

**WHAT WE KNOW AND DON'T YET KNOW ABOUT THE GROWTH-  
MODULATING ROLES OF INSULIN-LIKE GROWTH FACTOR-  
BINDING PROTEINS (IGFBPs) IN FISHES**

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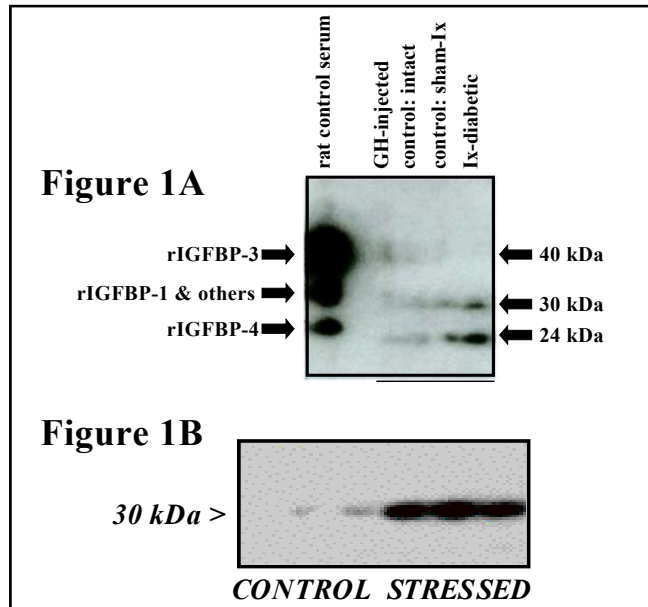
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IGFBPs, unrelated to IGF receptors, are derived from a superfamily of cysteine-rich growth factors (Kelley et al., 2002). Having a central position between IGF ligand and receptor, IGFBPs are 'growth integrators', having important influences on the distribution and bioavailability of IGF in the cell and physiological environments. IGFBPs exhibit an array of specialized properties, including interaction with proteins that post-translationally modify them, cell membrane association, direct IGF-independent cellular actions, and intracellular nuclear actions (Mohan and Baylink, 2002). Up to 6 IGFBP genes are expressed in a given tissue or cell type, with a diversity of factors regulating their individual expression, meaning that different physiological circumstances will result in a characteristic local IGF bioactivity.

Mammalian IGFBP-3 carries >90% of serum IGFs, and a ~140 kDa 'ternary' complex of IGFBP-3 (45 kDa), IGF (7 kDa), and a bound 'acid-labile subunit' (ALS; 90 kDa) predominates the serum IGF-binding capacity of mammals. Growth hormone (GH) stimulates the production of all three (IGF-I, IGFBP-3, ALS) and their levels in the circulation are positively correlated with growth rate. The large size of the IGFBP-3 ternary complex functions to maintain a substantial, functionally important reservoir of IGF within the blood circulation. In fishes, however, it has not been possible to demonstrate any large IGFBP

complex in serum, while the levels of all IGFBPs are much lower than those of mammals (Figure 1A compares IGFBPs present in equal volumes (4  $\mu$ l) of serum from rat versus the goby *Gillichthys mirabilis*). Indeed, sera from all fish species tested to date exhibit similarly low relative levels of IGFBPs, as well as lower IGF-I concentrations (Kelley et al., 2002).

