REVIEW

The acid-labile subunit (ALS) of the 150 kDa IGF-binding protein complex: an important but forgotten component of the circulating IGF system

Y R Boisclair, R P Rhoads, I Ueki, J Wang and G T Ooi¹

Department of Animal Science, Cornell University, Ithaca, New York 14853, USA

¹Prince Henry's Institute of Medical Research, Clayton, Victoria 3168, Australia

(Requests for offprints should be addressed to Y R Boisclair, 259 Morrison Hall, Department of Animal Science, Cornell University, Ithaca, New York 14853, USA; Email: yrb1@Cornell.edu)

Abstract

The insulin-like growth factors-I and -II (IGFs) are involved in a wide array of cellular processes such as proliferation, prevention of apoptosis, and differentiation. Most of these effects are mediated by the IGF-I receptor, although at higher IGF concentrations the insulin receptor can also be activated. As the expression of both the IGFs and their receptors is widespread, IGFs are thought to have autocrine/paracrine modes of actions also, particularly during foetal life. The endocrine component of the IGF system is recognised to be important after birth, with IGF-I mediating many of the effects of growth hormone (GH), and linking anabolic processes to nutrient availability. Consideration of ligands and receptors, however, is insufficient to provide a complete understanding of the biology of IGF. This is because IGFs are found in binary

complexes of 40–50 kDa with members of a family of IGF-binding proteins (IGFBPs-1 to -6) in all biological fluids. In addition, in postnatal serum, most IGFs are sequestered into ternary complexes of 150 kDa consisting of one molecule each of IGF, IGFBP-3 or IGFBP-5, and acid-labile subunit (ALS). Despite evidence that ALS plays an important role in the biology of circulating IGFs, it has received only limited attention relative to the other components of the IGF system. This review provides an overview on the current knowledge of ALS protein and gene structure, organisation and regulation by hormones, and insights from novel animal models such as the ALS knockout mice.

Journal of Endocrinology (2001) **170**, 63–70

Introduction

Insulin-like growth factors (IGFs)-I and -II play crucial roles in growth and development. IGF-I is synthesised by a variety of cell types, and is involved in linear growth, cell proliferation and differentiation, and in apoptosis. Most of these effects are mediated by both endocrine and autocrine/paracrine mechanisms through the IGF-I receptor (Werner *et al.* 1991). In circulation, almost all the IGFs are present as 150 kDa ternary complexes comprising of one molecule each of IGF, IGF-binding protein-(IGFBP)-3 (the predominant IGFBP in serum) or IGFBP-5, and a 85 kDa glycoprotein, the acid-labile subunit (ALS) (Rechler 1993, Baxter 1994, Ooi & Boisclair 1999). Plasma also contains lower molecular mass complexes of ~50 kDa which are made up of several IGFBP species (IGFBPs-1, -2, -4, and -6) that are

incompletely saturated with IGFs leaving virtually no free IGFs in circulation (Jones & Clemmons 1995, Stewart & Rotwein 1996). Unlike free IGFs and IGFs bound to the ~50 kDa binary complexes, which can cross the vascular endothelium, formation of the ternary complexes restricts the IGFs to the circulation, prolongs their half-lives and allows them to be stored at high concentration in plasma to facilitate their endocrine actions and to minimise their local effects due to their intrinsic insulin-like activities such as hypoglycaemia (Zapf *et al.* 1995). Despite this important role of ALS in determining the endocrine effects of IGFs on target tissues, it has historically received limited attention compared with the other members of the circulating IGF system, such as the IGFBPs and proteases. ALS, synthesised exclusively by the liver, is predominantly stimulated by growth hormone (GH), as are both IGF-I and IGFBP-3. Presence of ALS after birth is coincident

with increased responsiveness to GH resulting from an increase in GH secretion and hepatic GH receptors, and is an important factor driving the formation of the 150 kDa complex. After the initial increase in ALS after puberty, ALS concentrations largely remain unchanged throughout adulthood, thereby ensuring that IGFs are constantly sequestered as the 150 kDa complexes. With the recent characterisation of the ALS gene in many species, including structure–function relationships, and elucidation of the regulation of ALS synthesis, future research efforts can now be devoted to understanding the significance and health benefits of the recruitment of IGFs into ternary complexes during postnatal life. This review summarises recent work by others and us on the biochemical properties of ALS, its gene structure and organisation, the regulation of its synthesis by GH and the molecular mechanisms underlying this transcriptional regulation. New information obtained from our studies on the ALS knockout mice will also be discussed.

ALS gene and protein structure

The *ALS* gene was first cloned in 1996, in the mouse (Boisclair *et al.* 1996). The mouse gene covers approximately 3.3 kilobases (kb) of chromosomal DNA, and is comprised of two exons separated by a 1126 base pair (bp) intron. Exon 1 encodes the first five amino acids of the signal peptide, and exon 2 encodes the remaining 22 amino acids of the signal peptide and the 576 amino acid residues of the mature protein. This chromosomal structure is conserved across species, as shown by the subsequent descriptions of the gene in rat, man and sheep (Delhanty & Baxter 1997, Rhoads *et al.* 2000, Suwanichkul *et al.* 2000). *ALS* is a single-copy gene, and was mapped to bands A2–A3 of mouse chromosome 17, and to the short arm of human chromosome 16 at p13.3 (Boisclair *et al.* 1996, Suwanichkul *et al.* 2000).

In all species studied so far, transcription of the *ALS* gene is controlled by a TATA-less promoter and produces mRNAs of ~2.2 kb (Boisclair *et al.* 1996, Rhoads *et al.* 2000, Dai & Baxter 1992, Leong *et al.* 1992, Delhanty & Baxter 1996). These mRNAs encode proteins ranging in size from 603 amino acids in the mouse to 611 residues in the sheep. Identity of mature ALS is 93% between mouse and rat, 79% between mouse and human, and 73% between mouse and sheep. Structural features almost completely conserved across species include the presence of 12–13 cysteine residues, six or seven asparagine-linked glycosylation sites, and 18–20 repeating leucine-rich domains of 24 amino acids. These leucine-rich domains account for approximately 75% of the mature protein, and organise ALS into a doughnut-shaped structure (Janosi *et al.* 1999b). ALS belongs to the superfamily of leucine-rich repeats – a family characterised by their ability to participate in protein–protein interactions.

