This Week's Citation Classic ®

Ullrich A. Shine J, Chirgwin J, Pictet R, Tischer E, Rutter W J & Goodman H M. Rat insulin genes: construction of plasmids containing the coding sequences. *Science* 196:1313-9. 1977: **Ullrich A, Coussens L, Hayflick J S, Dull T J,** Gray **A, Tam A W, Lee J, Yarden Y, Libermann T A, Schlessinger J, Downward J, Mayes E L V, Whittle N, Waterfield M D & Seeburg P H.** Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. *Nature* 309:418-25, 1984; and **Ullrich A, Bell J R, Chen E Y, Herrera R, Petruzzelli L M, Dull T J, Gray A, Coussens L, Liao Y-C, Tsubokawa M, Mason A, Seeburg P H, Grunfeld C, Rosen O M & Ramachandran J.** Human insulin receptor and its relationship to the tyrosine kinase family of oncoaenes. *Nature* 313:756-61, 1985.

[Dept. Biochem. and Biophys. and Dept. Med.. Metab. Sect., VA Med. Or., University of California. San Francisco, CA; Depts. Mol. Biol. and Protein Biochem., Genentech, South San Francisco, CA: Dept. Chem. Immunol.. Weizmann Inst. Sci., Rehovot. Israel; Protein Chem. Lab., Imperial Cancer Res. Fund, London, England; and Dept. Mol. Biol. and Virol.. Memorial Sloan Kettering Cancer Ctr., New York, NY]

These papers reported the first cloning of a mammalian peptide hormone, insulin, and the complete sequences of the human epidermal growth factor (EGF) receptor and the human insulin receptor. The EGF receptor gene was found to be amplified and apparently rearranged in A431 cells, and our 1984 paper established its extensive homology with the *v-erb-B* oncogene. The 1985 paper reported the insulin receptor's similarities to the EGF receptor and provided the molecular basis for the understanding of insulin action. [The *SCI*® indicates that these papers have been cited in more than 920, 1,300, and 1,180 publications, respectively.]

Molecules That Generate Biological Signals

Axel Ullrich
Department of Molecular Biology
Max-Planck-Institut für Biochemie
8033 Martinsried
Germany

In many ways the first cloning of a cDNA encoding insulin, a peptide hormone of clinical relevance, and the structural characterization of the first two signal-generating cell surface molecules, the receptors for epidermal growth factor (EGF) and insulin, exemplify the principles that are essential for the rapid progress of modern science: communication and cooperation. For me personally, the history of this work began during my undergraduate studies at the University of Tubingen in Germany, where I was exposed to efforts to create a better insulin molecule by synthetic peptide chemistry in G. Weitzel's Institute of Physiological Chemistry. This massive premolecular modeling and pregenetic engineering endeavor was not blessed with much success, but impressed on mea lasting interest in applied research in general and insulin in particular.

Upon my arrival at the University of California in San Francisco (UCSF) in 1975, shortly after the groundwork for genetic engineering technology had been laid at Bay Area universities, I decided to attempt to clone a DNA copy of insulin mRNA. Under rather exciting circumstances, which are very well described in Stephen Hall's book *Invisible Frontiers,*[1] this effort led to success in 1977 and to the first characterization of a cDNA encoding a mammalian peptide hormone. With respect to the insulin molecule, this represented another "first," as it was already the first hormone discovered, the first natural peptide used as a therapeutic agent, the first peptide hormone sequenced, the first prohormone discovered, the first protein of which a three-dimensional structure was established, the first biologically active peptide hormone chemically synthesized, and, after the cloning, it would become the first human recombinant pharmaceutical to be marketed and used in diabetes therapy. For me, this success led to an interest in gene evolution and therefore to the other members of the insulin gene family, insulin-like growth factors I and II (IGF-II). I pursued these interests after moving from UCSF to Genentech, the first company founded on the theoretical promises of recombinant DNA technology.

