

Multidrug Resistance in Cancer

An ancient pump protein that flushes toxins out of cells may be to blame when cancer chemotherapy fails. It's identification offers hope that multidrug-resistant cancers might be made vulnerable again

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Ms. Smith had complained of cramping abdominal pain to her family doctor. After a thorough examination she was referred to a local cancer clinic, where she was diagnosed as having an abdominal tumor and was immediately booked for surgery. Removal of the primary tumor was successful but, as is often the case, the cancer had already spread widely to other tissues. The patient underwent chemotherapy with a combination of anticancer drugs, the method of choice for delocalized tumors that are untreatable by surgery or radiation, and the response was miraculous. By all available diagnostic methods she was free of disease. Three months later, during a routine follow-up, Ms. Smith was found to have relapsed: tumors had emerged in several organs. She underwent a second course of chemotherapy but responded poorly. A month later a third course of chemotherapy, with different drugs, had no effect on the growing tumors. Three weeks after that Ms. Smith died.

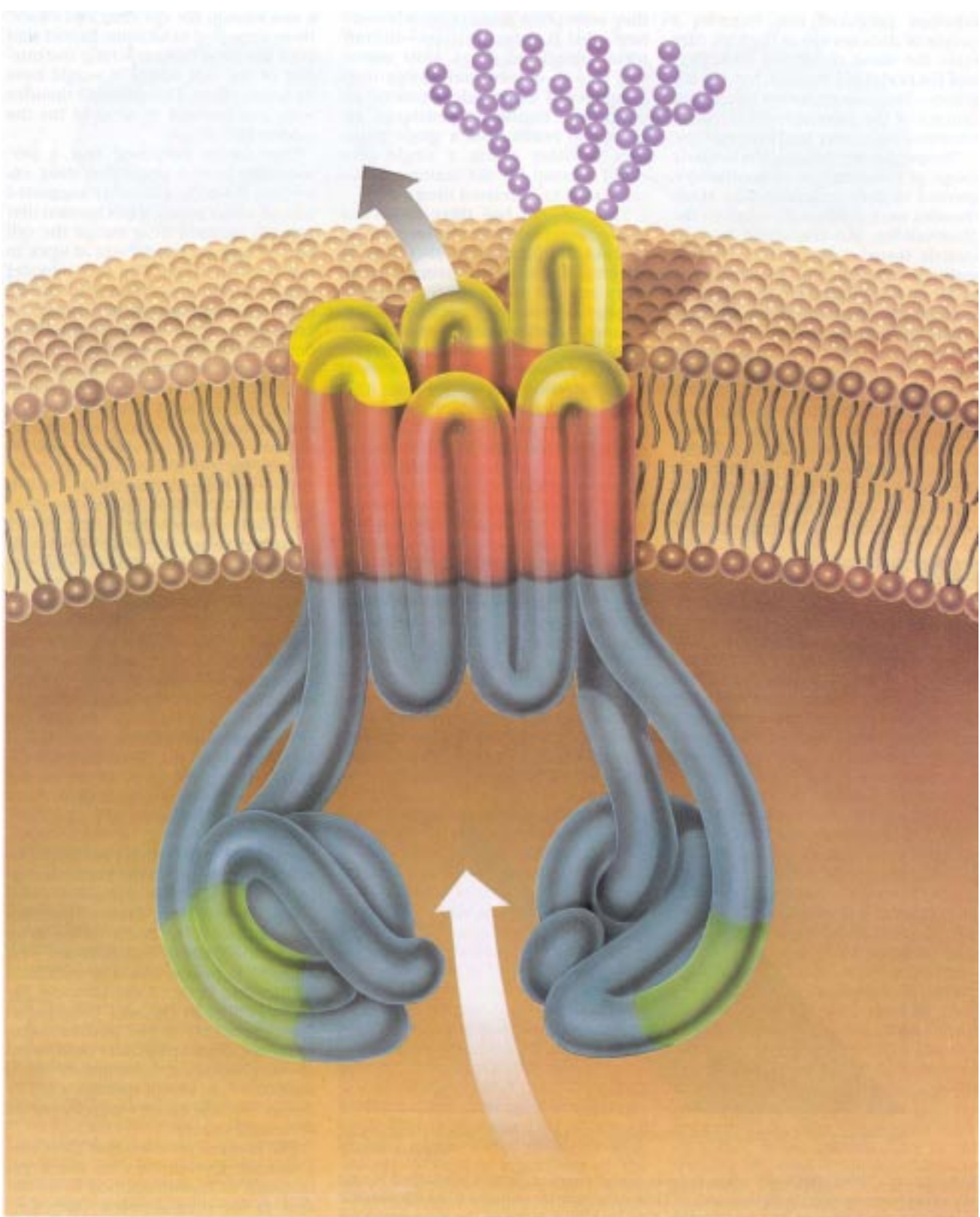
Why did chemotherapy eventually fail Ms. Smith when at first it had appeared to be highly successful? Why are some cancers curable by chemotherapy alone, whereas others are unaffected by drugs and are apparently incurable? These are not new questions. Indeed, the resistance of parasites and infectious-disease organisms to antibiotics is as old as chemotherapy itself. The German chemist Paul Ehrlich, the father of chemotherapy, had envisioned "magic bullets," drugs that would cure many of the diseases that plague mankind, but after decades of experience with antimicrobial drugs he lamented that drug resistance had followed the development of new drugs "like a faithful shadow."

