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REVIEW PAPER

Insulin-like growth factor-binding protein-1: an evolutionarily conserved fine tuner of insulin-like growth factor action under catabolic and stressful conditions

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The insulin-like growth factor-binding proteins (IGFBPs) are evolutionarily conserved components of the insulin-like growth factor (IGF) system. The six forms of IGFBPs (IGFBP-1–6) bind the IGF ligands (IGF-1 and -2) with high affinity and regulate the IGFs available to their receptors, therefore providing additional flexibilities in regulating IGF signalling. IGFBP-1, the first identified member of the IGFBP family is highly inducible under a variety of catabolic conditions, such as food deprivation, malnutrition, stress, injury and hypoxia. Recent *in vivo* studies have indicated that the induced IGFBP-1 serves as a molecular switch by restricting IGF signalling and diverts the limited energy resources away from growth and development towards those metabolic processes essential for survival. This article reviews the recent understandings of the molecular basis of IGFBP-1 regulation and its biological functions, as revealed through research in mammalian and fish models.

Key words: development; fish; growth; IGF; signalling; stress.

ROLES OF INSULIN-LIKE GROWTH FACTOR-BINDING PROTEINS IN CONTROLLING IGF ACTIONS

Insulin-like growth factors (IGF-1 and -2) have been established as key molecules that regulate a variety of cellular processes including proliferation, differentiation, survival and migration. The special importance of IGF signalling in embryonic development is demonstrated by studies showing that targeting disruption of the IGF-1, IGF-2 and/or IGF-1 receptor gene in mice or natural mutations in human patients results in severe dwarfism (DeChiara *et al*., 1990; Baker *et al*., 1993; Liu *et al*., 1993; Woods *et al*., 1996; Abuzzahab *et al*., 2003; Dupont & Holzenberger, 2003). The biological actions of IGFs are mainly mediated through type-1 (IGF-1) receptor on the cell surface, leading to tyrosine

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phosphorylation of the beta-domain of the receptor and subsequent signalling cascades such as the mitogen-activated protein kinase (MAPK), phosphoinositol-3-kinase and protein kinase B (Akt) signalling pathways (Le Roith *et al*., 2001; Nakae *et al*., 2001). In contrast, the type-2 IGF receptor, identified as the cation-independent mannose-6-phosphate receptor, lacks recognizable catalytic domains and presumably works for clearance of IGF-2 (Braulke, 1999).

The molecular interactions of IGFs with their receptor are tightly regulated by six distinct forms of IGF-binding proteins (IGFBPs), referred to as IGFBP-1 through IGFBP-6. IGFBPs are secreted proteins with molecular masses of 24–44 kDa that share highly conserved N-terminal and C-terminal domains and a variable linker (L)-domain. Since IGFBPs bind IGFs with greater affinities (K_D *c.* 0·1 nM) than IGF receptors (K_D *c.* 1 nM), over 95% of IGFs in extracellular fluids form complexes with IGFBPs. In addition, the majority of IGFBP-3 and -5 in the circulation are associated with a glycoprotein known as acid-labile subunit to form 130–150 kDa of ternary complex. It has been considered that the roles of the IGF/IGFBP complex are (1) to prevent potential hypoglycemia resulting from cross-activation of the insulin receptor, (2) to mediate the efflux of IGFs from the vascular space to the cell surface and (3) to prolong their half-lives in circulation by inhibiting proteolytic degradation (Clemmons, 2001; Duan, 2002; Firth & Baxter, 2002; Duan & Xu, 2005). Importantly, these IGFBPs provide an additional level (temporal and spatial) of flexibility for co-ordinating the delivery of 'biologically active' IGFs to specific target cells. Despite the fact that the six IGFBPs share structural homology, each IGFBP has distinct characteristics and plays different roles in controlling IGF signalling, depending on the physiological context and the cell types (Fig. 1). For example, IGFBP-3 and -5 have a heparin-binding motif that binds to the glycosaminoglycan side chains and other components in the extracellular matrix (ECM). The binding of the IGF/IGFBP complex to the ECM reduces the affinity of the IGF–IGFBP interaction, causing release and delivering of free IGFs to the target cells. Thus, these IGFBPs potentiate the IGF actions (enhancing effect). In contrast, several IGFBPs, including IGFBP-1, inhibit IGF-1 activity by competing for the same binding surface of IGF-1 with IGF-1R (Jansson *et al*., 1998). Proteolysis of IGFBP-1 by the IGFBP protease decreases the binding affinity; therefore, the released IGFs are able to interact and activate IGF-1R (inhibitory effect). In addition, a new mode of IGFBP action, *i.e.* ligand-independent effect, has been reported. Despite the fact that all the IGFBPs are secreted proteins, IGFBP-3 and -5 are localized in the nucleus through their nuclear localization signal in the C-domain (Firth & Baxter, 2002; Xu *et al*., 2004). The nuclear localized IGFBP-3 has been reported to interact with the retinoid-X receptor α and that interaction is important for the IGFBP-3-induced apoptosis in prostate cancer cells (Liu *et al*., 2000). More recently, IGFBP-3 has been reported to interact with RNA polymerase II binding subunit 3, implying its possible involvement in gene transcription (Oufattole *et al*., 2006). A recent study has also shown that the nuclear IGFBP-5 is in a complex containing histone H3 and that the N-domain of both IGFBP-3 and -5 possess transactivation activity in a ligand-independent manner (Zhao *et al*., 2006). Therefore, the IGFBPs also provide the novel interface with the transcription machinery independent from the ligands binding, in

