DNA's Extended Domain

Sightings of cell-surface DNA turn scientific orthodoxy inside out

By INGRID WICKELGREN

Subject to the push of scrutiny, all scientific suppositions sit at truth's precarious edge. And now DNA's presumed place in the cell is beginning to sway. Although most scientists still think of this vital nucleic acid as residing only within cellular confines, accumulating evidence — some nearly 20 years old — indicates some DNA exists outside that domain, securely anchored to cell membranes.

In the 1970s, a handful of research groups reported discoveries of DNA on mammalian cell membranes, but most scientists dismissed their results. "When you find nucleic acids appearing in a strange new place, you're really shocking the scientific community," says biophysicist Barnett Rosenberg of Michigan State University in East Lansing. "As clever as our experiments were, we couldn't convince others as well."

Lacking funding and peer support, these researchers abandoned their work on membrane DNA by the end of the decade. In the past few years, however, others have stumbled upon surface DNA and have identified a specific protein that binds it at the cell surface, paving the way for a convincing proof of its existence.

Scientists cannot agree on whether the "out-of-place" nucleic acid — believed to represent about 1 percent of a cell's total DNA — emerges from within the cell itself or arrives at the membrane as blood-borne cellular debris. The surface DNA fragments do not appear to undergo replication or perform any genetic coding function. Nor has anyone demonstrated any role such fragments play in sickness or health. But the data supporting their existence have prompted numerous suggestions regarding their possible physiological importance.

The 1970s experiments hinted that cell-surface DNA might help explain how tumor cells and viruses evade the immune system and how certain cancer drugs work. More recent results suggest surface DNA may play a destructive role in autoimmune diseases and a useful role in drug treatment for psoriasis and a cancer called cutaneous T-cell lymphoma.

Surface DNA's external location puts it in an ideal position to interact with the immune system. If scientists can clearly delineate an immunologic role, membrane-bound DNA might someday prove important in diagnosing and treating a variety of diseases.

The first sighting of DNA on cell membranes occurred in 1970 at the Research Institute of Scripps Clinic in La Jolla, Calif. While analyzing the chemical composition of some human lymphocyte membranes under an electron microscope, Richard A. Lerner and his co-workers spotted what looked like DNA bound to the cell membranes.

"We found it and didn't know quite what to make of it," Lerner says. The team reported the observation in 1971 in PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, then abandoned the work.

But it wasn't long before others sighted membrane nucleic acid. In 1974, Rosenberg and his colleagues stained normal and cancerous cells from humans and rodents with a platinum dye that reacts avidly with nucleic acids. Using an electron microscope, they observed that the stain appeared not only in the nuclei, ribosomes and mitochondria — all known to contain RNA and/or DNA — but also on the cell surfaces.

Staining other cell types, the Michigan group found surface DNA on tumor cells and immune-stimulated lymphocytes but not on other cell types. From these results, reported in PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES in 1975, Rosenberg theorized that surface DNA helps a tumor cell avoid immunologic surveillance. He also speculated that surface DNA — which researchers in Lerner's lab later found, using Rosenberg's dye, on virus-infected lymphocytes — might somehow allow viruses within cells to hide from the immune system. Normally, viral proteins on cell surfaces alert the immune system to the invader's presence.

Rosenberg thinks surface DNA may exert an immunologic influence by masking molecules on tumor cells that would otherwise provoke an immune response, or by adding enough negative charge to the cell membrane that it electrically

repels lymphocytes. He reported experimental evidence in 1982 showing that surface DNA increases a cell's negative charge by 30 percent. That increase may be sufficient to prevent a lymphocyte from coming close enough to a DNA-coated cell to trigger an immune response, he told SCIENCE NEWS.

