REVIEW

IGF-binding protein-5: flexible player in the IGF system and effector on its own

M R Schneider, E Wolf, A Hoeflich and H Lahm

Institute of Molecular Animal Breeding, Gene Centre of the University of Munich, Feodor-Lynen-Strasse 25, D-81377 Munich, Germany

(Requests for offprints should be addressed to M R Schneider; Email: ms2278@columbia.edu)

(M R Schneider is now at Department of Genetics and Development, College of Physicians and Surgeons, Columbia University, 1150 St Nicholas Av., 10032 New York, NY, USA)

(H Lahm is now at Immunology-Molecular Biology Laboratory (IML), Thoraxklinik Heidelberg gGmbH, Amalienstrasse 5, D-69126 Heidelberg, Germany)

Abstract

The multiple activities of IGF-I and -II are modulated by a family of IGF-binding proteins (IGFBP-1 to -6). Although structurally related, each IGFBP has unique properties and exerts specific functions. IGFBP-5 is the most conserved IGFBP across species and was identified as an essential regulator of physiological processes in bone, kidney and mammary gland. In addition, IGFBP-5 appears to play a decisive role in the control of proliferation of specific tumour cell types. In many situations IGFBP-5 exerts biological activities in the absence of IGFs, indicating the existence of IGF-independent actions. This concept was supported by the unexpected localisation of IGFBP-5 in the nucleus and the description of IGFBP-5-specific membrane-bound IGFBP-5 receptor(s). The scope of this review is to summarise the available information about the structure of IGFBP-5 and the regulation of its expression. Furthermore, the potential significance of IGFBP-5 in the regulation of physiological processes will be critically analysed in the light of recent experimental data.


Introduction

The insulin-like growth factors (IGF-I and IGF-II) are the major growth-promoting factors present in the circulation. As true peptide growth factors they are secreted by a great variety of cells and have a multifunctional nature, stimulating the survival and promoting the proliferation and differentiation of many cell types (Stewart & Rotwein 1996). The A and B domains of these single-chain peptides are ~50% identical to the A and B chains of insulin, explaining the origin of this nomenclature (Daughaday & Rotwein 1989). The IGFs elicit their effects through binding to the type I IGF receptor (IGF-I R), a heterotetrameric protein complex with a tyrosine kinase signal transduction pathway (De Meyts et al. 1994). The structurally distinct IGF-II R lacks tyrosine kinase activity and is actually identical to the cation-independent mannose 6-phosphate receptor (Kornfeld 1992). The IGFs elicit their effects through binding to the type I IGF receptor (IGF-I R), a heterotetrameric protein complex with a tyrosine kinase signal transduction pathway (De Meyts et al. 1994). The structurally distinct IGF-II R lacks tyrosine kinase activity and is actually identical to the cation-independent mannose 6-phosphate receptor (Kornfeld 1992). It is involved in the degradation of IGF-II and the sorting of lysosomal enzymes in the Golgi apparatus (Wang et al. 1994). The essential importance of the IGFs and their receptors for normal embryonic, fetal and postnatal development was highlighted by gene-targeting experiments (Efstratiadis 1998).

The observation that most of the IGFs present in serum migrate in higher molecular mass fractions, while the molecular mass of free IGFs is approximately 7.5 kDa, led investigators to propose the existence of carrier proteins. The initial interpretation of the function of such binding proteins was that they would prolong the half-life of the IGFs in the circulation and inhibit their metabolic effects by preventing them from binding to the receptors (Zapf et al. 1979), which would be an essential property as the IGFs are also able to interact with the insulin receptor and are present in serum at a concentration 1000 times higher than that of insulin (Daughaday & Rotwein 1989). Intense research in this area led to the sequential discovery of six different IGF-binding proteins (IGFBPs). They were isolated from different tissues in several species and are characterised at the molecular level. Data obtained from several in vitro and in vivo experiments, and more recently also from transgenic animal models (Schneider et al. 2000), indicate that the IGFBPs are far more than mere binding proteins. These highly conserved proteins are secreted in a...
tissue- and developmental stage-specific manner, they are present in different concentrations in different body compartments, they can modulate IGF bioactivity positively or negatively and they also exert IGF-independent effects (Kelley et al. 1996, Rajaram et al. 1997). Additionally, at least three mechanisms have been shown which alter the affinity of IGFBPs for the IGFs: proteolysis, phosphorylation and adherence to either cell-surface proteins or the extracellular matrix (ECM) (Clemons 1997). Further proteins with structural and functional similarities to the IGFBPs have recently been discovered. This has led to the proposal of an IGFBP superfamily, comprising the six IGFBPs (Hwa et al. 1999). Despite their structural similarity, each IGFBP has unique characteristics and functions. IGFBP-5, the subject of this review, is considered to be rather a stimulatory IGFBP that appears to counteract the inhibitory actions of IGFBP-4 in systems like bone (Mohan et al. 1995b) and cultured vascular smooth muscle cells (Duan & Clemons 1998). Across species barriers IGFBP-5 is the most conserved IGFBP. Its amino acid sequence is 83% identical between human and chicken (Allander et al. 1997) and more than 97% identical between human, mouse and rat (James et al. 1993). It plays an important role in several biological processes including bone, ovary, mammary gland and kidney physiology (Kelley et al. 1996). Several unexpected and unique characteristics of this binding protein have been reported in the last years. In a dogma-breaking discovery it was recently identified as one component of a ternary complex with IGF-I or -II and the acid-labile subunit (ALS) in the human circulation (Twigg & Baxter 1998, Twigg et al. 1998, 2000). Also surprising was the recent discovery of IGFBP-5 being localised in the nuclear compartment of human breast cancer cells (Schedlich et al. 1998, 2000). Furthermore, a growing number of reports support the idea that IGFBP-5 is involved in the regulation of proliferation of cancer cells of different histogenetic origin (Higo et al. 1997, Gregory et al. 1999, Nickerson et al. 1999, Parisot et al. 1999, Rozen & Pollak 1999, Miyake et al. 2000a,b). IGFBP-5 is one of the IGFBPs that displays IGF-independent effects. Accordingly, potential signalling receptors for IGFBP-5 have been described (Andress 1995, 1998, Leal et al. 1999). The aim of this review is to present and critically discuss our current knowledge about the structural properties and biological functions of IGFBP-5.

