Guardians of the Genome?

Two breast cancer genes may safeguard DNA

By JOHN TRAVIS

Historic discoveries are often followed by frustratingly long periods in which scientists struggle to understand the meaning of what they have found.

Take the famed Rosetta stone, uncovered in Egypt in 1799 by soldiers of Napoleon Bonaparte's army. The slab, chiseled with the same message in three languages, proved the key to deciphering the Egyptian hieroglyphs.

Yet as they raced to decode the ancient writing, Egyptologists argued fiercely over the stone, often ridiculing each other's theories. In 1822, more than 20 years after the discovery, French scholar Jean-François Champollion finally deciphered the hieroglyphs.

In the world of breast cancer research, the genes *BRCA1* and *BRCA2* may prove to be a Rosetta stone. Mutations in this pair of genes, identified in 1994 and 1995, respectively, account for the large majority of inherited breast and ovarian cancers.

Though discovery of the two genes occasioned great fanfare and raised hopes of new treatments for breast and ovarian cancer, *BRCA1* and *BRCA2* are only slowly giving up their secrets. Biologists have again encountered the hard truth that identification of a cancer gene does not immediately reveal the function of the protein it encodes or how a mutation in the gene triggers tumors.

Indeed, until recently, the discovery of *BRCA1* generated little more than confusion, as several research groups offered utterly different proposals concerning the gene's protein. The field was in such turmoil last year that Richard Klausner, director of the National Cancer Institute in Bethesda, Md., called in members of the competing teams and urged them to cooperate to unravel the issue quickly.

The solution to this biological Rosetta stone may now be near. While the details are far from complete and some controversy remains, a rash of papers in the last few months strongly suggests that the proteins encoded by *BRCA1* and *BRCA2* play a role in a cell's ability to detect or repair damaged DNA. If so, these genes would join a select group of other cancer genes that have earned the title Guardians of the Genome.

Since the discovery that many families plagued with breast and ovarian cancer have *BRCA1* mutations, scientists have pursued several leads to the gene's role. The first came in 1995, when a team led by Wen-Hwa Lee of the University of Texas Health Science Center at San Antonio argued that the gene's protein, BRCA1, normally stays in the nucleus, the sac housing a cell's genes. In cancer cells, the protein sits solely in the cytoplasm outside the nucleus or in both places, the investigators reported (SN: 11/18/95, p. 334).

Lee's group suggested that the abnormal location of BRCA1 lies at the heart of why a healthy cell turns cancerous. Even more provocative, the researchers contended that the misplacement might explain noninherited cases of breast and ovarian cancer in which *BRCA1* is not mutated.

These sporadic cases account for more than 90 percent of all breast cancer, and Lee's group offered evidence that breast and ovarian cancer cells from such cases also have BRCA1 in the cytoplasm. The researchers theorized that mutations in other genes were turning cells cancerous by changing where BRCA1 appears.

This hypothesis landed on the front page of the New York Times, but a competing theory soon surfaced. The protein encoded by *BRCA1* does not function in the nucleus or in the cytoplasm, countered Roy A. Jensen and Jeffrey T. Holt, both of Vanderbilt University School of Medicine in Nashville, Mary-Claire King of the University of Washington in Seattle, and their colleagues. Instead, BRCA1 is a secreted molecule belonging to an obscure family of proteins known as granins, the investigators said (SN: 3/2/96, p. 132).

How could researchers come to such starkly different conclusions? The conflict stems largely from the difficulty scientists face in determining the location of proteins in cells. Scientists normally manufacture antibodies that bind tightly to a selected target protein and then use several well-established methods to spot those antibodies. By using different antibodies, teams can generate conflicting results, however. Moreover, such experiments can be misleading if the antibodies bind to more than their target protein.

Indeed, most scientists outside the San Antonio and Seattle-Nashville teams have now concluded that both research groups were mistaken and that BRCA1 is neither secreted nor found in the cytoplasm of cancer cells.

"I'm comfortable that it's a nuclear protein," says Cindy A. Wilson of the University of California, Los Angeles, who has conducted an independent analysis with the BRCA1 antibodies used by various research groups.

The most recent break in the *BRCA1* story occurred early this year, when scientists reported an association between the gene's protein and a protein called RAD51.

