Regeneration Biology
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W2L9_Asexual Reproduction- Fragmentation. Morphallaxis, Epimorphosis, lessons from Hydra

Hello, everyone. Welcome back for another session of regenerative biology. So, in the last class, we did not complete the mechanism. So, I have given two titles. One is the mechanism of regeneration in Hydra, which is ongoing, and then comes asexual reproduction, where we discuss fragmentation, morphallaxis, epimorphosis, and also a few lessons that you can learn from Hydra.

So yesterday in the previous class, what we were understanding or trying to learn was how Hydra makes use of various molecules for their regeneration to benefit their regeneration. So, we will continue with the same section. One gene called pedibin is needed for specifying the food, as you can see here, and it is expressed both in the food of hydra and in the base of the tentacles, which means it is present near the head region and at the base. This, as you can see here in panel B, is the sense control RNA in situ hybridization.

normal antisense pedibin signal. You can see that this is a higher magnification and that this is the base of the foot. And you can see both in cross-section and in whole mount in situ; the pedibin is highly expressed in the foot and the tentacle bases of Hydra. And its expression of pedibin transcripts is detected using hormone preparations on cryosections and also using digoxigenin-labeled riboprobes. Riboprobes mean RNA probes.

They can go and bind because riboprobes will be the antisense probe. It will go and bind to the sense RNA probe. And later you can detect this digoxigenin using the antibody, and the antibodies will have alkaline phosphatase. Enzyme, and once you put the substrate for this alkaline phosphatase, you will end up getting a proper signal that will appear. So, the food, as you can see here, has a heavy signal that is expressed, and also at the base of the tentacles, you can see the signal, and in the rest of the bottom panel, it is.

The cryo sections for those who want to know more detail can be found at this DOI URL given; it is from a published paper. Apart from this peptide, there is another peptide known as Hym-323, which is a 16-amino-acid-long peptide that shares no structural similarity to that of Hym-346, which we discussed in the previous class. It is expressed in

both ectodermal and endodermal epithelial cells throughout the body, except for the basal disc and the head region. So this Hym-323, is capable of providing positional identity; it is expressed both in the ectodermal and endodermal epithelial cells, and it contributes to the positional identity. When you conduct the transplantation and regeneration experiments, they indicate that upon initiation of food formation, the stored Hym-323 means it's a peptide.

16-amino-acid peptide the store Hym-323 will be released from the epithelial cells and induce differentiation of the basal disc cells of the foot, just like if you have a wound in your hand; if it is exposed to the air, it will clot. But before that, the platelets explode upon contact with the air and release a factor called PDGF, platelet-derived growth factor, which goes and binds to the receptors of PDGF, PDGFR, which are present in every cell and will stimulate cell proliferation. In the same way, Hym-323 is also stored in the cells, and whenever there is an insult that occurs to the cell, it will be released, and it can kickstart the feed formation, not just the cell proliferation. In the same way, another protein is called anklet. which is a protein.

Anklet means, I guess all of you know, where ladies wear ornaments on their feet. It's easy to remember. Anklet is a protein with a perforin domain and an EGF domain. EGF is an epidermal growth factor domain, which mediates foot-specific differentiation. Now, the previous, whatever this factor, Hym-323, etc.

can initiate foot formation, but they cannot make foot. So it's just like, you know, purchasing land and making a house. But land is not a house. Land is a place where you can build a house. So someone has to build a house in order to start living there.

So these anklets ensure that this destined area will now give rise to the differentiation of these cells into proliferating cells with foot-specific morphology. So, anklet protein is functionally involved in the transition of the ectodermal epithelial stem cells into food-specific basal disc epithelial cells. Anklet, which has a signal sequence at its end terminus, has two domains. They are called MAC domains.

PF domain. MAC stands for membrane attack complex, PF stands for perforin domain, and it also contains the EGF domain. What do these domains signify? They can interact with other proteins and attract other proteins, which will interact with these domains and can drive gene expression events in a specific manner. So in a food-regenerating hydra, ankylitis was first expressed in the newly differentiated basal disc. Basal disc initiation happened because of the peptides we discussed earlier, and the basal disc cells now start expressing the ankylate cells at the regenerating basal end; then the expression becomes restricted to the lowest region of the peduncle. You don't want other parts of the body,

such as the belly region or head region, to form from food; you want food to remain in the food.