Genomic organisation of the IGFBP-5 gene

In the mouse, the IGFBP-5 gene has a length of 17 kb and is localised on chromosome 1 (Kou et al. 1994). In humans it spans 33 kb and was identified on chromosome 2 (Allander et al. 1994). The IGFBP-5 gene is located on the same chromosome as the IGFBP-2 gene but orientated in a tail-to-tail fashion (opposite transcriptional direction). The distance between both genes comprises only 20 kb in humans (Allander et al. 1994) and 5 kb in mice (Kou et al. 1994). Similarly, the IGFBP-1 and -3 genes are tightly linked and are positioned in a tail-to-tail orientation on chromosome 7 in humans (Ehrenborg et al. 1992). The IGFBP-4 and -6 genes are located on separate chromosomes. The genomic distribution and the close relationship between certain IGFBPs suggest that these proteins have developed after duplication of an ancestral IGFBP. The resulting gene pair might then have been dispersed to different chromosomal locations (Allander et al. 1994). Since IGFBP-6 is the most divergent IGFBP it appears likely that it represents the binding protein which is most similar to the ancestral proto-IGFBP (Reinecke & Collet 1998).

The IGFBP genes are associated with the homeobox (HOX) genes, which are widely expressed in multicellular organisms and encode transcription factors that are crucial for early development. IGFBP-1 and -3 are localised on the same chromosome as the HOXA cluster, the IGFBP-2 and -5 genes map to the same chromosomal region as the HOXD cluster while the IGFBP-4 gene is found in the vicinity of the HOXB genes. Finally, IGFBP-6 and HOXC genes are found on the same chromosome. This suggests that IGFBPs and HOX genes were probably linked prior to the initial duplication event, implying that the IGFBPs are phylogenetically ancient molecules (Allander et al. 1994). Supporting this concept, several proteins that are able to bind to the IGFs were detected in the serum of reptiles, of the agnathan lamprey and in bony fish (reviewed in Reinecke & Collet 1998).

The IGFBP-5 gene has a conserved structure of four exons separated by three introns in human, mouse and rat. The first intron has a length of several kilobases, encompassing more than 50% of the gene. The promoter region has a simple structure, typical for regulated eukaryotic genes. Conserved TATAA and CAAT consensus sequences are present upstream of the transcription start in human (Allander et al. 1994), mouse (Kou et al. 1995) and rat (Zhu et al. 1993). The essential promoter activity seems to reside in the proximal 200 bp (Kou et al. 1995). In addition, an AP-2 recognition sequence was identified 5’ of the TATA box, which explains at least in part the responsiveness of this gene to agents that increase intracellular cAMP levels (Duan & Clemons 1995). The stimulation of IGFBP-5 transcription by progesterone was demonstrated to be mediated by a CACCC sequence in the proximal promoter (Boonyaratanakornkit et al. 1999).

IGFBP-5: a protein harbouring multiple functional domains

Although it is almost impossible to define exactly when IGFBP-5 was detected for the first time, there is a
consensus in the literature that it was first purified and correctly identified almost at the same time in adult rat serum (Shimasaki et al. 1991), human bone extract (Bautista et al. 1991) and conditioned medium of human osteoblast-like cells (Andress & Birnbaum 1991). In the same year, full-length cDNA clones for rat and human IGFBP-5 were isolated (Kiefer et al. 1991, Shimasaki et al. 1991).

After cleavage of a 20 amino acid signal peptide, the mature IGFBP-5 consisting of 252 amino acids and with a molecular mass of approximately 29 kDa is secreted in humans and mice. The most important functional elements of human IGFBP-5 are depicted schematically in Fig. 1. Comparison of the amino acid sequence of all mammalian IGFBPs reveals that they share a common organisation with three distinct domains of similar size. While the N-terminal and the C-terminal domains are conserved, the midregion is highly variable. The positions of 18 cysteines in IGFBP-1, -2, -3 and -5 are highly conserved, 12 being located in the N-terminal region and the remaining six in the C-terminal domain. IGFBP-4 contains two additional cysteines in the midregion while in IGFBP-6 only 16 cysteines are found.

**The N-terminal domain**

The clustering of an even number of cysteines in this region of the IGFBP molecules suggests a complex structure with six possible intradomain disulphide bonds rather than a formation of interdomain disulphide bridges with the cysteines in the C-terminal domain (Hwa et al. 1999). Indeed, it was demonstrated that the last four cysteines of the N-terminal domain form overlapping disulphide linkages in human IGFBP-5. Nuclear magnetic resonance spectroscopy of this subdomain revealed a rigid, globular structure that consists of a centrally located three-stranded...
Table 1 IGFBP-5-specific proteolytic activities

