wider distribution. This difference alone, according to Jablonski's formula, would be enough to predict the relative eclipse of the marsupials and the relative good fortune of the placentals when the crunch came.

Evolution in the classic Darwinian sense favors advantageous adaptations through natural selection, provided they are heritable. This process is the bedrock of times of background extinction. But when a mass extinction occurs selectivity applies at a level above the species and is blind to individual adaptations. Now, it is clearly of some interest to know whether the characteristic that confers survival advantage on a clade is itself heritable. In other words, do geographically dispersed clades give rise to clades that are also preferentially cosmopolitan?

If clade distribution were in fact heritable, one might expect that selection through a series of mass extinctions would favor the emergence of species that combined traits that were advantageous during background extinction with those that improved survivability through major extinctions. Such a combination would be a sure route to success through the history of life. Jablonski believes he can identify some groups of species that appear to have achieved such a combination and are therefore particularly persistent and diverse, but it seems not to be a general phenomenon. His preliminary assessment, therefore, is that geographic distribution of individual clades is not a heritable trait.

Jablonski's observations on the Cretaceous/Tertiary extinction are echoed in preliminary examinations of other major extinctions, although there are some clear differences too. And, as Steven Stanley of Johns Hopkins University points out, there are certain to be many more factors involved in mass extinctions than are mentioned here, any of which might be emphasized during different events. Overall, however, he describes Jablonski's analysis as an extremely useful approach and one that is consistent with some of his own observations in more recent parts of the fossil record.

If the inference of qualitative differences between major and background extinction holds up generally, a new perspective on earth history emerges. "Currently evolutionary history is formulated almost exclusively in terms of pattern and process during background times," Jablonski notes, "but if mass and background extinctions are qualitatively as well as quantitatively different in their effects, then it is the alternation of background and mass extinction regimes that shapes the large-scale evolutionary patterns in the history of life."

The qualitative difference between the two extinction regimes also speaks to the nature and potential cause of mass extinctions. "They are clearly global phenomena," he says, "probably involving worldwide change in climate, seasonality and productivity." Such events are consistent with, but do not prove, catastrophic impacts with extraterrestrial objects. ■ **ROGER LEWIN**

ADDITIONAL READING

D. Jablonski, "Background and mass extinctions: The alternation of macroevolutionary regimes," *Science* **231**, 129 (1986).

Why Do Cancer Cells Resist Drugs?

Cancer cells that become resistant to one drug frequently become resistant to several other unrelated ones

IT happens all too often. A cancer patient will be given a drug such as doxorubicin or Adriamycin and will go into remission. Then, the patient will relapse and will no longer respond to the drugs that originally destroyed the tumor cells. "The basic question," says David Housman of the Massachusetts Institute of Technology, "is, Why does the patient no longer respond to these drugs?"

What scientists suspect is happening in many instances is that the cancer cells that grow back have learned how to foil the drugs. In tissue culture systems developed to study this problem, the drug-resistant cells apparently turn on and amplify genes that allow them to pump the drugs out as fast as the drugs get in. Moreover, once the cultured cancer cells become resistant to one of a group of unrelated drugs, they are resistant to the others as well. This despite the fact that the only thing these drugs, which include Adriamycin, *Vinca* alkaloids such as vindesine and vincristine, and actinomycin D, have in common is that all are poorly soluble in water. Other than that, they are totally different. They are not similar in chemical structure and they act in different ways to kill cells.

This picture of the biochemistry of multidrug resistance is the product of a new consensus among researchers. Several groups of investigators independently studied this problem, using different methods and with different sorts of results. On 9 and 10 December, they met at a workshop at the National Institutes of Health* to compare notes. The conclusion was that they had all come across basically the same molecular explanation of drug resistance.