You don't want any other places. So that's why the anklet expression is very much restricted to the extreme end of the peduncle, where the animal's end exists. So, the suppression of the anklet gene—what if you get rid of the anklet gene, knock it down? What will happen? Lead to a smaller foot. Foot doesn't look like a foot. And a significant decrease in the basal disc size.

So this would be, say for example, your foot may be 30 centimeters or 20 centimeters long from... from the tip of your toe to the back of your feet. What if it is made like that of a small baby? You will struggle to balance, won't you? So food has to be a specific size if it is to anchor.

So, due to the decrease in the basal disc size and during foot regeneration, there is a delay in the basal disc regeneration. If you knock down or suppress the anklet function, you will end up with a deformed or malformed foot region either during regeneration or from the structure that is being formed by a newly formed hydra. So, regeneration is via communication. That is, signal transaction pathways in hydra regeneration are very important to discuss. Without cell-cell communication, regeneration would not occur.

Cell-cell communication basically means that if one cell is not able to communicate with another cell, it can be similar to the mechanisms observed in liver regeneration. If one cell is priming, one hepatocyte is priming the other hepatocyte. If that doesn't happen, the regeneration cannot happen either. Hydra regeneration communicates exogenous signals to the transcriptional machinery. Exogenous means something released from a cell that acts on another cell's receptor.

A classic example is wnt-beta-catenin signaling. Wnt binds down to the frizzled, and the beta-catenin gets stabilized inside the cell, where it goes to the nucleus and starts its action. So these signals must get translated into a recipient cell that will influence the transcriptional machinery. Hydra's regeneration tissue shows remarkable conservation of the signaling pathways used by the developing vertebrate embryo. Vertebrates are highly advanced organisms.

Hydra, in comparison to a vertebrate, is very primitive in terms of organismal complexity. But the signaling mechanisms are very similar to those of a developing vertebrate embryo. One example is BMPs 5 to 8. BMP stands for bone morphogenetic protein, which is a very predominant signaling event that occurs in the development of every organism. And BMP 5, BMP 6, BMP 7, and BMP 8 can be activated by the

respective receptor target.

BMPs and TGF beta are somewhat like cousins; I won't say siblings, but you can compare them to cousins. Cousins are genetically related, but they are not as related as siblings. So these orthologs of BMP 5 to 8 and the members of one of the most complex groups of cytokine superfamilies consist of the TGF beta isoforms and other family members such as Activin A. Activin, Nordin, and BMP are all required for the activation of various regenerative as well as developmental pathways.

And we have orthologs. Orthologs mean you have a gene identified from an organism. You are trying to identify a functionally similar gene in another organism. Not structurally similar. Functionally similar, we often call it an ortholog. But they can also have structural similarity.

So, there are BMP 5-8 orthologs present in the hydra. So regeneration via communication occurs when this Hydra BMP 5 to 8 is activated, which is essential for tentacle formation. Tentacle you know if a new head is formed in a decapitated Hydra new head is formed it should have the tentacles also and tentacle formation is dependent a lot on this Hydra specific BMP 5 to 8. In patterning the lower end of the body axis, the specification is also contributed by the BMPs; hydra BMPs, BMP antagonists, and agonists are common terms used in cell signaling. Even so, an antagonist is something that opposes it.

Say you want to speak. And someone encouraging you to speak is an agonist, while someone telling you not to speak or someone putting cotton in your mouth is an antagonist. So this is the signaling pathway: BMP has an antagonist called gremlin, which is a Cystein-knot protein that is present abundantly in vertebrates and antagonizes preferentially BMP2 and BMP4 on average. It is one of the factors involved in head regeneration. In Hydra, for the head to regenerate, you also need to have the BMP antagonist. Like I already told you, if you want to drive a car, you need to have not just an accelerator.

