

Multidrug Resistance Protein Pumps: Nature's Common Currency

Nearly a decade ago scientists discovered MDR within cancer cells. Scientists are now finding MDR pumps in a wide variety of organisms and throughout these organisms MDR plays a surprising role in defending a cell against toxins.

by Alison Davis

As it tunnels through the salty mudflats of Elkhorn Slough, California, the marine worm *Urechis caupo* collects food by spinning a sinewy, mucous net. But the muddy cocktail trapped within the net contains more than lunch: within lies a virtual potpourri of chemical toxins, some natural and some man-made, that aren't meant to be eaten. Yet as soon as the poisons find their way into

the worm's belly, they are spit back out into the mire.

At a nearby hospital, a breast cancer patient is losing her battle against cancer because the tenaciously dividing cancer cells in her body are using the same molecular gadgetry to jettison chemotherapy drugs back into her bloodstream, preventing them from attacking her tumor.

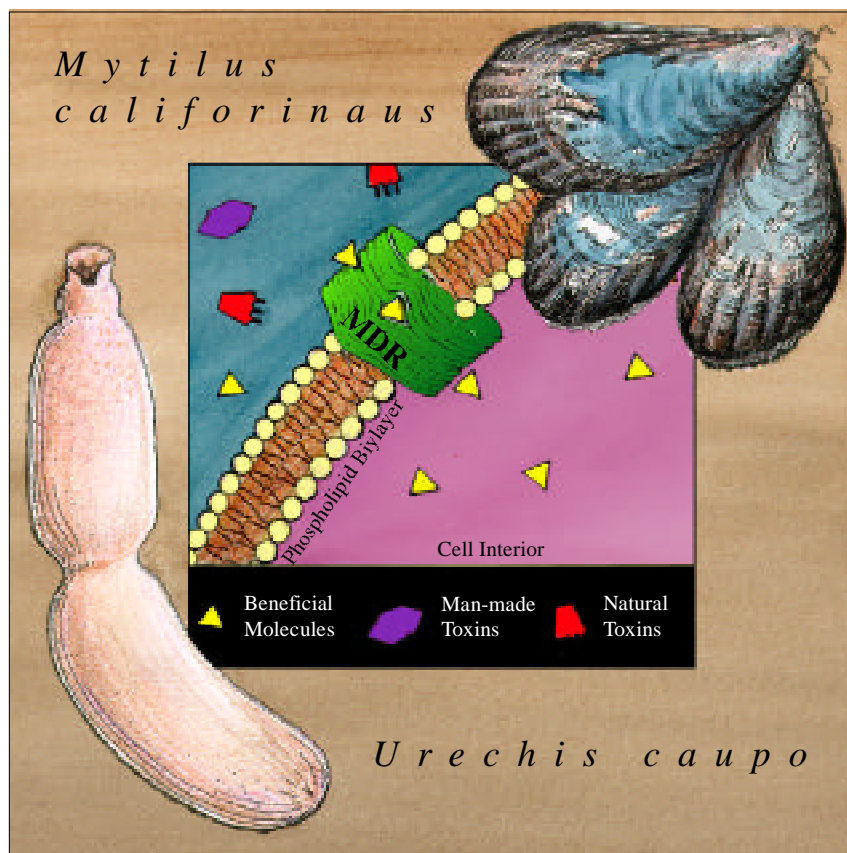
Nature's common currency is a

protein called a "multidrug resistance," or MDR, pump. Embedded within a cell's membrane, this protein protects a cell by ejecting a variety of molecules — in many cases, toxins — on contact. The cell might be a bacterium, in which case the "toxins" are antibiotics. With cancer cells, the "poisons" are chemotherapy drugs. MDR pumps are being discovered in almost every organism searched, including *Urechis caupo* that calls polluted, toxin-laden mud home, says Stanford University embryologist David Epel, who has also found the pumps studding the flapping gills of mussels. Detecting traces of MDR in such a wide array of organisms is leading scientists like Epel to believe that this line of defense has persisted for a long, long time — in biology, usually the signature of a very important molecule.