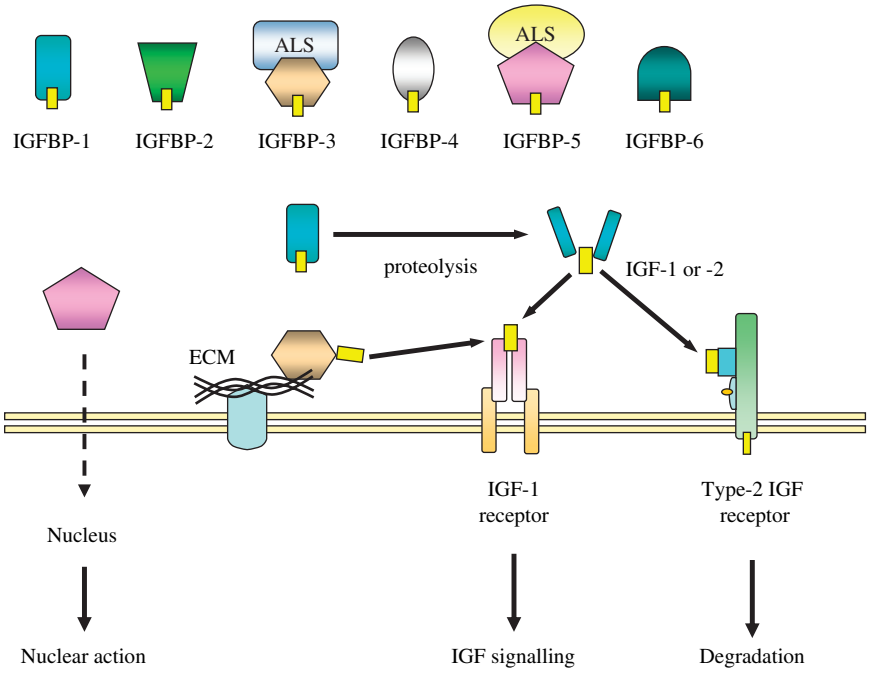

FIG. 1. The insulin-like growth factor (IGF) system. Two IGF ligands (IGF-1 and -2), two receptors (IGF-1 receptor and type-2 IGF receptor) and six forms of IGF-binding proteins (IGFBPs) are shown. IGFBP-3 and -5 form ternary complexes with acid-labile subunits (ALS) that stabilize the IGFBP/IGF complex in circulation. Three modes of IGFBP action are proposed: (1) enhancing effects of IGF actions, (2) inhibitory effects of IGF actions and (3) ligand-independent action. See text for detail. ECM, extracellular matrix.

addition to their conventional functions as IGF modulators. For comprehensive reviews on the structure perspectives of the IGFBP family, readers are referred to several recent reviews (Clemmons, 2001; Firth & Baxter, 2002; Duan & Xu, 2005). For a comparative overview of IGFBPs, see two review articles by Kelley *et al*. (2002) and Wood *et al*. (2005).

