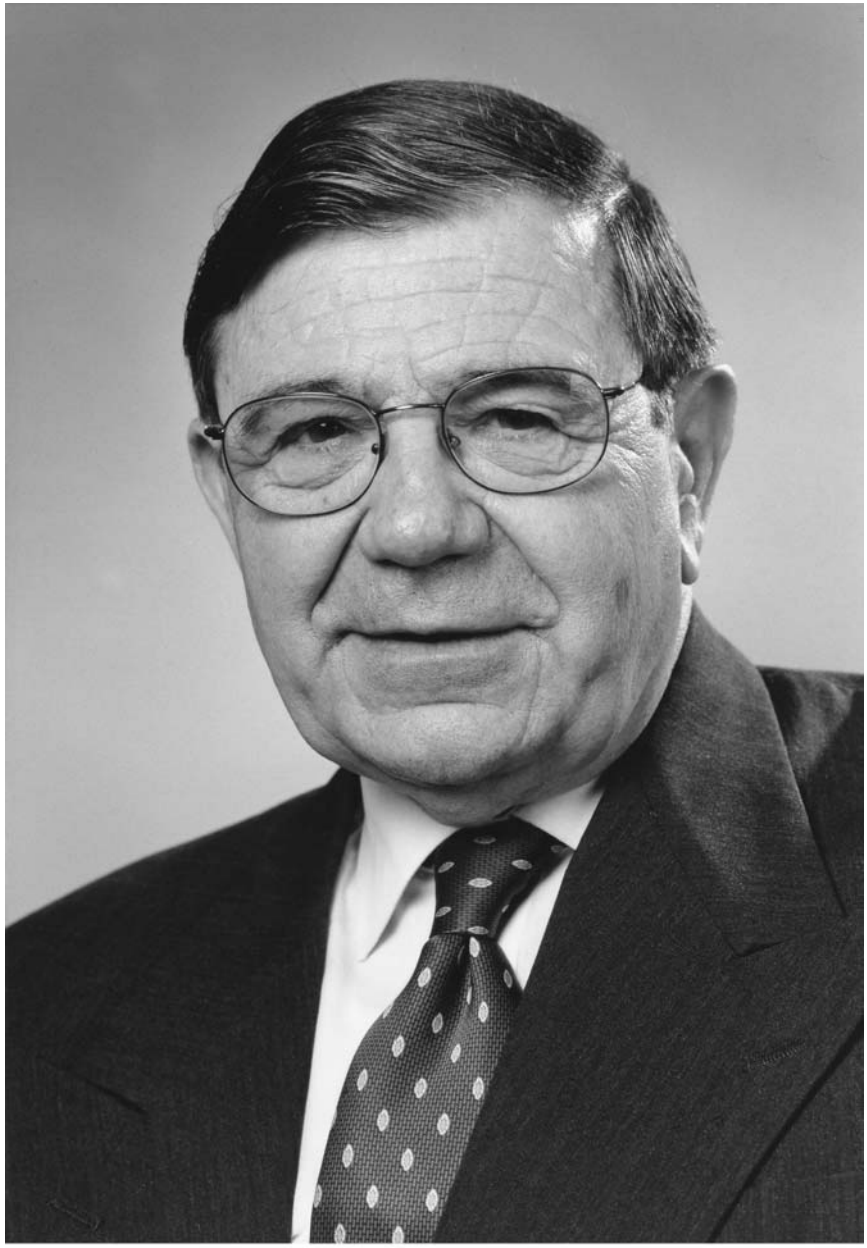

David Sarnoff

In Awe of Subcellular Complexity: 50 Years of Trespassing Boundaries Within the Cell

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glutaraldehyde fixation, membrane-bound ribosomes, signal hypothesis, MDCK cells, epithelial cell polarity

Abstract

In this review I describe the several stages of my research career, all of which were driven by a desire to understand the basic mechanisms responsible for the complex and beautiful organization of the eukaryotic cell. I was originally trained as an electron microscopist in Argentina, and my first major contribution was the introduction of glutaraldehyde as a fixative that preserved the fine structure of cells, which opened the way for cytochemical studies at the EM level. My subsequent work on membrane-bound ribosomes illuminated the process of cotranslational translocation of polypeptides across the ER membrane and led to the formulation, with Gunter Blobel, of the signal hypothesis. My later studies with many talented colleagues contributed to an understanding of ER structure and function and aspects of the mechanisms that generate and maintain the polarity of epithelial cells. For this work my laboratory introduced the now widely adopted Madin-Darby canine kidney (MDCK) cell line, and demonstrated the polarized budding of envelope viruses from those cells, providing a powerful new system that further advanced the field of protein traffic.

Contents

MY ARGENTINE BEGINNINGS AND THE ENIGMA OF ORGANELLES

The educational system that prevailed in Argentina in the late 1940s, when I finished high school, required that I then choose a specific university track in which to continue my studies. I found this an extremely difficult decision to make, as I had a wide range of interests both within the sciences and the humanities. I decided to enter medical school, not moved by a passionate yearning to be a physician, but rather because I believed that the study and practice of medicine offered almost limitless opportunities for both scientific exploration and a full appreciation of the human condition.

I obtained my education as a physician in Rosario, then the second most populous city in Argentina, where I graduated with an M.D. from the University of Litoral in 1954, a few months before the forced end of Peron's populist regime, brought about by a military coup that occurred in September of 1955. The beginning of my scientific career was made possible by that event because (following years of obscurantism and intolerance) after the fall of Peron, Argentine universities went into a sort of academic renaissance, and teaching positions were again, for a time, opened to all those qualified, without regard for political affiliation.

In medical school I had read several excellent classical text books, including the histology ones by Giuseppe Levy, Alexander Maximow, and William Bloom, and those on pathology by Herwig Hamperl, H. Ribbert, and G. Roussy, and became interested in the structure and function of cells. At such an early stage of my scientific life I also became acquainted with E.B. Wilson, through his marvelous introduction to the 1925 edition of his book *The Cell in Development and Heredity*, which I found at the medical school library. I was soon fascinated by questions such as whether the mysterious different cellular organelles illustrated in those books, although apparently cooperating with each other to sustain the activity of the cell, had a certain life of their own. Or, to phrase it in a more scientific manner, by what mechanisms did they perpetuate themselves as distinct structures within the cell and from one cellular generation to another, while retaining their individuality. So little was known about these questions that I used to think of them as somewhat akin to those concerning the structure of matter itself. I made some failed attempts to begin a research career in Rosario, and in 1956 I decided to move to Buenos Aires.

The late 1950s were splendid and exciting times at the University of Buenos Aires, and I was very fortunate to become an instructor there in 1956, at the Institute of General Anatomy and Embryology of the School of Medicine. Under the direction of Eduardo De Robertis—a pioneer electron microscopist and the discoverer of synaptic vesicles, as well as the author of the first textbook entitled *Cell Biology*, which was published in English in 1948[1]—this institute was later to become a celebrated center of cell biology research and training in South America. I had little experience in research and obtained my position through an open competition, involving written and oral examinations, that was announced in the newspapers and was intended to recruit aspiring scientists to the basic science departments to which the professors who had been forced out of the universities in the previous decade were now returning. I look back with amazement at the fact that at that time—following an academically regressive political regime that lasted for 12 years—it was possible by those methods to assemble at the University of Buenos Aires a group of very capable young scientists, many of them physicians with an interest in basic research, that included several individuals who would later become highly successful researchers. Most of them, regrettably, because of subsequent political events, are now scattered throughout the world.

Some of the outstanding physiologists and biochemists who left their university positions during the Peronist era, such as Bernardo Houssay and Luis Leloir (who received Nobel prizes in 1947 and 1970, respectively) or Eduardo Braun Menendez (the discoverer of hypertensin, now called angiotensin), had continued their research in Argentina because they were able to establish private laboratories, outside the official academic system, using their own financial resources, as well as those from private donors.[2]

Other investigators, who had been cast out of the universities, for example, Eduardo De Robertis, had taken the route of exile, which, unfortunately, was also to be well traveled by the next generation of Argentine scientists during the military regimes that, with sporadic interruptions, dominated the political landscape until the end of the 1980s. De Robertis—who had worked in the United States at the University of Chicago (1939–1941) with R.R. Bentley, a pioneer in cell fractionation, and subsequently developed a brilliant scientific career in Argentina—left Buenos Aires in 1947. He joined the laboratory of F.O. Schmitt, the founder of the Biology Department at the Massachusetts Institute of Technology, who had created the first electron microscopy (EM) facility devoted to the study of cellular ultrastructure. After gaining international recognition for his contributions to cellular neurobiology, De Robertis settled in Montevideo in 1949, at the Institute directed by Clemente Estable (a disciple of the Spanish School of Ramon y Cajal, and also a patrician figure in Latin American Science) where he found the generous hospitality and tolerance for which the natives of Uruguay are well known to Argentines.

As junior faculty members, we waited anxiously in Buenos Aires for almost a year until De Robertis was able, with the help of the Rockefeller Foundation, to establish at the Institute the first EM facility in the country, to which he could transfer a functional laboratory from Montevideo. I owe a great debt to De Robertis, without whom, most likely, I would not be a scientist today. His keen powers of observation and technical skills set for us standards of world-class caliber for EM and laboratory experimentation. He was

[1]The first Spanish edition, entitled *Citologia General*, was published in 1946.

[2]Some of those laboratories, including the laboratory established by the Fundacion Campomar for Leloir's group, have prospered and survived to this day. This was an unwanted and beneficial effect of the previous regime.

also enterprising, imaginative, and capable of remarkable insights. Most importantly, he rejected the notion that the quality or significance of research carried out in Latin America could be curtailed by environmental limitations. He was stern and highly valued discipline and hard work, but he also cared deeply for those whose commitment to science was demonstrated in the laboratory. He instilled in his students lofty ambitions, and a good measure of his expectations was apparent from the high caliber of the laboratories to which he chose to send them abroad for further research experience. In sum, I consider my training with "el maestro," as we all called him affectionately among ourselves, comparable in its rigor and excitement to what I could have had in the best of laboratories abroad. In De Robertis's laboratory I carried out extensive EM studies on the adrenal gland and began to study secretion in both the adrenal medula and cortex (De Robertis & Sabatini 1958, 1960; Sabatini & De Robertis 1961; Sabatini et al. 1962). In fact, as far back as 1960 we proposed a role for the Golgi apparatus in the formation of the cathecolamine-containing granules of the adrenal medulla and noted that the granules release their content at the cell surface by an exocytotic membrane fusion event (De Robertis & Sabatini 1960) (**Figure 1**). These notions, although a component of the dogma of cell biology today, were not widely accepted at the time.

