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Genetics, Chemistry, and Function of the IGF/IGFBP System

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Abbreviations

IGF — insulin-like growth factor
IGFBP — insulin-like growth factor binding protein
IGFBP-RP — insulin-like growth factor binding protein related proteins
BP-Pr — insulin-like growth factor binding protein proteases
IGF-I-R — type 1 IGF receptor

Introduction

The insulin-like growth factors (IGFs), insulin-like growth factors binding proteins (IGFBPs), and the IGFBP proteases *(BP-Pr)* are involved in the regulation of somatic growth and cellular proliferation, in vivo and in vitro. IGFs are potent mitogenic agents, and their actions are determined by the availability of free IGFs to interact with the IGF receptors. The rate of IGF production, clearance, and degree of binding to the IGFBPs modulate the levels of free IGFs in a system. There are six insulin-like growth factors binding proteins that bind to IGFs with high affinity and specificity (IGFBPs). IGFBPs not only regulate IGFs bioavailability but also have IGF-independent actions. IGFBPs are produced by a variety of different tissues, with each tissue having specific levels of several IGFBPs. Additionally, several enzymes capable of proteolyzing IGFBPs have been identified *(BP-Pr)*. The cleavage of IGFBPs by BP-Pr plays a key role in modulating free IGF and IGFBPs levels and actions. There are, at the time of submission of this manuscript, six insulin-like growth factors binding proteins like molecules that bind to IGFs with low affinity. They are called IGFBP-related proteins (IGFBP-RPs) (**Fig. 1**).

The IGFBPs have several functions: (1) prolongation of IGFs half-life in the circulation, (2) prevention of IGF-induced hypoglycemia, (3) regulation of the passage of IGFs from the intravascular to the extravascular space, (4) limitation of the bioavailability of free IGFs to interact with the IGF receptors, (5) enhancement of IGFs actions by the formation of a pool of slow release IGFs, and (6) direct cellular actions mediated through their own receptors, acting independently of IGFs (**Fig. 2**).

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IGFs and IGFBPs in Serum

The growth effects of growth hormone are mediated primarily through the hepatic production of IGF-I. In serum, most of the IGF-I and IGF-II are found in the ternary complex, formed by IGFs, IGFBP-3, as well as IGFBP-5 and the glycoprotein known as the acid labile subunit (ALS) *(1,2)*. Only small amounts of IGFs are carried by IGFBPs as a binary complex, and less than 1% circulate in the free form *(1)*. The ternary complex does not cross the capillary barrier, and ALS is found only in the intravascular space *(3)*. The formation of the ternary complex protects and consequently prolongs the half-life of both IGFBP-3 and IGFs. The half-life of unbound IGFBP-3 is between 30 and 90 min and the half-life of free IGF-I is less then 10 min, while the half-life of the 150 kDa complex is approx 12 h *(4)*. The binding of IGFs to IGFBP-3 and ALS maintains IGFs in the intravascular space for steady delivery of IGF-I in contrast to the pulsatile levels of growth hormone (GH) *(1)*. IGFBP-3 not only extends the serum half-life of IGFs, but also has an important role in the distribution of IGFs. It affects the localization of IGF-I in kidneys and, as a binary complex with IGF-I, functions as a transporter of IGF-I to the extravascular space. IGFBP-5 also forms a ternary complex with IGFs and ALS, although IGFBP-3 has twice the potency to form such complexes. At the tissue level, owing to the absence of ALS, most of the IGFs are bound to the IGFBPs as heterodimers, with only a small amount found in the free form.

The liver is the main source of circulating IGFs even though there is physiologically important production in other tissues with various autocrine and paracrine functions *(5)*. The liver is also the main source of circulating IGFBP-3 and ALS *(3)*, although different components of the liver produce different components of the ternary complex. IGFBP-3 is produced by the hepatic endothelia and

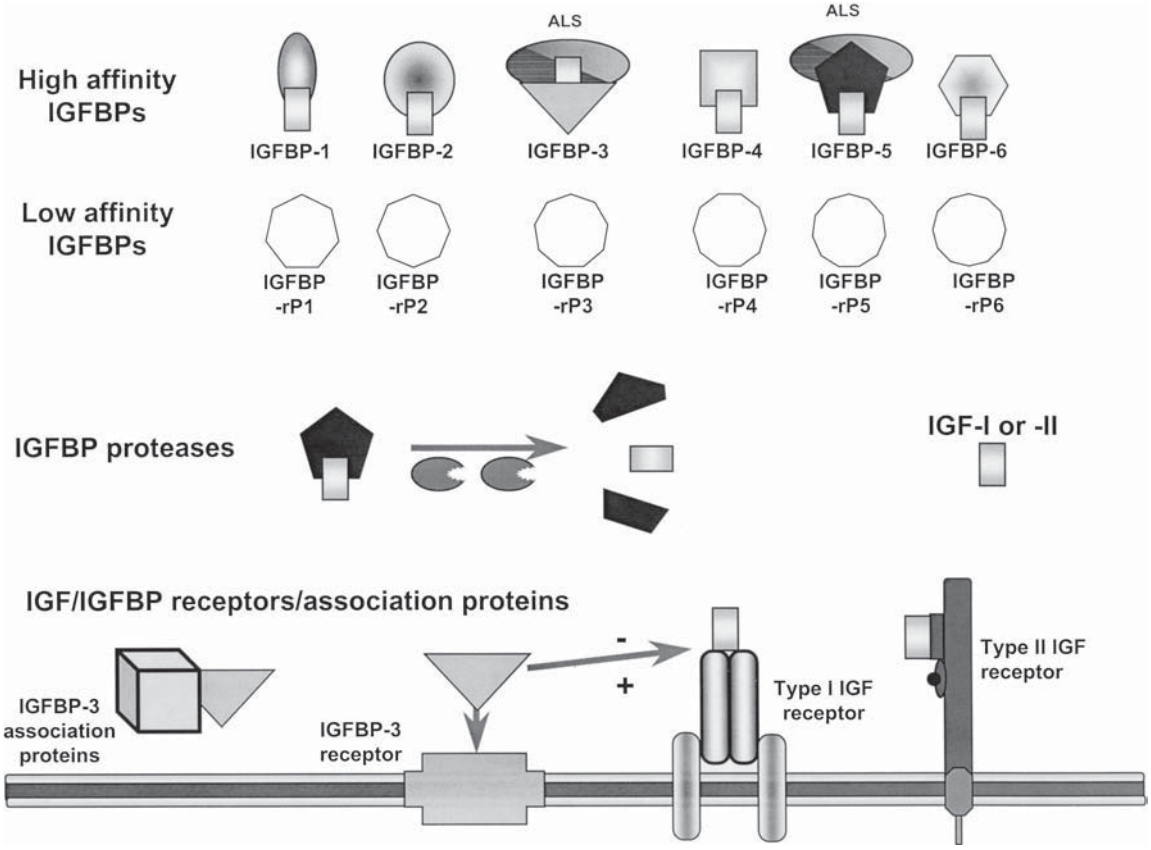

Fig. 1. The components of the IGF axis. See text for details.

