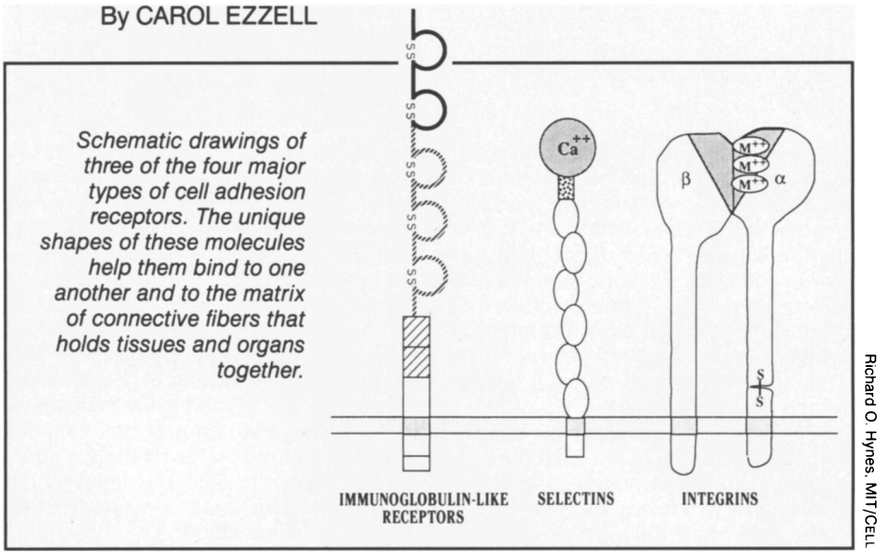
Sticky Situations

Picking apart the molecules that glue cells together

By CAROL EZZELL



Schematic drawings of three of the four major types of cell adhesion receptors. The unique shapes of these molecules help them bind to one another and to the matrix of connective fibers that holds tissues and organs together.

Richard O. Hynes, MIT/CELL

Some of their names sound like the fuel systems of fancy sports cars: VCAM, ICAM and NCAM. But these cell-surface molecules — which poke through the outer membranes of cells — have nothing to do with Porsches, Ferraris or Lamborghinis. Instead, they constitute the adhesive that literally glues together all multicellular organisms.

Several different classes of these cell adhesion molecules, or CAMs, form the appendages cells use to clasp each other and to grip the scaffolding of connective fibers that laces organized tissues and organs. Some also help migrating cells, such as those moving in to close a wound, to haul themselves past one another and into the correct position.

Recently, cell and molecular biologists have found that CAMs enable white blood cells to home in on injured tissues or those infected by disease-causing microbes. Within the past several months, researchers have also uncovered new evidence that cell adhesion molecules play a role in such diverse processes as embryonic development, learning and memory, viral infection and the spread of cancer cells throughout the body. Armed with this information, drug companies are now turning to CAMs for clues to new treatments for cancer, infectious diseases and autoimmune disorders.

The body contains four major types of cell adhesion molecules, which scientists have classified according to their overall structures. One type consists of a single string of protein that protrudes through the cell's outer membrane as a series of loops, like the decorative icing on a wedding cake. Because these loops resemble the business end of an antibody molecule, or immunoglobulin, which sticks to and inactivates invading microbes, researchers have named this class of molecules the immunoglobulin-like adhesion receptors.

These molecules usually bind to another type of CAM, the integrins, which contain two protein strings that sprout independently from the cell's outer membrane and then mesh together at the top, like closely planted trees. A third type, the selectins, pierces the outer cell membrane in the form of a single zigzag. A fourth type of adhesion molecule, the cadherins, penetrates the cell membrane in a straight line. These relatively simple receptors help hold like cells together.

Last year, Michael B. Lawrence and Timothy A. Springer of the Center for Blood Research at Harvard Medical School in Boston found that selectins protruding from the cell lining of blood vessels slow white blood cells in the bloodstream, much as the thick nap of a shag rug would slow a rolling ball. Curbing the speed of white blood cells — which normally whiz through the blood — gives these immune-system cells time to recognize and respond to SOS signals broadcast by damaged or infected tissue nearby.

