Eric Jensen: Hi everybody. Let's go ahead and get started. I'm Eric Jensen and professor in the physics and astronomy department here and I'm really excited about our colloquium talk today. It's our second-to-last colloquium. Just to remind you, on Wednesday of next week, we are also going to have a talk. Sean Andrews will be talking about planet formation. So that'll be Wednesday at 4:30.

Today we have professor Eva-Maria Collins. I'm super excited to have her here. As we've done before, I'm not going to do quite as detailed an academic bio for her, because I'm going to ask her to talk a little bit about how she got to where she wants, but very briefly, she got her PhD in Germany and then spent some time both at Princeton and UC San Diego before we were very fortunate, about two years ago, to have her join the faculty here. And it's been really great to have her here as part of what I've seen over my time here as a growing connection between physics and biology, and the life sciences more generally and [supplementary physics 00:01:01]... the physics department and thinking about the interface between those two fields. So it's great to have somebody here who represents that, and not just somebody, but Eva-Maria specifically, who has done great work and has connected with our department and with our students on some of the research projects that she's doing. So I will leave it at that and ask you to help me welcome professor Eva-Maria Collins.

Eva-Maria: Thank you Eric. Thank you for having me, and I also want to thank the physics department for welcoming me not just now, but ever since I arrived here and giving me keys so I can go to the stock room and get great demos and all this kind of good stuff, I was very happy about. So, Eric asked me to tell you a little bit about how I got here, and I don't think he meant how I walked over from [Markmont 00:00:01:54], but this is me. As you can tell from the crazy hair, today it's sort of okay, but most of the time it's [something else 00:00:02:01].

Pre-college I tried to figure out what I wanted to do with myself, and I had this vision that I wanted to become an astronaut. I was very fortunate, and I'm not going to go into the details, but I got connected with some people who were training German astronauts from the German space agency and basically they told me this: all German astronauts were physicists at that time. That's not true anymore today, but they were like, "If you want to become an astronaut, you've got to study physics." So that's why I went to study physics.

And then... If you would look at my CV, which you could find online, this is sort of the history of things here. It turns out that while this looks very linear, in a way, it wasn't really linear. It was more like this. I have a tendency to always... I like talking to people, and people can have a big influence on what I do because I get excited very easily about a variety of things. And so I originally thought I studied physics, then I had a fantastic math professor, so I thought, "Okay, that sounds great. I'm going to do mathematics." So I got my bachelor degree in math and physics.

After that, my professors weren't as inspiring, so I dropped mathematics again and I was like, "It's just going to be physics." I didn't want to become an astronaut anymore at some point in time because I realized I'm really claustrophobic. That's probably not good for an astronaut, so there was that. And then I was very fortunate to do part of my diploma research at Princeton University. Again, I just talked to somebody, that was sort of an offer that came up, I was like, "Great, that sounds great." That's how I got into biophysics. I was much more interested in astrophysics originally, but that's how I changed course. And then I ended up doing my PhD between two [Muksblank 00:03:46] institutes, one theoretical physics and one experimental biology. Same thing happened, I was asked to apply for the Lewis-Sigler Fellow position at Princeton, I did. I ended up getting that job, so I moved there. After that I was at UC San Diego and I thought I would never leave California again, but I'll tell you why I did in the end. So I was very happy doing that.

Baby number one happened. So, like with many other things, that just meant something new and that changed what I was doing, because I was worried while I was doing experiments, how would that affect the developing baby when I worked with all these chemicals, and I realized there wasn't that much knowledge about developmental neurotoxicology. And at the same time I had a major catastrophe happening in my lab that my freshwater animals weren't so happy about, a filter change in the water system, and so I had a big wipe out of my population. So these two things came together and we started developing planarians, which I'm not going to talk about today, as a new system for developmental neurotoxicology. And this is one of the major research areas of my lab now.

And then baby number two came about, and at that point we realized that we really wanted to be closer to family. My husband's company is in Princeton, New Jersey, so he was flying back and forth all the time, and so with one child it was still okay, with two... adorable little children, we decided we're going to be moving back to the East coast, and I was lucky that there was a position in systems biology opening, and so that's how I ended up here. And so, instead of an astronaut, I'm now a professor in the biology department, despite the fact that I never studied biology. And I'm just telling you this as an example. What Eric said is that things might look rather linear on paper, but often they sort of go in a very different way, and you end up in very different places and you're still very happy. And I'm actually happy I'm not an astronaut because I'm so claustrophobic.