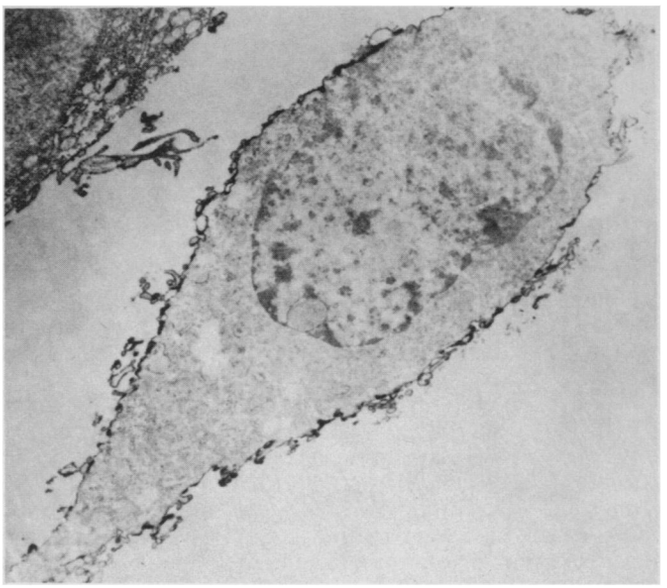
At about the same time as Rosenberg began speculating about an immunologic role for cell-membrane DNA, the first *evidence* for such a role emerged in the lab of Edward S. Golub, then at Purdue University in West Lafayette, Ind. In the mid-1970s, while investigating a link between leukemia and immunosuppression in mice, Golub and his colleagues found that leukemic cells taken from immunosuppressed mice, when mixed with a variety of normal immune cells, prevented the normal cells from initiating an immune response.

In subsequent work, Golub and graduate student James Russell treated leukemic cells with the DNA-chopping enzyme DNase, attempting to rid their cell preparations of unwanted debris. They discovered, to their surprise, that DNase destroyed the leukemic cells' ability to suppress an immune attack, Golub says. The researchers were stumped. Since DNase could not enter a cell, it must be acting on the cell surface, they reasoned. But that couldn't be the case, Golub recalls thinking, because "DNA is not on the surface."

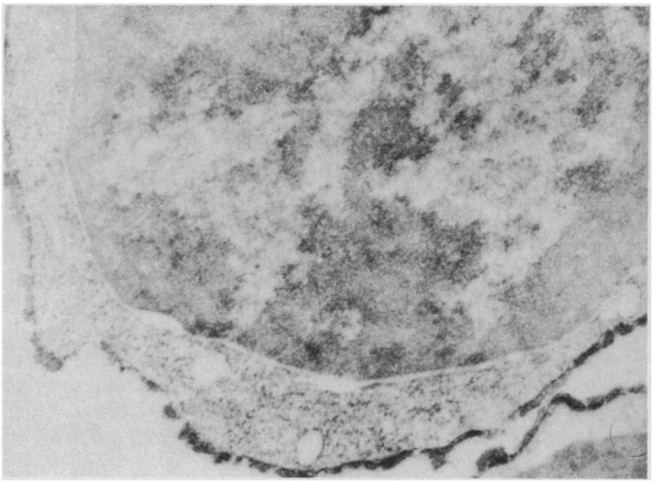
In their next experiment, Golub and Russell passed malignant mouse thymus cells through a plastic column filled with gel-like beads coated with antibodies that preferentially bind to DNA molecules. Only the cells that had stuck to the beads inside the column (and so presumably had DNA on their surfaces)—and not those cells that slid through — could suppress an immune response, they observed. The team further discovered that treating the malignant suppressor cells with DNase destroyed their immunosuppressive ability.

On the basis of these findings, published in PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES in 1978, Golub suggested that a small percentage of DNA-bearing leukemic cells might prevent a patient's immune system from destroying the entire tumor. A paper refining the team's original results appeared in 1980 in JOURNAL OF IMMUNOLOGY. After that, Golub says, he couldn't get a grant to pursue this line of research. He now directs the Johnson & Johnson Laboratories at Scripps.

