REVIEW

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

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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.

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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

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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 remains 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.* 1999*b*). 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.* 1999*a*). 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.* 1999*b*).

Regulation of ALS synthesis

ALS is found in high concentration almost exclusively in postnatal serum (Baxter 1990*a*, 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 1990*a*, 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 1990*a*, 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.* 1999*c*). 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 1990*a*). 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 1990*a*, 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)-1β 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

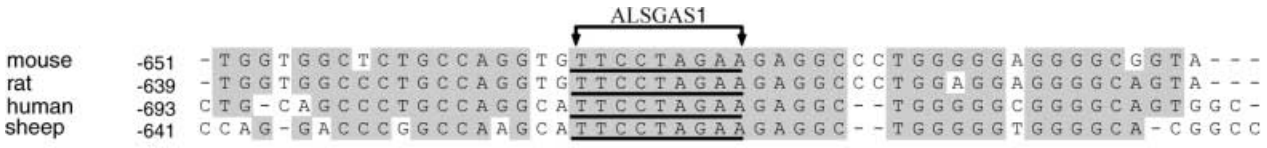

Figure 1 The GH response element of the *ALS* gene is conserved in various species. A 50 bp nucleotide sequence is presented for the mouse, rat, sheep and human ALS promoter (Boisclair *et al.* 1996, Delhanty & Baxter 1997, Rhoads *et al.* 2000, Suwanichkul *et al.* 2000). The position of the most 5′ 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-1β 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-1β (Wolf *et al.* 1996, Thissen & Verniers 1997). Using ALS as a model of GH-regulated gene transcription, IL-1β 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 1990*b*, 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 (Toretsky & 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.

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