At Genentech, I applied my cloning experience to IGF-II and expanded my interests to other growth factors such as nerve and EGF. While working on the analysis of EGF precursor cDNA sequences, Mike Waterfield, then at the Imperial Cancer Research Fund in London, visited Genentech and with his protein-analytical eye helped to shed some light on the 1,217 amino-acid long prepro EGF sequence. This first contact led to a phone call in 1983, in which Mike asked whether I was interested in collaborating on the structural characterization of the receptor for EGF. With the newly developed cDNA "long probe" approach, in combination with *X* phage libraries, I felt ready to approach any cloning project, no matter how scarce the mRNA! The next phone call from London in the fall of

1983 not only reported the first EGF receptor peptide sequences, but also the realization that they were highly homologous to amino acid stretches within the recently characterized *v-erb-B* oncogene.[2] This connected, for the first time, a retrovirai gene product with proven cancer-inducing activity to a normal cell protein with known functions, EGF binding and the generation of a mitogenic signal. At a collaborator "summit" meeting in London in December 1983, I met for the first time Yossi Schlessinger, a biophysicist who had actually initiated the project at the Weizmann Institute in Israel and whose expertise complemented Waterfield's protein chemistry and my molecular biology expertises. The collaboration with Schlessinger, which started under such exciting circumstances, has endured to this day and has been an extraordinarily productive alliance in terms of our continued investigation of EGF receptor-mediated signaling mechanisms.[3]

The 1984 *Nature* publication of the complete sequence of the EGF receptor not only fully characterized for the first time the primary structure of a signal-generating cell surface receptor, but also provided valuable tools for a rapidly expanding research area. The v-erb-8/EGF receptor relationship also had an immensely stimulating effect on research into the molecular basis of cancer. While intense efforts to detect v-erb-S-like molecular lesions in cancer tumors yielded no interesting findings, the surprising observation of EGF receptor gene amplification in A431 cervical carcinoma tumor cells, reported in this paper, paved the way for later discoveries of high frequency gene amplification of the EGF receptor gene, as well as that coding for the EGF receptor-related receptortyrosine kinase HER-2/neu(or *c-erbB2)* in both mammary and ovarian carcinoma, which was the outcome of a collaboration with Dennis Slamon, an oncologist at UCLA.[4]

Similarly, in continuation of the insulin-related work, yet another fruitful collaboration, between my lab group and J. Ramachandran's protein sequencing group at Genentech and the late Ora Rosen at the Sloan Kettering Cancer Center, enabled the rapid completion of the insulin receptor cloning project. Interestingly, our *Nature* paper appeared one month before a splice variant of the same receptor was reported in *Cell* by my collaborator on the rat insulin cloning paper, Bill Rutter.[5] Here again the sequence information provided in combination with cDNA tools fueled tremendous progress in a research area struggling with problems related to the molecular basis of insulin action and, most importantly, the understanding of non-insulin-dependent diabetes mellitus (NIDDM). Today the insulin receptor gene, due to the intense efforts of clinical diabetologists, is among those for which the most "natural" mutations are known, all of which cause various forms of insulin resistance. Unfortunately, in contrast to cancer, understanding the effects of molecular defects in this signaling system is unlikely to lead to new therapies. The next frontier—insulin signal controlling elements—is awaiting our efforts and may provide new possibilities for the eventual benefit of the patient.

In summary, the *Science* publication and the two *Nature* papers represent the result of very successful collaborations between international groups in academia and industry. While the *Science* article was the first and crucial step towards the development of the first recombinant therapeutic, human insulin, the molecular characterization of the EGF and insulin receptors opened new avenues towards the understanding of biological signaling mechanisms of vital importance and provided insights into the molecular defects that are the basis of human diseases such as cancer and diabetes. To have made contributions to discoveries that not only provided new scientific perspectives but also led to the development of therapeutic strategies in the treatment of diabetes and cancer is the most satisfying aspect of my professional life.

***[Editor's note-. An interview with Axel Ullrich[6] was published in the March 1993 issue* of Science Watch®.]**

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Received March 26, 1993