Biochemical attributes of ALS

Human ALS has an apparent molecular weight of 84–86 kDa after purification and of 66 kDa after enzymatic deglycosylation (Baxter *et al.* 1989, Baxter & Dai 1994). ALS has no affinity for free IGF-I or IGF-II and very low affinity for uncomplexed IGFBP-3. It, however, readily binds to binary complexes of IGF and IGFBP-3 (Baxter & Martin 1989, Twigg & Baxter 1998). Affinities of ALS for binary complexes are 300–1000-fold lower than those of IGFBP-3 for IGF-I or -II (Holman & Baxter 1996). As suggested by its name, the ability of ALS to form ternary complexes is irreversibly destroyed under acidic conditions (pH < 4.5; Holman & Baxter 1996).

IGFBPs-1, -2, -4 or -6 cannot substitute for IGFBP-3 in forming the ternary complex with ALS. In contrast, IGFBP-5, the member of the IGFBP family most closely related to IGFBP-3, is able to form ternary complexes with ALS, and circulates predominantly in a high molecular weight form characteristic of a ternary complex with IGF-I or -II and ALS (Twigg & Baxter 1998). Ternary complexes containing IGFBP-5 account at best for ~10% of total ternary complexes in serum, and their significance remains unclear. In addition, unlike IGFBP-3, IGFBP-5 is able to associate weakly with ALS in the absence of IGFs, raising the possibility that a large fraction of the high molecular weight complexes containing IGFBP-5 do not carry any IGF (Twigg *et al.* 1998).

Structurally, IGFBPs-1 to -6 share homologous amino- and carboxy-terminal domains but have unique central domains (Rechler 1993, Ooi & Boisclair 1999). Domain swapping experiments with IGFBP that are unable to form ternary complexes (i.e. IGFBPs-2 and -6) have demonstrated that the carboxy-terminal domains of IGFBPs-3 and -5 are important for binding ALS (Hashimoto *et al.* 1997, Twigg *et al.* 1998). Binding ability was further mapped to a conserved region of 18 amino acid residues corresponding to residues 201–218 in IGFBP-3, and residues 215–232 in IGFBP-5 (Firth *et al.* 1998, Twigg *et al.* 1998). This region is composed of mostly basic and positively charged amino acid residues. More recently, the central domain of IGFBP-5 was also shown to bind ALS, even in the absence of the carboxy-terminal domain (Twigg *et al.* 2000).

In the case of ALS, recent studies have sought to determine the role played by the sugar residues. Removal of the negatively charged sialic acid from the glycan chains of ALS reduces the affinity of ALS for the IGF-I and -II binary complexes, but does not eliminate complex formation (Janosi *et al.* 1999a). Independent mutations of each of the seven *N*-linked glycan attachment sites of human ALS do not eliminate its ability to form ternary complexes with IGFBP-3, but complete deglycosylation does. Overall, these data are consistent with a model in which the positively charged, conserved domain of 18 amino acid present in IGFBP-3 and -5 interacts with negatively

charged regions of ALS. This is supported by molecular modelling of ALS, which predicts two densely negatively charged regions, the first one created by the clustering of six of the seven *N*-linked sugar chains, and the second by the amino acids present at the internal surface of the doughnut-shaped protein (Janosi *et al.* 1999b).

Regulation of ALS synthesis

ALS is found in high concentration almost exclusively in postnatal serum (Baxter 1990a, Khosravi *et al.* 1997). Typical concentrations in human and rat serum are 230 and 570 nM respectively. ALS circulates in excess over the other components of the ternary complex, with 50–60% of serum ALS found in free form (Baxter 1990a, Baxter & Dai 1994, Khosravi *et al.* 1997). When total RNA is analysed by northern blot in rat, primate and sheep, *ALS* gene expression can be detected only in liver (Dai & Baxter 1994, Delhanty & Baxter 1996, Rhoads *et al.* 2000). Synthesis in liver is confined to parenchymal cells (Chin *et al.* 1994). Immunoreactive ALS is also present at very low concentration in cerebrospinal fluid, amniotic fluid, milk and lymph, and at low to medium concentrations in peritoneal, synovial, ovarian and blister fluid (Baxter 1990a, Xu *et al.* 1995, Cwyfan Hughes *et al.* 1997, Khosravi *et al.* 1997, Labarta *et al.* 1997). Serum is probably the source of most of this extravascular ALS, although local synthesis may occur in some tissues. Using sensitive methodologies (e.g. *in situ* hybridisation, generation of expressed sequence tags), *ALS* gene expression is detected in some extrahepatic tissues, including kidney, developing bone, lactating mammary gland, thymus and lung (Chin *et al.* 1994, Janosi *et al.* 1999c). *ALS* mRNA has also been detected in the theca and granulosa cells of the porcine ovary (Wandji *et al.* 2000). Extravascular ALS may be particularly significant in the ovary, because IGFs are found almost exclusively in 150 kDa complexes in human follicular fluid (Cwyfan Hughes *et al.* 1997). Irrespective of its origin, extravascular ALS can modulate local IGF action through sequestration of IGFs into ternary complexes, as recently shown by the ability of ALS to potentiate the inhibitory effects of IGFBP-5 on thyroidal cell proliferation (Twigg *et al.* 1999). This inhibitory role of ALS on IGF actions is consistent with the observation that generalised overexpression of ALS caused 13% growth retardation in 4- and 8-week-old mice (Modric *et al.* 1999).