STRUCTURAL AND BIOCHEMICAL FEATURES OF IGFBP-1

IGFBP-1, the authors focus in this review, is the first identified member of the IGFBP family, initially purified from human amniotic fluid (Drop *et al*., 1984*a*, *b*, also see review by Lee *et al*., 1997). Human IGFBP-1 consists of 234 amino acids with 12 cysteine residues in the N-domain and 6 cysteine residues in the C-domain [Fig. 2(a)]. Both the N- and the C-domains of IGFBP-1 directly interact with IGF-1 but not with insulin, and the L-domain acts as a hinge. The L-domain is rich in proline, glutamate, serine and threonine (PEST), known to increase the susceptibility to proteolysis. The instability of IGFBP-1 through the PEST sequence allows quick turnover (Lee *et al*., 1997). Another characteristic motif of IGFBP-1 is the Arg–Gly–Asp (RGD) sequence in the C-domain. It has been demonstrated that IGFBP-1 binds *via*

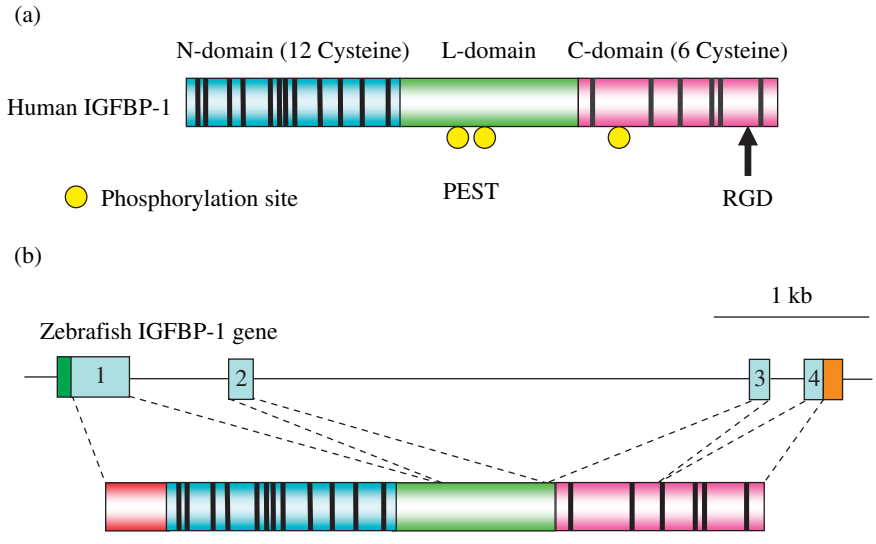

FIG. 2. Structural and biochemical features of IGFBP-1. (a) Structure of human IGFBP-1 (234 amino acids). It can be divided into three domains, *i.e.* the amino-terminal domain (N-domain) containing 12 cysteine residues, the linker domain (L-domain) and the carboxyl-terminal domain (C-domain) containing 6 cysteine residues. The proline, glutamate, serine and threonine (PEST) sequence in the L-domain and the Arg–Gly–Asp (RGD) motif in the C-domain are shown. Three phosphorylation sites are indicated. (b) Structure of the zebrafish IGFBP-1 gene. It spans 5 kb in the genome, composed of four exons and three introns. Note that all the 18 cysteine residues are located in conserved position with mammalian counterparts; however, the PEST, RGD motifs and phosphorylation sites are not conserved.

the RGD motif to $\alpha_5\beta_1$ integrin and stimulates cell migration (Jones *et al.*, 1993*c*). Since the C-domain of IGFBP-1 or IGFBP-1 without IGF-1-binding ability is still able to stimulate cell migration, this effect is considered to be ligand independent (Jones *et al.*, 1993*c*; Sala *et al.*, 2005). The interaction of IGFBP-1 with $\alpha_5\beta_1$ integrin leads to the activation of focal adhesion kinase dephosphorylation, and consequently, induces cell detachment and apoptotic cell death in human breast cancer cells (Perks *et al.*, 1999).