<table>
<thead>
<tr>
<th>Origin of the fragment</th>
<th>Molecular weight (kDa)</th>
<th>Cleavage site</th>
<th>Protease(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human pregnancy serum</td>
<td>22 and 15</td>
<td>ND</td>
<td>Gelatinase</td>
<td>Kübler et al. (1998)</td>
</tr>
<tr>
<td>Rat granulosa cells</td>
<td>19-5 and 17-5</td>
<td>ND</td>
<td>Non-MMP Zn²⁺ metalloproteinase</td>
<td>Resnick et al. (1998)</td>
</tr>
<tr>
<td>Human seminal plasma</td>
<td>20 and 14</td>
<td>ND</td>
<td>PSA and unknown protease</td>
<td>Lee et al. (1994)</td>
</tr>
<tr>
<td>Human fibroblasts</td>
<td>22, 20 and 17</td>
<td>ND</td>
<td>?</td>
<td>Nam et al. (1994)</td>
</tr>
<tr>
<td>Human osteoblasts</td>
<td>18–20</td>
<td>ND</td>
<td>?</td>
<td>Camacho-Hubner et al. (1992)</td>
</tr>
<tr>
<td>Human amniotic fluid</td>
<td>22 and 15</td>
<td>ND</td>
<td>?</td>
<td>Busby et al. (2000)</td>
</tr>
<tr>
<td>Human fibroblasts</td>
<td>23</td>
<td>ND</td>
<td>67 and 167 kDa proteases</td>
<td>Kanzaki et al. (1994)</td>
</tr>
<tr>
<td>Human fibroblasts</td>
<td>22</td>
<td>ND</td>
<td>Complement C1s</td>
<td>Andress &amp; Birnbaum (1992)</td>
</tr>
<tr>
<td>Human fibroblasts</td>
<td>23</td>
<td>ND</td>
<td>?</td>
<td>Besnard et al. (1996a)</td>
</tr>
<tr>
<td>Human osteoblasts</td>
<td>19</td>
<td>ND</td>
<td>Metallo- and serine proteases</td>
<td>Sunic et al. (1998b)</td>
</tr>
<tr>
<td>Mouse osteoblasts</td>
<td>22 and 14</td>
<td>ND</td>
<td>Serine protease</td>
<td>Imai et al. (1997)</td>
</tr>
<tr>
<td>Porcine smooth muscle cells</td>
<td>22</td>
<td>Arg¹³⁸-Arg¹³⁹</td>
<td>?</td>
<td>Thralkill et al. (1995)</td>
</tr>
<tr>
<td>Porcine smooth muscle cells</td>
<td>19</td>
<td>Lys¹₂⁰-His¹₂¹</td>
<td>Thrombin</td>
<td>Claussen et al. (1994)</td>
</tr>
<tr>
<td>Mouse osteoblasts</td>
<td>22</td>
<td>Lys¹⁵⁶-Ile¹⁵⁷, Arg¹⁹²-Alα¹⁹³</td>
<td>Arg¹³⁸-Lys¹³⁹ motif in IGFBP-5 (Imai et al. 1997). IGFBP-5 proteolytic activity was identified in biological fluids and in conditioned media of cell cultures. In some cases specific proteases have been characterised (Conover 1999). Table 1 presents some of the IGFBP-5-specific proteolytic activities described so far. In some instances the responsible proteases have been identified; however, it remains to be elucidated whether all of the other described activities represent distinct molecules.</td>
<td>Zheng et al. (1998)</td>
</tr>
<tr>
<td>Porcine smooth muscle cells</td>
<td>24, 23 and 20</td>
<td>Lys¹⁴³-Lys¹⁴⁴</td>
<td>PAPP-A2</td>
<td>Overgaard et al. (2001)</td>
</tr>
</tbody>
</table>

ND = not determined; PSA = prostate-specific antigen; MMP = matrix metalloproteinase; PAPP-A2 = pregnancy-associated plasma protein-A2.

anti-parallel β-sheet whose scaffold is further stabilised by these disulphide bonds (Kalus et al. 1998). Using the IGFBP-5 fragment Ala⁴⁰-Ile⁹² these authors also demonstrated that this region contains the primary binding site for IGFs (Fig. 2) comprising Val⁴⁹, Tyr⁵⁰, Pro⁶² and Lys⁶⁸–Leu⁷⁵, which form a hydrophobic patch on the surface of the protein. In vitro mutagenesis of the intact IGFBP-5 confirmed residues 68, 69, 70, 73 and 74 as being essential for high-affinity binding to IGF-I (Imai et al. 2000). However, the complete N-terminus has substantially lower affinity than the intact protein and sequences in the C-terminus, although not physically interacting with the IGFs, are indispensable for stable and high-affinity binding of the ligands.

Also located in the N-terminal domain of the IGFBPs (except IGFBP-6) is a GCGGCCxxxC motif (Fig. 1). It is also highly conserved in the IGFBP-rPs and can be found in several other unrelated proteins. Its significance, however, remains unknown at present (Hwa et al. 1999).

**Proteolysis**

All IGFBPs can be cleaved by specific proteases, a phenomenon that results in reduction or loss of IGF-binding activity. Most proteolytic sites identified so far are located in this non-conserved region (Fig. 1), suggesting a potential mechanism by which IGF activities can be regulated in a tissue-specific manner (Clemmons 1997). The cleavage site of a serine protease secreted by porcine smooth muscle cells was identified as the di-basic Lys¹³⁸/Lys¹³⁹ motif in IGFBP-5 (Imai et al. 1997). IGFBP-5 proteolytic activity was identified in biological fluids and in conditioned media of cell cultures. In some cases specific proteases have been characterised (Conover 1999). Table 1 presents some of the IGFBP-5-specific proteolytic activities described so far. In some instances the responsible proteases have been identified; however, it remains to be elucidated whether all of the other described activities represent distinct molecules.

**Glycosylation**

Analysis of U-2 human osteosarcoma conditioned medium by Western ligand blot showed IGFBP-5 as an IGF-binding triplet of 29, 32 and 34 kDa. Sequential treatment with neuraminidase and O-glycanase, but not with N-glycanase or endoglycosidase F, reduced all bands to the 29 kDa core protein, suggesting that IGFBP-5 was O-glycosylated (Conover & Kiefer 1993). Similar 29–34 kDa forms were identified in conditioned media of human fibroblasts and osteoblasts. O-glycosylation of Thr¹⁵² was detected in IGFBP-5 fragments isolated from human serum (Ständker et al. 1998). Thus, it appears that all predicted glycosylation sites (Thr¹⁰³, Thr¹⁰⁴ and Thr¹¹¹) (Conover 1999) are used in IGFBP-5, although this modification might happen in a tissue-specific manner. In IGFBP-6, the other...
O-glycosylated IGFBP, the glycosylation sites are also clustered in the midregion, and this modification makes the molecule less susceptible to proteolysis (Bach 1999).

**Phosphorylation** The IGFBP-5 molecule has 12 potential phosphorylation sites (Fig. 1). Such sites are also present in all other IGFBPs but only phosphosisoforms of IGFBP-1, -3 and -5 have been reported so far (Coverley & Baxter 1997). IGFBP-5 secreted by human fibroblasts can be highly phosphorylated (Jones et al. 1992). Phosphorylation of IGFBP-1 increases its affinity for IGF-I substantially (Jones et al. 1993). Therefore, this post-translational modification might also affect the property of IGFBP-5 to modulate IGF bioactivity. In addition, phosphorylation might alter the susceptibility for proteolysis. Conover (1999) suggested that phosphorylation might be important for the affiliation of IGFBP-5 with hydroxyapatite in bone. Nevertheless, phosphorylation is a common mechanism to modulate the activity of intracellular proteins and it may be important for the interaction of IGFBP-5 with cellular proteins on its way to the nucleus (see below).