Because cancer chemotherapy has its roots in the antimicrobial chemotherapy that has been under development since the turn of the century, clinical resistance to anticancer drugs was not entirely unexpected. A decade before cancer chemotherapy came to the clinic soon after World War II, experiments with tumors transplanted into mice had already demonstrated the development of progressive resistance to experimental drugs. Since then experimental tumors resistant to every class of anticancer drugs have been isolated. All organisms, including the cells within a cancer patient's tumor, seem to have the capacity to become resistant to drugs that would otherwise kill them.

The underlying cause of progressive drug resistance, whether in infectious diseases or in cancer, is the same. Spontaneous genetic mutations occur in all living cells, giving rise to heritable traits that are passed on to succeeding generations. In any cell population, mutants that are resistant to a given drug occur at a frequency of somewhere between one in 10⁵ and one in 10⁸ cells. How can such a rare event have an impact on the outcome of chemotherapy? To answer this question, one needs first to consider the microscopic scale of the cell and the limitations of methods for the early detection of cancer.

A tumor of average detectable size, say one centimeter in diameter, already contains hundreds of millions of cells, some of which are likely to be drug-resistant. Hence, in spite of the rarity of mutations that produce drug resistance, tumors containing some drug-resistant cells at the time of diagnosis seem likely to be the norm. The outcome of treating such a tumor with a single drug can be predicted. At first a remission will be seen, in which the tumor shrinks to an undetectable size as a result of the killing of the predominant drug-sensitive cells. Yet the remaining drug-resistant cells, all of whose progeny are also drug-resistant, continue to multiply; they eventually dominate the cell population of the tumor, which grows to a size that results in the death of the patient. It has been confirmed that even a single drug-resistant cell introduced into an otherwise curable tumor transplanted into a mouse will eventually multiply in the course of chemotherapy and dominate the tumor-cell population, resulting in an incurable and ultimately fatal disease.

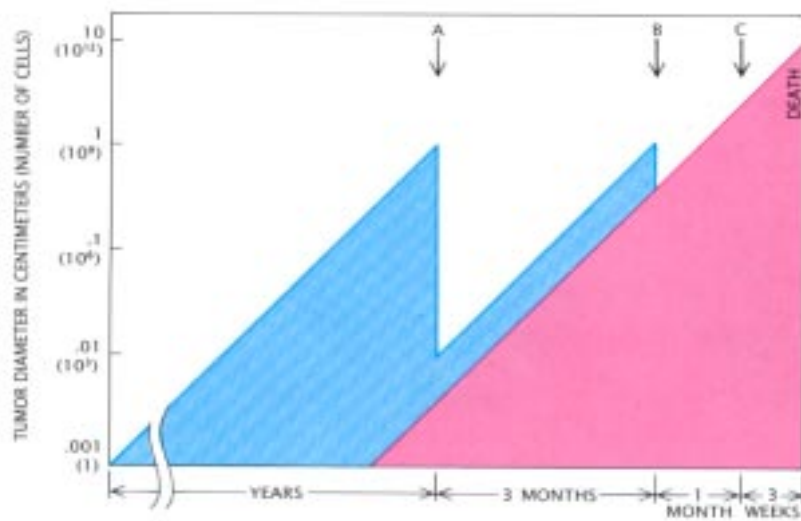
The solution to the problem would seem to be rather simple. In theory, treatment at the outset with a combination of drugs that act differently should preclude the outgrowth of a drug-resistant tumor, because the probability that two or more different drug resistances would arise spontaneously in the same cell is very small. Combination chemotherapy seemed to be the answer.



P.GLYCOPROTEIN resides in the cell membrane, where it may act to pump toxins out of the cell. The painting shows a model of the protein's structure that is based on its known sequence of amino acids. The protein chain is thought to snake back and forth 12 times across the lipid bilayer of the membrane forming a 12-sided pore. The pan of the protein outside the cell bears sugar chains (purple); two large and nearly identical domains protrude into the cell. They include regions (green) that bind the cellular energy-carrying compound ATP, which probably provides the energy that drives the efflux (arrows).

Recognizing the need for an arsenal of drugs and the futility of the single magic bullet for the treatment of advanced infectious disease was arguably one of the conceptual landmarks of pharmaceutical research in the first half of this century. Most of the tenets of antimicrobial chemotherapy were adopted wholeheartedly by cancer clinicians, who demanded research into the production of novel drugs and developed protocols for administering drugs in combination. Newly developed drugs and combination chemotherapy produced real victories a couple of decades ago in the high cure rates for some childhood leukemias and for Hodgkin's disease, but the big killers-lung cancer, breast cancer and cancers of the gastrointestinal tract- remained refractory to chemotherapy.

The perplexing failures, particularly those of combination chemotherapy, seemed to defy understanding. Many theories were proposed to explain the observations. but few could be adequately tested. Early in the development of experimental chemotherapy in mice it was recognized that simultaneous resistance to a number of drugs was an unexpectedly common occurrence. Yet research focused on the more easily understood phenomenon of resistance to single agents. It was not until the late 1960's, when investigators began to do experiments with drug-resistant tumor cells in vitro, that the issue of multiple drug resistance resurfaced and the first insights into what is now known as the multidrug resistance phenotype were gained.



Failure of Chemotherapy often follows initial success. In this simplified model of cancer progression the first course of chemotherapy (A) appears to be successful: it reduces the tumor to an undetectable size by killing most of the drug-sensitive cells (blue). It has no effect, however, on a tiny population of drug-resistant mutant cells (red), which grows exponentially as the drug-sensitive population recovers. Detection of the recurrence leads to a second course of chemotherapy (B). This course again reduces the drug-sensitive population, but it has no effect on the fraction - by now sizable - of drug-resistant cells. a final course of chemotherapy (C) has no apparent effect, and soon unchecked tumor growth kills the patient.

