CANCER RESEARCH

A Second Breast Cancer Susceptibility Gene Is Found

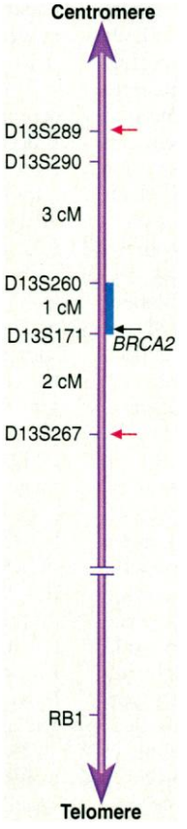
For several years, researchers looking for the genes underlying the 10% or so of breast cancers that run in families experienced more frustration than success. That's one reason why the discovery 15 months ago of the gene known as *BRCA1*, which causes about half of all hereditary breast cancers when it is mutated, was greeted with great excitement by cancer researchers and the public alike. Now they have another reason to cheer.

A large international team, led by Richard Wooster and Michael Stratton of the Institute of Cancer Research (ICR) in Sutton, U.K., and Andrew Futreal of Duke University School of Medicine, has just uncovered a second breast cancer susceptibility gene, called *BRCA2*. "It's a very important discovery. [*BRCA2*] probably accounts for the majority of the familial breast cancer pedigrees that didn't turn out to be due to *BRCA1*," says Francis Collins, director of the National Center for Human Genome Research at the U.S. National Institutes of Health.

Indeed, the discovery, reported in the 21/28 December issue of *Nature*, is sufficiently important to have touched off an apparent patent fight. CRC Technology, a technology transfer company set up by the Cancer Research Campaign, which funded the ICR research, has filed for a patent on *BRCA2* in the United Kingdom. And in a press release issued on 20 December, Myriad Genetics Inc., the Salt Lake City biotech firm and a co-holder of the *BRCA1* patent, also claims to have found *BRCA2* and says it has filed a U.S. patent application on the gene. After seeing the published *Nature* paper, says Peter Meldrum, Myriad's president and CEO, "we believe it's the same gene."

A patent could prove lucrative. Among women having either *BRCA1* or *BRCA2* mutations, 80% to 90% get breast cancer, and *BRCA1* mutations also carry a high risk of ovarian cancer. The new gene, as well as *BRCA1*, should provide the basis for tests to screen for cancer-causing mutations in women from families with a high incidence of these cancers. In fact, Myriad plans to offer such a test for *BRCA1* mutations next year.

The gene discoveries may have broader biomedical and commercial possibilities as well. Researchers hope that both genes will be keys to understanding the biochemical changes that bring about the development of breast cancer in the first place, information that could speed improved cancer therapy. That task is shaping up to be difficult, however, because it's not yet clear what these genes do or whether they even play a role in the 90% of breast cancers that apparently don't run in families.



Zeroing in. A deletion in a pancreatic cancer *(blue shading)* helped locate *BRCA2* within the much larger zone *(red arrows)* where it was originally mapped.

Wooster, Stratton, and their colleagues took their first big step toward finding *BRCA2* in 1994. Based on the inheritance of chromosomal markers in families with hereditary cancers not linked to *BRCA1*, they mapped its location to region q12-13 on chromosome 13, a development they reported in *Science* on 30 September 1994, just a week before the paper announcing that *BRCA1* had been found. At the time, though, the researchers still had their work cut out for them, as the DNA containing *BRCA2* spanned roughly 6 million base pairs, enough to hold perhaps 100 genes. Over the next several months, they narrowed the gene's location to about 600 kilobases of DNA, still a lot of DNA to search. But at that point, the team took a gamble.

Scott Kern and his colleagues at Johns Hopkins University School of Medicine had found a pancreatic cancer in which the tumor cells had a deletion of about 300 kilobases of DNA in the suspect region of both copies of chromosome 13. Such deletions often signal the locations of tumor suppressor genes, whose loss or inactivation can lead to cancer. And because so far all of the known cancer susceptibility genes have turned out to be tumor suppressors—possibly including *BRCA1* itself—the ICR team decided to focus on the deleted DNA as the site of the *BRCA2* gene, even though no one had previously suspected any link between the gene and pancreatic cancer.

