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Human Organ Transplantation: Background and Consequences

Joseph E. Murray

The story of the renal transplant program of the Peter Bent Brigham Hospital (now the Brigham and Women's Hospital) in Boston weaves together three distinct threads: the study of renal disease, the phenomenon of skin grafting in twins, and the development of surgical procedures ultimately leading to the use of chemical immunosuppression. The common leitmotiv is one of a single event or report proving to be decisive. Unanticipated consequences of successful human organ transplantation include the reorganization of clinical and nonclinical disciplines, national and international cooperation in organ preservation and distribution, tissue-typing as a marker for disease, redefinition of death in terms of brain function, better understanding of disease processes, and new health care quandaries that result from the scarcity of organ donors.

Although renal transplantation had been performed sporadically during the first half of this century (*1*, *2*), planned programs for human organ transplantation started only in the late 1940s. At that time, clinicians in Paris, London, Edinburgh, and Boston began renal transplantation in nonimmunosuppressed human recipients, in spite of warnings and pessimistic predictions of many scientists and experienced clinicians. Both L. Loeb (*3*) and P. B. Medawar (*4*) claimed that human allotransplantation would never be possible because the roots of individuality were so deep and impenetrable.

Bioscientists had difficulty understanding the determined optimism of clinicians who were willing to evaluate any type of treatment that might help terminally ill uremic patients, most of whom were young and otherwise healthy. Tantalizing reports of functioning human renal transplants had surfaced from time to time; these hints of success were further encouragement. Some researchers, working independently in Paris, had produced temporary function of human renal allografts (*5*, *6*), and Lawlor and co-workers in Chicago actually published "success" in a patient (*7*), which was later rescinded.

The first two physicians-in-chief at the Peter Bent Brigham Hospital (the Brigham), H. Christian (1912 to 1939) and S. Weiss (1939 to 1942), were interested in renal disease. When G. W. Thorn became chief in 1943, he and his associate, J. P. O'Hare, shared this interest, especially with regard to the relation of renal disease to hypertension. Although the association of high blood pressure with renal disease had been known for over a century, there was no effective treatment for kidneys damaged by hypertension. After World War II, Thorn invited W. Kolff from the Netherlands to demonstrate a dialysis machine that he had developed during his forced confinement by the Germans (*8*). C. W. Walter helped to improve the design (*1*), and thus the Kolff-Brigham "artificial kidney" was devised. It was first used in patients in 1948 and set the stage for extensive, innovative approaches to both acute reversible renal disease and end-stage kidney failure.

Because renal dialysis was to be only a temporary substitute for renal function, it was logical to seek a more permanent therapy. Chronic dialysis was developed 10 years later in 1958 in Seattle (*9*). Earlier, in the 1940s during a Grand Rounds at the Brigham, Thorn stated that the best way to treat hypertension was to remove both kidneys. The entire audience gasped. The seed for the Brigham renal transplant program had been planted.

Skin Grafting in Twins

This thread in the story involves the biological phenomena of monozygotic (identical) and dizygotic (fraternal) twinning. The monozygotic twin experience starts with the treatment of burns, and the dizygotic twin story begins with freemartin cattle.

In 1932, E. C. Padgett of Kansas City reported the use of skin allografts from family and unrelated donors to cover severely burned patients who had insufficient unburned donor sites for the harvesting of autografts (*10*). Although none of these skin allografts survived permanently, they could be lifesaving by remaining long enough to control infection and fluid loss and thus gaining time for the donor sites to

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re-epithelialize. It was difficult to determine accurately the duration of survival of any one allograft; some seemed to melt away slowly and be replaced by adjacent skin, whereas others seemed to be rejected rapidly (*10*).

Skin grafts from family members seemed to survive longer than those from unrelated donors. But even after observing hundreds of skin allografts, one could not be certain about their survival time. One certainty was established when J. B. Brown of St. Louis, in 1937, achieved permanent survival of skin grafts exchanged between monozygotic twins (*11*). This single observation, although restricted in application, was the only ray of light in the problem of tissue and organ replacement until T. Gibson and P. B. Medawar demonstrated that a second allograft from the same donor was rejected more rapidly than the first (*12*). This clear description of the "second set" phenomenon established that the rejection process was not immutable; instead, it implied an allergic or immunological process which potentially might be manipulated.