**The C-terminal domain**

Six cysteines are found in this region of the IGFBPs at strictly conserved positions (Fig. 1). Consistent with the formation of intradomain disulphide bridges in the N-terminal region, there is evidence that these cysteines also interact with each other (Hwa et al. 1999). Although it is not the primary IGF-binding site, this domain was demonstrated to be essential for high-affinity and stable IGF binding. Site-specific mutagenesis of the strictly conserved amino acids Gly203 or Glu209 dramatically reduced the IGF binding affinity of rat IGFBP-5 (Bramani et al. 1999, Song et al. 2000). In addition, it was demonstrated that N-terminal fragments of IGFBP-5 have a 10- to 200-fold lower affinity for the IGFs compared with the full-length protein (Kalus et al. 1998), consistent with the reduced IGF affinity observed in C-terminally truncated fragments (Andress & Birnbaum 1992, Andress et al. 1993). The region comprising the last five cysteines of IGFBP-5 shares 37% similarity with the thyroglobulin-type I domain. The function of this domain, also found in several other unrelated proteins, is unknown (Hwa et al. 1999). Finally, these basic regions found in the C-terminal domain of IGFBP-3, -5 and -6 were shown to be able to inhibit IGFBP-4 degradation (Fowlkes et al. 1997).

Several investigations using amino acid substitution and peptide competition experiments have identified a highly basic region between amino acids Arg201 and Arg218 to which several important functions were ascribed. These functions include binding to the ECM and binding to ALS and cell membranes and they are described in the sections below.

**Binding to ECM** Early experiments have shown that IGFBP-5 has the ability of binding to the ECM and that this interaction results in reduced affinity for the IGFs and stimulation of IGF activities (Andress & Birnbaum 1992, Jones et al. 1993). This was confirmed by the observation that co-incubation of IGFBP-5 with glycosaminoglycans (GAGs) reduced the IGF affinity 17-fold (Arai et al. 1994). In addition, GAGs inhibited proteolysis of IGFBP-5 (Arai et al. 1994). The basic region between residues 201 and 218 was identified as being important for the binding to heparin and to other components of the ECM (Arai et al. 1994, 1996, Parker et al. 1996, 1998, Campbell & Andress 1997, Nam et al. 1997, 2000, Rees & Clemmons 1998, Song et al. 2000). It was hypothesised that binding of heparin to this domain would produce a conformational change that might reduce the affinity for the IGFs (Arai et al. 1994, 1996). A systematic mutational analysis of this region identified Arg207 and Arg214 as the most critical amino acids for ECM binding (Parker et al. 1998). A helical wheel prediction places these residues adjacent to Gly203, which was demonstrated to be critical for IGF binding, while Glu209, another amino acid essential for IGF binding, is flanked by two other basic residues of the wheel (Parker et al. 1998, Bramani et al. 1999). In addition, mutation of the basic residues 201, 202, 206 and 214 resulted in attenuated heparin binding but only in a small reduction of the affinity for the IGFs (Song et al. 2000). This strongly suggests that ECM and IGF binding sites are located in close proximity to each other and may even overlap, providing an alternative explanation for the reduced IGF affinity of ECM-bound IGFBP-5.

**Binding to ALS** Until recently IGFBP-3 was believed to be the only IGFBP able to participate in the formation of a ternary complex with ALS and IGF-1 or -II. For IGFBP-3 the C-terminal region is essential for the interaction with the ALS. This is of interest for the study of IGFBP-5 because it was recently demonstrated that this IGFBP can also form a ternary complex of about 130 kDa with ALS and one IGF molecule (Twigg & Baxter 1998). IGFBP-3 and -5 share a high degree of similarity in the C-terminal region (54%) and the sequence 201–218 appears to be the primary binding site of IGFBP-5 for ALS (Twigg et al. 1998) and Lys211, Arg214, Lys217 and Arg218 were identified as the key residues for ALS binding (Firth et al. 2001). In addition, mutation of specific residues in the midregion resulted in a small decrease in the binding affinity for ALS (Firth et al. 2001).

**Binding to cell membrane proteins** In 1995 it was demonstrated that IGFBP-5 binds to and is internalised by a 420 kDa membrane protein of mouse osteoblastic cells which is probably not a proteoglycan (Andress 1995). The highly basic region 201–218 appears to be important for...
this binding, which can be modulated by GAGs. The fact that this protein is rapidly downregulated by IGFBP-5 suggested that it may function as a receptor. Further analysis revealed that intact IGFBP-5 as well as IGFBP-51–169 and IGFBP-5201–218 were able to stimulate the phosphorylation of this protein (Andress 1998), a clear example of an IGF-independent action. It remains to be elucidated whether this protein is identical to the 400 kDa type V transforming growth factor-β receptor which has been shown to interact with IGFBP-3, -4 and -5, although the effect of these molecules on DNA synthesis remained rather minor (Leal et al. 1999). The IGFBP-51–169 fragment does not contain a known heparin-binding domain, but the existence of a cluster of basic amino acids between residues 133 and 143 may explain the ability of the IGFBP-5 fragment to bind to the membrane protein (Andress 1995). A similar membrane protein was identified in mesangial cells (Abrass et al. 1997) and it was demonstrated that IGFBP-5201–218 was able to stimulate Cdc42 GAP aggregation and filopodia formation in migrating mesangial cells by binding to a serine/threonine kinase receptor (Berfield et al. 2000).

Nuclear localisation

Potential nuclear localisation signals are present in IGFBP-3 and -5 (Radulescu 1994). Consistent with this was the finding that recombinant IGFBP-3 and -5, but not IGFBP-1 and -2 are translocated to the nucleus of human breast cancer cells (Schedlich et al. 1998). Further analysis showed that this occurs by a nuclear localisation signal-dependent pathway and is mediated mainly by the importin-β nuclear transport factor (Schedlich et al. 2000). The significance of the intranuclear localisation of these IGFBPs is not clear at present, but may be linked to IGF-independent activities.