The first phase of the work began in 1971 when June Biedler and her colleagues at the Sloan-Kettering Institute for Cancer Research grew cancer cells and exposed them to actinomycin D and selected the cells that became resistant. They obtained cells that are resistant to other drugs as well. At the same time, and independently, Victor Ling of the Ontario Cancer Institute in Toronto unexpectedly obtained similar results in the course of trying to select cells with mutations affecting their microtubules. He exposed cells to drugs—*Vinca* alkaloids and colchicine—that bind to microtubules and ended up with cells that are resistant to a variety of anticancer drugs. Since these results echo what happens in patients, Biedler and Victor Ling began pursuing the problem of determining just what is happening biochemically when cells become resistant.

Researchers soon began seeing evidence that these multidrug-resistant cells may not be accumulating the drugs as sensitive cells do. They found that when they put the drug-resistant cells into a drug-free medium, the drugs pour out of the cells more quickly than they are released from sensitive cells. And if they poisoned the drug-resistant cells by giving them substances that prevent them from pumping chemicals across their membranes, the anticancer drugs remain in the cells. If the researchers then remove these poisons, the anticancer drugs come out of the cells. For these reasons, they concluded that the anticancer drugs enter the resistant cells but are then quickly pumped out before they can do any damage.

Meanwhile, Ling was looking for biochemical changes in the cells that corre-

*The workshop was sponsored by the National Cancer Institute's Division of Cancer Treatment and the General Motors Cancer Research Foundation.

spond to their drug resistance. Since the cells do not accumulate drugs inside their membranes, he looked for changes in the membranes. What he found was a 170,000 molecular weight membrane protein, which he called P (for permeability) glycoprotein. Biedler, William Beck of St. Jude Children's Hospital in Memphis, who worked with human leukemia cells, and others also found changes in membrane proteins as well as in cellular proteins that seem to go along with multidrug resistance.

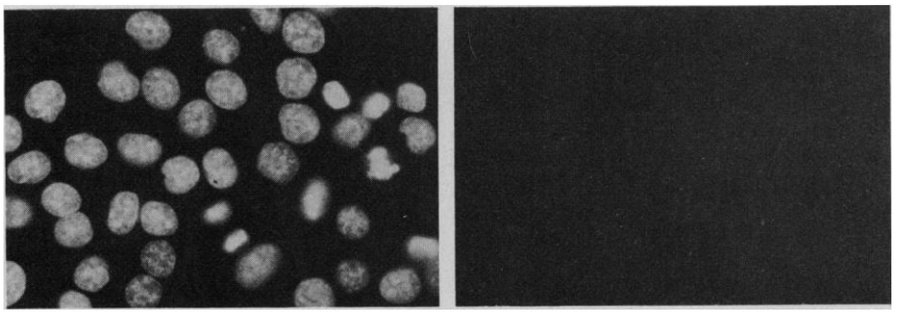
Ling decided to focus on P-glycoprotein, trying to show that its presence correlates with multidrug resistance and that the more of this protein there is, the more resistant the cells are. Recently, he made monoclonal antibodies to the protein and used them to clone the gene that codes for the protein the antibodies recognize. He found that the gene, which he calls P170, is amplified—the cells have multiple copies—in resistant cells.

Independently of Ling and the others who were focusing on membrane proteins, Igor Roninson and Phillippe Gros of MIT and Housman were looking directly for genetic changes in resistant cells. Working with hamster cell lines, they studied resistant cells and then used a technique developed by Roninson that allows them to find and clone amplified genes. They found a large gene or gene family that is amplified 20 to 60 times and that codes for a messenger RNA (mRNA) that is about 5 kilobases in length. But they were not at all sure how, or even whether, their gene was related to Ling's gene and corresponding membrane protein.

Other researchers decided to focus on human cells to see if what holds for cultured hamster cells also holds for cells from humans. Ira Pastan and Michael Gottesman of the National Cancer Institute began by selecting a human carcinoma cell line, KB, that is a HeLa cell variant. Then they and Shin-Ichi Akiyama selected multidrug-resistant mutants. At this point, Pastan and Gottesman began collaborating with Gros, Housman, and Roninson. Gros, Housman, and Roninson had isolated a piece of hamster DNA that contains drug-resistance genes. The question was, Are these same gene sequences present and amplified in the resistant human cells? The answer is yes. And it was confirmed independently by another means.

