

QnAs with Richard O. Hynes, Erkki Ruoslahti, and Timothy A. Springer: Winners of the 2022 Albert Lasker Basic Medical Research Award

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The 2022 Albert Lasker Basic Medical Research Award was jointly awarded to Richard O. Hynes, Erkki Ruoslahti, and Timothy A. Springer for their discoveries concerning proteins called integrins. Integrins are transmembrane receptors involved in cell–cell adhesion and in adhesion between cells and the extracellular matrix (ECM) surrounding cells. When integrins bind ligands, they activate numerous signaling pathways and play key roles in cellular physiology and diseases such as cancer.

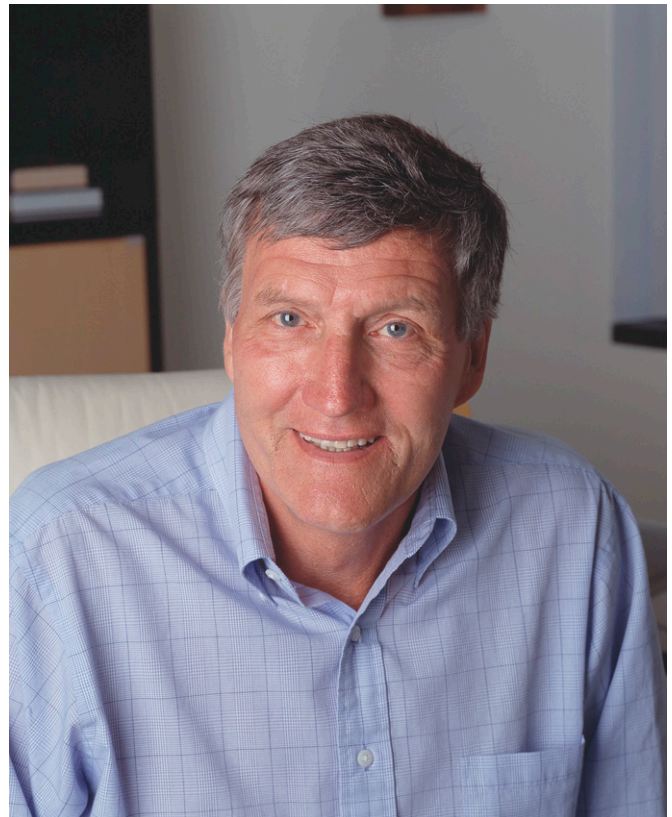
Richard O. Hynes, a member of the National Academy of Sciences, is a professor and cancer researcher at the Koch Institute and the Massachusetts Institute of Technology. Hynes codiscovered fibronectin, published in *PNAS* (1), and cloned and named some of the first integrins, published in *Cell* (2, 3). He has made significant contributions to the scientific understanding of ECM–integrin interactions and the role of integrins in cancer progression and metastasis, with potential applications in developing treatments for cancer and other ailments, such as thrombosis and fibrosis (4, 5).

Erkki Ruoslahti, a member of the National Academy of Sciences, is an emeritus professor at Sanford Burnham Prebys Medical Discovery Institute and has performed pioneering work on cell adhesion. He was one of the discoverers of fibronectin and identified the three-amino acid RGD (arginine-glycine-aspartate) sequence within integrins that is responsible for attachment to cells, work that was partly published in *Nature* (6, 7). By isolating the cell surface receptors for RGD, he discovered some of the first members of the integrin family. Ruoslahti's research eventually led to the development of RGD-based antiplatelet and anticancer drugs (8). More recently, he has worked on using peptides for targeted delivery of drugs and nanoparticles to tumors and disease sites (9).

Timothy A. Springer, a member of the National Academy of Sciences and professor at Harvard Medical School, is recognized for his discovery of some of the first integrins and the first intercellular adhesion molecules, partly published in *Nature* and *Cell* (10–12). Springer has helped elucidate the role of cell adhesion molecules in the immune system, and his work has formed the basis for the development of multiple therapeutics for autoimmune disorders. More recently, he has been working on deciphering details of the molecular basis of integrin function.