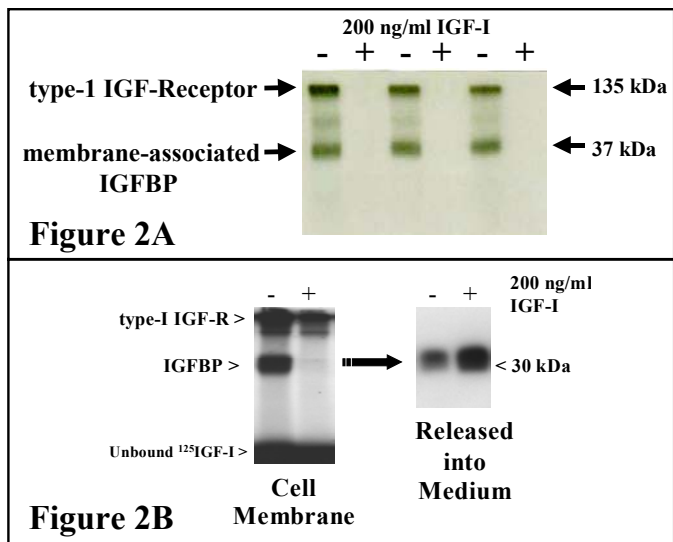


Thus, at least under basal physiological conditions, IGFBPs and IGFs are far less prominent features of fish serum as compared with that of mammals. However, if provoked physiologically (e.g., hormone injection or stress induction), levels of one or more particular IGFBPs will be substantially altered. As shown in Figure 1A, low levels of the 40 kDa IGFBP are seen in control gobies, but are increased after injection of growth hormone (GH). Alternatively, the 30 and 24 kDa IGFBPs are up-regulated under catabolic circumstances, when body growth is inhibited (e.g., diabetic fish, Fig. 1A). Based on such findings, we propose that in fishes, enhancement (or inhibition) of growth is somewhat like “filling an empty glass”, in that the serum requires “addition” of growth factors (IGF & IGFBP) from low basal levels, in contrast to mammals that maintain a standing reservoir of these growth factors.

In catabolic fish (induced by stress, fasting, or diabetes), levels of a 30 kDa IGFBP (& 24 kDa, IGFBP in some species) are increased in serum in

association with elevated cortisol levels and a suppression of growth (3). In Figure 1B, serum IGFBP in jack mackerel (*Trachurus symmetricus*) is seen to be substantially increased after 60 min of handling stress (along with 8-fold increased serum cortisol). Thus, our findings to date indicate a physiology comparable to that of mammalian IGFBP-1, a key growth inhibitor via its high-affinity binding of IGF (blocks cell receptor binding). The connection between stress and growth inhibition in fishes, via alterations in IGFbps, has raised an interesting possibility: that measurements of IGFbps, which require only a few  $\mu$ l of serum, can serve as a useful biomarker of the impacts of stress on growth (Kelley et al., 2001). For instance, the physiological impacts of “catching and releasing”, a practice intended to protect fisheries, may well depend on the degree to which stress and IGFbps are altered, a subject about which very little is known.

It is now understood that IGFbps are produced by a wide variety of cell types in fishes, as in mammals, and therefore that a “local IGF/IGFBP axis” exists in fish tissues. There are only a very limited number of studies on the properties of IGFbps at the cellular level using non-mammalian models. This is a critical yet unanswered issue, as the dynamics of IGFbps in the microenvironment of mammalian cells are essential in IGF-regulated processes. Until recently, it was not known whether IGFbps from lower vertebrates utilized cell surface association, or whether such properties were important in influencing IGF-mediated growth. We have found that there are apparent cell surface-associated IGFbps both in goby muscle membrane preparations and in heart cells of green iguana. In Figure 2A, affinity crosslinking of  $^{125}$ I-IGF-I identifies two binding sites in goby muscle cells, the 135-kDa alpha-subunit of the IGF receptor and a 30-kDa IGFBP. Both sites bind specifically, as excess unlabeled IGF-I competitively inhibits label binding. In monolayer cultures of iguana heart cells (Fig. 2B), a 30 kDa IGFBP is present both as a soluble form in the medium as well as localized to the cell membranes. Interestingly, when IGF-I is added, it results in a rapid, complete release of the IGFBP from the membrane into the medium (compare left and right panels in Fig. 2B). Cells in which membrane-associated IGFBP has been removed experimentally show a greater proliferative response to IGF-I, leading to the hypothesis that the IGFBP serves a growth-inhibitory role. Based on our studies to date, it appears that IGFBP membrane association occurs in fish and reptilian cells, with relevance to whether IGF activates its cell receptor to stimulate growth.



## References

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