Their bet quickly paid off. By early November, they found a promising mutation in a short segment of cloned DNA from the same region in a breast cancer patient from one of the families they are studying. Another helpful development came a few days later: On 23 November, genome sequencers at the Sanger Centre in Cambridge, U.K., and Washington University School of Medicine in St. Louis put on the Internet a 900-kilobase sequence of DNA thought to contain *BRCA2*.

Their goal, says Washington University's Robert Waterston, was to "establish a trend of academic scientists releasing their sequences immediately for everyone to use." The *BRCA2* searchers wasted no time in putting the sequence to use: It was, Wooster says, "very useful" for identifying adjacent sequences of protein-coding DNA that might belong to the gene. These could then be screened in the families to see if they, too, were mutated in breast cancer patients. By the time the paper was submitted to *Nature* on 5 December, the team had found five more mutations, leaving no doubt that the gene was *BRCA2*. "It was delightful to see how rapidly discovery of additional mutations took place," says Waterston. "It does illustrate the impact the sequence can have."

Since then, the team has identified another five mutations, a result suggesting that *BRCA2*, like *BRCA1*, may be subject to a great many different cancer-causing mutations. As both genes are very large, that will make screening difficult, although not impossible. The BRCA1 protein contains more than 1800 amino acids, and even though the published *BRCA2* sequence is incomplete, with perhaps 20% of the gene missing, the portion sequenced so far is still big enough to encode a protein 2339 amino acids long. Meanwhile, Myriad's Meldrum says that his company has obtained the sequence of the entire gene, which they deposited in the GenBank database on Thursday, 21 December.

Still, a good many questions remain to be answered about both *BRCA1* and *BRCA2*. No one knows yet what their normal functions are or how their mutation or loss might lead to cancer. The protein structures inferred from the sequences of *BRCA1* and *BRCA2* have provided little help in this regard. "There is no similarity [of *BRCA2*] to anything identified so far, no motifs that give any hints as to its function," Wooster says. While there is a slight resemblance to *BRCA1*, its significance is unclear, he notes.

Also unclear are what roles, if any, *BRCA1* and *BRCA2* mutations play in the development of the great majority of breast cancers that are "sporadic"—apparently not hereditary. Most other tumor suppressor genes play a role in both hereditary and sporadic tumors, but early failure to detect *BRCA1* mutations in most sporadic breast cancers suggested that it might be an exception. But those studies didn't see the whole picture, says Mary-Claire King of the University of Washington, Seattle. Her own group has found that the BRCA1 protein is often not made in breast tumors, an indication

that loss of the protein may have contributed to the cancer development. Other work hints that if the protein is produced, it may end up in the wrong place in the cell. In the 3 November issue of *Science*, Wen-Hwa Lee's team at the University of Texas Health Science Center in San Antonio reported that the BRCA1 protein is located in the nucleus of normal breast epithelial cells, but in the cytoplasm of cells derived from tumors.

Exactly what that means is not yet known, but it suggests that something has gone wrong in *BRCA1* function in the tumor cells. And now that researchers have *BRCA2*, they can begin asking similar questions about that gene's role in sporadic cancers.

The research puzzles aren't the only ones swirling about this new breast cancer susceptibility gene. There's also the matter of who will win the patent on the gene. Meldrum maintains that Myriad should have the edge in the United States because it has the complete sequence. But Guy Heathers, CRC Technology's business manager, says their patent application also claims the whole gene, or at least the coding region. It's far too early to know who will win out. But one thing is certain: The potential payoffs—for science, medicine, and industry—are so high that *BRCA2* will be getting a lot of attention. As King puts it, its discovery "is only the end of the beginning."

–Jean Marx

BIOCHEMISTRY

Flexing Muscle With Just One Amino Acid

The workings of muscles interest more than just athletes: For years, scientists have been tracing the cascade of molecular events that trigger muscle contraction. One key player in the sequence is a protein in muscle cells known as troponin-C, which responds to a chemical signal—the release of calcium ions from within the cell—by changing its shape. The contortion alters its chemical interactions with neighboring proteins, and these interactions eventually lead to cell contraction. But just why troponin-C undergoes this crucial shape change has remained murky.