Expression of IGFBP-5 in vivo

Tissue-specific and developmental expression

Preimplantation mouse embryos transcribe IGFBP-1, -2, -3, -4 and -6 but not IGFBP-5 mRNA (Hahnel & Schultz 1994, Liu et al. 1997), whose expression is detectable as early as day 10·5 of gestation in the rat embryo (Green et al. 1994) and at day 11 of gestation in the mouse (Schuller et al. 1993). In contrast, IGFBP-5 mRNA expression was detected by RT-PCR in bovine blastocysts (Winger et al. 1997) and shown to be upregulated by exogenous IGF peptides (Prelle et al. 2001). Initial studies identified the meninges, vertebrae, lung, kidneys and intestine as the major sites of IGFBP-5 expression in the late gestation mouse conceptus (Schuller et al. 1993) and kidney, lung, heart, brain, muscle, ovary and testes in the adult animal (James et al. 1993, Schuller et al. 1994). Table 2 summarises the major sites of IGFBP-5 expression in embryonic and adult tissues of different species. IGFBP-5 is present at different levels in slow and fast rat muscles, and denervation dramatically upregulates transcription of its mRNA (Bayol et al. 2000). In agreement,

### Table 2 Major sites of IGFBP-5 expression in different species

<table>
<thead>
<tr>
<th>Species</th>
<th>Expression site</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Trabecular meshwork</td>
<td>Wirtz et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>Bone</td>
<td>Bautista et al. (1991)</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>Allen et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>Ovary</td>
<td>Zhou &amp; Bondy (1993a)</td>
</tr>
<tr>
<td>Mouse</td>
<td>Limbs (e)</td>
<td>Van Kleffens et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>Kidney (e)</td>
<td>Lindenbergh-Kortleve et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>Lung (e)</td>
<td>Schuller et al. (1995)</td>
</tr>
<tr>
<td></td>
<td>Ovary</td>
<td>Adashi et al. (1997), Wandji et al. (1998)</td>
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<tr>
<td></td>
<td>Spinal cord</td>
<td>Arnold et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>Skeletal tissues</td>
<td>Wang et al. (1995)</td>
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<td></td>
<td>Mammary gland</td>
<td>Wood et al. (2000)</td>
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<tr>
<td>Rat</td>
<td>Pancreas (e)</td>
<td>Hill et al. (1999)</td>
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<tr>
<td></td>
<td>Kidney (e)</td>
<td>Matsell et al. (1994)</td>
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<td></td>
<td>Ovary (e)</td>
<td>Erickson et al. (1992)</td>
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<td>Lung (e)</td>
<td>Van de Wetering et al. (1997), Wallen et al. (1997)</td>
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<td>Kidney</td>
<td>Price et al. (1995)</td>
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<td>Forebrain</td>
<td>Stenvers et al. (1994)</td>
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<td>Eyes</td>
<td>Burren et al. (1997)</td>
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<td></td>
<td>Liver</td>
<td>Zimmerman et al. (2000)</td>
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<td></td>
<td>Pituitary</td>
<td>Bach &amp; Bondy (1992), Gonzalez-Parra et al. (2001)</td>
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e=embryonic stages.
overloading mouse skeletal muscle decreased IGFBP-5 expression to one-third of the control value, while unloading by hind limb suspension doubled this value (Awede et al. 1999). In contrast, the expression of the IGFs and IGFBP-5 was not altered acutely by nutrients or insulin in human skeletal muscle (Schimke et al. 1999). This suggests that IGFBP-5 may play a role in muscle long-term adaptation to changes in loading. In the involuting rat mammary gland (Tonner et al. 1997), prostate (Thomas et al. 1998) and thyroid gland (Phillips et al. 1994) IGFBP-5 expression was demonstrated to be upregulated, suggesting that it may be involved in the regulation of apoptosis-mediated involution of these organs.

### IGFBP-5 in serum and hormonal regulation

The most important regulator of IGFBP-5 expression *in vivo* is IGF-I. This is underlined by the upregulation of IGFBP-5 in the brain of IGF-I–transgenic mice (Ye & D’Ercole 1998) and consistent with the observation that both proteins are spatially and temporally co-expressed during brain development (Bondy & Lee 1993). In normal adult human serum, IGFBP-5 levels are positively correlated with IGF-I concentrations (Mohan et al. 1996). As mentioned previously, IGFBP-5 in human serum is capable of forming a ternary complex of 130 kDa with ALS and IGF-I or -II. The mean percentage of IGFBP-5 in ternary complexes was 58-2% in normal human serum (Baxter et al. 2000). However, since IGFBP-5 is a minor IGF-carrier, the physiological significance for this phenomenon remains to be determined. The expression of IGFBP-3, ALS and IGF-I is strictly regulated by growth hormone (GH). This is also true for IGFBP-5 under some circumstances (Mohan et al. 1995a, Ono et al. 1996, Thoren et al. 1998, Ulinski et al. 2000). It is plausible that for IGFBP-5 the ALS binding is a characteristic conserved during evolution after the gene duplication event that originated IGFBP-3 and -5, but not of outstanding physiological significance at the systemic level. Liver is the source of serum ALS, but extrahepatic expression was detected in bone and renal cortex (Chin et al. 1994), also sites of high IGFBP-5 expression.

IGFBP-5 serum levels increase in puberty and decrease with ageing (Rajaram et al. 1997). The skeletal content of IGFBP-5 was also demonstrated to decrease by 28% between the age of 20–29 years and 54–64 years, which correlates positively with both IGF-I and -II levels (Mohan et al. 1995a). The report that the intrinsic capacity of human bone cells to produce these IGF components is largely preserved with age illustrates the complex interplay of molecules in the bone environment (Pfeilschifter et al. 2000). However, since Kveiborg et al. (2000) observed an age-related impairment in the production of components of the IGF system by osteoblasts *in vitro*, the reduction observed *in vivo* can either reflect their secretion levels by osteoblasts or the serum level.

### Expression of IGFBP-5 *in vitro*

There is some information on signals which modulate IGFBP-5 expression *in vivo*, e.g. prolactin (PRL) in the mammary gland (Tonner et al. 1997). However, most of our knowledge about regulation of IGFBP-5 expression is derived from *in vitro* studies. Many cell types express IGFBP-5 mRNA and serve as biological systems to identify molecules which alter IGFBP-5 expression.