PNAS: How did you discover integrins?

Hynes: During the early days of [research on the] molecular cell biology of cancer, I became interested in the question of what was different about the surfaces of normal



Erkki Ruoslahti. Image courtesy of Sanford Burnham Prebys.

and tumor cells. That difference was revealed by surface labeling, and that was how we found fibronectin in 1973 (1). Fibronectin promoted cell adhesion, migration, and organization of the actin cytoskeleton. That then led to our search for its receptor, and eventually to the discovery of integrins (2–4). These molecules played an important role in the change in behavior of tumor cells, which change their integrin functions and lose contact with the matrix and move around much more than normal, well-anchored cells do. That whole idea of how cells are anchored to one another, to the matrix, and to other substrates is what's driven my interest in this field. It's been a very exciting and collaborative field from the beginning, and hundreds of [laboratories] have worked on integrins, and we benefited from and built upon the work of many others and, of course, our own research has depended on many excellent

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Published September 28, 2022.



Timothy A. Springer. Image courtesy of Caitlin Faulds (Institute for Protein Innovation).

postdocs and graduate students, and they'll all be pleased by this recognition of the field.

Ruoslahti: When I started my own [laboratory], there was very little known about how cells recognize their surroundings and how they knew where they should go. I started studying the surface proteins of fibroblasts, and working with colleague Antti Vaheri, we made antibodies against material peeled off the fibroblasts. We found antibodies against one protein, which we later named fibronectin (6). We then started zeroing in on the cell attachment function of fibronectin until we reduced it to the tripeptide RGD and reproduced the cell attachment function with synthetic peptides. That gave us the tools to go after the receptor with affinity chromatography.

The peptide was coupled to a column and we found a protein that bound to it, but it didn't bind to fibronectin, which was puzzling. We then used a large fragment of fibronectin on the column and eluted with the peptide and that gave us a different protein that bound to fibronectin (13). It was the fibronectin receptor. In the meantime, we had cloned another protein that behaved like fibronectin, which we had named vitronectin, and we realized that the first receptor we had discovered was primarily a vitronectin receptor. We then made antibodies against these proteins and cloned the α -subunit (14). Around the same time, Richard Hynes cloned the β -chain of the primary



Richard O. Hynes. Image courtesy of Kalman Zabarsky.

fibronectin receptor, integrin $\alpha 5\beta 1$, and we cloned the α -chain of the vitronectin receptor, and then eventually both of us cloned the whole thing, and Tim Springer cloned the chains of the lymphocyte receptors he was working on (2, 15). The real revelation was that we had all these receptors that recognized RGD but in different contexts. That integrins would end up being this important family, of course, there was no way of predicting that. Also, I did not necessarily expect this, but integrins turned out to be important signaling molecules.

Springer: When I started working on this [topic] in the 1970s, we knew nothing about the molecular basis of how one kind of immune cell would bind to a target cell or to another immune cell. Immunologists knew that antigen-specificity was required in targeting of cytotoxic T cells. But the interaction was magnesium-dependent, which made me think there would be additional types of molecules involved. I set out to make monoclonal antibodies to identify such molecules, and found a molecule with two chains that was required not only for antigen-specific responses but also other kinds of responses. I called this a lymphocyte function-associated (LFA) molecule (10).

Previously I had identified a different molecule on macrophages [immune cells that fend off pathogens] that had a similar composition of two polypeptide chains. I was intrigued by the similarity between these two types of surface molecules, and I proved that the smaller chain, which I called the β -subunit, was identical between these two molecules, and the α -subunits were distinct (16). I did some sequencing and found that the α -subunits were related to one another, and realized it must have been a family of genes that was duplicated. This was the first evidence for family relationships among integrins. I went on to find more integrins and showed that they required magnesium to bind their ligands (11). At the same time, Richard Hynes and Erkki Ruoslahti were working on extracellular matrix ligands and realized that these must have receptors on the cell. When they identified the receptors, their sequences were homologous to the ones I had identified on the lymphocytes, further expanding the family. It was exciting to realize that we were working on related molecules, and the family kept expanding and now has 24 members.