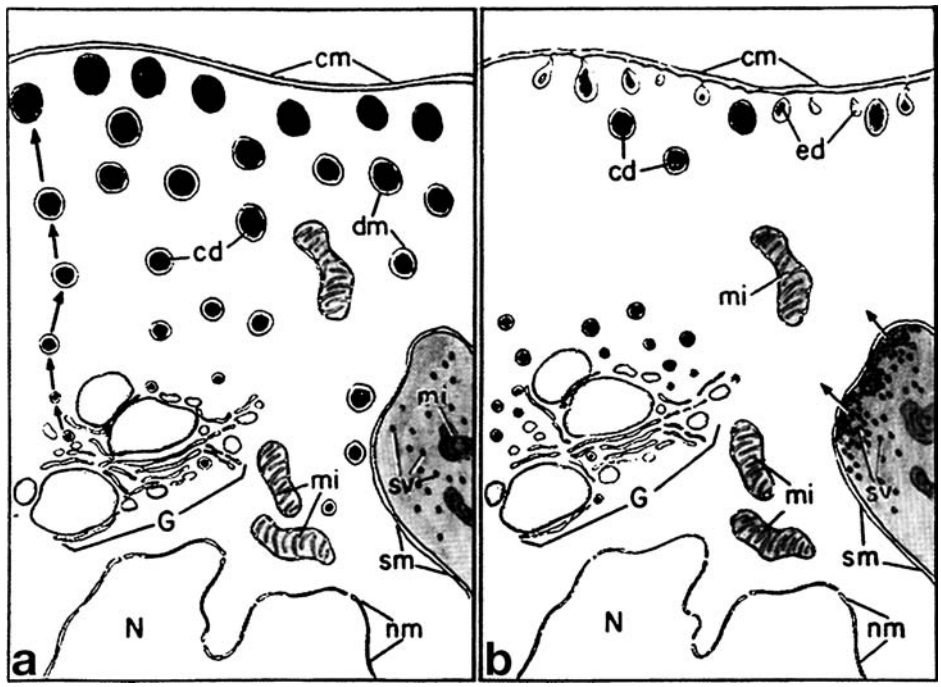

Figure 1

A diagramatic interpretation of the secretory process in a chromaffin cell of the adrenal medulla. **(*a*)** A chromaffin cell in the resting state. Cathecolamine-containing granules (cd) bounded by a membrane (dm) form (*arrows*) from the Golgi apparatus (G). The cell makes a synapse (sm) with a nerve ending containing synaptic vesicles (sv) and mitochondria (mi). (*b*) A chromaffin cell after electric stimulation of the splanchnic nerve. An exocytic discharge of cathecolamines takes place at the intercellular cleft resulting from fusion of the granule membranes with the plasma membrane (cm). Drawing from E.D.F. De Robertis, D.D. Sabatini. 1960. With permission from *Fed. Proc.* 19:70–78.

THE LURE OF PROTEINS, THEIR ORIGIN, AND TRAVAILS

By the end of the 1950s, when my first educational stage as a physician and a budding scientist was being completed in Buenos Aires, the grounds had already been set in the United States and Europe for the explosive developments that, during the remainder of the century, were to transform the biological sciences. As my turn was coming in De Robertis's group to seek training abroad—to which we all looked forward as necessary for the ripening of our scientific careers—I could scan an ample horizon of emerging fields in my search for a laboratory in which to invest the coming years in a foreign land.

A decade was closing that had witnessed a revolution in genetics and the explosive growth of molecular biology that followed the discovery of the double helix in 1953. Proteins were, of course, known to be the final products of gene expression, and their diversity as building blocks and as molecular machines had been recognized as responsible for the myriad of metabolic and physiological processes that sustain life. The first primary sequence of a protein, insulin, had already been determined by F. Sanger (who had visited our laboratory in Buenos Aires) and the first glimpses at the three-dimensional structure of some proteins by L. Pauling, M. Perutz, and J. Kendrew were making it clear that much would soon be learned about the particular architectural features that endowed proteins with specific functional activities. I could see that chemistry and crystallography held the key to unlock these secrets, and was attracted to these fields.

On the other hand, electron microscopy studies, initially concerned with macromolecular complexes, had by the mid-1950s already revealed a previously unimaginable complexity in the organization of the cytoplasm of the eukaryotic cell. The notion that the distinct compartments or organelles, whose existence had long intrigued me, defined chemical compositions and carried out specific cellular functions was also being established by a combination of histochemistry, electron microscopy, cell fractionation, and biochemical and enzymatic analysis. This added considerably to the fascination the eukaryotic cell exerted over those, like myself, who had begun to peek at it through the powerful eyes of the electron microscope.

Cell biology pioneers such as T. Caspersson and J. Brachet had already demonstrated a relationship between the protein biosynthetic activity of the cell and its capacity for RNA synthesis, manifested in prominent nucleoli and the intense basophilic staining of regions of the cytoplasm. The process by which protein themselves were synthesized, however, remained a mystery that was also alluring to me, as it lay at the boundary of biochemistry and cell biology. By 1960 it had been recognized that protein synthesis took place in ribosomes, but the puzzle of how these particles carried out the decoding of genetic information had not been solved.

Of course at that time few were concerned with the even greater mystery of the mechanisms and pathways that ensure that proteins, once synthesized, become the building blocks of organelles or become functional within them. Although our knowledge of the properties of membranes was meager, it was indeed difficult to imagine how proteins, once released from ribosomes, could freely traverse the ubiquitous intracellular membrane barriers to find their correct destination within an organellar lumen or in the membrane that limited a specific compartment. For anyone whose interests straddled the boundaries of cell biology and biochemistry this should have seemed an insurmountable problem to tackle.

In 1959 I had to make the difficult decision of whether I would get training in protein crystallography—which promised to give the ultimate understanding of biological processes at the molecular and even the atomic level, and therefore go to Great Britain, the undisputed mecca of crystallography at that

time—or go to the United States to work with Palade and Siekevitz, who were beginning to unravel the pathway responsible for protein secretion, one of the most complex cellular processes.

Cytological studies extending back to the nineteenth century had made it clear that secretion requires the concerted and probably sequential action of several organelles, and efforts to increase our understanding of the secretory process were moving toward the vanguard of biological research. During the 1950s Palade and Siekevitz had pioneered in developing an approach to study the secretory pathway that combined the techniques of cell fractionation with biochemical and morphological analyses, which today is still the underpinning for most of the work being carried out in the field of molecular cell biology. I believe that it is fair to say that it is from those studies that the field of organelle and membrane biogenesis and the broad current concern with protein traffic sprung to life.

Moreover, the work of Palade and Siekevitz had focused foremost on the protein biosynthetic role of the endoplasmic reticulum (ER), an organelle unknown before EM. The discovery that the rough, ribosome-studded portions of the ER (Palade 1955) corresponded to the highly basophilic ribonucleoprotein-rich areas of the cytoplasm, characteristic of cells with intense protein biosynthetic activity, had placed the ER at center stage in the emerging field of cell biology.

Palade had discovered the ribosome in 1953 (Palade 1953, 1955) as a small particulate component of the cytoplasm visible in his electron micrographs, and Siekevitz had a long-standing interest in protein synthesis since his times in Zamecnik's laboratory in Boston, where he had pioneered in demonstrating that a microsomal fraction was capable of carrying out the process in vitro (Siekevitz 1952). The identification of the ribosome as a molecular machine that carries out protein synthesis had begun with the finding that polypeptide chains, labeled in vivo during short incubations of *Escherichia coli* with radioactive amino acid precursors, were recovered in association with ribosomes when these particles were purified from the remains of disrupted bacteria (McQuillen 1959). Yet, until 1961 (the same year I began to work at Rockefeller), when Jacob & Monod (1961) proposed the mRNA hypothesis—derived primarily from observations made on ribosome reprogramming in *E. coli* after mating and after bacteriophage-infection—the prevalent notion was that individual ribosomes were "congenitally specialized" to synthesize specific proteins.

I decided to apply for a postdoctoral position with Palade, expecting that in Siekevitz's laboratory, where I had asked Palade to place me, I would be able to investigate the function of the membrane-bound ribosomes that characterize the rough ER. Meanwhile, with the support of De Robertis and Houssay, I obtained a Rockefeller Foundation Fellowship. I am still impressed by the fact that I was interviewed twice by officers of the Foundation who came to Buenos Aires and devoted a substantial amount of their time to assess my potential as a scientist. Palade agreed to take me into his laboratory, but he wrote that it would be best if I could postpone my arrival to New York for six months, which was planned for January 1961, because Keith Porter and his group were leaving the Rockefeller Institute for Harvard and more space would then become available. Since my Rockefeller Foundation Fellowship had been arranged to begin in January, Palade suggested that I first spend a few months at Yale with Russell Barrnett, a well-known histochemist with whom he had previously collaborated. I knew of Barrnett's work, since he had developed with Seligman a histochemical technique for the detection of sulfhydryl groups at the light microscope level, and in my first paper I had used a related method to localize cysteine-rich neuropeptides in the toad hypothalamus (Lasansky & Sabatini 1957).

A CYTOCHEMICAL INTERLUDE: THE POWER OF DIALDEHYDES

On January 26th of 1961 my wife and I arrived in New Haven during a severe snow storm to find that Barrnett was out of town for a week. We were snowbound, confined to the now extinct Taft Hotel, and in danger of running out of funds, when we were rescued by Mrs. E. DeVane, a daughter of the then Yale University President, who somehow, learning of the plight of the stranded South American couple, kindly brought us to her home and helped us find an apartment.

My stay in Barrnett's lab, short as it was by the usual standards, was immensely productive. I was the most experienced electron microscopist in his group and was coming from a laboratory using cutting edge technology not yet widely adopted in the United States. Thus in Argentina we had already replaced methacrylate with epoxy resin embedding and had added to our old RCA EMU2 EM a new Siemens Elmiskope. Barrnett had inherited an RCA EMU3 from Sanford Palay, a former Yale professor who had trained with Porter and Palade and had recently moved to Harvard, and I was put in charge of that instrument.

The focus of Barrnett's laboratory was on the application of histochemical techniques to electron microscopy, something that was being eagerly attempted in several labs, but with little success. Since the 1940s histochemistry had been a thriving field of research and a powerful tool for histophysiological studies at the light microscope level, but its application to EM faced considerable challenges. The fine structure of unfixed tissues and cells sadly decayed to almost an unrecognizable state during the harsh conditions of incubation needed for some histochemical reactions. Conversely, prefixation in OsO_4—the universal fixative for EM, mainly used in a veronal-acetate buffered form introduced by Palade (1952)—rapidly and irreversibly inactivated the enzymatic activities that histochemists wanted to relate to subcellular structures. This was also the case with other metal-containing reagents, such as $KMnO_4$ and $K_2Cr_2O_7$, that were used to increase contrast in the EM.

At Yale, with my Fieser & Fieser *Organic Chemistry* textbook (Fieser & Fieser 1956) open on my desk, I decided to examine the usefulness as fixatives of a series of dialdehydes, agents that in a single molecule incorporate a double dose of the group responsible for the efficacy of formaldehyde, the preferred fixative for light microscopy. I hoped that some dialdehydes would serve as good cross-linking reagents that, at appropriate concentrations, could prevent the disintegration of subcellular structures, while perhaps maintaining some enzymatic or cytochemical activities. By a great coincidence, I found a source of glutaraldehyde nearby. Klaus Bensch—a young resident in pathology who was a frequent visitor to Barrnett's lab and was working with Don King, then a pathology professor at Yale—had used it following an industrial recipe to produce gelatin microcapsules containing *E. coli* DNA, which was administered to cultured mammalian cells in attempts to transform them into amino acid prototrophs. My very first experiments showed that glutaraldehyde gave an excellent preservation of cellular structures (**Figure 2**), while allowing the demonstration in situ of several specific enzymatic activities. I soon extended my search for new fixatives to other dialdehydes and related reactive organic compounds, but none was better than glutaraldehyde. It was a simple matter to choose concentrations and conditions that gave a compromise between acceptable tissue preservation and retention of enzymatic activities. The first paper we published (Sabatini et al. 1963) had 38 figures and illustrated the cytochemical detection at the EM level of the products of more than a dozen enzymes. Glutaraldehyde had distinct advantages over OsO_4 as a fixative in that it penetrated rapidly and deeply into tissues and was not nearly as noxious and difficult an agent to handle as OsO_4. However,