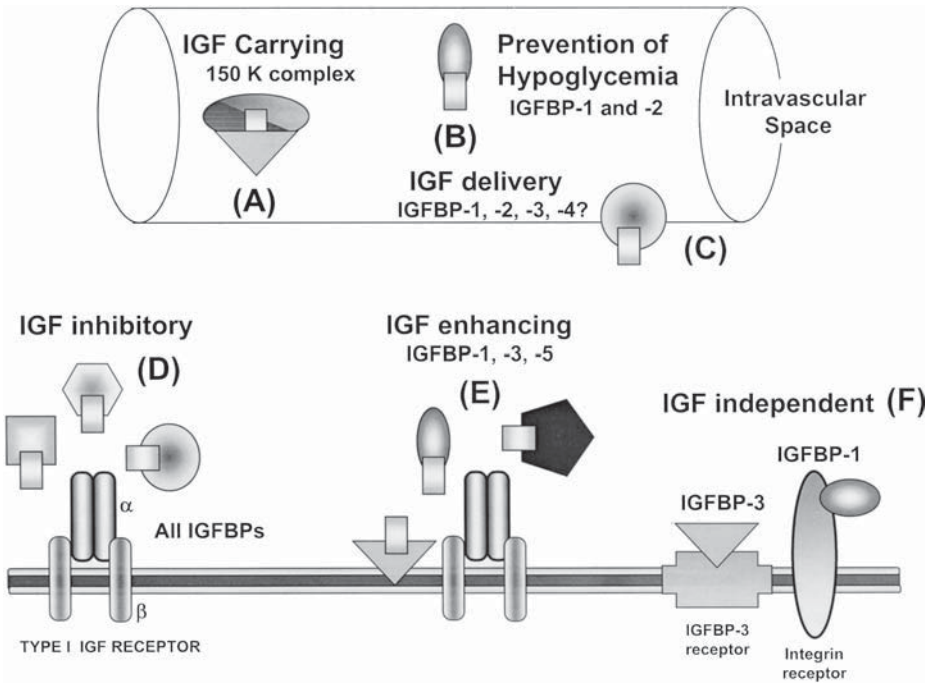

Fig. 2. IGFBP functions. (A) prolongation of IGFs half-life in the circulation, (B) prevention of IGFs-induced hypoglycemia, (C) facilitation of the passage of IGFs from the intravascular to the extravascular space, (D) limitation of bioavailability of free IGFs to interact with the IGF receptors, (E) enhancement of IGFs actions by the formation of a pool of slow release IGFs and presentation of IGFs to the IGF receptor, and (F) direct cellular effect mediated through their own receptors.

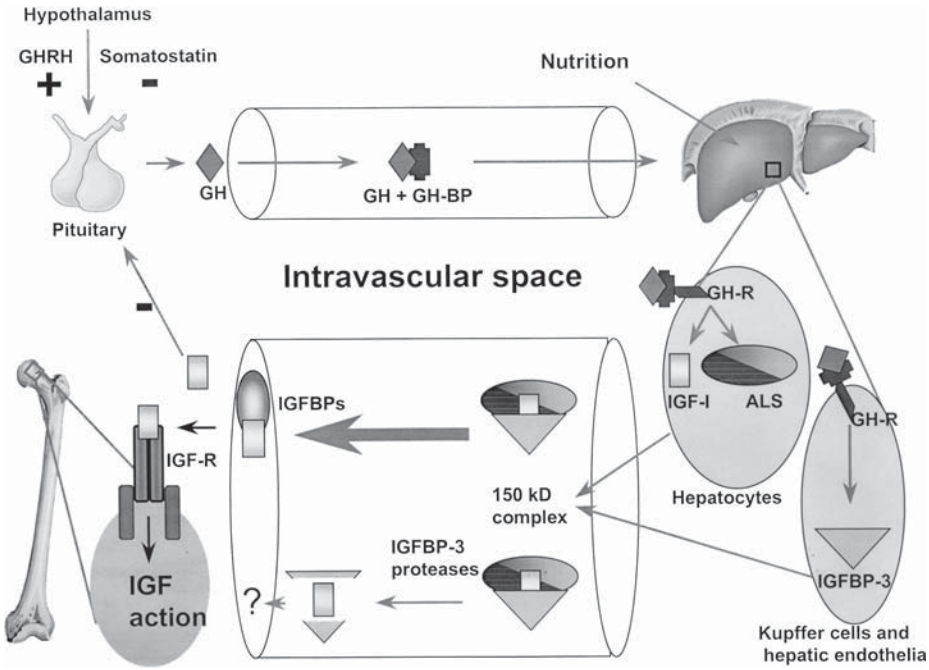

Fig. 3. The GH-IGF axis. See text for details.

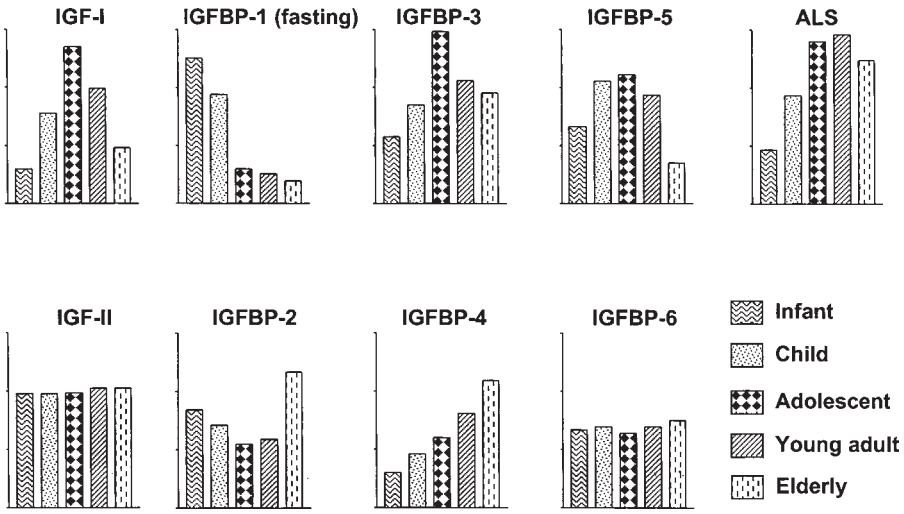

Fig. 4. Age dependent levels of the IGF axis components. See text for details on the developmental aspects of the IGF axis.

by Kupffer cells, and ALS and IGF-I are produced by hepatocytes *(6–8).* The hepatic production of all three components of the 150 kDa complex is regulated by growth hormone *(9),* with reduced serum levels of all three components of the 150 kDa complex in growth hormone deficiency and/or resistance and elevated levels in growth hormone excess *(9–14)* (**Fig. 3**).

IGFBP-3 is the most abundant IGFBP in postnatal serum and its levels do not change acutely *(15).* IGFBP-1 and -2 levels are variable during the day, depending on the metabolic state *(14,16).* The regulators of IGFBP-4 through -6, in serum, have not been well studied at the present time; however, their levels have been shown to be age-dependent *(17–19)* (**Fig. 4**). Serum levels of IGFBP-4 increase with age and correlate with PTH while the serum levels of IGFBP-5 decrease with age, correlating with serum IGF-I levels. IGFBP-4 and IGFBP-5 levels are also increased by GH *(20)*. IGFBP-3 is the predominating IGFBP, with levels (2000–5000 ng/mL) which are an order of magnitude higher than the levels of the other IGFBPs. After feeding, IGFBP-1 has the lowest levels (5–20 ng/mL), 10-fold lower then IGFBP-2, -4, -5, and -6.

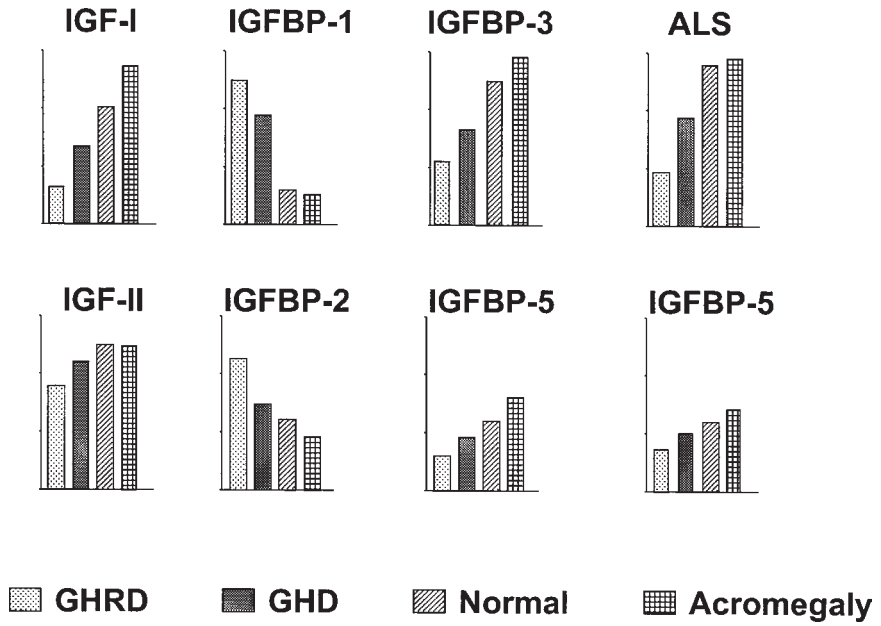

Fig. 5. Serum levels of IGF axis components are regulated by growth hormone levels. Serum levels of IGF-I, IGF-II, IGFBP-3, IGFBP-5 and ALS rise simultaneously with the elevation of growth hormone levels. IGFBP-1 and IGFBP-2 levels are inverse to the growth hormone levels.