At a Keystone (Colo.) Symposium on integrins in April, Springer described an "area code hypothesis" to explain how white blood cells tell when and where to leave the superhighway of the bloodstream and answer the emergency call of a tissue in distress. According to this now widely accepted hypothesis, a white blood cell must recognize three specific signals in a particular sequence in order to attach to and penetrate the blood vessel wall and reach an injured or infected tissue.

The first digit of the area code represents one of the three types of selectins. Lawrence and Springer demonstrated in the May 31, 1991 CELL that selectins cause white blood cells to slow down and roll along a blood vessel wall.

Once slowed, a white blood cell can pick up the trail of one of 10 chemical attractants secreted by clotting blood, marauding bacteria or tissues in danger. This signal serves as the second digit in the area code.

Finally, as the code's third digit, the white blood cell uses its integrin receptor to latch onto one of five or six different immunoglobulin-like adhesion receptors that cells in the vessel wall make during a crisis. One of these receptors is ICAM-1, intercellular adhesion molecule-1. In a process not well understood by researchers, binding the integrin to ICAM-1 somehow causes the white blood cell to flatten against the vessel wall and feel around for a microscopic gap between the cells that make up the wall. Once it gains such a foothold, the white blood cell can squeeze its way through the wall and out into the surrounding tissue.

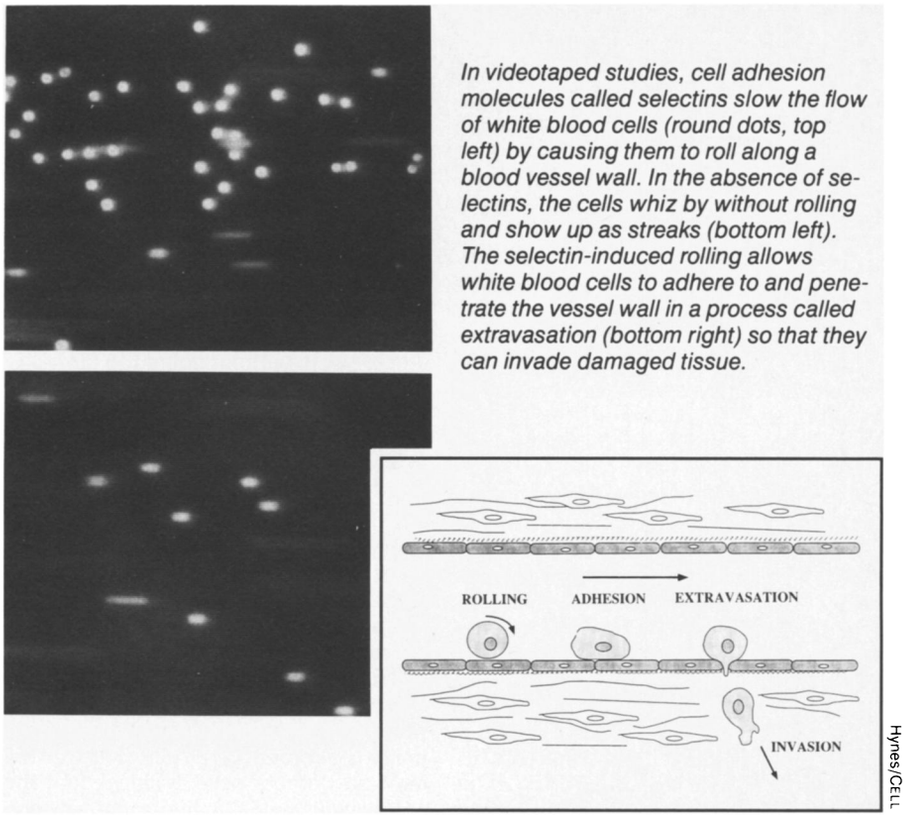
"There are three different steps, like an area code, and there are multiple stimuli that can be selected at each step, just as each digit in an area code can be one of 10 numbers," explains Springer. "Not only that, but the digits must be dialed in the correct sequence."