The dizygotic twin story is more circuitous and led ultimately to the experimental production of acquired immunological tolerance. In 1779, J. Hunter, always curious about experiments of nature, presented before the Royal Society of London his observations of the physical characteristics of freemartin cattle (*13*). Freemartins, known by the ancient Greeks and Romans, are females of male-female twin pairs in which the male is always normal and the female is almost always sterile. Although sporadic descriptions of these cattle dizygotic twins appeared subsequently, no additional knowledge accrued until 1917, when F. R. Lillie, not content with mere descriptions, dissected the placentas of several pairs of freemartin cattle. He noted and described the placental intermingling of blood between these differently sexed twins (*14*). Because of the sterility of the female, it was natural that most subsequent studies related to endocrine aspects of the freemartin.

Twenty-nine years later, R. D. Owen noted the coexistence of different blood types in these twin cattle and published on the tolerogenic consequences of placental intermingling of circulation (*15*), citing Lillie as the key reference. This brought freemartins to the attention of the immunologists. In 1951, D. Anderson and coworkers reported successful experimental skin allografts between the freemartin and the normal male (*16*).

The freemartin story culminated in the report of R. E. Billingham, L. Brent, and P. B. Medawar in 1953 that described acquired immunological tolerance in mice (*17*). They injected cells from one mouse strain into neonatal recipients of a genetically disparate strain. When these recipient mice became adults, they could accept skin grafts from the cell donor strain but not from other strains. Thus, a specific immunological tolerance was produced. The researchers acknowledged that their experimental protocol was a counterpart to the twin cattle model of R. D. Owen (*17*).

Although not applicable to the clinical situation, their experimental breaching of the immunological barrier was another impetus for optimism in the quest for successful human renal transplantation. M. F. A. Woodruff, the pioneer transplant surgeon in Edinburgh, confirmed the freemartin concept in humans when he found a pair of twins—one male, the other female—who shared each other's differing red cell types. Postulating a shared placental circulation between the two, he cross–skin-grafted them successfully (*18*).

Surgical Developments

In the early 1900s, A. Carrel, a French surgeon working at the Rockefeller Institute, developed techniques for suturing blood vessels in dogs. Carrel and co-worker C. C. Guthrie then transplanted kidneys and even entire heads in these animals. Although they recognized that autografts survived longer than allografts or xenografts (*19*), they did not conceptualize the rejection process but noted that loss of function of the transplants was not a result of infection or infarction (*20*).

Other surgeons adapted Carrel's techniques for their own investigations. In 1916, W. C. Quinby from Boston used Carrel and Guthrie's canine renal autograft model to study renal function after total denervation of the kidney (*21*). A decade later, others studied the different survival times between canine renal autografts and allografts (*22*). These researchers noted the longer survival of the autografts but like Carrel and Guthrie did not pursue their long-term function. After World War II, W. J. Dempster (*23*) and M. Simonsen and co-workers (*24*) published extensively on canine renal transplantation, concentrating on the biology and biochemistry of allograft rejection. They demonstrated that skin and kidney allografts possess a common antigen that could sensitize a recipient to a subsequent allograft of either tissue from the same donor. In these reports, there was the tacit assumption that renal autograft function would ultimately deteriorate, possibly because of lack of nerve supply, lymphatics, or both.

From a physiological view, if human renal transplantation were to be successful, researchers needed to establish that renal transplants in the absence of an immunological barrier could function permanently. In the course of many laboratory experiments on canine renal transplantation, I had developed a reproducible operation that could connect the blood vessels of the donor kidney to those within the abdomen of the recipient, with implantation of the ureter directly into the urinary bladder (*25*). This intra-abdominal operation has become the standard renal transplant. Critical functional studies of some of these autografted kidneys 2 years after transplantation proved that they functioned completely normally (*25*).