As important as the accelerator is, the brake is also crucial because driving is not a one-sided affair. You also want to stop driving. If you cannot, then you will end up getting into an accident. In the same way, this antagonist does as important a job as an agonist or the activator itself. Another putative cytokine in Hydra is the gene encoding insulin-related protein.

Remember, Hydra also has protein similar to that of vertebrate insulin, which led to the identification of three candidate genes for insulin from Hydra, which we know from the

insulin-related insulin-related gene basically means it is not exact insulin protein. But it is mimicking the function and role of insulin even though we have insulin-like growth factor, which we call IGF. So, the identification and characterization of the hydra insulin receptor, which is called HTK7, support this view that the hydra use insulin signaling. Normally, insulin signaling is not just for the absorption of glucose into the cell, but it plays a major role in cell proliferation. Also, in various reprogramming stages, insulin is a multifaceted signaling molecule.

So that also contributes to the regenerative events of the hydra. Now, let us see what morphallaxis and epimorphosis are, which are extensively discussed in various stages, but we will revisit them once again. Any isolated fragment of the hydra body, which is larger than a few hundred epithelial cells, any part of the body you take and just smash—smash means not to break the cells, just to decimate them into a few hundred epithelial cells—can regenerate into a miniature version of the new animal, so we can call this an Asexual reproduction. Asexual reproduction means there is no involvement of gametes. Although Hydra is capable of sexual reproduction, it can create a new Hydra from a small tissue sample or a few hundred cells from its body.

And this process is called Morphylaxis. Morpholaxis refers to the type of regeneration that occurs in the absence of cellular proliferation and involves the transformation of the existing body parts or tissues into newly organized structures. Say you took a small portion of the hydra from its belly area. That means the belly region doesn't have a head, doesn't have a foot, doesn't have tentacles, or doesn't have many other organs. But these 100 cells will be arranged in such a way that they will form a miniature hydra, a very small hydra. So that means the belly cell will now be distributed as a head, as a tentacle, as a foot, etc.

It is repurposing, although it is not formed by active proliferation. 100 cells remain as 100 cells, but you arrange them in such a way that they form a miniature organism. Now it will start proliferation, so this arrangement of the cells into a miniature organism without involving proliferation is very important. Without involving cellular proliferation, that is called morphelaxis.

But that miniature is useless. You can't do anything with this miniature organ. It's basically a model. If you have a model of a Mercedes Benz car, you can't put petrol in it and start driving. All you know is that it is a model.

It is an exact copy, but it's a miniature model. Many people will have miniature models of the Taj Mahal, miniature models of the Eiffel Tower, etc. They are not, but they do mimic. You can put it in your showcase. This animal, although it is alive, cannot perform

the duties or job that a hydra is supposed to do. In that situation, this now has to increase the size to that of a typical hydra.

How is it possible? By cellular proliferation. So first arrange the by morphallaxis, then you undergo epimorphosis. Epimorphosis refers to regeneration that requires active cell proliferation. So now you think about how you have got a tiny Mercedes-Benz car with you, a very small one.

It is suddenly getting big. A big-sized car. Now you can put petrol if the engine and other things are there. You put petrol in it, and you can ride it. Something like that. That is what happens in Hydra's regeneration. Early regenerative processes in Hydra, however, always occur in the absence of DNA synthesis, as the morphallactic process is powerful enough.

The cells from the gastric region differentiate into head and food-specific cells. I just explained. Gastric region, or belly region, you take it. The same region of cells can now give rise to a head, food, or whatever you want.

But it's actually not that cell. It is basically mimicking. It is basically fooling around. However, in subsequent proliferation, the induction events are powerful enough that the food will become actual food. The head will become the actual head.

Tentacle will become an actual tentacle. There is no; it is not a... Knock-off copy or a non-functional; it's not a dummy or something. Now, morphallaxis and epimorphosis, if you see in the correct term what we should know when a hydra is cut.

