 kb, and there are differences in the relative abundance of different size classes in different tissues (Han et al., 1988b; Rotwein, 1986). To date, however, there is very little information about the mechanism that gives rise to these different mRNA size classes.

IGF-II Genes, mRNAs, and Precursors

IGF-II cDNAs and Precursor

IGF-II cDNAs have been characterized in human, rat, and mouse (Bell et al., 1984; Dull et al., 1984; Whitfield et al., 1984; Stempien et al., 1986). In each of these species, the cDNAs encode a precursor peptide that consists of a 24 amino acid signal peptide, IGF-II (BCA and D domain) and an 89 amino acid carboxyl terminal precursor (E domain or trailer peptide). There is a high degree of sequence homology across the IGF-II precursors from different species (Fig. 7). The highest sequence homology is in the IGF-II sequence, but significant homology exists in both the signal peptide and the E domain sequences (Fig. 7). In humans, a "variant" IGF-II cDNA has been described that has three additional amino acids in the B domain resulting from the use of an alternate splice site (Jansen et al., 1985; Fig. 7).

IGF-II Genes

Our understanding of IGF-II and mRNA structure in human and rat is much more complete than for IGF-I. As with IGF-I, a single rat or human IGF-II gene gives rise to a complex family of IGF-II mRNAs. Utilization of different promoters, alternate exon splicing, and different polyadenylation sites account for these mRNAs.

The rat IGF-II gene contains at least six exons with three 5' exons (exons 1,2, and 3), each encoding three different 5'UTs and exons 4,5, and 6 encoding the IGF-II precursor and 3'UT (Frunzio et al., 1986; Soares et al., 1986; Ueno et al., 1987, 1989). Splicing of the alternate 5'UTs to

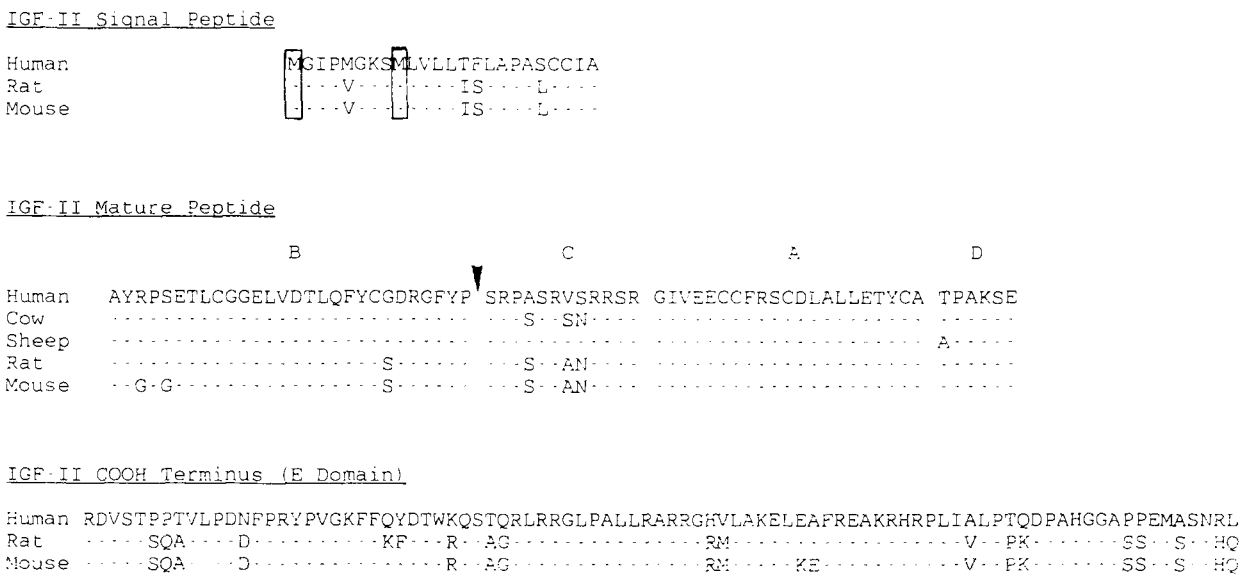

Fig. 7. Comparison of amino acid sequences of IGF-II precursors from several species. Dashes indicate identical amino acids compared with the human precursor. Human, rat, and mouse precursor sequences are based on characterization of cDNAs. For cow and sheep, only the IGF-II sequence is known and was derived from analyses of the proteins. In the signal, peptide methionine residues, conserved across species, represent potential initiators. In the human IGF-II sequence, the arrow indicates the replacement of the serine residue with four amino acids (RLPG) in a variant human IGF-II precursor that was predicted based on analyses of a cDNA and also isolated from serum.

exons 4,5, and 6 leads to three different IGF-II mRNA types. The 5' most promoter in rat is different from the 5' most promoter in humans, whereas the two other promoters show sequence homology across human and rat. All three of the of the rat promoters are coordinately regulated during development (Table 1; Graham et al., 1986; Gray et al., 1987; Ueno et al., 1988). There are alternate polyadenylation sites within exon 6 of the rat IGF-I gene that gives rise to IGF-II mRNAs of different size (Figs. 8 and 9; Chiariotti et al., 1988; Ueno et al., 1989).

The human IGF-II gene consist of nine exons and is similar in structure to the rat IGF-II gene (Fig. 10; de Pagter-Holthuizen et al., 1987,1988; Gray et al., 1987; Holthuizen et al., in press). The first six human exons specify 5' untranslated sequence, and the last three are coding exons for the human IGF-II precursors. Exons 1,2, and 3 are spliced together to form one 5'UT, whereas exons 4, 5, and 6 each encode different 5'UTs (Figs. 10 and 11). Exons 1,4, 5, and 6 each have promoters that are directly adjacent, providing the possibility for independent regulation of the corresponding mRNAs. The promoters are active in tissue and developmentally specific manner, with the 5' most promoter being active only in adult liver, and the other promoters being expressed in fetal liver and nonhepatic tissues (Table 1; de Pagter-Holthuizen et al., 1988; Holthuizen et al., in press). Exons encoding the different 5'UTs are each spliced to the last three exons (exons 7, 8, and 9) to produce the mature IGF-II mRNAs. Exon 9 encodes a portion of the IGF-II precursor E domain and a long 3'UT. Within this long 3'UT there are different polyadenylation sites that give rise to IGF-II mRNAs of different size.

Multiple size classes of human and rat IGF-II mRNA detected in northern blots can now be accounted for based on alternate splicing of 5' exons, as well as utilization of alternate polyadenylation sites (Table 1). In addition, a 1.8-kb rat IGF-II mRNA and a 2-kb human IGF-II mRNA are detected on northern blots using 3'IGF-II probes, but not probes to the IGF-II coding

Table 1
IGF-II mRNAs

Human IGF-II mRNAs		
Promoter	mRNA size	Tissue/Developmental Expression
P1	5.3 kb	adult liver
P2	5.0 kb	fetal liver
P3	6.0 and 2.2 kb	fetal and adult nonhapatic tissues
P4	4.8 kb	fetal and adult nonhapatic tissues
Rat IGF-II mRNAs		
Promoter	mRNA size	Tissue/Developmental Expression
P1	3.8 kb	fetal and neonatal tissues, adult brain
P2	4.7 and 2.2 kb	fetal and neonatal tissues, adult brain
P3	3.9 and 1.2 kb	fetal and neonatal tissues, adult brain
P6	1.8 kb	fetal and neonatal tissues, adult brain (mRNA does not encode IGF-II)

Table to show sizes of IGF-II mRNAs derived from different promoters in human and rats. It should be noted that slightly different estimated sizes are reported by different groups in the field. Sizes of human IGF-II mRNAs are from Sussenbach, 1989 and Holthuizen et al., in press. Sizes of rat IGF-II mRNAs derived from usage of P2 and P3 promoters are data from our laboratory, and the size of the mRNA derived from the P1 and P6 promoter are from Ueno et al., 1988 and Matsuguchi et al., 1989 respectively.

sequence. These mRNAs are known to derive from transcription of a portion of the 3' exon that specifies 3'UT in IGF-II mRNAs (Figs. 8 and 10; de Pagter-Holthuizen et al., 1988; Chiariotti, et al., 1988). Thus, the 3' end of the IGF-II gene appears to contain an alternate transcription unit. The mRNAs derived from use of this transcription unit appear, however, to be coregulated with IGF-II mRNAs and have a long open reading frame encoding a protein distinct from the IGF-II precursor. It is not yet established whether this mRNA is translated in vivo to generate protein product and whether these putative protein products are biologically active.

Regulation of IGF mRNAs

Studies performed primarily in rat demonstrate that regulation of IGF mRNAs and, therefore, IGF synthesis is multifactorial. As discussed in the preceding sections, subtypes of the complex family of IGF-I and IGF-II mRNAs are expressed in a tissue and cell specific manner. This suggests that tissue and cell specific mechanisms may regulate the promoter, exon splicing mechanisms, and use of multiple polyadenylation sites that give rise to the mRNA subtypes, although the precise mechanisms are still to be defined.

There are also alterations in IGF mRNA abundance during development, in response to different hormonal stimuli and in response to altered nutritional status. In most cases, such changes have not yet been linked to specific functional consequences, but provide a starting point for future studies aimed at understanding the function of the IGFs. Since liver probably serves as the major source of circulating IGFs for possible endocrine actions in the nervous system, regulation of liver IGF synthesis will be reviewed here together with information on regulation of IGF synthesis in the nervous system.

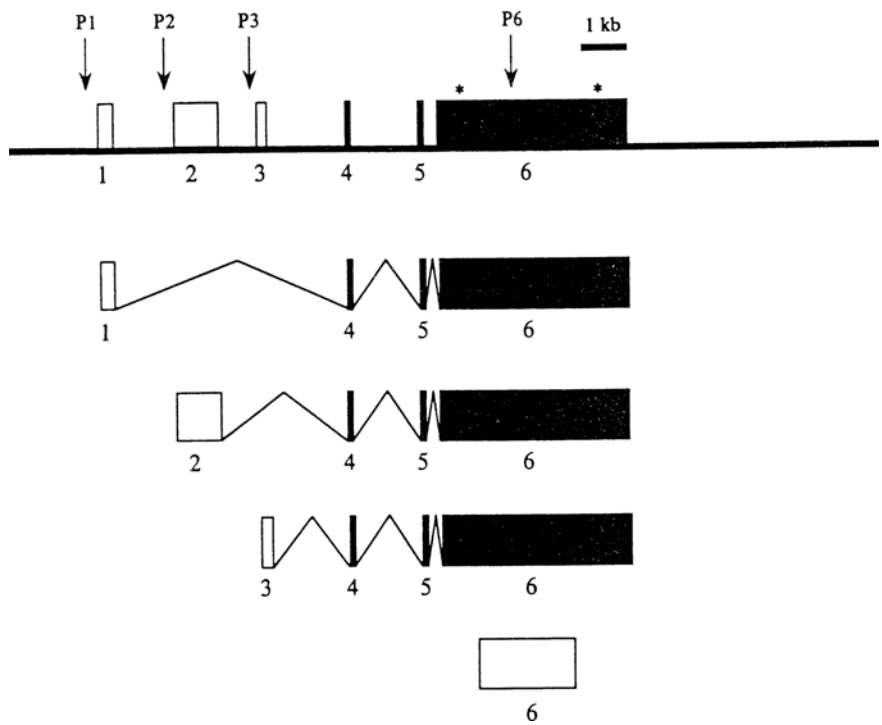

Fig. 8. Schematic of the rat IGF-II gene. Black boxes indicate exons common to all rat IGF-II mRNAs. Open boxes indicate exons that are alternately spliced. P1, P2, and P3 indicate promoters associated with these exons. Asterisks above exon 6 represent polyadenylation sites mapped by nuclease protection. The first three panels below the gene indicate alternate exon splicing mechanisms that result in IGF-II mRNAs with different 5' ends. The lower most panel indicates an alternate transcription unit in exon 6 that results in an mRNA without IGF-II coding sequence. P6 denotes a promoter associated with this alternate transcription unit (Matsuguchi et al., 1989). Note that a variety of numbering systems have been used in the literature to describe IGF-II exons. A 5'-3' numbering system has been adopted here.