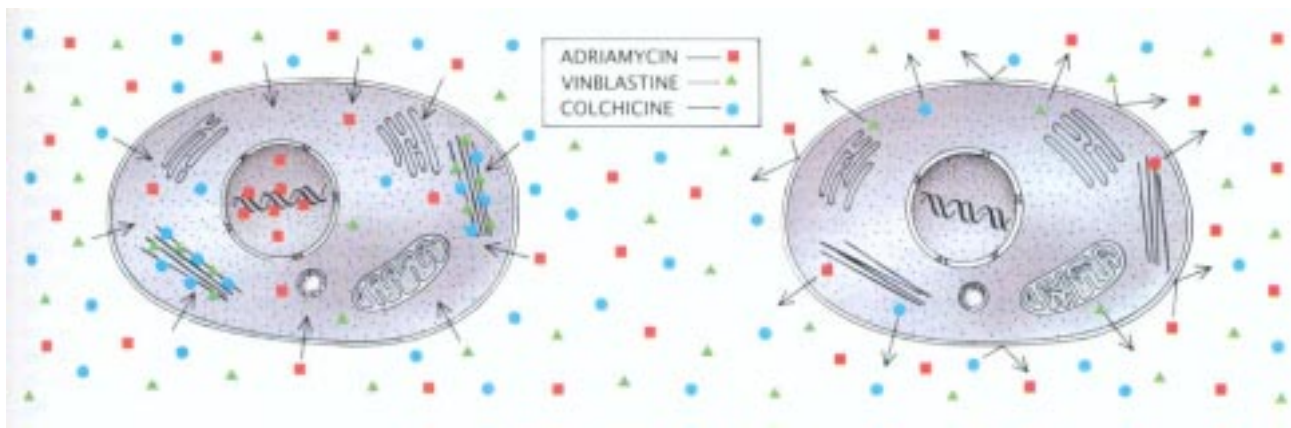
The observations defined the fundamental properties of multidrug resistance. Although drug resistant mutants were selected by means of a single anticancer drug, they were often simultaneously resistant - that is, cross-resistant - to completely unrelated drugs. Most important was an observation arising from a number of independent genetic experiments: multidrug resistance appeared to result from a single mutation. In other words, a single gene could account for the multiple cross-resistance to unrelated drugs.

That concept had three important consequences. It spurred research to find the multidrug-resistance gene in experimental tumors, it stimulated inquiry into the gene's effect and it provided a rational explanation for failures of combination chemotherapy. Since a single drug-resistance mutation is a rare event, the acquisition of multiple mutations in the same cell, yielding resistance to unrelated drugs, is an

occurrence that would be highly improbable. The multidrug-resistance phenotype that resulted from a single mutation explained how resistance to combination chemotherapy could be a common occurrence. But how could a single gene have such a broad effect?

Working with various systems, investigators had found that cells that were resistant to a drug somehow excluded it. This observation suggested a mechanism for the drug resistance: there appeared to be some barrier that kept the drug from reaching the interior of the cell, where it would have its lethal effect. Two possible theories were put forward to account for the evidence.

One theory proposed that a permeability barrier prevented drug entry into the cells. The other suggested that an efflux pump, a mechanism that actively pumped drug out of the cell once it had got inside, was at work in the resistant cells. The latter model was based on observations of the kinetics of drug flow into and out of the cells. It was found that when a resistant cell was temporarily poisoned with cyanide to inhibit energy production, the cell behaved like a drug-sensitive one: it could not keep out the drug. When the cyanide was washed out and normal metabolism was restored, the cell could once again exclude the drug. Furthermore, the cell was then able to pump out the drug that had accumulated while



MULTIDRUG RESISTANCE enables a cell to withstand the effects of toxic molecules that vary in size, structure and site of action in the cell. A common anticancer drug, adriamycin, acts in the nucleus of a drug-sensitive cell (left), interfering with the transcription of DNA and its synthesis during cell division.

Two other toxic compounds used in chemotherapy and studies of drug-resistant cells, vinblastine and colchicine, affect microtubules, which play an important role in cell division. Either a passive barrier or an active pump in the cell membrane could explain simultaneous resistance to such diverse agents (right).

it was poisoned. Hence an energy-dependent drug-efflux pump seemed to be the simplest explanation.

Whatever the actual mechanism, two points seemed clear. One was that the process of keeping drugs out of the cell would need to be rather nonspecific, that is, able to cope with drugs of diverse molecular structure. The other was that because the cell's surface membrane (the plasma membrane) is the first line of defense against the entry of drugs, the difference between drug-sensitive and drug-resistant cells would probably be found there.

Perhaps the first direct evidence of a specific alteration of the plasma membrane in multidrug resistance came from our studies of Chinese hamster cells that were resistant to the drug colchicine, done in collaboration with Rudolph L. Juliano and later with John R. Riordan, both of the Hospital for Sick Children in Toronto. We separated components of the plasma membranes of Chinese hamster cells by gel electrophoresis, a technique in which molecules are drawn through a gel by an electric field and are thereby sorted according to size.

The process revealed that there was a unique glycoprotein in the drug-resistant cells that seemed to be absent in the drug-sensitive ones. Glycoproteins are complex molecules, made up of protein and carbohydrate, which are usually associated with the plasma membrane. This glycoprotein was rather large in size (its molecular weight was roughly 170,000), and it turned out to be associated specifically with the plasma membrane. We named it P-glycoprotein for its association with the apparent permeability barrier to drugs that accompanied multidrug resistance.