The wnt pathway is activated in the lower portion. Normally the wind signaling gets usually activated in the portion that is in the bottom most portion of the body until it can, it is capable of, so as soon as the head is cut, head is removed. So you have the body part and there, The tip of the body of the existing amputated body. So remember, Hydra is there; its head is cut.

So now head is gone. That is not attached. The bottom is just below that head now. That is the topmost part of the existing body. Hydra is cut. The Wnt pathway is activated.

That means the wind is somehow contributing. Lower portion. That is the portion that will eventually form the new head. So, in other words, Wnt signaling marks the place where the new head has to form. Without that, you cannot get a head. If the cut is made close to the head, just below the hypostome, wnt3 in the epithelial cells will cause the remodeling of the existing cells to form a new head; hence, it is called morphallactic

regeneration. That means if the cut is made very close to the head, just below the hypostome, the wind can influence the epithelial cells, causing the remodeling of the existing tissue and starting the formation of a new head that is morphallaxis to start with.

However, if the hydra is cut in the mid-section, the lower half is a little bit lower than the head. The cells derived from the interstitial cells; that is where the neurons, nematocytes, gonad cells, etc. are found. are there, they will undergo apoptosis immediately below that cut site.

You made a cut just below it. They will undergo apoptosis. Why? Because you don't want those cells. You don't want... a you don't want a neurons or nematocytes there, you want to make either head or foot in that region because it's an extreme now.

Before dying, these cells produce a burst of Wnt3. Wnt is stored in the cells in these cells they are not released because of your mechanical damage they rupture just like the release of the pdgf happen from the platelets of your blood They release, and every cell that has a frizzled receptor will bind with this WNT3. So WNT3 activates beta-catenin in the interstitial cells. Actually, there is no plan for the formation of a head. Why has the plan started now? Because of the WNT.

Who released the WNT? The cells ruptured, and they released the WNT. They will start to make a head, causing a wave of cell proliferation. The interstitial cell that is far away from the head is specific; they released. Now the neighboring cells are losing their identity and starting to proliferate in these cells. as well as the remodeling of the epithelial cells. So whichever cell has a Frizzled receptor will undergo beta-catenin stabilization, go to the nucleus, and have regeneration, which we now call epimorphic.

So understand that in the first situation where the head is cut, the newly formed region is a rearrangement of cells that is morpholactic. But whereas there is an active proliferation involved when you make the same cut towards the bottom region, not near the head, but the bottom region. Now these cells have to rearrange the same wnt signaling. Wnt signaling is contributing in both scenarios, but in one scenario it is morpholactic, whereas a little bit lower, where you don't have any head-specific signals that exist, it will undergo epimorphic.

It has to make a lot of new cells. There are no head activators. There is no head inhibitor. It is far away from this entire zone. And this property makes use of its sexual reproduction, such as fragmentation, which we will see in a short while. So Hydra is a small freshwater hydrozoan which we have already seen that is native to tropical and temperate regions. It normally occurs in freshwaters, and they don't seem to age or die of

old age.

Quite understandable because their stem cells divide infinitesimally, and they continue to divide. And because of this, they have the ability to constantly replace lost cells. If you constantly replace the lost cell, we can say that the animal is immortal. Like one of the earlier classes we mentioned, if an organism has extreme regenerative potential, we can call that organism immortal because it is not dying.

Like I told you, your skeleton's lifespan is seven years. If every... Seven years you replenish it, say you have no plan of replenishing; you decided not to replenish your skeleton. That means your lifespan is seven years. Your skeleton disappearing means you are not there before that itself; you will die. Maybe by four or five years you will die. You are not dying because your skeleton is being replenished; that means your body also has the ability to replenish.

Like your skin gets replenished in old age, people who don't have adequate skin stem cells start peeling off their skin; it will be almost like potato skin. You may have seen some new potatoes coming into the market; you see their skin peeling automatically, right? Like that, in adult persons, some people—not everyone—shouldn't think that everyone who is in their 90s will have peeling skin. Some people who don't have adequate stem cells, for some reason, have overworked stem cells.

