



How cells grow, the discovery of Insulin like growth factors

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As discussed in the section on history of cell culture, serum was used for several decades as a source of nutrients, hormones and growth factors, the nature of which was largely unknown. The first growth factor to be identified, nerve growth factor (NGF) was discovered in 1953 at Washington University in St Louis (1) by Rita Levi-Montalcini, an Italian-born physician (born in 1909 and now a still very active centenarian).

She purified it with the help of Stanley Cohen (born 1922) who went on to become professor of biochemistry at Vanderbilt University where he discovered in 1962 and extensively characterized epidermal growth factor (EGF) (2).

Levi-Montalcini and Cohen shared the 1986 Nobel Prize in Physiology and Medicine.

NGF was sequenced in 1971 (3) and EGF in 1972 (4).

The following discovery of the insulin-like growth factors I and II took nearly three decades of sometimes confusing research that stemmed from early studies of the mechanism of action of pituitary growth hormone on somatic growth.

In 1957, W.D. Salmon Jr and William H. Daughaday at Washington University in St Louis provided compelling evidence that the effects of growth hormone in stimulating the incorporation of radioactive sulfate into chondroitin sulfate in cartilage *in vivo* and *in vitro* was not direct, but mediated by a factor circulating in serum that they named "sulfation factor" (5). In the early 70's, many investigators started to search for the factor(s) mediating growth hormone effects that by consensus became known as "somatomedins" (6). Different groups used different target cell systems and different isolation and purification methods, resulting in a multiplicity of somatomedins of which the similarities and differences were unclear.

Somatomedin A was identified as a factor that promotes labelled sulfate uptake by chicken cartilage (7). Somatomedin B was identified as a factor that stimulates DNA synthesis in human glial cells (8,9). Future Nobel laureate Rosalyn Yalow developed in 1975 a radioimmunoassay for somatomedin B (10). Somatomedin C was a more basic peptide than somatomedin A or B and stimulated uptake of labelled sulfate into rat cartilage (11).

At about the same time, future Nobel laureate Howard Temin and coworkers isolated a new growth factor, which they named Multiplication Stimulation Activity (MSA), from serum-free medium that had been conditioned by a Buffalo rat liver cell line (BRL-3A) (12).

A different angle into the developing story came from the work of Rudolph E. Froesch and coworkers at the University of Zürich, who initially were studying, not as the groups above the mitogenic properties of serum components, but their metabolic properties. Before a radioimmunoassay for insulin had been developed in 1960 by Rosalyn Yalow and Solomon A. Berson, insulin in serum had been assayed in *in vitro* bioassays using e.g. rat diaphragm or rat fat pads, later collagenase- isolated rat or mouse adipocytes. When the radioimmunoassay became available, it became clear that the plasma immunoreactive insulin (IRI) represented less than 10% of the total insulin-like activity. Moreover, specific guinea-pig anti-insulin serum produced only a small suppression of the insulin-like effects of human serum. Therefore the large fraction of insulin-like material could not be identical to insulin, and was called "non suppressible insulin-like activity" (NSILA). The fraction of it that was soluble in acid-ethanol was called NSILA-S (13,14). It became clear that NSILA also had growth-promoting properties, in fact at much lower concentrations than the metabolic properties, and therefore was primarily a growth factor (14). NSILA cross-reacted in the radioreceptor assay for somatomedins A and C, and therefore it became clear that NSILA's components were somatomedins (14). It took the Froesch group 20 years of a relentless effort led by René E. Humbel in the Institute of Biochemistry at the University of Zürich, starting with 11 kilos of an acetone powder from 6 tons of a Cohn fraction of human plasma (containing a and b globulin) obtained from Hoffmann-La Roche in Basel, to purify enough material (5 μ mol) to sequence the two polypeptides that made up NSILA. It became evident that their sequence was closely related to insulin's sequence and they were therefore called insulin-like growth factors I and II (15-17). Their characteristics will be described in more detail in the section on the insulin peptide family. 3D modelling by Tom L. Blundell (a former member of Dorothy Hodgkin's team that solved the crystal structure of insulin in 1969) and Sri Bedarkar at Birkbeck College showed that the two IGFs likely had the same tertiary structure as insulin (18).

A few years later, the other groups also managed to sequence their favourite somatomedin. Somatomedin A (19) and C (20) turned out to be IGF-I. A rat basic somatomedin was also IGF-I (21). In contrast, the active component of MSA turned out to be IGF-II (22), which is not properly speaking a somatomedin since it is not growth hormone-dependent.

Somatomedin B was sequenced in 1978, the same year as the IGFs, and turned out to be completely unrelated to the above peptides, with characteristics that make it dubious that it is a somatomedin at all or even a growth factor. The sequence of somatomedin B showed it to be a novel 44 amino acid peptide with 8 cysteines, with protease-inhibiting activity (23), although still claimed to be growth hormone dependent. This peptide later turned out to be a N-terminal proteolytic fragment of vitronectin, now called somatomedin B domain (24), responsible for binding to the urokinase receptor and the plasminogen activator inhibitor-I. It is unclear how this relates to the initially reported growth promoting activities of somatomedin B. However, vitronectin has been shown to have synergistic effects with growth factor signalling (24), including IGF-I. Zee Upton at Queensland University in Australia has provided evidence for multimeric complexes between vitronectin, IGF-I and IGF binding proteins (25) with potential applications in wound healing.

In the 80's, the cDNAs for the IGFs were cloned and sequenced as well (26-28). In the 90's and early 2000's, both NMR and crystal structures of the IGFs were solved (29-34), confirming the early predictions of structure similarity to insulin's (18).

In the end, an apparent multiplicity of supposedly cell-type specific somatomedins turned out to be just the two but ubiquitous IGF-I and IGF-II. This helps explain why insulin, which at high concentrations binds to and stimulates the IGF-I receptor that binds IGF-I and II, is such a nearly universal growth factor in cell culture.

Further reading: for a contemporary update on the original "somatomedin hypothesis", see ref. 35.

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Insulin-Like Growth Factor-I. Although rhIGF-I seems to be a logical treatment for growth failure in pediatric CRF, because of the increased IGFBP in this condition, clinical studies with rhIGF-I have not been performed in children outside of patients with GH insensitivity syndrome (GHIS). Studies in animal models of CRF (5/6 nephrectomy) show that the growth response after rhIGF-I treatment is almost comparable to that after GH treatment (56). After the discovery of ghrelin and its receptor, it is possible that new indications for GHRP will be discovered. In renal failure, in which GH secretion is already increased, it seems unlikely that GHRP would be a preferred form of therapy. GH-Receptor Antagonists and Somatostatin Analogues.