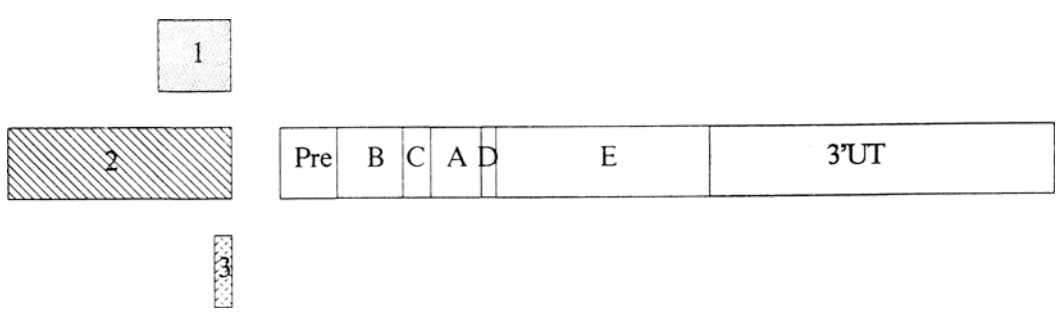

Fig. 9. Schematic of rat IGF-II mRNAs. Bars labeled 1, 2, and 3 represent alternate 5' sequences. The middle bar (Pre, B, C, A, D, E) represents an identical sequence reported in all rat IGF-II cDNAs. Shaded bar represents the 3'UT shared by all rat IGF-II cDNAs, although alternate polyadenylation signals result in varying amounts of 3'UT

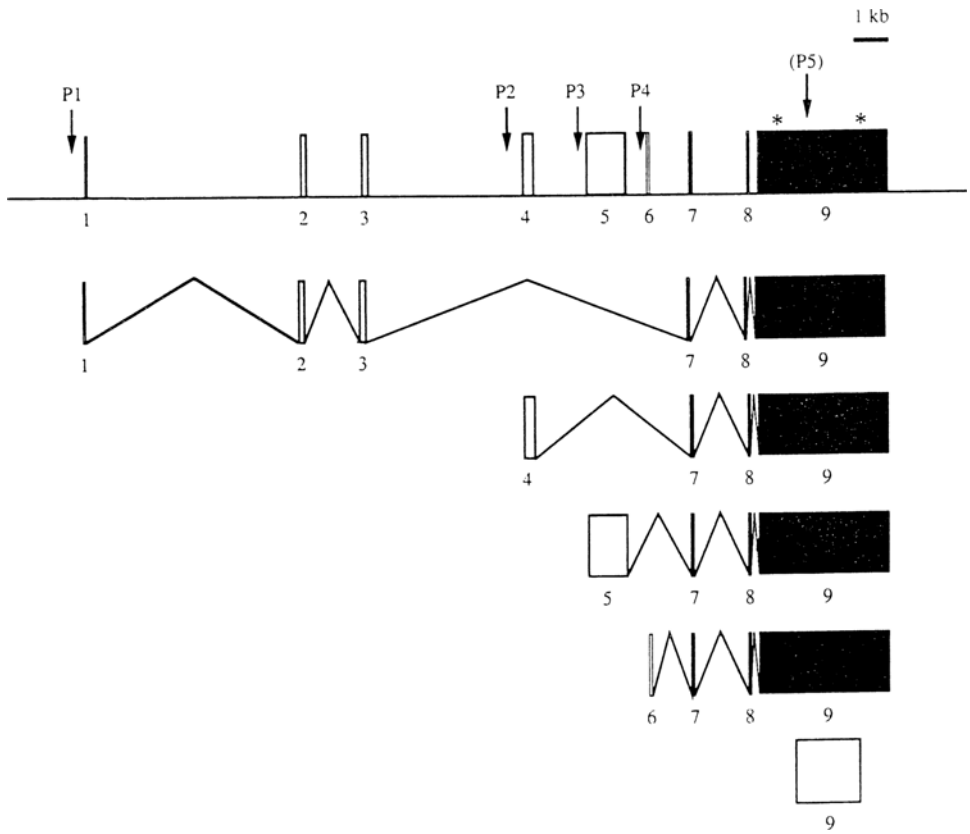

Fig. 10. Schematic of the human IGF-II gene. Black boxes indicate exons that are found in all characterized human IGF-II cDNAs. Asterisks above exon 9 represent polyadenylation sites predicted by nuclease mapping and that result in IGF-II mRNAs of different size. Open boxes indicate exons that are alternately spliced. P1–P4 are promoters associated with alternately spliced 5' exons. (P5) has yet to be defified but may constitute a promoter that regulates the alternate transcription unit in exon 9. The first three panels below the gene indicate alternate exon splicing mechanisms that result in IGF-II mRNAs with different 5' ends. The lower most panel indicates an alternate transcription unit in exon 6 that results in an mRNA without IGF-II coding sequence.

Development

IGF-I

IGF-I mRNAs are detected in rat liver during fetal development. Postnatally, the abundance of IGF-I mRNAs rises gradually, reaching highest abundance in the mature rat (Fig. 11; Lund et al., 1986; Hoyt et al., 1988; Norstedt et al., 1988; Adamo et al., 1989b). In rat brain the levels of IGF-I mRNAs are highest during fetal development. Abundance of brain IGF-I mRNAs decreases postnatally to low but significant levels in mature rats (Fig. 5; Rotwein et al., 1988; Adamo et al., 1989b).

IGF-II

IGF-II mRNA abundance is highest in liver of fetal rats and rapidly declines postnatally to levels that are barely detectable (Fig. 5; Soares et al., 1985; Gray et al., 1987; Levinovitz and Norstedt, 1989). In rat brain, also, abundance of IGF-II mRNAs is high in the fetus and decreases postnatally, but IGF-II mRNAs are detected in the brain of adult rat in significant amounts (Fig. 5; Rotwein et al., 1988). Since most tissues in adult rat contain undetectable or barely detectable IGF-II mRNA, the brain is unusual in its continued synthesis of IGF-II in the adult animal.

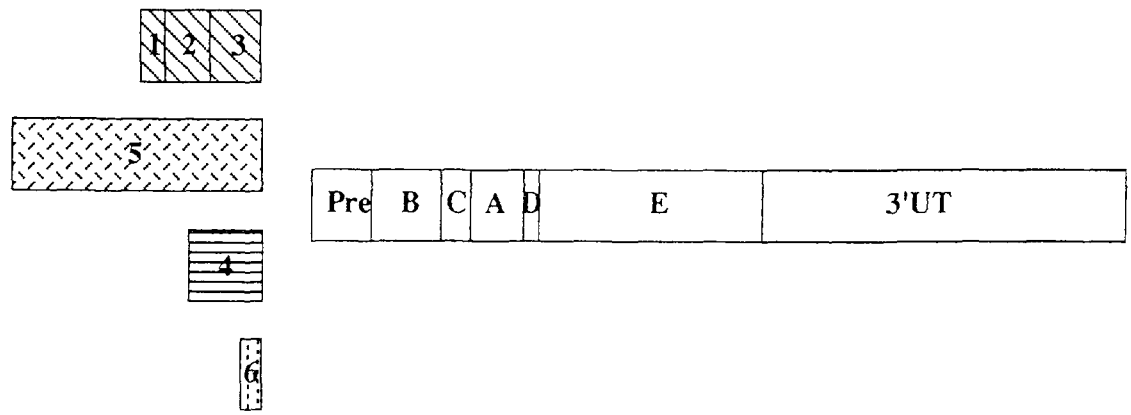

Fig. 11. Schematic of human IGF-II mRNAs. Diagonally striped bar labeled 1, 2, 3 represents an alternate 5' sequence that is derived from the splicing together of exons 1,2, and 3. Bars labeled 4, 5, and 6 represent additional alternate 5' sequences; that are derived from exons 4, 5, and 6 respectively. The middle bar (Pre, B, C, A, D, E) represents an identical sequence reported in all rat IGF-II cDNAs. Bar labeled 3'UT represents the 3'UT shared by all human IGF-II cDNAs, although alternate polyadenylation signals result in varying amounts of 3'UT.

Hormonal Regulation of IGF Expression

Growth Hormone

Growth hormone is a primary regulator of serum concentrations of IGF-I and IGF-I mRNAs in rat liver (Roberts et al., 1986; Mathews et al., 1986; Murphy et al., 1987a; Hynes et al., 1987). GH regulation of IGF-I mRNAs has been documented also in a number of nonhepatic tissues (Roberts et al., 1987a; Mathews et al., 1986; Murphy et al., 1987a; Hynes et al., 1987), but the role of GH in regulating IGF-I expression in the nervous system is less well established. Most evidence suggests that GH does not cross the blood-brain barrier. Studies by Hynes et al. 1987 indicate, however, that brain IGF-I mRNA abundance is influenced by GH status. IGF-I mRNA abundance is fourfold lower in hypophysectomized rats than in intact age matched rats, and IGF-I mRNA was restored to normal abundance within 4 h of GH administration into the lateral ventricle (Fig. 12; Hynes et al., 1987).

IGF-I is expressed in the pituitary, and it is thought that this may represent a mechanism for short loop feedback control of GH release, since it is known that IGF-I can inhibit GH gene expression at the level of the pituitary (Berelowitz et al., 1981; Goodyer et al.; 1984, Yamashita and Melmed, 1986). GH may also control expression of IGF-I in the pituitary since GH upregulated IGF-I mRNA expression in GH_3 cells, a rat pituitary tumor cell line (Fagin et al., 1989).

GH-dependent regulation of IGF-II mRNAs is less well established. Most studies of GH effects on IGF mRNAs have been carried out in adult rats where IGF-II mRNA levels are low. In brain of hypophysectomized rats, IGF-II mRNA abundance is lower than in intact animals, suggesting that GH status influences brain IGF-II synthesis (Hynes et al., 1987). Administration of GH either intraperitonally or into the lateral ventricle results in an increase in IGF-II brain mRNA abundance towards normal, further suggesting some level of GH control of brain IGF-II mRNAs (Hynes et al., 1987). A slower time course of GH dependent increase in brain IGF-II mRNAs compared with brain IGF-I mRNAs, as well as observations that IGF-II mRNAs were not restored to normal after GH treatment, indicates different GH regulatory mechanisms for brain IGF-I and IGF-II. One intriguing possibility, based on the more rapid GH-dependent regulation of IGF-I vs IGF-II mRNAs (Fig. 12), is that IGF-I may mediate the action of GH on brain IGF-II mRNAs. To date,

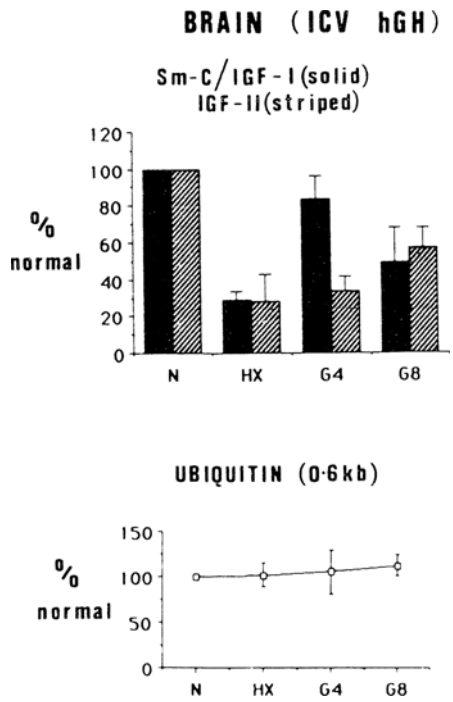

Fig. 12. Abundance of brain IGF-I and IGF-II mRNAs after hypophysectomy and Icv GH. The histograms at the top show the abundance of the 7.5-kb IGF-I mRNA (■) and IGF-II mRNAs (▨) in poly A+ RNAs from brains of normal (N) hypophysectomized (HX), and hypophysectomized rats at 4 h (G4) or 8 h (G8) after icv hGH. Abundance of a 0.6-kb ubiquitin mRNAs is shown at the bottom for comparison. Abundance in each case is expressed as percentage of signal intensity observed in poly A+ RNAs from normal brain. Each estimate of mRNA abundance shown is the mean and SD of four different poly A+ RNA preparations from four different animals. (From Hynes et al., 1987, with permission.)

however, effects of IGF-I on brain IGF-II mRNAs have not been analyzed.

Glucocorticoids

In children, glucocorticoids can inhibit growth (Loeb, 1976) and brain growth and development (Devenport and Devenport, 1985), but the mechanism by which this occurs is not clear. Recent studies indicate inhibitory effects of glucocorticoids on IGF-I synthesis, raising the possibility that growth inhibitory effects of glucocorticoids involve IGF-I. In pituitary intact rats, dexamethasone decreases abundance of IGF-I mRNAs in liver, and in hypophysectomized rats, dexamethasone attenuates the increase in liver IGF-I mRNAs induced by GH (Murphy and Luo, 1989).

Rat liver IGF-II mRNA abundance is also downregulated by glucocorticoids. Neonatal rats given daily subcutaneous injections of dexamethasone have reduced abundance of liver IGF-II mRNAs compared with saline injected controls. This effect is specific to dexamethasone, since estrogen and testosterone did not reduce liver IGF-II mRNA abundance (Levinovik and Norstedt, 1989).

To date, little is known about the effects of dexamethasone on IGF-II mRNAs in the nervous system. In cultured glial and neuronal cells, dexamethasone decreases IGF-I mRNA abundance, suggesting that brain IGF-I synthesis in the nervous system also may be inhibited by glucocorticoids (Adamo et al., 1988).

Insulin

A number of observations implicate insulin in regulation of serum IGF-I concentrations and IGF-I mRNAs in liver. Addition of insulin to perfused rat liver promotes IGF-I release (Shapiro et al., 1978). In rat, serum IGF-I concentrations and IGF-I mRNA abundance in liver are significantly reduced in experimentally induced diabetes (Murphy, 1988; Goldstein et al., 1988). This effect of insulin does not appear to be the result of indirect effects on GH, because administration of exogenous GH does not normalize serum IGF concentrations, and GH binding sites appear to be normal in the diabetic rat (Maes et al., 1986). Thus, in rats with insulin deficiency, there seems to be a defect in IGF-I gene activation of unknown origin. Effects of insulin on IGF-I in the nervous system have not been examined, but given the established neurological effects of diabetes, this is likely to be an important area of investigation.

One study has examined the effects of insulin on IGF-II in brain. In adult rats given long-term (4-d) insulin treatments, relative abundance of IGF-II mRNA in decreased is most hypothalmic

nuclei (Lauterio et al., 1990), suggesting inhibitory effects of insulin on IGF-II synthesis in some regions of the hypothalmus.

Estrogen

Acute treatment with estrogen does not affect IGF-I mRNA in rat liver, but increases IGF-I mRNA in rat uterus (Murphy et al., 1987b; Murphy and Friesen, 1988). Chronic estrogen treatment does, however, decrease the degree to which GH can increase rat liver IGF-I mRNA abundance (Murphy and Friesen, 1988). Thus, effects; of estrogen on IGF-I synthesis appear to be complex. The effects of estrogen on IGF expression in the nervous system have yet to be examined, but studies by Torran-Allerand et al. 1988 *(see below)* indicate an interaction between estrogen and the IGFs in the nervous system.