In the toxic battleground of the ocean, creatures that can cope with what their opponent throws at them are more likely to survive. "It's a space war, and the weapons are chemicals," says Epel, "Organisms use them to compete for the best real estate." The MDR protein, he says, is one way animals have evolved to escort toxins out of a cell. He and other researchers are finding that creatures of all types rely on the pump as a first line of defense — and it's a particularly advantageous one at that. "MDR doesn't even let the things get in the cell," he says.

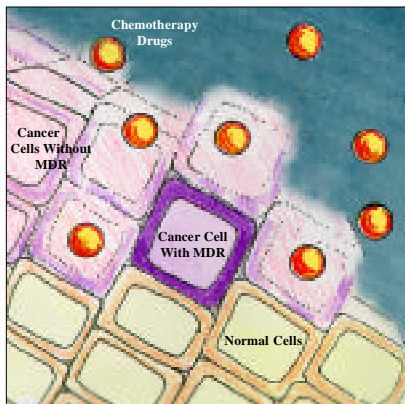
MDR History and Function

The MDR protein got its name from the place it was originally discovered roughly a decade ago: in the membranes of cancer cells. A huge



The marine worm, *Urechis caupo* and the California mussel, *Mytilus californianus* are examples of two marine organisms utilizing MDR pumps. Both live in muddy coastal areas that contain harmful man-made and naturally occurring toxins. The MDR protein pump embedded in the cell walls of these organisms, allows beneficial molecules to pass into the interior of the cell while preventing the entrance of harmful toxins.

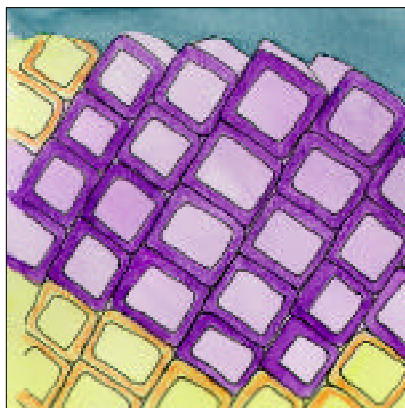
snake-like protein, the MDR pump threads through a membrane like a needle through a piece of cloth, back and forth across the membrane a dozen times. It uses packets of energy made by the cell, called ATP, to force out toxins. In cancer cells, the MDR pump ejects cell-killing molecules such as chemotherapy drugs — a victory for



Chemotherapy drugs are introduced and transported into the interior of cancer cells that lack MDR. As a result, these cells die. Those cancer cells with MDR prevent the drugs from entering the cell and survive.



Cancer cells with MDR continue to replicate and maintain their immunity to the drugs.



Cancerous cell growth continues and can spread unchecked.

the cell, but of course not for the patient, who must then rely on some other form of treatment to keep the cancer at bay.

Many important chemotherapy drugs are denied entry into MDR-laden cancer cells, says Stanford University oncologist Brandy Sikic, who for years has studied multidrug resistance in cancer. “The pump is active in a broad spectrum of human cancers, including lymphoma, leukemia, and breast, lung and ovarian cancers,” says Sikic. And in most cases, he says, how much MDR is present in tumor cells can predict how well the patient fares treatment.

Scientists don’t yet fully understand why some cancer cells are packed with MDR and some appear to have little or none at all, but they do know that chemotherapy drugs themselves can sometimes prod MDR production within a select group of cancer cells in the body. When that happens, those cells have a distinct growth advantage and quickly take over, rendering the drug useless for its original purpose: attacking the tumor.