PNAS: Why are integrins important?

Hynes: These receptors have so many roles to play and are responsible for an enormous amount of the organization and functioning of the body. How cells know where to stick and why they don't always stick and how they let go are important questions in biology and in pathology. Platelets are a prime example of how control of adhesion is vital. If you have too much adhesion, you get thrombosis; if you have too little, you bleed to death. In many of these systems, the final adhesion comes from integrins, they're very good adhesion receptors. Most migratory cells also use integrins to know where to go. So, the front end of a neuron, when it's growing, has integrins on it that determine its path. And when neurons eventually synapse with a target, they use adhesion molecules including integrins to make sure they make the right connections. Come the

1990s, we started making mouse models for human diseases involving various integrin and ECM mutations, such as platelet bleeding disorders, various leukocyte adhesion disorders, and we were able to figure out what integrins do in vivo. When genomes came along, it was interesting to find out how widespread they were. Most cells in all metazoa have integrins, and they're responsible for making sure cells make the correct connections with their environment and with their neighbors. If we didn't have things like integrins and cadherins, we'd be a puddle of cells on the floor.

PNAS: What are some applications of research on integrins?

Hynes: Clearly, these are good molecules to target, as they're on the outside of cells, there's a finite number of them, and we understand them pretty well at the biochemical and biophysical level (5). There are ways to tweak them, and the monoclonal antibodies that we and others used to figure out how they function were also the beginning of therapeutic agents. The RGD sequence that Ruoslahti and his colleagues initially discovered opened up another way of approaching things with peptidomimetics. So they're very well suited for treating disease, and that's well underway. Integrin targets in hemostasis, thrombosis, inflammation, autoimmunity, and the like are already in the clinic.

Ruoslahti: One of the integrins is the main receptor in platelets that makes platelets aggregate in blood clotting, and we showed that it also was an RGD receptor. Pharmaceutical companies made chemical compounds that mimic the RGD peptide sequence and used them in preventing what is called reocclusion: when an artery gradually closes again after it has been opened up with a balloon. It turned out that reocclusion could be prevented by RGD peptides and RGD-derived compounds. Another area where these peptides hopefully will be useful is to target drugs in tumors. Cancer blood vessels are rich in αV integrins, so we developed an RGD peptide that seeks out tumors because it binds to the αV integrins in tumor vessels. It goes deep into tumor tissue, and it can take a payload with it. What's

remarkable is that you don't have to couple the peptide to the drug, you just give them at the same time and the endothelial cells take in whatever is there in the blood next to the cell, including the drug (9). So that's now just entering into phase 2 trials.

Springer: These molecules mediate most of the migration of cells in the body. Leukocytes circulating in the bloodstream cross the vessel wall to accumulate at sites of inflammation and autoimmune disease. [Our team] discovered that integrins are also required for that exit from the bloodstream. A company I founded, LeukoSite, followed up on these discoveries and started developing an antibody to block an integrin that's important for certain white blood cells to enter the gut. In 1996, LeukoSite published that such an antibody could treat ulcerative colitis in a monkey model (17). That antibody, Entyvio, was approved for humans in 2014 for treatment of ulcerative colitis and Crohn's disease. It is now a very important drug that's treating around 100,000 patients a year. Other approved therapeutics, including ones used to prevent thrombosis, one to treat dry eye disease, and another to treat multiple sclerosis, all block integrins.

PNAS: Where do you see this field going in the future?

Springer: One of the remaining mysteries is that cells, in order to migrate and use these integrins, have to use force. These forces help the cells crawl through tissues, they're exerted through the integrins, and have a very big impact on integrin function. I'm very interested in understanding the molecular basis for how forces transmitted through integrins regulate their conformation and the cytoskeletons assembled downstream of integrins.

Hynes: One goal has been to see how one could use integrins to combat cancer. There have been lots of singles, but no home runs yet because it's so complicated. But it's beginning to pay off. Beyond that, studying integrins covers the whole of biology, so one certainly doesn't get bored, there's always more to discover.

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