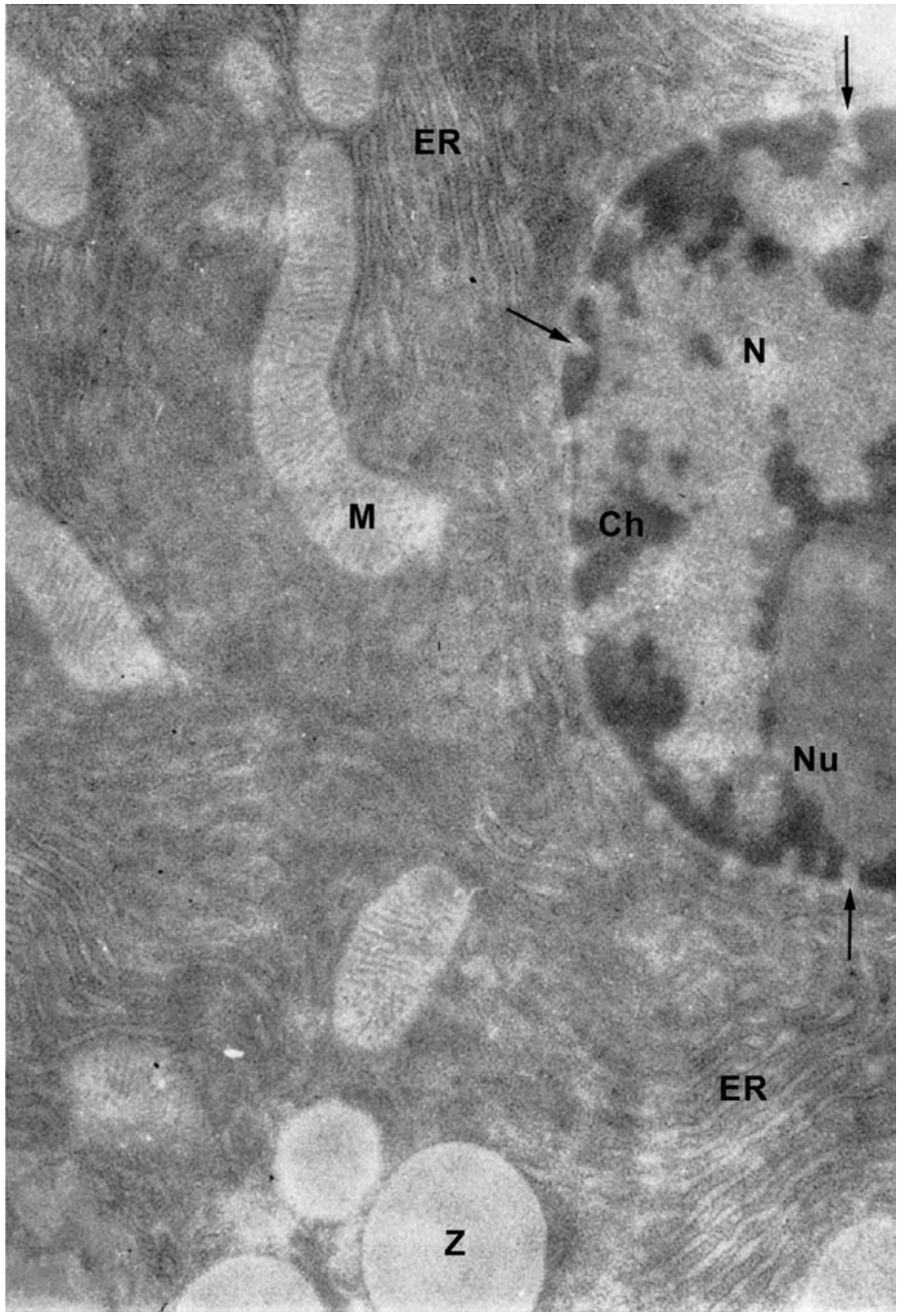

Figure 2

Partial view of a pancreatic acinar cell fixed in glutaraldehyde alone. The structure of the nucleus and of cytoplasmic elements, such as mitochondria and rough endoplasmic reticulum and zymogen granules, is well preserved. Membranes, however, are not visible unless the sample is post-fixed in OsO_4. From D.D. Sabatini, K. Bensch, R. Barnett. Reproduced from *J. Cell Biol.* 1963, 17:19–59 by copyright permission of the Rockefeller Univ. Press.

glutaraldehyde by itself provided little electron contrast (**Figure 2**), a drawback easily remedied by the introduction of a post-fixation step with OsO_4 that could be carried out after the histochemical reaction had been completed and be followed by staining with the usual contrast-enhancing uranyl or lead-containing reagents. Post-fixation in OsO_4 not only revealed membranes and stained other lipid-containing structures that were

seen as negative images after glutaraldehyde fixation alone, it also reacted with some of the organic deposits generated during the histochemical reactions, making their products more visible under the electron beam. The advantages of the double-fixation procedure I developed to preserve fine structural details were well illustrated in a second paper (Sabatini et al. 1964) in which I included primarily the work I did at Rockefeller in collaboration with Fritz Miller, then a visiting scientist in Palade's laboratory who, on his return to Europe, assumed the Chair of Histology in Munich.

I was later particularly gratified that one of the main bonuses of glutaraldehyde was its preservation of cytoplasmic microtubules, which, as components of the cytoskeleton, Keith Porter first described in detail in doubly fixed specimens (Ledbetter & Porter 1963, Porter 1965). I believe that the warm and amiable life-long relationship I had with Keith owed much to his appreciation of the very indirect contribution I made to one of his many important discoveries.

In 1961, just before I moved to Rockefeller, I presented our results with glutaraldehyde fixation at a meeting of the New York Society for Electron Microscopy, which was held at an NYU town house in Washington Square. The other presenter that night was Alex Novikoff, a leading cytochemist at the Albert Einstein College of Medicine, and a fiery speaker and equally challenging critic. I had great respect for Novikoff, who was already an impressive figure in the emerging field of cell biology. While at the University of Vermont (from which he was dismissed during the McCarthy period and from which he received an honorary degree not too long before his death), he had been a pioneer in cell fractionation, one of the first to use some of the same marker enzymes we now employ to define subcellular compartments. While visiting Christian De Duve's laboratory in Belgium, he had obtained the first electron micrographs of subcellular fractions containing lysosomes (Novikoff 1956). These organelles were at first pure mental constructs of De Duve's brilliant analytical mind, which had deduced their existence from biochemical experiments demonstrating the compartmentalization and latency of acid hydrolases. De Duve and his collaborators later further purified the subcellular particles that contained the enzymes. Novikoff, who examined them at the EM, concluded that they corresponded to a type of electron-dense body that he and others had recognized in many cell types and, in hepatocytes, were abundant near bile canaliculi (Beaufay et al. 1956, Novikoff 1956). After my presentation at Washington Square, Novikoff engaged me and Barrnett in a heated discussion on whether the small blocks of tissue that we had used for some of the histochemical reactions were more prone to generate artifacts, owing to diffusion of the reaction product within the block, than the thin frozen sections that he preferred. Amusingly, after returning to New Haven, Barrnett wrote to Novikoff to admonish him for mercilessly challenging in public a novice like me who had an imperfect command of English. But Novikoff responded by praising my vigorous defense and insisted that I should not be pitied. This first encounter inured me to Alex's sometimes intimidating attacks and marked the beginning of a life-long friendly relationship with him.

Not long after I arrived in New Haven, I met Palade for the first time, when he came to present a lecture on his work on capillary permeability, and I was fortunate to be invited to the dinner honoring him that night, where I also met Jon Singer, then a Yale faculty member. Thus, in a single day, as a beginning postdoctoral fellow, I had the chance to meet and talk with two pioneers in cell biology whose discoveries were to shape our field, and I was deeply impressed by both. Singer was then laying the basis for his development of immunoelectron microscopy and was introducing a procedure to link ferritin molecules to antibodies, which, thus marked, could be used to detect specific proteins within the cell (Singer 1959). He was later to

develop the fluid mosaic model for membrane structure (Singer & Nicolson 1972) that has almost completely passed the test of time and inspired much of our later thoughts on membrane biogenesis. In collaboration with Tokuyasu (Painter et al. 1973, Tokuyasu & Singer 1976), Singer also advanced the technique of cryo-ultramicrotomy, which made immunoelectron microscopy the key tool for molecular cell biology studies that it is today.[3]

GETTING STARTED AT ROCKEFELLER

At the Rockefeller I was assigned a desk and a bench in Siekevitz's laboratory in the fifth floor of the South Lab, since renamed the Bronk Lab. This was a wonderful place to do science. Siekevitz's enthusiasm was infectious, and he set a marvelous example with his own intense involvement in experiments, readiness to discuss novel ideas, and willingness to give his junior associates considerable freedom in any direction they took. In the same room, Yutaka Tashiro, a Rockefeller Foundation fellow from Japan, who has since made many contributions to cell biology and became president of the Kansai University Medical College in Japan, was already working. David Luck, an M.D., who was to make seminal discoveries on mitochondrial biogenesis and to become a professor at Rockefeller, was finishing a Ph.D. thesis that he had begun with Keith Porter on the association of glycogen particles, so abundant in hepatocytes, with glycogen synthetase, the enzyme that carries out their synthesis. Len Sauer, another M.D. working toward his Ph.D. with Siekevitz, was studying the regulation of electron transport and oxidative phosphorylation in isolated mitochondria. In other laboratories on the same floor Lucien Caro was perfecting and applying autoradiography to tissues of animals injected with radioactive amino acids, attempting to trace the intracellular pathway followed by newly synthesized secretory proteins after their segregation in the ER. James Jamieson, a student from Canada with an M.D. degree, was attempting to isolate the secretory granules of heart muscle that we now know contain the atrial natriuretic factor. He was later to develop with Palade the system of pancreatic tissue slices, which in pulse-chase labeling experiments, employing autoradiography at the EM level and cell fractionation, allowed the elucidation of the steps involved in the transfer of newly synthesized proteins from the ER to zymogen granules (see Palade 1975). Faculty members in the cell biology group directed by Palade included Walter Stoeckenius, Sam Dales, and Marilyn Farquhar, who held a visiting appointment at that time. Stoeckenius was elucidating the arrangement of lipid and protein molecules within the bilayer of natural and synthetic membranes at the highest level of resolution attainable with the electron microscope. Sam Dales was using EM to study virus-cell interactions, as well as viral replication and assembly in infected tissue culture cells, which represented an avant garde approach at that time. Marilyn Farquhar, who as a postdoctoral fellow with Palade had studied the glomerular capillaries of the nephron, was embarking on the landmark work (Farquhar & Palade 1963) that revealed the organization and structural details of the components of the junctional complexes that hold together epithelial cells and allow intercellular communication between them. Marilyn and I became good friends and, because of our shared interest in fine structure and histochemistry, we met frequently for chats during lunch, and I was privileged to be one of the first to see some of her new and exciting findings.

[3] Upon my arrival at the Rockefeller Institute in the summer of 1961, I used Singer's procedure to successfully attach ferritin molecules to the outer surface of isolated liver mitochondria. After disrupting the surface-labeled mitochondria by sonication, I was able to separate the denser ferritin-bearing outer membrane fragments from those derived from the inner membrane. Unfortunately, I failed to preserve and detect biochemically any of the activities that we now know characterize the outer mitochondrial membrane, and dropped the project.

