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The 1984 Nobel Prize in Chemistry

As Bruce Merrifield stepped off the elevator on the fourth floor of Flexner Hall at The Rockefeller University on Wednesday morning, 17 October, he was informed by a laboratory assistant that the Royal Swedish Academy of Sciences had awarded him the 1984 Nobel Prize in Chemistry. The informal way in which Dr. Merrifield learned of his award was entirely consistent with his modest and self-effacing manner. Subsequent to the announcement, comments of delight were frequently heard at Rockefeller, inspired not only by this recognition of Dr. Merrifield's superb scientific accomplishments but also by widespread appreciation of his outstanding qualities as a human being.

R. Bruce Merrifield was born in Fort Worth, Texas, on 15 July 1921. He received both his B.A. (1943) and his Ph.D. (1949) degrees from the University of California at Los Angeles, the latter degree as a student of Professor Max Dunn. He joined The Rockefeller Institute for Medical Research (as The Rockefeller University was then known) in 1949 and rose through the ranks, becoming Professor in 1966 and being named John D. Rockefeller Jr. Professor in 1983.

Dr. Merrifield was cited by the Swedish Academy for his development of a methodology for chemical synthesis on a solid matrix. The application of his methodology in the fields of peptide and protein chemistry, his own area of research, has opened up completely new vistas in biochemistry, pharmacology, and medicine. His synthetic approaches have also had a major impact on nucleic acid chemistry, where the developments arising out of the application of his ideas by others have contributed greatly to the techniques of site-directed mutagenesis and gene synthesis so important to molecular biology. His conceptual approaches have been used in the preparation of solid matrix–bound organic and inorganic reagents and have even been applied to the detection of highly reactive intermediates.

In his early years at Rockefeller, in the laboratory of the late D. W. Woolley, Merrifield worked on the isolation and characterization of biologically active peptides and encountered the need to synthesize structural analogs of such peptides. The synthesis of peptides, then based on extensions of the pioneering work of Emil Fischer at the turn of the century, was extremely laborious. Merrifield recognized the great importance of finding alternative approaches that would open up the possibility of rapid and convenient peptide synthesis. In May of 1959, an entry appeared in his research notebook under the heading "A New Approach to the Continuous Stepwise Synthesis of Peptides." It read: "There is a need for a rapid, quantitative, automatic method for the synthesis of long chain peptides." By developing a simple and ingenious method for obtaining peptides and proteins through synthesis on a solid matrix, Bruce Merrifield was to fulfill this need.

The crucial step in peptide synthesis is the formation of the peptide bond. To achieve this, it is necessary to activate the carboxyl group of one amino acid so that it will react with the free amino group of the other. Because amino acids often contain reactive functional groups in addition to their amino and carboxyl groups, all of the groups except those directly involved in making the bond must be chemically protected against the formation of undesirable combinations if a specific pure peptide of known structure is to result. Even in the simplest case, the coupling of two amino acid units to form a dipeptide, the amino group of one unit and the carboxyl group of the other must be protected. (In some cases the carboxyl of one unit can be selectively activated, eliminating the necessity for protecting the carboxyl of the other.) Before a second peptide bond can be formed, the protecting group from one of the blocked groups of the original dipeptide must be removed.

Prior to the Merrifield solid-phase method, a major problem in peptide synthesis was that the peptides resulting from each coupling step had to be isolated and purified before chemists could proceed to the formation of the next peptide bond. Biologically important and complex peptides had been synthesized by classical synthetic methods. An example is the ground-breaking synthesis of the hormone oxytocin, a nonapeptide, by Vincent du Vigneaud, who received the Nobel Prize in Chemistry in 1955. However, the synthesis of important long chain peptides such as insulin or secretin by classical methods has involved many years of arduous effort.

