Regeneration Biology
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W2L8_Mechanisms of regeneration in Hydra

Hello, everyone. Welcome back to another study class on the regeneration of Hydra. And today we will try to understand the mechanisms of regeneration in Hydra. So mechanisms basically mean how this individual or this organism is orchestrating various gene expression events that lead to a restored body part. So cellular and structural changes during regeneration in hydra are seen in this picture. As you can see here in the left extreme, before amputation, there is a head region and also the tentacles you can see.

And the scissors show there is the amputation plane. And soon after amputation, what you see is that there is a retraction of this blue-colored extracellular matrix, or the so-called mesoglial structure, which has been withdrawn. And you could see the cells that were elongated. These are endodermal cells, light yellow, and the brown color represents the ectodermal cells.

So the endodermal cells became spherical in shape. And then, after around one hour, what you see are these spherical cells, because the beauty of this sphere is that it can move in any direction. So it will allow it just like a football does. So it formed a dome-shaped structure and it merged with. And so this is nothing but a healing wound.

It healed the wound. And now you can see that after some time the endoderm cells started becoming elongated again. So if you look at the ultra-structural level, this is what you see: at one to four hours, the cells become rounded in shape, and then it starts around four to seven hours. It started producing this matrix protein, and after around seven to twenty hours, it began filling them with interstitial matrix. As you can see, the key on the left-hand side, the interstitial matrix, which is fibrillar collagen, fills it up, and the basal lamina, which is nothing but the cells secreted by these epithelial and endothelial cells.

And these ectodermal cells are brown in color, and endodermal cells are light brown in color. So by around 7 to 20 hours, it restored the normal system. Now what is left? It has to make the tentacles again. This is what is needed for the. Head to form.

So the ECM is retracted. That is very important. It is retracted immediately so that it gives freedom for the cells to move around because there is no matrix, which means it is free for all. It is just like you had a string of beads, a garland or a chain you had which had so many beads and you suddenly pulled the beads out of the string what will happen it will go randomly so this is what happens the basal lamina is retracted upon decapitation so followed by change in the ectoderm endodermal cell shape and then comes this is succeeded by the wound closure and the secretion of ecm component the moment the wound is closed again it will restore the basal lamina and also the interstitial matrix in this way what you can do is you can restore the dome shape And the dome shape was very much there. Only difference was they had extra tentacles and this dome shape is restored now here and it will start making the tentacles.

So now, if you look closely, the term "cellular dynamics" refers to the movement of the cell. So no matter whether there is an injury or not, the hydra body keeps moving; the cells keep producing. Or in other words, the hydra's body is constantly regenerating whether or not there is a necessity for it. So cells undergo continuous proliferation and movement in both directions along the oral-aboral axis. The empty cells denote proliferative cells, and the blue ones are the terminally differentiated ones, as you can see in this picture.

So the number of days taken for the cells to reach their respective destinations is as buds or as hypostome. The hypostome is nothing but a mouth. And the peduncle termini are shown in the red direction of the red colored arrow. So the arrows depict the direction of cell movement. Like you see here, this is a tentacle and the tentacles are terminally differentiated cells.

That means they don't have a movement backward. So from around the neck region, it takes about four days to reach the tip of the tentacle, and from the tip of the tentacle, it will fall off; it is not going to stay back there, and constantly new cells keep coming. Like in the case of what you may have seen about the teeth, even for elephants and sharks, new layers of teeth keep coming from the back, and elephants also keep getting them just like a train from the back; they keep coming unlike us, so the cells keep coming from this neck region towards the tip. Whereas in the hypostome region, it takes around 20 days. Whereas this long distance tentacle takes only four days.

And recently you can guess it because the mouth is moving minimally. Tentacles are constantly moving. So, wear and tear are very high. So it needs to be repaired. And then you can see near the neck region, there is little to hardly any movement.

And you can assume this might be the production site. Either it will go towards the

tentacle region, or it will move towards the body region or the basal region. Where it will come down, in this case a new hydra is being formed, and that is called budding. Here it is trying to make another bud, so this is a special zone in which we call that spot the zone of budding. Below this zone is basically the basal peduncle, where the foot is attached.