Several studies have reported the post-translational modifications of IGFBP-1 including polymerization and phosphorylation that affect its function (Busby *et al.*, 1989; Sakai *et al.*, 2001). According to Sakai *et al.* (2001), IGFBP-1 forms multimers upon the stimulus of transglutaminase type 2. Non-phosphorylated IGFBP-1 polymerizes more rapidly and to a greater extent, leading to the attenuation of the IGFBP-1's inhibitory effects on IGF-1-stimulated protein synthesis. However, phosphorylation at Ser101, Ser119 and Ser169 of human IGFBP-1 enhances the binding affinity to IGF-1 by six-fold (Jones *et al.*, 1991, 1993*a*, *b*). Gibson *et al.* (2001) have reported that placental alkaline phosphatase, presumably produced in trophoblast, dephosphorylates the phosphorylated forms of IGFBP-1. Since the non-phosphorylated form is more susceptible to the proteolysis degradation, the phosphatase is able to regulate the availability of IGFs by controlling the amount of local IGFBP-1 through post-translational modifications. In addition, the relationship between IGFBP-1

phosphorylation and pathological condition has been suggested (Frost *et al*., 1994; Westwood *et al*., 1994; Gibson *et al*., 1999). However, unlike human IGFBP-1, rat IGFBP-1 phosphorylated at Ser107 and Ser132 retains its affinity to IGF-1 regardless of the phosphorylation status (Peterkofsky *et al*., 1998).

In teleosts, it has been known that the serum from several species contains at least three major IGFBPs around 20–25, 30 and 40–45 kDa (Kelley *et al*., 1992, 2001; Anderson *et al*., 1993; Fukazawa *et al*., 1995; Siharath *et al*., 1996; Shimizu *et al*., 1999, 2000; Park *et al*., 2000; Kajimura *et al*., 2003; Riley *et al*., 2004; Davis & Peterson, 2006; see also the reviews by Kelley *et al*., 2002 and Wood *et al*., 2005). Based on the molecular sizes and the hormonal regulations of mammalian counterparts, one of the IGFBPs under 30 kDa was speculated to be fish IGFBP-1 (Kelley *et al*., 1992, 2001, 2002; Siharath *et al*., 1996; Shimizu *et al*., 1999, 2000; Park *et al*., 2000; Bauchat *et al*., 2001; Kajimura *et al*., 2003; Riley *et al*., 2004; Peterson & Small, 2005; Shepherd *et al*., 2005). Bauchat *et al*. (2001) purified a 30 kDa IGFBP from the cultured rainbow trout liver cells. Based on the structural homology and biochemical properties, this IGFBP is the trout homologue of human IGFBP-1. Shimizu *et al*. (2005) purified a 22 kDa IGFBP from Chinook salmon serum and characterized it as IGFBP-1. To date, the full-length IGFBP-1 sequence has been reported for zebrafish (Maures & Duan, 2002), rainbow trout (Kamangar *et al*., 2006) and a partial sequence for longjaw mudsucker (Gracey *et al*., 2001). The zebrafish IGFBP-1 gene spans 5 kb in the genome (Kajimura *et al*., 2005). It is composed of four exons and three introns located in a tail-to-tail position with the IGFBP-3 gene, as reported in mammals [Fig. 2(b)]. Although fish IGFBP-1 shows the highest homology to human IGF-1 among the IGFBP family, the actual identity is modest (36–40%), perhaps due to the low homology in the L-domain (15–20%). Fish IGFBP-1 also shares significant sequence identity to human IGFBP-4 (34–39%). Its identities with other human IGFBPs are substantially lower. All the 18 cysteine residues are located in conserved positions in fish IGFBP-1; however, the PEST and RGD motifs are not found. Furthermore, none of the phosphorylation sites, identified in human and rat, are conserved in fish IGFBP-1. Consistent with the structural features of fish IGFBP-1, it has been shown that fish IGFBP-1 by itself had no effects on the cell proliferation of zebrafish embryonic cells (Bauchat *et al*., 2001; Kajimura *et al*., 2005).

REGULATION OF IGFBP-1

From earlier studies that measure IGFBP-1 levels in a number of physiological conditions, it has been revealed that circulating IGFBP-1 displays dynamic kinetics. The rapid and highly inducible profile distinguishes IGFBP-1 from other IGFBPs. The half-life of IGFBP-1 in circulation is estimated in the range of 7–13 min (Lee *et al*., 1997). In addition to the protein instability through the PEST sequence, the instability of mRNA contributes to the high turnover of IGFBP-1. The 3′-untranslated region (3′-UTR) of IGFBP-1 mRNA contains multiple ‘ATTTA’ sequences, known to play a role in transiently expressed mRNAs (Gay & Babajko, 2000).