Tito Fojo in Pastan and Gottesman's lab used Roninson's technique to pull out amplified DNA sequences in the KB cells. Those sequences are the same as the ones identifed by Roninson's probe.

Using Roninson's probe from the resistant hamster cells, Gottesman and Pastan went on to show that the mRNA corresponding to the drug resistance genes is overexpressed in the human cells, mirroring the results previously obtained by others working with hamster cells. And, as in hamster cells, when the human cells are just slightly resistant to the anticancer drugs, the mRNA is overexpressed, as though the gene activity is turned up. When the cells become more resistant, the genes are present in multiple copies.



Mark Willingham, NCI

Drug resistance in human cancer cells

Human carcinoma cells, KB, are treated with the anticancer drug daunomycin, which is fluorescent. Then they are fixed so that only their nuclei remain. Any cell that has daunomycin in its nucleus will appear as a bright spot. The cells on the left are sensitive to the drug, which gets to the cell nuclei. The cells on the right are resistant—virtually no daunomycin gets to the nuclei.

But these experiments still do not tell whether the amplified gene is necessary for drug resistance. To show that, Ling and, independently, Housman and Gros, transferred DNA or chromosomes containing their hamster drug resistance gene to mouse cells and found that, in doing so, they made the cells drug resistant. Pastan and Gottesman transferred their human drug resistance genes to mouse cells and showed that the mouse cells then became resistant. Moreover, Ling's monoclonal antibodies to the membrane protein he identified detected the expression of these drug resistance genes. "All the coincidental evidence indicates we are all probably looking at the same gene," Pastan says.

Still, there also is evidence indicating that the situation may not be quite so simple. It may be that more than one gene is involved in drug resistance and that there are various ways to control the levels of drug resistance in different cells.

Gros finds two pieces of DNA and two mRNA's associated with resistance, indicating that there may be at least two genes or that a single gene may be spliced. Piet Borst of the Netherlands Cancer Institute in Amsterdam presented evidence at the workshop that as many as five different DNA segments, possibly corresponding to five drug resistance genes, are amplified in resistance cells.

Borst and others emphasize that the additional genes may not be directly involved in resistance—they may simply go along as passengers when the drug resistance gene is amplified. As evidence for that hypothesis, they note that the only mRNA that is always amplified in all drug resistance lines is the mRNA for P170. But the other alternative is that more than one gene may be necessary for drug resistance. Which members of a family of genes are amplified may determine the relative resistance of the cells to the different anticancer drugs.

Of course, there are as yet many unanswered questions. One experiment that a number of researchers want to do is to try and isolate the specific DNA segment that codes for the multidrug resistance gene and tranfer it to sensitive cells. If that one gene makes the cells resistant to the panoply of drugs, then that would be evidence that one gene alone is necessary and sufficient for multiple drug resistance.

Another question is whether what occurs in cell lines also occurs in drug-resistant human tumors. The research thus far has involved cell lines almost exclusively. But now that investigators have probes for the DNA segments involved in drug resistance, they can look to see whether human tumor cells also start out by overexpressing the genes and then amplifying them. Ling has preliminary data suggesting that some drug-treated human tumor cells express P170.

And then there is the question of what, if anything, this means to patients. If the mechanisms of multiple drug resistance are understood, can the resistance be overcome? Pastan, for one, is optimistic. "Since a surface protein seems to be involved, it may be possible to make an antibody to it that will stop it from functioning. If we can get rid of that protein, the cell presumably might be sensitive to drugs again." In any event, it can only be helpful to know the molecular mechanism of multiple drug resistance, and most investigators at the workshop seem to agree that they are almost there. ■

GINA KOLATA