Okay. So, that's the story about that. So now I get to tell you about Hydra. How many of you are familiar with the myth about Hydra? So some, okay. So Hydra was this multi-headed monster that lived in Lake Lerna, so possibly that lake was the entrance to the underworld, and it was one of Heracles' 12 labors to kill the Hydra. What he did, depicted here in this beautiful drawing, he had a sword and he kept on cutting off the heads and every time he cut off one head, two new heads came back. So basically, he couldn't defeat the monster. And then he did what we often do in science when we're sort of stuck, we find a collaborator who hopefully has some expertise that we don't have. So he asked his nephew, and his nephew had the brilliant idea to just use a flame to cauterize the stumps after they were cut and that prevented the Hydra from regenerating the heads and eventually the Hydra was defeated. So very nice story.

So, the real Hydra looks more something like this. Rather inconspicuous, but I want to convince you that it's actually much better than the fiction, because it can do some quite cool things. One of them is, when you look at how this animal feeds. So this is a top-down view of the Hydra. This is its head with its mouth, and it actually has a closed epithelial sheet. And so every time it wants to eat, it basically has to rupture a new hole. And this looks more like a whole new universe opening up here as it's trying to eat the entire cover slip of that glass part where it's mounted.

So that's pretty cool. And that mouth will become relevant again later, but even cooler, and which would have been rather detrimental for Heracles, probably, is that you can take the Hydra and you can make a soup of cells and it will regenerate. And so you take the Hydra, put it in a blender, get Hydra soup, that Hydra soup will self-organize, and that's what we're going to talk about today, and eventually you get a new Hydra. You should say, "Wow, so much better than the gross story." I think it is. And so that's what's one of these things, I heard a talk about Hydra when I was in San Diego and I was so inspired. That's why I started working on this. So, that's usually what happens to me. Get too excited. So I think it's a unique system to study spatial patterning, and in fact there's two things I want to talk about.

The majority of my talk will be about this first part, because that's more of the physical part. We're going to see how physics plays in here. We have the two populations of cells, they somehow self organize into first a solid sphere, eventually this hollow sphere. And then in the second part of my talk, I'm going to tell you a little bit about symmetry breaking, and that's one of the things we are actively working on now, the question of how do you get from a system that seems to be homogeneous, so you have this sphere here, to a system that actually has an axis with a head and a foot at the end. And so that's the question is, "How can physical laws and processes account for the [denormal 00:08:46] formation of spatial patterning in this system?"

Before we can talk about the process, we've got to talk a little bit about the systems, so I'll give you some background. So here's the Hydra. You can basically think of Hydra as an intestinal tube. So, it's a hollow tube, consists of two layers of cells, an outer ectoderm and an inner endoderm, and it has two ends, a foot and a head. So these are distinct, but otherwise it's just a hollow cavity. We have three stem cell populations, so ectoderm cells always make more ectoderm cells, endoderm cells more endoderm cells, and then we have an interstitial cell population that gives rise to more specialized cell types, but we're largely interested in ectoderm and endoderm. The arrangement is important here. Ecto, as I said, is on the outside, endo is on the inside. We have tools now that allow us to label these different cell types, so different proteins in the cell, by fluorescently tagging them. And so the cell line we're going to use for most of this talk, it's called the watermelon cell line because the inside cells, the endoderm, have a red flourophore, which I'm going to show in magenta, and the outside have a green flourophore. And so that's like a watermelon.

We have tools that can take advantage of the regenerative capabilities of the system and, for example, do grafts where we introduce parts of a Hydra into another Hydra and use that to induce a new axis. And we can also completely separate the tissues, stick them back together, and they will regenerate into a new Hydra, so we have tools to look at these different components individually. Furthermore, we can reduce the system to just two components, and I think that's important when we think about the cell sorting processes. So everything I'm going to tell you about hasn't been done on systems that were reduced to two components, but it has been shown that you can do that and you get the same outcomes. So the reason why we didn't worry about these other cell types is simply because they are so small in number they're not important, but it's good to know that you can reduce it to a two component system.

And the way we do it is that we treat it with a chemical called colchicine, that kills the interstitial stem cells, and so all progeny that come from these interstitial stem cells, these are basically all cells that are not ectoderm and endoderm, will eventually go away. And these Hydra are therefore called epithelial Hydra, because they only have these two epithelial tissues and they look a little bit funny, they look like this. They can regenerate and they can bud and propagate in the lab, but they can't feed. And here's a video that shows you how we feed these animals in the lab and that's what's done. Undergrads in my lab at UCC... Cassidy Tran, she will basically force feed these animals. You hold them and move the foot end and then you take little shrimp and you sort of stuff it in. A little bit like they make foie gras. So you stuff it in and eventually you also have to burp them again, and let it come out because they can't open their mouth. That's going to become important later on, so we'll use those animals later on. Okay, so that's the tools that we have in the system.