Thus, all the elements for a multidisciplinary renal transplant program were in order: experienced knowledge in renal disease, availability of dialysis, and skilled, imaginative surgeons under F. D. Moore, Moseley Professor of Surgery at Harvard Medical School. To minimize morbidity, the first human kidney allografts in the nonimmunosuppressed recipients were added as a third kidney under local anesthesia. D. Hume, the surgeon for these patients, connected the renal vessels of the graft to the femoral vessels of the recipient. The ureter was brought out on the surface of the thigh as a skin ureterostomy, allowing the urine to be collected in a bag strapped to the thigh (*26*). Several of these human allografts functioned better than experimental canine allografts would have predicted. Possible explanations include an immunosuppressive effect of uremia or a beneficial effect of the acute tubular necrosis that occurred regularly in these inadequately preserved donor kidneys. One thigh transplant functioned for almost 6 months with return of the patient's biochemical profile and blood pressure to normal, which demonstrated that transplants could correct multiple manifestations of terminal renal disease.

A historic Brigham renal transplant performed in 1945, before the development of renal dialysis, deserves special comment because it has been misinterpreted historically. The patient was a young woman who had obstetrical complications leading to complete renal shutdown—that is, acute tubular necrosis. A kidney from an unrelated donor was joined to the blood vessels in her arm under local anesthesia. The purpose was to provide temporary renal function to allow time for her own kidneys to recover. The transplanted kidney did produce some inconsequential amounts of urine for a few days. Meanwhile, the patient's own kidneys did recover, and she left the hospital. This episode has been considered erroneously by some as a "success." Although a functional failure, this event did kindle and solidify interest in transplantation throughout the hospital as a dramatic treatment for renal disease (*27*).

Fig. 1. Kidney transplant patient Edith Helm (right) with her two children in 1962. Her identical twin sister Wanda Foster and her three children are on the left. In 1956, Wanda Foster donated a kidney to her sister Edith, now a grandmother and the longest surviving renal transplant recipient.

The Trails Merge

In the fall of 1954, D. Miller of the U.S. Public Health Service referred to J. P. Merrill, the nephrologist in charge of the medical aspects of the Brigham transplant program, a patient with severe renal disease and suggested there might be the opportunity for transplantation of a kidney because the patient had a healthy identical twin brother. Our transplant team was interested in the possibility of transplanting a genetically compatible kidney, and we were ready to apply our medical skills and the laboratory-tested surgical technique to humans.

The only remaining problem was the ethical decision concerning the removal of a healthy organ from a normal person for the benefit of someone else. For the first time in surgical history, a normal, healthy person was to be subjected to a major surgical operation for someone else's benefit. After many consultations with experienced physicians within and outside the Brigham and with the clergy, we felt it reasonable to offer the operations to the recipient, the donor, and their family. We discussed in detail the preparations, anesthesia, operations, possible complications, and anticipated result. At the conclusion of our last preoperative discussion, the donor asked whether the hospital would be responsible for his health care for the rest of his life if he decided to donate his kidney. The surgeon for the donor said that the hospital would not be, but he then asked the donor if he thought that anyone in the room would ever refuse to take care of him if he needed help? It was clear that his future medical care depended on our sense of professional responsibility rather than on legal assurances. Because the donor was expected to survive normally, once the patients and the team decided to proceed with the transplant, an extra professional burden fell on the surgeon performing the donor nephrectomy. In contrast, the surgeon who performs a transplant is operating on a patient otherwise doomed to die, and the nephrologist caring for these critically ill patients cannot be faulted for failure to cure.

In this case, the transplanted kidney functioned immediately, with a dramatic improvement in the renal and cardiopulmonary status of the recipient. This success was a demonstration that organ transplantation could be lifesaving. We had spied into the future because we had achieved our long-term goal by bypassing, although not solving, the issue of biological incompatibility (*28, 29*).

Subsequent Laboratory and Clinical Study

Our success stimulated worldwide laboratory attempts to breach the immunological barrier. Experimental protocols included total body x-ray treatment, after which new bone marrow cells were infused; immunoparalysis by consecutive graftings; immunological enhancement or adaptation by exposure of the host or graft to antigen before the transplant; matching of donor and recipient by red or white cell typing; and the use of drugs such as toluene and nitrogen mustard as immunosuppressants.