Nutrition and IGF Expression

It is well established that fasting or protein deprivation decreases serum IGF-I concentrations (Phillips, 1986; Underwood et al., 1986). IGF-I mRNAs in liver decrease in response to fasting (Elmer and Schalch, 1987). This decrease appears to be dependent on decreased protein intake and decreased caloric intake (Phillips, 1986; Underwood et al., 1986; Kato et al., 1989; Lowe et al., 1989; Moats-Staats et al., 1989). Fasting decreases IGF-I mRNA abundance in brain, but not to the same extent as in liver (Lowe et al., 1989).

Sites of Synthesis of IGFs in the Nervous System

Early in vitro studies suggested IGF synthesis in cultures of neural derived tissue. Insulin-like material (these studies did not distinguish between IGF-I and IGF-II) was detected in media collected from organ cultures of pituitary, hypothalmus, cerebral cortex, and cerebellum (Binoux et al., 1981).

Since then, a number of approaches have been used to localize sites of IGF synthesis in the nervous system, including analysis of proteins and mRNA from different brain regions, different cells in culture, and use of immunocytochemistry and *in situ* hybridization to localize cellular sites of synthesis. There are some discrepancies between localization of immunoreactivity and mRNA, as well as differences in results obtained in vitro and in vivo. Thus, the precise sites of synthesis of IGFs in the nervous system are still not defined. Available information will be reviewed here. It is relevant to note that data obtained by immunocytochemistry might be complicated by crossreactivity of IGF antibodies with IGF-I and II and by the possibility that immunoreactivity may arise via uptake rather than *de novo* synthesis. Negative data obtained by *in situ* hybridization must be interpreted with caution, since IGF mRNAs might be expressed at levels below the detection level of the *in situ* hybridization procedure.

IGF-I

IGF-I mRNAs are found in all brain regions in the adult rat, and abundance of IGF-I mRNAs is highest in the olfactory bulb. IGF-I mRNA abundance is also high in the spinal cord of adult rat (Rotwein et al., 1988). In human fetal brain, low levels of IGF-I mRNAs have been detected in hypothalmus and cortex (Han et al., 1988b). Recent studies using *in situ* hybridization histochemistry demonstrate expression of IGF-I mRNAs in neurons of the olfactory bulb and the hippocampus (Werther et al., 1990).

IGF-I has been detected in low abundance in the rodent brain by RIA and immunocytochemistry (D'Ercole et al., 1984; Norguchi et al., 1987; Andersson et al., 1988). In 40-d-old mouse, IGF-I immunoreactivity was detected in the anterior pituitary and in neuronal forebrain structures, such as the hippocampus, but no immunoreactivity was detected in the cerebral cortex (Norguchi et al., 1987). Using immunocytochemistry, IGF-I immunostaining is barely detectable or undetectable in cerebellum and retina of adult rats (Andersson et al., 1988; Hansson et al., 1988). In the developing cerebellum, IGF-I immunoreac-

tivity was detected transiently in glial cells from postnatal d 3 to d 21 and in neurons from postnatal d 5 to d 28 (Andersson et al., 1988). In developing retina, IGF-I immunostaining was seen primarily in glial cells (Hansson et al., 1989). The times during which IGF immunoreactivity was detected in cerebellum and retina correspond to the times of proliferation and differentiation (Andersson et al., 1988; Hansson et al., 1988).

Very low levels of IGF-I immunoreactivity are detected in adult human brain and human CSF by RIA (Haselbacher et al., 1985; Backstrom et al., 1984).

A variant, truncated 67 amino acid IGF-I, lacking the fifirst 3 amino acids of the B domain was recently isolated from human brain (Sara et al., 1986; Carlsson-Skwirut et al., 1986). This discovery has major implications for IGF-I action in the nervous system since the truncated IGF-I does not associate with IGF-I binding proteins and is more biologically potent than the 70 amino acid IGF-I (Carlsson-Skwirut et al., 1989; Sara and Hall, 1990). Furthermore, the tripeptide that may result from cleavage fo IGF-I to truncated IGF-I also may act as a neuroactive peptide on NMDA receptors (Sara et al., 1989). Further analysis of the tripeptide, truncated IGF-I and the biosynthetic pathways from which they derive should provide new insights into the role of IGF-I in the nervous system.

In cultures derived from embryonic rat brain, IGF-I mRNA was detected in both neuronal and glial cell types (Rotwein et al., 1988; Adamo et al., 1988).

IGF-II

In fetal and adult rat, *in situ* hybridization studies indicate that the major sites of synthesis of IGF-II mRNA in brain are the choroid plexus, pia, and meniges (Fig. 13; Hynes et al., 1988; Stylianopoulou et al., 1988; Ichimiya et al., 1988; Beck et al., 1988). As would be predicted from these data, IGF-II immunoreactivity is found in the cerebral spinal fluid (CSF) elaborated by the choroid plexus (Haselbacher and Humbel, 1982). In

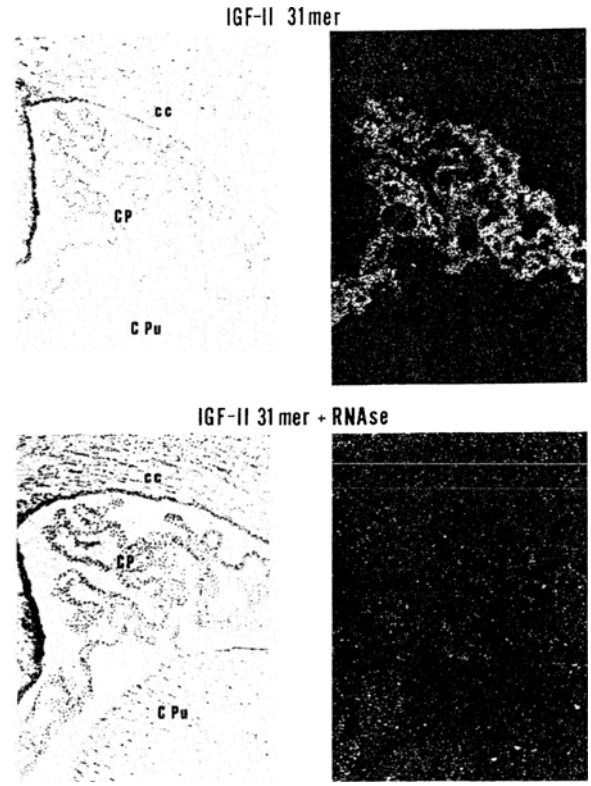

Fig. 13. IGF-II mRNA in rat choroid plexus. *Top*, photomicrographs of a portion of a coronal section of rat brain hybridized with ^{32}P labeled 31 base oligomer complementary to a rat IGF-II mRNA. *Bright fifield* photograph (left) shows the choroid plexus (CP) within the lateral ventricle, and adjacent corpus callosum (cc) and caudate putamen (CPu). *Dark field* shows accumulation of silver grains (indicative of hybridized probe) over the choroid plexus, and only randomly distributed silver grains over other brain regions. *Bottom*, photomicrographs of an adjacent section to the one shown at the top that was pretreated with 20μg/mL ribonuclease A (Sigma, St. Louis, MO) for 10 min at 37°C before hybridization with the IGF-II 31 mer. *Bright fifield* (left) shows the same brain regions as in top, and *dark fifield* illustrates that RNAse pretreatment abolished hybridization to choroid plexus. Exposure of sections to photoemulsion was for 14 d at 4°C. Scale bar=200μm. (From Hynes et al., 1988, with permission.)

contrast to IGF-I, quantitation of IGF-II mRNA in discrete brain regions suggests no major regional variations in abundance of IGF-II mRNAs (Rotwein et al., 1988). This would be predicted if choroid/pia/meniges were attached to various regions and represented the source of IGF-II

mRNA. In humans, IGF-II mRNA was localized to the sclera of the eye (Han et al., 1988b) suggesting synthesis in mesenchymal elements of the eye.

Using immunocytochemistry, IGF-II immunoreactivity is detected in human brain and also at high levels in the anterior pituitary (Haselbacher and Humbel, 1985). IGF-II immunoreactivity is detected in human CSF at much higher concentrations than IGF-I (Haselbacher et al., 1982; Backstrom et al., 1984). IGF-II has also been isolated from human brain and is identical in amino acid sequence to serum IGF-II (Carlsson-Skwirut et al., 1986). Immunocytochemistry in adult rat brain revealed a pattern of immunostaining consistent with localization of IGF-II to neurons (Laurterio et al., 1990). Since this is in contrast to localization of IGF-II mRNA primarily to choroid plexus, it is possible that the immunostaining reflects uptake rather than *de novo* synthesis or that IGF-II mRNAs expression in neurons at levels below detection by *in situ* hybridization. In cultures of embryonic rat brain, IGF-II mRNA was detected in cultured glial, but not neuronal cells (Rotwein et al., 1988).

Biological Actions of IGFs in the Nervous System

Binding Proteins

An emerging and rapidly expanding area of investigation is the characterization of IGF binding proteins in serum and tissue, and analyses of their relevance to IGF action. The IGFs circulate in serum bound to proteins that protect the IGFs from degradation. The half-life of IGFs in the bound form is 3–4 h, whereas the half-life of IGFs in the unbound form is 10 min (Cohen and Nissley 1976). Findings that IGF binding proteins are produced in cells in culture and tissues in vivo, and that IGF binding proteins can modulate IGF actions suggest that the binding has additional roles than the prolongation of plasma half-life of the IGFs. Since recent reviews of IGFBPs have appeared in the literature (Baxter and Martin, 1989; Sara and Hall, 1990), the focus here will be what is known about IGFBPs in the nervous system and the potential relevance to IGF actions in the nervous system.

Until recently, a large and confusing array of different molecular forms of serum and tissue IGF binding proteins was described in the literature based on cross-linking studies and molecular weights on SDS polyacrylamide gels. Recent cloning of three IGFBPs has provided their primary molecular structure and has led to the adoption of a standard nomenclature for IGFBPs (Ballard et al., 1989). Availability of the IGFBP cDNAs has also allowed analysis of their sites of expression. IGFBP3 appears to be expressed in a wide range of tissues and predominates postnatally (Baxter and Martin, 1986). IGFBP2 is also expressed in a wide range of tissues and is predominant prenatally (Brinkman et al., 1988; Margot et al., 1989; Brown et al., 1989). IGFBP1 is predominant in the fetus, and appears to be expressed primarily in placenta and decidua (Brown et al., 1989; Drop et al., 1984).

IGF Binding Proteins Found in Brain

The first indication that IGF binding proteins might be synthesized in the nervous system was the discovery of IGF binding proteins in media from cultured explants of rat pituitary and brain (Binoux et al., 1981). Subsequent studies have confirmed and expanded these initial findings. Rat astroglial and neuronal cultures produce multiple binding proteins, one of which is most likely rIGFBP2 (Han et al., 1988a; Ocrant et al., 1989; Burgess et al., 1987). Evidence supporting this contention includes recognition by an antibody to rIGFBP2, as well as similar results with rIGFBP2 and a protein isolated from astrocyte and neuronal cultures when used in affinity labeling studies and crosslinking. In addition, the protein derived from astrocytes has similar affinity for IGF-I and IGF-II, as does rIGFBP2 (Han et al., 1988a).

Studies in both human and rat have demonstrated IGF binding proteins in CSF (Rosenfeld

et al., 1989; Binoux et al.,1982; Hossenlopp et al., 1986; Tseng et al., 1989). In rat CSF, a 30-kD binding protein is recognized by an antibody to rIGFBP2, indicating that the major CSF binding protein is rIGFBP2 (Tseng et al., 1989). In human CSF, multiple IGF binding proteins are detected with apparent *Mr* ranging from 24,000–41,500, with the 34 kD being predominant (Hossenlopp et al., 1986) Competitive binding, structural, and immunological studies of the human CSF IGF binding proteins suggest that they are distinct from hIGFBP1 (Rosenfeld et al., 1989). In addition, the 34-kD human CSF binding protein is recognized by antibodies to rIGFBP2 indicating that it is most likely the human homolog of rIGFBP2 (Romanus et al., 1989). There may be a species differences in the major CSF binding proteins, since the human CSF binding protein has a higher affinity for IGF-II than IGF-I, whereas the rat CSF binding protein has similar affinity for IGF-I and IGF-II (Tseng et al., 1989).

Consistent with the finding of rIGFBP2 in CSF, recent studies demonstrate that the major site of synthesis of rIGFBP2 in rat brain is the choroid plexus (Tseng et al., 1989). The rIGFBP2 mRNA is developmentally regulated. As with IGF-II, levels of the rIGFBP2 mRNA decreased markedly in liver postnatally, but persisted in adult brain (Tseng et al., 1989; Margot et al., 1989).

Future studies using cDNAs from the other recently cloned binding proteins will be helpful in providing additional information about the spectrum of IGF binding proteins that are expressed in the nervous system, how they are regulated, and their roles in the nervous system.

Biological Actions of IGF Binding Proteins

In glial cultures, addition of purified rIGFBP2 reduced incorporation of tritiated thymidine supporting a hypothesis that IGF binding proteins regulate availability of free IGFs for action on target cells (Han et al., 1988a). In other systems, however, IGFBPs can either inhibit or potentiate the growth promoting action of the IGFs. In some instances, the incubation conditions, coincubation of IGFBP and IGF vs preincubation of IGFBP and IGF, result in inhibition or potentiation of IGF activity, respectively (DeMellow and Baxter, 1988; Blat et al., 1989). In the case of IGFBP1, one form of IGFBP1 inhibited IGF-mediated mitogenesis, whereas another form potentiated it (Elgin et al., 1987). The potentiating form of IGFBP1 differed from the inhibiting form only in its ability to multimerize and bind to the cell surface. Cell surface binding was attributed to an RGD sequence in IGFBP1, which appears to be essential for binding of proteins.

