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The Trick Of The Tryp: [Dr. George Cross]

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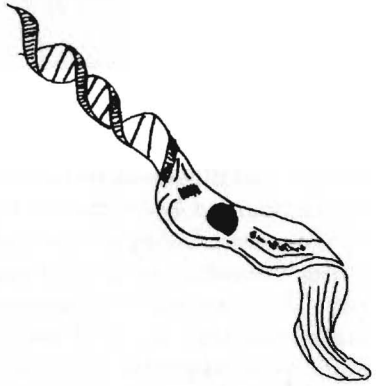


THE ROCKEFELLER UNIVERSITY

RESEARCH PROFILES

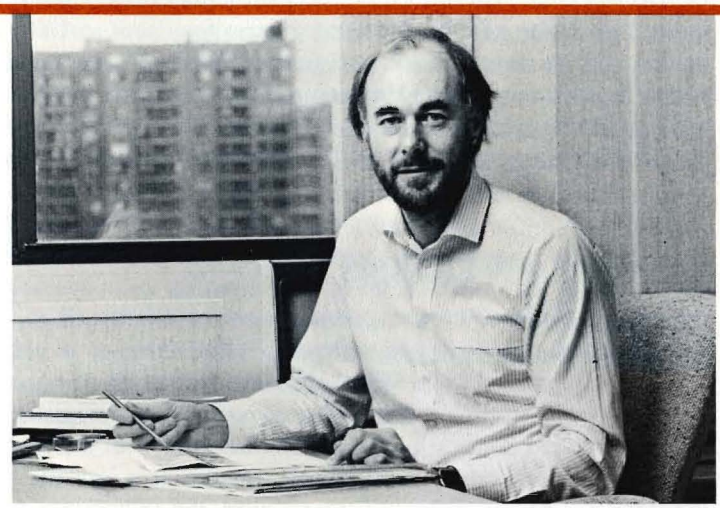
WINTER 1983/84

The Trick of the Tryp

Francis Crick, codiscoverer of the double helix structure of DNA—the molecule of the genes—has been credited with defining molecular biology as anything that interests molecular biologists. What interests George Cross is parasites; in particular, certain African species of trypanosomes, parasitic protozoa that are transmitted to animal hosts by tsetse flies. In human beings and domestic cattle, trypanosome infection causes sleeping sickness, for which there are as yet no preventive measures or totally safe treatment. On a continent gripped by catastrophic food shortages, the four million square miles where the tsetse fly ranges are a precarious environment for raising cattle.

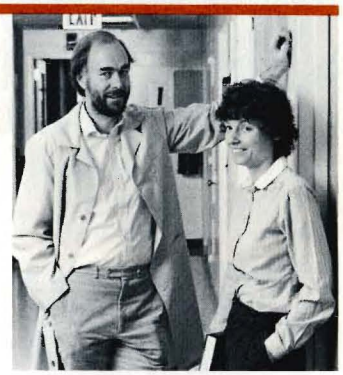
Trypanosomes possess a unique mechanism called antigenic variation. Like the wily archvillain Moriarty switching disguises to confound Sherlock Holmes, trypanosomes can switch their molecular identity to fool detective-defenders of the mammalian immune system. Dr. Cross has been studying the "trick of the tryp," as he calls it, for more than a decade. What he has been learning may eventually lead to more effective treatments for sleeping sickness. It has already inspired a new respect for parasites as subjects for basic research.

"Until recently," Dr. Cross says, "parasites have been largely ignored by molecular biologists. The reasons are both scientific and social. Parasites have complex life cycles and they're expensive, difficult, or next to impossible to maintain in the laboratory. Socially and politically they have been ignored because the control of parasitic diseases remains primarily a problem of the developing world."



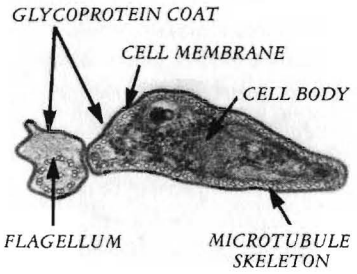
George Cross

A native of England, Dr. Cross came to Rockefeller University a year ago to establish a laboratory of molecular parasitology. Shortly after, he was named to the newly endowed André and Bella Meyer professorship. At 41, he is a world leader in his field. His work has recently earned him the Chalmers Medal of the Royal Society of Tropical Medicine and Hygiene and the Paul Ehrlich and Ludwig Darmstaedter Prize of the Paul Ehrlich Foundation in Germany. His appointment continues a long tradition of parasitology at Rockefeller, which includes some of the earliest studies of sleeping sickness.