Although I came to the laboratory as a postdoctoral fellow, after one year I was seduced by the opportunity to join the Ph.D. program at Rockefeller, following the example of other young physicians, who had already done or were doing so, and later developed stellar careers, such as H. Rasmussen, Gerald Edelman, Chuck Stevens, Ed Reich, David Luck, James Jamieson, Scott Grundy, and others. Palade supported my application and, after an interview with Detlev Bronk, the Rockefeller University President, I was admitted into the program with Palade as my mentor. This change of status gave me the opportunity of repairing some of my educational deficiencies by arranging for some excellent tutorials in math (with E. Kogbetliantz), chemistry (with T.P. King and W. Agosta), and physical chemistry (with D. Yphantis). It also provided for much coveted free xeroxing privileges but did not change the direction of my research. What it did, however, was to greatly enrich my intellectual and personal life, as I entered into daily contact with the exceptional group of brilliant aspiring young scientists who were my classmates, including David Baltimore, Robert Barlow, Tony Cerami, Bert Hille, David Hirsch, Bob Klug, Harvey Lodish, Dan Rifkin, and others.

It is impossible for me to transmit in a few pages the extraordinarily stimulating environment that prevailed at the Rockefeller when I arrived, or to dwell on the scientific achievements that in 1974 brought George Palade the Nobel Prize, together with his former mentor, Albert Claude—a pioneer in biological EM and in the development of cell fractionation procedures—and his Belgian colleague at Rockefeller, Christian De Duve—the discoverer of lysosomes and peroxisomes. I also regret not being able to dwell, as I did in a published profile (Sabatini 1999), on Palade's admirable personal attributes that make him one of the most admired and beloved figures of today's scientific scene. Palade had an awesome capacity to assimilate and elaborate new ideas, and every discussion I had with him increased my enthusiasm for the work we were doing, helping to reinforce my conviction that nowhere else could I find such a fertile environment to make innovative discoveries about membrane and organelle biogenesis. My later career as a research mentor also benefited greatly from the example Palade set by always being available and eager to communicate with his younger associates and being able to maintain a calm, patient, and reassuring attitude, even when one's optimistic expectations were being dashed by the dismal reality of experimental results.

ENTERING THE ROUGH ENDOPLASMIC RETICULUM

In Siekevitz's laboratory I became a close friend and admirer of Tashiro. He was a very experienced biochemist from whom I had much to learn. He and I shared a profound interest in ribosomes, which he had begun to study in Japan using the analytical ultracentrifuge. Tashiro was interested in the molecular architecture of the ribosome and was using the analytical centrifuge in the laboratory of David Yphantis, a major contributor to technical improvements of that instrument, to characterize rat liver ribosomes. He introduced me to the intricacies of the technique of analytical centrifugation, as well as to the use of the sucrose gradient centrifugation methods. These had been recently applied to the study of ribosomes by Richard Roberts at the Carnegie Institution, and with them we could separate and obtain preparative amounts of ribosome monomers and subunits. We frequently visited the laboratory of Mary Peterman at the Sloan Kettering Institute, who was also studying the physical and chemical properties of eukaryotic ribosomes, and we followed closely the work of J.D. Watson and his colleagues at Harvard, who had shown earlier that *E. coli* ribosomes were composed of two unequal subunits (Tissieres et al. 1959) and that the larger one contained the nascent polypeptide chain (Gilbert 1963).

The workers at Harvard had taken advantage of the fact that *E. coli* ribosomes spontaneously dissociate into functional subunits when Mg^{2+} ions are removed by dialysis. Tashiro, on the other hand, had to use the Mg^{2+} chelating agent ethylenediaminetetraacetic acid (EDTA) to induce the dissociation of rat liver monomeric ribosomes into the subunits (Tashiro & Siekevitz 1965, Tashiro & Yphantis 1965). Unfortunately, this treatment also caused the partial unfolding and irreversible inactivation of the subunits. Nevertheless, Tashiro was able to demonstrate that in rat liver ribosomes nascent polypeptide chains are also associated with the large subunits (Tashiro & Siekevitz 1965).

I was impressed by the asymmetry and obvious specialization of the two sub-ribosomal domains and asked myself whether the association of the ribosomes with the ER membrane that characterizes the rough portions of the ER involves one or both ribosomal subunits, and how this might relate to the function of the ribosomes. I wondered if EDTA treatment of rough microsomes would also dissociate the bound ribosomes into subunits and whether, in that case, one or the other subunit would remain bound to the membrane.

The first experiments I did worked like a charm (Sabatini et al. 1966). At low concentrations, EDTA preferentially released small ribosomal subunits, whereas most large subunits remained associated with the membrane. When microsomes containing radioactive nascent chains labeled after very short pulses in vivo were treated with EDTA, I found that the ribosomal subunits recovered after detergent dissolution of the membranes contained a higher level of radioactive nascent chains than those subunits removed by EDTA. I had also begun an EM study of free ribosomes and of rough microsomes using the technique of negative staining. It was clear that ribosomal particles contained a cleft that divided them into two unequal regions and that, in general, in membrane-bound ribosomes the cleft lay parallel to the microsomal membrane to which the larger region was adjacent (**Figure 3**). Of course, these observations immediately suggested that the nascent chain in the large subunit might play a role in anchoring the ribosome to the membrane. At that time, however, we cautiously stated, "It seems plausible to consider the possibility that strong attachment of 'active ribosomes' is related to passage of the protein through the membrane. However, our work does not indicate which of the following possibilities holds. (*a*) The ribosomes which are strongly attached are, because of this situation, more active in protein synthesis and, therefore, become labeled in vivo. (*b*) The presence of the product of protein synthesis on the ribosomes is what makes them stick to the membrane" (Sabatini et al. 1966).

The initial evidence for a role of membrane-bound ribosomes in the synthesis of secretory proteins had been provided by Siekevitz & Palade in 1960. They reported that very soon after the injection of a radioactive amino acid into an animal, the purified pancreatic secretory enzyme chymotrypsinogen, having the highest specific radioactivity, was found in association with the ribosomes, which could be isolated by sedimentation from rough microsomes treated with detergent to dissolve the membranes. At later times after the injection, however, chymotrypsinogen was found in the detergent-soluble microsomal subfraction, which included the content of the microsomal lumen. Curiously, the route by which the chymotrypsinogen molecules released from the membrane-bound ribosomes found their way to the lumen of the cisterna was not considered, perhaps because it was regarded as a problem to be dealt with later. Indeed, a scheme drawn in 1961 (Palade et al. 1961) showed the site of passage of the protein through the membrane at some distance from the ribosomal membrane junction, as if it could be effected by an independent transport mechanism that operated on completed polypeptides released from the ribosome.

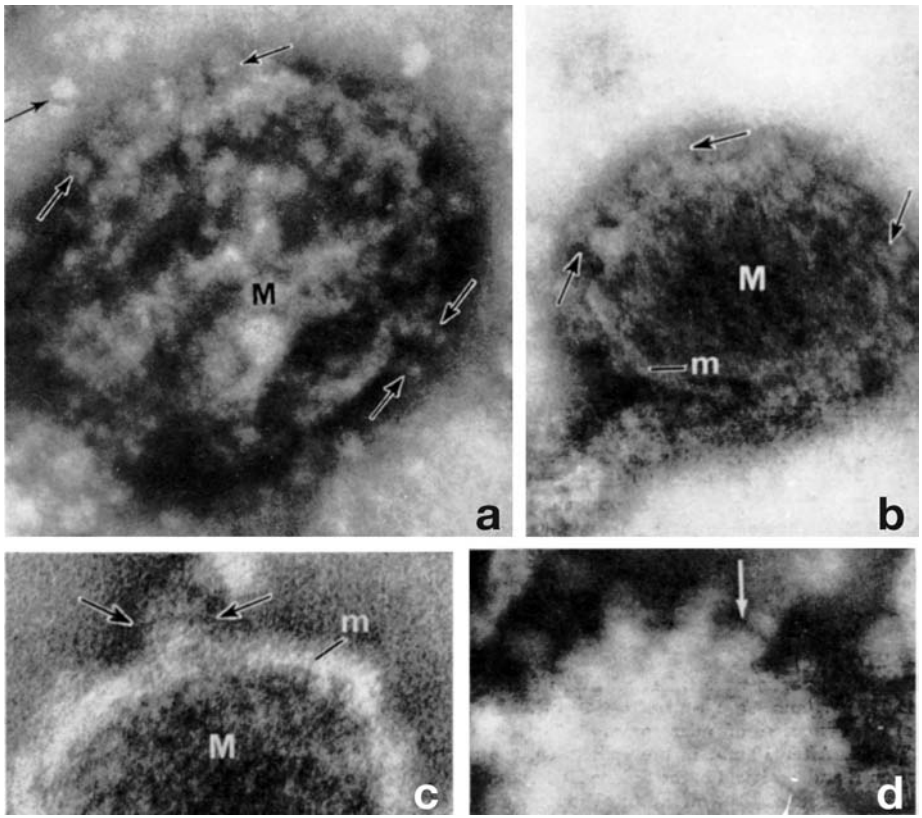

Figure 3

Ribosomes in rough microsomes are bound to the membrane through the large ribosomal subunit. Electron micrographs of rat liver rough microsomes examined after negative staining. Many ribosomes show a groove, penetrated by the stain (*arrows*), parallel to the membrane surface. The large ribosomal subunit, which contains the nascent chain, lies close to the membrane. M: microsome; m: microsomal membrane. Reprinted from *J. Mol. Biol.* 19, D.D. Sabatini, Y. Tashiro, G.E. Palade. On the attachment of ribosomes to microsomal membranes, pp. 503–24. Copyright 1966 with permission from Elsevier.

LAYING THE BASIS FOR THE SIGNAL HYPOTHESIS

It was left to Colvin Redman, who joined the Siekevitz and Palade laboratory in 1964, after obtaining his Ph.D. in Canada with Lowell Hokins—the scientist who first discovered the involvement of phosphoinositides in controlling secretion—to demonstrate that completed polypeptides, in this case amylase, which was synthesized in vitro by pigeon pancreas microsomes, were not released into the incubation medium but remained associated with the microsomes, most likely sequestered in their luminal cavities (Redman et al. 1966). These experiments, however, could not exclude that polypeptides discharged from the ribosomes into the medium were rapidly taken up into the microsomes by an uptake and transporting mechanism. Therefore, I, in collaboration with Redman, decided to examine the fate of in vitro-labeled incomplete polypeptides that were synthesized in a system of liver microsomes and were released from the ribosomes by the action of puromycin. This aminoglycoside antibiotic substitutes for aminoacyl tRNA and its incorporation at the C-terminal end of a nascent polypeptide causes premature termination of