We're going to first talk about self sorting, and I was not completely honest with you, just for the sake of effect, so we don't put one Hydra in and get one Hydra out and we don't use a blender. You might've guessed that. If you looked at the scale bar they're only roughly a few millimeters long, so we don't put them in a real blender. We use roughly a hundred Hydra. A hundred Hydra go in, we have a little Eppendorf tube where they get ground up, roughly 30 get out. So there's some loss in the procedure. It's just not easy to keep all the cells. But that process is true, so they do self organize into this tissue. That's what you see here. So again, we use these transgenic animals, where the outer layer is green, the inner layer is magenta, and this is the course of cell sorting that you can see here.

And so what I'm going to tell you about is work that we've published a few years ago in a biophysical journal, I'm not going to go into all the details, but if you're interested in more of the details, this is where you can find them. So the question we wanted to address was, "How does it work, what's the mechanism that actually drives the sorting in this system?" And this is a question that was first posed more than 40 years ago, in 1972, and it was work by actually another German physicist, Alfred Gierer, in the lab of Trenkner, where they figured out that you could even do that experiment, that you can take the Hydra and you can do this dissociation-reaggregation experiment. And so given that this was so long ago, it might be surprising that we have not had an answer of how that actually works.

There were two prominent theories, up until our work a few years ago. One was differential motility and the other one differential adhesion. So one said basically that you could have differences in motility between the two cell types, and due to these differences it will get sorted. And when you look in the literature, depending on whether you look at experimental papers or theoretical papers, they actually disagree about which one of the cell populations has to be faster to be on the inside versus the outside. But that was one possible mechanism. The other possible mechanism was that you might have differences in adhesion, which ultimately translate into differences in surface tension, which then cause the sorting. And I'm going to explain what I mean by this in detail later. I want to first look at this one, which is sort of intuitive.

Okay, so we took advantage of these transgenic animals that we didn't have a few years back and we created what we call mosaic aggregates. These are aggregates where most of the Hydra are not labeled, and then some of the Hydra have these fluorescent proteins, and so that means we can see a subpopulation of the cells. You have to think about it a little bit like this: if I would label all the cells, I have one big glowing ball, so it's very difficult to track individual cells. But if most of my ball is invisible and only a few cells in there are visible, it makes it very easy to track these in three dimensions, and that's basically what we wanted to do. So we used two-photon microscopy for that because we wanted to get in the inside of the aggregate and not necessarily just see the cells on the outside, and so that allowed us to have deeper penetration. And then we use particle tracking to basically reconstruct the trajectories of the cells. And once we have the positions of the cells over time, then we can calculate parameters such as speed, directionality, mean squared displacement, and ask what these cells are doing. And so that's basically what we did.

Here you see some example tracks, mean speeds, units are micro meter over hours as a function of time. And what we see is there's basically no difference between the two cell populations. We can look at directionality by calculating the end to end distance and the path lines, D over L, and when you look at this, there is no difference here either, so it's not that one cell type moves much more directed than others. And for those of you who are familiar with mean squared displacement, you basically get all that information by just calculating the mean squared displacement. So by looking at these different parameters, what we said was, it's definitely... There's no differences in motility. So, that cannot explain what's happening.

So that means we were looking at this other question. And so I'm going to introduce this now in detail, because now that becomes relevant. The basic underlying idea of talking about differential adhesion as a driving mechanism is that you can draw an analogy between liquids and tissues. And the analogy is as follows: for liquids, the subunits would be molecules. Think about water for example, water molecules. The mobility of the molecules is due to thermal motion, so you have Brownian motion here. And you have interaction between these water molecules while under Lorentz forces. Tissues, the subunits would be cells. The mobility is not thermal, the mobility is due to ATP. They are continuously consuming ATP, which allows them to propel themselves, so it's active motion. And the cohesion between the cells is via glycoproteins that are expressed in cell membranes, and that allows them to have specific interactions.

So that's the analogy you can draw. To give you a little bit more biology background, you don't have to worry about the details here. So cells at the interface express these adhesion molecules, they're called cadherins, and there's some specificity associated with these cadherins. So different cell types can express different cadherin molecules and there may or may not be a possibility for them to interact and cross bind, and also different cell types can express different amounts of these cadherin molecules, and therefore you can modulate the interaction strengths. So the more cadherin molecules you have, the more strongly you can bind to each other. If you express completely different cadherin molecules, you might not be able to interact with each other. So, that's the bit of biology that is important here.

