Very simply, the argument runs as follows. The evolutionary interests of single cells are to vary and proliferate as much as possible, and this is fine as long as they are single-celled organisms. But once cells are part of a multicellular organism, unbounded variation and proliferation become inimical to the individual. The resolution has been progressively to isolate germline cells and embryological development from the individual, a tactic that has not only rescued the individual from danger but also has constrained variation. "I think this reduced variation is part of the explanation of the lack of phylum-level evolution in the post-Permian," says Buss.

The issue of differences in evolutionary innovation at different levels of the genealogical hierarchy arises not just in temporal comparisons, like that between the Cambrian and Permian, but also in spatial comparisons. For instance, Jablonski and Bottjer analyzed evolutionary innovation in benthic marine organisms and discovered a distinct—and unexpected—pattern. Higher taxa—orders—preferentially arise in shallow-water, onshore environments. "Expectations might have put originations in more stable environments offshore," they noted recently. "Or, if successful innovation is largely a matter of the chance combination of novelty and opportunity, in a bathymetrically random distribution."

Moreover, taxa below orders in the hierarchy—families and genera—arise preferentially in offshore environments. "We could not have predicted the pattern at the ordinal level from the pattern shown by genera and families." Jablonski and Bottjer were able to show that the pattern is not an artifact of preservation: it is real and therefore must be saying something about evolutionary mechanisms. The most obvious message is that a simple extrapolation from one level to another is an unlikely explanation of evolutionary innovation at the different levels.

Currently there is a multitude of possible explanations for this pattern, none of which is more compelling than any other. For now, however, the major point is as Jablonski and Bottjer state: "In terms of the ecology of their evolutionary origins, higher taxa seem to have properties all of their own." In fact, higher taxa may have several properties all of their own, and evolutionary theory must strive to accommodate this.

■ ROGER LEWIN

ADDITIONAL READING

L. W. Buss, *The Evolution of Individuality*. (Princeton Univ. Press, Princeton, NJ, 1987).

D. H. Erwin *et al.*, "A comparative study of diversification events," *Evolution* **41**, 1177 (1988).

D. Jablonski and D. J. Bottjer, "The ecology of evolutionary innovation," in *Evolutionary Innovations*, M. H. Nitecki, Ed. (Univ. of Chicago Press, in press).

Eye Cancer Gene Linked to New Malignancies

Retinoblastoma gene loss may contribute to the development of breast cancer and small cell lung cancer in addition to the relatively rare retinoblastomas

A FEW YEARS AGO, researchers learned that retinoblastomas, highly malignant tumors that arise in the retina of the eye, are caused by the loss or inactivation of a particular gene, known as the retinoblastoma (RB) gene. More recent research suggests that RB gene inactivation may also contribute to the development of two additional types of cancer, namely breast cancer and small cell lung cancer, that occur much more frequently than the uncommon retinoblastomas.

"This gene, and others like it, may have a fundamental role in the genesis of many tumors, not just the rare eye tumor," says J. William Harbour a medical student who is a Howard Hughes Medical Institute Scholar at the Navy Medical Oncology Branch of the National Cancer Institute (NCI) in Bethesda, Maryland. Retinoblastoma afflicts only about 1,000 patients every year in the United States, whereas some 130,000 individuals develop breast cancer and another 30,000 get small cell lung cancer annually.

The new results may eventually have implications for treating breast cancer and small cell lung cancer, and also for predicting who will get the malignancies. Moreover, the RB gene studies are providing a better understanding not just of carcinogenesis, but of normal cell growth as well.

In particular, they lend further credence to the view that growth inhibitory forces may be just as important to the life of the cell as growth stimulatory forces. The assumption is that the protein encoded by the RB gene normally suppresses cell division. Its loss then causes retinal cells to grow out of control and become cancerous.

One reason why cancer researchers find the RB gene so interesting is that its inactivation may be an early, perhaps even an initiating, event in carcinogenesis. This appears to be the case for retinoblastoma at least.