Like other researchers, Ralph Scully of the Dana-Farber Cancer Institute in Boston and his colleagues had used fluorescent antibodies to identify the location of BRCA1. They observed that the protein's location in the nucleus forms a distinctive pattern—a collection of dots. Cells with these nuclear dots, the researchers discovered, have just entered S-phase, the period in a cell's life cycle during which it synthesizes a second copy of its DNA in preparation for dividing into two cells.

A search through the scientific literature for other proteins forming similar dots ultimately revealed RAD51, a human version of a bacterial protein known to help fix breaks in the DNA double helix. Scientists believe that RAD51 performs a similar function in mammalian cells.

"The evidence that it repairs breaks directly is very strong," says Paul Hasty, a RAD51 researcher at Lexicon Genetics in The Woodlands, Texas.

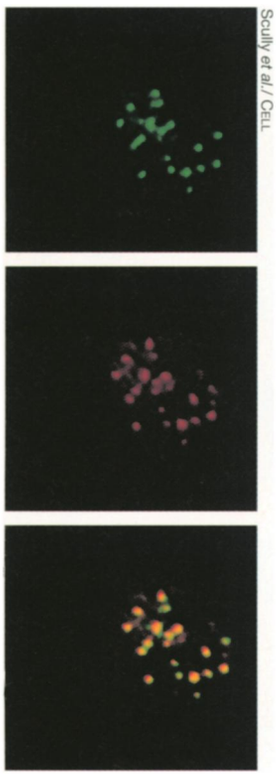
In the Jan. 24 CELL, Scully and his colleagues reported several coincidences leading them to believe that BRCA1 works in concert with RAD51. The nuclear dots formed by the two proteins often, though not always, overlap. Moreover, mice with mutations in both their copies of either *BRCA1* or *RAD51* die at roughly similar points in embryogenesis, apparently from a lack of cell proliferation.

Furthermore, when the scientists fished BRCA1 out of cells with antibodies, RAD51 often came along. The proteins may not bind to each other, but they are probably part of a complex of proteins, says Scully.

In addition to normal DNA repair, RAD51 aids in the shuffling of genes among pairs of chromosomes during meiosis, the process by which a cell readies itself for sexual reproduction. Scully and his colleagues found that BRCA1 and RAD51 both appear in synaptonemal complexes, complicated DNA-protein structures formed by chromosomes undergoing meiosis. Scully notes that

DNA probably breaks and anneals in these complexes, which adds to the circumstantial case that BRCA1 works with RAD51 in ensuring DNA integrity.

At a recent lecture at the National Institutes of Health in Bethesda, Md., Scully's colleague David M. Livingston described unpublished findings further supporting that view. His research team has damaged the DNA in cells and observed that those cells respond by quickly adding a phosphate group to BRCA1, a method commonly employed to activate a protein to carry out a specific duty. Livingston's group also had evidence that after DNA damage, the dot pattern disappears, apparently because BRCA1 moves to sites in the nucleus where DNA is being synthesized.



Scully et al./ Cell

The cellular locations of BRCA1 (green) and RAD51 (red) often overlap (yellow).

"The cell biology observations, meager as they are, are strongly consistent with the notion that *BRCA1*, for at least part of its life, possibly only part of its tumor-suppressive life, operates as a guardian of the genome. How it actually does that is very much open to speculation," says Livingston.

Remarkably, *BRCA1* is not the only breast cancer gene being linked to DNA repair and RAD51. Just before Scully and his colleagues published their report in Cell, Hasty had found a protein that bound to RAD51. It turned out to be BRCA2.

Not far down the road from Hasty, his former boss, Allan Bradley, founder of Lexicon and a Howard Hughes Medical Institute investigator at Baylor College of Medicine in Houston, had begun to study the activity of *BRCA2* by creating mice without a functioning copy of the gene. These so-called knockout mice invariably died about a week into their embryonic development. Indeed, the *BRCA2* knockout mice more closely resembled *RAD51* knockouts than did *BRCA1* knockout mice. The latter mice tended to die a day earlier in their development than the other two strains.

Another link observed between *BRCA2* and *RAD51* involved radiation-induced DNA damage. Cells from *RAD51* knockout embryos are extremely susceptible to death from ionizing radiation, presumably because the absence of the protein prevents the proper repair of DNA. As Bradley, Hasty, and their colleagues report in the April 24 Nature, cells from the *BRCA2* knockout mice exhibit a similar vulnerability. "We've clearly shown BRCA2 is involved in the DNA repair pathway," asserts Bradley.