In most people, this process works just fine. But in inflammatory disorders, such as rheumatoid arthritis, inflammatory bowel disease or psoriasis, rampaging white blood cells somehow dial the wrong number and end up mistakenly invading healthy tissue.

Advances in understanding this molecular misdialing have prompted scores of biotechnology and pharmaceutical companies to examine whether targeting errant white blood cells might reverse

rheumatoid arthritis, prevent organ-transplant rejection (SN: 2/29/92, p.132) or check the tissue-injury response that sometimes gets out of hand following a heart attack or stroke.

Cytel Corp. in San Diego, for example, has begun developing antibodies and other drugs to block an integrin called VLA-4, which company researchers have found at elevated levels on the cells of rheumatoid arthritis patients. They are also working to design compounds to inactivate a selectin called ELAM-1 that sometimes gets overzealous in summoning white blood cells to injured tissues.



Springer/CELL

Hynes/CELL

In videotaped studies, cell adhesion molecules called selectins slow the flow of white blood cells (round dots, top left) by causing them to roll along a blood vessel wall. In the absence of selectins, the cells whiz by without rolling and show up as streaks (bottom left). The selectin-induced rolling allows white blood cells to adhere to and penetrate the vessel wall in a process called extravasation (bottom right) so that they can invade damaged tissue.

Drug companies might soon have another objective for their cell adhesion molecule research: cancer. Several recent studies indicate that different CAMs can help or hinder the spread, or metastasis, of cancer cells throughout the body.

At the Keystone meeting on integrins, cell biologist Randy Kramer of the University of California, San Francisco (UCSF), reported finding an association between a particular type of integrin molecule ($\alpha_7\beta_1$) and an aggressively metastasizing form of melanoma. Melanoma strikes the skin's pigmented cells, called melanocytes. Kramer and his colleagues isolated the integrin from melanoma cells but could not detect it in normal melanocytes. Surprisingly, however, the melanoma cells with the most integrin on their surfaces formed tumors that spread more slowly in mice than melanoma cells with little integrin.

"That was the curious thing," Kramer says. "This particular integrin's [presence] was inversely correlated with the metastatic potential of the cells."

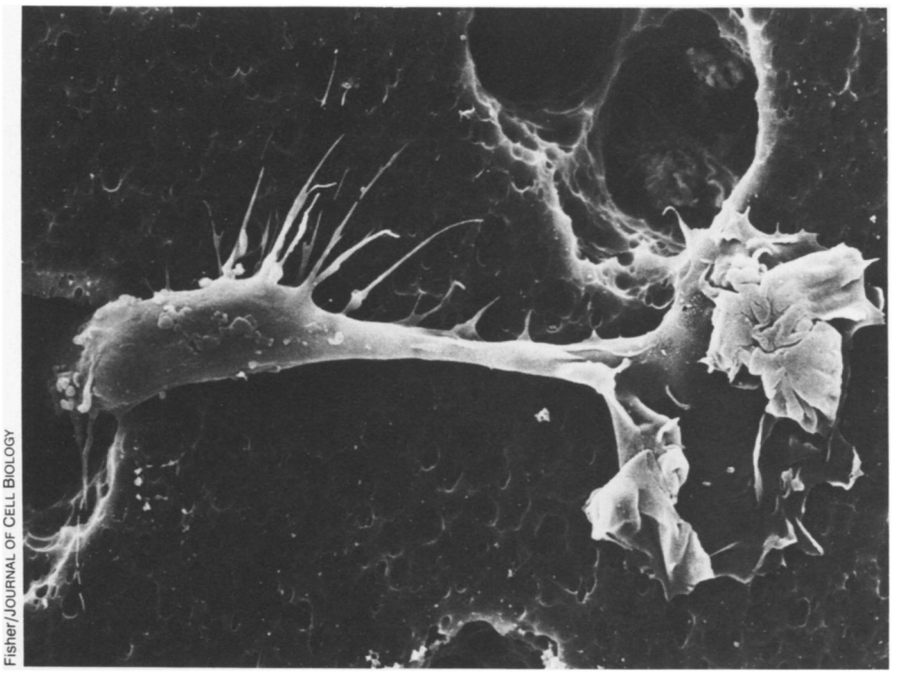
When Kramer's group probed further, they found another, closely related integrin that was present only in melanoma cells, this time at elevated levels in the *faster*-spreading tumors. Both of the integrins are known to bind to laminin, a connective-tissue fiber that constitutes the so-called basement membrane encasing tissues and organs.