Not long after the first report of P-glycoprotein in Chinese hamster cells, similar findings were reported by other groups. Each group was working with a different tissue-culture system. A variety of mouse, hamster and human cells were selected for resistance to anyone of a variety of drugs: adriamycin, colchicine, daunomycin, vinblastine, vincristine and so on. All these systems showed extensive cross-resistance to unrelated drugs, reduced intracellular accumulation of the drugs involved and alterations in the cell's surface membrane. The most consistent of the observed alterations was the appearance of a high-molecular-weight cell-surface glycoprotein similar in size to the P-glycoprotein. These findings prompted us to ask whether the various phenomena were in some way related.

To answer that question more specific tools were required, and so we decided on an immuno-chemical approach. The technique involves creating traceable antibodies that adhere to a specific molecule such as P-glycoprotein, so that it can be isolated and studied. To develop the highly specific antibodies called monoclonal antibodies, we first injected purified plasma membranes from multidrug-resistant cells into mice. Then, by fusing spleen cells from those immunized mice with immortal tumor cells, we produced clones of identical antibody-secreting hybrid cells. We isolated the clones that secreted monoclonal antibodies to P-glycoprotein.

We then sought to determine whether an increased amount of P-glycoprotein was correlated with a high degree of cross-resistance. To do so we employed a technique known as immunoblotting, in which the antibodies served to identify P-glycoprotein in a complex mixture of proteins and glycoproteins that had been separated by gel electrophoresis. Not

surprisingly, the immunoblots showed that there was very little P-glycoprotein in the drug-sensitive Chinese hamster cells; progressively more P-glycoprotein appeared in cell lines that were found to be increasingly resistant to the drug colchicine.

We had foreseen that result. When we applied the same method to a variety of cell lines supplied by other groups, however, we were astonished to see that Chinese hamster, Syrian hamster, mouse and human cell lines selected for resistance to a variety of drugs all showed components like P-glycoprotein in their plasma membranes. These components not only were indistinguishable in size but also reacted with the same antibodies that were highly specific for P-glycoprotein in Chinese hamster cells.

It had become clear that P-glycoprotein was a conserved molecule: a molecule that has retained its structural identity across different mammalian species. Moreover, regardless of the species of origin or the drug of selection, the drug-resistant cells all exhibited a large elevation in P-glycoprotein expression in concert with the development of drug resistance. Conservation of structure in biological molecules is usually indicative of an important functional role; this premise, and the concept of the universality of p-glycoprotein expression in concert with multidrug resistance, was of key importance in establishing the direction we would take later.

Clearly P-glycoprotein seemed to play an important role in multi-drug resistance. We therefore turned to tools of molecular biology that could give us a very close look at the structure and ultimately the function of the molecule itself. The most powerful way to determine the predicted structure of a protein is to find the genetic sequence that encodes it. The appropriate tool is complementary DNA, or cDNA, which corresponds to the DNA that encodes the protein whose properties are being studied.

Our cDNA was developed in collaboration with Riordan, who had established a cDNA library from highly colchicine-resistant Chinese hamster cells. A cDNA library is a living repository for genetic material. This one consisted of a mixture of bacteria, each infected by a virus called a bacteriophage. These particular bacteriophages were recombinant, that is, their genetic material contained an inserted fragment of foreign DNA. The foreign DNA was cDNA derived from messenger RNA that was being actively translated into various proteins by the drug-resistant cells. (Messenger RNA, or mRNA, is the intermediary in the transfer of information from DNA to proteins.) The cDNA fragments were inserted into a viral gene coding for the enzyme beta-galactosidase. When the viral gene infiltrated the bacteria's genetic material, the bacteria expressed an altered beta-galactosidase that contained an additional protein fragment, one encoded by the cDNA.

To find the cDNA that corresponded to P-glycoprotein in dishes containing tens of thousands of bacterial colonies, each one producing a different protein fragment, would seem equivalent to finding a book in the stacks of the University of Toronto library by looking at each volume- But with a radioactively labeled monoclonal antibody acting as a "magnet" specific for P-glycoprotein, we quickly identified the right cells. Growing the selected colony, in which all bacteria originated from a single cell that carried a single cDNA fragment, resulted in the isolation of a cloned fragment of P-glycoprotein cDNA. This cloned fragment could then serve as a probe with which to perform blot hybridization, one of the powerful analytical methods of modern molecular biology.