Two weeks ago, however, a group of Canadian researchers, flexing some investigatory muscle of their own at the 1995 International Chemical Congress of Pacific Basin Societies in Honolulu, Hawaii, may have cleared up this mystery. The researchers, led by Brian Sykes, a biochemist at the University of Edmonton in Alberta, Canada, unveiled new studies showing that a single amino acid—glutamic acid, the 41st amino acid in the protein chain—controls this shape change by dragging a section of the protein toward a newly bound calcium ion; mutant proteins without this amino acid didn't budge. Troponin-C comes in two forms, one in skeletal and one in cardiac muscle. These studies were done on the skeletal form, but researchers believe the information may aid the design of drugs, known as calcium-sensitizing drugs, intended to treat heart attack victims by strengthening the contractions of undamaged heart muscle cells.

"It's a very nice study," says Walter Chazin, a molecular biologist at the Scripps Research Institute in La Jolla, California. The new result is the first to suggest that a large shape change can be controlled by the identity of just a single amino acid, he says. But although Chazin finds the evidence compelling, he cautions that some uncertainty remains, because the mutation could also be altering the protein's response by affecting the way it binds calcium.

Sykes's group has been pursuing the relationship between troponin-C's structure and function for some time. Their new result comes just 3 months after they completed the first description of the protein in its calcium-bound state. Past x-ray crystallography studies of the unbound protein had shown that the amino acids in the key regulatory section of the molecule are woven into a series of five connected helixes and a pair of loops. Researchers suspected that the calcium ions were bound inside the loops and played a role in the protein's shape change. But scientists were unable to confirm these suspicions, because they couldn't crystallize the protein in its calcium-bound configuration to study it with x-rays.

B. SYKES

Shaping up. Models of the muscle protein troponin-C show that glutamic acid *(red)* is usually attracted to lysine *(purple)*. But a calcium ion *(white)* pulls it away—bending the protein.

But in the September issue of *Nature Structural Biology*, Sykes and his colleagues Stephane Gagné, Sakae Tsuda, Monica Li, and Larry Smillie addressed the question with a different structure-determining technique, known as nuclear magnetic resonance (NMR) spectroscopy, that doesn't require crystallization. The NMR technique, which determines the position of atoms within a protein from the way they resonate in a magnetic field, confirmed that calcium binding takes place inside the two loops.

The bound structure also revealed a clue to how this might trigger the shape change. The calcium-bound structure showed that a calcium ion in one loop was sitting near a glutamic acid in a neighboring helix; in the unbound structure, the two are farther apart. That difference suggests that upon binding to the protein, the positively charged calcium attracts the negatively charged glutamic acid, which pulls the helix along with it, forcing the protein to change its shape, says Sykes.

But this circumstantial evidence didn't eliminate the possibility that other amino acids were involved in the shape change as well. So in their latest study, the Alberta researchers created a mutant version of the protein in which the key glutamic acid was changed to an alanine, another amino acid—but one with a neutral charge. When the team studied the structure of the mutant protein, they found that it no longer altered its shape even after the two calcium ions bound inside their loops. With no electronic attraction, Sykes suggests, there is nothing forcing the helix to change its position.

The structural information may provide clues to shape changes in the cardiac form of the protein, and thus help to design drugs that strengthen heart muscle contractions by keeping that form in its calcium-bound position, says R. John Salero, a physiologist studying such drugs at the University of Illinois, Chicago. But structural studies on the cardiac form have yet to be completed.

Chazin also notes one complication in studies of the skeletal form. The glutamic acid is normally a key link in the protein's framework that holds the calcium in place, and the mutational change may change the way calcium sits in the binding pocket. That in turn could prevent the shape change by altering the way in which the calcium interacts with neighboring atoms.

But so far Sykes sees no sign of that. "I would say our data show the binding pocket is still as it was," says Sykes. "And there isn't any evidence that the calcium is binding anywhere else." And Chazin says that seeing the full NMR data of the new protein once it's published should help settle the matter. "If he's got the right answer," says Chazin, "then he's on to something very big."

–Robert F. Service