Onset of ALS synthesis is one of the last events in the development of the circulating IGF system. In humans, ALS is undetectable in foetal serum at 27 weeks of gestation, but is present at term (Lewitt *et al.* 1995). Serum concentrations of ALS increase fivefold from birth to puberty, and decline somewhat in older individuals (Baxter 1990a). Studies in rats have shown that an induction of *ALS* gene expression in liver is responsible for this increase

in plasma ALS in early life (Baxter & Dai 1994, Dai & Baxter 1994, Frystyk *et al.* 1998). In the sheep, abundance of *ALS* mRNA is also low before birth, but increases abruptly within 7 days of postnatal life (Rhoads *et al.* 2000). The functional consequence of this pattern of *ALS* expression in the sheep is that IGFs circulate primarily in 50 kDa complexes before birth, and in 150 kDa complexes 1 week after birth (Butler & Gluckman 1986).

GH is by far the most potent inducer of *ALS* mRNA in liver and of *ALS* in plasma (Baxter 1990a, Baxter & Dai 1994, Ooi *et al.* 1997, Olivecrona *et al.* 1999). The importance of this regulation is underlined by the near complete absence of *ALS* in GH-deficient states (Zapf *et al.* 1989, Gargosky *et al.* 1994, Aguiar-Oliveira *et al.* 1999), and by the temporal correlation between appearance of *ALS* mRNA and functional GH receptor in liver of both sheep and rats (Gluckman *et al.* 1983, Tiong & Herington 1992). These effects of GH in liver are direct and occur at the level of *ALS* gene transcription (Ooi *et al.* 1997, 1998).

A variety of conditions has been shown to reduce serum ALS in rats and humans. They include fasting, undernutrition and catabolic diseases such as diabetes, burn injury and cirrhosis (Dai & Baxter 1994, Bereket *et al.* 1996, Lang *et al.* 1996, 2000, Oster *et al.* 1996, Fukuda *et al.* 1999, Moller *et al.* 2000). Negative regulation of *ALS* synthesis occurs at both transcriptional and post-transcriptional levels. Dexamethasone, cAMP and epidermal growth factor decrease secretion of ALS in primary rat hepatocytes, primarily by reducing the abundance of *ALS* mRNA (Dai *et al.* 1994, Delhanty & Baxter 1998). Increases in factors such as glucocorticoid and cellular cAMP could explain the marked decrease in *ALS* synthesis observed during thermal injury and liver failure (Lang *et al.* 2000, Moller *et al.* 2000). In contrast, insulin deficiency may be the primary defect causing decreased concentrations of serum ALS during fasting, undernutrition and diabetes. This effect of insulin occurs post-translationally, because insulin increases ALS secretion in the absence of any change in *ALS* mRNA abundance in primary hepatocytes (Dai *et al.* 1994). Finally, decreased *ALS* synthesis could also occur secondarily to the development of GH resistance in liver. Recent studies have shown that most of the negative actions of cAMP and the inflammatory cytokine interleukin (IL)-1 β on *ALS* synthesis occur via the induction of a GH-resistant state in liver cells (Delhanty 1998, Boisclair *et al.* 2000).

Transcriptional regulation of *ALS* gene in liver

In vivo, GH is the predominant determinant of *ALS* synthesis, underlying the importance of the IGF system in mediating the growth-promoting effects of GH (Daughaday & Rotwein 1989, Etherton & Bauman 1998). Using rat liver cells, a GH-responsive promoter was

Figure 1 The GH response element of the *ALS* gene is conserved in various species. A 50 bp nucleotide sequence is presented for the mouse, rat, sheep and human *ALS* promoter (Boisclair *et al.* 1996, Delhanty & Baxter 1997, Rhoads *et al.* 2000, Suwanichkul *et al.* 2000). The position of the most 5' nucleotide (relative to ATG, +1) is given on the left of each sequence. Areas of identity between sequences are shaded. ALSGAS1, the GH response element (TTCCTAGAA) of the mouse *ALS* promoter, is underlined. This sequence is conserved in position and in sequence across species.

identified in the genomic fragment corresponding to nt -2001 to nt -49 (relative to A₊1TG) of the mouse *ALS* gene (Ooi *et al.* 1997). The GH-response element of the mouse promoter was located by deletion and mutation analysis to a 9 bp sequence located between nt -633 and -625 (Ooi *et al.* 1998). This sequence was called ALSGAS1 because of its resemblance with the consensus sequence for γ -interferon activated sequence (GAS) (Schindler & Darnell 1995). The effects of GH on the *ALS* gene are mediated by the JAK-STAT pathway: the tyrosine kinase JAK2 is recruited to the activated GH receptor complex and phosphorylates signal transducers and activators of transcription (STAT)-5a and STAT-5b (Schindler & Darnell 1995, Carter-Su *et al.* 1996). After dimerisation, STAT5 isomers translocate to the nucleus, and activate *ALS* gene transcription by binding to the ALSGAS1 element (Ooi *et al.* 1998). The GH signalling pathway leading to increased *ALS* gene transcription is critically dependent on the activation of STAT5 isomers, and is independent of RAS activation, as cells co-transfected with either dominant negative STAT-5a or STAT-5b completely abolished GH stimulation, whereas co-transfection with either dominant negative RAS or constitutively active RAS had no effect (Boisclair *et al.* 1998).

Validation of this mechanism of GH activation of the *ALS* gene was obtained from studies of the human and sheep *ALS* genes. Despite limited homology between their proximal sequence, the mouse, rat, sheep and human genes share complete conservation in sequence and position of the ALSGAS1 element (Fig. 1) (Rhoads *et al.* 2000, Suwanichkul *et al.* 2000). Sheep and human *ALS* promoters are also GH-responsive when transfected in liver cells, and this responsiveness also requires the presence of the ALSGAS1 element (Rhoads *et al.* 2000, Suwanichkul *et al.* 2000). Overall, these observations suggest conservation of the mechanism by which GH stimulates transcription of the *ALS* gene.

IL-1 β blocks the GH-dependent induction of *ALS* and IGF-I mRNA in primary hepatocytes (Wolf *et al.* 1996, Thissen & Verniers 1997, Delhanty 1998, Barreca *et al.* 1998, Boisclair *et al.* 2000). Some of these effects have been attributed to the down-regulation of the GH receptor by IL-1 β (Wolf *et al.* 1996, Thissen & Verniers 1997).