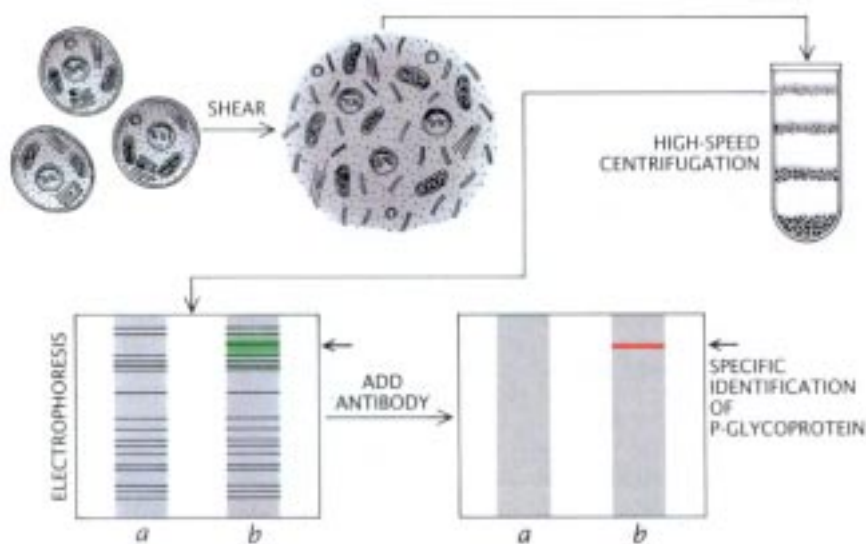
Blot hybridization, in which a cDNA probe serves to pick out corresponding sequences in DNA or RNA that has been separated by electrophoresis, provided insight into both the nature of the P-glycoprotein molecule and how it functions. Two types of blot hybridization were done. In the first one, known as Northern blotting, the isolated cDNA was used to probe mRNA derived from different cell lines- It became clear that an mRNA species about 4.5 kilobases long (an mRNA including about 4,500 of the chemical subunits known as bases) was associated with various multi- drug resistant cells and Dot with their drug-sensitive counterparts; the more resistant the cells were, the larger the amount of the specific mRNA species was. It was reasonable to suppose this RNA was associated with the production of P-glycoprotein.

A second method, known as Southern blotting, employed the cDNA to probe genomic DNA, the DNA of the cell nucleus. From the results of this work it became apparent that the increased amount of P-glycoprotein seen in multidrug-resistant cells arose through a process of gene amplification: an increase in the number of copies of a gene. As many as 60 copies of the same P-glycoprotein gene could be seen in resistant cells. This observation confirmed independent evidence that multidrug resistance resulted from gene amplification. In our system Southern blots revealed multiple bands in both drug-sensitive and drug-resistant cells instead of the one or two bands expected for a single gene. The simplest explanation was that there was more than one multidrug-resistance gene in the normal genetic makeup of the cell-not identical genes, but very closely related ones, constituting what is known as a multi gene family.

While that work was in progress, other groups were looking into multidrug resistance. Different approaches were being taken by Igor B. Roninson of the Massachusetts Institute of Technology and Piet Borst of the Netherlands Cancer Institute in Amsterdam. They cloned ONA fragments associated with multidrug resistance by independent means. Regardless of the methodology, their work confirmed our results: gene amplification and the overexpression of a 4.5-kilobase mRNA were seen in each case. The size of the mRNA yielded a clue to the protein it encoded; it was the length expected or an mRNA coding for a protein the size of P-glycoprotein.

Monoclonal antibodies enabled us to identify our cDNA probe with P-glycoprotein beyond any doubt. The partial gene product made by the bacteria fitted three independent monoclonal antibodies that recognized different sites on P-glycoprotein. An exchange of partial DNA sequence information made it clear that all three groups had independently, and by quite different approaches and rationales, cloned P-glycoprotein genes. A functional, causative role for P-glycoprotein in the multidrug-resistance phenotype seemed assured.

From a rigorous scientific point of view such circumstantial evidence was not entirely satisfying; direct proof was needed, and it was sought. This direct proof came from the laboratory of Philippe Gros at McGill University. Gros took a piece of cDNA that contained the full length or the coding region for P-glycoprotein from a drug-sensitive mouse cell and inserted it into a normal, drug-sensitive hamster cell by a process known as gene transfection. When the progeny of the transfected cell grew in the presence of a selecting drug, Gros could conclude that they were drug-resistant. He isolated DNA and mRNA from the cells and probed them by means of blot hybridization. He found that the hamster cells contained multiple copies of the mouse P-glycoprotein gene and were expressing the gene. Since no other changes had occurred in what was originally a drug-sensitive cell, it appeared that the elevated levels of P-glycoprotein expression alone could account for the drug resistance.



CELL-SURFACE COMPOSITION distinguishes drug-sensitive and drug-resistant cells. The surface proteins are extracted by shearing the cells and placing their components in a sucrose solution whose density increases from top to bottom. When the solution is spun in a centrifuge, components of the cell membrane, which have a low density, form a band near the top. Gel electrophoresis then separates the membrane proteins by size. Multidrug-resistant cells (ti) yield a protein (green band) that is apparently absent in drug-sensitive cells (a). The development of a specific antibody that labels the protein-P-

When the hamster cells were tested for resistance to unrelated drugs, it was found that they displayed the same extensive cross-resistance seen in spontaneously arising multidrug-resistant cells. Since only a single gene, a single member of the P-glycoprotein multigene family, had been inserted, it became clear that a single type of P-glycoprotein molecule could account for the extensive cross-resistance to unrelated drugs that is characteristic of multidrug resistance.

How could a single molecular species such as P-glycoprotein accomplish such an apparently complicated task? The first steps toward understanding the details of how P-glycoprotein works were taken by deducing the full primary amino acid sequence of the protein. Sequencing DNA has become a routine practice, and so the base sequence of the cDNA that represents the full-length mRNA coding for P-glycoprotein was soon determined. Once the coding sequence was known it could be translated into an amino acid sequence. That is possible because the genetic code is known and every three-base codon of the DNA sequence codes for a specific amino acid. The so-called primary sequence turned out to be about 1,280 amino acids long. With the primary sequence available, one could search the sequence for certain structural features known to have specific functions. Reliance on computers and an extensive protein-sequence database makes this a relatively simple task.