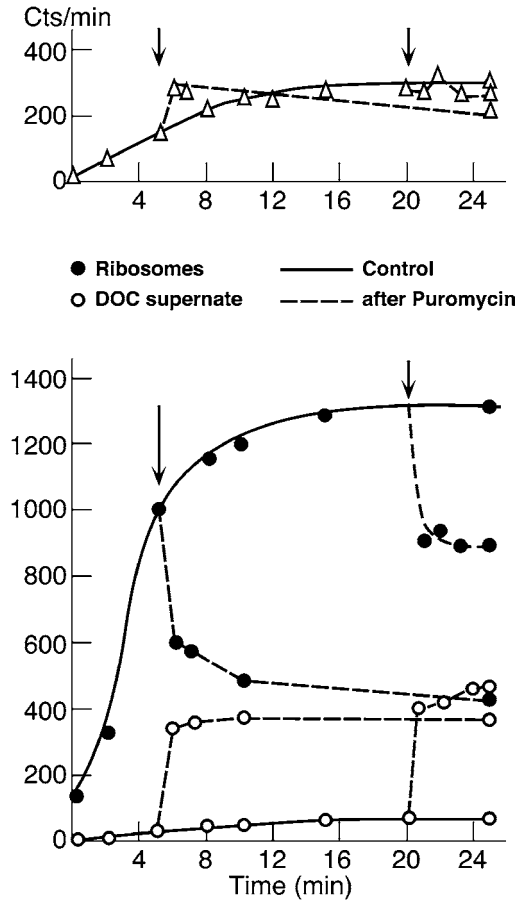

Figure 4

Nascent polypeptides released from membrane-bound ribosomes after incubation with puromycin in vitro are vectorially discharged into the microsomal lumen. Microsomes were incubated for protein synthesis with ^{14}C-leucine. Puromycin was added to the system at the times indicated by the arrows. At various times thereafter the microsomes were separated from the incubation medium by sedimentation and resuspended. The microsome suspension was treated with the detergent sodium deoxycholate (DOC) to solubilize the membranes and release the content of the microsomal lumen. A subsequent centrifugation separated the ribosomes from the final supernatant. The top panel shows that a small fraction of the radioactivity is recovered in the incubation medium obtained after sedimenting the microsomes and that that fraction does not increase after puromycin treatment. In the bottom panel, the closed circles represent the radioactivity associated with the ribosomes, which decreases rapidly (*dashed lines*) when the nascent chains are released by puromycin. The open circles correspond to the radioactivity present in the detergent-soluble fraction (DOC supernatant after ribosome removal). This fraction, which increases rapidly after puromycin treatment, represents nascent chains translocated into the lumen or inserted into the membrane. Drawing from C.M. Redman, D.D. Sabatini, 1966. Vectorial discharge of peptides released by puromycin from attached ribosomes. *Proc. Natl. Acad. Sci. USA* 56:608–615.

polypeptide synthesis, purging the ribosomes of nascent chains, which are released in the form of peptidyl-puromycin molecules. We found that the peptidyl-puromycin molecules lost from the ribosomes were not released into the surrounding medium, but remained associated with the microsomes, from which they could be recovered in soluble form after dissolution of the membranes by detergent and sedimentation of the ribosomes (Redman & Sabatini 1966) (**Figure 4**).

These observations, together with the finding that a significant fraction of the peptidyl puromycin molecules could also be released from the microsomes by a mild sonication procedure, suggested that such molecules had become sequestered within the microsomal lumen. This was confirmed by the finding that they could also be released using a low concentration of detergent that permeabilized the membranes without causing their extensive disassembly. This method to dissect the microsomes, which worked much better with liver microsomes than with the pigeon pancreas microsomes used by Redman, had been introduced by Lars Ernster (Ernster et al. 1962), a distinguished Swedish biochemist who had spent a sabbatical year in Siekevitz's laboratory.

We suggested that nascent chains synthesized in bound ribosomes become engaged with a translocation machinery in the membrane before their synthesis is completed and, therefore, the site of translocation must be in close proximity to the ribosome, most likely at the ribosome-membrane junction (Redman & Sabatini 1966). Hence, transport across the membrane appeared to be effected by a nondiscriminating mechanism that does not distinguish between complete and artificially terminated incomplete chains, when either one is released from the ribosome. I had become convinced that translocation was a cotranslational phenomenon and that it resulted from the fact that in a membrane-bound ribosome growth of the nascent chain occurred in an environment that precludes its release into the surrounding medium, which in the cell

is represented by the cytosol. We described a model, illustrated in a later paper (Sabatini & Blobel 1970) that explained the vectorial, unidirectional discharge of the polypeptide into the lumen of the ER as a result of the polypeptide growing in a tunnel or central cavity within the large ribosomal subunit (**Figure 5**). We suggested that because the ribosome sits on the membrane, this tunnel is made continuous with the ER cisternal space through a permanent or intermittent discontinuity in the microsomal membrane. We wrote, "As visualized at present the transfer mechanism relies primarily on release from the large subunit and on structural restrictions at the ribosome membrane junction and, hence, is non discriminatory and possibly passive" (Redman & Sabatini 1966). In a note to this paper we indicated, "Unpublished electron microscopic observations by D. Sabatini, Y. Tashiro, and G.E. Palade are the basis of this model. The existence of a channel is suggested by electron microscopy of negatively stained large subunits. Discontinuities in the membrane under the large subunits can be detected in some instances in sectioned specimens." Electron micrographs of isolated large ribosome subunits in which a central depression or cavity was apparent because it accumulated negative stain (as expected from the entrance to a tunnel in the subunit) were published in 1971 (Sabatini et al. 1971). The passive character of the transport of the peptidyl puromycin molecule into the microsomal lumen was supported by a paper in which Redman showed that ATP was not required for this process (Redman 1967).

The two papers representing my work on membrane-bound ribosomes became the basis for my doctoral thesis at Rockefeller, where I received a Ph.D. in 1966. I felt that my work had opened an important avenue of cell biology research, which I very much wanted to follow, hoping to provide a molecular description of the relationship between the ribosome and the translocation apparatus that I presumed existed in the membrane. Of course, very little was known about the basic organization of cellular membranes

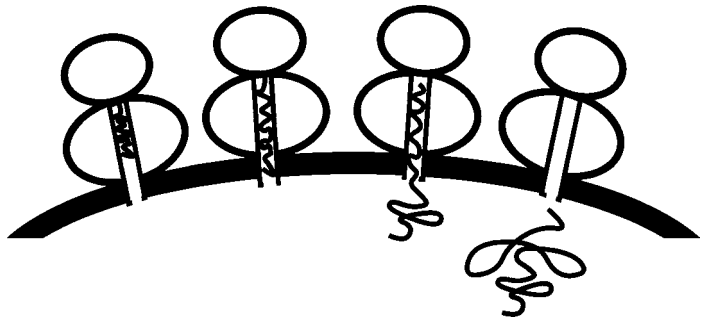

Figure 5

A model depicting the basic structural features of the ribosome-membrane junction. The relationship of nascent polypeptides and ribosomes, with the membranes of the endoplasmic reticulum, account for the protection of the polypeptides from the attack of added proteases. The structural arrangement proposed is also compatible with the known features of the process of transfer of secretory polypeptides into the cisternal cavity. Drawing from D.D. Sabatini, G. Blobel. Reproduced from *J. Cell Biol.* 1970, 45:146–57, by copyright permission of the Rockefeller Univ. Press.

at that time, but it seemed most likely that the passageway or pore in the membrane through which the nascent polypeptide was transported was of proteinaceous nature or was constructed of protein subunits, although given our meager knowledge of membrane structure, I was hesitant to present such a speculation. Obviously, this was a topic of great interest to Palade, and I was, therefore, delighted when he offered me an Assistant Professorship and a laboratory being vacated by Sam Dales, who was leaving Rockefeller to take a position at the Public Health Institute of New York.

In my new laboratory I began to examine more directly the notion that nascent polypeptides indeed grow within a ribosomal tunnel and, in the case of those synthesized in the ER, upon emerging from the ribosome enter in a relationship with the underlying membrane that precludes their release into the medium or its cellular equivalent, the cytosol, as proposed in the model I had generated. I was successful with my first grant application and had started to determine to what extent nascent polypeptides in free ribosomes and in microsomes, which I could label in vivo or in vitro, were protected from the attack of exogenous proteolytic enzymes, when

a new postdoctoral fellow, Günter Blobel, an M.D. from Germany who had recently completed his Ph.D. in Wisconsin with Van Potter (Siekevitz's old mentor), joined the Palade and Siekevitz laboratory.

GÜNTER AND I AND THE FORMULATION OF THE HYPOTHESIS

During his Wisconsin sojourn, Günter had worked on rough microsomes and was, therefore, already interested in the subject of my research. He was a frequent visitor to my laboratory and we soon began to hold many animated discussions on this somewhat esoteric subject. I was pleased to find that he had read my two papers on membrane-bound ribosomes carefully and that he was anxious to collaborate with me in experiments where I had begun to examine the accessibility to exogenous proteases of nascent polypeptide chains in free and membrane ribosomes. Together (Blobel & Sabatini 1970) we found that nascent polypeptides labeled in vitro in free ribosomes were easily and completely digested when the ribosomes were incubated with a mixture of trypsin and chymotrypsin at 37°C. But when the incubation was carried out at 0°C, most of the label incorporated in a brief pulse was protected from proteolysis and appeared in protease-resistant fragments of approximately 39 amino acids in length that remained associated with the partially proteolyzed ribosomes. Pulse-chase and continuous labeling experiments indicated that the protected segment contained the growing end of the nascent chain, which Dintzis had demonstrated several years before was at its C terminus (Dintzis 1961). Everyone recognized that this end of the nascent chain should be intimately associated with the ribosome because it serves as the substrate to which the peptidyltransferase, an integral component of the ribosome, adds amino acids to the growing chain. Similar results on a ribosome-protected fragment of the growing polypeptide had been obtained in Alex Rich's laboratory at M.I.T. (Malkin & Rich 1967) with polyribosomes from rabbit reticulocytes, which produce almost exclusively globin chains that could be labeled in vivo. All these findings, together with the hydrodynamic properties of large subunits examined by analytical centrifugation (Petermann & Pavlovec 1969), provided strong support for the notion that a cavity or tunnel within the large ribosomal subunit contains the nascent polypeptide, as I had proposed for membrane-bound ribosomes to explain the vectorial discharge of the incomplete polypeptides released by puromycin (Redman & Sabatini 1966).

We suggested that the nascent polypeptide chain in the membrane-bound ribosomes would remain protease inaccessible as it grew beyond the 39-amino acid length sequestered within the ribosome provided that the exogenous protease did not break the membrane barrier (Redman & Sabatini 1970, Blobel & Sabatini 1970).

Omura, Sato, and their collaborators (Ito & Sato 1969, Omura et al. 1967) had already studied the effect of proteolytic enzymes on microsomes, showing that they were capable of digesting or dissecting out from the cytoplasmic face of the membranes several sets of proteins—later shown to be anchored in the membrane by C- or N-terminal segments. Their work had also shown that microsomal vesicles incubated with proteolytic enzymes at 0°C remain largely intact and apparently impermeable to the proteases.

Günter and I found that during proteolytic digestion of microsomes at 0°C, the bound ribosomes were released from the membranes (Sabatini & Blobel 1970). Concomitantly, the individual nascent polypeptides that they contained underwent cleavage, generating two sets of segments that remained largely protected from proteolysis. One set of segments remained associated with the detached ribosomes, which, although bearing partially proteolyzed ribosomal proteins, still sedimented as intact particles. These segments were of the same length as those protected in free ribosomes and corresponded to the C-terminal

portions of the polypeptides. The other set of segments generated by proteolysis corresponded to the N-terminal portions of the growing polypeptides, and these were of variable length, mostly larger than the ribosome-protected ones. The N-terminal segments remained associated with the membranes and were also inaccessible to the proteases as long as the membranes remained intact.

This work was originally submitted to the *Journal of Cell Biology* as a single paper, but the editor found it too long and asked that we split it into two, which we did. One paper dealt specifically with free ribosomes (Blobel & Sabatini 1970); the second (Sabatini & Blobel 1970) presented evidence for the protection of the N-terminal portions of the nascent polypeptides by the microsomal membrane. In this paper, the model proposing the existence of a tunnel within the ribosome that is continuous with a passageway for the nascent polypeptide through the membrane was graphically illustrated (**Figure 5**).