IGF Receptors

IGF Receptor Types

The importance of the IGF receptors in the nervous system has been discussed in a recent review (Adamo et al., 1989a) and will be considered only briefly here. Both IGF-I and IGF-II can bind to two IGF receptor types (type 1 and type 2) as well as insulin receptors, although with widely differing affinities (Table 2).

Structure of the IGF Receptors

The insulin and type 1 receptors are structurally similar glycoproteins that consist of two α and two β subunits (Czech 1989). The extracellular alpha subunit contains the receptor binding domain. The transmembrane spanning beta subunit contains tyrosine kinase activity which mediates the growth promoting actions of the type I receptor (Czech, 1989). Because the type 1 receptor has high affinity for both IGF-I and IGF-II, it is thought to mediate the growth promoting actions of both of the IGFs.

The type 2 receptor is a single chain transmembrane glycoprotein composed primarily of extracellular domain and a relatively short cytoplasmic domain. The type 2 receptors binds both mannose 6-phosphate and IGF-II (Kiess et al., 1988; Tong et al., 1988; Braulke, et al., 1988). Analysis of cDNAs has established that the human type 2 IGF receptor has a high degree of

Table 2
Relative Affinities of Insulin and IGF Receptors

Receptor	Insulin	IGF-I	IGF-II
Insulin	high	low	low
Type 1	low	high	high
Type 2	none	low	high

homology to the cation independent bovine mannose 6-phosphate receptor (Morgan et al., 1987; Lobel et al., 1987). The precise function of the type 2 receptor is not known, although the mannose 6-phosphate receptor is known to target lysosomal proteins to the lysosomes. In neural plasticity, there is remodeling of synapse morphology. This presumably involves alterations of synaptic proteins. Although purely speculative, it is possible that the type 2 receptor may play a role in targeting synaptic proteins for lysosomal degradation or intracellular recycling during remodeling of the synapse.

IGF Receptors in the Nervous System

Evidence suggests differences in the IGF receptors found in neuronal vs nonneuronal tissues. The α subunit of the type 1 receptor found in brain appears to be somewhat smaller than the α subunit in other tissues because of differences in glycosylation (Burgess et al., 1987; Shemer et al., 1987; McElduff et al., 1988; Heidenreich et al., 1986). Fetal rat neurons in culture also appear to exhibit a smaller type 1 receptor, whereas the receptor isolated from glial cultures is the same size as that isolated from placenta (Burgess et al., 1987). Similarly, the type 2 receptor found in brain also has lower molecular weight than the liver type 2 re-ceptor, apparently because of differences in glycosylation (McElduff et al., 1987). The functional significance of these differences is not known.

Studies of IGF receptors in cells derived from the nervous system are complicated by the cross-reactivity of receptors with IGF-I, IGF-II, and insulin, and by the possibility of binding to tissue binding proteins distinct from the IGF receptors. Nonetheless, many in vitro studies suggest the existence of the type 1 or type 2 receptors in cells derived from the nervous system as summarized in Table 3. Neurons and astrocytes express both the type 1 and type 2 receptors. Oligodendrocytes express the type I receptor, but it is not clear whether they express the type 2 receptor.

In Vivo Analysis of IGF Receptors

Binding sites for IGF-I and IGF-II are found throughout the brain, indicating widespread distribution of type 1 and 2 receptors. Autoradiographic ligand binding studies have found the highest levels of IGF-I and IGF-II binding in the choroid plexus, median eminence, and pituitary (Bohannon et al., 1986, 1988; Lesniak et al., 1988; Smith et al., 1988; Araujo et al., 1989). Since an IGF-I binding protein, IGFBP2, is expressed in the choroid plexus, this may contribute to the high level of binding. Other brain regions show lower binding of both IGF-I and IGF-II, and patterns of binding for IGF-I and IGF-II are different. In the olfactory bulb, for example, IGF-I binding is found in the olfactory nerve, glomerular layer, and external plexiform layer, whereas IGF-II binding is absent from the olfactory nerve and external plexiform layers, but is found in the glomerular and mitral cell layers (Lesniak et al., 1988). Distinct localization of binding sites for IGF-I and IGF-II suggest distinct functional roles for the two peptides.

Transport of IGFs Across the Blood Brain Barrier

Binding sites for IGF-I and IGF-II have been found on the membranes that make up the blood-brain barrier (BBB) in both rat and bovine (Frank et al., 1986; Rosenfeld et al., 1987). IGF-II and IGF-I are internalized by endothelial cells, which is consistent with transport of the IGFs across the BBB. The human BBB was reported to have only a single binding site that bound IGF-II with higher affinity than IGF-I and did not bind insulin (Duffy et al., 1988).

Table 3
IGF Receptors In Vitro

	Type 1	Type 2	References
Astrocytes neonate rat, fetal rats	+	+	Gammeltoft et al., 1985; Han et al., 1987; Ballotti et al., 1987; Ocrant et al., 1988
Oligodendrocytes neonate rats	+	?	McMorris et al., 1986
Neurons fetal rat	+	+	Burgess et al., 1987; Shemer et al., 1987; Ocrant et al., 1988

In humans with pituitary disorders, a comparison of IGFs in serum and CSF has revealed no significant correlation. Individuals with elevated levels of serum IGF-I resulting from acromegaly, for example, do not have higher levels of IGF-I or IGF-II in CSF when compared to GH-deficient individuals (Backstrom et al. 1984). Thus, increases in plasma concentrations of IGF-I apparently do not result in increased IGF-I in CSF. These findings provide evidence that circulating IGF-I is not transported across the BBB, at least in individuals with these pituitary disfunctions.

In Vitro Action of the IGFs

In vitro studies have provided insights into the roles of the IGFs in the nervous system (Table 4). Since IGF-I and IGF-II have only recently become readily available in sufficient amounts for biological testing, many actions of the IGFs have been inferred based on the effects of supraphysiological levels of insulin that acts on the type 1 IGF receptor (Table 2). Most studies have not documented the receptor that mediates the response examined. In vitro studies have used cloned cell lines, primary cultures of neuronal and glial cells, and brain explants. Studies in vitro (Table 4) provide strong evidence for a role of the IGFs to

1. Promote neurite formation
2. Promote survival of cells derived from nervous tissue
3. Promote differentiation of nervous system derived cells.

Neurite Promoting Effects of IGFs

Evidence for neurite promoting effects of the IGFs has been derived from three independent systems: the human neuroblastoma SH-SY5Y cell line, primary cultures of neurons, and cultured explants of CNS tissue. Insulin and IGF-II enhance neurite formation in SH-SY5Y cells in serum-free media (Mill et al., 1985; Recio-Pinto et al., 1984; Recio-Pinto et al., 1986; Recio-Pinto and Ishii, 1988; Ishii et al., 1985) and also increase tubulin mRNA apparently by stabilization of the tubulin mRNA (Mill et al., 1985; Fernyhough et al., 1989) Tubulin is an important cytoskeletal protein in axons and dendrites. The dose-response curve for the induction of tubulin mRNA levels by insulin was very similar to that for increased neurite formation.

Insulin and IGF-II can stimulate neurite formation in serum-free, primary neuronal cultures of chick dorsal root ganglia sensory neurons, parasympathetic ciliary ganglion neurons, and chick sympathetic neurons (Collins and Dawson, 1983; Bothwell, 1982; Recio-Pinto et al., 1986). Although these studies have the advan-

Table 4
In Vitro Actions of the IGFs

Actions	Cells	References
DNA Synthesis	Fetal rat brain cells Neonatal rat brain cells Human neuroblastoma SH-SY5Y Neonate rat astroglial cells Neonate rat neuronal cultures	Lenoir and Honegger, 1983; Enberg et al., 1985; Han et al., 1987; Shemer et al., 1987
RNA synthesis	Fetal rat neuronal cells	Burgess et al., 1987
Neurite formation	Human neuroblastoma SH-SY5Y Chick parasympathetic ciliary neurons Chick sensory neurons Chick sympathetic neurons Mouse brain explant (insulin)	Bothwell 1982; Collins and Dawson, 1983; Recio-Pinto and Ishii, 1984; Recio-Pinto et al., 1984; Recio-Pinto et al., 1986; Mill et al., 1985; Toran-Allerand et al., 1988
Oligodendrocyte proliferation & differentiation	Neonate rat brain cells Oligodendrocyte enriched cultures Rat glial progenitor cells	McMorris et al., 1986; McMorris and Dubois-Dalcq, 1988; Saneto et al., 1988; van der Pal et al., 1988
Growth promoting	Rat fetal hypothalmic cell lines Rat cortical neuronal cultures Rat neuroblastoma cell line (B104) Fetal rat brain (ODC) Rat sympathetic neuroblast (mitotic cycle)	Yang et al., 1981; Aizenman and deVellis, 1987; DiCicco-Bloom and Black, 1988; Orlowski et al., 1989
Differentiative	Fetal quail DRG	Xue et al., 1988

tage that the cells are not transformed, insulin and the IGFs are required survival factors for these cells, and therefore, it is difficult to establish whether the neurite promoting effects are distinct from the survival effects. When effects of insulin and NGF on neurite formation are compared, they are not additive, indicating that these hormones are probably acting on the same subset of cells. The neurite promoting effect of IGF and NGF appears to be specific, since EGF, FGF, and angiotensin do not stimulate neurite formation in cells in which insulin and NGF are active (Collins and Dawson, 1983).

Supraphysiological concentrations of insulin induce neurite formation in CNS explants (Toran-Allerand et al., 1988). Insulin at supraphysiological concentrations potentiates the estrogen induced neurite formation in explants from brain regions known to contain the estrogen receptor. Detailed analysis of the effects of IGFs in this system has not yet been reported.

IGFs as Survival Factor

Insulin in high concentrations or IGF-I in lower concentrations is required for survival of neuronal and glial cells in serum free media (Aizenman and de Vellis, 1987; Torres-Aleman et al., 1989; Snyder and Kim, 1980). This property may relate to the demonstrated growth promoting effects of the IGFs, including induction of DNA and RNA synthesis and proliferation of rat sympathetic neuroblasts (Shemer et al., 1987; DiCicco-Bloom and Black, 1988; Sara and Carlsson-Skwirut, 1988). In cultures of fetal rat brain, the

IGFs induce ornithine decarboxylase activity, an enzyme thought to be a marker for cell proliferation (Yang et al., 1981).

IGFs and Differentiation

IGF-I has differentiative effects on glial cells in culture. Oligodendrocytes are the glial cells in the CNS that make myelin. IGF-I promotes oligodendrocyte development in culture (McMorris et al., 1986; van der Pal et al., 1988) apparently by stimulating proliferation of a precursor cell population and a "commitment" of the precursors into cells that are positive for oligodendrocyte markers (McMorris and Dubois-Dalcq, 1988). Myelin basic protein (MBP) is a major protein component of myelin and is a marker for myelin producing glial cells. In cultures of oligodendrocyte progenitor cells treated with IGF-I or insulin, MBP concentrations are elevated compared to controls. Insulin was the only component of oligodendrocyte-defined media (ODM: serum free medium, insulin, transferrin, and basic FGF) to increase levels of MBP (Saneto et al. 1988). Based on these findings, McMorris et al. have hypothesized that IGFs are important in oligodendrocyte development and myelination (McMorris et al., 1986). Serum IGF-I levels are decreased in malnutrition (Underwood et al., 1986). Hypomyelination is associated with malnutrition at critical times in the postnatal period (Wiggins, 1982). In addition, the critical period for hypomyelination resulting from malnutrition corresponds to the period of oligodendrocyte development and not to the period of actual myelin synthesis (Wiggins, 1982). Together these data suggest a role for IGF-I in oligodendrocyte development and myelination.

Although IGF-I may be capable of inducing oligodendrocyte formation, it is probably not the only factor responsible for oligodendrocyte development in vivo. Rats made hypothyroid by treatment with propylthiouracil have normal serum concentrations of IGF-I and normal concentrations of immunoreactive IGF-I in brain, but still have 52% of the normal 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNP) activity, a standard marker for myelin (King et al., 1988). Thus, myelin formation in vivo may involve an interplay between IGFs, thyroid hormone, and possibly other factors.

As well as effects on oligodendrocytes, IGF-I is a differentiative factor for catecholaminergic precursors in cultured cells from DRG of embryonic quail (Xue et al., 1988) These cells will differentiate in response to chick embryo extract (CEE). Only insulin and IGF-I and not NGF, FGF, and EGF could replace CEE in this activity (Xue et al., 1988).

In Vivo Actions of the IGFs

In vivo studies point to a role of the IGFs in synaptogenesis, repair and regeneration, disease of the nervous system, and regulation of the hypothalmic pituitary axis.

Synaptogenesis

Recently, it has been reported that increased IGF-II mRNA abundance correlates with synaptogenesis in skeletal muscle (Ishii, 1989). In rat skeletal muscle, IGF-II mRNA abundance increases during polyneuronal innervation and decreases during synapse elimination. Muscle denervation results in increases in IGF-II mRNAs concomitant with new synapse formation (Ishii, 1989).

Some evidence indicates a role for IGF-I in neuronal repair. In rat sciatic nerve after crushing or freezing, IGF-I increases sciatic nerve responsiveness and stimulates neurite growth (Kanje et al., 1989; Sjoberg and Kanje, 1989). Increases in IGF-I immunostaining are also detected in regenerating sciatic nerves of adult rats (Hansson et al., 1986). Observations in rat in vivo that nerve regeneration is inhibited by perfusion with monoclonal antibodies to IGF-I provide additional evidence for involvement of IGF-I in neuronal repair (Sjoberg and Kanje, 1989; Kanje et al., 1989). IGF-I is rapidly trans-

ported in both anterograde and retrograde directions immediately after nerve crush (Hansson et al., 1987), suggesting that IGF-I is transported to the site of damage.

