Are we alone? I want to tell you the formula for success in developmental biology. It's a six-word mantra that will help you plan experiments, write grants and pass exams with ease. Find it, Lose it, Move it. Find it, the correlation of a molecule or structure with a function. The molecule has to be present at the right time and place. Lose it, the loss of function if you get rid of the molecule or structure by mutation or by inhibitors. So when you lose the molecule, do you lose the function? And Move it. If you put the molecule or structure in a new place, where it's never been before, do you get the function appearing? So this is critical. Find it, Lose it, Move it.

It's the basis of developmental biology research. This strategy has been essential in the studies of fertilization. Now you remember that the part of the sperm that adheres to the egg initially is the acrosome. The acrosome is put out from the sperm by the polymeration of actin molecules, and it's the inner membrane of the acrosomal vessicle, and it adheres to the egg. Now bindin was found as an insoluble molecule and was thought that it might be on the acrosome. Now to find it, immunohistochemical localization of bindin was demonstrated. What they did was, they took an acrosome-reacted sperm, they took sperm with acrosomes coming out and they put an antibody against bindin onto the sperm such that if the sperm had bindin anywhere, the antibody would bind to it. Not only that, the antibody was conjugated to a protein which would take diaminobenzadine, DAB, in the presence of hydrogen peroxide, and turn it into a dense, blue-black precipitate, so everywhere there's bindin, the antibody would bind. Everywhere the antibody would bind, there would be a chemical reaction to turn DAB, a colorless chemical, into a black-blue precipitate.

As you could see, these investigators, Vacquier and Moy, found that there was bindin present on the acrosome of the sperm and that when the sperm bound to the egg through the acrosome, they could see the bindin there. So that was the first part, that was Find it. What about Lose it? Here what the investigators did was they took inhibitors of bindin, they took soluble pieces of bindin, which would inhibit the egg binding to the sperm's bindin. And what they did is they put this inhibitor into solution with the sperm and the egg, and the more of this inhibitor they used, the less sperm were able to bind

to the egg. So if this was a specific inhibitor for bindin, they were able to show that inhibiting bindin inhibited sperm-egg adhesion. So that was Lose it.

Now Move it, what they did here was they put bindin particles, no sperm, just bindin particles into solution and they put eggs with the bindin particles, and the bindin particles were either of the purpuratus species or the franciscana species. The bindin from purpuratus sperm agglutinated the eggs of Strongylocentrotus purpuratus, so the correct bindin matched the correct eggs. The franciscana's bindin, that agglutinated the eggs of franciscanas but did not agglutinate the eggs of purpuratus. So the bindin particles, without even being on sperm, were capable of producing the function. So the evidence that bindin mediates sperm-egg adhesion in sea urchins is Find it, which is the correlation, bindin is found on the acrosome at the right time and place. It's found on the place where the sperm contacts the egg. Lose it, this is negative inference. This is the loss of function where inhibitors against bindin inhibit sperm-egg interaction, they inhibit the adhesion of sperm and egg. And then Move it, positive inference, the gain of function evidence, is that bindin beads will aggregate the eggs together, and indeed, it's species-specific. So that's the first Find it, Lose it, Move it evidence.

Another set of Find it, Lose it, Move it evidence comes from modern experiments, experiments done in 2014, looking to see what is the protein on the egg that binds mammalian sperm. Now it's been known that mammalian sperm has a molecule called Izumi. Izumi is the molecule on the acrosome of the mammalian sperm that adheres to the egg. The question is, what is Izumi binding to? What is the receptor for Izumi?

Well, they found a protein called Juno. Juno is at the right place and time for binding Izumi, and actually this picture here is a picture of green fluorescent protein-labeled Izumi. If you lose it, if you use antibodies against Juno or against Izumi, and then you look to see if the sperm adheres to the egg, you find that when you inhibit the Izumi-Juno binding, you inhibit fertilization. You could do it by inhibiting Juno, you could do it by inhibiting Izumi. So that's the Lose it evidence. You get rid of the molecule, you get rid of fertilization. Then there's Lose it again. We can make mutants which lack the Juno

protein, and we can ask, "Do sperm bind to eggs that lack the Juno protein?" And the answer is not very well. There's some non-specific binding, but for the most part, you need Juno to have sperm-egg binding in mammals. So that's another piece of Lose it evidence. What about Move it evidence? Okay, you take Juno and you label some human embryonic kidney cells with Juno. In other words, you express Juno in the cell membranes of human embryonic kidney cells which never express Juno. So you then take another set of human embryonic kidney cells and you express Izumi in them. So you have one set of kidney cells with Juno, one set of kidney cells with Izumi. What happens when you put them together? They adhere to each other at the Izumi-Juno boundary.

So you have Find it, Lose it, Move it. Find it, the correlative evidence, Juno is on the egg cell membrane, it binds to Izumi, a sperm acrosomal protein that is known to bind to eggs. It's there at the right time and place. Lose it, the negative inference evidence, the loss of functions where antibodies to Juno block sperm-egg binding. Juno-deficient mutants don't bind sperm well. And then Move it, the positive inference, the gain of function where human embryonic kidney cells will adhere through the Juno-Izumi junctions on their cell membranes.

There's more evidence, there's more ways that Find it, Lose it, Move it has been used in fertilization. As you've read in the textbook, the evidence that calcium activates the sea urchin egg. Find it. The calcium ions spread through the egg at fertilization and not before it. They're there at the right place and time. Lose it, if you inhibit calcium ions with EDTA, there is no egg activation. And Move it, if you gain that function, if you add calcium ions to unfertilized eggs or release the calcium ions from endoplasmic reticulum, the egg is activated without sperm. So the mantra, Find it, Lose it, Move it. This is critical in experimentation throughout developmental biology.