To metastasize from one part of the body to another, cancer cells must traverse their own basement membrane, hitch a ride in the bloodstream and cross another basement membrane to take up residence in a new tissue. But oddly, Kramer's team found that cells with the first, less metastatic integrin bound to laminin more tightly than those with the second, more metastatic integrin.

"It could be that the melanoma cells with [the first integrin] bind to laminin but become glued on and can't go anywhere," speculates Kramer. "But I suspect that [binding of the first integrin] confers a more global effect on the cells. It may be a tumor-suppressor signal that has developed over time to prevent death from melanoma, even once tumors have started."

Kramer adds that physicians might one day use these two integrins as markers for identifying those melanomas most likely to metastasize and therefore requiring more rigorous treatment to eradicate. "It could be used as a diagnostic tool . . . that would be predictive of whether you've got an aggressive tumor," says Kramer. "That would be very useful clinically."

But he cautions that blocking the bind-



Fisher/JOURNAL OF CELL BIOLOGY

A human embryonic cell from a trophoblast — the early precursor of placenta — uses cell adhesion molecules called integrins to crawl through the pores of a gel matrix.

ing of the more metastatic integrin—with antibody-based drugs, for example — would probably not work as a melanoma therapy. He suggests the antibodies might instead stimulate metastasis by signaling to melanoma cells that they are stuck to a basement membrane and that they should push their way through.

While some researchers look for ways to obstruct cell adhesion molecules involved in disease processes, developmental biologists are finding that the molecules play a vital role in embryos. UCSF placentologist Susan J. Fisher told an April Keystone Symposium on tissue engineering that another laminin-binding integrin helps shape the human placenta.

She and UCSF cell biologist Caroline H. Damsky study the trophoblast, the outer layer of embryonic tissue that implants into the uterine wall. Developing trophoblast cells must crawl through and meld with maternal tissue and blood vessels to form the placenta, which allows the embryo to receive nutrients from the mother while preventing their blood from mixing.

"These cells behave much like tumor cells," says Damsky. "They are able to penetrate basement membranes and blood vessels, and they make many of the same [protein-digesting enzymes] that tumor cells make."

Fisher and Damsky developed a way to grow human trophoblasts in laboratory cultures in order to study how the cells accomplish implantation. When the researchers added antibodies against a laminin-binding integrin to the trophoblast cultures, they found that the antibodies reduced the implantation capabilities of the trophoblasts by half. But when they added antibodies against another integrin — this time one that binds to another connective-tissue molecule called fibronectin — the trophoblasts invaded nearly three times faster than usual.

"We think that the net invasiveness of the trophoblasts depends on a balance between the effects of these two integrins [throughout the tissue]," concludes Damsky. "One is a brake on invasion, and the other is promoting invasion."

Like Kramer, Damsky hypothesizes that the integrins transmit signals to the cell about its surroundings. "The interaction of the integrins with the [connective-tissue] matrix is informational," she asserts. "It's not just 'stick' or 'don't stick.'"