We continued with both clinical and laboratory studies. In conjunction with the Department of Pathology at the Brigham under G. J. Dammin, we studied a series of volunteer uremic patients and noted a prolonged, but not permanent, survival of skin allografts, which suggested that the uremic state itself was immunosuppressant (*30*). We tried to test this hypothesis in dogs and established a state of renal insufficiency by partial removal of renal mass, by infusion of toxins directly into the renal artery, by temporary ischemia, or by thermal insult. Graft survival, however, was not prolonged. Treatment of the hosts with steroids, anticoagulants, or both also failed (*31*). We used mice and rabbits to study the x-irradiation–bone marrow protocol, which seemed to have the best potential for human application. Sublethal or lethal doses of total body x-rays that were followed by marrow infusions from single or multiple donors could prolong the survival of a limited number of skin allografts (*32*).

During the 1950s, identical twins, one dying and the other healthy, were being referred in increasing numbers for transplantation. One twin transplanted in 1956 completed a pregnancy 2 years later (*33*) (Fig. 1). She is now a grandmother and the longest surviving renal transplant recipient. Her donor, also a grandmother, is also in perfect health. It is estimated that at least 50 patients worldwide have received transplants from their identical twins.

Several patients in end-stage renal disease or who had lost their solitary kidney were treated with an x-irradiation–bone marrow protocol—that is, they received total body x-rays, an infusion of bone marrow, and a renal allograft. In most of the patients, the transplanted kidneys functioned immediately and continued to do so for several weeks, but in only one of twelve patients did function persist beyond three months. The one success was in 1959 with our third patient, a dizygotic twin who received a sublethal dose of total body x-rays that did not necessitate an infusion of bone marrow, after which we transplanted a kidney from his twin brother. After a complicated postoperative course, he recovered to lead a fully active, normal life. He was the first successful renal allograft patient and was the enticement and stimulus for us to continue this method of procedure until immunosuppressive drugs became available (*34–36*). J. Hamburger in Paris subsequently had similar success with a dizygotic twin recipient after sublethal x-ray treatment. Using the same protocol, he succeeded in attaining long-term survival in more patients by using a sibling and a first cousin as donors (*37*). R. Kuss and co-workers, also in Paris and using a similar protocol in patients receiving kidneys from nonrelated donors, had two survivors for over 1 year (*38*).

The First Successful Cadaveric Transplant in Humans

We searched for a regimen that could substitute for total body irradiation. In 1958, using rabbits, we tested without success the anticancer drug triethylenethiophosphoramide as a possible immunosuppressive drug (*39*). The breakthrough came, however, with the introduction of immunosuppressive drugs by R. Schwartz and W. Dameshek in 1959 (*40*). They prevented rabbits from producing antibody to human serum albumin by treating them for 2 weeks with the antimetabolite 6-mercaptopurine (6-MP). This drug-induced tolerance remained after drug treatment was stopped, even though the animals could produce reactions to another protein antigen, bo-

Fig. 2. Normal-appearing and normal-acting immunosuppressed dogs living for over a year on solitary renal allografts.

vine gamma globulin. Thus, the tolerance seemed to be specific for an antigen introduced at the time of drug administration. R. Y. Calne in London (*41*) and C. Zukoski and co-workers in Virginia (*42*) tested this drug in the canine renal transplant model and had encouraging results.

On the advice of P. B. Medawar, in 1960 Calne came to Boston to work in the Department of Surgery, under F. D. Moore, at Harvard and the Brigham. Calne introduced us to G. H. Hitchings and G. B. Elion of the Burroughs Wellcome laboratories, who became enthusiastic collaborators. After Calne's arrival, and with drugs from Burroughs Wellcome, the improvement in allograft survival was rapid and dramatic; we soon had bilaterally nephrectomized dogs living on solitary renal allografts that survived for years (Fig. 2). One recipient produced a normal litter sired by a drug-treated allografted male. Another was able to recover from a severe infection of the mandible, which indicated that he was not an immunological cripple, a state we feared might result from prolonged use of the drugs (*43*). Of other drugs provided by Hitchings and Elion, B-W 322, the imidazole derivative of 6-MP, seemed to have the best therapeutic index. This drug is now known as azathioprine, or Imuran, and for the next 20 years was used throughout the world to support organ transplantation. Now, more effective drugs with less toxicity are available, but azathioprine is still widely used as an essential immunosuppressive drug.