In the Merrifield solid-phase method, the first amino acid of a peptide chain is attached through its carboxyl group to a polymeric support. In subsequent stages, the remaining amino acids of the desired peptide chain are added stepwise in the proper order. A polystyrene support crosslinked with divinylbenzene has typically been used in Merrifield's own research. This continues to be the support most commonly used by others in solid-phase peptide synthesis. Because the solid support is insoluble in the various solvents used, the peptide species produced as intermediates and held on the insoluble support during the course of the synthesis can be readily separated from the solvents, which contain reagents needed for the peptide-bonding steps. Thus, it is possible to use excess reagents, allowing each step to be driven close to completion. At the end of the coupling steps, the excess reagents and by-products can be washed away. Once the required peptide chain has been assembled, it can finally be removed from the solid support, and purification methods can then be applied to isolate the desired product in a homogeneous state. Because it is not necessary to isolate the intermediate peptide products at each

R. Bruce Merrifield [The Rockefeller University]

stage, an enormous amount of time can be saved in the synthesis of a peptide. Also, the use of excess reagents makes it possible to obtain the yields with values as high as 99.5 percent or better, a remarkable achievement for organic synthesis and one that is necessary if long peptide chains are to be assembled in respectable overall yield.

The first results of peptide synthesis using a solid polymeric support were reported by Merrifield at a meeting of the Federation of American Societies of Experimental Biology in 1962, and the synthesis by this method of the important peptide bradykinin, a hormone, was reported by him in the *Journal of the American Chemical Society* in 1963. This achievement was rapidly followed by his synthesis of angiotensin, carried out in collaboration with his first graduate student, Garland Marshall, and reported in 1965. Having shown that peptide synthesis on a solid support was feasible, Merrifield was ready for the next step toward the goal he had set in his laboratory notebook in 1959. Since the need to isolate and purify peptide intermediates had been eliminated, it was clear to him that the steps in solid-phase synthesis could potentially be carried out automatically with the use of a suitably constructed machine. Working with John Stewart in a home basement, he completed the first operating model of an automatic peptide synthesizer. This machine had three basic elements: a series of reservoirs for amino acids, reagents, and solvents; a reaction vessel that had suitable plumbing for the introduction of amino acids and chemicals in the right order at the right times and that permitted excess chemicals to be readily removed; and a programmer that controlled all the operations. Automated peptide synthesis was described by them in articles in *Nature* and in *Science* in 1965.

The enzyme ribonuclease is a catalytic protein that has played a considerable role in the history of The Rockefeller University. It was isolated by Dubos in 1938 at Rockefeller and crystallized there by Kunitz in 1940. The peptide sequence of ribonuclease was elucidated by Hirs, Stein, and Moore (Stein and Moore were Nobel winners in 1972), and in 1969 Merrifield capped this long history of ribonuclease research at Rockefeller by the total synthesis of the enzyme using the solid-matrix method. This work, done together with Bernd Gutte, required 369 chemical reactions and 11,391 steps with the automated peptide synthesizer. A synthesis of ribonuclease by a different approach was reported at the same time by a group at Merck Sharp & Dohme Research Laboratories led by R. Hirschmann and R. G. Denkewalter, but it is the Merrifield procedure that has been adopted by most investigators in their syntheses of peptides.

With the synthesis of ribonuclease by the Merrifield solid-phase method, the full potential of this approach was recognized worldwide. However, the application of the solid-phase method to peptide synthesis was not immediately and universally accepted. While the elimination of the need to isolate intermediates in the course of a peptide synthesis contributed greatly to the high yields and to the rapidity with which the synthesis could be achieved, there were those who felt that these features of the solid-phase method also posed significant problems. In particular, although the yields at each stage might be very high, even an exceedingly small degree of incomplete coupling or of a side reaction at each stage could add up to an appreciable degree of impurity in the final product of a long synthesis.

Merrifield, of course, recognized this point, and it is interesting to note what he wrote about it.

> The pessimist says that a pure product cannot be isolated and that, even if it were, its purity could not be demonstrated. Since this attitude produces no progress, we prefer the pragmatic approach of being aware of the problem of purity and simply using the best methods currently available during synthesis, isolation and characterization of the products. Improvements in separation methods are appearing regularly; what cannot be achieved today may seem simple tomorrow.

His statement was indeed prophetic. The purification methods for peptides have progressed enormously since the solid-phase method was first described. Perhaps first among the advances has been the development of high-performance liquid chromatography (HPLC), which has had a revolutionary effect on our ability to purify peptides. In addition, the use of highly sensitive analytical techniques, including HPLC, and major improvements in automated sequencing techniques have all contributed to the establishment of stringent criteria for the purity of peptides prepared by the Merrifield method.