So the key concept, then, is this idea that tissues are a fluid and that's a little bit hard to imagine at the beginning, especially if you think about the steak you had on your grill, or if you pull on your skin. Obviously nothing is flowing anywhere. But if you think about embryonic tissues or tissues during regeneration that need to undergo large scale, morphogenetic changes, it's important for them to flow. It's not enough to just stretch, you actually have to go places in order to get there.

The analogy I like is silly putty, and I've been holding this in my hand to sort of warm this up. How many of you know what silly putty is? Oh yeah, a good amount. Good. So right, silly putty acts like a rubber ball on short timescales, it's very elastic, but on long timescales you can get flow. And so this is a movie I took with a piece of silly putty hanging from this table, so you have gravity pulling on it, and I forgot how long it takes. It takes a pretty long time. Eventually, the silly putty actually flows downward. That's what you see in the movie and then it will break... There it goes.

So it does act like a liquid, and I can pull this up here and we can see whether that actually will do anything by the end of the talk. Same holds true for tissues, embryonic tissues especially. We'll sort of look back and forth. You can cut it into pieces, but if you wait, then eventually that piece of tissue will roll up like a ball. That means on short timescales it acts like a solid, and on long timescales it acts like a fluid. Again, not all tissues do that, but embryonic tissues as well as Hydra regenerative tissues have to do that in order to get to different places.

So, for especially the students who haven't thought about this, I thought it might be useful to conceptualize a little bit how we think about these kind of tissues. The way we like to think about them when we model them is in terms of springs and dashpots. We can look at a simple scenario where you have a step stress that you're applying to these different things here: springs, dashpots, and then a combined model, and what you will get if you just have an elastic spring is that it reacts instantaneously. You get a certain deformation, that's basically Hooke's law, and then you release the stress and it goes back. If you have a viscous element, that's what the dashpot symbolizes, then it doesn't react immediately. You have the slow deformation that happens, and it doesn't go back once you release the stress. And then you can have these combined models where you get a combined effect. The way we look at tissue often is, at least in this case, what we have is sort of a Maxwell model. What you actually have is a spring that is in parallel with the spring in the dashpot.

I'm not good at drawing this. I will draw this differently. This is better.

Okay, so that's the way we can think about this and then we can model the tissues according to these behaviors. Oh, before I go into this, one nice exercise that maybe is worth talking about. If we believe this fluid analogy, once this thing is a ball in this movie, what are the cells doing in the ball? Once it's rounded up, what are the cells doing in the ball?

Speaker 3: They're shifting around inside of the ball?

Eva-Maria: Yeah, they're basically diffusing in the ball, because otherwise it wouldn't be a liquid anymore. So, the cell positions are not fixed. You basically have diffusion of the cells inside of the ball. And, if you wanted to... you could actually calculate a diffusion constant of the cells. So you could track the cells in the ball, and you could calculate a diffusion constant and you could then... So this is for an exercise I like to do with students sometimes to convince them of this fact that this is active motion. It's not thermally-driven motion. So, you could write down the Einstein-Smoluchowski equation, where you have the diffusion constant, the drag force, the Boltzmann constant, and the temperature. And you could calculate the temperature that you would need in order to get diffusion here using Stokes' drag, where this is the viscosity, and this is the cell radius.

And when you go through this exercise, what you get is more or less the temperature of the sun. It would be outrageous temperatures, so if it's hard to visualize intuitively why this cannot be thermally-driven motion at this point, you get to why this can't be. So it's ATP-driven motion. Yeah?

Speaker 4: Is gravity playing a key role in things here anymore or not?

Eva-Maria: No. Gravity is absolutely not important.

Speaker 4: [inaudible 00:23:41].

Eva-Maria: Okay, good. So that was as a side note, nice exercise. So what do liquids in contact do? Yes, they fuse, driven by surface tension. They're going to fuse, and they're going to make a bigger ball. And that's sort of what you see here. So this is olive oil swimming on water, right, less dense, so it's on top of the water. And you can get the same with tissues. This is going to loop because I had problems with the movie, so it's an animated image file. I'll have to go back to the beginning. There we go. Two balls of tissue, each one of them, like 10 thousands of cells, they fused. The time scales are very different. This is on the order of like eight hours. So it's a very slow process, but it does the same behavior. So it's driven by surface tension and it's opposed by the viscosity of these fluids, tissues, in a sense.