Reassured by our laboratory results with dogs, we attempted to use these drugs for immunosuppression of humans. The first renal transplant recipient to receive azathioprine was an adult transplanted with an unrelated kidney in March 1961. The transplant functioned well for over 1 month, but the patient died of drug toxicity because the dosage required in dogs was toxic in humans. Our second patient also died of drug toxicity in spite of receiving only half of the dose used for our first patient. We were able, however, to reverse the rejection process, a previously unknown phenomenon (*44*). When we discontinued the drug because of too few circulating white blood cells (leukopenia), rejection began. As the patient's leukopenia improved, we restarted the drug, the rejection process reversed, and renal function improved. Nevertheless, this second patient also succumbed to drug-induced sepsis within a month.

Our third patient, who received a transplant in April 1962, was treated with azathioprine after a cadaveric renal allograft. He survived over 1 year and was the first successful unrelated cadaveric transplant (*45*, *46*). W. E. Goodwin and co-workers at the University of California in Los Angeles almost immediately introduced corticosteroids as a further adjunct to the treatment (*47*). Subsequently, several transplantation groups worldwide began their own productive transplantation programs.

By 1965, 1-year survival rates of allografted kidneys from living related donors approached 80%, and survival rates of kidneys from cadavers approached 65%. Regional and national donor procurement programs were established along with a Human Renal Transplant Registry (*48*). Optimism and enthusiasm were high as new drugs and other methods of immune suppression were tested along with refinements in tissue typing and improved organ preservation. Antilymphocyte serum and globulin prepared in horse, sheep, and rabbit, along with thoracic duct drainage of lymphocytes, were among the more promising regimens tested. Currently, more than 250,000 human renal transplants have been performed worldwide.

Other Organs

The success with renal allografts naturally led to attempts to transplant other organs. F. D. Moore and co-workers developed a surgical technique for orthotopic canine liver transplantation (*49*), as did T. E. Starzl and co-workers, who subsequently performed the first successful human liver allografts (*50*). Calne, returning to Cambridge in England, also developed an extensive human liver transplantation experience. For almost 15 years, both Starzl and Calne and their co-workers performed most of the world's human liver transplants (*51*). Today, transplantation of the liver is done around the world and is the second most frequently performed transplant operation.

After the liver, the next organ to be transplanted was the heart. R. Lower and N. Shumway had developed the surgical technique in dogs in 1960 (*52*) and were planning a careful program for cardiac transplantation in humans. After C. N. Barnard's first human cardiac transplant in South Africa in 1967, many other cardiac surgeons with little or no immunological background rapidly accumulated large numbers of heart-transplanted patients only to witness them all die of rejection within a few months. This period, from 1968 to 1970, was transplantation's darkest hour because of the careless application of technical procedures with insufficient laboratory background. The sole redeeming feature in heart transplantation was the continuation of Shumway's program at Stanford, which achieved permanent success in 1970 (*53*). Today, with the development of newer drugs, cardiac transplantation is a recognized and accepted form of treatment.

Single and double lung transplants have followed, as well as combined heart-lung transplants. Transplantation of the pancreas, with or without an accompanying renal graft, is now possible for some patients. Multiple organ transplants in combination with liver and parts of the intestinal tract have also been successful. In 1989, there were 8890 kidney, 2160 liver, 1673 heart, 413 pancreas, and 67 heart-lung transplants performed in the United States alone (*54*).

Ironically, allografts of skin, the tissue used classically in most of the early studies of transplantation, have proven to be the most difficult to transplant. Skin is the ultimate protection of the individual against the environment and, therefore, over time has evolved into our strongest barrier against foreign proteins. The earlier conventional wisdom was that the fate of skin allografts predicted the results of other transplants. Commenting on the contrasting survival rates of skin and kidney allografts in immunosuppressed dogs, Medawar proclaimed with his customary flair that the success of organ transplantation has "overthrown the doctrinal tyranny of skin grafts" (*55*).

Consequences

In less than 40 years, organ transplantation has produced exciting insights about complex biological and clinical problems. Bench scientists have become more interested in clinical problems, and clinical in-

vestigators have increased their understanding and activity in basic studies. The boundaries between immunology, microbiology, genetics, cellular and molecular biochemistry, and pharmacology have become porous. Cooperation between bench and bedside has led to progress on many fronts—for example, more effective immunosuppressive agents, increased understanding of autoimmune disease, and the association of the immunosuppressed state with neoplasia.