IGFs in Disease

Abnormal levels of IGFs have been noted in diseases of the CNS. IGF-II is decreased in the CNS in alcoholism (Tham et al., 1986). Neurofibromatosis is associated with increased IGF-I immunoreactivity (Hansson et al., 1988). Increased levels of IGFs have also been associated with abnormal growth in the nervous system. IGF-II was increased in CSF of a patient with macrenecephaly (Schoenle et al., 1986).

Hypothalmic-Pituitary-IGF Axis

IGF-I exerts negative feedback effects at the levels of the pituitary and the hypothalmus via effects to decrease GH release and increase SRIF release (Berelowitz et al., 1981; Tannenbaum et al., 1983; Abe et al., 1983; Yamashita and Melmed, 1986). IGF-I inhibits GHRH-elicited GH release from anterior pituitary cells in culture (Ceda et al., 1987).

The hypothalmus-pituitary-IGF axis has been implicated in control of feeding (Phillips, 1986). In rats, intracerebroventricular (ICV) administration of IGF-II, but not IGF-I or insulin, results in significant decreases in *acute* food intake and body weight (Lauterio et al., 1987). This is distinct from the effects of insulin where chronic ICV administration of insulin decreases food intake (Woods et al., 1979).

Transgenic Models

Transgenic mice represent an emerging and useful in vivo model for analyses of the in vivo roles of hormones, growth factors, and the hypothalmic-GH-IGF-axis in the nervous system. Two general approaches have been commonly used in generation of transgenic mice. In one approach, a strong constitutive promoter, the mouse metallothionein promoter, was used to elicit elevated and widespread expression of different components of the hypothalamic/pituitary/IGF system. This approach has been used to generate animals overexpressing GRF and GH, resulting in a "giant" phenotype (Palmiter et al., 1982; Hammer et al., 1985). Animals overexpressing human IGF-I (Mathews et al., 1988) were also generated using this approach, resulting in enhanced overall body growth and overgrowth of a number of organs, including brain *(see below)*.

Another approach has been to ablate a component of the hypothalmic/GH/IGF system. This has been accomplished by linking the growth hormone promoter to a toxin gene, such as diphtheria toxin (Behringer et al., 1988). Transgenic mice with these genes integrated into their genome specifically express the toxin in the pituitary somatotrophs leading to toxin mediated ablation of somatotrophes during development. These animals exhibit dwarf phenotype and reduced brain weight (Behringer et al., 1988, 1990). In a more recent approach, homologous recombination was utilized to destroy the endogenous mouse IGF-II gene and generate IGF-II deficient animals. As with GH-deficient animals, these animals exhibit dwarf phenotype (DeChiara et al., 1990).

Behringer et al. analyzed body and organ weight in wild-type mice, mice over expressing IGF-I via metallothionein promoter driven over expression of a human IGF-I cDNA, GH-deficient dwarf mice generated by GH promoter driven diphtheria toxin mediated ablation of somatotrophes, and crossbreeds of the IGF-I overexpressers and GH deficient dwarfs. Analysis of brain weight in these animals revealed significant decreases in brain weight of dwarfs, significant elevation of brain weight in IGF-I overexpressors, and normalization of brain weight in dwarfs crossed with IGF-I expressors. These findings indicate an effect of IGF-I on brain weight, independent of GH (Fig. 14; Behringer et al., 1990).

McMorris et al. studied the myelin content in these transgenic mice. In animals overexpressing

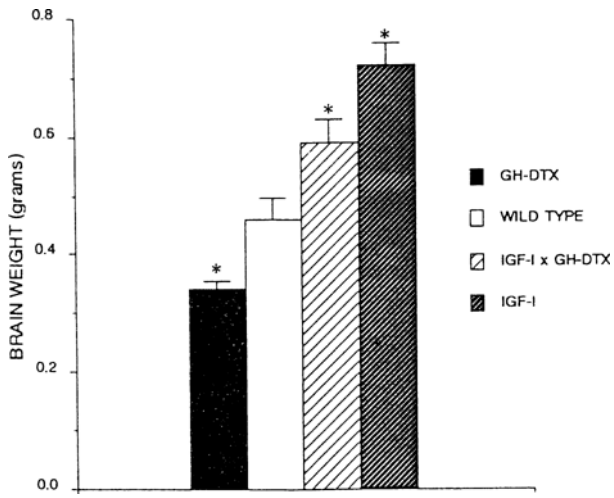

Fig. 14. Histogram of brain weight in wild type and transgenic mice. Brain weight is represented on the abscissa in grams. Mice with the following genotypes: GH-DTX, growth hormone promoter linked to the diphtheria toxin gene (■) wild type (□) IGF-I transgene and GH-DTX gene (IGF-I × GH-DTX, ▨), and IGF-I transgene (▨) are represented by the columns. Each brain weight is the mean of a minimum of five mice. Standard deviation is shown at the top of the column. Asterisks indicate significant difference compared with wild type.

IGF-I, relative myelin content is 20–30% higher than in wild-type litter mates and total myelin content is 90–100% higher (Carson et al., 1988). In contrast, dwarf transgenic mice with GH cells ablated show hypomyelination in the cerebrum. The crossbreeds of the IGF-I overexpressors and GH-ablated dwarf mice exhibit a 90% higher myelin content in cerebrum compared with GH-deficient littermates (Carson et al., 1988, 1989). These results suggest that the alteration in brain weight in transgenic animals of different GH and IGF-I status is via effects on myelination. In addition, these data indicate IGF-I is capable of inducing myelin formation in vivo in the absence of GH.

As well as for myelin formation, these different transgenic lines provide model systems to address many questions, including feedback interaction among the components of the hypothalmic-GH-IGF system, effects of GH and the IGFs on behavior or aging, and relative roles of IGF-I and IGF-II in nervous system function. In the future, it seems likely that other transgenic lines will be developed with altered IGF receptors and binding proteins. Such models will rapidly expand our information base about IGFs and the nervous system.

Conclusions

In the past ten years, molecular biology approaches have provided the structures of multiple components of the IGF system, IGF peptides, receptors, binding proteins, and the mRNAs and genes encoding them. Progress has been made toward understanding which components of the IGF system are expressed in the nervous system, their cellular localization, and their regulation. The molecular studies also provide the potential for availability of pure components of the IGF system for testing of biological effects and interactions. Emerging information from these approaches together with the applications of gene transfer approaches in cells in culture and transgenic animals suggest that the next few years will provide exponential increase in our information and understanding of the precise roles of the IGF system in nervous system function.

Acknowledgments

The authors would like to thank S. Burgess, A. J. D'Ercole, B. Kay, A. Willard, L. McIlwain, and J. J. Van Wyk for their critical reading of the manuscript. This work was supported by NIH grant AM 1022.

References

Abe H., Molitch M., Van Wyk J., and Underwood L. (1983) Human growth hormone and somatomedin C suppress the spontaneous release of growth hormone in unanesthetized rats. *Endocrinology* **113**, 1319–1324.

Adamo M., Raizada M. K., and LeRoith D. (1989a) Insulin and insulin-like growth factor receptors in the nervous system. *Mol. Neurobiol.* 3, 71–98.

Adamo M., Lowe W., LeRoith D., and Roberts C. (1989b) Insulin-like growth factor I messenger ribonucleic acids with alternative 5'-untranslated regions are differentially expressed during development of the rat. *Endocrinology* **124**, 2737–2744.

Adamo M., Werner H., Farnsworth W., Roberts C. T. J., Raizada M., and LeRoith D. (1988) Dexamethasone reduces steady state insulin-like growth factor I messenger ribonucleic acid levels in rat neuronal and glial cells in primary culture. *Endocrinology* **123**, 2565–2570.

Aizenman Y. and de Vellis J. (1987) Brain neurons develop in a serum and glial free environment: effects of transferrin, insulin, insulin-like growth factor-*I* and thyroid hormone on neuronal survival, growth and differentiation. *Brain Res.* 406, 32–42.

Andersson I. K., Edwall D., Norstedt G., Rozell B., Skottner A., and Hansson H. A. (1988) Differing expression of insulin-like growth factor I in the developing and in the adult rat cerebellum. *Acta Physiol. Scand.* **132**, 167–73.

Araujo D. M., Lapchak P. A., Collier B., Chabot J. G., and Quirion R. (1989) Insulin-like growth factor-I (somatomedian-C) receptors in the rat brain: distribution and interaction with the hippocampal cholinergic system. *Brain Res.* 484, 130–138.

Backstrom M., Hall K., and Sara V. (1984) Somatomedin levels in cerebrospinal fluid from adults with pituitary disorders. *Acta Endocrinologica* **107**, 171–178.

Ballard J., Baxter R., Binoux M., Clemmons D., Drop S., Hall K. I, Hintz R., Rechler M., Rutanen E., and Schwander J. (1989) On the nomenclature of the IGF binding proteins. *Acta Endocrinologica* **121**, 751–752.

Ballotti R., Nielson F. C., Pringle N., Kowalski A., Richardson W. D., Van Obberghen E., and Gammeltoft S. (1987) Insulin-like growth factor I in cultured rat astrocytes: expression of the gene, and receptor tyrosine kinase *EMBO J.* 6, 3633–3639.

Baxter R. and Martin J. (1986) Radioimmunoassay of growl hormone-dependent insulin like growth factor binding protein in human plasma. *J. Clin. Invest.* **78**, 1504–1512.

Baxter R. C. and Martin J. L. (1989) Binding proteins for the insulin-like growth factors: structure, regulation and function. *Progress in Growth Factor Research* **1**, 49–68.

Beck F., Samani N. J., Byrne S., Mogan K., Gebhard R., and Brammer W. (1988) Histochemical localization of IGF-I and IGF-II mRNA in the rat between birth and adulthood. *Development* **104**, 29–39.

Behringer R. R., Mathews L., Palmiter Richard D., and Brinster R. L. (1988) Dwarf mice produced by genetic ablation of growth hormone-expressing cells. *Genes & Development* **2**, 453–461.

Behringer R., Lewin T., Quaife C., Palmiter R., Brinster R., and D'Ercole A. (1990) Expression of insulin-like growth factor I stimulates normal somatic growth in growth hormone-deficient transgenic mice . *Endocrinology* **127**, 1033–1040.

Bell G., Merryweather J., Sanchez-Pascador R., Stempien M., Priestly L. S., and Rall L. (1984) Sequence of a cDNA clone encoding human preproinsulin-like growth factor II. *Nature* **310**, 775–777.

Bell G., Stempien M., Fong N., and Rall L. (1986) Seqences of liver cDNAs encoding two different mouse insulin-like growth factor I precursors. *Nucleic Acids Research* **14**, 7873–7882.

Berelowitz M., Szabo M., Frohman L., Firestone S., Chu L., and Hintz R. (1981) Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalmus and the pituitary. *Science* **212**, 1279–1281.

Binoux M., Hardouin S., Lassarre C., and Hossenlopp P. (1982) Evidence for production by the liver of two IGF binding proteins with similar molecular weights but different affinities for IGF-I and IGF-II. Their relations with serum and cerebrospinal fluid IGF binding proteins. *J. Clin. Endoc. and Metab.* **55**, 600–602.

Binoux M., Hossenlopp P., Lassarre C., and Hardouin N. (1981) Production of insulin-like growth factors and their carrier by rat pituitary gland and brain explants in culture. *FEBS Lett.* **124**, 178–184.

Blat C., Delbe J., Villaudy J., Chatelain G., Golde A., and Harel L. (1989) Inhibitory diffusible factor 45 bifunctional activity. *J. Biol. Chem.* **264**, 12449–12454.

Bohannon N. J., Corp E. S., Wilcox B. J., Figlewicz D. P., Dorsa D. M., and Baskin D. G. (1988) Localization of binding sites for insulin-like growth factor-I (IGF-I) in the rat brain by quantitative autoradiography. *Brain Res.* **444**, 205–213.

Bohannon N. J., Figlewicz D. P., Corp E. S., Wilcox B. J., Porte D., and Baskin D. G. (1986) Identification

of binding sites for an insulin-like growth factor (IGF-I) in the median eminence of the rat brain by quantitative autoradiography. *Endocrinology* **119,** 943–945.

Bothwell M. (1982) Insulin and somatomedin MSA promote nerve growth factor-independent neurite formation by cultured chick dorsal root ganglionic sensory neurons. *J. of Neurosci. Res.* **8,** 225–231.

Braulke T., Causin C., Waheed A., Junghans U., Hasilik A., Maly P., Humbel R., and von Figura K. (1988) Mannose 6-phosphate/insulin-like growth factor II receptor: distinct binding sites for mannose 6-phosphate and insulin-like growth factor II. *Biochem. Biophys. Res. Comm.* **150,** 1287-1293.

Brinkman A., Groffen C., Kortleve D., Geurts van Kessel A., and Drop S. (1988) Isolation and characterization of a cDNA encoding the low molecular weight insulin-like growth factor binding protein (IB-I). *EMBO J.* **7,** 2417–2423.

Brown A., Chairotti L., Orlowski C., Mehlman T., Burgess W., Ackerman E., Bruni C., and Rechler M. (1989) Nucleotide sequence and expression of a cDNA clone encoding a fetal rat binding protein for insulin-like growth factors. *J. Biol. Chem.* **264,** 5148–5154.

Bucci C., Mallucci P., Robers C., Frunzio R., and Bruini C. (1989) Nucleotide sequence of a genomic fragment of the rat IGF-I gene spanning an alternate 5 non coding exon. *Nucleic Acids Research* **17,** 3596.