Other work, however, began to yield results consistent with Golub's findings. Ariel C. Hollinshead of the George Washington University Medical Center in Washington, D.C., observed that cells from human lung tumors contained membrane DNA that acted as "inhibitory antigens," suppressing a variety of immune reactions. Her results appeared in PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH in 1979.



Electron micrograph shows a human cancer cell that Rosenberg and his colleagues stained with a platinum-thymine blue dye. The dye, which reacts specifically with nucleic acids, shows up clearly on the cell surface as well as in the nucleus and ribosomes.



The same stain reveals membrane DNA on a mouse T-lymphocyte that has been primed to initiate an immune response.

Photos: Richard W. Wagner, Surinder K. Aggarwal

Then, in a paper in the September 1982 CANCER RESEARCH, Rosenberg and David A. Juckett reported that a variety of antitumor drugs, including the widely used cisplatin, remove surface DNA from tumor cells. In addition to anticancer drugs' known effect on nuclear DNA, their ability to remove surface DNA "may be a major component in the specific anticancer activity in chemotherapy," Rosenberg told SCIENCE NEWS. "This could be the mechanism by which [anticancer drugs] can selectively kill cancer cells without significant damage to normal cells."

Critics of this early work remained unconvinced that the DNA seen on cell membranes was stably and specifically bound. It could have come from broken, dead cells in the bloodstream and randomly stuck to the membranes examined, they argued. Some scientists were especially skeptical of Lerner's experiments because he had deliberately broken up cells to isolate their membranes, Rosenberg says.

But Rosenberg calls random, passive DNA attachment to cell surfaces "a physically unreasonable" explanation of the surface DNA observations. It's true that DNA, an electrically "sticky" molecule, can temporarily attach itself to any positively charged molecule it encounters. But since both DNA and cell membranes possess a negative charge, Rosenberg says, electrostatic forces should make them repel each other. Skeptics counter that patches of positively charged proteins on the cell surface could have an affinity for DNA and so bind to it in a random fashion. If this were the case, however, one might wonder how cisplatin, shown to remove surface DNA, and DNase, known to break up only the DNA outside a cell, could increase the cell's susceptibility to immune attack.

The proposed immunologic implications drew even more criticism than the experiments themselves. Few scientists accepted Rosenberg's and Golub's immunosuppression theories. "It was just too fantastic that there was DNA on the cell

surface that was doing something [physiologically]," Golub says.

Researchers trying to document the existence and role of cell-surface DNA bear a heavier burden of proof than scientists who happen upon more plausible discoveries. Golub, who recalls this period in his work as a sort of "midlife crisis," says, "I learned not to talk about it, but to talk about my more respectable, boring stuff."

But he had enough faith in his data to predict that securely bound surface DNA would be rediscovered. And so it has.

In the early 1980s, Robert M. Bennett and his co-workers at Oregon Health Sciences University in Portland stumbled across surface DNA while looking for a cell-membrane receptor for the iron-transporting protein lactoferrin. They realized the surface molecule they were seeking was in fact DNA, Bennett says, when they found that the protein-digesting enzyme trypsin did not prevent lactoferrin's binding to white blood cells, whereas DNase did.

The Oregon group then began looking for a receptor that holds this oddly placed nucleic acid on the cell membrane. While trypsin had failed to block the binding of lactoferrin, they discovered that it did prevent *DNA* from binding to the cell membrane, suggesting the presence of a membrane protein that binds to DNA. They later isolated this receptor and found that nonradioactive DNA could prevent radioactive DNA from binding to the receptor, whereas other molecules resembling DNA could not. This, they concluded, suggests the receptor is specific for DNA.

Bennett and his colleagues further demonstrated that the DNA binds to the receptor in the same way hormones bind to their cell-surface receptors. But instead of activating the cell as a hormone does, the DNA and its receptor are rapidly engulfed into the cell interior, where the DNA breaks into smaller pieces, they reported in the December 1985 JOURNAL OF CLINICAL INVESTIGATION.