National and international collaborations have been established for the preservation and distribution of organs and have spawned vital forums for the exchange of ideas. With clinical success came the need for better organ preservation, and today donor organs can be preserved long enough to be shipped worldwide, if necessary (*56*).

Another unforeseen result of transplantation was the central role that histocompatibility antigens, originally recognized as markers for animal and human transplantation, play in many unrelated diseases. For example, the histocompatibility antigen DR2 is linked to narcolepsy and B27 to ankylosing spondylitis. Susceptibility to juvenile-onset diabetes is linked to A1, B8, DR3, and DR4, whereas resistance is linked to DR2.

Successful transplantation is most likely when donor organs are in excellent condition. The former criteria for death—cessation of spontaneous breathing and heartbeat—prevented organ use before their function began to deteriorate. The concept of brain death, formulated by a committee at Harvard Medical School in 1968 in direct response to the needs of transplant teams, now guides these decisions not only for transplant centers but also for intensive care units worldwide (*57*).

Liver transplants have allowed the treatment and "cure" of inborn errors of metabolism, such as α-1-antitrysin defect, Wilson's disease, and tyrosinemia. Liver replacement not only can cure liver failure but can also correct the various extrahepatic symptoms that are the results of metabolic aberrations. Liver transplantation now is being done in patients with liver-based metabolic diseases that produce severe generalized symptoms, even if the liver is otherwise normal in function and appearance (*58*).

The very success of transplantation has created a scarcity of donor organs that in turn has led to their unethical allocation. In some areas, the buying and selling of organs has become acceptable (*59*). The solution to this unexpected and, by most standards, degrading situation does not lie in ethics, politics, or even religion but in the professional standards of surgical and medical care and in the cultural environment of the region.

Animal research has been absolutely indispensable for the development of clinical organ transplantation. The first twin transplant was a complete surgical success only because it was perfected in operations on hundreds of dogs. Without the experience derived from genetically pure strains of mice, human tissue-typing almost certainly could not have been possible or at best would have lagged for several decades.

Although thousands of lives have already been saved by the use of various immunosuppressive regimens, serious complications still occur as a result of treatment. An increased incidence of de novo neoplasia in long-term survivors has been reported, a result presumably of decreased immune surveillance on the part of the recipient (*60*). The ultimate aim in transplantation is to achieve an immunological tolerance between donor and recipient, eliminating entirely the need for drugs. There are signs both in the laboratory and in humans that the liver itself may produce tolerogenic factors that may reduce or eliminate the need for immunosuppression (*61*).

Organ transplantation has progressed from the impossible to the commonplace. The complementary roles of clinical and laboratory research have produced profound changes in patient care and laboratory disciplines, and transplantation teams with clinical and laboratory expertise now exist worldwide. Although kidney transplantation began this progression, subspecialties have developed for liver, heart, lung, pancreas, intestine, and marrow. Pediatric transplantation, for example, requires special skills and facilities. Our increased understanding of cellular and humoral immunity, autoimmunity, and human tissue-typing, combined with imaginative and skillful surgical experimentation, has revolutionized patient care. This cascade of progress began with an apparently simple, clear-cut aim: to find a way to replace a destroyed or missing organ.

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RESEARCH ARTICLES

Unusual Resistance of Peptidyl Transferase to Protein Extraction Procedures

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Peptidyl transferase, the ribosomal activity responsible for catalysis of peptide bond formation, is resistant to vigorous procedures that are conventionally employed to remove proteins from protein–nucleic acid complexes. When the "fragment reaction" was used as a model assay for peptide bond formation, *Escherichia coli* ribosomes or 50*S* subunits retained 20 to 40 percent activity after extensive treatment with proteinase K and SDS, but lost activity after extraction with phenol or exposure to EDTA. Ribosomes from the thermophilic eubacterium *Thermus aquaticus* remained more than 80 percent active after treatment with proteinase K and SDS, which was followed by vigorous extraction with phenol. This activity is attributable to peptidyl transferase, as judged by specific inhibition by the peptidyl transferase–specific antibiotics chloramphenicol and carbomycin. In contrast, activity is abolished by treatment with ribonuclease T1. These findings support the possibility that 23*S* ribosomal RNA participates in the peptidyl transferase function.