Burgess S., Jacobs S., Cuatrecasas P., and Sahyoun N. (1987) Characterization of a neuronal substype of insulin-like growth factor I. *J. Biol. Chem.* **262,** 1618–1622.

Cao Q., Kunguay S., Plisetskaya E., Steiner D., and Chan S. (1989) Nucleotide sequence and growth hormone-regulated expression of salmon insulin-like growth factor I mRNA. *Mol. Endocrinol.* **3,** 2005–2010.

Carlsson-Skwirut C., Jornvail H., Holmgren A., Andersson C., Bergman T., Lundquist G., Sjogren B., and Sara V. R. (1986) Isolation and characterization of variant IGF-I as well as IGF-2 from adult human brain. *FEBS Lett.* **201,** 46–50.

Carlsson-Skwirut C., Lake M., Hartmans M., Hall K., and Sara V. R. (1989) A comparison of the biological activity of the recombinant intact ani truncated insulin-like growth factor I (IGF-I). *Biochimica et Biophysica Acta* **1011,** 192–197.

Carson M., Behringer R., Mathews L., Palmiter R., Brinster R., and McMorris F. (1988) Myelin content increased in transgenic mice producing elevated levels of insulin-like growth factor-I (IGF-I). *Society for Neuroscience Abstracts* **14,** 119.

Carson M., Behringer R., Mathews L., Palmiter R., Brinster R., and McMorris F. (1989) Hypomyelination caused by growth hormone deficiency is reversed by insulin-like growth factor I in transgenic mice. *Trans. Am. Soc. Neurochem.* **20,** 286.

Casella S., Smith E., Van Wyk J., Joseph D., Hynes M., Hoyt E., and Lund P. (1987) Isolation of rat testis cDNA encoding an insulin-like growth factor I precursor. *DNA* **6,** 325–330.

Ceda G., Davis R., Rosenfeld R., and Hoffman A. (1987) The growth hormone (GH)-releasing hormone (GHRH)-GH somatomedin axis: Evidence for rapid inhibition of GHRH-elicited GH release by insulin-like growth factors I and II. *Endocrinology* **120,** 1658–1 662.

Chiariotti L., Brown A., Frunzio R., Clemmons D., Rechler M., and Bruni C. (1988) Structure of the rat insulin-like growth factor II transcriptional unit: heterogeneous transcripts are generated from two promoters by use of multiple polyadenylations sites and differential ribonucleic acid splicing. *Mol. Endocrinol.* **2,** 1115–1126.

Clemmons D. R., and Shaw D. S. (1986) Purification and biologic properties of fibroblast somatomedin. *J. Biol. Chem.* **261,** 10293–10298.

Clemmons D. (1989) Structural and functional analysis of insulin-like growth factors. *British Medical Bulletin* **45,** 465–480.

Cohen K., and Nissley S. (1976) The serum half-life of somatomedin activity: evidence for growth hormone dependence. *Acta Endocrinologica* **83,** 243–258.

Collins F., and Dawson A. (1983) An effect of nerve growth factor on parasympathetic neurite outgrowth. *Proc. Natl. Acad. Sci. USA* **80,** 2091–2094.

Czech M. P. (1989) Signal transmission by the insulin-like growth factors. *Cell* **59,** 235–238.

Daughaday W. H., Hall K., Raben M. S., Salmon W. D. J., Van den Brande J. L., and Van Wyk J. J. (1972) Somatomedin: a proposed designation for the "sulfation factor." *Nature* **235,** 107.

Daughaday W. H., Hall K., Raben M. S., Salmon W. D. J., Van den Brande J. L., and Van Wyk J. J. (1989) On the nomenclature of the somatomedins and insulin-like growth factors. *J. Clin. Endoc. Metab.* **5,** 15.

Daughaday W. H., and Rotwein P. (1989) Insulin-like growth factors I and II. Peptide, messenger

ribonucelic acid and gene structures, serum, and tissue concentrations. *Endocrine Reviews* **10**, 68–91.

DeChiara T. M., Argiris Efstratiadis, and Robertson E. J. (1990) A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature* **345**, 78–80.

DeMellow J., and Baxter R. (1988) Growth hormone-dependent insulin-like growth factor (IGF) binding protein both inhibits and potentiates IGF-I stimulated DNA synthesis in human skin fibroblast. *Biochem. Biophys. Res. Comm.* **156**, 199–204.

de Pagter-Holthuizen P., Jansen M., van der Kammen R. A., van Schaik F. M. A., and Sussenbach J. S. (1988) Differential expression of the human insulin-like growth factor II gene. Characterization of the IGF-II mRNAs and an mRNA encoding a putative IGF-II associated protein. *Biochim. Biophys. Acta* **950**, 282–295.

de Pagter-Holthuizen P., Jansen M., van Schaik F., van der Kammen R., Oosterwijk C., Van de Brande J., and Sussenbach J. (1987) The human insulin-like growth factor II gene contains two developmental-specific promotors. *FEBS Lett.* **214**, 259–264.

D'Ercole A., Stiles A., and Underwood L. (1984) Tissue concentrations of somatomedin C: Further evidence for multiple sites of synthesis, and paracrine or outocrine mechanisms of action. *Proc. Natl. Acad. Sci. USA* **81**, 935–939.

Devenport L., and Devenport J. (1985) Adrenocortical hormones and brain growth: reversibility and differential sensitivity during development. *Expt. Neurol.* **90**, 44–52.

DiCicco-Bloom E., and Black I. B. (1988) Insulin growth factors regulate the mitotic cycle in cultured rat sympathetic neuroblasts. *Proc. Natl. Acad. Sci. USA* **85**, 4066–4070.

Drop S., Kortieve D., Guyda H., and Posner B. (1984) Immunoassay of a somatomedin-binding protein from human amniotic fluid: levels In fetal, neonatal, and adult sera. *J. Clin. Endoc. Metab.* **59**, 908–915.

Duffy K., Pardridge W., and Rosenfeld R. (1988) Human blood brain barrier insulin-like growth factor receptor. *Metabolism* **37**, 136–140.

Dulak N., and Temin H. M. (1973) A partially purified polypeptide fraction from rat liver cell conditioned medium with multiplication-stimulation activity for embryo fibroblasts. *J. Cell. Physiol.* **81**, 153–160.

Dull T. J., Gray A., Hayflick J. S., and Ullrich A. (1984) Insulin-like growth factor II precursor gene organization in relation to insulin gene family. *Nature* **310**, 777–781.

Elgin R., Busby W., and Clemmons D. (1987) An insulin-like growth factor (IGF) binding protein enhances the biologic response to IGF-I. *Proc. Natl. Acad. Sci. USA* **84**, 3254–3258.

Elmer C., and Schalch D. (1987) Nutritionally-induced changes in hepatic insulin-like growth factor I (IGF-I) gene expression in rats. *Endocrinology* **120**, 832–834.

Enberg G., and Hall K. (1984) Immunoreative IGF-II in serum of healthy subjects and patients with growth hormone disturbances and uremia. *Acta Endocrinol.* **107**, 164–170.

Enberg G., Tham A., and Sara V. R. (1985) The influence purified somatomedins and insulin on foetal rat brain DNA synthesis in vitro. *Acta Physiol. Scand.* **125**, 305–308.

Fagin J., Fernandez-Mejia C., and Melmed S. (1989, Pituitary insulin-like growth factor-I gene expression: regulation by triiodothyronine and growth hormone. *Endocrinology* **125**, 2385–2391 .

Fernyhough P., Mill J., Roberts J., and Ishii D. (1989) Stabilization of tubulin mRNA by insulin and insulin-like growth factor I during neurite formation. *Mol. Brain Res.* **6**, 109–120.

Francis G., McNeil K., Wallace J., Ballard F., and Owens P. 1989) Sheep insulin-like growth factors I and II: sequences, activities and assays. *Endocrinology* **124**, 1243–1173.

Frank H. J., Pardridge W. M., Morris W. L., Rosenfeld R. G., and Choi T. B. (1986) Binding and internalization of insulin and insulin-like growth factors by isolated brain microvessels. *Diabetes* **35**, 654–661.

Froesch E., Burgi H., Ramseier E., Bally P., and Labhart A. (1963) Antibody-suppressible and non-supressible insulin-like activities in human serum and their physiologic significance. *J. Clin. Invest.* **42**, 1816–1834.

Frunzio R., Chiariotti L., Brown A. L., Graham D. E., Rechler M. M., and Bruni C. B. (1986) Structure and expression of the rat insulin-like growth factor II (rIGF-II) gene. *J. Biol. Chem.* **261**, 17138–17149.

Fryklund L., Skottner A., Sievertsson H., and Hall K. (1976) Somatomedins A and B: isolation, chemistry and in vivo effects, in *Growth Hormone and Related Peptides.*, Excerpta Medica, Amsterdam, pp. 156–168.

Gammeltoft S., Haselbacher G., Humbel R., Fehlmann M., and Obberghen E. (1985) Two types of receptors

for insulin-like growth factors in mammalian brain. *EMBO J.* **4,** 3407–3412.

Goldstein S., ASertich G. J., Levan K. R., and Phillips L. S. (1988) Nutrition and somatomedin. XIX. Molecular regulation of insulin-like growth factor-I in streptozotocin-diabetic rats. *Mol. Endocrinol.* **2,** 1093–1099.

Goodyer C. G., De Stephano, L., Lai W. H., Guyda H. J., and Posner B. I. (1984) Characterization of insulin-like growth factor receptors in rat anterior pituitary, hypothalamus, and brain. *Endocrinology* **114,** 1187–1195.

Graham D., Rechler M., Brown A, Frunzio R, Romanus J, Bruni C., Whitfield H., Nissley S., Seelig S., and Berry S. (1986) Coordinate developmental regulation of high and low molecular weight mRNAs for rat insulin-like growth factor II. *Proc. Natl. Acad. Sci. USA* **83,** 4519.

Gray A., Tam T. J., Haflick J., Pintar J., Cavenee W. K., Koufos A., and Ullrich A. (1987) Tissue-specific and developmentally regulated transcription of the insulin-like growth factor 2 gene. *DNA* 6, 283–295.

Hall K. (1972) Human somatomedin: determination, occurrence, biological activity and purification. *Acta Endocrinol.* **163 (suppl.),** 1–45.

Hammer R. E., Brinster R. L., Rosenfeld M. G., Evans R. E., and Mayo K. E. (1985) Expression of human growth hormone-releasing factor in transgenic mice results in increased somatic growth. *Nature* **315,** 413–416.

Han V. K., Lauder J. M., and D'Ercole A. J. (1987) Characterization of somatomedin/insulin-like growth factor receptors and correlation with biologic action in cultured neonatal rat astroglial cells. *J. Neuroscience* **7,** 501–511.

Han V. K., Lauder J. M., and D'Ercole A. J. (1988a) Rat astroglial somatomedin/insulin-like growth factor binding proteins: characterization and evidence of biologic function. *J. Neuroscience* 8, 3135–3143.

Han V. K. M., Lund P. K., Lee D. C., and D'Ercole A. J. (1988b) Expression of somatomedin/insulin-like growth factor messenger ribonucleic acids in the human fetus: Identification, characterization, and tissue distribution. *J. Clin. Endoc. Metab.* **66,** 422–429.

Hansson H. A., Dahlin L. B., Danielsen N., Fryklund L., Nachemson A. K., Polleryd P., Rozell B., Skottner A., Stemme S., and Lundborg G. (1986) Evidence indicating trophic importance of IGF-I in regenerating peripheral nerves. *Acta Physiol. Scand.* **126,** 609–614.

Hansson H. A., Holmgren A., Norstedt G., and Rozell B. (1989 Mar) Changes in the distribution of insulin-like growth factor I, thioredoxin, thioredoxin reductase and ribonucleotide reductase during the development of the retina. *Exp. Eye Res.* **48,** 411–420.

Hansson H. A., Lauritzen C., Lossing C., and Petruson K. (1988) Somatomedin C as tentative pathogenic factor in neurofibromatosis. *Scand. J. Plast. Reconstr. Surg. Hand Surg.* **22,** 7–13.

Hansson H. A., Rozell B., and Skottner A. (1987) Rapid axoplasmic transport of insulin-like growth factor I in the sciatic nerve of adult rats. *Cell Tissue Res.* **247,** 241 –247.

Haselbacher G., and Humbel R. (1982) Evidence for two species of insulin-like growth factor II (IGF-II and big IGF-II) in human spinal fluid. *Endocrinology* **110,** 1822– 1824.

Haselbacher G., Schwab M., Pasi A., and Humbel R. (1985) Insulin-like growth factor II (IGFII) in human brain: Regional distribution of IGF-II an of higher molecular mass forms. *Proc. Natl. Acad. Sci. USA* **82,** 2153–2158.

Heidenreich K. A., Freidenberg G. R., Figlewicz D. P., and Gilmore P. R. (1986) Evidence for a subtype of insulin-like growth factor I receptor in brain. *Regul. Pept.* **15,** 301–310.

Hepler J. E., Van Wyk J. J., and Lund P. K. (1990) Different half-lives of insulin-like growth factor I mRNAs that differ in length of 3' untranslated sequence. *Endocrinology* **127,** 1550–1552.

Holthuizen P., van der Lee F., Ikejiri K., Yamamoto M., and Sussenbach J. (in press) Identification and initial characterization of a fourth leader exon and promoter of the human IGF-II gene. *Biochimica Biophysica Acta.*

Honegger A., and Humbel R. (1986) Insulin-like growth factors I and II in fetal and adult bovine serum. *J. Biol. Chem.* **261,** 569–575.

Hossenlopp P., Seurin D., Segovia-Quinson B., and Binoux M. (1986) Identification of an insulin-like growth factor-binding protein in human cerebrospinal fluid with a selective affinity for IGF-II. *FEBS Lett.* **208,** 439–444.