Okay. Let's look at surface tension, then, because surface tension clearly seems to be a force that allows us to change shape in the system. The rounding up, same thing. It's driven by surface tension. The movie I showed you earlier. So what's surface tension? It's basically an energy cost per unit of area. So I'd have to do work in order to expand the surface area of this drop, because inside the water drop the forces more or less cancel out because I have the same number of molecules acting on this guy here, versus for a molecule on the surface, I have no neighbors up here, so that means I experience a net force inwards. So, surface tension is an energy per area or force per length. And the reason the drop doesn't collapse, in a sense, become smaller and smaller and smaller, is because I have the Laplace pressure, which basically balances the surface tension. So A is the radius of the drop, this is the pressure difference between outside and inside, and this is the surface tension. And so that means that smaller drops have higher pressure. The units we usually use are fun units, if you don't work in this regime. It's dynes per centimeter. And so, one dyne is 10 micronewtons, just to give you some sort of sense of scale.

Okay, how can we broaden this, for the tissues, now we can do the same thing. So instead of an air-liquid interface, we have a cell-medium interface in this case. This still holds true, this is just a reminder of the analogy we were working out earlier. And that means there's a penalty for cells to be on the surface, because they lack neighbors with which they could have contact due to these adhesion proteins that allows them to bind. Turns out that when you look at this image, what you might realize is here we talk about a lot of molecules or whatsoever. Here we actually talk about extended objects.

A cell is not just some hard sphere, it's actually a squishy little ball by itself, and deforming the cell, which... The cell has a cortex... Actually costs energy. So, it turns out that we can't simply say, "The more adhesion I have, the higher the surface tension." That is not really true, because there is a penalty for the cells to deform. I don't want to go into those because the actual derivation of the energy is rather complicated. But in case you're interested, this was work I did a long time ago, we did explicitly calculate the energy use of cells in the bulk and cells at the surface, and what you find is that the surface tension depends on the ratio of the cortical tension of the cell to the adhesion it has.

So it's a rather complicated thing, but for the sake of the talk, we're simply going to work with this concept that the more adhesive the cells are, the higher the surface tension. As I said, this is just a disclaimer, it's not complete. So how can we measure surface tension? We talked about the Laplace pressure a couple of slides back, we can take advantage of this relationship in order to calculate surface tension. There's two general principles of how to do it. One is that you apply a known force. In this case that will be gravity, right? When we do a pendant drop method, and we look at the change in geometry. The geometry is here, surface tension is here, pressure is here. So we can use one of them that's controlled, measure the other one and we can calculate the surface tension.

The alternative is we can change the geometry. So you can take two plates, for example, you can squish your tissue aggregate, hold it in there. You record the geometry at the same time as measuring the force. So these are different ways of getting surface tension. One of the techniques that we use a lot, so we use this a lot, we also use this in a slightly modified way. You can aspirate a tissue, so you have a micro pipette, you start sucking on the tissue. Once the tissue starts flowing in your micro pipette, then you use that pressure. That works better than the pendant drop method, but it's the same idea. So either apply a known force or apply a known geometry and then use that relationship to calculate it. So, that means we can quantify things. Now how does that relate to sorting? So we can measure the interfacial tension between the tissue and the outside medium, right?

But now what we wanted to explain was, "What happens if I have two different kinds of tissues that are mixed together?" The way you can understand cell sorting is that you can have this different equilibrium configuration. So the system as a whole still undergoes the same process. It wants to minimize the overall energy of the system, and depending on what the surface tensions are of your components, and what the cadherins are that they expressed, whether or not they can interact, that will set the sorting pattern.

If I have two types of cells that express either the same cadherin in the same amount, or a different cadherin that can bind with the same energies, they measure the same surface tension, they're not going to sort from each other, because it doesn't matter which cells they make contact to. If I have two types of tissues that can interact with each other, but one has a higher surface tension than the other, and there's some terms of that I was neglecting, you would get full engulfment, because that minimizes the energy of the whole system. Depending on how big the difference is, you might also just get partial engulfment.

And if you have two tissue types, no matter what their surface tensions are, if they can't interact, they just going to stay separate and make balls on their own. So it's really just a purely energetic argument, we're not worried about the dynamics of how they get there. We're only worried about the equilibrium state. In the paper I was citing earlier, that biophysics journal paper, we are looking at the dynamics, and you can actually show that the dynamics of sorting are exactly what you would expect from liquids that you have intermixed. So you get exactly the same kind of sorting, so that is pretty nice.