Circulating IGFBP-1 levels are greatly influenced by nutritional status and insulin levels, implying their involvement in glucose metabolism. In fact, insulin is one of the major endocrine regulators of IGFBP-1 transcription. The promoter region of the mammalian IGFBP-1 gene contains a palindrome sequence of insulin response elements [IRE: T(G/A)TTT(T/G)(T/G)] that mediate the inhibitory effects of insulin (Suwanickul *et al*., 1993). A forkhead transcription factor family, named hepatic nuclear factor-3, was initially shown to bind to the IRE (Unterman *et al*., 1994; Allander *et al*., 1997). However, subsequent studies have demonstrated that the forkhead box O1 (FoxO1) (previously described as FKHR) is the bona fide protein that binds to the IRE in the human IGFBP-1 gene and mediates the insulin effects (Durham *et al*., 1999; Nakae *et al*., 2006). Glucocorticoid is another potent endocrine regulator of IGFBP-1expression. Glucocorticoid induces IGFBP-1 at the transcription level, although insulin acts in a dominant manner to suppress basal- and glucocorticoid-induced IGFBP-1 expression (Suwanichkul *et al*., 1994; O'Brien *et al*., 1995). The biological effect of glucocorticoid is mediated through the direct binding of the glucocorticoid receptor (GR) to the GR response elements (GRE) on the human IGFBP-1 promoter, located contiguous to the IREs (Goswami *et al*., 1994; Gan *et al*., 2005). It should be noted that, FoxO proteins also act cooperatively to mediate the glucocorticoid-induced IGFBP-1 expression through recruiting cAMP response element-binding protein /p300 and the steroid receptor coactivator complex (Nasrin *et al*., 2000). Furthermore, several endocrine factors are reported to regulate IGFBP-1 gene expression including glucagon (Kachra *et al*., 1994), progesterone (Gao *et al*., 1994; Gao & Tseng, 1997), interleukin-6 (Leu *et al*., 2001) and vitamin D (Matilainen *et al*., 2005). The *cis*-elements of these factors are identified as cAMP response element (CRE), progesterone receptor response element, hepatocyte nuclear factor-1-binding site and vitamin receptor response element on the IGFBP-1 promoter region, respectively.

IGFBP-1 is also induced by a variety of catabolic conditions. For example, fasting, malnutrition and protein restriction rapidly induce IGFBP-1 at the transcription level (Busby *et al*., 1988; Ooi *et al*., 1990; Murphy *et al*., 1991; Straus *et al*., 1993; Takenaka *et al*., 1993). Interestingly, depletion of a single amino acid (arginine, cysteine and each of the essential amino acids) is sufficient to induce IGFBP-1 expression *in vitro* (Jousse *et al*., 1998; Takenaka *et al*., 2000). According to Takenaka *et al*. (2000), the promoter region containing both IRE and GRE on the rat IGFBP-1 gene is required for the IGFBP-1 induction in response to amino acid depletion. Averous *et al*. (2005) have also reported that amino acid depletion stabilizes IGFBP-1 mRNA through its 3′-UTR region. Furthermore, Patel *et al*. (2002) have demonstrated that rapamycin inhibits the mammalian target of rapamycin (mTOR), an essential integrator of nutrient signalling, and abolishes the inhibitory effect of insulin on IGFBP-1 expression. Since amino acid depletion *per se* downregulates the mTOR signalling, the mTOR pathway through IRE appears to be involved in the nutrition-dependent IGFBP-1 regulation in conjunction with the glucocorticoid-mediated pathway. The underlying molecular mechanisms of how single amino acid depletion regulates IGFBP-1 remain unclear at this time.

Other catabolic conditions regulating IGFBP-1 expression include endoplasmic reticulum (ER) stress and hypoxia. Under ER stress, unfolded proteins are