In the years since the ribonuclease synthesis, many important peptides including hormones, hormonal antagonists, neuropeptides, protein growth factors, and toxins have been prepared by the Merrifield method. The peptide hormones ACTH (adrenocorticotropic hormone) and calcitonin have been produced on a commercial scale by solid-phase synthesis. Many laboratories are now engaged in the study of the effects of synthetic antigenic peptides on the production of specific antibodies, research which has had important implications for the prevention of various diseases. Syntheses of peptides up to approximately 50 amino acids in length using the Merrifield method now appear frequently in the literature. Such syntheses by the

solid-phase approach take weeks rather than years as they would if performed by classical methods.

The refinement of the chemistry and technology of solid-phase peptide synthesis remains a major concern of the Merrifield laboratory. In recent years Professor Merrifield and his co-workers have made substantial progress in developing new protecting groups and in improving the conditions for coupling amino acids when building peptide chains. Through the analysis of the mechanisms of side reactions, they have devised ways to eliminate many of these, thus preventing the formation of undesired by-products. Merrifield has also enhanced the effectiveness of the solid-phase process by improving the separation methods for the final purification of the desired peptide chain. While the synthesis of peptides greater than 100 amino acids in length remains a difficult challenge, even by the solid-phase method, Merrifield is applying improvements developed over the last several years to the synthesis of such large molecules. With his wife, Elizabeth, who has been working with him for the past few years, he has made major strides in the synthesis of an interferon (166 amino acids in length).

Bruce Merrifield's research at Rockefeller has had the objective of understanding the relations between the chemical structures of peptides and proteins and their physical and biological properties. Through his development and use of solid-matrix synthetic methodology, he has demonstrated that many questions can best be answered by chemical synthesis of these compounds and appropriate structural variants. His "simple and ingenious" methodology not only has increased the power of basic research but has also enriched the promise of peptide chemistry for medical applications.—EMIL THOMAS KAISER

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Molecular Indices of Cell Lineage Specification in Sea Urchin Embryos

Robert C. Angerer and Eric H. Davidson

Summary. The origins of several of the differentiated cell lineages of the advanced sea urchin embryo are well defined. Cytological application of molecular probes to three lineages, those responsible for the formation of the skeleton, the gut, and the aboral ectodermal wall of the late embryo, has demonstrated expression of lineage-specific genes long before overt morphological differentiation. These observations lead to useful generalizations regarding the processes of gene regulation that underlie the molecular biology of cell lineage specification in the embryo.

In the normal development of many species each of the sets of cells that form the diverse structures of the embryo descends from a specific group of cleavage-stage blastomeres. Where the cell lineages are known, embryogenesis can be regarded as the sum of the processes by which these lineages are initially specified and then induced to express their different properties. The sea urchin embryo invites this form of cellular and molecular analysis. Thus, within a few days of fertilization the discrete cell lineages of this embryo differentiate into the larval gut, skeleton, ectoderm, and other tissues and cell types. The blastomeres from which the prominent lineages arise were long ago identified and in some, although not all, cases the number of cells constituting each lineage at every point in early development is also known, at least for certain species. In this article we use the term cell lineage to denote a set of embryonic cells of given function that descends from a restricted group of early progenitor cells. The major experimental advantages of the sea urchin embryo such as its availability, the ease of preparative fractionation of some embryonic cell types, the relatively small number of embryonic cells, and the growing repertoire of molecular probes for specific genes and gene products have been described (*1*). New and sensitive cytological methods in which such molecular probes are used are now being applied to the study of the sea urchin embryo, with consequent elucidation of the process of cell lineage differentiation.

Normal Cell Lineage Along the Animal-Vegetal Axis

One of the embryonic axes of symmetry, the animal-vegetal axis, is preformed in the egg at the time of fertilization. This was initially demonstrated by Boveri (*2*), who reported several features of the unfertilized sea urchin egg that bear an invariant relation to the future orientation of the embryo. The polar bodies are extruded at the future animal pole, and Boveri also noted at this pole a minute channel in the external coat of the egg called the "jelly canal." He studied the eggs of the Mediterranean species, *Paracentrotus (Strongylocentrotus) lividus*, and described, following earlier observers (*3*), a subequatorial band of pigment granules that is orthogonal to the planes of the first two cleavages, and to the future animal-vegetal axis of the embryo. These observations have now been confirmed and extended to other species (*4*). The preformed animal-vegetal organization of the egg is not affected by the point of sperm penetration, which may occur at any locus on the egg surface (*4*). After fertilization, the egg cytoplasm is divided equally by two successive meridional cleavages intersecting at right angles

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