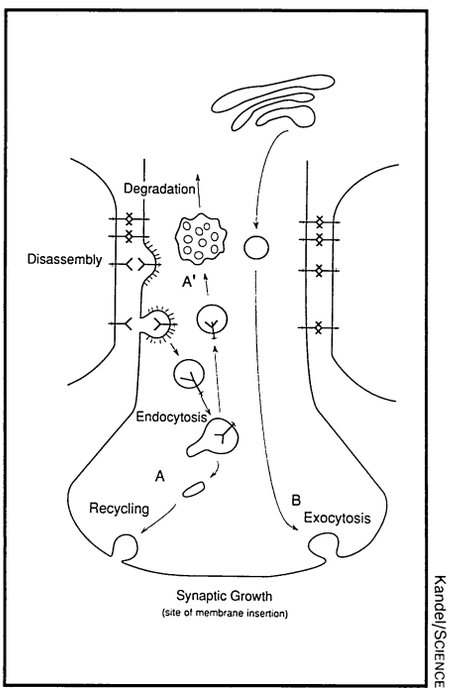
Damsky suggests that trophoblast cells receive a signal to migrate when one integrin binds to laminin but are told to stop migrating when another integrin binds to fibronectin. She concludes that such information may help trophoblast cells navigate to the correct depth in the uterine wall, without going too far.

But how does integrin binding tell a cell to hit the brakes or keep chugging along? Many researchers believe the answer lies in a biochemical connection between integrins and the cytoskeleton, the network of proteins that gives a cell its shape.

Alan F. Horwitz reported at the Keystone integrins meeting that he and his colleagues at the University of Illinois at Urbana-Champaign have isolated a set of proteins that may link integrins to actin, a major constituent of the cytoskeleton. His team and others have evidence that the cytoplasmic tails of integrins — which dangle in the cell's watery interior, or cytoplasm — bind to these proteins. And, in turn, the proteins bind to actin.

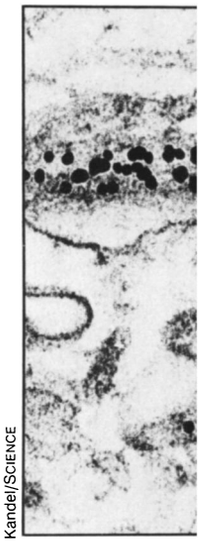
Jonathan C. R. Jones of Northwestern University Medical School in Chicago also told the meeting that he and his colleagues have found a particular integrin at the core of cell structures called hemidesmosomes. These biochemical snaps form microscopic bridges between the cytoskeletons of cells and connective tissue fibers.

Researchers say that proteins and structures like these might serve as molecular clutches to engage or disengage the cell's transmission, thereby regulating its movement.



Kandel/SCIENCE

Nerve cells unsnap themselves from their neighbors by gobbling cell adhesion molecules in a process called endocytosis. Once inside a nerve cell, the molecules can be degraded by enzymes (pathway A') or recycled to a growing part of the nerve cell to form new connections called synapses (A). Nerve cells can also grow by making new membrane (B) and sending it to the synapse through a process called exocytosis.



Kandel/SCIENCE

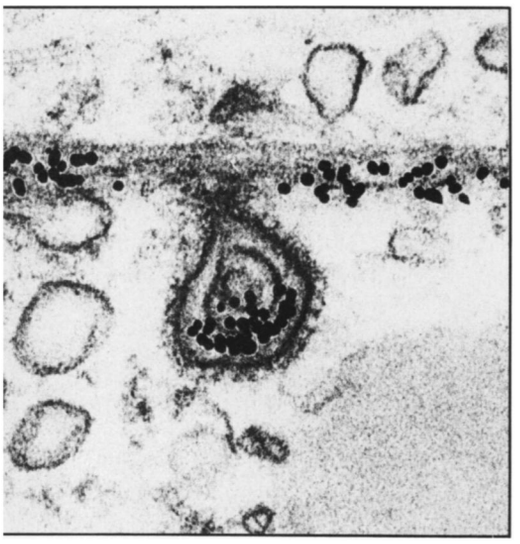
Electron microscopy shows a nerve cell breaking its bond with a neighboring cell by internalizing the cell adhesion molecules (labeled by black dots) that hold the two cells together. In this instance, the breakaway cell has even gobbled a portion of the other cell's membrane (inner blob coated with black dots) in order to free itself.