Insulin-Like Growth Factors

Biochemistry, Molecular Biology, and Regulation

IGF-I, originally known as sulfation factor and/or somatomedin-C, is the actual mediator of somatic growth. The IGF-I gene is located in the long arm of chromosome 12 *(21)*. IGF-I is a 70 amino acid peptide with a molecular weight of 7.5 kDa and a structure 70% homologous to IGF-II structure and 50% homologous to proinsulin *(22)*. The liver is the main source of serum IGF-I even though there is a physiologically important production of IGF-I at the tissue level *(23)*. IGF-I has not only endocrine but also important autocrine and paracrine functions. Hormones like estrogens, PTH, and glucocorticoid have effects on the IGF-I production *(15,24)*. Growth hormone is the main regulator of hepatic IGF-I production, with low levels in growth hormone deficiency and/or resistance and elevated levels in growth hormone excess (**Fig. 5**). Serum levels of IGF-I are age-dependent with low levels at birth, peak levels during puberty, and steady lowering levels with age *(15,25)*.

In vitro studies showed that IGF-I stimulates cell growth and differentiation in nearly all systems *(26)*. In vivo, IGF-I stimulates growth in the pre- and postnatal periods. Knockout animals demonstrated that IGF-I is also important for prenatal growth and for neurologic development. These mice were small at birth and had neurological abnormalities *(27,28)*. This was later proved to be true in humans by the report of one child with IGF-I deficiency *(29)*. This defect caused low birth weight, progressive failure to thrive, deafness, and mental retardation. Children with growth hormone deficiency or with growth hormone receptor deficiency have normal birth weight, low serum levels of IGF-I, and postnatal growth failure. Replacement of growth hormone and IGF-I, restores growth. These findings demonstrate that during the prenatal period, IGF-I is growth-hormone-independent and is crucial for normal development and growth of many tissues including the brain.

IGF-I has important functions not related to somatic growth. It acts synergistically with insulin in the post-prandial period, as a hypoglycemic hormone. This function is regulated by IGFBP-1, which in turn is regulated by insulin *(30,31)*. It also regulates renal function; increasing renal blood flow, glomerular filtration rate, and proximal tubule re-absorption of phosphate *(32–34)*. Studies in Japanese postmenopausal women showed positive correlation of IGF-I and IGFBP-3 with bone mineral density and a negative correlation with the incidence of spine fractures *(35)*.

Several studies indicate that IGF-I involvement is present not only in normal growth but also in tumor growth. Abnormalities in the IGF axis causing increased levels of free IGF-I are found in dysregulated cell growth (as in malignancies) *(36)*. The association between plasma IGF levels and prostate cancer risk was demonstrated when men with plasma IGF-I levels in the highest quartile had a relative risk to develop prostate cancer of 4.3 when compared with men in the lowest quartile *(37)*. At the present time there are no data to determine if this finding is a cause or an effect. It could be that the elevated IGF-I for many years stimulates the growth of cancer cells, but it could also be that the higher IGF-I is a marker of a system that is developing a tumor.

IGF-II is a 67 amino acid peptide with 7.5 kDa. The IGF-II gene is located on the short arm of chromosome 11 *(22).* IGF-II production is also mainly in the liver. Even though postnatal IGF-II levels are not as dependent on growth hormone as IGF-I, IGF-II levels decrease by 20% in growth hormone-deficient states, probably owing to the decreased levels of binding proteins (Fig. 5). The main regulators of the IGF-II levels are still unknown. Levels are low at birth, increase in the first weeks of life, and then remain stable (Fig. 4). Efstradiatis and his group showed that IGF-II is the most important fetal growth factor but has no effect on postnatal somatic growth *(38).* It is also important in the placental growth, where high levels of IGF-II exist. In vitro studies showed that even though IGF-II binds to IGF-I receptor with 2–15-fold less affinity than IGF-I, IGF-II produced most of its effects through the type I IGF receptor *(26).*

Similar to IGF-I, IGF-II is linked to several tumors. Adrenal cortical malignant tumors have 10 times higher IGF-II concentration than benign tumors or normal glands *(39).*

IGFs Receptors

The type I IGF receptor, a heterotetramer with a structure similar to the insulin receptor, mediates most of the effects of IGFs. It is a tyrosine kinase receptor with two transmembrane beta subunits that are linked to two extracellular alpha subunits by disulfide bonds (Fig. 1). Disulfide bonds also link the two alpha subunits. The alpha subunits have areas rich in cysteine giving them the specificity to bind IGFs. The IGF-I receptor has a molecular weight of 440 kDa and even though it has a 50% homology to the insulin receptor, the affinity of this receptor to insulin is two orders of magnitude smaller than to IGF-I *(26).* The demonstration of hybrid receptors, with one alpha and one beta subunit from the insulin receptor and another half from the type I IGF receptor explain the difficulties in trying to differentiate the effects of each receptor *(26).*

The IGF-II receptor (mannose-6-phosphate receptor) is structurally different from the type I IGF and insulin receptors and its physiologic functions are still under investigation. Most of the studies demonstrate that this receptor functions mainly as a scavenger, facilitating the degradation of IGF-II. It binds IGF-I poorly and does not bind insulin. The IGF-II receptor has been shown to be deleted in some cancers, indicating that it may serve as a tumor suppressor by removing IGF-II.

Evolutionary and Physiological Aspects of Insulin-Like Growth Factor Binding Proteins (IGFBPs)

There are six high-affinity IGFBPs identified to date, and at least four IGFBP-RP have been proposed. All six IGFBPs have at least 50% homology among themselves and 80% homology between different species *(13,40).* Most of the homology is conserved in the N- and C- terminal regions, while the middle region has little similarity between the different IGFBPs *(1).* The IGFBPs have a highly conserved set of at least 16 cysteines, which shape their structure *(15,23).*

IGFBP genes are in close proximity to the Homeobox gene clusters (Hox A through Hox D) and appear to have evolved together. It is speculated that both Hox and IGFBP genes originated from single genes that underwent coordinated duplications and translocations several times *(41–43).* The Hox genes are DNA binding proteins, and like some of the IGFBPs, are transcriptionally regulated by retinoic acid. Chromosomes 2 and 7 encode two binding proteins each and all of these four binding proteins have 18 cysteines *(26,43).* IGFBP-1 and IGFBP-3 genes are in chromosome 7, next to the Hox-A gene. IGFBP-1 has an Arg-Gly-Asp (RGD) sequence and is related to carbohydrate metabolism while IGFBP-3 is involved primarily in growth *(23).* Similarly, on the long arm of chromosome 2, next to Hox-D, IGFBP-2 also has an RGD sequence and correlates with the metabolic state while IGFBP-5 functions, as expected owing to its high homology with IGFBP-3, are primarily growth-related. The IGFBP-6 gene, with 16 cysteines and next to Hox-C, is found on chromosome 12 *(41–44),* and IGFBP-4, with 20 cysteines, is localized at the long arm of chromosome 17, next to Hox-B *(26,41–44).*

Insulin-Like Growth Factor Binding Protein 1

Biochemistry, Molecular Biology, and Regulation

IGFBP-1 is a 25 kDa protein with an RGD sequence in its structure *(40,45).* RGD is a recognition sequence for membrane integrin receptors, which suggests the possibility of an IGF-independent action via these receptors *(45).* The effects of IGFBP-1 are still under investigation. However, both inhibitory and stimulatory activity have been reported. IGFBP-1 has serine residues that can be phosphorylated. Phosphorylation of IGFBP-1 substantially increases its affinity for IGF-I and is probably involved in the regulation of IGFBP-1 function. Phosphorylated forms, in general, inhibit IGF-I action while the de-phosphorylated forms appear to have stimulatory activity *(46,47).*

