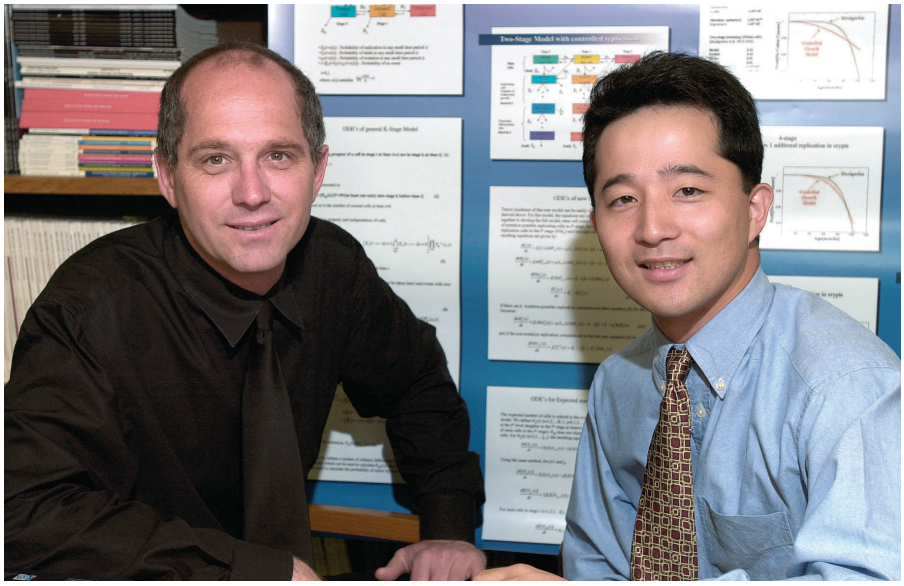
Decoding the Cryptic Origins of Colon Cancer

Cancers of the colon (large intestine) will be diagnosed in more than 130,000 Americans this year, and an estimated 58,000 deaths are expected. Colon cancers are the fourth most commonly diagnosed cancers and rank second among cancer deaths in the United States. The scientific models used today to describe development of colon cancer are deterministic, suggesting that certain mutations in cells will surely lead to cancer. But National Toxicology Program researchers Christopher J. Portier and Hiroyoshi Toyoshiba had a different idea about the probability of cancer developing. They have used computers to mathematically "visualize" what must physically happen within the colon in order for cancer to develop. They show, for the first time, that the biology of the large intestine and the location of errant cells are key factors in whether colon cancer will develop, even if a cell is mutated.



(left to right) Christopher J. Portier and Hiroyoshi Toyoshiba

"All cancer models now being used don't pay attention to the structure of the tissue in which the cancer develops," says Portier, director of the Environmental Toxicology Program. "They act like the cells are floating in space, independent of each other and unrelated to the tissue around them."

The new model offers evidence confirming what biologists had already thought to be true regarding a rare inherited form of colon cancer known as familial adenomatous polyposis (FAP): what they found is mathematical validation that a single gene mutation can result in FAP. Geneticists have long theorized that just a single mutated gene (the *APC* gene on chromosome 5) is to blame for FAP, in which thousands of polyps form in young patients, some of which inevitably turn into cancer. But no cancer model to date had been able to show how that could happen, says Portier.

Portier and Toyoshiba, a visiting fellow in the NIEHS Laboratory of Computational Biology and Risk Analysis, unveiled their new cancer model at the Keystone Symposia Genomics and Genetics of Senescence and Cancer meeting, held 22–27 January 2002 in Keystone, Colorado. Although not yet published or validated, the model has won praise from researchers who have seen it.

"The authors' new model appears to explain the incidence of colon cancer far more accurately than previous models," says Johns Hopkins Medical Institute oncologist Bert Vogelstein, who codiscovered which gene is responsible for FAP. "It should stimulate several interesting lines of investigation, and it will be important to see if it applies to other tumor types as well."

Taking a Stochastic Approach

The Portier–Toyoshiba model incorporates the idea that the growth kinetics of a cell and the structure of a tissue influence the development of cancer. Because these physical factors can either inhibit or promote carcinogenesis, the model introduces the idea of probability. Cancer may develop in these tissues, or it may not; it is not a certainty, Toyoshiba says. The model is, therefore, not deterministic, as many previous colon cancer models are, but stochastic.

"In a stochastic model, you follow the individual cells and work with the probability of a cancer occurring," Portier explains. "In a deterministic model, you deal basically with expectations rather than the probabilities themselves."