You can see that the movement of cells is pretty low, and it takes around 20 days to travel. Minimal days, somewhat like those of the hypostome. So extreme and hypostome, and in the basal region, the movement of cells is minimal. But in the body region, the movement is very intense.

You can. Assume that this could be because of the active movement around the tentacle region and also in the body region, unlike what you see in the hypostome and the basal region. So the different cell movements that take place during the maintenance of the Hydra body plan are mentioned in this picture. So now, if you see, to study gene expression, in order to understand how Hydra is regenerating, we need to understand which genes are contributing to this regeneration. So, for that, scientists use a lot of techniques. It can be RNA in situ hybridization or immunofluorescence, which all depend on some chemical mechanism.

That means if you have the antibody with an alkaline phosphatase enzyme and you put the substrate for that BCIP/NPT, it will produce a colored reaction. So wherever there is a colored reaction that indicates there was an antibody, and if the antibody was there, that indicates a substrate was present, and the substrate is your subject of interest. So wherever the protein or the RNA is, it will go and bind, and now you are detecting it using an antibody. So that is what you see here. So the major head inducer of the hypostome organizer is a set of Wnt proteins acting through the canonical beta-catenin pathway.

The beta-catenin pathway is one of the most important signaling events that occur in every organism, starting from hair growth to skin growth or even the regrowth of your nails, and whether you had a mosquito or an ant bite on your skin, it has to grow back. Everything requires Wnt signaling. And the Wnt signaling occurs when the ligand Wnt binds to the receptor, frizzled. And this causes the activation of this receptor, which will attract a component from the cytoplasm that is disabled. Disabled was part of a destruction complex.

Destructing what? Destructing beta-catenin. So in every cell, beta-catenin is produced abundantly and is needed for cell-cell communication, which we discussed in one of the earlier classes. But in any case, it has to be degraded. Otherwise, it will go to the nucleus and turn on pro-mitotic genes, and you don't want unwanted proliferation at any given

time. Because of this, this excess beta-catenin will be degraded by the destruction complex.

But if the wind is bound to its receptor, frizzled on the cell surface, it will recruit disheveled, which is one of the major players in this destruction. As a result, the beta-caterine is no longer degraded. It will go to the nucleus, and with the help of collaborators like TCF, it will turn on. So what you are seeing here is that the growth of a new hydra from the budding zone definitely requires the Beta-catenin pathway. The Wnt beta-catenin pathway means Wnt is the ligand and beta-catenin is the actual messenger that goes to the nucleus and binds to the DNA and turns on the gene expression events.

So the wind expression during the hydra budding is what you are seeing in this purple-colored reaction, which is the detection of the Wnt proteins. And you can see here, extreme end. In other parts, there is no purple color. It is only the extreme end that is determining the head region. As you can see in this picture, this is the mother hydra and this is the daughter hydra.

Both of them have the Wnt signal expressed in the hypostomal region. These Wnt proteins are seen in the apical end of the early bud, defining the hypostome region as the bud elongates from its source of production. If GSK3, which is one of the members of the destruction complex, like I told you, beta-catenin is degraded, and disheveled is a protein that is recruited by the Wnt ligand-bound Frizzled. But if there are other methods, can we think of blocking this degradation by some other method? Let the disabled be there.

Let there be no Wnt. Frizzled, there's no need to come into the picture. All you can do is stay in a given cell, even if you prevent the destruction. And one of the major enzymes that contribute to this destruction of beta-catenin is GSK3 beta glycogen synthase kinase 3. And this GSK3, if you block it, what will happen is that the beta-catenin is no longer phosphorylated, which means it will not be degraded. So if you prevent GSK3 from functioning, you will stabilize beta-catenin because beta-catenin is not degraded.

It will go to the nucleus. It will look as if there is a Wnt signaling. Although there is no Wnt, there is no binding to the frizzled, but what you did was play something inside the cell by blocking the GSK3. So if your GSK3 is inhibited throughout the body axis, ectopic tentacles form at all levels, and each piece of the trunk has the ability to stimulate the outgrowth of new heads, this hydra will now have heads budding out from everywhere on its body because wherever beta-catenin is getting stabilized, you will end up getting multiple heads instead of one head. This signaling is very powerful and is capable of driving the formation of multiple hydras from one organism itself. So now, if you look into the head inhibition gradients, we already saw that the head has the ability to

form a new head.