IGFBP-1 is produced in the liver, decidua, and kidneys and is the most abundant IGFBP in amniotic fluid. Serum IGFBP-1 levels are regulated mainly by metabolic factors. After meals, IGFBP-1 levels fall to less than 10 ng/mL, while during fasting, IGFBP-1 levels rise to over 100 ng/dl *(15,23).* In children with ketotic hypoglycemia who underwent diagnostic fasting studies, the IGFBP-1 levels were as high as 700 ng/dl at the time of the hypoglycemia *(30).* Insulin and corticosteroids are the main regulators of serum IGFBP-1 levels, through the transcriptional control of the hepatic production of IGFBP-1 *(6).* Insulin inhibits the synthesis of IGFBP-1 resulting in elevated levels during low insulin states such as intrauterine growth retardation, fasting, or poorly controlled type 1 diabetes *(14,16,48–51).* The inverse has also been shown, with decreased levels in

conditions of hyperinsulinemia, such as the post-prandial period, obesity, large-for-gestational-age babies, insulinomas, and congenital hyperinsulinism with hypoglycemia *(30)*. Glucocorticoids and glucagon stimulate IGFBP-1 production in synergism with low levels of insulin *(31,51–55)*. In chronic renal failure, owing to increased renal production, IGFBP-1 levels rise *(15)*. In diabetes and renal failure high IGFBP-1 levels are thought to have a pathophysiological role, presumably decreasing the levels of free IGFs to interact with the IGF-I receptor, and may be responsible for the decreased linear growth seen in these conditions. Some cytokines (TNF-, IL-1, and IL-6) are also involved in the regulation of IGFBP-1 *(56)*. The increase in serum and hepatic concentrations of IGFBP-1 caused by these pro-inflammatory agents may be involved in the up-regulation of IGFBP-1 in catabolic states *(56)*.

IGF Inhibiting Actions

Transgenic mice, which overproduce IGFBP-1, have significantly lower birthweights, poorer postnatal weight gain, and disproportionately smaller brains compared to wild-type *(57)*. These transgenic mice also manifest fasting hyperglycemia, impaired glucose tolerance, and reduced fertility, suggesting that IGFBP-1 inhibits metabolic as well as growth-promoting effects of IGFs *(57)*. Another transgenic mouse, which had equally high levels of serum IGFBP-1, did not confirm the growth retardation observed in the first model *(58)*.

IGFBP-1, in vivo, helps to protect the organism from the hypoglycemic effects of IGF-I. Without this mechanism, IGF-I could cause hypoglycemia during fasting. IGFBP-1 levels rise in the fasting state, suppressing the hypoglycemic effect by binding to IGFs and decreasing the levels of free IGF-I. The administration of IGFBP-1 to rats caused a transient elevation in blood glucose, corroborating this theory *(59,60)*. Another proposed inhibitory function for IGFBP-1 may occur during fetal development in certain conditions. In fetuses with poor placental supply, low availability of nutritional factors cause insulin levels to be low and consequently levels of IGFBP-1 to rise. The rise in IGFBP-1 levels sequesters free IGF-I from the circulation so the use of nutrients for fetal growth will decrease in detriment to survival. Similarly, large-for-gestational-age fetuses that have low serum IGFBP-1 (due to high insulin) have a decreased inhibition of IGFs and thus are large.

Consistent with the in vivo effects of IGFBP-1 inhibiting the hypoglycemic effects of IGF-I, several studies in vitro have shown that IGFBP-1, when added to cells in culture, inhibits IGF-I stimulated growth. The inhibition of IGF-I action by IGFBP-1 does not occur when IGF-I analogues with low affinity to the IGFBPs are added to the system, demonstrating an IGF-dependent mechanism of inhibition *(26)*.

IGF Stimulating Actions

Even though IGFBP-1 inhibits mitosis by removing free IGF-I from the extracellular space, in some in vitro systems IGFBP-1 stimulates growth. IGFBP-1, in the presence of low concentrations of platelet-poor plasma and IGF-I, stimulated DNA synthesis in porcine aortic smooth muscle cells, chick embryo fibroblasts, and mouse embryo fibroblasts *(61,62)*. Koistinen et al. concluded that IGFBP-1 caused slow and steady release of IGF-I when they found that concentrations that can inhibit IGF-I binding to its receptor sometimes enhance IGFs-stimulated thymidine incorporation. The inhibition did not occur when IGFBP-1 was added without IGF-I, suggesting an effect due to the slow release of IGF-I and not due to a direct effect of IGFBP-1 *(63,64)*. Gagliano et al. demonstrated the stimulatory effect of IGFBP-1 in vivo. IGFBP-1 stimulated healing in rabbit ears when added with IGF-I. They also demonstrated that this effect is dependent on the binding of IGFBP-1 to the integrin receptor *(65)*.

IGFBP-1 is involved in IGF-I transport through the capillary barrier, a process that in certain tissues appears to involve an insulin-dependent mechanism *(60,66)*.

Insulin-Like Growth Factor Binding Protein 2

Biochemistry, Molecular Biology, and Regulation

IGFBP-2 is a 31 kDa protein *(40)*. Like IGFBP-1, IGFBP-2 has an RGD sequence although it has not been demonstrated to bind integrin-type receptors. However, IGFBP-2 has been shown to be cell-surface-associated via other, unknown, mechanisms *(67)*. IGFBP-2 is neither phosphorylated nor glycosylated.

The levels of IGFBP-2 are age-dependent, with high levels seen in infancy and older age and low levels in young adults *(68)* (Fig. 4). IGFBP-2 is the major IGFBP in cerebrospinal fluid, owing to its production by multiple neural tissues *(69)*, and IGFBP-2 levels are elevated in the spinal fluid of patients with some forms of CNS tumors *(70)*. The concentration of IGFBP-2 in seminal plasma is greater than that of any IGFBP in any biological fluid, about 10,000 ng/mL *(68)*. Growth hormone deficiency causes an increase in the levels of IGFBP-2 in serum *(15,71)* (Fig. 5), even though growth hormone seems to have no direct effects on IGFBP-2 gene expression in cultured cells. The regulation of IGFBP-2 levels is also dependent on the metabolic state and the insulin level albeit, to a lesser degree than IGFBP-1 *(72)*. IGFBP-2 levels increase with prolonged fasting and are more sensitive to protein restriction than to caloric restriction, being elevated in malnutrition and anorexia nervosa *(16)*. In patients with low levels of insulin, such as untreated insulin-dependent diabetes mellitus, levels of IGFBP-2 are elevated and return toward normal with chronic insulin therapy *(50)*. The levels of IGFBP-2 are

also altered in some tumors, including prostate cancer. The levels are higher in malignant adrenal tumors, owing to the post-transcriptional regulation, than in benign adrenal tumors *(39). In vitro*, IGFBP-2 is produced in multiple cell systems *(70,72).* The in vitro regulators in most cell systems remain largely unknown; however, in human neuroblastoma cells, retinoic acid down regulated IGFBP-2 expression *(59).*

IGF Inhibiting Actions

IGFBP-2 has mainly inhibitory effects on IGF-mediated functions. Studies performed with multiple cell lines demonstrated that IGFBP-2 inhibits cell growth and multiplication *(26).* In IEC-6 cells, the inhibition of IGFBP-2 mRNA expression causes stimulation of growth *(73)* and the reverse also occurred, since the inhibition of growth by TGF-β is associated with the induction of IGFBP-2 mRNA *(74).* In prostatic stromal cells from patients with benign prostatic hyperplasia, the IGFBP-2 levels are reduced, probably causing increased levels of free IGFs, facilitating tissue growth *(75).* The mechanism by which glucocorticoids inhibit lung growth is in part through the induction of IGFBP-2 production *(76).* Nevertheless, targeted disruption of the IGFBP-2 gene in mice did not result in an altered phenotype *(77).*