Using *ALS* as a model of GH-regulated gene transcription, IL-1 β was also shown to interfere with the activation of STAT5 (Boisclair *et al.* 2000). This interference is mediated by the intracellular suppressor of cytokine signalling (SOCS)-3, an inhibitor of the JAK-STAT pathway. This may be an important mechanism that contributes to an apparent GH-resistance seen in inflammatory diseases.

Physiological role of *ALS* in the circulating IGF system

In adult animals, serum IGFs reach concentrations that are ~1000 fold that of insulin. *ALS* is a critical component that contributes to the development of this large reservoir by extending the half-lives of IGFs from 10 min when in free form, and 30–90 min when in binary complexes, to more than 12 h when bound in ternary complexes (Guler *et al.* 1989, Zapf *et al.* 1995). Given this large reservoir of bioactive IGFs, a second important role of *ALS* is the prevention of the non-specific metabolic effects of the IGFs, such as causing severe hypoglycaemia (Zapf *et al.* 1995). *ALS* is able to contain these metabolic effects because IGFs in ternary complexes cannot traverse capillary endothelia and activate the insulin receptor, whereas free IGFs and IGFs bound as binary complexes can do so. Incorporation of IGFs into ternary complexes therefore completely restrains the intrinsic insulin-like effects of the IGFs (Zapf *et al.* 1995). An important correlate of this model is that specific mechanisms must exist to release IGFs from ternary complexes for their actions on target cells. Proteolytic attack of IGFBP-3 and interactions of the ternary complex with proteoglycans have been shown to release IGFs (Baxter 1990b, Lee & Rechler 1996). It is also possible that much of the released IGFs is the product of the equilibration between the ternary complex and its individual components in serum.

These roles of *ALS* have been inferred from short-term studies of GH-deficient animals (Zapf *et al.* 1989, Gargosky *et al.* 1994). However, in these animals, the concentration of all the components of the ternary complex are simultaneously decreased, making it difficult to delineate the separate roles of *ALS* from those of IGF-I and

IGFBP-3. Moreover, studies covering the entire life of GH-deficient animals are usually not feasible. For these reasons, we have generated an ALS-null mouse model in which the ternary complexes are absent due to the inactivation of the *ALS* gene (Ueki *et al.* 2000). Interestingly, null ALS mice have dramatically reduced circulating IGF-I and IGFBP-3 concentrations compared with their wild-type siblings (62 and 88% reductions respectively). These changes occur despite the absence of any reductions in IGF-I or IGFBP-3 synthesis, as expression of both these genes in liver, the predominant site of synthesis, was normal. These results proved that ALS is absolutely necessary for the serum accumulation of both IGF-I and IGFBP-3 and that, without ALS, induction of IGF-I and IGFBP-3 synthesis after birth would cause only a modest increase in their plasma concentrations (Albiston & Herington 1992, Kikuchi *et al.* 1992).

Under normal circumstances, ALS is usually not considered to play a role in regulating serum IGFs, because it circulates in large excess over the concentrations of IGFs and IGFBP-3. This notion needs to be reconsidered in view of the low association constant of ALS for the binary complexes of IGFBP-3 and IGF (Holman & Baxter 1996). Mice with a single null ALS allele provide an example of this phenomenon. They secrete less ALS, which results in significant reductions in serum IGF-I (17%) and IGFBP-3 (40%) (Ueki *et al.* 2000). Another example is provided by GH treatment of normal animals. A greater concentration of serum IGF-I probably represents the combined effect of increased hepatic synthesis of IGF-I and ALS, whereas greater concentration of IGFBPs-3 and -5 must primarily reflect stabilisation by ALS (Cohick *et al.* 1992, Powell *et al.* 1999).

Despite these disturbances in the circulating IGF system, null ALS animals suffered only a 13% growth deficit by adulthood. This modest effect is surprising, given the central role postulated for plasma IGF-I in regulating postnatal growth (Baker *et al.* 1993, Louvi *et al.* 1997, Etherton & Bauman 1998). However, it is consistent with the observation that abrogation of IGF-I synthesis only in liver, which results in a reduction in plasma IGF-I similar to that of the null ALS mice, does not alter postnatal growth (Sjogren *et al.* 1999, Yakar *et al.* 1999). A revised somatomedin hypothesis that accommodates these findings is one in which the primary function of liver is to supply the IGF-I needed to respond to various challenges. In this model, ALS plays a critical role by capturing liver-derived IGF-I into long-lived ternary complexes. This model would predict that null ALS mice with limited retention of liver derived IGF-I would not respond as well to GH therapy or would have greater muscle wasting after exposure to endotoxin (Clark *et al.* 1995, Frost & Lang 1999). In support of this hypothesis, IGF-I therapies are more effective when ternary complexes formation is enhanced by co-administration of GH or IGFBP-3 (Kupfer *et al.* 1993, Bagi *et al.* 1995).

The null ALS mice have normal concentrations of plasma glucose and insulin, although ternary complexes cannot form (Ueki *et al.* 2000). This is surprising, given the hypoglycaemic potential of IGFs. One reason why hypoglycaemia does not develop in ALS-null mice may be the near absence in mice of serum IGF-II (Wolf *et al.* 1994), a much more potent insulin receptor agonist than IGF-I (Frasca *et al.* 1999). In mammals with high concentration of serum IGF-II, such as humans, the presence of ALS and formation of ternary complexes is likely to play a more important role in containing the intrinsic and more potent insulin-like effects of IGF-II. Indeed, hypoglycaemia in humans suffering from non-islet tumour hypoglycaemia is associated with high concentration of incompletely processed IGF-II and depressed ternary complex formation (Daughaday & Kapadia 1989, Baxter *et al.* 1995). Finally, protection afforded by ALS may extend to the mitogenic effects of IGFs also. Evidence consistent with this hypothesis includes increased incidence of tumours in transgenic mice overexpressing IGF-II (Rogler *et al.* 1994), defects promoting greater concentrations of IGF-II in some cancer cells (Toretzky & Helman 1996, De Souza *et al.* 1997), and the positive association between concentrations of plasma IGF-I and incidence of prostate and breast cancers in human populations (Rosen & Pollak 1999). Although these studies do not establish a cause-and-effect relationship, it is possible that the larger pool of bioavailable IGFs in ALS-null mice would promote excessive cell proliferation and, perhaps, the development of cancers.