MDR in Marine Organisms

The presence of MDR pumps in mussels might be an indicator that certain natural and man-made chemicals are present in the sea water, Epel says. And, in fact there may be more than previously suspected because of the way toxins have traditionally been measured. For more than a decade, a biomonitoring effort called the California State Mussel Watch Program has been gauging water pollution concentrations by measuring levels of contaminants in mussel tissues. Mussels were thought to be ideal for the job. Since they eat by filtering water through their gills, they have a high exposure to whatever is in the water, says Nancy Eufemia, a graduate student in Epel’s lab. Also, they are basically stuck in place, she says, so they can’t swim away from something noxious like a fish can.

Epel and his group tested eight compounds on the Program’s list of common pollutants — half of them were spit right out by the MDR pump, suggesting that water levels of some chemicals could be underestimated, Epel says.

Bacteria and Drug Resistance

MDR pumps don’t always work toward the common good. They can bestow deadly power — in the form of resistance to antibiotics — to tiny organisms that can only be seen through the eyes of a microscope: bacteria. While bacterial MDR genes are not identical to those from mollusks and people, the three-dimensional shape of all pumps is much the same, and they all perform a similar task: preventing potentially harmful molecules from setting up shop inside their own quarters, the cell’s interior.

Microbiologist Kim Lewis of the University of Maryland at Baltimore, studies MDR pumps in “gram-negative” bacteria, a group of microbes that includes the recently notorious *E. Coli*. One member of this microbial family, *S. aureus*, can skillfully evade antibiotic-containing hospital handsoaps by immediately rejecting their antiseptic weaponry. “It’s a pretty dangerous pest,” says Lewis.

Many MDR-like pumps also confer resistance against first-line drugs used to treat pathogenic yeasts and parasites, says Lewis. Resistance to drugs called antifungals that are used to treat yeast infections is increasing rapidly, in particular affecting immune-



Top: *Escherichia coli*
Middle: Yeast cells
Bottom: *Staphylococcus aureus*

crippled AIDS patients, who can't fight back, he says. And the latest victim that may succumb to MDR is saquinavir, one of a class of anti-AIDS drugs called protease inhibitors, a recent study suggests. Some bacteria, such as *P. aeruginosa*, have a virtual armamentarium of different MDR pumps. "They extrude practically every [drug] we know of," says Lewis.

Bacteria probably aren't alone in that regard — it is likely that there are probably many as yet unidentified MDR pumps lurking in membranes of all kinds of cells, says Sikic. The latest estimates pin 2,000 of the estimated 100,000 human genes to be part of the family of proteins to which the MDR pump belongs, says Sikic, adding that this huge family also includes proteins that gate the passage of charged ions in and out of cells, called channels. Only a fraction of those genes and the proteins they encode have been studied, Sikic says.

While there are no doubt many more MDR-like proteins to be discovered, what puzzles scientists is the broad spectrum of chemicals, called substrates, that the pumps repel. The list of such substrates includes a wide variety of compounds that are all very different from each other. Some are naturally occurring substances, such as alkaloids in plants or antibiotics in dirt, and some are man-made chemicals, such as certain dyes and some pesti-

cides. The only common thread is that all of them are relatively hydrophobic (they don't dissolve easily in water).

All of this is leading scientists to believe that there are likely a variety of pumps that each deals with a group of its own, similar substrates. But it also leads researchers such as Lewis to suspect that there might also be an as yet unidentified natural chemical substrate that has kept the pumps around throughout evolution.

Epel agrees. While it may be that the primordial role for these proteins was to protect organisms from natural toxins, he suggests, MDR pumps might also transport "normal" substances, such as steroids and some fats, out of cells. For instance, in yeast cells, some MDR-like proteins pump out hormones involved in mating, he says. Yet, in mammals, MDR pumps are positioned so as to encounter potentially harmful substances face-to-face — in important places like the intestine, the placenta, and the blood-brain and blood-testes barriers, making them an excellent first line of defense, he says.