Hoyt E., Van Wyk J., and Lund P. (1988) Tissue and development specific regulation of a complex family of insulin-like growth factor I messenger ribonucleic acids. *Mol. Endocrinol.* **2,** 1077–1086.

Hoyt E. C., Hepler J. E., Van Wyk J. J., and Lund P. K. Structural characterization of exon 5 of the rat IGF-I gene encoding an unusually long 3'untranslated region. Manuscript in preparation.

Humbel R. E. (1990) Insulin-like growth factors I and II. *Eur. Biochem.* **190,** 445–462.

Hynes M. A., Brooks P. J., Van Wyk J. J., and Lund P. K. (1988) Insulin-like growth factor II messenger ribonucleic acids are synthesized in the choroid plexus of the rat brain. *Mol. Endocrinol.* **2,** 47–54.

Hynes M. A., Van W. J., Brooks P. J., D'Ercole A. J., Jansen M., and Lund P. K. (1987) Growth hormone dependence of somatomedin-C/insulin-like growth factor-I and insulin-like growth factor-II messenger ribonucleic acids. *Mol. Endocrinol.* **1,** 233–242.

Ichimiya Y., Emson P. C., Northrop A. J., and Gilmour R. S. (1988) Insulin-like growth factor II in the rat choroid plexus. *Brain Res.* **464,** 167–170.

Ishii D. N. (1989) Relationship of insulin-like growth factor II gene expression in muscle to synaptogenesis. *Proc. Natl. Acad. Sci. USA* **86,** 2898–2902.

Ishii D. N., Recio-Pinto E., Spinelli W., Mill J., and Sonnenfeld K. H. (1985) Neurite formation modulation by nerve growth factor, insulin, and tumor promoter receptors. *Intern. J. Neuroscience* **26,** 109–127.

Jansen M., Van Schaik F., Ricker A., Bullock B., Woods D., Gabby K., Nussbaum A., Sussenbach J., and Van Den Brande J. (1983) Sequence of cDNA encoding human insulin-like growth factor I precursor. *Nature* **306,** 609–611.

Jansen M., Van Schaik F., Van Tol H., Van de Brande J., and Sussenbach J. (1985) Nucleotide sequence of cDNAs encoding precursors of human insulin-like growth factor II (IGF-II) and an IGF-II variant. *FEBS Lett.* **179,** 243–246.

Kajimoto Y., and Rotwein P. (1989) Structure and expression of a chicken insulin-like growth factor I precursor. *Mol. Endocrinol.* **3,** 1907–1913.

Kajimoto Y., and Rotwein P. (1990) Evolution of Insulin-like Growth Factor I (IGF-I) Precursor from *Xenopus laevis*. *Mol. Endocrinol.* **4,** 217–226.

Kanje M., Skottner A., Sjoberg J., and Lundborg G. (1989) insulin-like growth factor I (IGF-I) stimulates regeneration of the rat sciatic nerve. *Brain Res.* **486,** 396–398.

Kato H., Miura Y., Okoshi A., Umezawa T., Takahashi S., and Noguchi T. (1989) Dietary and hormonal factors affecting the mRNA level of IGF-I in rat liver in vivo and in primary cultures of rat hepatocytes, in *Molecular and Cellular Biology of Insulin-Like Growth Factors and Their Receptors,* (LeRoith D. and Raizada M. K., eds.) Plenum Press, New York, pp. 125–128.

Kiess W., Blichenstaff G. D., Sklar M. M., Thomas C. L., Nissley S. P., and Sahagian G. G. (1988) Biochemical evidence that the type II insulin-like growth factor receptor is identical to the cation-independent mannose 6-phosphate receptor. *J. Biol. Chem.* **263,** 9339–9344.

King R., Smith R., Meller D., Dahlenburg G., and Lineham J. (1988) Effect of growth hormone on growth and myelination in the neonatal hypothyroid rat. *J. Endocr.* **119,** 117–125.

Klapper D. G., Svoboda M. E., and VanWyk J. (1983) Sequence analysis of somatomedin-C: confirmation of identity with insulin-like growth factor I. *Endocrinology* **112,** 2215–2217.

Lauterio T., Aravich P. F., and Rotwein P. (1990) Divergent effects of insulin on insulin-like growth factor-II gene expression in the rat hypothalmus. *Endocrinology* **126,** 392–398.

Lauterio T. J., Marson L., Daughaday W. H., and Baile C. A. (1987) Evidence for the role of insulin-like growth factor II (IGF-II) in the control of food intake. *Physiol. Behav.* **40,** 755–758.

Lenoir D. and Honegger P. (1983) Insulin-like growth factor I (IGF I) stimulates DNA synthesis in fetal rat brain cell cultures. *Brain Res.* **283,** 205–213.

Lesniak M. A., Hill J. M., Kiess W., Rojeski M., Pert C. B., and Roth J. (1988) Receptors for insulin-like growth factors I and II: Autoradiographic localization in rat brain and comparison to receptors for insulin. *Endocrinology* **123,** 2089–2099.

Levinovik A. and Norstedt G. (1989) Developmental and steroid hormonal regulation of insulin-like growth factor II expression. *Mol. Endocrinol.* **3,** 797–804.

Lobel P., Dahms N. M., Breitmeyer J., Chirgwin J. M., and Kornfeld S. (1987) Cloning of the bovine 215-kDa cation-independent mannose 6-phosphate receptor. *Proc. Natl. Acad. Sci. USA* **84,** 2233–2237.

Loeb J. (1976) Corticosteroids and growth. *N. Eng. J. Med.* **295,** 547–552.

Lowe W., Adamo M., Werner H., Roberts C., and LeRoith D. (1989) Regulation by fasting of rat insulin-like growth factor I and its receptor. *J. Clin. Invest.* **84,** 619–626.

Lowe W., Lasky S., LeRoith D., and Roberts C. (1988) Distribution and regulation of rat insulin-like growth factor I messenger ribonuleic acids encoding alternative carboxyterminal E-peptides: Evidence for differential processing and regulation in liver. *Mol. Endocrinol.* **2,** 528–535.

Lowe W., Roberts C., Lasky S., and LeRoith D. (1987) Differential expression of alternative 5' untranslated regions in mRNAs encoding rat insulin-like growth factor I. *Proc. Natl. Acad. Sci. USA* **84,** 8946–8950.

Lund P., Hoyt E., and Van Wyk J. (1989) The size heterogeneity of rat insulin-like growth factor-I mRNAs is due primarily to differences in the length of 3'-untranslated sequence. *Mol. Endocrinol.* **3,** 2054–2061.

Lund P. K., Moats-Staats B. M., Hynes M. A., Simmons J. G., Jansen M., D'Ercole A. J., and Van W. J. J. (1986) Somatomedin-C/insulin-like growth factor-I and insulin-like growth factor-II mRNAs in rat fetal and adult tissues. *J. Biol. Chem.* **261,** 14539–14544.

Maes M., Underwood L. E., and Ketelslegers J. M. (1986) Low serum somatomedin-C in insulin-dependent diabetes: evidence for a postreceptor mechanism. *Endocrinology* **118,** 377–382.

Margot J., Binkert C., Mary J., Landwehr J., Heinrich G., and Schwander J. (1989) A low molecular weight insulin-like growth factor binding protein from rat: cDNA cloning and tissue distribution of its messenger RNA. *Mol. Endocrinol.* **3,** 1053–1060.

Marquardt H., Todaro G., Henderson L., and Oroszlan S. (1981) Purification and primary structure of a polypeptide with multiplication-stimulation activity from rat liver cell cultures. *J. Biol. Chem.* **256,** 6859–6865.

Martin D., Renold A., and Dagenais Y. (1958) An assay for insulin-like activity using rat adipose tissue. *Lancet* **275,** 76–77.

Mathews L. S., Hammer R. E., Behringer R. R., D'Ercole A. J., Bell G. I., Brinster R. L., and Palmiter R. D. (1988) Growth enhancement of transgenic mice expressing human insulin-like growth factor I. *Endocrinology* **123,** 2827–2833.

Mathews L. S., Norstedt G., and Palmiter R. D. (1986) Regulation of insulin-like growth factor I gene expression by growth hormone. *Proc. Natl. Acad. Sci. USA* **83,** 9343–9347.

Matsuguchi T., Takahashi K., Ueno T., Endo H., and Yamamoto M. (1989) A novel transcription unit within the exon sequence of the rat insulin-like growth factor II gene. *Biochemistry International* **18,** 71–79.

McElduff A., Poronnik P., and Baxter R. C. (1987) The Insulin-like growth factor-II (IGF II) receptor from rat brain is of lower apparent molecular weight than the IGF II receptor from rat liver. *Endocrinology* **121,** 1306–1311.

McElduff A., Poronnik P., Baxter R. C., and Williams P. (1988) A comparison of the insulin and insulin-like growth factor I receptors from rat brain and liver. *Endocrinology* **122,** 1933–1939.

McMorris F. A., and Dubois-Dalcq M. (1988) Insulin-like growth factor I promotes cell proliferation and oligodendroglial commitment in rat glial progenitor cells developing in vitro. *J. Neurosci. Res.* **21,** 199–209.

McMorris F. A., Smith T. M., DeSalvo S., and Furlanetto R. W. (1986) Insulin-like growth factor I/somatomedin C: a potent inducer of oligodendrocyte development. *Proc. Natl. Acad. Sci. USA* **83,** 822–826.

Mill J. F., Chao M. V., and Ishii D. N. (1985) Insulin, insulin-like growth factor II, and nerve growth factor effects on tubulin mRNA levels and neurite formation. *Proc. Natl. Acad. Sci. USA* **82,** 7126–7130.

Moats-Staats B., Brady J., Underwood L., and D'Ercole A. (1989) Dietary protein restriction in artificially reared neonatal rats causes a reduction of insulin-like growth factor-I gene expression. *Endocrinology* **125,** 2368–2374.

Morgan D., Edman J., Standring D., Fried V., Smith M., Roth R., and Rutter W. (1987) Insulin-like growth factor II receptor as a multifunctional binding protein. *Nature* **329,** 301–307.

Moses A., Nissley S., Short P., and Rechler M. (1980) Immunological cross-reactivity of multiplication-stimulating activity polypeptides. *Eur. J. Biochem.* **103,** 401 –408.

Murphy L. (1988) Impaired estrogen-induced uterine insulin-like growth factor-I gene expression in the streptozotocin diabetic rat. *Diabetologia* **31,** 842–847.

Murphy L., and Friesen H. (1988) Different effects of estrogen and growth hormone on uterine and hepatic insulin-like growth factor I gene expression in the ovariectomized hypophysectomized rat. *Endocrinology* **122,** 325–332.

Murphy L., and Luo J. (1989) Dexamethasone inhibits growth hormone induction of insulin-like growth

factor-I (IGF-I) messenger ribonucleic acid (mRNA) in hypophysectomized rats and reduces IGF-I mRNA abundance in the intact rat. *Endocrinology* **125,** 165–171.

Murphy L. J., Bell G. I., Duckworth M. L., and Friesen H. G. (1987a) Identification, characterization, and regulation of a rat complementary deoxyribonucleic acid which encodes insulin-like growth factor-I. *Endocrinology* **121,** 684–69

Murphy L. J., Murphy L. C., and Friesen H. G. (1987b) Estrogen induces Insulin-like growth factor-I expression in the rat uterus. *Mol. Endocrinol.* **1,** 445–450.

Nissley S., Adams S., Acquaaviva A., Yang Y., Bruni C., Angust G., White R., Foley T. J., Moses A., Cohen A., and Rechler M. (1983) Multiplication stimulating activity for cells in culture, in *Insulin-Like Growth Factors/Somatomedin: Basic Chemistry, Biology, Clinical Importance,* Walter de Gruyter, Berlin, New York, pp. 31–48.

Noguchi T., Kurata L. M., and Sugisaki T. (1987) Presence of a somatomedin-C-immunoreactive substance in the central nervous system: immunohistochemical mapping studies. *Neuroendocrinology* **46,** 277–282.

Norstedt G., Levinovitz A., Moller C., Eriksson L., and Andersson G. (1988) Expression of insulin-like growth factor I (IGF-I) and IGF-II mRNA during hepatic development, proliferation and carcinogenesis in the rat. *Carcinogenesis* **9,** 209–213.

Ocrant I., Pham H., Oh Y., and Rosenfeld R. (1989) Characterization of insulin-like growth factor binding proteins of cultured rat astroglial and neuronal cells. *Biochem. Biophys. Res. Comm.* **159,** 1316–1322.

Ocrant I., Valentino K. L., Eng L. F., Hintz R. L., Wilson D. M., and Rosenfeld R. G. (1988) Structural and immunohistochemical characterization of insulin-like growth factor I and 11 receptors in the murine central nervous system. *Endocrinology* **123,** 1023–1034.

Orlowski C. C., Chernausek S. D., and Akeson R. (1989) Actions of insulin-like growth factor-I on the B104 neuronal cell line: effects on cell replication, receptor characteristics, and influence of secreted binding protein on ligand binding. *J. Cell. Physiol.* **139,** 469–476.

Palmiter R. D., Brinster R. L., Hammer R. E., Trumbauer Myrna E., Rosenfeld M. G., Birnberg N. C., and Evans R. M. (1982) Dramatic growth of mice that develop with metallothionein-growth hormone fusion genes. *Nature* **300,** 611–615.

Phillips L. S. (1986) Nutrition, somatomedins, and the brain. *Metabolism* **35,** 78–87.

Pierson R., and Temin H. (1972) The partial purification from calf serum of a fraction with multiplication-stimulating activity for chicken fibroblasts in cell culture and with non-suppressible insulin-like activity. *J. Cell. Physiol.* **79,** 319–330.