Conclusion

Progress on the biology of ALS has been rapid in the past few years. Significant advances include characterisation of the *ALS* gene in many species, resolution of important structure–function relationships and identification of primary mechanisms regulating ALS synthesis. The challenge is now to understand the functional significance of ternary complexes and, more broadly, the role of plasma IGFs after birth. Future efforts need to take into account recent experiments in the mouse showing that liver-derived IGF-I is dispensable for postnatal growth under idealised conditions (Sjogren *et al.* 1999, Yakar *et al.* 1999). These results suggest that the benefits of a large and stable reservoir of plasma IGFs are likely to be more subtle than originally thought, and to vary according to species, development and disease.

Acknowledgements

This work was supported in part by NIH grant DK-51624 and by the Cornell University Agricultural Experiment Station (Y R B, R P R, I U, J W), and by the National

Health and Medical Research Council Grant No. 983212 (G T O).

References

- Aguiar-Oliveira MH, Gill MS, de A Barretto ES, Alcantara MR, Miraki-Moud F, Menezes CA, Souza AH, Martinelli CE, Pereira FA, Salvatori R, Levine MA, Shalet SM, Camacho-Hubner C & Clayton PE 1999 Effect of severe growth hormone (GH) deficiency due to a mutation in the GH-releasing hormone receptor on insulin-like growth factors (IGFs), IGF-binding proteins, and ternary complex formation throughout life. *Journal of Clinical Endocrinology and Metabolism* **84** 4118–4126.
- Albiston AL & Herington AC 1992 Tissue distribution and regulation of insulin-like growth factor (IGF)-binding protein-3 messenger ribonucleic acid (mRNA) in the rat: comparison with IGF-I mRNA expression. *Endocrinology* **130** 497–502.
- Bagi CM, DeLeon E, Brommage R, Rosen D & Sommer A 1995 Treatment of ovariectomized rats with the complex of rhIGF-I/IGFBP-3 increases cortical and cancellous bone mass and improves structure in the femoral neck. *Calcified Tissue International* **57** 40–46.
- Baker J, Liu J-P, Robertson EJ & Efstratiadis A 1993 Role of insulin-like growth factors in embryonic and postnatal growth. *Cell* **75** 73–82.
- Barreca A, Ketelslegers JM, Arvigo M, Minuto F & Thissen JP 1998 Decreased acid-labile subunit (ALS) levels by endotoxin *in vivo* and by interleukin-1 beta *in vitro*. *Growth Hormone and IGF Research* **8** 217–223.
- Baxter RC 1990a Circulating levels and molecular distribution of the acid-labile α subunit of the high molecular weight insulin-like growth factor-binding protein complex. *Journal of Clinical Endocrinology and Metabolism* **70** 1347–1353.
- Baxter RC 1990b Glycosaminoglycans inhibit formation of the 140 kDa insulin-like growth factor-binding protein complex. *Biochemical Journal* **271** 773–777.
- Baxter RC 1994 Insulin-like growth factor binding proteins in the human circulation: a review. *Hormone Research* **42** 140–144.
- Baxter RC & Dai J 1994 Purification and characterization of the acid-labile subunit of rat serum insulin-like growth factor binding protein complex. *Endocrinology* **134** 848–852.
- Baxter RC & Martin JL 1989 Structure of the Mr 140,000 growth hormone-dependent insulin-like growth factor binding protein complex: determination by reconstitution and affinity-labeling. *PNAS* **86** 6898–6902.
- Baxter RC, Martin JL & Beniac VA 1989 High molecular weight insulin-like growth factor binding protein complex. *Journal of Biological Chemistry* **264** 11843–11848.
- Baxter RC, Holman SR, Corbould A, Stranks S, Ho PJ & Braund W 1995 Regulation of the insulin-like growth factors and their binding proteins by glucocorticoid and growth hormone in nonislet cell tumor hypoglycemia. *Journal of Clinical Endocrinology and Metabolism* **80** 2700–2708.
- Bereket A, Wilson TA, Blethen SL, Sakurai Y, Herndon DN, Wolfe RR & Lang CH 1996 Regulation of the acid-labile subunit of the insulin-like growth factor ternary complex in patients with insulin-dependent diabetes mellitus and severe burns. *Clinical Endocrinology* **44** 525–532.