Bennett's team found additional evidence for the receptor's DNA specificity upon discovering that the cell would stop sequestering DNA when they exposed it to a chemical that inhibits protein synthesis. The chemical presumably prevented the cell from producing new DNA receptors to replace those it had engulfed.

Although the Oregon researchers presented compelling evidence for the receptor, some scientists say they will remain skeptical until they see genetic proof. Several biologists, upon learning of Bennett's work from SCIENCE NEWS, have expressed doubt that the DNA-binding protein represents a specific receptor for DNA. More likely, they say, it's a protein with a different purpose that also happens to bind to DNA.

Bennett and molecular biologist Michael A. Forte have isolated a particular stretch of DNA that they suspect contains the gene coding for the protein, and are now trying to clone the gene for the DNA receptor, Forte told SCIENCE NEWS. If they can clone the gene and use it to direct cells lacking the DNA receptor to make a functional DNA-binding protein, this would constitute virtual proof that a specific surface-DNA receptor exists, according to Woodruff Emlen of the University of Colorado Health Sciences Center in Denver.

So far, Bennett reports, he and his colleagues have found the DNA receptor on human liver, kidney and white blood cells (macrophages and T- and B-lymphocytes) and on kidney, liver and spleen cells from mice. And last year, Emlen and his colleagues also reported finding a trypsin-sensitive, DNA-binding protein on the membranes of certain liver cells from mice. Emlen described the discovery in the October 1988 AMERICAN JOURNAL OF PATHOLOGY.

Although Bennett's and Emlen's results suggesting that surface DNA appears on many cell types seem inconsistent with earlier findings, Bennett says this may be because DNA's surface interactions are dynamic rather than static, as the earlier researchers had assumed. Because cells rapidly internalize their surface DNA, he explains, it may appear only on fresh cells or on cells exposed to a lot of DNA debris from dead cells (as may accumulate around a cancer). Thus, early investigators looking at normal cells could easily have missed it.

After finding the proposed DNA receptor, Bennett began to wonder if cell-surface DNA might play some role in systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome and other autoimmune disorders involving connective tissues. These are all incurable diseases in which patients produce antibodies against their own tissues — including, in the case of lupus, antibodies that bind to DNA.

Examining isolated white blood cells from lupus patients, Bennett and his coworkers observed that they would not bind to or internalize added DNA. After noticing that the cells regained their DNA-binding ability after overnight incubation in a nonserum solution, the researchers concluded that the defect sprang from some blood serum component in lupus patients. They went on to identify an antibody against the DNA receptor in serum from lupus patients, reporting their findings in the October 1987 JOURNAL OF EXPERIMENTAL MEDICINE.

In mice, Bennett and immunologist Steven H. Hefeneider at the Portland Veterans Administration Medical Center have also produced a DNA-receptor antibody that prevents DNA from binding to human white blood cells. They described the work in the May 1, 1988 JOURNAL OF IMMUNOLOGY.

Using this monoclonal antibody to purify the DNA-binding protein, Bennett and Hefeneider went on to look for antibodies to the protein in blood samples taken from autoimmune-diseased individuals, from their apparently healthy relatives and from normal, unrelated controls. They found the "antireceptor antibodies" in 22 of 48 lupus patients and in 32 of 61 patients with other connective-tissue autoimmune diseases. The antibodies also turned up in 13 of 20 apparently healthy relatives of lupus patients, whereas only 6 of 256 unrelated individuals had them.

"This receptor seems to be the site of an autoimmune attack in connective-tissue disease," Bennett suggests, adding that the antibody made against it "may be a general marker for such diseases." Although the screening results clearly show that autoimmune patients and their relatives harbor elevated levels of the antibody, Hefeneider says the "quick and dirty" nature of these preliminary tests probably led him and Bennett to underestimate the actual percentages of the patients and relatives carrying them.