Insulin-Like Growth Factor Binding Protein 3

Biochemistry, Molecular Biology, and Regulation

The molecular weight of IGFBP-3 in its nonglycosylated form is 29 kDa *(78).* IGFBP-3 has 3 glycosylation sites and is present in the circulation in the glycosylated state, with a molecular weight between 40 and 44 kDa *(23).*

Serum levels of IGFBP-3, as well as IGFs, are age-dependent, being low at birth and increasing during childhood to reach a peak during puberty, after which levels start to decrease *(23,25)* (Fig. 4). Nutritional status plays an important role in controlling serum IGF-I with low IGF-I levels observed in chronic diseases and malnutrition *(14,16,49).* The levels of IGFBP-3, however, are less affected by these conditions *(14).* Estrogens, parathyroid hormone (PTH), and glucocorticoids are other regulators of IGFs and IGFBPs in the circulation *(15,24).*

The mechanism by which growth hormone stimulates IGFBP-3 production is still under investigation. The three proposed mechanisms are (1) a direct effect of growth hormone on Kupffer cells, (2) an indirect effect mediated by IGF-I, and (3) stimulation of nonhepatic tissues. Support for the first mechanism came from studies of human hepatocarcinoma cells that demonstrated that GH increased IGFBP-3 gene expression independently of IGF-I *(79).* In vivo studies also supported the first mechanism. A child with IGF-I deficiency, owing to a partial deletion of the IGF-I gene, had elevated levels of GH, undetectable levels of IGF-I, and normal levels of IGFBP-3. In another study, in a large population of growth hormone receptor deficient patients from Ecuador, the administration of IGF-I stimulated growth, but did not change IGFBP-3 levels by RIA *(9,80).* The second mechanism is supported by other studies, in vivo and in vitro. In a similar population of growth hormone receptor-deficient children (from Israel), IGF-I therapy caused increased levels of IGFBP-3 by Western ligand blotting; however, IGFBP-3 levels by RIA were not performed *(81).* One of the explanations for this finding is that IGF-I did not stimulate the production of IGFBP-3 but instead protected IGFBP-3 from proteolysis. In some animal models, IGF-I induces the serum levels of IGFBP-3 detected by ligand blotting *(82). In vitro* studies have shown production of IGFBP-3 in liver cells after IGF-I but not after growth hormone stimulation *(83).* The in vitro support for the third mechanism came from studies in bone cells. Both unchanged levels of IGFBP-3 in response to growth hormone *(84)* and an increase in IGFBP-3 expression after growth hormone (but not IGF-I) stimulation were found *(85).* Depending on the tissue studied, IGFBP-3 expression has been shown to be regulated in vitro by interleukin-1 *(86),* tumor necrosis factor-alpha (TNF-α) *(86,87),* transforming growth factor-beta (TGF-β) *(88),* retinoic acid *(89),* PTH, osteogenic protein-1 *(90),* estradiol *(91),* prostaglandin E_2 *(92),* glucocorticoids *(24),* and p53 *(93).* Though considered predominantly a serum and extracellular protein, IGFBP-3 has been shown to localize to nuclei of lung cancer cells *(94).*

IGFBP-3 can promote or inhibit growth both in vivo and in vitro. Effects of IGFBP-3 can be either IGF-mediated or IGF-independent. Transgenic mice have been developed that express a human IGFBP-3 transgene in small bowel, colon, and kidney as detected by Northern analysis, but do not have increased serum IGFBPs by Western ligand blotting *(95).* Of note, the majority of serum hIGFBP-3 in these transgenic mice was not associated with ALS. Compared to genetically related, wild-type mice and to nontransgenic littermates, the transgenic mice demonstrated selective organomegaly affecting heart, liver, and spleen *(95).* Birth, body, brain, and kidney weights as well as litter size did not differ significantly between transgenic mice and nontransgenics *(95).* In a different model, rat ventral prostate following castration rapidly increased gene expression of IGFBP-2 through -5 in association with apoptosis *(96).* In another model, the hIGFBP-3 gene was transfected into a cell line derived from an IGF receptor "knockout" mouse, which is nonIGF responsive *(97).* Cell growth decreased significantly in this model, proving definitively that IGFBP-3 inhibits cell growth independently of the type 1 IGF receptor (IGF-I-R) *(97).* Later in this review we will detail further support for IGFBP-3 as an IGF-independent modulator of cell growth.

IGF Inhibiting Actions

Different cell systems have shown different effects of IGFBP-3 on IGF-I function. Multiple in vitro studies showed that IGFBP-3 in solution decreases the stimulatory effects of the IGFs, in most of the cases by preventing IGFs from binding to their receptors since the affinity of IGF to IGFBP-3 is higher than to the IGF-I receptor *(98).* The addition of IGFBP-3 to a variety of cells, in competition with IGFs, results in attenuation of IGF-mediated mitosis as well as metabolic actions *(5,9).* The inhibitory effects of several hormones such as TGF-β and retinoic acid may be mediated by stimulation of IGFBP-3 *(87).*

IGF Stimulating Actions

Studies by DeMellow and Baxter and by Conover et al. showed that even though, in the same cell system, the addition of IGFBP-3 to culture media caused inhibition of cell growth, pre-incubation with IGFBP-3 followed by its removal caused potentiation of the IGF-I effects *(98,99).* The conclusion was that the presence of large amounts of IGFBP-3 caused reduction in the free IGF levels, while small amounts of IGFBP-3 protected IGFs, intensifying their effects. Conover et al. also showed that IGFBP-3, when added to the conditioned media, inhibits IGF-I action, probably owing to removal of free IGF-I. When cells were pre-incubated with IGFBP-3 and then washed, low-molecular-weight forms of IGFBP-3, probably representing proteolyzed fragments, were bound to the cell membrane. In this condition, the IGF-I effects were enhanced *(98).* The proposed mechanism for the difference between the effects of pre-incubated and soluble IGFBP-3 is that the IGFBP-3 fragments bound to the cell membrane have an order of magnitude with a lower affinity to IGF-I than the intact soluble IGFBP-3, which has a higher affinity to the IGFs than the IGF-R. In fact, the affinity of IGF-I to the membrane-bound IGFBP-3 fragment is lower than that of the type I IGF receptor. Thus, IGFBP-3 might function as a reservoir of IGF-I, presenting and slowly releasing IGF-I to interact with its receptor, while protecting the receptor from down-regulation. In support of this latter concept, Conover and Powell showed that the IGF-I receptor down-regulation induced by IGF-I can be prevented by IGFBP-3 by regulating the availability of IGF-I to bind to its receptor *(100).*

In vivo studies have shown that the topical use of IGFBP-3 in association with IGF-I causes better wound healing then the use of IGF-I alone *(101).* In another study, the administration of IGFBP-3 and IGF-I to growth hormone-deficient rats caused better growth than the administration of IGF-I alone *(102).* In the same study, however, IGFBP-3 protected the rats from the hypoglycemic effects of IGF-I. Thus, it appears that IGFBP-3 targets IGF-I toward growth provocation and away from the insulin-sensitive glucose-consuming tissues.

Insulin-Like Growth Factor Binding Protein 4

Biochemistry, Molecular Biology, and Regulation

IGFBP-4 is found with its predicted molecular weight of 24 kDa or in the glycosylated form with a molecular weight of 28 kDa *(15).* IGFBP-4 has been identified in all biological fluids *(5).* The serum levels increase with age (Fig. 4). Different cell types produce IGFBP-4 locally, including fibroblasts, neuroblastoma cells, prostate cells, and bone cells *(70,103).* There is demonstrable binding of IGFBP-4 to cell membranes (the function of which is unknown) but IGFBP-4 is found mainly in the extracellular soluble form *(4).* The regulatory mechanisms affecting IGFBP-4 expression are still poorly understood, but at least in bone, IGFBP-4 is regulated by vitamin D and PTH *(4,103),* while in neuroblastoma cells, retinoic acid inhibits IGFBP-4 *(69).*

IGF Inhibiting Actions

All studies using IGFBP-4 in different cell lines reported so far showed an IGF inhibitory effect *(46,103,104).* IGFBP-4, in solution, binds to IGF-I competitively, decreasing its binding to the type I IGF receptor. It does not affect IGF-induced cell proliferation when IGF-I analogs with low affinity for IGFBP-4 but with normal affinity for the IGF-I receptor are used *(103).* The inhibition of IGF activity by IGFBP-4 was confirmed to be a consequence of the diminution of free IGF-I from the extracellular environment by the addition of recombinant IGFBP-4 *(46,103,105)* and by the transfection of IGFBP-4 into cell lines *(104).* The regulation of the IGF inhibitory IGFBP-4 function is protease-dependent, as discussed below.