Dr. Cross and colleague Christine Clayton in the hallway outside their laboratories which are appropriately housed in Theobald Smith Hall. The building is named for a pioneer Rockefeller scientist whose work helped to lay the foundation for understanding insect-transmitted and other infectious diseases. Dr. Clayton, a transplant from England like Dr. Cross, has recently resumed parasitological research after several years of studying the molecular biology of viruses and drug resistance in cancer.

Scanning electron micrograph of Trypanosoma brucei. *Life size is about one thousandth of an inch.*



An electron micrograph through a trypanosome cell section showing surface coat.

As often happens in science, Dr. Cross's introduction to what was to become his major work was fortuitous. As a student at Cambridge University, his interest was biochemistry. Later, as a doctoral candidate, he studied the process by which eukaryotic cells (those of higher animals and protozoa, in contrast to bacterial cells) make proteins. He chose a simple parasitic protozoan of the trypanosome family because it happened to be available in the microbiology laboratory in which he was working. Not until he'd completed his Ph.D. and was invited to join a new biochemical parasitology unit at Cambridge did he think about parasites as disease organisms rather than merely as experimental models of general cellular activity. He turned his attention to *Trypanosoma brucei,* a species of African trypanosome that infects cattle and humans.

ELUSIVE ANTIGENS

Animals, including humans, recognize an infectious cell as a foreign invader by means of molecules called antigens on the alien's surface membrane. When the host detects the foreign presence, it sorts through its own inventory of molecular weapons, called antibodies, to find a molecule with a shape that fits the distinctive shape of the antigen, as a key fits a lock. If a prototype antibody is pinpointed, the cells that produce it multiply clonally. (Clones are asexually generated offspring, identical to the parent cell.) The clones pour forth antibodies which bind to the antigens and may succeed in killing the foreign cells before they can proliferate and damage or kill the host cells. When *Trypanosoma brucei* invades, the immune system fails. "While the host antibodies are busy killing off most of the tryps," Dr. Cross explains, "a small number of tryps are busy *changing* antigens. Because the new antigens are not recognizable to the antibodies manufactured for the original antigens, the immune system must go through the whole selection and production process again. Tryps can switch antigens essentially indefinitely. The antibodies never catch up and the animal usually dies long before the tryps run through their antigenic repertoire."

The ability of trypanosomes to elude immune responses was noted long ago and the involvement of surface antigens suspected, but no one had been able to find them. It was assumed that if they existed, they were scarce and chemically unstable. Applying "straight biochemical analysis and a few tricks of my own," Dr. Cross found antigens in abundance—millions of molecules to the cell. He found that they were in fact stable, and he identified them as glycoproteins: proteins with a section of carbohydrate attached. Proteins are large molecules composed of smaller molecules called amino acids. Some twenty amino acids comprise the alphabet from which proteins are put together.

"What we wanted to do, first of all," Dr. Cross says, "was to find out what properties of the variant surface glycoproteins (VSGs) were responsible for the variation we saw in the living cell. After we determined that antigenic variation was a function of the protein and not the carbohydrate content, we began studying the sequence of the amino acids to develop a hypothesis for the genetic basis of antigenic variation. We wondered whether the structure of trypanosome variant antigens might be made up of variable and constant regions. It turned out, instead, that the *entire* molecule is variable."

A THOUSAND GENES

What proteins a cell makes are determined by the genes on the chromosomes within its nucleus. Genes, made of units of DNA (deoxyribonucleic acid), contain subunits—nucleotides—that code for specific amino acids. When a cell needs a particular protein, the gene or genes that hold the instructions for its manufacture are turned on or "expressed," as scientists say. (When genes are not at work they are "silent.")