He describes the difference using the example of a pipe to illustrate mathematical modeling of continuous versus random events. "A pipe with water running through it can have the flow of water controlled by a valve. Unless you close off the valve completely, there is always something flowing in the pipe, and this is fairly constant. Deterministic models work like this," he says.

"Now, take that same pipe out into your backyard and hold an open end up to the sky during a rainstorm. In this case, instead of a constant flow through the pipe, you have a probability of a raindrop falling into a pipe, and you count drops rather than the amount of water per unit time. That's stochastic."

Portier says the mathematics become more difficult when moving from the continuous deterministic approach to the stochastic approach. "However," he says, "cells are individual units, and cancers are actually rare, given all the cells in the body. Several authors, including myself, have shown that when studying cancer, using a deterministic model rather than a stochastic model for tumor incidence can lead to different results."

Crippled Crypts?

To understand what dictates the probability that cancer will develop, it is necessary to model the colon itself, the researchers say. The colon is a very structured organ. Shaped like a large question mark, the colon is about five feet long and an inch or two in diameter. Its job is to reclaim the excess water from intestinal waste and produce a solid sludge that is later expelled. Doing all that work are a million or so deep crevices called the crypts of Lieberkühn that look like test tubes in a rack. Each crypt constantly produces new cells, which move to the top of the crevice, do their job, and die. When cells within the crypts divide abnormally, however, the mass forms a mushroom-like polyp that can become cancerous.

Much is already known about the crypts. Portier likens these holes in the colon tissue to a shower drain. They extend 40 cells down and form a little sac, and in this sac, colon cancer cells can replicate and grow, and move to the top of the colon epithelium. These cells are continuously replenished in a birth-and-death process that takes 10–12 days.

The cells that repeatedly drive the renewal process are just nine stem cells that lie at the bottom of each crypt, according to the researchers. These stem cells are

Arnold Greenwell/EHP

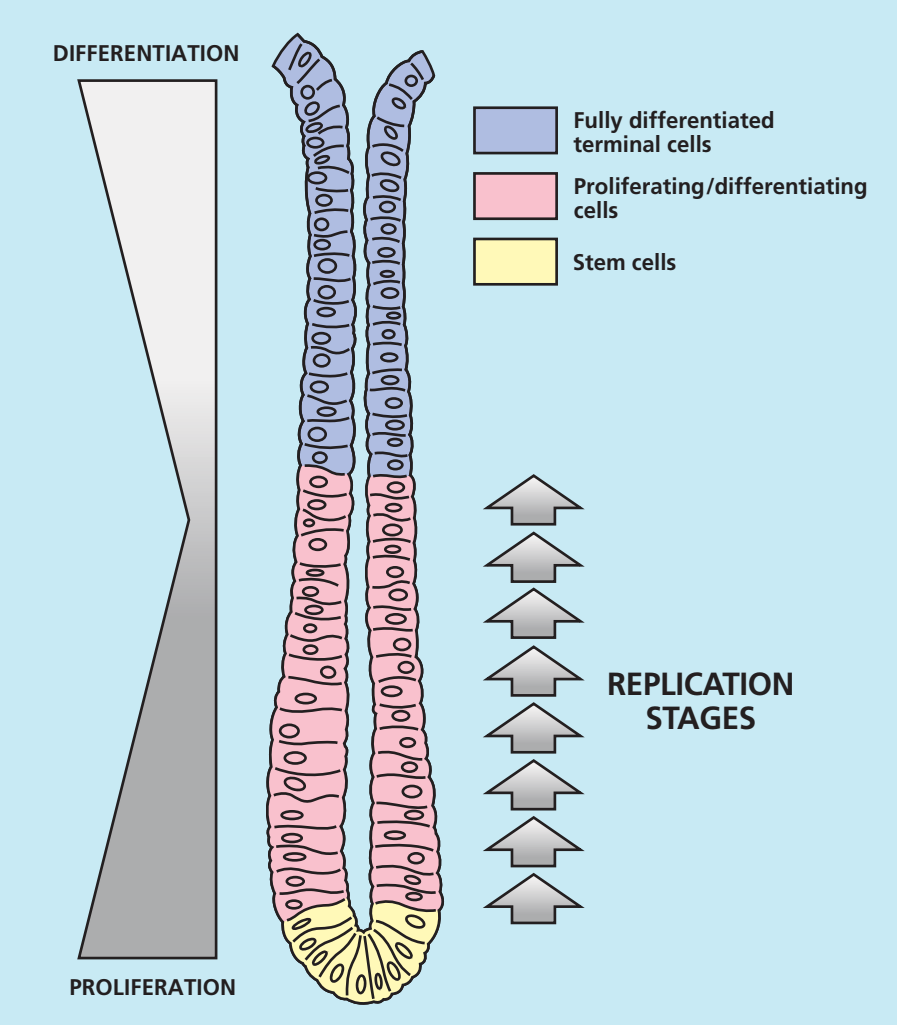
anchored there, and their job is to continually produce daughter cells that then further divide nine times, together generating about 2,000 cells per iteration. As this new small balloon of cells is being formed, it pushes older cells to the surface of the colon, where they reabsorb water and eventually die, and are finally sloughed off in the waste moving through the gut.