Insulin-Like Growth Factor Binding Protein 5

Biochemistry, Molecular Biology, and Regulation

IGFBP-5 has a molecular weight of 29 kDa and it can be found in several glycosylation forms with molecular weight between 29 and 32 kDa *(15).* Like IGFBP-3, IGFBP-5 levels decrease with age, starting after puberty (Fig. 4). The levels in older women are approx 30% of teenagers *(18,19).* Growth hormone treatment of growth hormone-deficient children or adults increases the serum levels of IGFBP-5 (Fig. 5). In adults, the change in serum IGFBP-5 correlated with lumbar bone mass density and total alkaline phosphatase activity. Fetal tissues have high levels of IGFBP-5 during rapid growth *(25),* while levels in adult tissues vary. CSF and connective tissues have substantial concentrations of IGFBP-5, and IGFBP-5 is the main IGFBP expressed in the kidneys *(15).* Unlike the others IGFBPs, IGFBP-5 strongly binds to bone cells due to its high affinity for hydroxyapatite *(72).* Like IGFBP-3, IGFBP-5 binds to endothelial cell monolayers and is found in large concentrations in the extracellular matrix. The binding to the endothelial cells is competitively inhibited

by heparin and heparan sulfate, and alterations in the C-terminal region of IGFBP-5 inhibit the binding to the cells but not to the extracellular matrix *(106)*. The binding of IGFBP-5 to the extracellular matrix is on an ionic basis. When bound to the extracellular matrix the affinity of IGFBP for IGF-I is reduced seven- to twelve-fold when compared to the intact IGFBP-5 in solution *(46,107)*.

The mechanisms by which IGFBP-5 is regulated are still being unraveled. Bone cells produce large amounts of IGFBP-5 and levels decrease with the progression of maturation due to protease activity, as will be discussed subsequently. Treatment of osteoblastic cells with fibroblast growth factor, transforming growth factor beta (TGF-β), platelet-derived growth factor BB, IGFBP-2, and IGFBP-3 cause a decrease in the IGFBP-5 expression *(108)*. In the same cell systems, treatment with IGF-I and retinoic acid cause the inverse effect, with increased levels of IGFBP-5 mRNA *(109)*. The levels of IGFBP-5 in fibroblasts and osteoblast-like cells decreased when glucocorticoids were added to the cells *(24,110)*. In smooth muscle cells, IGFBP-5 mRNA expression is stimulated by IGF-I by a mechanism which is time- and dose-dependent and cell type specific *(111)*.

IGF Inhibiting Actions

IGFBP-5 inhibits the growth of a variety of cells. In granulosa cells, the levels of IGFBP-5 rise during the degenerative phase *(72)*. Since IGF-I promotes maturation and proliferation of the follicles, it was speculated that IGFBP-5 inhibits IGF-I action *(72)*. It has also been shown that FSH promotes granulosa cell proliferation by reducing intact IGFBP-5 levels (in a process which involves induction of a specific binding protein protease) *(72)*. Ling showed that IGFBP-5 inhibited granulosa cell steroidogenesis stimulated by IGF-I *(112)*. In the kidneys, IGFBP-5 may also inhibit growth, since it is expressed inversely with renal tissue growth status *(72)*. IGFBP-5 inhibits glycogen and DNA synthesis in osteosarcoma cells, a process that is IGF-I-dependent *(105)*. Porcine smooth muscle cells produce IGF-I, IGFBP-5, and an IGFBP-5 protease. The use of a protease-resistant IGFBP-5 inhibited IGF-I effects. This demonstrates that IGFBP-5 proteolysis serves to regulate IGF-I action *(113)*.

IGF Stimulating Actions

Several in vitro studies showed that IGFBP-5 stimulated IGF-I actions when compared to IGF-I alone *(46,114)*. The IGF-enhancing actions of IGFBP-5 are particularly evident in bone cells. Studies suggest a need for IGFBP-5 to be bound to the cell membrane or extracellular matrix to cause this potentiating effect *(46,114)*. IGFBP-5, when bound to the extracellular matrix, has a lower affinity for IGF-I than the free form but has a prolonged half-life *(47,107)*. The binding of IGF-I to the matrix-bound IGFBP-5 facilitates subsequent binding of IGF-I to its receptors *(47,107)*.

Insulin-Like Growth Factor Binding Protein 6

Biochemistry, Molecular Biology, and Regulation

IGFBP-6 is an O-glycosylated protein *(115)* with a predicted molecular weight of 34 kDa *(5)*. In humans, IGFBP-6 is found predominantly in CSF and serum *(15)*. It is the only IGFBP that preferentially binds to IGF-II by over two orders of magnitude better than to IGF-I *(115–117)*. In osteoblastic cells, the addition of an excessive concentration of IGFBP-6 inhibited IGF-II-stimulated DNA and glycogen synthesis but had minimal effects on inhibiting IGF-I actions *(116)*. IGFBP-6 is expressed in ovarian cells, prostatic cells, fibroblasts and other cells *(115)*. IGF-II and other hormones regulate the expression of IGFBP-6. Studies in breast carcinoma cell lines showed that, in the cells studied, only estrogen receptor negative cells produced IGFBP-6, and that its expression was not changed by IGF-I but was enhanced by retinoic acid *(118)*. In human osteoblast cells, retinoic acid increases IGFBP-6 expression by more than 1000% *(119)*.

The levels of IGFBP-6 in children with chronic renal failure are at least five times higher than the levels in normal controls. These levels correlated with glomerular filtration rate and did not change with hGH therapy *(120)*.

IGF Inhibiting Actions

In vitro studies have shown that when added to tissue culture media, IGFBP-6 inhibits IGF-II binding, suppresses IGF-II-dependent myoblast differentiation and proliferation, but does not affect IGF-I-dependent functions *(121,122)*. In osteoblastic cells, the addition of an excessive concentration of IGFBP-6 also inhibited IGF-II-stimulated DNA and glycogen synthesis but had only a relatively lower effect on inhibiting the IGF-I effects *(1)*.

IGFBP Proteases

An extensive review of IGFBP proteolysis is beyond the scope of this review. The proteolysis of the IGFBPs is probably an essential piece of the intricate and complex regulation of IGF action. In general, fragmented binding proteins have decreased affinity for IGFs, causing release of free IGFs to interact with its receptor. In serum, the proteolysis of IGFBP-3 releases IGF-I and may allow IGF-I to be transported to the extravascular space. In the extravascular space, IGFs are bound to various IGFBPs and specific proteases presumably promote the release of IGFs at the tissue level. As mentioned earlier, the IGFBP-3 bound to the cell membrane that causes stimulatory effects over IGF action, is a fragment, due to local proteolysis, with lower affinity for IGFs *(98)*.