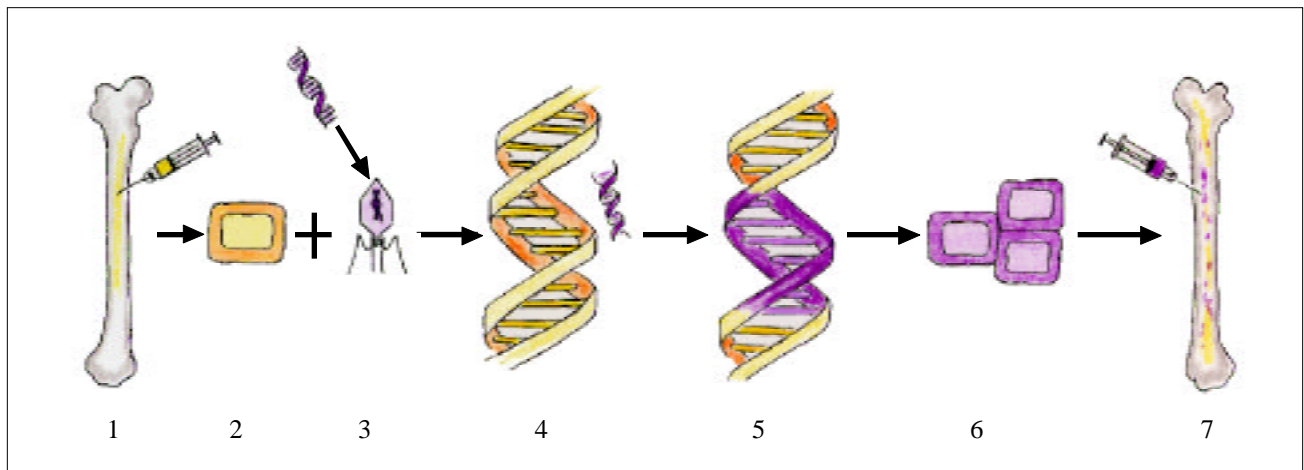
Fighting Cancer with MDR

Cancer researchers are now trying to outwit Mother Nature with her own weaponry: MDR. Some cells in the body, including those in the bone marrow called stem cells, have little or no MDR in their membrane.

For that reason, they are very sensitive to many cancer-killing drugs: indeed, one of the major drawbacks to chemotherapy is bone marrow toxicity, a serious complication that can leave the patient defenseless against deadly infections. Now, scientists are devising ways to equip the helpless stem cells with MDR armor.

By using gene therapy, in which an MDR gene is "pasted" into the DNA of dividing stem cells in the bone marrow, researchers hope to give stem cells a chance to stand up to the doses of chemotherapy required to kill tumors elsewhere in the body. Though that's easier said than done, says Sikic, the strategy has promise. Also in progress are clinical trials to determine whether blocking the MDR pump in cancer cells will allow much-needed chemotherapy drugs easy access that might otherwise be denied entry, says Sikic, who is currently testing such a drug called Valspodar in leukemia and lymphoma patients.

Meanwhile, researchers around the globe continue to search by land and by sea for the MDR signature: indeed, bottom-dwelling flounder are among the most recent club members. As scientists better understand how and why cells universally use MDR pumps, they may well be able to beat nature at its own game. ■



Because of its lack of MDR, bone marrow tends to be highly susceptible to cancer-killing drugs. Scientists have proposed a possible method of gene therapy that would place the genetic code of MDR into the stem cells of the patient. In turn, this would help increase bone marrow resistance to chemotherapy drugs. 1: Stem cells are removed from the patient. 2: These cells do not contain MDR protein pumps. 3: A piece of RNA with the genetic code for MDR is placed within a retrovirus which is then added to the stem cell sample from the patient. 4: The retrovirus attaches to the stem cell and feeds the RNA into the cell. 5: Using reverse transcriptase, the MDR genetic code is incorporated into the DNA of the stem cell. 6: As this cell reproduces the code for MDR is passed on to each new generation of stem cell. 7: The genetically enhanced stem cells can then be re-introduced to the patient. As the stem cells reproduce the number of cells containing MDR would increase, thus increasing the patient's bone marrow resistance to chemotherapy treatment.