So what you can do is take a small piece near the head region from a donor hydra and transplant it into the head region of another hydra. Earlier, we saw that if you put it towards the belly region, you will get a new head that is being formed. But here, if you put near the head region, you don't see a head that is being formed. What happened to it? If you put the same thing towards the belly region, you will get a new head.

But nothing happens here. On the other hand, if you take the same thing and put it into another recipient hydra whose head has been removed, then what will happen? Not only will its lost head appear, but this new transplanted disc will also give rise to one new head. That means you will end up getting a two-headed hydra. One is the replacement for the old head. The other is the formation of a new head. So, what is the difference? The only difference is in the first case, the recipient had a proper head.

Whereas in the second case, the recipient had no head. You decapitated. Before it is regenerated, you should transplant the disc. So you end up with two heads.

So this gives you an idea, an existing head. Functional heads prevent the formation of one more head. It does not want one more head. That means some inhibitors are being secreted. In the same way, we have seen this in an intact host. Like, if you take a graft, put it in the basal region.

This you saw in the previous class towards the belly region where you put it. No matter whether the host has a head or not, a new head will be formed. So subhyperstomal tissue does not generate a new head when placed close to the existing head. That is the top panel. In the B panel, what you see is that subhyperstomal tissue generates a head if the existing host head is removed.

And third, the subhyperstomal tissue generates a new head when placed away from the existing head, which means that closer to the head there is a zone of no response or some zone of surveillance from the existing head; it doesn't want one more head. It is just like in a lion pride: if one male lion is there, you don't want one more male lion to come, either from within the pride or from outside, so they will fight. One pride needs only one lion; rarely will two be there, which will be mainly brothers. So the host head appears to produce an inhibitor that prevents the grafted tissue from forming a secondary axis in the new head. The identity of the new head inhibitor remains unknown.

It appears to be a labile substance that has a half-life of only 2 to 3 hours. How do we know? If you decapitate, immediately you put.

.. If they graft the tissue, it will not form a head. But if you wait for two or three hours, then you put it, a new head will be formed. So that means the inhibitory signal is a volatile substance, or it can sublimate easily into the atmosphere. So you end up getting ahead only if you do it for two to three hours. That means it's a short-lived molecule constantly sending signals. And the same short-lived molecule, when it reaches the belly region, diffuses off.

Hence, the belly region is most welcome for the formation of a new head anytime, provided you transplant it with new donor tissue from the head region. It is thought that the head activator, which is nothing but wind, as we have seen, and the head inhibitor, the nature of which we do not know, but we are assuming it is a flammable, evaporatable, or sublimable substance, are both made in the hypostome. Both sources have to be from the same place, but the head inhibitor gradient falls off more rapidly than that of the head activator. The head activator has a long life; that is why you took it from a donor and put it into a recipient. Still, it is forming a head, which means the designation or the message to form a head is present in that donor tissue.

Also, if it is a sublimable substance, it would have disappeared. The place where the head activator is uninhibited by the head inhibitor, becomes the new budding zone. So in a spontaneously budding, asexual reproducing zone, the inhibitor signal is minimal. Therefore, it has the potential to form a new head. So what prevents the bottom of the hydra from forming a new head? Normally, you can think, "Oh, an inhibitor has faded off from the bottom," so why don't heads start appearing from the base? It doesn't form from the base; it forms from somewhere in the midway lower region.

If you cut the hydra into three pieces—piece one, piece two, and piece three—it will come between the piece two and piece three regions, which means the lower two-thirds region forms. So you can see in this picture, several small peptides could have been contributing to this. Do we have any evidence? Let us see the bud location as a function of head and foot inhibition gradient. That is what is depicted here. As you can see in this picture, you can assume that if there is a head determinant, there will also be a tail determinant.

In young adult hydras, the gradients of head and foot inhibitors appear to block bud formation. There is a head gradient coming from the hypostome region, and there is a foot gradient coming from the basal region. So somewhere midway, they get nullified. It won't be exactly midway.

It depends on the quality of the molecule that is inhibiting. So the foot gradient

diminishes quickly and the head gradient diminishes slowly. As a result, you have somewhere two things; that is what you can say: head inhibition and foot inhibition. There is a midway, as you can see in this red. In a young individual, the newly developed bud is pretty close. As a young adult, there will be little bone change, and in budding adulthood, what happens is kind of neutralized.