Beyond the connection to RAD51, other data imply that the *BRCA1* and *BRCA2* genes share functions, whether DNA repair or as-yet-undiscovered duties. Scientists have recently found that *BRCA2* is particularly active during embryogenesis in tissues that experience large amounts of cell proliferation and differentiation. This pattern of gene activity largely mirrors that of *BRCA1*, they discovered.

"It's quite striking the degree to which the genes get turned on in the same organs, in the same cells within those organs, and at the same developmental time point as each other. You just don't find many genes that behave in such a similar manner," says Lewis A. Chodosh of the University of Pennsylvania School of Medicine in Philadelphia. Chodosh is an author of the study, which appears in the April 15 Developmental Biology.

Chodosh notes that the activity of the two cancer genes is not identical. Moreover, the discrepancies may help explain why mutations in the two genes pose different cancer risks, he notes. For example, *BRCA2* mutations are much more frequently found in male breast cancer than *BRCA1* mutations are.

Scientists are still struggling to make sense of all the latest findings. "My feeling is that there may be a large complex that includes *BRCA1*, *BRCA2*, *RAD51*, and maybe some other things," says Bradley.

This hypothetical complex may monitor the integrity of DNA in rapidly proliferating or differentiating cells, propose Bradley and other scientists. That action would resolve an apparent paradox. *BRCA1* and *BRCA2* are considered tumor-suppressor genes. The absence of other genes in that category stimulates cell growth and division. Yet mice lacking the breast cancer genes die as embryos because their cells do not proliferate when they should. The *BRCA1* knockout mice, for example, die at gastrulation, usually a period of tremendous cell proliferation. "Cells are basically dividing every 5 hours [during gastrulation]," says Tak W. Mak of the University of Toronto.

The paradox would vanish if mutations in *BRCA1* or *BRCA2* do not trigger cell proliferation by themselves, observe researchers. In this scenario, the normal role of the proteins encoded by the two genes is to repair DNA. Cells that have mutations in those repair genes but are otherwise healthy, such as cells in the knockout embryos, would sense accumulating DNA damage and stop dividing, causing the embryos to die.

Occasionally, particularly in older organisms, cells with defective *BRCA1* or *BRCA2* genes would also experience mutations in the genes needed to arrest the cell cycle. Such cells are then free to divide out of control, generating a tumor.

Additional evidence connecting the mutated genes and cell cycle arrest has recently come from Mak's research group and one led by Argiris Efstratiadis of Columbia University. The groups have eliminated *BRCA1* or *BRCA2* from mice that do not have either of the cell cycle genes *p53* or *p21*. The mice die at least 1 day later than normal *BRCA1* and *BRCA2* knockout mice, indicating that disrupting the cell cycle control in such mice allows their cells to proliferate a bit longer.

Linking *BRCA1* and *BRCA2* to DNA repair may explain why so few sporadic cases of breast and ovarian cancer involve one of those genes. For such cancers, at least three mutations would have to occur: Both copies of either *BRCA1* or *BRCA2* would have to go bad, and additional mutations would have to strike genes regulating the cell cycle. In contrast, people who inherit mutations in either *BRCA1* or *BRCA2* have an unfortunate head start on this road to tumor formation.

Researchers stress that they have just started to make inroads into understanding the roles of the proteins encoded by *BRCA1* and *BRCA2*. Moreover, old controversies linger. Lee maintains that BRCA1 shows up in the cytoplasm of cancer cells, noting that new antibodies generated by his group confirm the earlier results. In addition, according to other researchers at a recent meeting on *BRCA1*, the Seattle-Nashville collaboration reiterated its belief that the gene's protein is a secreted granin. (Members of the collaboration did not return calls from Science News.)

Another unresolved issue is whether BRCA1 or BRCA2 directly influences the activity of genes. Several studies have hinted that the proteins have this ability, and their structures suggest they should be able to bind DNA. Supporting that notion, Scully and his colleagues report in the May 27 Proceedings of the National Academy of Sciences that BRCA1 may be part of RNA polymerase II holoenzyme, a large collection of proteins used to create strands of RNA, the first step in assembling a gene's protein.

Whether the recent clues linking BRCA1 and BRCA2 to DNA repair can lead to a new cancer therapy remains the most pressing question. Researchers caution that the proteins are large, so they probably do more than repair DNA. "I'd be very surprised if there's just a single function for these things," says Chodosh. □