There is much evidence to support the view that ribosomal RNA (rRNA) participates directly in protein synthesis (*1*, *2*), and it has even been argued that the fundamental mechanism underlying translation may be RNA-based (*3*, *4*). Indeed, demonstration of the ability of RNA to perform enzymatic catalysis in other biological contexts (*5*, *6*) has drawn increased attention to the functional potential of rRNA. However, apart from the well-established role of the 3′ terminus of 16*S* rRNA in mRNA selection, direct proof of this has been elusive. For example, efforts to carry out steps of protein synthesis with protein-free preparations of rRNA have not been successful [but see (*7*)], possibly because billions of years of co-evolution of ribosomal proteins and rRNA have led to a requirement for ribosomal proteins to achieve proper folding and function of the rRNA (*8*–*10*).

Localization of peptidyl transferase to the large ribosomal subunit. In our efforts to study the biological activity of rRNA, we have chosen as a model system the peptidyl transferase reaction, which is the source of the catalysis of peptide bond formation, and is also the single catalytic activity that has unambiguously been shown to be an integral part of the ribosome structure (*11*). In spite of many attempts by several laboratories, peptidyl transferase activity has never been detected in RNA-free preparations of ribosomal proteins. An important attraction of peptidyl transferase is that it can be monitored with a simplified assay known as the "fragment reaction" (Fig. 1), which measures the transfer of *N*-formyl-methionine from a short fragment of tRNA to the amino group of puromycin to form a model peptide bond (*12*). The fragment reaction requires only the large ribosomal subunit, appropriate ionic conditions, and 33 percent methanol or ethanol, in addition to the f-Met-oligonucleotide and puromycin substrates. Thus, there is no requirement for the small ribosomal subunit, mRNA, protein factors, guanosine triphosphate (GTP), or even complete tRNA molecules. The authenticity of the model reaction is supported by the stereochemical specificity of the substrates and highly specific inhibition of the reaction by antibiotics that are known peptidyl transferase inhibitors (*13*).

Earlier studies showed that this system can be simplified even further by stepwise removal of ribosomal proteins from the 50S subunit with high concentrations of salt (*14*–*16*). In one study, removal of approximately half of the proteins from the 50S subunit resulted in loss of peptidyl transferase functions; full activity was restored by reconstitution of the resulting core particles with purified protein L16 (*16*). These same preparations of purified L16 showed no detectable peptidyl transferase activity, however. Another study provided evidence for an L16-dependent, conformational change in similar protein-deficient 50S core particles (*17*). The temperature dependence of the kinetics of this process corresponds to an activation energy of about 30 kcal/mol, suggesting the occurrence of a fairly substantial structural rearrangement. These experiments indicate that protein L16 plays an important role in proper assembly of the core particle. Reconstitution experiments, in which individual components were omitted, showed that proteins L2, L3, L4, L15, L16, and L18, as well as 23S rRNA were essential for reconstitution of peptidyl transferase activity (*18*); of this group, L18 could also be excluded on the basis of other studies (*19*). This list most likely represents an overestimate of the number of proteins actually needed for ca-

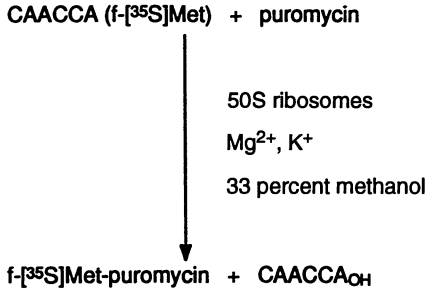

Fig. 1. The "fragment reaction." Peptidyl transferase activity is measured by formation of f-[^{35}S]Met-puromycin from reaction of the CAACCA(f-[^{35}S]Met) oligonucleotide fragment, derived from the 3′ end of f-[^{35}S]Met-tRNA by RNase T1, with puromycin, in the presence of 33 percent methanol (*12*). The oligonucleotide fragment and puromycin serve as peptidyl-tRNA and aminoacyl-tRNA analogues, respectively.

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