The BP-Prs were first described in pregnancy serum as a proteolytic activity against IGFBP-3 *(123).* Since then, BP-Prs have been described in many other clinical situations, in various body fluids, and have been shown to cleave IGFBP-2 through –6 with varying specificity. The molecular nature of some of these proteases is being unraveled and at least three classes of BP-Prs have been recognized. These include kallikreins *(124,125),* cathepsins *(126–129),* and matrix metalloproteinases proteases (MMPs) *(130–132).*

Kallikrein-like serine proteases, which cleave IGFBP-3, include PSA, gamma-nerve growth factor, and plasmin *(133).* In fact, plasmin degrades multiple IGFBPs *(133).* Thrombin, another serine protease, cleaves IGFBP-5 at physiologically relevant concentrations (i.e., within one order of magnitude of fibrinogen, its natural substrate) *(134).*

Cathepsins are intracellular proteinases which activate under acidic conditions and may be relevant to certain physiologic and pathological processes including neoplastic infiltration. Under these conditions in vivo, a high release of hydrogen ions may provide an acidic environment for extracellular cathepsin action which through interaction with the IGF axis may be related to cell growth rates *(135).*

MMPs (also called matrixins) comprise a family of peptide hydrolases (2,800 to 92,000 kDa) responsible for the degradation of extracellular matrix components, such as collagen and proteoglycans, in processes involving tissue remodeling. These peptide hydrolases require a metal ion for their catalytic activity and thus are inactivated by metal chelators as well as by specific inhibitors. An IGFBP protease in mouse pregnancy serum belongs to the MMP family, as does a zinc-dependent protease produced by dermal fibroblasts. MMPs have been identified in prostatic fluid and cells. The IGFBP protease induced in airway smooth muscle cell culture by inflammatory agents (leukotriene D4 and interleukin 1-β) has been identified as MMP-1 by immunoblotting and immunoprecipitation techniques *(136).*

Pregnancy serum has elevated levels of proteases, more specifically, MMPs *(130).* Protease activity regulating the IGF-IGFBPs axis is present from early pregnancy *(130)* with MMPs and serine proteinases secretion being documented during trophoblast invasion *(130).* During pregnancy, serum levels of MMPs progressively increase, causing reduction in the levels of intact IGFBP-3. IGFBP-2, -4, and -5 also undergo proteolysis during pregnancy *(137–139).* The increased activity of BP-Prs during pregnancy probably causes increased levels of free IGF-I, and consequently, stimulation of placental and fetal growth. When chick embryo fibroblasts were exposed to IGF-I and IGFBP-3, the IGFBP-3 inhibitory effect on IGF-I action was more pronounced in normal serum than in pregnancy serum. The authors concluded that the fragmented IGFBP-3 released IGF-I faster *(140,141).*

IGFBP proteases are also important autocrine/paracrine growth regulators. They have been implicated in physiologic processes such as ovarian follicular growth and atresia *(142).* IGFBP proteases may be the critical elements in malignant and benign proliferative diseases including prostate cancer and airway smooth muscle hyperplasia of long-standing asthma *(132).*

IGFBP-4 proteases have been identified in neuroblastoma cells, smooth muscle cells, and fibroblasts *(46).* The IGF enhancing activity of IGFBP-4 proteases was also demonstrated using protease-resistant forms of IGFBP-4. IGFBP-4 proteases are activated by the IGFs, predominantly by IGF-II *(143,144).* In vitro studies using porcine vascular smooth muscle cells showed that elevation in the glucose concentration of the conditioned-media induced IGFBP-4 proteolysis, which resulted in increased availability of IGF-I to interact with the receptor *(145).* This mechanism was postulated as one of the causes for some of the abnormalities in diabetes mellitus. Recently, a novel IGFBP-4 protease was cloned *(146).*

IGFBP-5 activity is also modulated by proteases. Similar to IGFBP-3 and unlike IGFBP-4, IGFBP-5 is protected from protease activity when bound to IGFs. IGFBP-5 is found in high concentrations in the extracellular matrix. When bound to the matrix, IGFBP-5 is protected from BP-Pr activity *(46).*

IGFBP-6 glycosylation protects the protein from proteolysis *(147).*

Regulation of IGFBP proteolysis is a new area of investigation, and recent papers have revealed intriguing observations. *In vivo,* the IGFBP-3 protease activity detectable in the serum of newly-diagnosed youngsters with insulin-dependent diabetes mellitus appears to be reversible by insulin *(148).* IGFs stimulate but IGFBPs inhibit an IGFBP-4 protease of MC3T3-E1 osteoblasts *(149).* Similar findings have been demonstrated in dermal fibroblasts *(150).* The IGFBP-3 protease secreted by MCF–7 breast cancer cells can be inhibited by IGFs, suggesting a unique loop by which IGFs can regulate their own activity *(151).* Such findings implicate the relative proportion of IGFs to IGFBPs to be a critical regulator of IGFBP proteases.

IGFBPs with IGF Independent Function

Several studies have shown a direct effect of the IGFBPs on cell growth, which is independent of the IGFs. At the present time, only IGFBP-1, -3, and -5 have been shown to have independent functions but such actions are postulated for IGFBP-2 and -4 as well *(152,153).*

The discovery of IGF-independent modulation of growth by IGFBPs provided evidence for the presence of specific cell-surface IGFBP receptors and added a further layer of complexity to the IGF axis. An accumulating body

of evidence has revealed that IGFBP-3 has important IGF-independent effects in vitro on growth regulation in multiple cells and tissues. Unfortunately, in vivo studies to date (including those in transgenic animals or with infusions into human subjects) have not shown direct, inhibitory, IGF-independent effects of IGFBP-3, even though such models produced high serum levels of free IGFBP-3.

The existence of cell-surface IGFBP-3 association proteins/receptors was first suggested by Oh, et al., who demonstrated specific, dose-dependent binding of IGFBP-3 to breast cancer cell surface proteins of 20, 26, and 50 kDa *(94,153).* In these estrogen-receptor-negative breast cancer cells, the inhibitory effects of IGFBP-3 on growth were shown to be dose-dependent and diminished by co-incubation with IGFs but not by IGF analogs with reduced affinity for IGFBP-3.

We have also shown that IGFBP-3 had independent inhibitory effects on cell growth, utilizing a cell transfection system *(152).* Valentinis et al. showed inhibition of growth by IGFBP-3 in cells derived from an IGF-I receptor "knockout" mouse, and demonstrated that the IGF-IGF receptor interaction is not involved in this IGFBP-3 effect *(154).* In addition, these cells demonstrated marked growth inhibition when transfected with a vector containing the IGFBP-3 gene *(154).* This growth inhibition correlated with the magnitude of IGFBP-3 expression in these clones *(154).* Because these cells did not express IGF receptors, the growth-inhibiting effects of IGFBP-3 clearly were mediated, not through an IGF-I-R pathway, but presumably through a novel, IGFBP-3-specific pathway.

In cultured ovarian granulosa cells, DNA synthesis is stimulated by follicle-stimulating hormone. In these cells, IGFBP-3 inhibited DNA synthesis independently of the presence of IGFs. To further exclude the possibility of an effect mediated through IGFs, IGFBP-2, which should also bind IGFs, was added, but similar inhibition was not obtained *(155).* The synthesis of DNA by embryonic fibroblasts is inhibited by the addition of IGFBP-3 even in the presence of high concentrations of insulin, which should provoke effects mediated through the type I IGF receptor. The authors concluded that IGFBP-3 had inhibitory action, independent of the IGFs *(156).* Transfection of the IGFBP-3 gene into murine fibroblasts inhibited cell growth by a mechanism that was not reversible by the addition of excess insulin. Even though insulin has mitogenic activity in these cells, it does not bind IGFBP-3, and would presumably saturate the IGF-I-R *(152).* Lalou et al. generated a 16-kDa fragment of rhIGFBP-3 (by proteolysis with plasmin) with negligible binding affinity for IGF-I and presumably none for insulin *(157).* This IGFBP-3 fragment inhibited insulin- and IGF-I-stimulated DNA synthesis in chick embryo fibroblasts *(157).* The same fragment also inhibited mitogenesis in murine fibroblasts with a defective IGF-I-R that could respond to bFGF but not IGF, epidermal growth factor, or platelet-derived growth factor (PDGF) *(158).*

Jones et al. showed a direct effect of IGFBP-1 stimulating cell migration in a monolayer-wounding assay. The direct effect of IGFBP-1 and some of the other stimulatory activity of IGFBP-1 are dependent on the interaction of the RGD sequence with the integrin receptor in the cell membrane *(159).*