- Boisclair YR, Seto D, Hsieh S, Hurst KR & Ooi GT 1996 Organization and chromosomal localization of the gene encoding the mouse acid labile subunit of the insulin-like growth factor binding complex. *PNAS* **93** 10028–10033.
- Boisclair YR, Bassal S, Rechler MM & Ooi GT 1998 Regulation of ALS gene expression by growth hormone. In *Molecular Mechanisms to Regulate the Activities of Insulin-like Growth Factors*. Eds K Takano, N Hizuka & S-I Takahashi. Amsterdam: Elsevier.
- Boisclair YR, Wang J, Shi J, Hurst KR & Ooi GT 2000 Role of the suppressor of cytokine signaling-3 (SOCS3) in mediating the inhibitory effects of interleukin-1 β on the growth hormone-dependent transcription of the acid-labile subunit gene in liver cells. *Journal of Biological Chemistry* **275** 3841–3847.
- Butler JH & Gluckman PD 1986 Circulating insulin-like growth factor-binding proteins in fetal, neonatal and adult sheep. *Journal of Endocrinology* **109** 333–338.
- Carter-Su C, Schwartz J & Smit LS 1996 Molecular mechanism of growth hormone action. *Annual Reviews in Physiology* **58** 187–207.
- Chin E, Zhou J, Dai J, Baxter RC & Bondy CA 1994 Cellular localization and regulation of gene expression for components of the insulin-like growth factor ternary binding protein complex. *Endocrinology* **134** 2498–2504.
- Clark RG, Mortensen DL & Carlsson LMS 1995 Insulin-like growth factor-1 and growth hormone (GH) have distinct and overlapping anabolic effects in GH-deficient rats. *Endocrine* **3** 297–304.
- Cohick WS, McGuire MA, Clemmons DR & Bauman DE 1992 Regulation of insulin-like growth factor-binding proteins in serum and lymph of lactating cows by somatotropin. *Endocrinology* **130** 1508–1514.
- Cwyfan Hughes SC, Mason HD, Franks S & Holly JMP 1997 The insulin-like growth factors (IGFs) in follicular fluid are predominantly bound in the ternary complex. *Journal of Endocrinology* **155** R1–R4.
- Dai J & Baxter RC 1992 Molecular cloning of the acid-labile subunit of the rat insulin-like growth factor binding protein complex. *Biochemical and Biophysical Research Communications* **188** 304–309.
- Dai J & Baxter RC 1994 Regulation *in vivo* of the acid-labile subunit of the rat serum insulin-like growth factor-binding protein complex. *Endocrinology* **135** 2335–2341.
- Dai J, Scott CD & Baxter RC 1994 Regulation of the acid-labile subunit of the insulin-like growth factor complex in cultured rat hepatocytes. *Endocrinology* **135** 1066–1072.
- Daughaday WH & Kapadia M 1989 Significance of abnormal serum binding of insulin-like growth factor-II in the development of hypoglycemia in patients with non-islet cell tumours. *PNAS* **86** 6778–6782.
- Daughaday WH & Rotwein P 1989 Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. *Endocrine Reviews* **10** 68–91.
- Delhanty PJD 1998 Interleukin-1 β suppresses growth hormone-induced acid-labile subunit mRNA levels and secretion in primary hepatocytes. *Biochemical and Biophysical Research Communications* **243** 269–272.
- Delhanty P & Baxter RC 1996 The cloning and expression of the baboon acid-labile subunit of the insulin-like growth factor binding protein complex. *Biochemical and Biophysical Research Communications* **227** 897–902.
- Delhanty PJD & Baxter RC 1997 Cloning and characterization of the rat gene for the acid-labile subunit of the insulin-like growth factor binding protein complex. *Journal of Molecular Endocrinology* **19** 267–277.
- Delhanty PJD & Baxter RC 1998 The regulation of acid-labile subunit gene expression and secretion by cyclic adenosine 3',5'-monophosphate. *Endocrinology* **139** 260–265.
- De Souza AT, Yamada T, Mills JJ & Jirtle RL 1997 Imprinted genes in liver carcinogenesis. *FASEB Journal* **11** 60–67.
- Etherton TD & Bauman DE 1998 Biology of somatotropin in growth and lactation of domestic animals. *Physiological Reviews* **78** 745–761.
- Firth SM, Ganeshprasad U & Baxter RC 1998 Structural determinants of ligand and cell surface binding of insulin-like growth factor-binding protein-3. *Journal of Biological Chemistry* **273** 2631–2638.
- Frasca F, Pandini G, Scalia P, Sciacca L, Mineo R, Costantino A, Goldfine ID, Belfiore A & Vigneri R 1999 Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. *Molecular and Cellular Biology* **19** 3278–3288.

