# Hidden Gems

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## Shining a light into the past for the articles that continue to shape our diabetes clinical practice today

This issue: Goeddel DV, Kleid DG, Bolivar F et al (1979) Expression in *Escherichia coli* of chemically synthesized genes for human insulin. *Proc Natl Acad Sci U S A* **76**: 106–10

*The first use of bacteria to produce synthetic human insulin*

Early formulations of insulin were associated with allergic reactions. These occurred largely in response to non-insulin impurities rather than the insulin itself. Bovine insulin only differs from the human form by three amino acids, and porcine insulin differs by just one. In 1972, a form of porcine insulin was developed that was structurally identical to the native human hormone. However, a breakthrough came at the end of that decade when *Escherichia coli* was used to produce human insulin synthetically using recombinant DNA technology. In this article, Goeddel et al describe the technique, which heralded a new era of insulin therapy. Whilst synthetic human insulin is not without its own problems, and a number of people with diabetes still opt for the older formulations, these products were adopted by the majority of insulin-treated individuals. The technique was later developed as a basis for insulin analogue production.

Progress in science often arises not through linear, incremental footsteps but through non-linear, entirely novel approaches arising from some unrelated field. The development of Teflon during the 1960s Apollo programme is an often (although erroneously) cited example, used to help justify the cost of the politically motivated space race. During the decade that followed, techniques involving recombinant DNA technology were producing a new solution to innumerable old problems, including the impurity of insulin derived from animals (usually pigs and cattle). Evident from the very first injection into a patient (14-year-old Leonard Thompson) on 11 January 1922, the potential for insulin to trigger allergic responses in human recipients led to a range of purification techniques aiming to effectively remove the contaminant allergens still present from the animal source (Bliss, 2009).

Insulin is a highly conserved peptide that is found in almost all animals, even invertebrates. The use of recombinant DNA techniques to clone synthetic insulin from bacteria offered not only a molecule identical to the native human peptide (which in fact had already been achieved through modification of porcine insulin; Ruttenberg, 1972), but also a freedom from the non-insulin contaminants in animal extracts. It also served to meet the increasing demand for insulin as diabetes prevalence and the proportion of people with type 2 diabetes requiring insulin expanded globally.

### The Hidden Gem

In their paper, Goeddel and colleagues describe in technical detail the sequence of steps taken to produce the insulin. To summarise it briefly, the technique involved the following:

- Isolation of *Escherichia coli* K-12 strains 294 and D1210, each used separately to produce, respectively, the 21-amino-acid A-chain and the 30-amino-acid B-chain of the insulin molecule.
- Purification of the necessary enzymes, including DNA ligase, T4 polynucleotide kinase and restriction endonucleases.
- Isolation of plasmids, including pBR322 as a cloning vehicle.
- Assembly of the insulin hybrid plasmids and attachment of the insulin genes to the gene for beta-galactosidase.
- Expression of the cloned genes and production of high volumes of beta-galactosidase with the insulin chain attached to it.
- Cleaving of these hybrids using cyanogen bromide to release the insulin chains.
- Joining of the A- and B-chains to produce the human insulin hormone.

The insulin gene was the second human gene to be cloned (the first being the somatostatin gene), and human insulin was the first product of recombinant DNA technology to go into commercial manufacture. Subsequent developments included the use of the yeast

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*Saccharomyces cerevisiae* as an alternative to *E. coli* bacteria.

## Why it still shines today

Improvement in the purity of insulin formulations has been a significant development in modern diabetes management. Genetically engineered human insulin was only one step in this process, but an important one. Prior to the late 1970s, other purification techniques had made serious allergic reactions relatively rare. Such reactions may still occur with synthetic human insulin, and it was not obvious that the new source would necessarily be superior. Synthetic insulin does not include a C-peptide fragment, and there is some evidence of biological activity of this peptide *in vivo* (Nordquist et al, 2008). The marketing of synthetic formulations during the 1980s was followed by reports of other reactions, including loss of hypoglycaemia awareness (Teuscher and Berger, 1987), and some opted to stick with the older, animal-derived products, although evidence for this effect was difficult to confirm (Colagiuri et al, 1992). So the impact of synthetic insulin on diabetes management is still difficult to determine and certainly less than expected (Richter and Neises, 2005). In a commentary, Gale (1989) stated that it was "sad to say the brilliance underlying its creation has not been matched by corresponding clinical benefit".

An interesting twist to this story occurred through the development of insulin analogues in the 1990s. The first of these was insulin lispro, produced by Eli Lilly from 1996, and was followed by insulins aspart, glulisine, glargine, detemir and degludec. These products also result from the same recombinant DNA technology but, unlike the first synthetic insulin, they are structurally modified to differ from the native molecule. These differences produce useful absorption characteristics (either rapid onset or long duration of action) that can be used and combined to replicate physiological insulin profiles. It is ironic that a technique developed in the 1970s that was motivated partly by the desire to exactly replicate the native molecule was later modified to produce analogues that are deliberately different.

Like human insulin itself, the superiority of these analogues over their forebears is a matter of controversy, with only minor benefit reported in a number of systematic reviews (Siebenhofer et al, 2006; Horvath et al, 2007). Nevertheless, they have been actively promoted and are displacing synthetic human insulin itself in the market. At the same time, animal-sourced insulin has actually become scarce, causing concern among those who still choose to use it.

Whatever the conclusion regarding the benefits of synthetic insulin and its descendants, this paper makes a fascinating read for anyone wishing to appreciate, in Gale's words, "the brilliance underlying its creation". ■


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