About half of retinoblastoma patients have an inheritable form of the tumor in which they acquired one bad or deleted copy of the RB gene from their mother's egg or father's sperm. This almost guarantees that retinoblastoma will develop. Some 90% of these individuals get the eye cancer at an early age, usually before they are 3 years old. Another mutation to knock out the second copy of the gene in retinal cells is also required, but this is apparently a frequent event.

Researchers began looking at the RB gene in breast cancer cells partly because of observations about the inheritance patterns of the malignancy. Over the past several years, improved therapies have greatly increased the survival rate of the retinoblastoma patients, and clinicians began to find that children who had been successfully treated for the inheritable form of the disease developed other types of cancer, especially sarcomas such as osteosarcoma (a bone cancer), at higher than expected rates.

Moreover, clinicians are seeing more cases of breast cancer in the survivors of inheritable retinoblastoma, although it is too early to tell whether this represents a true increase in the incidence of the disease in the group. The mothers of children with osteosarcoma do have an increased risk of developing breast cancer, however.

These observations suggested that the same RB gene defect that confers susceptibility to retinoblastoma might increase susceptibilities to the other cancers as well. This was soon confirmed for osteosarcoma. In retinoblastoma cells, both copies of the RB gene are either deleted or so badly rearranged that they cannot be functional. The same thing was happening to the gene in osteosarcoma cells.

Two groups have now shown that comparable RB gene abnormalities occur in breast cancer cells. In a report published in the 8 July issue of *Science* (p. 218), Eva Lee, Wen-Hwa Lee, and their colleagues at the University of California School of Medicine at San Diego describe results showing that two of nine lines of breast cancer cells have the abnormalities and also fail to make detectable RB protein. In addition, Yuen-Kai Fung of the University of Southern California School of Medicine and his colleagues have found RB gene deletions or other abnormalities in 5 of 16 lines of

breast cancer cells.

Moreover, the researchers are finding the RB gene defects in at least a small percentage of primary breast cancers. The Fung group has detected them in 3 of 41 primary tumors and the Lee group in 1 of 15. Webster Cavenee's group at the Ludwig Institute for Cancer Research in Montreal also has evidence that some primary breast cancers have the RB gene defects.

Small cell lung cancer, unlike retinoblastoma or breast cancer, does not appear to have a hereditary component, but is associated with heavy cigarette smoking. Nevertheless, Harbour, John Minna, Frederic Kaye, and their colleagues at the NCI–Navy Medical Oncology Branch have found RB gene abnormalities, comparable to those in retinoblastoma, in about 20% of the 22 small cell lines that they examined (see p. 353). The researchers also found the defects in one of eight primary small cell lung cancers and in cells derived from a related lung tumor, known as pulmonary carcinoid.

They may not have detected all the mutations that inactivate the RB gene in small cell and pulmonary carcinoid lines, however. Measurements of the messenger RNA transcribed from the gene indicated that its expression is greatly reduced or shut off completely in about 80% of the cell lines. Harbour and his colleagues speculate that the RB gene, which is very large—about 200 kilobases—may be especially susceptible to mutation by the chemical carcinogens in tobacco smoke.

As mentioned earlier, the loss of a functional RB gene is apparently sufficient to put retinal cells on the path to cancerous transformation, although additional genetic mishaps may be required for progression to full malignancy. If RB gene inactivation is also the first step toward producing breast and small cell lung cancer, then it might be possible to treat the malignancies by replacing the lost gene and restoring normal growth control. Attainment of that goal is far in the future, however.

A more readily achievable clinical application of the work on the RB gene might be the identification of women who are at high risk of getting breast cancer. Having a mother or sister who had the malignancy roughly doubles a woman's risk of getting the disease herself. It will be interesting to see whether this increased susceptibility is related to an RB gene defect. If so, it should be possible to identify those women who carry the defect and need to be carefully monitored so that breast cancer can be detected early if it develops.