Similar to the *in vivo* conditions, IGF-I is the most important regulator of the expression of IGFBP-5 in a large number of cell types in different species *in vitro*. Depending on the cell type this can occur in two different ways. The first is direct stimulation of the transcription of the gene (Conover & Kiefer 1993, Matsumoto et al. 1996) (Table 3). The second occurs post-translationally by interaction with the secreted protein; when bound to IGF-I, IGFBP-5 is protected from proteolysis (Camacho-Hubner et al. 1992, Matsumoto et al. 1996). IGF-I-mediated transcription of the IGFBP-5 gene is generally believed to occur after the activation of the IGF-I R, but how these two phenomena are linked together in detail is poorly defined. In porcine vascular smooth muscle cells this activation of the IGFBP-5 gene was demonstrated to be dependent on the activation of the PI 3-kinase-PKB/Akt-p70S6k signalling cascade but not on the MAPK pathway (Duan et al. 1999). Likewise, IGF-II and insulin can stimulate transcription of the IGFBP-5 gene (see Table 3). Interestingly, the expression of IGFBP-5 is also affected by cell density in porcine aortic smooth muscle cells (Duan & Clemmons 1998) and ovine granulosa cells (Monget et al. 1998). Apart from these components of the IGF-system, IGFBP-5 can be regulated by hormones, cytokines and other molecules in a cell-specific manner. Table 3 gives an overview of these molecules.

### Physiological role of IGFBP-5

Since neither the overexpression nor the genetic ablation of IGFBP-5 in animal models has been published our knowledge about the function of IGFBP-5 *in vivo* remains restricted. In that situation *in vitro* assays are the almost exclusive source to gather information about the physiological activities of IGFBP-5 and the use of cellular systems has enabled us to link IGFBP-5 activity with IGF-mediated cell proliferation, differentiation and motility. In addition, cell lines have clearly indicated that IGFBP-5 also triggers events which occur independently of IGFs. Some of these activities are listed in Table 4. However, specific effects of IGFBP-5 *in vivo* which correspond with *in vitro* data suggest that IGFBP-5 plays a significant role in the regulation of organ function, including the development of the central nervous system (Lee et al. 1995, Ye & D’Ercole 1998), involution of the mammary gland (Tonner et al. 1997) and bone physiology (Richman et al. 1999, Miyakoshi et al. 2001).
Soon after its molecular characterisation IGFBP-5 was identified as an essential regulator of IGF activities in bone cells. As a consequence, this property became a target of extensive studies. Expression of IGFBP-5 in bone cells is tightly regulated by the IGFs and several other hormones and growth factors (Table 3). In addition, not only the expression, but also the effect and the fate of IGFBP-5 are different in distinct bone cell lines (Schmid et al. 1995).

In contrast to IGFBP-4, which exerts exclusively inhibitory actions on bone cells both in vitro (Mohan et al. 1989, 1995b) and in vivo (Miyakoshi et al. 1999), IGFBP-5 was identified as a stimulator of osteoblast mitogenesis (Andress & Birnbaum 1991, 1992, Bautista et al. 1991, Andress et al. 1993, Mohan et al. 1995b). The stimulatory

### Table 3: Effect of different agents on the expression of IGFBP-5 in vitro

<table>
<thead>
<tr>
<th>Agent</th>
<th>Cell type</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Human chondrocytes</td>
<td>s</td>
<td>Matsumoto et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>Rat chondrocytes</td>
<td>s</td>
<td>Matsumoto et al. (1996), Koedam et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>Ovine chondrocytes</td>
<td>s</td>
<td>Sunic et al. (1998a,b)</td>
</tr>
<tr>
<td></td>
<td>Human breast cancer cells</td>
<td>s</td>
<td>Sheikh et al. (1993), Shemer et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>Rat intestinal smooth muscle</td>
<td>s</td>
<td>Zimmerman et al. (1997), Hou et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>Mouse myoblasts</td>
<td>s</td>
<td>Rouse et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>Human osteosarcoma</td>
<td>s</td>
<td>Conover &amp; Kiefer (1993)</td>
</tr>
<tr>
<td></td>
<td>Mouse pituitary cells</td>
<td>s</td>
<td>Fielder et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>Rat Schwann cells</td>
<td>s</td>
<td>Cheng et al. (1999)</td>
</tr>
<tr>
<td></td>
<td>Rat thyroid follicular cells</td>
<td>s</td>
<td>Backeljauw et al. (1993)</td>
</tr>
<tr>
<td>IGF-II</td>
<td>Human aortic smooth muscle cells</td>
<td>s</td>
<td>Duan et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>Rat chondrocytes</td>
<td>s</td>
<td>Matsumoto et al. (1996), Koedam et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>Mouse myoblasts</td>
<td>s</td>
<td>McCarthy et al. (1994)</td>
</tr>
<tr>
<td></td>
<td>Mouse pituitary cells</td>
<td>s</td>
<td>Fielder et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>Rat thyroid follicular cells</td>
<td>s</td>
<td>Backeljauw et al. (1993)</td>
</tr>
<tr>
<td>Insulin</td>
<td>Human aortic smooth muscle cells</td>
<td>s</td>
<td>Duan et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>Rat chondrocytes</td>
<td>s</td>
<td>Koedam et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>Mouse myoblasts</td>
<td>s</td>
<td>Rouse et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>Mouse pituitary cells</td>
<td>s</td>
<td>Fielder et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>Rat thyroid follicular cells</td>
<td>s</td>
<td>Backeljauw et al. (1993)</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>Rat chondrocytes</td>
<td>i</td>
<td>Koedam et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>Human fibroblasts</td>
<td>i</td>
<td>Conover et al. (1995)</td>
</tr>
<tr>
<td></td>
<td>Mouse pituitary cells</td>
<td>i</td>
<td>Fielder et al. (1993)</td>
</tr>
<tr>
<td>GH</td>
<td>Rat osteoblasts</td>
<td>s</td>
<td>McCarthy et al. (1994)</td>
</tr>
<tr>
<td>Interleukin-1α</td>
<td>Ovine chondrocytes</td>
<td>s</td>
<td>Sunic et al. (1998a)</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>Rat osteoblasts</td>
<td>s</td>
<td>Franchimont et al. (1997)</td>
</tr>
<tr>
<td>Osteogenic protein-1</td>
<td>Rat fetal calvarial cells</td>
<td>i</td>
<td>Yeh et al. (1998), Yeh &amp; Lee (2000)</td>
</tr>
<tr>
<td>Parathyroid hormone</td>
<td>Rat osteosarcoma cells</td>
<td>s</td>
<td>Torring et al. (1991), Conover et al. (1993), Nasu et al. (1997, 1998)</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>Rat osteoblasts</td>
<td>s</td>
<td>Dong &amp; Canalis (1995)</td>
</tr>
<tr>
<td></td>
<td>Human breast carcinoma</td>
<td>i</td>
<td>Shemer et al. (1993)</td>
</tr>
<tr>
<td>TSH</td>
<td>Rat thyroid follicular cells</td>
<td>i</td>
<td>Backeljauw et al. (1993)</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>Rat osteosarcoma cells</td>
<td>s</td>
<td>Nasu et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>Rat osteoblasts</td>
<td>s</td>
<td>Schmid et al. (1996)</td>
</tr>
</tbody>
</table>