A similar process might work in neurons, or nerve cells, according to recent studies. Several researchers have found evidence that neurons in the brain make new connections, or synapses, with other neurons during the learning process. In this phenomenon — referred to as synaptic plasticity — the neurons' finger-like projections, called neurites, must move around and stick to one another.

Several years ago, researchers discovered that a molecule named NCAM (neural cell adhesion molecule) constitutes one of the glues that can hold neurites together. Now, a group led by Eric R. Kandel of Columbia University in New York City has found that a similar cell adhesion molecule might help hard-wire a learned reflex into the neurites of a species of sea snail named *Aplysia californica*. The researchers call the new molecule — which falls within the immunoglobulin-like cell adhesion molecule family — apCAM. They report their finding in the May 1 SCIENCE.

Kandel's team studies sea snails because they can be trained to draw in their gills following a tap on their siphons. To learn this task, the snails increase the number of synapses between their siphon neurons and their gill neurons. Kandel and other researchers have already shown that these neurons also form new synapses in cell culture when treated with serotonin, one of the neurotransmitters that convey messages between neurons in the brains of animals.

Using fluorescently labeled antibodies, Kandel's team found that serotonin treatment reduced the amount of apCAM on the surfaces of sea snail neurites after one hour. Moreover, the researchers demonstrated that the apCAM later showed up



embedded in blebs of cell membrane floating around in the neurons' interiors.

On the basis of this evidence, they assert that a learning neuron unsnaps itself from its neighbor by gobbling the apCAMs that hold the two cells together. This frees the neuron to form new connections with other neurons.

The researchers suggest that the neuron moves toward other neurons by recycling the patch of membrane that once contained the apCAM. "Maybe what's happening is that the cell pulls in the [apCAM-containing] membrane at one point, where it's attached to another neuron, and moves that membrane to the active growth zone where it's forming new synapses," says Kandel. "Neurons might use this as a way to redistribute membrane."

Researchers have also found that some cell adhesion molecules render cells vulnerable to viruses. Three years ago, two teams – one led by Harvard's Springer – discovered that ICAM-1 is the conduit through which the cold-causing rhinoviruses gain access to cells (SN: 3/18/89, p.165). In the March 27 SCIENCE, Jeffrey M. Bergelson and his colleagues report that echovirus-1, one of a family of viruses that cause flu-like diseases, invades cells after binding to the integrin VLA-2.

Working in Robert W. Finberg's laboratory at the Dana-Farber Cancer Institute in Boston, Bergelson's group found that antibodies against VLA-2 prevented echovirus-1 from attaching to and infecting cells grown in laboratory cultures. Moreover, when the researchers inserted a gene for VLA-2 into cells that normally resist echovirus-1 infection because they lack the integrin, the cells became susceptible to the virus.

In adults, echoviruses cause the aches and fever of a particular flu-like disease that is common in the summer months. But newborn infants can develop a potentially fatal viral meningitis following echovirus infection. The Centers for Disease Control in Atlanta estimates that echoviruses are responsible for two-thirds of all U.S. cases of viral meningitis in infants and children.

Bergelson speculates that babies might have larger amounts of VLA-2 than adults. "One reason that infants might get echovirus infections in organs that aren't affected in adults is that the [VLA-2] receptor might be expressed in fetal life or early newborn life and then get shut off," he suggests. But he cautions that researchers have not yet confirmed that VLA-2 levels decrease after childhood. He adds that his group hasn't yet tested whether other echoviruses—particularly echovirus-11, which is known to be especially virulent – bind to VLA-2.

Fueled by these new developments, virologists, cancer researchers, cell biologists, neuroscientists and developmental biologists are constantly discovering new cell adhesion molecules and elucidating the CAMs' actions in normal and disease processes. These scientists have so far identified roughly 20 different integrins alone, spread throughout various tissues of the body. And the count is sure to get higher. □