The revolution in cellular and molecular research in recent years has been largely the result of the new technologies of gene splicing—recombinant DNA—and monoclonal antibodies. Explained simply (more simply than achieved), scientists can cut a sequence of DNA from the nucleus of a eukaryotic cell, using special enzymes as "shears," and insert it into the DNA of another cell, usually a bacterium. The bacterium will then possess and pass on to its progeny the ability to code for

the product of the inserted gene. Bacteria are simpler cells than eukaryotes. They have fewer genes and they reproduce quickly. Gene splicing provides a rich and easily retrieved source of the inserted genes and their products.

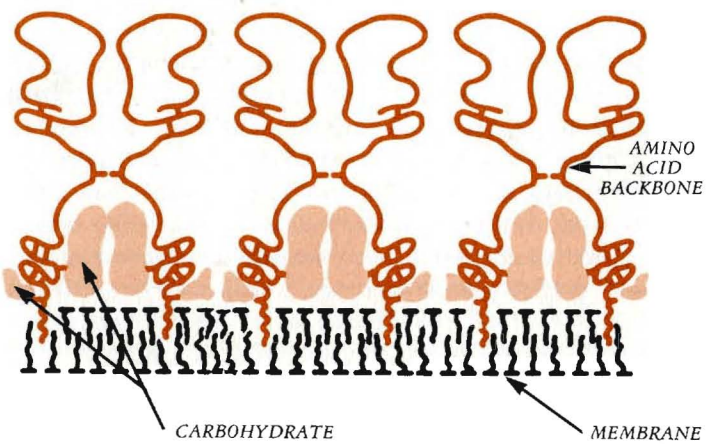
Monoclonal antibodies are produced in test tubes by cell fusion and cloning techniques. Each monoclonal is a single molecular species of antibody, arising from a single antibody-producing cell, and will usually have exquisite specificity for a single antigen. "This property," says Dr. Cross, "enables us to use monoclonals as unsurpassed reagents for the localization, assay, and extraction of the corresponding antigens."

In 1977 Dr. Cross accepted an offer to lead his own research team as head of the department of immunochemistry of the Wellcome Foundation Limited, at its main research facility in Kent, England. Wellcome is a multinational manufacturer of pharmaceutical and biological products. The profits of the company, administered by the Wellcome Trust, are used to support medical research throughout the world. At Wellcome, Dr. Cross supervised studies of bacterial, viral, and parasitic biology. His laboratory also began work toward a malaria vaccine and played a key role in the development of clinical-grade leukocyte interferon. (Interferons are small proteins, produced by cells infected by viruses, that have the ability to inhibit virus multiplication. They have been under intense study in recent years as potential therapeutic agents for various viral diseases and some cancers. Because the body produces interferons in such infinitesimal quantities, it was difficult and expensive to study them before the advent of gene-splicing technology.)

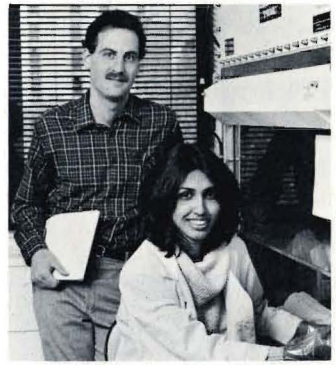
Reflecting the remarkable events unfolding in science at the time and Dr. Cross's vigorous leadership, the department expanded its scope and changed its name to molecular biology. When he joined, there were eight people in the group. By the time he left to come to Rockefeller, there were thirty-six. He himself continued working mainly on trypanosomes and began what proved to be an extremely productive collaboration with the molecular biology laboratory headed by Dr. Piet Borst at the University of Amsterdam.

"At the time I was setting up at Wellcome," says Dr. Cross, "we had neither the expertise nor the resources to start recombinant DNA studies ourselves. I asked Piet Borst if he'd be interested in working with us since he was also studying trypanosome gene function, although not antigenic variation. Together we were able to obtain clones coding for the variant glycoproteins and from that we could go on and get some idea of the genetic mechanisms." Dr. Cross and Dr. Borst discovered that the basis of antigenic variation lies in the ability of *Trypanosoma brucei* to express *alternative* genes for different antigens. What does this mean?