Günter and I soon learned that by using puromycin in a medium of relatively high ionic strength we could dissociate ribosomes within polysomes into subunits that, contrary to those obtained by chelation of Mg^{2+} with EDTA, remained properly folded and were capable of protein synthesis when reprogrammed with mRNA (Blobel & Sabatini 1971). This allowed Yoshiaki Nonomura, who came to my laboratory as a postdoctoral fellow from Ebashi's group in Tokyo, to undertake an EM study by negative staining of the structure of individual active ribosomal subunits, of the monomers formed by their association, and of the relationship of the subunits to mRNA within polysomes (Nonomura et al. 1971). This work was followed by a study by Takashi Morimoto (a former student of Tashiro who had joined my laboratory at Rockefeller and whom I later recruited to NYU where he became a valued member of the cell biology department) (Morimoto et al. 1972a,b) of the mechanism by which ribosomes assemble into the tetramers that Breck Byers, then a student of Keith Porter at Harvard, had shown form crystalline arrays in chicken embryos upon slow cooling (Byers 1967).

Soon after, we discovered the utility of puromycin to generate functionally capable ribosomal subunits. Mark Adelman—who had completed his Ph.D. in Chicago working with Ed Taylor in one of the first demonstrations of the presence of actin and myosin in non muscle cells—joined my laboratory as a postdoctoral fellow to study the role of the nascent chain in the association of the ribosome with the membrane. He developed a new procedure to obtain large amounts of highly purified rough microsomes from rat liver (Adelman et al. 1973a), which he used to study the role of the nascent chain in maintaining the association of ribosomes with the membrane. He showed that, even after the microsomes were treated with puromycin to release the nascent chains, the ribosomes remained associated with binding sites on the microsomal membrane. However, they now could be effectively detached from the membranes simply by raising the ionic strength, which was not possible without previous puromycin treatment (Adelman et al. 1973b, Sabatini et al. 1971). We concluded, therefore, that at least two molecular interactions are responsible for maintaining the ribosome-membrane junction: a direct one between the large subunit and a putative receptor in the membrane, which is disrupted by high salt treatment, and a second one that is provided by the nascent chain linking the polypeptide exit site in the large ribosomal subunit to the passageway in the underlying microsomal membrane, which leads to the microsomal lumen (**Figure 6**).

By 1971 it seemed clear to us that free and membrane-bound ribosomes were structurally identical and functionally interchangeable, something for which Nica Borgese, then a student in my laboratory, was accumulating evidence in subunit exchange experiments (Borgese et al. 1973). The only difference between free and membrane-bound ribosomes seemed to be that they were translating different classes of mRNAs, with the bound polysomes from secretory glands synthesizing

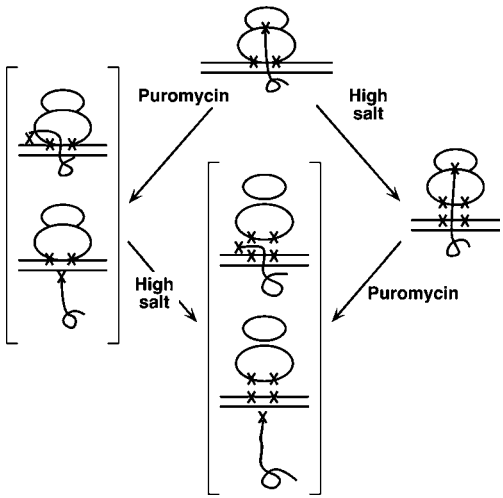

Figure 6

Interactions that maintain the association of active ribosomes with the ER membrane. Membrane-bound ribosomes are held on the membrane by two types of bonds: (*a*) ionic interactions between sites in the large ribosomal subunit and complementary sites in the membrane and (*b*) a link provided by the nascent polypeptide chain. The latter can be disrupted by puromycin, which releases the nascent chain from the ribosome. The ionic interactions are broken in media of high salt concentration. A combination of both treatments is necessary to release all ribosomes from the membrane. Drawing from D.D. Sabatini, N. Borgese, M. Adelman, G. Kreibich, G. Blobel. 1972. Studies on the membrane-associated protein synthesis apparatus of eukaryotic cells. RNA viruses/ribosomes. North Holland, Amsterdam. *FEBS Symp.* 27:147–71.

mainly secretory proteins. We had obtained a plethora of evidence implicating the N-terminal portion of the nascent polypeptide in establishing and maintaining the association of a bound ribosome with the membrane. Most salient were (*a*) the vectorial discharge resulting from puromycin treatment (Redman & Sabatini 1966), (*b*) the role of the membrane in protecting the N-terminal portion of the polypeptide from the proteolytic attack by exogenous enzymes (Sabatini & Blobel 1970), and (*c*) the fact that ribosomes containing nascent chains could not be detached by simply raising the ionic strength, which effectively dissociates from the membrane those ribosomes lacking nascent polypeptides (Adelman et al. 1973a, Sabatini et al. 1971, Sabatini 1972).

The remaining burning question concerned the features in the N-terminal region of a nascent secretory polypeptide that were responsible for the association of the ribosome-nascent chain complex with the membrane. In 1971, it was already established that, as was known for prokaryotes, methionine was the initiating amino acid for eukaryotic proteins. It then seemed reasonable to assume that because no known secretory protein retained an N-terminal methionine, the membrane-binding information that we postulated existed in the N-terminal sequence was removed during or after insertion of the polypeptide into the membrane. We considered the possibility that N-terminal amino acids were modified by acylation with fatty acids or by the addition of cholesterol or other hydrophobic moieties. There are now examples of such modifications in proteins, but none was known at that time other than the formylation of the initiator methionine, which occurs only in prokaryotes. In fact, we suspected that the feature in the nascent polypeptide that served to trigger the association of the ribosome with the membrane could be a stretch of hydrophobic amino acids, because we found that polyphenylalamine synthesized in vitro by microsomes programmed with poly U, upon termination with puromycin, remained membrane associated (Sabatini et al. 1971).

After many hours of enthralling argumentation in front of my office blackboard, Günter and I arrived at the first formulation of what was later to be known as the signal hypothesis (Blobel & Sabatini 1971) (**Figure 7**). We proposed that "all mRNAs to be translated on bound ribosomes have a common feature, such as several codons near their 5′ end, not present in mRNAs which are to be translated on free ribosomes. The resulting common sequence of amino acids near the N-terminal of the nascent chains or a modification of it (indicated by X) would then be recognized by a factor mediating the binding to the membrane. This binding factor could be a soluble protein, which recognizes both a site on the large ribosomal subunit and a site on the membrane. After release of the chain from

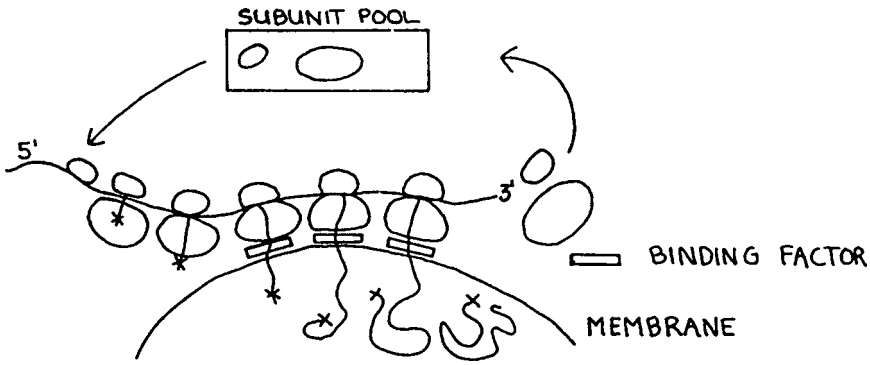

Figure 7

The first formulation of what came to be called the signal hypothesis. The crosses represent signal peptides at the N termini of the nascent chains, which were proposed to be recognized by a factor that initiates binding of the ribosome-nascent chain complex to the membrane and, therefore, has properties of SRP. In *Biomembranes*, Vol. 2, 1971, pp. 193–95. Ribosome-membrane interactions in eukaryotic cells. G. Blobel, D.D. Sabatini, with kind permission of Springer Science and Business Media.

the ribosome into the intracisternal space, the ribosomal subunits and perhaps the binding factor dissociate from the membrane and enter their respective soluble pools. The pool of ribosomal subunits would also be fed from subunits generated from free polysomes after chain completion and release and a new cycle would be started." This was a rather prophetic statement that predicted not only the presence of a signal peptide sequence at the N terminus of the nascent chain, but also the existence of a signal-binding factor with the behavior later demonstrated by Blobel and his associates and former disciples (Walter & Blobel 1981, Walter et al. 1981) for the signal recognition particle [SRP and its cognate receptor in the membrane (Gilmore et al. 1982a,b; Meyer et al. 1982)].

THE FULL FLOWERING OF THE INITIAL MODEL: MOLECULES AND MECHANISMS

Before leaving Rockefeller in 1972, my laboratory had nearly completed other studies that established the presence in ER membranes of a finite number of specific ribosome receptors to which ribosomes, even after being purged of their nascent chains, are capable of binding (Borgese et al. 1974). We deduced that such binding sites contained an essential protein component because the ribosome-binding capacity of ER membranes was sensitive to heat treatment and was abolished by mild proteolytic digestion, and proposed that those sites were spatially close to those through which cotranslational translocation occurs.

With the development of cell-free systems for the translation of natural mRNAs, several laboratories observed that the primary translation products of mRNAs for secretory proteins produced in systems lacking microsomal membranes were somewhat larger than those produced in microsomes (Kemper et al. 1974, Mach et al. 1973, Milstein et al. 1972, Tonegawa & Baldi 1973). It was also shown that the extra segments were at the N termini of the primary translation products. But, of course, it was Günter's laboratory, employing a reconstituted in vitro protein synthesizing system containing microsomal membranes and ribosomal subunits programmed with immunoglobulin mRNA, that provided definitive proof for the signal hypothesis (Blobel & Dobberstein 1975a,b). They demonstrated the role of a cleavable signal sequence in initiating insertion of nascent immunoglobulin light chains into the membrane, characterized the signal sequence, and revealed the action of a membrane-associated signal peptidase in its

cotranslational removal. In these experiments, the protease protection assay that we had developed was used to demonstrate the sequestration in the microsomal lumen of completed immunoglobulin light chains whose signal sequences were removed cotranslationally.

Over the next decade, in now classical experiments, Günter's laboratory went on to illuminate nearly all aspects of the complex molecular interactions that lead to the insertion of specific polypeptides into ER membranes. Years later, his laboratory provided electrophysiological proof for the existence of a protein-conducting channel in the microsomal membrane that appears to be opened by the signal peptide and to be kept in that state by the bound ribosome (Simon & Blobel 1991). Soon thereafter, the existence of an aqueous channel was also detected using microsomes with in vitro-synthesized, fluorescently labeled nascent chains whose accessibility to selective quenching procedures was assessed under various conditions (Crowley et al. 1994).

Major steps toward the molecular identification of the components of the translocation complex were made in the late 1980s in the laboratories of Randy Schekman and Tom Rapoport, beginning with the discovery that a yeast mutant (*sec61*) was defective in an early stage in the translocation of secretory proteins into the ER (Deshaies & Schekman 1987). Subsequently, cross-linking experiments showed that the Sec61p polypeptide of yeast, as well as its mammalian orthologue, was in close association with translocating nascent chains (Gorlich et al. 1992, Musch et al. 1992, Sanders et al. 1992). Soon thereafter, the Sec61 complex, which was found to consist of three polypeptides, was shown to be essential to confer translocation activity to reconstituted proteoliposomes (Oliver et al. 1995).

In 1977 we had carried out freeze-fracture studies of rough microsomes (Ojakian et al. 1977) that gave us a first glimpse at the size and distribution of the components of the protein translocation channel and associated ribosome-binding sites. These revealed the presence of intramembranous particles (IMPs), approximately 10 nm in diameter, that were associated with the bound ribosomes because they were displaced in the plane of the membrane with the ribosomes when the latter were forced to undergo aggregation on the membrane surface.