Powell D., Lee P., Chang D., Liu F., and Hintz R. (1987) Antiserum developed for the E peptide region of insulin-like growth factor IA prohormone recognizes a serum protein by both immunoblot and radioimmunoassay. *J. Clin. Endoc. Metab.* **65,** 868–875.

Prats H., Kaghad M., Prats A., Klagsbrun M., Lelias J., Liauzun P., Chalon P., Tauber J., Amalric F., Smith J., and Caput D. (1989) High molecular mass forms of basic fibroblast growth factor are initiated by alternative CUG codon-. *Proc. Natl. Acad. Sci. USA* **86,** 1836–1840.

Recio-Pinto E. and Ishii D. N. (1984) Effects of insulin, insulin-like growth factor-II and nerve growth factor on neurite outgrowth in cultured human neuroblastoma cells. *Brain Res.* **302,** 323–334.

Recio-Pinto E. and Ishii D. N. (1988) Insulin and insulin-like growth factor receptors regulating neurite formation in cultured human neuroblastoma cells. *J. Neurosci. Res.* **19,** 312–320.

Recio-Pinto E., Lang F. F., and Ishii D. N. (1984) Insulin and insulin-like growth factor II permit nerve growth factor binding and the neurite formation response in cultured human neuroblastoma cells. *Proc. Natl. Acad. Sci. USA* **81,** 2562–2566.

Recio-Pinto E., Rechler M. M., and Ishii D. N. (1986) Effects of insulin, insulin-like growth factor-II, and nerve growth factor on neurite formation and survival in cultured sympathetic and sensory neurons. *J. Neuroscience* **6,** 1211–9.

Renold A. E., Martin D. B., Dagenais Y., Steinke J., Nickerson R., and Sheps M. (1960) Measurement of small quantities of insulin-like activity using rat adipose tissue I. A proposed procedure. *J. Clin. Invest.* **39,** 1487–1498.

Rinderknecht E. and Humbel R. (1978a) The amino acid sequence of human insulin-like growth factor I and its structural homolgy with proinsulin. *J. Biol. Chem.* **253,** 2769–2776.

Rinderknecht E. and Humbel R. (1978b) Prima structure of human insulin-like growth factor II. *FEBS Lett.* **89,** 283–286.

Roberts C., Brown A., Graham D., Seelig S., Berry S., Gabbay K., and Rechler M. (1986) Growth hormone regulates the abundance of insulin-like growth factor I RNA in adult rat liver. *J. Biol. Chem.* **261,** 10025–10028.

Roberts C., Lasky S., Lowe W., Seaman W., and LeRoith D. (1987a) Molecular cloning of rat insulin-like growth factor I complementary deoxyribonucleic acids: Differential messenger ribonucleic acid processing and regulation by growth hormone in extrahepatic tissues. *Mol. Endocrinol.* **1,** 243–248.

Roberts C., Lasky S., Lowe W., and LeRoith D. (1987b) Rat IGF-I cDNAs contain multiple 5'-untranslated regions. *Biochem. Biophys. Res. Comm.* **14,** 1154–1159.

Romanus J., Tseng L., Yang Y., and Rechler M. (1989) The 34 kilodalton insulin-like growth factor binding proteins in human cerebrospinal fluid and the A673 rhabdomyosarcoma cell line are human homologues of the rat BRL-3A binding protein. **163,** 875–881.

Rosenfeld R., Pham H., Conover C., Hintz R., and Baxter R. (1989) Structural and immunological comparison of insulin-like growth factor binding proteins of cerebrospinal and amniotic fluids. *J. Clin. Endoc. Metab.* **68,** 638–646.

Rosenfeld R. G., Pham H., Keller B. T., Borchardt R. T., and Pardridge W. M. (1987) Demonstration and structural comparison of receptors for insulin-like growth factor-I and -II (IGF-I and -II) in brain and blood-brain barrier. *Biochem. Biophys. Res. Comm.* **149,** 159–166.

Rotwein P. (1986) Two insulin-like growth factor I messenger RNAs are expressed in human liver. *Proc. Natl. Acad. Sci. USA* **83,** 77–81.

Rotwein P., Burgess S. K., Milbrandt J. D., and Krause J. E. (1988) Differential expression of insulin-like growth factor genes in rat central nervous system. *Proc. Natl. Acad. Sci. USA* **85,** 265–269.

Rotwein P., Folz R. J., and Gordon J. I. (1987) Biosynthesis of human insulin-like growth factor I (IGF-I) *J. Biol. Chem.* **262,** 11807–11812.

Rotwein P., Pollock K., Didier D., and Krivi G. (1986) Organization and Sequence of the Human Insulin-like Growth Factor I Gene. *J. Biol. Chem.* **261,** 4828–4832.

Salmon W. D., and Daughaday W. (1957) A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. *J. Lab. Clin. Med.* **49,** 825–836.

Saneto R. P., Low K. G., Melner M. H., and deVellis J. (1988) Insulin/insulin-like growth factor I and other epigenetic modulators of myelin basic protein expression in isolated oligodendrocyte progenitor cells. *J. Neurosci. Res.* **21,** 210–219.

Sara V. R., and Carlsson-Skwirut C. (1988) The role of the insulin-like growth factors in the regulation of brain development. *Prog. Brain Res.* **73,** 87–99.

Sara V. R., Carlsson-Skwirut C., Andersson C., Hall E., Sjogren B., Holmgren A., and Jornvall H. (1986) Characterization of somatomedins from human fetal brain: identification of a variant form of insulin-like growth factor I. *Proc. Natl. Acad. Sci. USA* **83,** 4904–4907.

Sara V., and Hall K. (1990) Insulin-like growth factors and their binding proteins. *Physiological Reviews* **70,** 591–614.

Schoenle E. J., Haselbacher G. K., Briner J. B., Janzer R. C., Gammeltoft S., Humbel R. E., and Prader A. (1986) Elevated concentration of IGF-II in brain tissue from an infant with macrenephaly. *J. Pediatrics* **108,** 737–740.

Shapiro B., Waligora K., and Pimstone B. L. (1978) Generation of somatomedin activity in response to growth hormone and insulin from isolated perfused livers of normal and protein malnourished rats. *J. Endocrinology* **79,** 369–373.

Shemer J., Raizada M. K., Masters B. A., Ota A., and LeRoith D. (1987) Insulin-like growth factor I receptors in neuronal and glial cells. Characterization and biological effects in primary culture. *J. Biol. Chem.* **262,** 7693–769

Shimatsu A. and Rotwein P. (1987) Mosaic Evolution of the Insulin-like Growth Factors. *J. Biol. Chem.* **262,** 7894–7900.

Sjoberg J. and Kanje M. (1989) Insulin-like growth factor (IGF-1) as a stimulator of regeneration in the freeze-injured rat sciatic nerve. *Brain Res.* **485,** 102–108.

Smith M., Clemens J., Kerchner G. A., and Mendelsohn L. G. (1988) The insulin-like growth factor-II (IGF-II) receptor of rat brain: regional distribution visualized by autoradiography. *Brain Res.* **445,** 241–246.

Snyder E. Y. and Kim S. U. (1980) Insulin is it a nerve survival factor? *Brain Res.* 565–571 .

Soares M., Ishii D., and Efstratiadis A. (1985) Developmental and tissue-specific expression of a

family of transcripts related to rat insulin-like growth factor II mRNA. *Nucleic Acids Research* **13,** 1119–1134.

Soares M. B., Turken A., Ishii D. N., Mills L., Episkopou V., Cotter S., Zeitlin S., and Efstratiadis A. (1986) Rat insulin-like growth factor II gene. A single gene with two promoters expressing a multitranscript family. *J. Mol. Biol.* **192,** 737–752.

Spencer E., Ross M., and Smith B. (1983) The identity of human insulin-like growth factors I and II with somatomedins C and A and homology with rat IGFI and II, in *Insulin-Like Growth Factors/Somatomedins: Basic Chemistry, Biology, Clinical Importance,* Walter de Gruyter, Berlin, New York, pp. 81–96.

Stempien M. M., Fong N. M., Rall L. B., and Bell G. I. (1986), Sequence of a placental cDNA encoding the mouse insulin-like growth factor II precursor. *DNA* **5,** 357–361.

Stylianopoulou F., Herbert J., Soares M. B., and Efstratiadis A. (1988) Expression of the insulin-like growth factor II gene in the choroid plexus and the leptomeninges of the adult rat central nervous system. *Proc. Natl. Acad. Sci. USA* 85, 141–145.

Sussenbach J. S. (1989) The gene structure of the insulin like growth factor family. *Progress in Growth Factor Research* **1,** 33–48.

Tannenbaum G. S., Guyda H. J., and Posner B. I. (1983) Insulin-like growth factors: a role in growth hormone negative feedback and body weight regulation via brain. *Science* **220,** 77–79.

Tavakkol A., Simmen F., and Simmen R. (1988) Porcine insulin-like growth factor-I (pIGF complementary deoxyribonucleic acid cloning and uterine expression of messenger ribonucleic acid encoding evolutionarily conserved IGF-I peptides. *Mol. Endocrinol.* **2,** 674–681.

Tham A., Sara V. R., Borg S., and Wetterberg L. (1986) Circulating levels of insulin-like growth factors 1 and 2 and somatomedin B in alcoholic patients. *Psychiatry Res.* **18,** 301–308.

Tong P. Y., Tollefsen S. E., and Kornfeld S. (1988) The cation-independent mannose 6-phosphate receptor binds insulin-like growth factor II. *J. Biol. Chem.* **263,** 2585–2588.

Toran-Allerand C. D., Ellis L., and Pfenninger K. H. (1988) Estrogen and insulin synergism in neurite growth enhancement in vitro: mediation of steroid effects by interactions with growth factors? *Developmental Brain Research* **41,** 87–100.

Torres-Aleman I., Naftolin F., and Robbins R. J. (1989) Growth promoting effects of IGF-I on fetal hypothalamic cell lines under serum-free culture conditions. *Int. J. Dev. Neurosci.* **7,** 195–202.

Tseng L., Brown A., Yang Y., Romanus J., Orowski C., Taylor T., and Rechler M. (1989) The fetal rat binding protein for insulin-like growth factors is expressed in the choroid plexus and cerebrospinal fluid of adult rats. *Mol. Endocrinol.* **10,** 1559–1568.

Ueno T., Takahashi K., Matsuguchi T., Endo H., and Yamamoto M. (1987) A new leader exon identified in the rat insulin-like growth factor II gene. *Biochem. Biophys. Res. Commun.* **148,** 344–349.

Ueno T., Takahashi K., Matsuguchi T., Endo H., and Yamamoto M. (1988) Transcriptional deviation of the rat insulin-like growth factor II gene initiated at three alternative leader-exons between neonatal tissues and ascites hepatomas. *Biochimica et Biophysicca Acta* **950,** 411–419.

Ueno T., Takahashi K., Matsuguchi T., Ikejiri K., Endo H., and Yamamoto (1989) Multiple polyadenylation sites in a large 3'-most exon of the rat insulin-like growth factor II gene. *Biochimica et Biophysica Acta* **1009,** 27–34.

Underwood L. E., Clemmons D. R., Maes M., D'Ercole A. J., and Ketelslegers J. (1986) Regulation of somatomedin-C/insulin-like growth factor II by nutrients. *Hormone Res.* **24,** 166–176.

Uthne K. (1973) Human somatomedins: pruification and studies on their biological actions. *Acta Endocrinol.* **73 (suppl. 175),** 1–35.

van der Pal R., Koper J. W., van Golde L. M., and Lopes-Cadozo M. (1988) Effects of insulin and insulin-like growth factor (IGF-I) on oligodendrocyte-enriched glial cultures. *J. Neurosci. Res.* **19,** 483–490.

Van Wyk J., Underwood L., Hintz R., Clemmons D., Voina S., and Weaver R. (1974) The somatomedins: a family of insulin-like hormones under growth hormone control. *Rec. Progr. Horm. Res.* **30,** 259–318.

Werther G. A., Abate M., Hogg A., Cheesman H., Oldfield B, Hards D., Hudson P., Power B., Freed K., and Herington A. C. (1990) Localization of insulin-like growth factor-I mRNA in rat brain by in situ hybridization-relationship to IGF-I receptors. *Mol. Endocrinol.* **4,** 773–778.

Whitfield H. J.. Bruni C. B.. Frunzio R., Terrell J. E., Nissley S. P., and Rechler M. M. (1984) Isolation of a cDNA clone encoding rat insulin-like growth factor-II precursor. *Nature* **312,** 277–280.

Wiggins R. C. (1982) Myelin development and nutritional insufficiency *Brain Res.* **4,** 151–175.

Woods S., Lotter E., McKay L., and Porte D. (1979) Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature* **282,** 503–505.

Xue Z., Le Douarin N., and Smith J. (1988) Insulin and insulin-like growth factor-I can trigger the differentiation of catecholaminergic precursors in cultures of dorsal root ganglia. *Cell Differ. Dev.* 25, 1–10.

Yamashita S. and Melmed S. (1986) Insulin-like growth factor I action on rat anterior pituitary cells: suppression of growth hormone secretion and messenger ribonucleic acid levels. *Endocrinology* **118,** 176–182.

Yang J. W., Raizada M. K., and Fellows R. E. (1981) Effects of insulin on cultured rat brain cells: stimulation of ornithine decarboxylase activity. *J. Neurochem.* **36,** 1050–1 057.

Zapf J., Walter H., and Froesch E. (1981) Radioimmunological determination of insulin-like growth factors I and II in normal subjects and in patients with growth disorders and extrapancreatic tumor hypoglycemia. *J. Clin. Invest.* **68,** 1321–1330.