If you are working in the sunshine, etc., you will have a high turnover. So your skin cells will be peeled off because you don't have enough stem cells to support. But if you have adequate stem cells, like in the case of Hydra, we can say that Hydra does not die. Hydra reproduces asexually through budding and fragmentation. The two new methods it uses are budding, which you can see in this picture; this is a full hydra, and you can see a small miniature hydra that is being formed. We have also seen this; this is the place where the head gradient and the foot gradient kind of nullify, and this is the place where it actually gives rise to.

Give rise to a new place that means this region is neither favoring head nor favoring tail, so that area is a no-zone area or doesn't belong to either head or foot. There, the budding can happen; this we have seen in the earlier classes also. This picture shows that here the budding occurs, and this is one of the most prominent. A sexually reproducing mechanism of Hydra exists.

In both processes, the offspring are genetically identical to the parent. So any organism that is asexually reproducing, say a plant, you take a stick of a plant and plant it. So basically, you are making a clone of that animal. Clone in the sense of a genetic clone. In

the same way, if you are doing asexual reproduction, there is no way you will have any genetic variation, which is not very good for the animal; that is why hydra has the ability to reproduce sexually as well.

Normally, a bud forms on the body wall. There is a spelling mistake. It is written and should be read as 80 at the lower two-thirds region. And the bud grows into a miniature adult. As you can see, there is a miniature adult. The bud separates from the parent body when it is mature.

It will fall off, free float, and settle down in one place. The new organism is a clone of its parent. Clone means maturity. Morphologically, it is the same, and genetically, it has to be identical. This is what we refer to as a clone.

Hydra is capable of another way of reproduction that we call fragmentation. Fragmentation means the Hydra can undergo damage due to various reasons, such as its body getting cut, possibly because of sudden water movement or a predator. Predator means not just a hunter or something. A smaller animal came and hit it, etc. So it can break into pieces. So understanding the fragmentation means that the Hydra does not get into a fixed number of pieces.

It can come in two pieces, three pieces, four pieces, and so on. Random shape, randomly. The body of the Hydra breaks into two or more parts. Each part grows into a new individual. This is a simple form, as you can see in this picture. There is a Hydra, and the Hydra is cut into two, forming fragments by the loss of body parts.

So the upper part lost its foot. The lower part lost its head. And now it arranged itself into a miniature hydra, growing and regenerating fragments. So basically, fragmentation is nothing but regeneration. But since it's asexual reproduction, it is often referred to as fragmentation. This Hydra grows slowly by cellular proliferation, and it will become a new Hydra that is fully identical to the parent Hydra, genetically identical, and it can reproduce by budding, etc.

It can also do sexual reproduction by using the gametes. And this mechanism, sexual reproduction, is a process where an organism produces offspring without the need for fertilization; that means there is no egg and there is no sperm, but it is beneficial to sexually reproduce because it can bring in genetic variability. So that is why, in a given environment, if one particular genotype of the hydra is unfit, then.

.. Any adversary can wipe out all genotype-bearing Hydra. You would have seen during COVID that not everyone had the same kind of mortality rate. Some people, not just the

old or young, were more vulnerable. Even in some countries, people were more vulnerable because credit is given to their genotype. People who have a specific genotype cannot predict whether they are vulnerable or non-vulnerable in the same way; every organism does not need to have an infection in a given condition. A genotype that is not diverse enough invites trouble for that species' survival; hence, hydra can also follow sexual reproduction to achieve variation.

But in this fragmentation and budding, they do not happen. In a stable environment, asexual reproduction is effective when there is no challenge. There is absolutely no challenge, no trouble. Everything is in surplus. There is no need for any botheration. So it will undergo asexual reproduction, and it is effective because the offspring are adapted to that environment.

The environment has a nutrient and is well adapted. But in an unstable, unpredictable environment, what happens? We don't know the offspring. We want diversity. If there is some good diversity, then you will be able to make a living. So you keep randomly looking for a variation.

The offspring may not have the genetic variation needed to survive. Hence, sexual reproduction is preferred. A favorable environment promotes asexual reproduction. Unfavorable environment and sexual reproduction. We will learn more about the other species, which will be planaria, in the next class. Thank you.