- Frost RA & Lang CH 1998 Growth factors in critical illness: regulation and therapeutic aspects. *Current Opinion in Clinical Nutrition and Metabolic Care* **1** 195–204.
- Frystyk J, Gronbaek H, Skjaerbaek C, Flyvbjerg A, Orskov H & Baxter RC 1998 Developmental changes in serum levels of free and total insulin-like growth factor I (IGF-I), IGF-binding proteins-1 and -3, and the acid labile subunit in rats. *Endocrinology* **139** 4286–4292.
- Fukuda I, Hotta M, Hizuka N, Takano K, Ishikawa Y, Asakawa-Yasumoto K, Tagami E & Demura H 1999 Decreased serum levels of acid-labile subunit in patients with anorexia nervosa. *Journal of Clinical Endocrinology and Metabolism* **84** 2034–2036.
- Gargosky SE, Tapanainen P & Rosenfeld RG 1994 Administration of growth hormone (GH), but not insulin-like growth factor-I (IGF-I), by continuous infusion can induce the formation of the 150-kilodalton IGF-binding protein-3 complex in GH-deficient rats. *Endocrinology* **134** 2267–2276.
- Gluckman PD, Butler JH & Elliott TB 1983 The ontogeny of somatotrophic binding sites in ovine hepatic membranes. *Endocrinology* **112** 1607–1612.
- Guler H-P, Zapf J, Schmid C & Froesch ER 1989 Insulin-like growth factors I and II in healthy man. Estimations of half-lives and production rates. *Acta Endocrinologica* **121** 753–758.
- Hashimoto R, Ono M, Fujiwara H, Higashihashi N, Yoshida M, Enjoh-Kimura T & Sakano K 1997 Binding sites and binding properties of binary and ternary complexes of insulin-like growth factor-II (IGF-II), IGF-binding protein-3, and acid-labile subunit. *Journal of Biological Chemistry* **272** 27936–27942.
- Holman SR & Baxter RC 1996 Insulin-like growth factor binding protein-3: factors affecting binary and ternary complex formation. *Growth Regulation* **6** 42–47.
- Janosi JB, Firth SM, Bond JJ, Baxter RC & Delhanty PJ 1999a N-Linked glycosylation and sialylation of the acid-labile subunit. Role in complex formation with insulin-like growth factor (IGF)-binding protein-3 and the IGFs. *Journal of Biological Chemistry* **274** 5292–5298.
- Janosi JB, Ramsland PA, Mott MR, Firth SM, Baxter RC & Delhanty PJ 1999b The acid-labile subunit of the serum insulin-like growth factor-binding protein complexes. Structural determination by molecular modeling and electron microscopy. *Journal of Biological Chemistry* **274** 23328–23332.
- Janosi JB, Twigg SM, Firth SM, Baxter RC & Delhanty PJD 1999c Histochemical examination of the acid-labile subunit protein in human tissue. In *Proceedings of the 5th International Symposium on Insulin-like Growth Factors*, abstract P126, p 373.
- Jones JI & Clemmons DR 1995 Insulin-like growth factors and their binding proteins: biological actions. *Endocrine Reviews* **16** 3–34.
- Khosravi MJ, Diamandi A, Mistry J, Krishna RG & Khare A 1997 Acid-labile subunit of human insulin-like growth factor-binding protein complex: measurement, molecular, and clinical evaluation. *Journal of Clinical Endocrinology and Metabolism* **82** 3944–3951.
- Kikuchi K, Bichell DP & Rotwein P 1992 Chromatin changes accompany the developmental activation of insulin-like growth factor I gene transcription. *Journal of Biological Chemistry* **267** 21505–21511.
- Kupfer SR, Underwood LE, Baxter RC & Clemmons DR 1993 Enhancement of the anabolic effects of growth hormone and insulin-like growth factor I by use of both agents simultaneously. *Journal of Clinical Investigation* **91** 391–396.
- Labarta JI, Gargosky SE, Simpson DM, Lee PD, Argente J, Guevara-Aguirre J & Rosenfeld RG 1997 Immunoblot studies of the acid-labile subunit (ALS) in biological fluids, normal human serum and in children with GH deficiency and GH receptor deficiency before and after long-term therapy with GH or IGF-I respectively. *Clinical Endocrinology* **47** 657–666.
- Lang CH, Fan J, Frost RA, Gelato MC, Sakurai Y, Herndon DN & Wolfe RR 1996 Regulation of the insulin-like growth factor system by insulin in burn patients. *Journal of Clinical Endocrinology and Metabolism* **81** 2474–2480.
- Lang CH, Liu X, Nystrom GJ & Frost RA 2000 Acute response of IGF-I and IGF binding proteins induced by thermal injury. *American Journal of Physiology, Endocrinology and Metabolism* **278** E1087–E1096.
- Lee CY & Rechler MM 1996 Proteolysis of insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) in 150-kilodalton IGFBP complexes by a cation-dependent protease activity in adult rat serum promotes the release of bound IGF-I. *Endocrinology* **137** 2051–2058.
- Leong SR, Baxter RC, Camerato T, Dai J & Wood WI 1992 Structure and functional expression of the acid-labile subunit of the insulin-like growth factor binding protein complex. *Molecular Endocrinology* **6** 870–876.
- Lewitt MS, Scott FP, Clarke NM & Baxter RC 1995 Developmental regulation of circulating insulin-like growth factor-binding proteins in normal pregnancies and in pre-eclampsia. *Progress in Growth Factor Research* **6** 475–480.
- Louvi A, Accili D & Efstratiadis A 1997 Growth-promoting interaction of IGF-II with the insulin receptor during mouse embryonic development. *Developmental Biology* **189** 33–48.
- Modric T, Shi Z, Rajkumar K, Suwanichkul A, Durham SK, Powell DR & Murphy JJ 1999 Growth retardation of transgenic mice overexpressing insulin-like growth factor binding protein-3 and acid labile subunit. *Proceedings of the 5th International Symposium on Insulin-like Growth Factors*, abstract P196, p 393.
- Moller S, Juul A, Becker U & Henriksen JH 2000 The acid-labile subunit of the ternary insulin-like growth factor complex in cirrhosis: relation to liver dysfunction. *Journal of Hepatology* **32** 441–446.
- Olivecrona H, Hilding A, Ekstrom C, Barle H, Nyberg B, Moller C, Delhanty PJ, Baxter RC, Angelin B, Ekstrom J & Tally M 1999 Acute and short-term effects of growth hormone on insulin-like growth factors and their binding proteins: serum levels and hepatic messenger ribonucleic acid responses in humans. *Journal of Clinical Endocrinology and Metabolism* **84** 553–560.
- Ooi GT & Boisclair YR 1999 Molecular biology of the insulin-like growth factor binding proteins. In *Contemporary Endocrinology: the IGF System*, pp 111–139. Eds R Rosenfeld & JC Roberts. Totowa, NJ: Humana Press Inc.
- Ooi GT, Cohen FJ, Tseng LY-H, Rechler MM & Boisclair YR 1997 Growth hormone stimulates transcription of the gene encoding the acid-labile subunit (ALS) of the circulating insulin-like growth factor-binding protein complex and ALS promoter activity in rat liver. *Molecular Endocrinology* **11** 997–1007.
- Ooi GT, Hurst KR, Poy MN, Rechler MM & Boisclair YR 1998 Binding of STAT5a and STAT5b to a single element resembling a γ -interferon activated sequence mediates the growth hormone induction of the mouse acid-labile subunit promoter in liver cells. *Molecular Endocrinology* **12** 675–687.
- Oster MH, Levin N, Fielder PJ, Robinson IC, Baxter RC & Cronin MJ 1996 Developmental differences in the IGF-I system response to severe and chronic calorie malnutrition. *American Journal of Physiology* **270** E646–E653.
- Powell DR, Durham SK, Brewer ED, Frane JW, Watkins SL, Hogg RJ & Mohan S 1999 Effects of chronic renal failure and growth hormone on serum levels of insulin-like growth factor-binding protein-4 (IGFBP-4) and IGFBP-5 in children: a report of the Southwest Pediatric Nephrology Study Group. *Journal of Clinical Endocrinology and Metabolism* **84** 596–601.
- Rechler MM 1993 Insulin-like growth factor binding proteins. *Vitamins and Hormones* **47** 1–114.
- Rhoads RP, Greenwood PL, Bell AW & Boisclair YR 2000 Organization and regulation of the gene encoding the sheep acid