So here's one example from a paper by Foty and Steinberg from 1996, where they did this for a variety of cell types in culture that usually don't necessarily see each other, but they measured the surface tensions of each of these different cell types, and they pairwise mixed them. And you get this nice translational thing that you would expect from liquids also, so limb bud here has a surface tension of 20.1, pigmented epithelial has 12.6, so they sort in a way where pigmented epithelial surrounds limb bud. But if you mix these pigmented epithelial cells with heart cells that have a lower surface tension, then the pigmented epithelial goes to the center and the heart cells go around, so this is exactly what you would predict. It's a very nice paper that showed that.

So our Hydra aggregates did this kind of thing, we know they can interact, we already did look at the sorting. So based on this, the prediction would be that if we can explain the sorting due to differential adhesion, that endoderm should have a higher surface tension than ectoderm. That's basically what we're predicting, then we can measure this, and indeed what you find is you have roughly a twofold, not quite, but roughly a twofold difference between the two tissues. And so that was really nice, because we directly showed that differential adhesion, or differential surface tension, can explain how these cells sort.

The other thing that came out of this paper and that wasn't going in there, is that we used a Cellular Potts model to simulate the sorting in 3D and for the first time, what we did is we looked at the dynamics, which people most of the time don't look at. And what we found was that our sorting dynamics were way faster than the differential adhesion model would predict. And it turns out the reason for this is that the boundary conditions matter. And so, this was also the reason why previously there was always this dispute between the two models, because people didn't worry about the boundary conditions, they did 2D models. What we did was 3D models, but if you do differential adhesion modeling on a sphere, the sorting is very slow, compared to the experiments.

But it turns out that these Hydra aggregates, the way we make them is we centrifuge them, remember, I showed you these little centrifuge tubes. So they come out flat first, and then they fold up. As they sort, you also have rounding happening, and if you do your simulation in the same way, it seems like due to this folding process, the cells come together much quicker, and the sorting speeds up dramatically. And so once we ran the simulations in the same way, we could exactly recapitulate the experimental conditions, and what that means and where it has a broader implication beyond Hydra, is that we have to revisit models of cell sorting to take into account proper boundary conditions, because that changes the dynamics of the process dramatically.

Okay. So that was the story. Just for the sake of fun, because I think sometimes it's really useful to put numbers in context of things we're more familiar with, I wanted to give you a little bit of flavor of what kind of material we are actually talking about. So the surface tension we measured was on the order of like 10 dynes per centimeter. Viscosity is on the order of 10^4 pascal-seconds. So you might all be very familiar with water, which is like 70 PaS. Elastic modulus is on the order of like 40 pascals. So ultimately what the tissues are like is they're sort of similar in surface tension to something like olive oil... Water is 70 dynes per centimeter, or on the order. They have the viscosity of peanut butter, and they're basically as soft as Jello, really low percentage Jello. So it's a very weird material if you think about it in this way, and so that's why I like to just have that in context.

So that's what I was telling you. We started trying to figure out which one of these models this right and we narrowed it down to this second model, and for the remainder of my time I want to tell you a little bit about part two. There's less physics in it, but it's actually really cool. [Ria 00:35:02] is here, if you want to ask her more questions about that later. And the question was really, "What causes symmetry breaking and how are oscillations, that I'm going to show you in a second, related to the symmetry breaking event?" So I told you about this, so far. Turns out you can also cut tiny little pieces of tissue out of the body column. They will round up, they will also go from round to oblong, break symmetry and then become full Hydra. And people in the field have, for the longest period of time, treated those as equal because there was no obvious difference. So if you cut it small enough, it makes a sphere, it seems to break symmetry and then you get a Hydra. And as you might imagine, it's much easier to just cut pieces and do this process than going through this process here. This is rather painful and elaborate and it's a hundred animals, here you need one little piece to get this up.

And so, this is what I meant by oscillation. So when you cut these pieces, what happens is that these spheres undergo these mechanical oscillations of swelling and rupture, and the period of these oscillations is on the order of a few hours, so three to four hours. This guy has already broken symmetry, you see it's already a body axis. Eventually the head is going to form here. Tentacles are starting to come out, and it's going to get more and more wild because I think at that point it really wants to eat. And so it's starting to look around. There we go. So it's over the course of like three days, you get a full Hydra out. And when you look at these oscillations, so you can plot the radius as a function of time, and you get these sawtooth-like oscillations, and eventually here there seems to be a pattern shift where you go from these large amplitude, longer period oscillations to the shorter amplitude, shorter period oscillations.

