Scientists

Following the Membrane Potential

Paul Roepe

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Paul Roepe's research on drug resistance in tumor cells and malaria caught our attention. Curious to know more, *BioTechniques* contacted him to find out about the ambitions, character, and motivations that led to his success.

What do you believe has been your biggest contribution to your field so far?

If I wanted to pick just one thing, it would be expanding on the idea that altered subcellular physiology can promote drug resistance. Early on, it was understood that alterations in drug metabolism, mutations in drug targets, or changes in signal transduction could lead to resistance. We had an example of drug resistance in tumor cells that appeared not to be caused by any of these routes, but there were some signs that it may be due to altered organelle activity.

What prompted you to study subcellular physiology as a fourth possible cause for drug resistance?

Unlike most people in my field, my background is in physical chemistry, not molecular or cell biology or parasitology. When you're a physical chemistry student, you learn about chemical partitioning phenomena; the concept of electrochemical potential as a driving force for molecular transport is a very natural thing to a physical chemist. When drug resistance arises but is not caused by one of those three routes, we often see altered drug accumulation or differences in subcellular drug distribution. Once I started learning about membrane biology, I quickly realized that a cell could manipulate the movement of small molecules and ions by changing the electrochemical driving forces

for their passive diffusion. All eukaryotic cells have an electric membrane potential difference across the plasma membrane. Positively charged molecules want to come in. So a cell can change the degree of that internalization by changing the membrane potential. Because of my physical chemistry background, this popped into my head as a logical area to study when thinking about the movement of drugs into and within a cell.

What subcellular changes lead to drug resistance?

We probably haven't yet defined them all, but one example is found in malarial parasites. The parasites invade red blood cells and eat all of the hemoglobin, which is then broken down in the digestive food vacuole. The PfCRT protein resides in this vacuole, and when mutated, confers chloroquine resistance to the parasite. The protein is unique, with no known homologs or orthologs in other species, so we assume that it performs a unique function. One hemoglobin is one particle, but when degraded, it results in a thousand particles (amino acids and dipeptides plus four hemes). Every particle counts equally in terms of osmotic pressure, so there's a tremendous change in osmotic force during this phase of metabolism. Membranes are designed to regulate electrochemical potential, pH, and osmotic pressure by moving osmolytes in and out of various compartments to prevent them from blowing up like balloons or collapsing in on themselves. We believe that PfCRT is a transporter involved in regulating the osmotic environment of the digestive vacuole and the concentration of drugs within this organelle.

I understand that you originally studied tumor cell drug resistance. How did you make the move into malaria research?

Twenty years ago, I started working on multidrug resistance in tumor cells. The prevailing dogma is that human p-glycoprotein pumps the drugs out of the cell to cause resistance. At that time, the only way to overexpress the human p-glyco-

protein gene was to select transfectants with chemotherapeutic drugs. My question was that after conditioning and selecting with the chemotherapeutic drug, how could we know if the drug resistance was due to p-glycoprotein or to other effects of the drug selection? To answer this, we stably overexpressed p-glycoprotein to high levels in a drug-sensitive cell without using chemotherapeutic drug selection. When we examined the drug resistance of those transfectants, they actually weren't very resistant at all. I became less convinced that human p-glycoprotein was really as dominant a player in human drug resistance as was initially thought.

At that same time, a few papers by Tom Wellem's—a leading malarial geneticist at the National Institutes of Health—really caught my eye. He showed that there were specific mutations in a segment of chromosome 7 that cause chloroquine resistance in the malarial parasite *Plasmodium falciparum*. But the p-glycoprotein homolog for malaria is encoded by a gene on chromosome 5. In these papers, Tom showed that the genetic events that cause drug resistance in malaria don't involve the p-glycoprotein as much as was originally thought. This was analogous to what we were stumbling across in our tumor cells, so I started corresponding with Tom and began looking into malaria drug resistance 15 years ago.

My career started with studying multidrug-resistant tumor cells, but my fundamental interest remains how small molecules travel across membranes and what influences their rate of movement and accumulation. To follow this question, I had to understand the biology of ion channels, small molecule pumps and exchangers, gene regulation, etc. Before I knew it, I had become a biologist as well.

Interviewed by Kristie Nybo, Ph.D. Image courtesy of Jacqueline McCabe.

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