*i*=inhibition; *s*=stimulation; TSH=thyroid-stimulating hormone.
properties of IGFBP-5 were postulated to be mediated by its ability to bind to the cell membrane or ECM (Andress & Birnbaum 1992). Bautista et al. (1991) have previously reported that among the IGFBPs IGFBP-5 has the unique property of binding to hydroxyapatite, a component of the mineralised ECM of bone. The authors proposed an IGF-dependent mechanism by which IGFs are sequestered and concentrated in bone. Release of IGFs during bone remodelling or after injury would then stimulate proliferation of neighbouring osteoblasts (Fig. 3A). The age-related decline in the skeletal concentration of IGF-I and IGFBP-5 supports this model and provides an explanation for the age-related impairment of bone formation vs resorption (Mohan et al. 1995a). In addition, bone is the only tissue where IGF-II is stored in higher concentrations than in serum (Mohan et al. 1988).

Inhibitory actions of IGFBP-5 on bone cells were reported by Kiefer et al. (1992, 1993) and Conover & Kiefer (1993). IGFBP-5 also inhibited IGF activity on osteosarcoma cells (Schmid et al. 1995). The important factor responsible for this apparent divergence might be the localisation of IGFBP-5; stimulatory actions were associated with binding to the cell membrane (Bautista et al. 1991, Andress & Birnbaum 1992). In contrast, inhibitory activities were correlated with IGFBP-5, which was present exclusively in the culture medium (Conover & Kiefer 1993). Similarly, stimulation of growth of human fibroblasts was associated with IGFBP-5 located in the ECM. When present only in the medium, IGFBP-5 became rapidly degraded and did not affect proliferation (Jones et al. 1993).

An IGF-independent effect of IGFBP-5 on bone cells, firstly reported by Andress & Birnbaum (1992) was confirmed by Mohan et al. (1995b) and demonstrated to occur also in vivo (Richman et al. 1999, Miyakoshi et al. 2001). The identification of putative IGFBP-5 receptors in the membrane of osteoblasts (Andress 1995, 1998) and mesangial cells (Abrass et al. 1997) and the detection of IGFBP-5 in the nucleus (Schedlich et al. 1998, 2000) support the idea that IGFBP-5 functions also as an independent growth factor (Fig. 3A). From all these data it appears evident that IGFBP-5 is an essential molecule for the maintenance of a complex network of factors which guarantee normal bone physiology. Disturbance of this equilibrium may lead to pathological situations like osteoporosis (Rosen et al. 1994, Rosen & Donahue 1998, Rosen 2000) or renal osteodystrophy (Jehle et al. 2000).

**Mammary gland involution**

Travers et al. (1996) reported that the involution of the mammary gland of rats induced by the combined deficiency of PRL and GH could not be overcome by IGF-I treatment, a rather unexpected finding if we...
consider that GH actions are often mediated by IGF-I. The authors suggested that this may be due to an inhibitory IGFBP, which was present in increasing amounts in the involuting mammary gland. This inhibitory IGFBP increased 50-fold in the milk after 2 days of mammary gland involution and was shown to be IGFBP-5 (Tonner et al. 1997). PRL inhibits apoptosis and involution of the mammary gland and also the increase in IGFBP-5. Furthermore, IGFBP-5 interacted with the casein micelles (which possess calcium phosphate), similar to its interaction with hydroxyapatite in bone (Bautista et al. 1991). Therefore, Flint et al. (2000) proposed that IGFBP-5 functions as an inhibitor of cell proliferation and survival during mammary gland involution, preventing the interaction of IGF-I with its receptor on epithelial cells (Fig. 3B). In contrast, GH and PRL cooperatively inhibit involution; GH increases IGF-I synthesis and PRL inhibits IGFBP-5 production. This model of IGFBP-5-induced...

**Figure 3** Physiological activity of IGFBP-5 in different tissues. (A) Regulation of osteoblast proliferation. IGFBP-5 allows the storage of IGFs in bone ECM, which can be mobilised later for stimulating repair processes after injury (1). IGFBP-5 (or proteolytic fragments) can also stimulate cell proliferation directly by binding to a membrane receptor and activating specific signalling pathways (2). (B) Mammary gland involution. IGFBP-5 inhibits the binding of IGF-I to its receptor thereby promoting increased apoptosis (1). Through an IGF-independent action IGFBP-5 interacts with casein and/or plasminogen-activator inhibitor-1 (PAI-1). As a consequence, plasmin is generated from plasminogen and extensive tissue remodelling (a key event of mammary gland involution) occurs (2).
apoptosis through an IGF-dependent mechanism is supported by further experimental data; in conditional knock-out mice lacking Stat3 in the mammary gland, delayed involution of the organ is associated with a lack of increase in IGFBP-5 expression (Chapman et al. 1999). Increased IGFBP-5 levels were also associated with apoptosis and involution in the rat ovary (Liu et al. 1993), prostate (Guenette & Tenniswood 1994, Thomas et al. 1998) and thyroid (Phillips et al. 1994). In addition, IGFBP-5 co-localises with areas of apoptosis during mouse development (Van Kleffens et al. 1999, Allan et al. 2000).

Activation of plasminogen to generate plasmin is a key event for the extensive ECM remodelling that occurs during mammary gland involution. The observation that IGFBP-5 interacts with both casein (Tonner et al. 1997) and plasminogen activator inhibitor-1 (Nam et al. 1997) suggests that IGFBP-5 may be also involved in the regulation of tissue remodelling. Tonner et al. (2000) proposed that IGFBP-5 may coordinate both apoptosis and tissue remodelling during mammary gland involution through IGF-dependent and -independent mechanisms respectively (Fig. 3B).