accumulated within the cell, and the co-ordinated adaptive programmes are triggered to alleviate ER stress through several proteins. One of them is activating transcription factor-4 (ATF-4), a basic leucine zipper (bZIP) transcription factor that regulates ER-induced gene expressions including CCAAT enhancer-binding protein (C/EBP) homologous protein (Ohoka *et al*., 2005). Marchand *et al*. (2006) have demonstrated that a distal regulatory region of human IGFBP-1 contains an ATF-4-binding site [(R/C)TT(R/T)CRTCA, R = G/A] at −6481/−6473 to which ATF-4 specifically binds. Since mutation of the ATF-4-binding site or knockdown of endogenous ATF-4 blunts the induction of IGFBP-1 by ER stress, ATF-4 mediates the ER-stress-induced IGFBP-1 expression. Another example is the regulation of IGFBP-1 expression in a hypoxic environment. Hypoxia triggers the transcriptional changes in a number of genes that promote O_2 delivery and anaerobic respiration and suppress major energy-requiring processes (Guillemin & Krasnow, 1997; Huang *et al*., 2004; Seta & Millhorn, 2004). Many of these transcriptional responses to hypoxia are mediated by the hypoxia-inducible factor-1 (HIF-1), a heterodimeric complex composed of HIF-1α and aryl hydrocarbon receptor nuclear translocator. HIF-1α is subjected to the constant degradation *via* ubiquitin and the proteosome pathway when oxygen levels are ample. In a hypoxic environment, however, HIF-1α is stabilized and eventually accumulates within the nucleus, where it activates target gene expression by direct binding to the consensus *cis*-regulatory DNA sequence, named hypoxia response element (HRE; A/G CGTG) (Semenza, 1999; Bruick, 2003). Several *in vitro* and *in vivo* studies have shown that IGFBP-1 is a hypoxia-inducible gene (McLellan *et al*., 1992; Tazuke *et al*., 1998; Popovici *et al*., 2001). Furthermore, significantly higher levels of serum IGFBP-1 are found at higher altitude (Krampl, 2002). Tazuke *et al*. (1998) have identified a HRE located in intron 2 of the human IGFBP-1 gene responsible for the hypoxia response in cultured human hepatoma cells. Likewise, recent studies using zebrafish embryo show that the induction of IGFBP-1 gene expression by hypoxia is mediated through HIF-1 both *in vitro* and *in vivo*, and the HIF-1 pathway is established in early embryonic stages (Kajimura *et al*., 2006). Although the zebrafish IGFBP-1 promoter contains 13 potential HREs within a 2·1 kb region upstream of the translational start site, only one HRE positioned at −1090/−1086 is responsible for the hypoxia and HIF-1-induced IGFBP-1 expression. Intriguingly, an additional *cis*-element, HIF-1 ancillary sequence (HAS), is required for the responsiveness to hypoxia and to HIF-1 (Kajimura *et al*., 2006). Since HAS exists close to the authentic HRE site in the IGFBP-1 gene of mammalian species as well as in other hypoxia-responsive genes, including vascular endothelial growth factor, erythropoietin and lactate dehydrogenase A, the requirement of HAS for the hypoxia responses may be applicable not only to the IGFBP-1 gene but also to many, if not all, of the hypoxia-inducible genes (Kimura *et al*., 2001; Kajimura *et al*., 2006). The identification and functional characterization of HAS awaits further investigation.

The hormonal regulation of IGFBP-1 gene expression appears to be conserved between mammals and teleost species. IGFBP-1 or a lower molecular weight (20–30 kDa) IGFBP is induced by fasting, stress, hypoxia and salinity challenge (Siharath *et al*., 1996; Kelley *et al*., 2001, 2002; Kajimura *et al*., 2005, 2006; Shepherd *et al*., 2005; Davis & Peterson, 2006). In the cultured tilapia

hepatocyte, oestradiol-17β and 5α-dihydrotestosterone have been found to regulate the release of IGFBP-1 (Riley *et al*., 2004). Moreover, cortisol or dexamethasone treatment increases circulating IGFBP-1 and its gene expression (Kajimura *et al*., 2003; Peterson & Small, 2005; Pierce *et al*., 2006). Using a homologous radioimmunoassay of salmon IGFBP-1, Shimizu *et al*. (2006) demonstrated that circulating IGFBP-1 levels were inversely correlated with somatic growth in Chinook salmon. Although the functional contribution remains unclear, the promoter region of the IGFBP-1 gene in zebrafish contains multiple hormonal regulatory *cis*-elements including GRE and CRE, as found in mammalian species, suggesting conserved mechanisms to regulate IGFBP-1 under catabolic conditions. Pierce *et al*. (2006) have recently reported that insulin did not have inhibitory effects on IGFBP-1 gene expression nor protein levels in the salmon hepatocytes. The mechanisms underlying the lack of the inhibitory effects by insulin are unknown. The identification of the authentic *cis*-elements responsible for the hormonal/environmental stimuli is a future direction of study.