Certain features of P-glycoprotein were immediately apparent. Short sequences were found that constitute sites where sugar molecules are added to produce a glycoprotein. Another crucial clue emerged from the fact that different amino acids have different affinities for lipid or water. A so-called hydropathy plot can identify regions of a primary amino acid sequence that would be associated with the lipid bilayer of the plasma membrane. When these regions have a continuous length of about 21 amino acids, they are said to be transmembrane regions: they can span the membrane from the inside to the outside of the cell or vice versa. A number of such transmembrane regions were identified in the P-glycoprotein sequence.

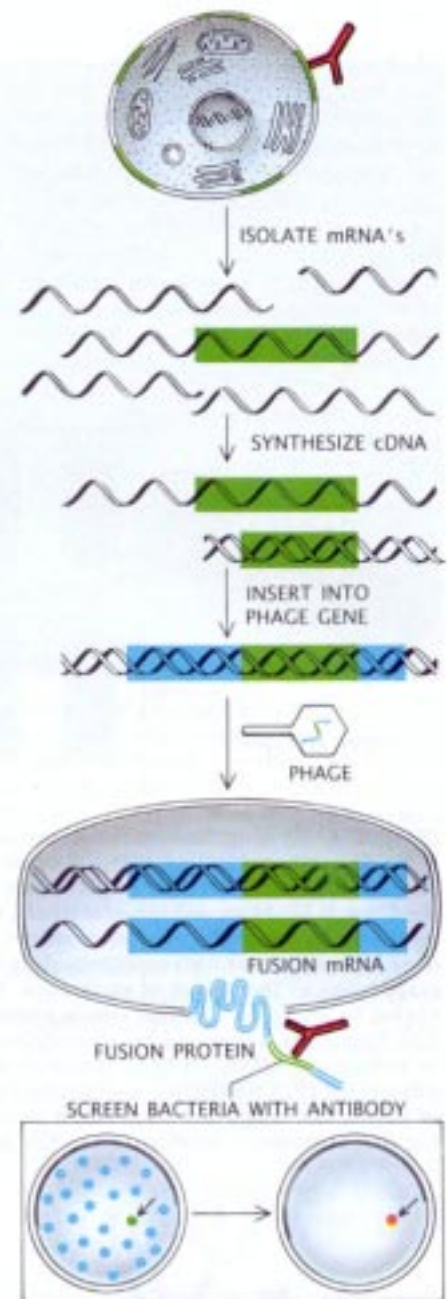
We began to understand the significance of these structural features by comparing their sequences with those of other regions within the same protein molecule and with sequences of other known proteins. In addition to showing that the P-glycoprotein sequences characterized by the different laboratories were very similar, these comparisons revealed some intriguing features of the P-glycoprotein structure. The P-glycoprotein molecule is internally duplicated: the first half of the protein sequence is very similar to the second half. This suggests that a simple ancestral gene was duplicated to give a tandem repeat yielding the more complex protein seen today.

Each half-sequence has six putative transmembrane regions, meaning that the P-glycoprotein molecule can snake back and forth across the membrane 12 times. Such a complex transmembrane configuration is characteristic of channel-forming, or pore-forming, proteins involved in the transport of nutrients, ions and cellular metabolites across the cell's surface membrane, either into or out of the cell.

Comparison with a protein database confirmed the suspicion that P-glycoprotein resembles a membrane transport protein. Regions of similarity with known transport proteins in organisms ranging from bacteria to insects were found. The primary region that showed a high degree of conservation among such widely divergent species turned out to be an ATP-binding region. ATP, or adenosine triphosphate, is a molecule that provides energy for biochemical activity. Both halves of P-glycoprotein contain a lengthy hydrophilic region, a region that is more likely to be in contact with an environment of water than one of lipid. This region was known to be on the inner side of the surface membrane. It was there that the ATP-binding site was situated.

The most surprising discovery on searching through the existing protein data base was that the homologous halves of P-glycoprotein greatly resembled a previously described protein known as hemolysin B. Hemolysin B is present in the surface membrane of certain bacteria and is responsible for transporting a protein called alpha-hemolysin out of the cells.

These amino acid sequence studies and comparisons with other proteins have led to a proposed model for P-glycoprotein structure, one that suggests possible ways the protein might provide multidrug resistance. It is likely that the 12 transmembrane regions of P-glycoprotein converge to form a 12-sided pore. On the outside of the cell there is little exposure of protein; this is the site where the sugar chains that make it a glycoprotein are attached. On the inside of the cell there are two large, homologous

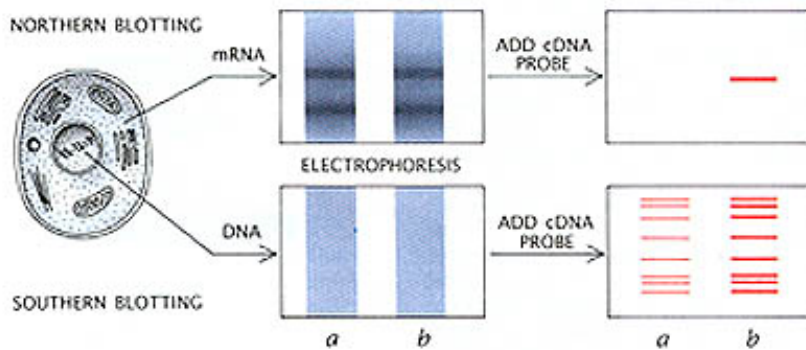


CLONING of P-glycoprotein DNA begins with the identification, with a monoclonal antibody (red), of a cell producing P-glycoprotein (green) and the isolation of messenger RNA's (mRNA's) coding for the cell's proteins. Double-strand complementary DNA (cDNA) is then synthesized for a portion of each mRNA; each cDNA is inserted into a gene (blue) of phage lambda, a virus that infects bacteria. An infected bacterium transcribes the resulting "fusion gene" into mRNA and translates the mRNA into a fusion protein that includes part of a protein from the original cell. Each bacterium carrying a fusion gene multiplies into a clone of genetically identical bacteria expressing the same fusion gene. The antibody identifies the clone bearing the fusion protein incorporating a portion of P-glycoprotein. That clone can then serve as a source of P-glycoprotein DNA.