Scientists know that anti-DNA antibodies cause lupus-linked kidney disease, says Bennett, but they do not know whether such antibodies contribute to the other physical manifestations of lupus, including skin rashes, arthritis, convulsions and heart disease. At present, researchers can only speculate on the role of the DNA-binding protein. A malfunctioning receptor could explain rheumatologists' observations that lupus patients have elevated levels of circulating DNA. The DNA excess, says Hefeneider, might result in more antibody-bound DNA that stimulates symptom-causing "inflammatory cascades." In addition, circulating DNA that later binds to antibodies might inflict damage through direct contact with kidney cells, he suggests.

Hefeneider, Bennett and Jane Siegel are now studying mouse models to determine when the antireceptor antibody is produced. If it arises early in the course of connective-tissue disease, screening blood serum for it might offer an early warning of such diseases, prompting physicians to monitor such patients closely, Hefeneider suggests. And if researchers someday develop effective cures, early intervention might increase the therapeutic success rate, he says.

Although many scientists seem unaware of the work done by Bennett and his colleagues, at least one has found the results convinc-

ing enough to apply them to a seemingly unrelated field. Photobiochemist Francis P. Gasparro of the Yale University School of Medicine is seeking cell-surface targets for psoralen – a treatment for psoriasis and cutaneous T-cell lymphoma – to help explain the drug's apparent ability to stimulate an immune response against abnormally dividing skin cells. He read Bennett's research reports and saw a possible connection.

Because Bennett's papers "made [cell-surface DNA] look real," Gasparro says he decided to investigate whether surface DNA might play a role in psoralen treatment. So far, his experiments have revealed that the light-activated drug binds to DNA on human lymphocyte membranes (SN: 7/1/89, p.5).

Gasparro, who presented the finding at a Yale photobiology symposium last June, says other researchers have reacted positively, albeit with surprise. He believes he has convincingly shown that surface DNA exists, but says additional experiments will be needed to determine its potential role in photobiology.

Most scientists initially react to the recent surface-DNA research with reservations, but then become "quite fascinated by it," Bennett says. The continuing flow of funds for such work, coupled with accumulating reports in prestigious journals, indicates the scientific community just might give surface DNA a chance to answer some provocative questions.

Where, for instance, does the membrane-bound DNA originate? Most of the scientists who pioneered studies in this area believe it's manufactured in the nucleus and somehow transported to the cell surface. But Gasparro and Bennett think it comes from dying cells that expel their nucleic acids into the bloodstream, where the DNA circulates until receptors on living cells "grab" it. "No one knows what happens to DNA in the body after it's released from [dead] cells," notes Bennett. "It's a black box that people have just ignored."

And how does surface DNA manage to survive the DNA-digesting enzymes in blood? Gasparro, basing his hypothesis on ultraviolet-spectral data showing that surface DNA has an unusual molecular composition, suggests it undergoes some chemical modification that protects it. Surface DNA may contain unusual nucleotide building blocks or added methyl groups, he speculates.

Perhaps most puzzling is the mystery of what purpose the DNA receptors evolved to serve. Bennett believes they provide a way for healthy cells to recycle DNA from dead ones, in "a salvage pathway for conserving DNA's building blocks."

Scientists do not know whether the DNA taken up by the receptor actually becomes part of a cell's genetic material or alters cell function in any way. But the receptor's existence provides great fodder for scientific imaginations. For example, Hefeneider says, "it would be very exciting for us" if this DNA receptor provided the entry route for "antisense" DNA – short DNA pieces that can bind to and cripple specific viral or cancer-causing genes inside a cell (SN: 6/10/89, p.360).

Alternatively or additionally, the DNA itself might exert an immunologic influence while still on a cell's surface, as Rosenberg and Golub have suggested. An immunologic role might have multiple medical implications. For instance, if immunosuppressed T-cells were shown to contain membrane DNA, scientists might be able to develop drugs that act on the DNA to boost immunity in AIDS patients – an idea Golub says Rosenberg once suggested to him.