PHYSIOLOGICAL FUNCTIONS OF IGFBP-1

A number of *in vitro* studies have shown that IGFBP-1 has an inhibitory effect on IGF activity. As described above, the inhibitory effects seem to be mainly through competing for the same binding site in IGF-1 for IGF-1R. Several *in vivo* models with gain of function approaches support this view. A transgenic mouse model expressing IGFBP-1 driven by the phosphoglycerokinase promoter causes growth retardation and hyperglycaemia (Rajkumar *et al*., 1995). Another transgenic mouse line under the control of a liver-specific promoter (antitrypsin promoter) also displays pleiotropic defects in several skeletal units, delayed mineralization, antenatal growth retardation and reproduction defects (Gay *et al*., 1997; Froment *et al*., 2002; Ben Lagha *et al*., 2006). Crossey *et al*. (2002) established a transgenic line using its own promoter that mimics the endogenous expression in foetal liver and decidual tissue. These mice show foetal growth retardation in midgestation and defects in placental development. Since the excess amounts of IGFBP-1 in amniotic fluid impaired foetal growth regardless of the foetal genotype, maternal IGFBP-1 causes the placental insufficiency. However, Watson *et al*. (2006) employed an α-foetoprotein gene promoter that directs the IGFBP-1 expression predominantly in the foetal liver without altering the circulating IGF-1 levels or nutrient supply to the foetus. These mice display foetal growth retardation resembling the intrauterine growth restriction phenotype. In zebrafish embryos, overexpression of IGFBP-1 reduced the embryo size to 83% of the control and also caused developmental delay (Kajimura *et al*., 2005). Therefore, the high level of IGFBP-1 in the embryo is sufficient to induce growth retardation. Overall, regardless of the difference in the amount, tissue and timing of the transgene expression, these studies confirmed the importance of IGFBP-1 in regulating embryonic growth through restricting IGF actions. Although most studies report the inhibitory effects of IGFBP-1 on IGF activity, several papers have shown the stimulatory effects of IGFs mitogenic action in the presence of low concentrations of platelet-poor plasma in some cell lines. It is speculated that IGF-1/IGFBP-1 complex sustains the release of IGF-1 to receptor, leading to the potentiation of IGF-1 actions (Jones & Clemmons,

1995). On the other hand, the loss-of-function approach in the mouse model does not provide the definitive evidence for IGFBP-1 as a growth regulator.

IGFBP-1 knockout mice were normal in growth and development during pre- and post-natal stages (Leu *et al.*, 2003*a*, *b*). This appears to be due to the compensatory effects by other IGFBPs that have redundancy in function and expression patterns. Similar findings have been reported in other IGFBP members. IGFBP-2 knockout mice also have minor phenotypic aside from a smaller spleen and a larger liver (Wood *et al.*, 2000). Knockout mice for IGFBP-3, -5 and -6 have also been generated, but these animals appeared normal in size and shape (Pintar *et al.*, 2001). Consistent with these findings, transient knockdown of IGFBP-1 by morpholino oligos did not cause any developmental defects in zebrafish embryos. However, under certain stressful conditions, loss of IGFBP-1 exhibits clear defects. IGFBP-1 null mice showed impaired DNA synthetic response and liver necrosis during liver regeneration after partial hepatoectomy, although liver mass restoration was normal (Leu *et al.*, 2003*a*). This impairment was associated with the reduced responses of MAPK/extracellular signal-regulated kinase and C/EBP β that are involved in the liver regeneration process. In addition, loss of IGFBP-1 leads to massive apoptosis in the liver after treatment with a Fas ligand or liver injury, and pretreatment with IGFBP-1 was able to rescue this abnormality (Leu *et al.*, 2003*b*). These results suggest that IGFBP-1 plays a critical role as a survival factor. Since the liver expresses IGF-1R at a very low level, the effects on hepatocyte cell proliferation may be IGF independent. A knock-in approach would be necessary to test this notion using IGFBP-1 mutants that do not interact with IGFs or mutants without the RGD sequence.