- labile subunit of the 150 kDa-binding protein complex. *Endocrinology* **141** 1425–1433.
- Rogler CE, Yang D, Rossetti L, Donohoe J, Alt E, Chang CJ, Rosenfeld R, Neely K & Hintz R 1994 Altered body composition and increased frequency of diverse malignancies in insulin-like growth factor-II transgenic mice. *Journal of Biological Chemistry* **269** 13779–13784.
- Rosen CJ & Pollak M 1999 Circulating IGF-I: new perspectives for a new century. *Trends in Endocrinology & Metabolism* **10** 136–141.
- Schindler C & Darnell J 1995 Transcriptional responses to polypeptide ligands: The JAK-STAT pathway. *Annual Review of Biochemistry* **64** 621–651.
- Sjogren K, Liu JL, Blad K, Skrtic S, Vidal O, Wallenius V, LeRoith D, Tornell J, Isaksson OG, Jansson JO & Ohlsson C 1999 Liver-derived insulin-like growth factor I (IGF-I) is the principal source of IGF-I in blood but is not required for postnatal body growth in mice. *PNAS* **96** 7088–7092.
- Stewart CEH & Rotwein P 1996 Growth, differentiation, and survival: multiple physiological functions for insulin-like growth factors. *Physiological Reviews* **76** 1005–1026.
- Suwanichkul A, Boisclair YR, Olney RC, Durham SK & Powell DR 2000 Conservation of a growth hormone-responsive promoter element in the human and mouse acid-labile subunit genes. *Endocrinology* **141** 833–838.
- Thissen J-P & Verniers J 1997 Inhibition by interleukin-1 β and tumor necrosis factor- α of the insulin-like growth factor I messenger ribonucleic response to growth hormone in rat hepatocyte primary culture. *Endocrinology* **138** 1078–1084.
- Tiong TS & Herington AC 1992 Ontogeny of messenger RNA for the rat growth hormone receptor and serum binding protein. *Molecular and Cellular Endocrinology* **83** 133–141.
- Toretsky JA & Helman LJ 1996 Involvement of IGF-II in human cancer. *Journal of Endocrinology* **149** 367–372.
- Twigg SM & Baxter RC 1998 Insulin-like growth factor (IGF)-binding protein 5 forms an alternative ternary complex with IGFs and the acid-labile subunit. *Journal of Biological Chemistry* **273** 6074–6079.
- Twigg SM, Kiefer MC, Zapf J & Baxter RC 1998 Insulin-like growth factor-binding protein 5 complexes with the acid-labile subunit. Role of the carboxyl-terminal domain. *Journal of Biological Chemistry* **273** 28791–28798.
- Twigg SM, Robinson BG & Baxter RC 1999 The acid-labile subunit potentiates the inhibitory effect of insulin-like growth factor (IGF) binding protein-5 on IGF-I induced cell proliferation on thyroidal cells *in vitro*. *Proceedings of the 81st Annual Meeting of the Endocrine Society*, San Diego, CA, p170.
- Twigg SM, Kiefer MC, Zapf J & Baxter RC 2000 A central domain binding site in insulin-like growth factor binding protein-5 for the acid-labile subunit. *Endocrinology* **141** 454–457.
- Ueki I, Ooi GT, Tremblay ML, Hurst KR, Bach LA & Boisclair YR 2000 Inactivation of the acid labile subunit gene in mice results in mild retardation of postnatal growth despite profound disruptions in the circulating insulin-like growth factor system. *PNAS* **97** 6868–6873.
- Wandji SA, Gadsby JE, Simmen FA, Barber JA & Hammond JM 2000 Porcine ovarian cells express messenger ribonucleic acids for the acid-labile subunit and insulin-like growth factor binding protein-3 during follicular and luteal phases of the estrous cycle. *Endocrinology* **141** 2638–2647.
- Werner H, Woloschak M, Stannard B, Shen-Orr Z, Roberts Jr CT & LeRoith D 1991 The insulin-like growth factor I receptor: molecular biology, heterogeneity, and regulation. In *Insulin-Like Growth Factors: Molecular and Cellular Aspects*, pp 17–47. Ed D LeRoith. Boca Raton: CRC Press.
- Wolf E, Kramer R, Blum WF, Föll J & Brem G 1994 Consequences of postnatally elevated insulin-like growth factor-II in transgenic mice: endocrine changes and effects on body and organ growth. *Endocrinology* **135** 1877–1886.
- Wolf M, Bohm S, Brand M & Kreymann G 1996 Proinflammatory cytokines interleukin 1 beta and tumor necrosis factor alpha inhibit growth hormone stimulation of insulin-like growth factor I synthesis and growth hormone receptor mRNA levels in cultured rat liver cells. *European Journal of Endocrinology* **135** 729–737.
- Xu S, Cwyfan-Hughes SC, Van der Stappen JWJ, Sansom J, Burton JL, Donnelly M & Holly JMP 1995 Insulin-like growth factors (IGFs) and IGF-binding proteins in human skin interstitial fluid. *Journal of Clinical Endocrinology and Metabolism* **80** 2940–2945.
- Yakar S, Liu JL, Stannard B, Butler A, Accili D, Sauer B & LeRoith D 1999 Normal growth and development in the absence of hepatic insulin-like growth factor I. *PNAS* **96** 7324–7329.
- Zapf J, Hauri C, Waldvogel M, Futo E, Hasler H, Binz K, Guler HP, Schmid C & Froesch ER 1989 Recombinant human insulin-like growth factor I induces its own specific carrier protein in hypophysectomized and diabetic rats. *PNAS* **86** 3813–3817.
- Zapf J, Hauri C, Futo E, Hussain M, Rutishauser J, Maack CA & Froesch ER 1995 Intravenously injected insulin-like growth factor (IGF) I/IGF binding protein-3 complex exerts insulin-like effects in hypophysectomized, but not in normal rats. *Journal of Clinical Investigation* **95** 179–186.

Received in final form 19 February 2001

Accepted 7 March 2001