Later cryoelectron microscopy studies employing image processing techniques, in which Günter collaborated with Joachim Frank (Beckmann et al. 1997), showed that the large ribosomal subunit, indeed, contains a tunnel that, when the ribosome is bound to Sec61p, is continuous with the transmembrane channel provided by this protein, as we had predicted decades earlier for bound ribosomes (Redman & Sabatini 1966, Sabatini & Blobel 1970, Sabatini et al. 1971).

A full elucidation of the structure of a eukaryotic translocation channel is yet to come. However, the structure of SecY, the archeabacteria equivalent of the main subunit (α subunit) of the eukaryotic Sec61 complex, has been solved through crystallography at a resolution of 3.2 Å (van den Berg et al. 2004). The deduced structure makes several tantalizing suggestions, including sites for signal sequence recognition, for the lateral exit into the bilayer of transmembrane segments of nascent membrane polypeptides, and for binding of ribosomes.

It is more than 30 years since Günter and I delineated the basic features of the signal hypothesis. We have now gained enough perspective to view that work—which began with a rather focused concern with the early stages of the secretory process—within a larger frame of reference, one that encompasses more general questions related to membrane and organelle biogenesis. From this we can derive considerable satisfaction as some of the concepts and ideas we put forward have had a wide impact in the field of protein traffic.

The years that followed the presentation and verification of the signal hypothesis revealed that the cotranslational mechanism

first envisaged for the passage of secretory polypeptides across the membrane also applies to resident luminal and integral membrane proteins of the entire endomembrane system, including the ER itself, the Golgi apparatus, the plasma membrane, endosomes, and lysosomes. It is also now clear that a wide variety of signal sequences exist in polypeptides that are inserted into ER membranes, which, however, are all decoded by the same translocation apparatus. Some such signal sequences are transient features of the polypeptide that serve only to initiate its insertion into the membrane, whereas others also serve as permanent transmembrane anchors responsible for specific transmembrane dispositions of the mature polypeptide. Moreover, studies, first on simple transmembrane proteins, and later on proteins that traverse the membrane multiple times, brought to light the signaling function of other largely hydrophobic, interior segments of translocating polypeptides that serve to arrest the translocation process and to determine the proper disposition of the polypeptide relative to the phospholipid bilayer in the membrane. Both Günter and I envisaged the complex topology of certain membrane proteins as resulting from the sequential action of insertion and halt transfer signals operating cotranslationally, before substantial evidence for this mechanism was obtained (Blobel 1980, Sabatini et al. 1982). Several subsequent papers from our group clarified important aspects of the nature of these signals (Finidori et al. 1987, Monier et al. 1988, Rizzolo et al. 1985).

The notion we introduced in 1971 of a signal peptide containing information decoded by soluble and membrane receptors that informs the cell of the polypeptide destination was a harbinger of the discovery of signals that determine the post-translational importation of polypeptides into other membranes and organelles, including chloroplasts, mitochondria, and peroxisomes. Moreover, the role of the signal peptide as the first sorting signal that initiates the journey of a variety of proteins toward diverse destinations, as distant from the site of their insertion into the ER as the surface of the cell or lysosomes, opened the way to the realization that other sorting signals and cognate receptors must exist that mediate the successive sorting events needed to steer each polypeptide along the one pathway that leads to its site of function.

In the light of current knowledge, our original model for cotranslational translocation, also illustrates, once more, how natural phenomena invariably are more complex than one initially imagines. As the translocation machinery whose existence we recognized was dissected into its molecular components, the model, rather stylish at the beginning, grew much more elaborate and ornate, with the inclusion of a host of additional proteins that either assist in the translocation or modify the nascent polypeptide during this process. The Sec61p protein that constitutes the channel consists of at least three polypeptide subunits (Gorlich & Rapoport 1993). Two other ER transmembrane polypeptides, ribophorins I and II, that we, along with Gert Kreibich, identified as putative ribosome receptors (Kreibich et al. 1978a,b) are closely associated with the translocation site (Yu et al. 1990). They are now known to constitute, with two other subunits, the oligosaccharyl transferase that transfers a glycan moiety to asparagine residues in nascent glycoproteins (Kelleher et al. 1992). Similarly, SRP—the complex factor that targets the ribosomes to the ER membrane—consists of an RNA molecule and six polypeptides (Walter & Blobel 1983), one of which is a GTP-binding protein (Bernstein et al. 1989) that is recognized by the SRP receptor in the membrane. The receptor itself consists of two subunits, both of which are also GTP-binding proteins (Miller et al. 1995).

Amazingly, a retrotranslocation machinery is now known to also function in the ER in cooperation with a cytosolic ATPase and to transfer misfolded polypeptides, already segregated in the lumen of the ER or incorporated into the membrane, back to the cytosol

for degradation by the proteasome (Lilley & Ploegh 2004, Ye et al. 2004). This machinery is an essential component of a quality control mechanism in the ER that employs luminal chaperones and an elegant transmembrane signaling system that controls levels of protein synthesis and the expression of genes whose products make this organelle a nearly perfect manufacturing plant. Remarkably, certain viruses have learned to evade the immune system by encoding proteins that promote the retrotranslocation and, hence, degradation of MHC class I molecules that otherwise would have presented viral antigens at the cell surface and thus called into action cytotoxic lymphocytes to eliminate the infected cell (Lilley & Ploegh 2004, Ye et al. 2004).

It is gratifying for me to think that our initial model provided a framework that facilitated further progress and that, although it has undergone considerable elaboration and fine details have been elucidated, its fundamental aspects are still in place.

A POST-TRANSLATIONAL TRANSFER TO NYU

In September 1972, a few months before Palade, Farquhar, Jamieson, and their groups left Rockefeller for Yale, my research group moved to the NYU School of Medicine, where I assumed the chairmanship of the Cell Biology Department—succeeding Howard Green, who had moved to M.I.T. I not only transferred my laboratory, but was able to appoint several new faculty members interested in protein traffic, including Milton Adesnik, who had finished postdoctoral training with Jim Darnell at Columbia, as well as Gert Kreibich and Takashi Morimoto, who had been postdoctoral fellows with me at Rockefeller. Jim Lake, who was working on ribosomal structure at Rockefeller, also decided to join us. With these young and generous people, I developed productive collaborations. With Gert Kreibich we continued for some time to characterize biochemically the membranes and content of microsomal vesicles, demonstrating that specific sets of proteins sequestered within the microsomal lumen, including serum and lysosomal proteins, are transient components of the ER (Kreibich et al. 1973, Kreibich & Sabatini 1974). We also showed that the numerous integral components of the ER membranes have characteristic transmembrane dispositions (Kreibich et al. 1974), with their mannose-rich oligosaccharide chains luminally disposed (Rodriguez Boulan et al. 1978). We also continued our research on ribosome structure (Ivanov & Sabatini 1981) and on the ribosome-membrane association (Kreibich et al. 1978a, 1982; Kruppa & Sabatini 1977; Lande et al. 1975; Lewis & Sabatini 1977; Marcantonio et al. 1982; Ojakian et al. 1977) as well as on the role of bound ribosomes in the synthesis of proteins other than secretory proteins, such as proteins of the ER itself (Bar-Nun et al. 1980, Chyn et al. 1979, Harnik-Ort et al. 1987, Monier et al. 1988, Okada et al. 1982, Rosenfeld et al. 1984), the plasma membrane (Colman et al. 1982, Finidori et al. 1987, Mentaberry et al. 1986, Sabban et al. 1981, Sherman & Sabatini 1983), or lysosomes (Croze et al. 1989, Nishimura et al. 1986, Rosenfeld et al. 1982). In that period we also saw the virtue of utilizing cultured cells infected with enveloped viruses, such as Sindbis, influenza or vesicular stomatitis virus (VSV), to investigate the post-ER sorting of plasma membrane proteins, for which other laboratories (Katz et al. 1977, Wirth et al. 1977) had shown viral glycoproteins serve as facile models.

MDCK CELLS, THE POLARIZED BUDDING OF VIRUSES, AND THE SORTING OF ORGANELLAR PROTEINS

Several years after I came to NYU, I benefited once more from the windfall of emigres that usually follows the repressive policies of military regimes. The distinguished physiologist Marcelino Cereijido, who was Dean of the School of Biochemistry in Buenos

Aires, and is now in Mexico, had to leave Argentina, and I invited him to become a visiting professor in my department. He was interested in epithelial physiology and, together, we sought to develop a cell culture system that could be used to study, with a combination of biophysical and cell biological techniques, the generation and maintenance of the transport properties of polarized epithelial monolayers. After some searching, we settled for a dog kidney-derived cell line (Madin-Darby canine kidney; MDCK), some of whose epithelial features had been recognized a few years earlier by J. Leighton, then Professor of Pathology at Pittsburgh (Leighton et al. 1970). Confluent monolayers of these cells exhibit some of the properties of distal convoluted tubules of the kidney, including the capacity of vectorially transporting water and electrolytes across the cell layer, which reflects the functional polarization of individual cells. We grew the cells on collagen-coated disks of a nylon mesh, which were then placed between two fluid compartments, and found that as the monolayers reached confluency, a transepithelial electrical resistance developed. We also showed that the establishment of the resistance occurs concomitantly with the development of an extensive system of tight junctions that restricts the passage of molecules and ions through the intercellular spaces and defines morphologically, biochemically, and functionally distinct apical and basolateral domains on the surface of the cells. Calcium ions were found to be necessary for maintaining the integrity of the junctions, which could be reversibly opened and closed by the removal of calcium by the chelating agent EDTA and calcium readdition. Our findings (Cereijido et al. 1978a,b) and the almost contemporaneous work from Pitelka's laboratory (Misfeldt et al. 1976) established that MDCK cells are a suitable system to study the sorting processes, which in polarized epithelial cells effect the segregation of specific polypeptides into the two plasma membrane domains, as well as to investigate other phenomena characteristic of epithelial cells, such as the formation of tight junctions, transcytosis, and the polarized organization of the cytoskeleton (see Griepp et al. 1983). Following our extensive characterization of the properties of MDCK cells, this cell line has become a widely adopted paradigm for studies of epithelial cell physiology and intracellular protein traffic in general.

Soon after we developed the MDCK cell system, Enrique Rodriguez-Boulan, a young Argentine physician-scientist who had begun his training in Argentina with Cereijido, joined my laboratory, where he initially studied the asymmetric disposition of integral membrane proteins in the ER (Rodriguez-Boulan et al. 1978). With him we later discovered a striking manifestation of the polarized nature of MDCK cells (Rodriguez-Boulan & Sabatini 1978). We found that when these cells were infected with enveloped viruses, the virions assembled selectively on either one or the other plasma membrane domain, from where the virus subsequently buds. We saw that influenza (**Figure 8**), Sendai, and Simian virus 5 budded exclusively from the apical surface of the cells, whereas other virions, such as vesicular stomatitis virus (VSV), assembled only on the basolateral regions of the plasma membrane (**Figure 9**). Enrique observed (Boulan & Pendergast 1980) that, before budding takes place, the viral glycoproteins accumulate in the respective cell surfaces, as expected if their site of accumulation determines the site of budding. With Michael Rindler and Ivan Ivanov we were able to show that in MDCK cells the glycoproteins are segregated in the *trans* region of the Golgi apparatus into different membrane-bound carriers that are directly delivered to the specific plasma membrane domain, which highlighted the role of the Golgi in the sorting of cargo proteins (Rindler et al. 1984, 1985). We also demonstrated that in single cells, attachment to a substrate is sufficient to trigger the expression of plasma membrane polarity, which is manifested in the asymmetric budding of viruses (Rodriguez-Boulan et al. 1983).