The loss of function approach has been successful in zebrafish embryos probably because of the lack of maternal compensation in gene knockout progeny *via* placental circulation. Chronic hypoxia treatment results in significant embryonic growth retardation and developmental delay, concomitant with a significant increase in IGFBP-1 mRNA and protein levels. The targeted knockdown of IGFBP-1 partially (43–65%) alleviated the hypoxia-caused growth retardation and developmental delay. Reintroduction of an MO-resistant IGFBP-1 to the IGFBP-1 knocked down embryos and restored the hypoxia effects. Furthermore, the inhibitory effect of IGFBP-1 on IGF-dependent cell proliferation was reversed in the presence of excess IGF-1 or IGF-2, whereas IGFBP-1 *per se* did not have any effects (Kajimura *et al.*, 2005). Therefore, the mode of IGFBP-1 action is primarily through reducing free/active forms of IGFs and the consequent suppression of IGF signalling (Fig. 3). These findings provide strong evidence supporting the hypothesis that IGFBP-1 plays a key role in hypoxia-caused growth retardation and developmental delay by binding foetal IGF and inhibiting their growth-promoting activities.

In summary, there is compelling evidence that IGFBP-1 is a fine tuner of IGF actions under a number of catabolic and stressful conditions. In the normal environment, the inhibition of IGF signalling by IGFBP-1 is in an 'off' mode, thus favouring fast growth and development. In a condition unfavourable to growth and development, highly induced IGFBP-1 is 'turned on' to restrict IGF signalling by binding free IGFs. This involvement of insulin/IGF signalling in a catabolic environment has also been indicated in invertebrates. For

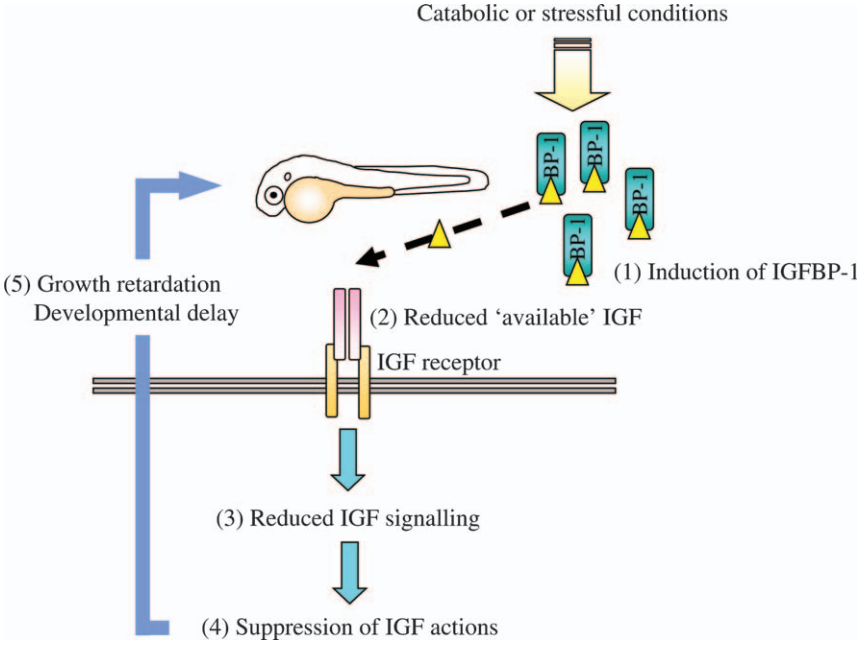

FIG. 3. A model of IGFBP-1 action. The IGFBP-1 gene is rapidly induced under a catabolic or stressful condition. Increased IGFBP-1 binds to IGFs and reduces their availability to the IGF-1 receptor. This leads to the reduction of IGFsignalling and subsequent suppression of IGF-dependent actions. This suppression of IGF signalling contributes to the embryonic growth retardation and developmental delay.

example, it has been demonstrated in the nematode *Caenorhabditis elegans* that the wild type does not survive in a hypoxic environment, whereas the *daf-2* mutant (insulin receptor mutation) is highly tolerant to hypoxia, mediated through an Akt-1/PDK-1/FKHR pathway overlapping with, but distinct from, signalling pathways regulating life span (Scott *et al.*, 2002). Because these stressful and catabolic conditions lead to adaptive changes in metabolic reorganization, such as the activation of the anaerobic ATP-generating pathway (glycolysis), the biological significance of IGFBP-1 may be to serve as a molecular switch by controlling the availability of IGFs to their receptors and to divert the limited energy resources away from growth and development towards those metabolic processes essential for survival.

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