IGFBP-4 may also have IGF-independent actions compatible with its membrane binding. When the IGFBP-4 gene was transfected into the colon cancer cell line HT–29, growth inhibition was observed that was not reversed by IGFs or serum *(104).* IGFBP-5 has been shown to stimulate in vitro bone cell proliferation both in the absence of IGFs and in the presence of IGF analogs that do not bind to IGFBP-5, demonstrating an IGF-independent function, probably involving cell surface binding sites *(91,114).*

Recently, a protein biochemically identified as the type V TGF-β receptor (by affinity cross-linking and immunoprecipitation techniques) was shown to bind IGFBP-3, and it may be another IGFBP-3 associated protein *(160).* However, this protein has not been characterized structurally and it has not been cloned, and its size is several-fold larger than other putative IGFBP-3 receptors *(160).*

IGFBP-3 and IGFBP-5 have recently been shown to be translocated into the nucleus compatible with having a nuclear localization sequence (NLS) in their mid-region *(97,161–163).* IGFBP-3 has also been shown to bind importin, a molecule that facilitates nuclear transport *(164).* Nuclear IGFBP-3 may directly control gene expression. Of note, IGFBP-3 interacts with a plethora of molecules which may regulate IGFBP-3 action on cells, including transferrin, heparin, and the latent TGF-β binding protein (LTBP-1) *(165–168).*

IGFBP-Related Proteins

The IGFBP superfamily also encompasses several IGFBP-related proteins (IGFBP-rPs) which bind IGFs with low affinity *(10).* The eclectic taxonomy of the IGFBP-rPs is a work in progress *(169–179).* IGFBPs and IGFBP-rPs share the highly conserved and cysteine-rich N-terminus, which appears to be crucial for several biological actions including their binding to IGFs *(181,182).*

IGFBP-rP1 (first named mac25) is a 31-kDa protein cloned originally from leptomeninges *(170)* and more recently from breast cancer cells *(169,173).* Proteins identical to IGFBP-rP1 have also been isolated from human diploid fibroblast cells (prostacyclin-stimulating factor or PSF) *(171)* and a bladder carcinoma cell line (tumor-derived adhesion factor or TAF) *(172).* IGFBP-rP1 is a secreted protein and has been identified in normal human urine, cerebrospinal and amniotic fluids *(182).* IGFBP-rP1 mRNA is down-regulated in several tumor cell lines and highly expressed in senescent mammary epithelial cells,

suggesting that IGFBP-rP1 has growth-suppressing activity *(183)*. Additional support for this hypothesis came from studies showing that loss of heterozygosity for IGFBP-rP1 expression was essential to the initiation and progression of *in situ* and infiltrating ductal breast cancer *(173)*. Conversely, TGF-β stimulates IGFBP-rP1 mRNA expression in the C2 skeletal myogenic cell line *(184)*, a line which transitions from actively dividing, undifferentiated myoblasts to nondividing myotubes. IGFBP-rP1 levels are markedly higher in dividing C2 myoblasts than in nondividing myotubes, suggesting that IGFBP-rP1 has proliferative actions *(184)*.

IGFBP-rP2 (previously called connective tissue growth factor, CTGF) is involved in human atherosclerosis and fibrotic disorders and can be identified in serum-free condition media of human breast cancer cells and in human biological fluids such as normal sera, pregnancy sera, and cerebrospinal, amniotic, follicular and peritoneal fluids *(183)*. Recent studies indicate that IGFBP-rP2 appears to be an important endocrine factor and one of the critical downstream effectors of TGF-β *(185)*.

IGFBP-rPs designated -3, -4, -5, and -6 have been proposed, though few reports of their structures and functions have been published to date. mRNA levels of the nephroblastoma overexpression gene (novH, the proposed IGFBP-rP3) and the Wilms' tumor suppressor gene (WT1) are inversely correlated in individuals with Wilms' tumors *(186)*. IGFBP-rP3 is a target for WT1 regulation, suggesting that IGFBP-rP3 may play an important role during normal nephrogenesis and in the development of Wilms' tumors. IGFBP-rP3 may serve as a marker both for renal podocytic differentiation and heterotypic mesenchymal differentiation in Wilms' tumors *(186)*.

The proposed IGFBP-rP4 is human CYR61. Sequence analysis reveals the presence of several distinct protein domains, which confer a mosaic structure to human CYR61, confirming it as a member of the IGFBP-rP family, which includes several proto-oncogene products *(177)*. CYR61, a new immediate early gene, may play a role in cell commitment during embryogenesis and, more generally, in the control of cell proliferation *(177)*.

The N-terminus of the L56 protein (the proposed IGFBP-rP5) contains an IGF-binding domain which may modulate its activity as a serine protease *(178)*. These data are consistent with the assumption that L56 is yet another protease regulating the availability of IGFs by cleaving IGFBPs.

Finally, a novel human endothelial cell-specific molecule (ESM–1) was recently cloned from a human umbilical vein endothelial cell (HUVEC) cDNA library and proposed as IGFBP-rP6 *(179)*. Time-dependent up-regulation of ESM-1 mRNA was seen after addition of TNF- or IL-1 β but not with IL-4 or interferon gamma (IFN-) alone *(179)*. The combination of IFN- and TNF- inhibited the TNF- induced increase of ESM–1 mRNA levels. These data suggest that the proposed IGFBP-rP6 may play an important role in vascular cell biology and human lung physiology *(179)*.

IGFBPs and Apoptosis

In addition to its role as an IGF modulator and its IGF-independent actions on cell growth, IGFBP-3 has been recently linked to the induction of apoptosis. The first link between IGFBP-3 and apoptosis was made when it was shown that IGFBP-3 is transcriptionally activated by the tumor suppressor gene p53 *(93)*. Recently, it has been shown that mutants of p53 which have lost the ability to activate IGFBP-3 and Bax expression, but maintained their activation of the cyclin-dependent kinase inhibitor p21, are able to induce cell cycle arrest, but are unable to induce apoptosis *(187,188)*. Rajah et al. demonstrated that addition of exogenous IGFBP-3 to PC-3 cells resulted in a dose-dependent increase in the apoptotic index which was only partially attenuated by the addition of IGF-I and unchanged by the addition of IGF analogs with reduced affinity to IGFBP-3. Confirmation of the direct action of IGFBP-3 on apoptosis was achieved by the induction of apoptosis by IGFBP-3 in IGF-I-R-negative (knockout) murine fibroblasts. Using specific antisense oligonucleotides and neutralizing, antiIGFBP-3 antibodies to block IGFBP-3 expression and action, IGFBP-3 was identified as the mediator of apoptosis induced by retinoic acid (RA) and transforming growth factor-β (TGF-β) in multiple cell types *(89,91,189–191)*. We also showed that the TGF-β-induced apoptosis in the p53 negative prostate cancer cell line PC-3 was prevented by co-treatment with IGFBP-3 neutralizing antibodies or IGFBP-3 specific antisense thiolated. This suggests not only that the TGF- (induced apoptosis in PC-3 cells was mediated by IGFBP-3 but also that IGFBP-3 induced apoptosis through a novel pathway, independent of p53.

Additionally, we showed that IGFBP-3 induces apoptosis in fibroblasts from a type 1 IGF receptor knockout mouse demonstrating that this effect is independent of IGFs and probably occurs through the activation of the IGFBP-3 receptor present in this and other cell lines. The effects of IGFBP-3 on apoptosis appear to involve the serine phosphorylation of bcl-2 and the activation of ICE proteases *(189)*.

IGFBP-5 is involved in growth arrest and apoptosis of ship granulosa cells. Castration-associated apoptosis of the prostate is accompanied by induction of IGFBP-2, -3, -4, and -5 *(96)*.

Conclusion

Over the last decade, there has been an explosion of data in the scientific literature regarding the various components

of the IGF axis. IGFBPs and related molecules are now believed to be critical elements in numerous cellular processes and key factors in several disease states related to abnormal tissue and somatic growth. Recently, the BP-Prs were included in this complex system and their importance is being unraveled. The upcoming years will undoubtedly bring even more information on the molecular biology of these key cellular regulators. These discoveries are likely to lead to better understanding of growth and cellular regulation, and to the development of novel therapeutic approaches to a variety of diseases.

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