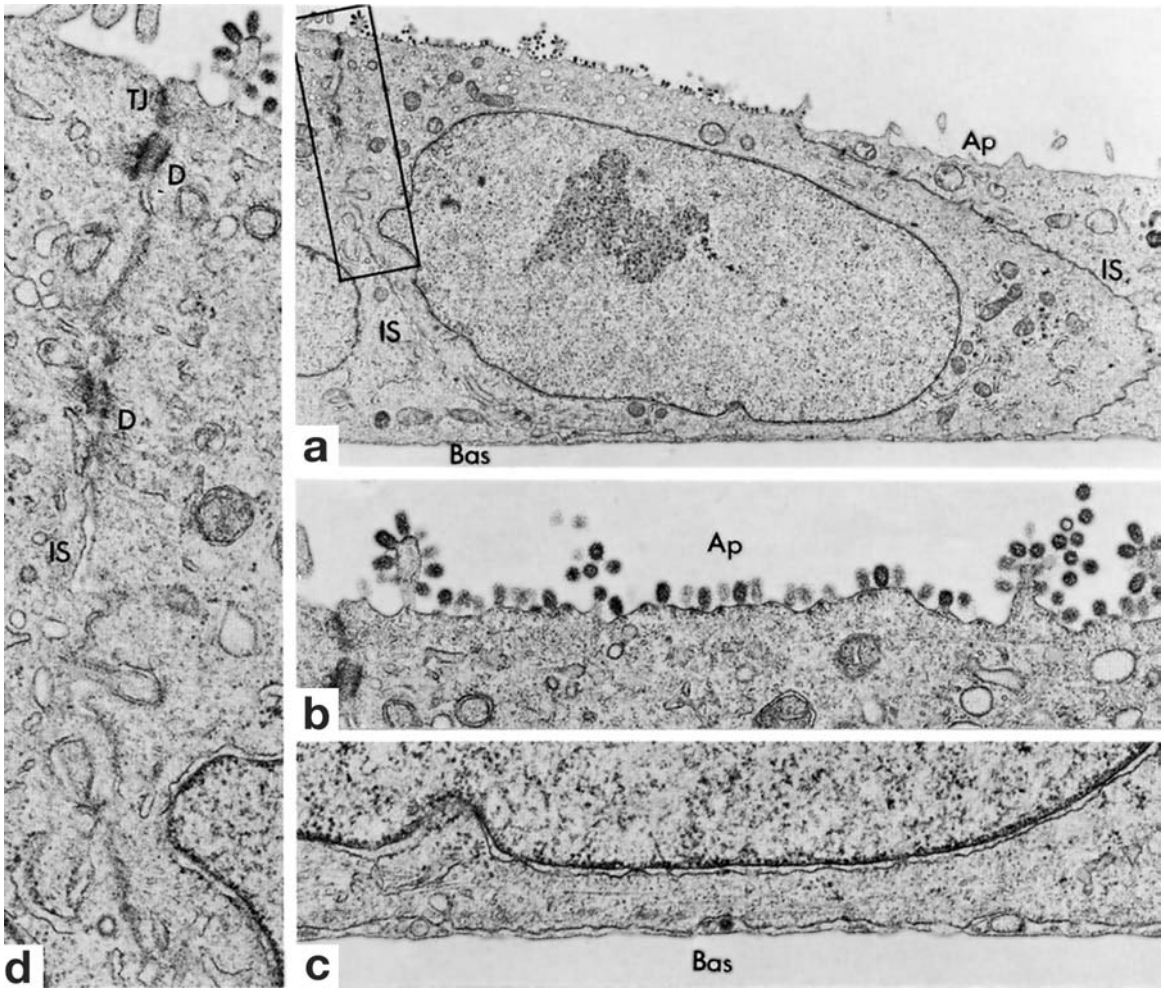

Figure 8

Exclusive budding of influenza virions from the apical surface of MDCK cells in a confluent monolayer. (*a*) View of one cell with abundant virion budding from the apical surface, shown at higher magnification in (*b*). Virus budding does not take place from the basal (*c*) and lateral surfaces (*d*) of the same cell. From E. Rodriguez-Boulan, D.D. Sabatini. 1978. Asymmetric budding of viruses in epithelial monolayers: A model system for study of epithelial polarity. *Proc. Natl. Acad. Sci. USA* 75:5071–75.

Soon it became possible to use transfected cells to express the individual viral glycoproteins in the absence of infection, which allowed us to demonstrate that the viral glycoproteins are effectively segregated to the appropriate cell surface domain even in the absence of other viral components (Gottlieb et al. 1986). This definitively proved that the sorting information necessary for their asymmetric distribution is contained in the glycoproteins themselves. Because we had also observed that truncated forms of the viral glycoproteins consisting only of their luminal domains were secreted in a nonpolarized form from MDCK cells, we concluded that their sorting information is contained within their membrane or cytoplasmic tails (Gonzalez et al. 1987).

The simple experimental system of virus-infected polarized MDCK cells that emerged

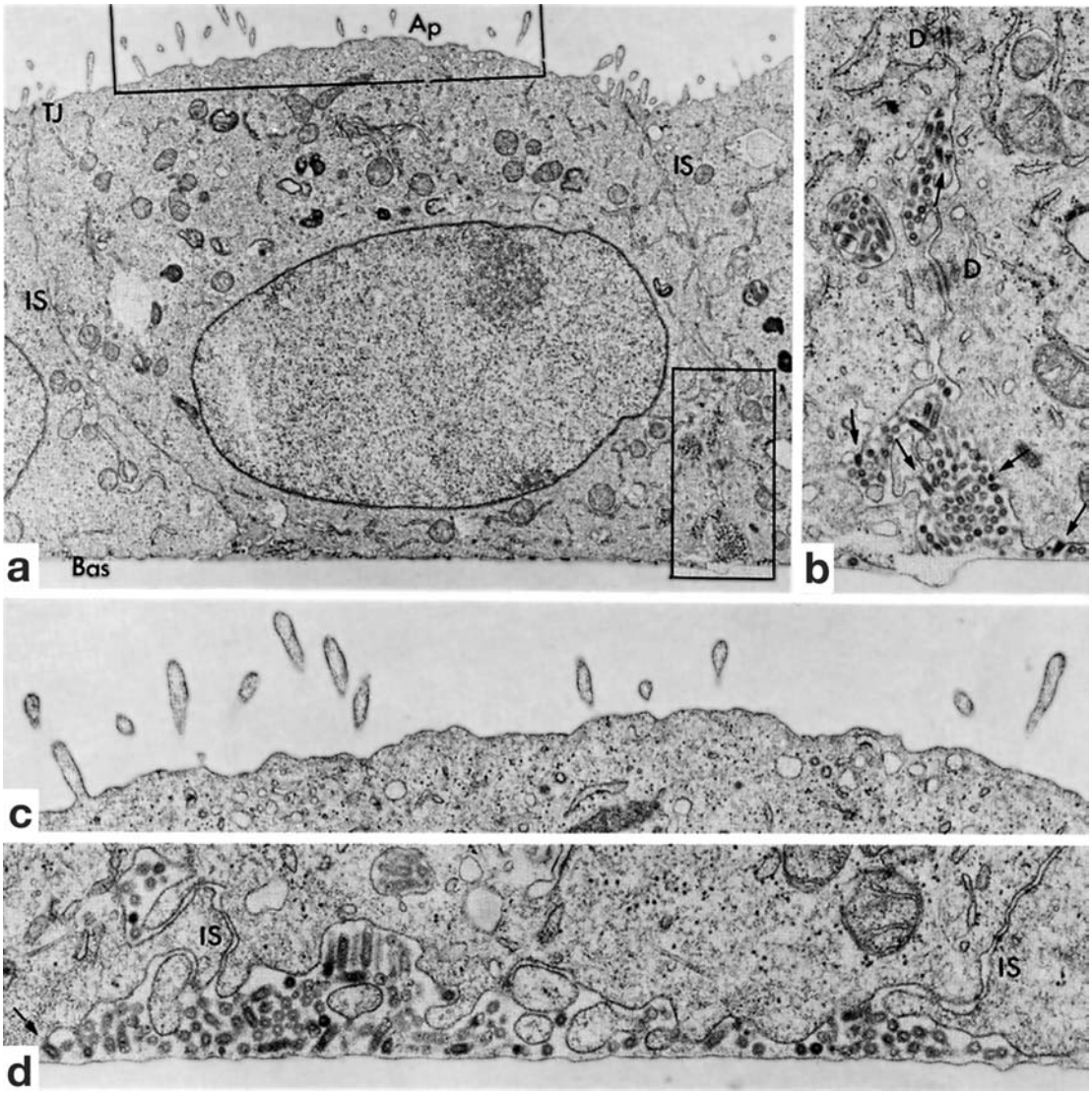

Figure 9

Exclusive budding of vesicular stomatitis virions from the basal and lateral surfaces of an MDCK cell in a confluent monolayer. Note that the apical surface of a cell (*a* and *c*), with abundant microvilli, shows no virus particles. On the other hand, virions are abundant in the intercellular space (*b*) between the lateral membranes of two adjacent cells and at the basal surface (*d*). The arrows show budding virions. From E. Rodriguez-Boulan, D.D. Sabatini. 1978. Asymmetric budding of viruses in epithelial monolayers: A model system for study of epithelial polarity. *Proc. Natl. Acad. Sci. USA* 75:5071–75.

from this work was soon adopted by many investigators to study the mechanisms of intracellular protein sorting and plasma membrane biogenesis and became the basis for the sprouting of a whole new area of cell biological investigation. In our own laboratory we showed that the integrity of the microtubule apparatus in MDCK cells was essential for the proper sorting of the influenza glycoprotein to the apical surface and the

subsequent polarized budding of the virus (Rindler et al. 1987). The polarized organization of MDCK cells also brought to the fore the existence of specialized endocytic machineries operating at the two surfaces. This was strikingly demonstrated when we found that the actin cytoskeleton plays a critical role in the process of apical endocytosis (which, for example, is required for infection with influenza virions), but plays no role in endocytosis from the basolateral surface (Gottlieb et al. 1993). We also discovered the curious fact that whereas the VSV-G glycoprotein, after reaching the cell surface, undergoes endocytosis, the influenza HA glycoprotein is not internalized to any significant extent (Gottlieb et al. 1986). Interestingly, we had obtained evidence from the behavior of chimeric proteins that the features determining the endocytic behavior of VSV-G are contained in the cytoplasmic domain of the protein (Rizzolo et al. 1985). The role of signals in the cytoplasmic tails of transmembrane proteins in determining the capacity of the proteins to be endocytosed was soon definitely established by the now classical studies (Davis et al. 1987) from Brown and Goldstein's laboratories on the LDL receptor.

Thanks to a partnership with my colleagues, Gert Kreibich, Takashi Morimoto, and in particular Milton Adesnik, with whom I have shared a common interest in protein traffic for over two decades and who helped me keep abreast of the new techniques of molecular cell biology, the work in my laboratory moved with the times. Now, using organelles isolated or prepared by variants of the classical techniques of cell fractionation pioneered at Rockefeller, we are engaged in attempts to reproduce in in vitro systems complex sorting and transport processes (Gravotta et al. 1990; Mayer et al. 1996; Simon et al. 1996a,b, 1998). We analyze the results with refined methods of immune-electron microscopy and biochemistry that I had the privilege of seeing born and evolving. We employ recombinant DNA-derived cargo molecules and similarly generated elements of the transport machinery and do not cease to be amazed by the detailed knowledge that is being attained about processes that seemed so impenetrable just a few decades ago.

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