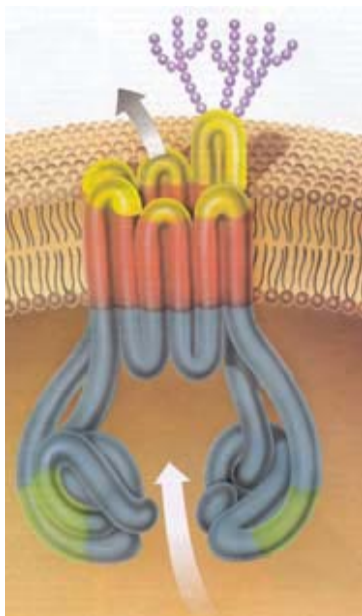
domains projecting into the cytoplasm, which bear the ATP-binding sites.

The sites that accept ATP on the P-glycoprotein molecule suggest that the protein has an energy-transducing function—such as the energy-dependent extrusion of toxic drugs from the cell.

It is likely that the P-glycoprotein pumps drugs out of the cell in one of two ways. Either it binds a variety of drugs and extrudes them directly through the membrane by way of its putative transmembrane pore, or a second molecule (a carrier protein) binds to the drug and the drug-carrier complex is extruded across the membrane. The latter possibility is based on the observation that P-glycoprotein resembles hemolysin B, which extrudes alpha-hemolysin across the bacterial cell membrane. No direct evidence of an ancillary carrier protein



GENETIC BASIS of multidrug resistance can be probed with cloned P-glycoprotein cDNA. In Northern blotting, mRNA is extracted from cells, separated by electrophoresis and transferred to filter paper. When radioactively labeled cDNA is applied to the filter, it binds to, and thereby labels, the corresponding mRNA—P-glycoprotein mRNA in this case. The procedure shows that whereas drug-sensitive cells (a) produce little mRNA for P-glycoprotein, drug-resistant cells (b) produce an amount corresponding to their level of resistance. The source of the increased mRNA is revealed by Southern blotting, which probes DNA rather than RNA. The DNA is cut into fragments, separated by electrophoresis, transferred to a filter and exposed to radioactive cDNA. In both sensitive and resistant cells the cDNA probe identifies eight DNA fragments, suggesting that P-glycoprotein is encoded by a family of genes. The fragments stain much more intensely in resistant cells (b), suggesting that resistance develops when the genes are amplified (copied many times).



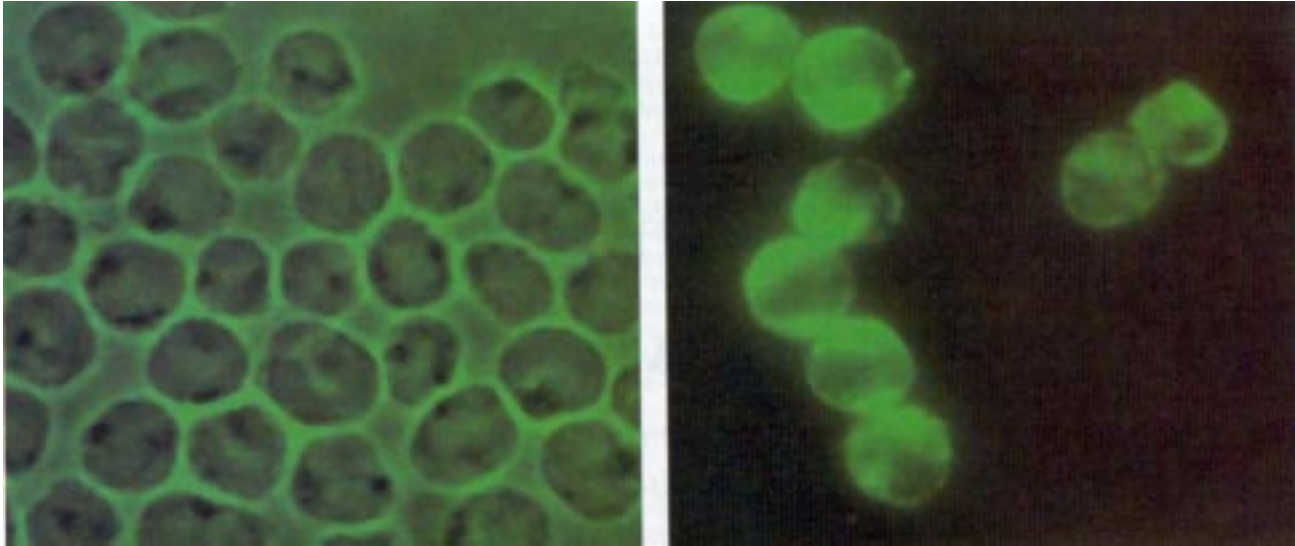
STRUCTURE OF P-GLYCOPROTEIN was inferred from its sequence of amino acids, which revealed that the protein chain (a) has two similar halves. Each amino acid was assigned a value for hydrophathy: affinity for a fatty environment such as the cell membrane rather than an aqueous environment such as the interior or exterior of the cell. A graph of hydrophathy (b) for the chain suggested that 12 separate segments (red) are embedded in the cell membrane. The sequence information also revealed some segments (green) likely to bind the energy-carrying molecule ATP and a region (purple) where sugar chains are likely to be attached. The information about the segments of the chain suggested the model for the protein's structure (left).