"Many microorganisms can vary antigenically," Dr. Cross explains. "Bacteria can and so can viruses. They change through the normal evolutionary process of mutation. Influenza viruses, for example, are continuously evolving new mutant forms, which is why immunization against one strain usually doesn't protect against another. The new strains have altered genes. What makes trypanosomes unique is that they have evolved a thousand different mutant genes along the line *and kept them all* imprinted in the genome, the genetic bundle, of every trypanosome cell. So far as we know, those species of trypanosomes with this capability represent the only situation in nature, outside of the system for producing antibodies in vertebrates, in which there is a large set of genes



Digrammatic representation of the molecular structure of the trypanosome surface coat. The glycoproteins are paired and closely packed to prevent antibodies from reaching the cell membrane.



Drs. Michael Wallach and Kasturi Haldar, who are studying the genes and membranes of malaria parasites.

Postdoctoral Fellows Gretel Lamont, Vivian Bellofatto, Michael Ferguson, and Doris Cully confer over coffee.

and gene products whose primary function is to exhibit variability. It's an extraordinary survival device."

In order to be expressed, a gene for a particular variant glycoprotein must first be copied into a special place on the chromosome called the expression site, where signals start the production process. "Until recently," says Dr. Cross, "we weren't able to obtain recombinant clones of an expressed copy of the gene, which made it difficult to characterize the expression site. Our friends in Amsterdam showed conclusively that the expressed copy is inserted in what is called the telomere, the end region of a chromosome. We got very excited when we found the expression site because we thought that we would soon find the promoter signal, the mechanism that initiates the transcription of the genes from DNA into RNA. (RNA is another nucleic acid which, in its "messenger" form, carries genetic instructions it has transcribed from DNA to the site in the cell where proteins are manufactured.) "We identified a short nucleotide sequence which is added to the beginning of all VSG RNA transcripts. We expected it would enable us to pinpoint the location of the promoter, which we assumed would be adjacent to the expression site. Unfortunately, that has turned out not to be the case. After two years of trying, we haven't yet discovered how the promoter is physically linked to the gene it's going to transcribe. Neither we nor anyone else understands exactly how VSG gene transcription is regulated. It's one of the key questions right now.

"There are a lot of things we don't know. We know nothing about how the gene switching is programmed: what determines when the gene in the expression site will be eliminated and replaced by a new gene. We don't know whether the tryps are triggered by an environmental event—something that happens to them from the outside—or whether there is an intrinsic timing mechanism that initiates gene-switching action at predetermined intervals, which is the answer most researchers in the field currently favor. We can't yet answer the most obvious question, which is the frequency with which gene replacement occurs.

"While we're trying to understand the genetics of antigenic variation," Dr. Cross continues, "we haven't lost sight of the antigens themselves. As antigenic variation pretty well precludes the possibility of immunization—of making a vaccine effective against every antigen form—it may turn out to be more practical to develop chemotherapy for the disease: to find ways to attack the antigens themselves, at the surface of the cell, rather than the genetic machinery; which is why we're continuing to study the biochemistry of the surface. We still don't have a complete understanding of the structure of the glycoproteins and the way in which they're attached to the cell surface, although since moving to Rockefeller we've made a major breakthrough in regard to the structural basis of the antigen-membrane linkage. This linkage is also novel to tryps and we see it as a potential point for therapeutic intervention."

DUAL SATISFACTION

Dr. Cross and his colleagues are also studying malaria parasites. They are following up observations about some unsuspected properties of the surface of those cells which first came to light in the Rockefeller laboratory of Professor William Trager. They came to light because in 1976 Dr. Trager succeeded in developing a method for cultivating and thereby observing the parasite in a test tube, something parasitologists had been trying to do for forty years. Unlike trypanosomal sleeping sickness, which has a limited geographical range, malaria is the premiere health problem in large areas of the world. "But like trypanosomes," says Dr. Cross, "malaria parasites exhibit unique phenomena which seem to be the consequences of novel gene products and gene rearrangements.

"In a nutshell, our focus includes both membrane and genetic phenomena. We're studying them in parasites, but they are two of the most basic aspects of cell biology no matter what cell you're studying. The membrane is the interface between the cell and its environment, and what happens at the membrane is controlled by the genes.

"What's so exciting about this field to me is that, if you choose the right parasites, you can have the dual satisfaction of working with organisms of major medical importance and on questions of fundamental biological interest." □

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