For now, membrane-bound DNA poses far more questions than answers – and those questions grow ever more intriguing as scientists improve their understanding of the controversial phenomenon. But if additional labs confirm Bennett's results, and if researchers can genetically reproduce the receptor in functional form, the nucleic outcast might someday code for a few clinical answers. □

Letters continued from p.227

'Better than chicken soup'

If you want to flush a number out of the bushes, just publish (as you did) a statement like "No numerical estimates exist for ... lycopene in foods" ("More veggies join fight against lung cancer," SN: 8/12/89, p.102).

The numbers exist; they just haven't been corralled. My "Father Nature's Farmacy" data base indicates that tomatoes contain 1 to 78 parts per million lycopene, the higher figure for the ripest tomato. Lycopene is also listed, but without quantification, for apricot, carrot, eggplant, grapefruit, papaya, pot marigold, stinging nettle, tea and watermelon. I'd like to hear from any other readers who have quantitative data on lycopene or lutein.

Your article makes tomato soup – or better yet, a 20-vegetable synergistic soup, seasoned with 10 antioxidant herbs and spices – look even better than chicken soup as a cancer preventive. What could be healthier for smoking Americans than switching from cancer sticks to carrrot sticks?

James A. Duke
Botanist
Germplasm Services Laboratory
USDA Agricultural Research Service
Beltsville, Md.

Stuck with the stuff?

"Making the Right Stuff" (SN: 8/12/89, p.108) both amazed and concerned me. I am amazed that such detailed and exacting technology exists with which to address specific problems. However, my concern is that this avenue of materials creation might contribute to the already unacceptable waste problems facing the world. If Mother Nature is not producing these materials, will she be able to reduce them?

Our friends in Washington would do well to consider legislation requiring all manufacturers to provide explicit information on how their products, once they've outlived their purpose, can be disposed of and/or recycled without endangering the environment.

Jan Eveleth
New Haven, Conn.

Superplants: Use and misuse

"Please Pass the Genes" (SN: 8/19/89, p.120) contains a statement that sounds like an agribusiness public relations release. It would be naive to think that genetically engineered, herbicide-resistant crop plants will be produced so that "farmers might someday be able to abandon the more damaging herbicides."

Indeed, by producing resistant crop strains, farmers will be able to use the more broadly damaging herbicides. The instances where some other environmental advantage is obtained, such as using an herbicide with faster degradation, will be the exception, not the rule.

Paul D. Morrell
San Francisco, Calif.

People have been "tinkering with genes" in plants since the dawn of agriculture, not just "since the early 1900s" as you state. Constant selection for high yield or better taste changed crop genetic makeup long before any knowledge of genetics. Genetic engineering is largely a more efficient method of producing superior plants, as a word processor is more efficient for writing than a quill pen.

But what are we going to do with high-lysine tobacco – make high-protein cigarettes?

David R. Hershey
Assistant Professor of Horticulture
University of Maryland
College Park, Md.

High-lysine tobacco has no commercial future, only research value. – I. Wickelgren

Cattle, sheep and cheat

As long as the cattle- and sheep-growers' associations dictate the grazing policies of the Bureau of Land Management and the U.S. Forest Service, cheat grass will win ("Combustible grass winning the West," SN: 8/19/89, p.127). It is an indicator of the overgrazing fostered by these agencies.

R. O. Baird
BLM Regional Range Examiner, retired
Tubac, Ariz.

CORRECTION

In "Cloudy Concerns" (SN: 8/12/89, p. 106), the statement that a 1,000-kilometer cloud system is "100 billion orders of magnitude" larger than a 10-micron water droplet should read "11 orders of magnitude larger" or "100 billion times as large." Orders of magnitude increase by a power of 10.

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