For P-glycoprotein has yet been found. There is evidence that some drugs may bind directly to P-glycoprotein, possibly as a first step in their ultimate transport across the surface membrane.

Further sequencing of various P-glycoprotein genes in various species by the increasing number of laboratories working in the field has allowed some comparisons to be made between genes, both within a species and among different species. Such comparisons have shed some light on the evolution of P-glycoprotein and the organization of its genes. The similar organization of coding sequences and intervening sequences in different P-glycoprotein genes from the same species suggests that the internal duplication of the ancestral gene occurred prior to the formation of a multigene family. Similarities in the organization of homologous members of the multigene family in different mammalian species suggest that the formation of a multigene family preceded the divergence of species, at the dawn of the evolution of mammals. The apparently long evolutionary history and conserved structure of P-glycoprotein beg two questions: What is the molecule's normal function and what are the details of its activity? As yet the answers are merely speculative, but two theories have been proposed. One is that P-glycoprotein does the same job in normal cells that it does in drug-resistant cells: it removes toxins from within the cell. A survival strategy that goes far back into evolutionary history is the secretion by an organism of toxic compounds to which it is immune in order to kill nearby competitive organisms. Some of the anticancer drugs in use today—and many

of the antibiotics- are, in fact, toxins produced by lower organisms for precisely that purpose. The evolution of a gene that protects an organism from such toxins would have provided a tremendous survival advantage. The P-glycoprotein gene could be a highly evolved descendant of such a primordial gene, protecting higher organisms from the natural toxins to which they are normally exposed through ingestion of food tainted by spoilage or contaminated by one or another of a myriad of toxic plants.

A second possibility is that P-glycoprotein is involved in some transport process critical to the physiology or development of a complex organism such as a mammal. With cDNA probes and monoclonal antibodies it has been determined that P-glycoprotein is normally expressed in the kidneys, adrenal glands, liver and parts of the gastrointestinal tract of the normal adult. These tissues are involved in the transport of nutrients and solutes and in secreting a variety of protein and steroid substances; perhaps p-glycoprotein plays a role in some of the processes.



LEUKEMIC CELLS, some of which are drug-sensitive and others of which are drug-resistant, are indistinguishable in a photomicrograph (left). When the same cells are exposed to a fluorescent antibody that binds specifically to P-glycoprotein, a fluorescence micrograph made under ultraviolet light reveals only the drug-resistant cells, which are rendered fluorescent because they bear high levels of P-glycoprotein (right). Such antibody testing can serve to identify drug-resistant cells in tumor biopsies, and the antibodies may someday serve as vehicles for delivering toxins capable of destroying drug-resistant cells. The micrographs were provided by Grace Bradley of the Ontario Cancer Institute and the University of Toronto.

The occurrence of P-glycoprotein in the tissues cited above does not preclude the first theory, since some of the organs are also involved in detoxification processes. It is interesting, as an aside, that such organs often give rise to tumors that are innately drug-resistant; that is, they are unresponsive to combination chemotherapy from the start. Possibly the normal expression of P-glycoprotein in these tissues is preserved in the cancerous cells that originate there. Whatever the normal function of P-glycoprotein may be, it seems likely that it plays some kind of membrane transport role, whether it is the extrusion of exogenous toxic substances or of endogenous, physiologically important cellular products.

Another important question is one that brings us full circle to the rationale for studying experimental drug resistance in the first place: Is P-glycoprotein relevant to the failure of chemotherapy in cancer patients? It has been established that in ovarian carcinomas, leukemia and a variety of sarcomas some of the tumors contain elevated levels of P-glycoprotein. In the small number of cases in which patient follow-ups have been possible, increased amounts of P-glycoprotein have been seen in concert with increasing unresponsiveness to chemotherapy; in perhaps 10 or 20 percent of the tumors tested, clear evidence of raised levels of P-glycoprotein has been obtained. Based on these preliminary studies, one can conclude that a significant fraction of treatment failures might be attributable to P-glycoprotein-mediated multi-drug resistance, although much further work needs to be done to substantiate this conclusion.

As more becomes known about multidrug resistance and the function of P-glycoprotein, ways to improve the effectiveness of drugs administered in chemotherapy will become clearer. Recently it has been found that a variety of compounds inhibit the function of P-glycoprotein, rendering multidrug-resistant tumor cells sensitive to drugs that would otherwise be ineffective. These compounds have been referred to as "chemosensitizers." Preliminary research suggests that some of them act by interfering with the binding of drugs to P-glycoprotein. Presumably the binding of a drug molecule to P-glycoprotein is an important first step in its transport out of the cell, so that blockade of the binding allows the drug to accumulate in the cell and kill it as intended. Subtler means of manipulating P-glycoprotein function may eventually come to light, making it possible to use anticancer drugs-which are often extremely effective in the absence of drug resistance-to their full potential.

Another possible way to defeat multidrug-resistant tumor cells may be to exploit the very fact that they contain P-glycoprotein. Perhaps monoclonal antibodies bearing a radioactive compound or a toxic drug could be targeted to P-glycoprotein in order to kill tumor cells that are untreatable by conventional means. Paul Ehrlich's vision of a magic bullet may yet be substantiated.