A good many issues concerning the RB gene remain to be resolved. For one, no one knows exactly how it acts to keep cell growth in check. Work from Wen-Hwa Lee's laboratory has shown that the RB protein is located in the nucleus and binds to DNA. This suggests that it might act to regulate gene expression, but more work will be required to confirm this possibility. The results linking RB gene inactivation to the development of breast cancer and small cell lung cancer will no doubt provide further impetus to research on the gene's mechanism of action because they indicate that its negative regulatory effects are not limited to retinal cells.

Two additional findings also point up the significance of the RB gene as an inhibitor of cell growth. Edward Harlow and Peter Whyte of Cold Spring Harbor Laboratory, in collaboration with Robert Weinberg's group at the Massachusetts Institute of Technology, have found that E1A protein of adenovirus forms a complex with three cellular proteins, one of which is the RB gene product.

"This gene, and others like it, may have a fundamental role in the genesis of many tumors, not just the rare eye tumor."

Adenovirus causes the cancerous transformation of some kinds of mammalian cells. The E1A protein participates in this transformation by immortalizing cells, that is, giving them the ability to divide indefinitely in culture. Harlow, Whyte, and their colleagues have found that RB protein binds to a region of the E1A molecule that had previously been shown to be necessary for its transforming activity. "If you believe that RB protein is part of a growth inhibitory pathway," Harlow says, "the result suggests that E1A is blocking the action of the RB protein."

In a similar vein, David Livingston and Eva Paucha of Harvard's Dana-Farber Cancer Institute, and their colleagues have found that another transforming protein, this one the large T antigen encoded by simian virus 40, also binds the RB protein. In this case, too, the RB protein binds to a region of the T antigen molecule that has been shown necessary for transformation. Adenovirus and SV40 may transform not by inactivating the RB gene but by binding the gene product and preventing it from performing its usual function.

What is intriguing about this result, Weinberg points out, is that two viruses, which are otherwise unrelated, have evolved the same mechanism for releasing the brake on cell growth. "It means that the RB protein is clearly sitting on a central node for growth regulation in the cell," he says.

To approach the problem of how the protein works, researchers are trying to transfer the RB gene into cells that lack it to see if it reverses their cancerous properties. It might then be possible to trace the specific effects of the gene. The transfer experiments also have obvious implications for eventual therapeutic strategies.

Another issue concerns why the percentage of primary breast tumors with inactivated RB genes is much smaller than the percentage of tumor-derived cell lines with the gene defects. One possibility is that abnormalities were actually present in more of the primary tumors, but escaped detection. Tumors are generally a mixture of cancer cells, which may be in the minority, with connective tissue, blood vessels, and other normal cells. The presence of the normal DNA from these cells may have masked any RB gene abnormalities in the cancer cells.

Another possibility is that the RB gene abnormalities in the cell lines are artifacts, the result of having established and maintained the cells in culture conditions and not related to the original induction of the cancers after all.

William Benedict of the University of Southern California School of Medicine in Los Angeles, who is also studying the RB gene, contends that this is unlikely, however. He says of the RB gene abnormalities found in the tumor cell lines, "I don't think that they are a fluke or cell culture artifact. Fung finds nearly 40% abnormalities in the cell lines, just as many as in retinoblastomas."

Moreover, the RB gene defects are not a general characteristic of all tumor-derived cell lines. The researchers looked for them in several other types, in addition to the breast cancer and small cell lines, and did not find them. For example, Harbour and his colleagues did not see the defects in lung cancer types other than the small cell carcinoma and pulmonary carcinoid. The findings suggest that RB gene inactivation leads to the formation of some specific cancers, but not to all types. ■ JEAN L. MARX

ADDITIONAL READING

P. Whyte *et al*, "Association between an oncogene and antioncogene: The adenovirus E1A proteins bind to the retinoblastoma gene product," in press in the 14 July issue of *Nature*.

J. A. DeCaprio *et al*, "SV40 large tumor antigen forms a specific complex with the product of the retinoblastoma susceptibility gene," *Cell* **54**, 137 (1988).