These oscillations are osmotically-driven. That's something that we confirmed, but that was published before we started. You can change the amount of sucrose in the medium and therefore change the osmotic pressure, and depending on the concentration you have in there, you can see that you can actually change the period of these oscillations and you can quantify the features of the oscillations, and so that's what we did. We classified them in two ways. We said there's long period oscillations, these are these ones, and there's short period oscillations, that's these guys, and they're significantly different from each other. You might not be surprised because when you look at this it's obvious that they are very different from each other.

Previous work associated that this change in oscillation pattern was directly linked to symmetry breaking. Because what they found was that at this point where it seems like you have a shift in pattern, you also get a change in the aspect ratio. So first you have this thing that's round, and at this point it becomes very, very asymmetric. And that was taken for granted, that this is what happened, and theoretical models that were trying to explain axis formation took this as an input into their modeling. So, that was a very important finding, that there is some timescale associated with symmetry breaking due to this work. So the question is, what do people really mean by symmetry breaking? They were just talking about symmetry breaking, and that previous block in a sense shows you that symmetry breaking might mean a change in aspect ratio.

There's morphological symmetry breaking, which is certainly true. You go from something that's round to something that's oblong. Then there is biochemical symmetry breaking, because this thing actually has a head and a foot. The two ends are not the same, so there is polarity to the system, and that polarity is set up by a specific molecular gradient. That molecule is called Wnt. So, there is a certain morphogen that diffuses from the head, and that sets the body axis. So this symmetry breaking is another form of symmetry breaking. And so the question is really what are we looking at? And the reason we got interested in this in the first place was because there were some models which started to link these two things, and said the oscillations might really be important for symmetry breaking, because what you could imagine is that as the sphere swells, you have tension on these cell membranes and that might influence the diffusion of these morphogens. So, there might be a feedback loop between these oscillations and the distribution of these morphogens, but that's all purely theoretical.

The other thing I need to tell you is that... Well, we haven't talked about at all because it wasn't relevant, Hydra has no real mesoderm, no muscles, but it has actin fibers that allow it to do its shape changes. And these have an orientation that's longitudinal, parallel to this head-foot-body axis in the ectoderm, and it's circular in the myoneme. We call this a hydrostatic skeleton. So they're basically filled with water in between, and whenever you contract in this way you get long in that way and vice versa. And it turns out there was a paper recently that showed that the tissue pieces actually inherit that orientation from the parents. So there's some symmetry that's broken in a way, because when you look at the orientation of the ectodermal myonemes, and you let this round up and you look at the regenerated Hydra, then you find that the orientation is conserved.

So they might still break morphological symmetry because they go from round to oblong, and they might still break by chemical symmetry, but they clearly don't break that symmetry anymore, because that's already broken. And so that somehow got us to think about, "Well, do we really know what's going on when the shift in oscillation pattern happens?"

Speaker 5: Miss Maria, just real quick, what's... What do those stripes represent, that's physically different, there? They're different cells or are they different-

Eva-Maria: Sorry, these are these myonemes. These are these weird muscle fibers. They're not cells, they're sort of external muscle fibers that are connected to the cells. And in here, I draw them continuous and they draw them continuous, but it's basically a cellular feature and they align and act together. So, thank you for asking why. Okay. The point is that if you cut a little piece of tissue, it will round up, but the new Hydra will always make the axis in this orientation but not in this orientation.

And so the question was, "Okay, what causes the shift?" And thinking back to what I told you, that Hydra has this mouth that can open and Hydra is a freshwater animal, so it has to regulate its osmotic pressure. In any other animal, that happens through the mouth. And the reason why these nerve-free animals look so weird and bloated, if you remember that picture, is because they don't open their mouth. They rupture from time to time. And so, what we thought is maybe the reason why these patterns change is because at the beginning, when you just cut a little piece of tissue and let it round up in a ball, it doesn't have a mouth. So it basically blows up when the pressure gets too large, it ruptures, and collapses again. But at some point it probably will develop a mouth and then it could actively release the pressure and therefore you wouldn't get these big amplitudes anymore.

So, that was our hypothesis. That the pattern shift actually has to do, somehow, with the function of the mouth. So if that's true, then our predictions would be that the rupture site at the beginning should be random because there is no mouth, so it should rupture anywhere. But eventually as you make a mouth, you should have a weak point that's conserved, and should only rupture at the mouth. And if I cut the little piece of tissue from the head of the animal that has a mouth, then I should only see these fast oscillations, because it can actively control the opening of the mouth. And last but not least, the question of course then is, "Is it only the mouth structure that's important, because you could think that's just a morphological defect, or is it this active opening of the mouth?" And there we can take advantage of these nerve-free animals that cannot open their mouth and distinguish between structure versus function. So that was the line of thought, and so let's look at the experiments that we did.