Ovarian physiology
The expression pattern of IGFBP-5 and its regulation were extensively studied in the rat ovary. Most studies concentrated on the expression of IGFBP-5 and its regulation by follicle-stimulating hormone (FSH) in vitro in granulosa cells (Adashi et al. 1990, 1991 1992, 1993, Liu et al. 1993). In situ hybridisation revealed that IGFBP-5 is expressed in a cell-specific manner: predominantly in granulosa cells of atretic follicles, to a lesser extent in interstitial cells, in corpora lutea and surface epithelium. This pattern is strongly affected by the stage of the oestrous cycle (Erickson et al. 1992).

The expression pattern of the IGF system in the mouse ovary and its hormonal regulation were found to be quite different from those of the rat (Adashi et al. 1997). While IGFBP-5 expression was associated with atretic follicles in the rat, it was linked to the survival of slowly growing and immature preantral follicles in the mouse (Wandji et al. 1998).

The regulation of expression and the potential function of IGFBP-5 and other components of the IGF system during follicular development and corpus luteum function were also investigated in large farm animals (Besnard et al. 1996a,b, Schams et al. 1999) and avian species (Onagbesan et al. 1999).

Kidney physiology and pathology
IGFBP-5 is abundantly expressed in the kidney. The specific expression pattern described in human (Matsell et al. 1994), mouse (Lindenbergh-Kortleve et al. 1997) and rat (Price et al. 1995) kidneys suggests specific roles in the development and physiology of this organ. Upregulation of IGFBP-5 expression was associated with the inhibited renal growth that follows hypophysectomy in rats (Hise et al. 1994) while reduced IGFBP-5 expression was reported after the onset of streptozotocin-induced diabetes (Landau et al. 1995). In fact, the whole renal IGF system undergoes massive alterations during the renal complications of diabetes (Flyvbjerg 1997). A stimulatory role was postulated for IGFBP-5 in children with chronic renal failure where the serum level of this IGFBP correlated with patient growth (Ulinski et al. 2000).

Muscle cell differentiation
A relationship between the secretion of specific IGFBPs and the differentiation of myoblasts was first reported by McCusker & Clemmons (1988). Tollefsen et al. (1989) observed that cultured muscle myoblasts secreted a single 29 kDa IGFBP during their terminal differentiation. This protein was later identified as IGFBP-5 and its expression was demonstrated to increase during the early stages of cell differentiation (James et al. 1993, Rotwein et al. 1995). Increased IGFBP-5 secretion was also observed in differentiating myoblasts that overexpress IGF-II (Stewart et al. 1996) or after treatment with insulin and IGFs (Ewton & Florini 1995). Direct evidence for a role of IGFBP-5 in myoblast differentiation came from transfection experiments; cells which overexpressed the construct in sense orientation failed to differentiate normally but had higher survival rates, while antisense RNA expression caused premature differentiation (James et al. 1996). These effects could be neutralised by the addition of exogenous IGFs. Another piece of evidence in this direction came from studies using L6A1 muscle cells; IGFBP-5 inhibited IGF-mediated cell proliferation and IGF-II-mediated differentiation, but potentiated IGF-I-stimulated differentiation under some circumstances (Ewton et al. 1998). The explanation for this dual role was proposed to be associated with the ability of IGFBP-5 to bind to the cell membrane. Finally, IGFBP-5 acts as a survival factor for differentiating myoblasts; IGFBP-5-overexpressing cells were protected from apoptosis induced by tumour necrosis factor-α (Meadows et al. 2000).

Even if it remains to be explained how the IGFs stimulate two almost mutually exclusive processes like proliferation and differentiation, it is clear that IGFBP-5 plays a major role in modulating these effects.

IGFBP-5 and cancer
The biological activity of IGFBP-5 on growth of neoplastic cells appears to be ambiguous even when assessed on a single cell line (Ewton et al. 1998). There is accumulating evidence that IGFBP-5 appears to be a protein with rather growth stimulatory functions also supported by the recent observation that it acts as a growth factor on bone cells.
IGFBP-5 stimulates growth of prostate cancer cells via IGF-dependent and -independent pathways in vitro (Gregory et al. 1999, Miyake et al. 2000a) and it promotes tumour growth in an animal model (Miyake et al. 2000b). Similarly, IGFBP-5 improves the survival of breast cancer cells. This function appears to be IGF-independent and to rely on an apoptosis-inhibiting effect triggered via a pathway which does not involve the mitochondria (Perks et al. 1999, 2000). However, breast carcinoma cells apparently are also susceptible to growth inhibitory signals of IGFBP-5 at certain stages if triggered by appropriate signals such as vitamin D (Rozen & Pollak 1999), anti-oestrogens (Parisot et al. 1999) or androgen deprivation (Nickerson et al. 1999). In addition, IGFBP-5 inhibits the proliferation of cervical carcinoma cells (Higo et al. 2000) and osteosarcoma cells (Kiefer et al. 1992, Conover & Kiefer 1993). In osteosarcoma cells it appears that at least some growth inhibition is mediated through IGF-independent mechanisms such as stimulation of differentiation which is accompanied by increased osteocalcin levels (Schneider et al. 2001). Thus, the effect of IGFBP-5 seems to be cell type-specific and the status of the cells of a certain tissue may also strongly influence and determine its sensitivity towards IGFBP-5. Future research has to unravel the molecular mechanisms of these biological pathways and may reveal potential therapeutic applications of IGFBP-5.

Conclusion and future perspectives

The molecular structure of IGFBP-5 was identified a decade ago and since then an enormous amount of data has been accumulated about the biological activity of this protein. Despite this progress, a number of central questions remain unanswered. Although cellular receptors have unambiguously been shown to exist, it remains completely unknown what happens after this interaction and to date no cellular partners of an IGFBP-5-specific signalling pathway have been identified. The localisation of IGFBP-5 in the nucleus suggests a role in the regulation of gene expression. Whether this happens through direct action of IGFBP-5 or whether it requires association with other proteins is also completely unsolved. Another major deficiency is the physiological function of IGFBP-5. Our present knowledge is mainly derived from cellular systems as no appropriate animal models exist. Although the knockout approach may be poorly informative due to the functional compensation assured by other members of the IGFBP superfamily, the overexpression of IGFBP-5 in selected tissues of transgenic models constitutes a promising strategy to reveal the role of this IGFBP in different systems. The establishment of such models and the unravelling of the molecular events which take place after ligand–receptor interaction are the most important challenges for researchers in this field in the near future.

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