Colon polyps form when the cells within the crypt mutate, producing excess cell replication. Cancer occurs when the downward pressure exerted by the polyp pushes mutant cells down to the layer of anchored stem cells, says Portier. When that happens, errant cells can join into the existing stem cell "communication pathway" and pass along faulty genetic information. "It's all location, location, location," he says.

In standard cancer models, all cells are equal, Portier says. There are no stem cells and no daughter cells, and mutations in any of the generations of cells produced are treated equally. In the Portier–Toyoshiba model, however, mutations have to occur in the bottom layer of stem cells or in the first 3–5 generations of cells in order for cancer to have a chance to develop. "After the fifth replication, any genetic mutation in a cell won't count," says Toyoshiba. "That cell will just be pushed up and sloughed off before it can cause damage."

And unlike other cancer models, which hypothesize that cells can go through an unrestricted number of replications, the researchers say their new mathematical model shows that the physical dimensions of the crypts limit the number of cell divisions of both mutant and normal cells. "The usual cancer model results in replication of cancer cells beyond the physiological capability of the living system," Portier says. While the number of division cycles can increase when mutations occur, the birth-and-death cycle is limited for stem cell progeny, and even when the birth rate exceeds the death rate in a normal crypt, the tissue will not grow out of control, says Portier. What results after the first mutation is a polyp that averages about 500,000 cells. This agrees with observations seen clinically, he says.

Using their model, the researchers were able to confirm that a single genetic mutation can lead to the thousands of polyps seen in FAP. This squares with what geneticists have been saying all along, says Portier, and also explains why not every polyp will become cancerous.



Encrypted message. Nine stem cells anchored at the bottom of each crypt continually produce proliferating/differentiating daughter cells, which replicate an estimated eight times, resulting in a bubble of cells that rises to the epithelial surface. According to the Portier–Toyoshiba model, mutations must occur either in the stem cells or within the first 3–5 replications of cells to cause cancer. Mutations occurring after the fifth replication in fully differentiated terminal cells will simply be pushed to the surface and sloughed off.

Christopher J. Portier, Hiroyoshi Toyoshiba, Matt Ray/EHP

Not Just Colons

The model is applicable to any organ that has a structure in which cells form in one area of the tissue and move to another area to serve their purpose and die, says Toyoshiba. That includes the lung, the kidney, and all parts of the gastrointestinal tract. It also explains cancer in organs that are layered, such as the skin. A cell that mutates on an upper layer can form a bump that pushes altered cells down to the lowest layer, where they become "immortalized" as stem cells, says Portier, who adds that he believes "stem cells are at the root of cancer."

The model can further be used to look at changes caused by aging, says Portier. "Aging may be a process in which stem cells just run out of oomph and can't contribute to the repair of the cellular structure," he says. "They are not necessarily mutant, but have slowed biochemical processes, and it may be possible to model that."

Bruce Boman, director of the Gastrointestinal Cancer Program of the Kimmel Cancer Center at Philadelphia's Thomas Jefferson University, says, "This is a novel study that addresses the predicament we are all trying to solve—how do genetic changes actually cause cancer as we know it?" Boman has published work on another computer model that simulates crypt cell dynamics. Using his model he provided evidence that proliferative abnormality in FAP crypts is due to stem cell overpopulation. But he says the Portier–Toyoshiba study contributes a new model for dynamics in the crypt that simulates the "real biological world."

"It's a tremendous effort that requires a lot of theorizing and a lot of computing," Boman says. "But it gives you a different and insightful perspective of mechanisms underlying cancer development."

Renée Twombly