The first one is that... And when I say "we", I largely mean Ria, of course, that's why I showed her picture at the beginning... We injected fluorescent beads into the cavity and see where they come out. So, you can inject them and then you can look at where the beads come out. What you find is when you quantify this, is that in the early stages of regeneration, when you have these large oscillations, the beads come out anywhere. So there is no conserved point, they can come out here or there, pretty much anywhere. But once they shift to these short oscillations, these low amplitude oscillations, then that's more or less conserved where the beads are coming out, so in agreement with this idea that you have a weak spot, and if you cut off this piece of tissue from the head that contains the mouth, then they are all conserved. They all come out at the mouth. And you can also look at the oscillations of this. They're all short oscillations.

And so that seemed to really show, directly, that indeed, there seems to be a weak point developing that's conserved. And then we took advantage of these nerve-free animals, that look so funny because they can't open their mouth. When you take tissue pieces out of the body column from this, then what you see is that these only show large scale oscillations, despite the fact that the aspect ratio changes and they regenerate. So they break symmetry, they regenerate, but they never show the shift. And if I cut a head piece from these nerve-free animals, here's the [wild type 00:44:43] again for illustration, this one shows the logical oscillations.

And so that means it's not the structure of the mouth as a defect that matters, it's this active controlling of the mouth that actually allows you to release the beads. So where did that put us? So basically, again, the starting point was this notion that some of these oscillations and especially the pattern shift, was linked to symmetry breaking. And what we found using both gain of function, as well as loss of function experiments, is that this really has nothing to do with symmetry breaking. It has to do with mouth function. So once I have a mouth, my symmetry is broken already. I already have a head, right? So obviously, symmetry breaking must happen much earlier than that, and I'm not showing you the data, but we have... So the reason why we stumbled over this was because we had plenty of animals that regenerated just fine, but never showed a shift of pattern. And so, somehow these animals seemed to develop their active mouth function relatively late, but they regenerated into normal-looking animals.

And so that's how we stumbled over this, skip, that it's not important. So what about that coupling of mechanics and biochemistry, right? What we told you is really about, does the shift in pattern matter, it doesn't really address this question of whether the oscillations themselves could somehow be coupled to the diffusion of these morphogens. The idea is the following. So you have osmotic pressure, which drives the oscillations. As the oscillations occur, you get tissue stretching in the swelling process. The idea is that the tissue stretching themselves changes the dynamics of morphogen diffusion. That in turn will feed back, so if you have clustering of the morphogens, you change the mechanical properties of your cells, so you lower the resistance to lateral tissue stretch, which then means you can reduce more stretching. So it's a very nice and conceivable model.

And so what we want to do now is induce, or control, the oscillations in the system and see whether there actually is any coupling. I want to say that you can get the pattern in using a modified Turing model, a reaction diffusion model, you can get patterning of the animal. You don't need any mechanics in the skin. So it's totally possible that all the oscillations it's doing is simply because it's a freshwater animal. It's basically like if you would stop breathing, you wouldn't do anything else. It needs to regulate its osmotic pressure, otherwise it wouldn't regenerate. It's totally possible it has no influence. But of course, it's a nice idea, it's a nice model. So we'll see whether there's anything to it, and this is one of these projects that will be available for the summer.

And so I hope with these stories I convinced you that indeed, reality is better than fiction, because Hydra can really do these amazing things and it's a fantastic model system to study these questions of "How do mechanics influence patterning process?" So we're really interested in how you make patterns. And the reason why Hydra is so good is because of all of these tools that we have available. We can label different components in any type we want. I haven't shown you some of Ria's latest and greatest experiments where she does all sorts of various chimeras, where you can stick things together with needles and test, how does mismatching of gradients, for example, affect the outcomes. And the nice thing is also because, in a sense, you're working with more or less just collections of cells, you can quantify everything they're doing, so you have full control over the system. On the other hand, you can always stick things back together and see how does it affect regeneration outcomes. So you're not stripping any components away, you always have the entire system to look at, and therefore you can always know whether or not what you did is actually relevant to the system. And so with that, I just want to acknowledge the people who did the work and all the funding we received for those. And I'm happy to take any questions you have.