So both there is a, you can call it like, you know, many countries, you would say the boundaries will have a no man's land. That means the middle of the two countries doesn't belong to anyone. So we call it a no man's land that doesn't belong to either country. In such a no man's land, budding can happen.

So that is roughly around two-thirds of the downward part. The trunk, when the levels of both inhibitors are minimal, is where the buds form. Several small peptides have also been found to activate foot formation. So the specification of cells as they migrate from the basal region through the body column may be mediated by the gradient of an enzyme that is a tyrosine kinase, and its name is Shinguard.

There are many enzymes. One of them is this one. So now if you look closely, the gene expression events and expression profile of wind and TGF beta are evident. TGF-beta, we also learned about it in liver regeneration, etc. Superfamily signaling components along the hydra body axis. So in this left panel, what you are seeing is the Wnt signaling events, and in the right panel, you are seeing the TGF-beta signaling, and what you see here are the molecular components that have been shown to be regulated by Wnt and the TGF-beta signaling pathway.

There are many genes; let us not focus on these genes. The colored bars represent the localization of gene expression. Along the body column of the hydra polyp. That is what it’s like; there’s a cartoon of a hydra that has been given, and if it has been shown here, that means up to here it is expressed, and if it is shown only here, near this narrow window, it is expressed only here. If this green one is shown up to here, and green basically means agonist of the signaling pathway.

Blue means the antagonist. Like when you say Wnt signaling, there are agonists and antagonists. Like Wnt, ligand is an agonist. There are many inhibitors for Wnt signaling; SFRP, DKK, WIF, and many other molecules are all antagonists of Wnt signaling. Many of these molecules are either agonists or antagonists, and you also have TGF-beta signaling. Agonist or antagonist are present, and you see the expression pattern, with some starting from the neck region, stating this particular thing until around the foot region where it is expressed, whereas some genes, like this particular gene, are expressed very closely to the hypostome region.

You can see that the blue color represents the antagonist, and you can also see that this terminology is mentioned here: ectoderm or endoderm, as you have a whole column that has got. Both ectoderm and endoderm are present. And its EC means ectoderm, and EN means endoderm.

So that is what has been mentioned here. Location is not important. This data is mainly presented to help you understand that, while many pathways exist, only two pathways have been examined here: Wnt and TGF-beta signaling, which include agonists and antagonists. It is a complex event, gene expression, or the orchestration of events that do happen. So the colored bars represent the localization of gene expression that you should understand. And the expression of genes in the ectoderm or endoderm is also specifically mentioned for both TGF beta signaling and Wnt signaling. So the hydra body plan is characterized by a defined body plan that consists of stem cells, a simple nervous system, and two differentiated tissue layers: ectoderm and endoderm.

And in the middle, you have a mesoglea, and also, you know, gametes, nerve cells, or some nematocytes, etc. So the Hydra is conscious of more than three active but separated stem cell communities: the ectodermal epithelial stem cells, endodermal epithelial stem cells, and interstitial stem cell lineage. So, these are all the main stem cell lineages. In Hydra, all three stem cell lineages are highly dynamic and all cells are continuously renewed every 20 to 30 days.

This is a unique feature that you can see in Hydra. This continuous proliferation of epithelial and interstitial stem cells occurs in the central part of the gastric body from which the cells migrate either to the upper or to the lower body column, or towards a growing bud. If the bud is growing, then there will be more migration towards the bud as part of asexual reproduction. At both ends, unipotent epithelial stem cells differentiate into head and foot somatic tissue, thereby losing the potential to initiate cell division. So multipotent interstitial stem cells differentiate not only into non-dividing somatic cells, for example, nematocytes and nerve cells, but also into gametes, that is, eggs and sperm. So this is the steady-state way of movement and migration of the cells in the body of a hydra.

You can see here that this has been depicted in the form of a cartoon. You have got endoderm, mesoglia, and ectoderm, and you can see the cells that are located in the ectodermal and endodermal epithelial stem cells. They keep cycling for three to four days, and they can differentiate into somatic ectodermal and endodermal tentacle or basal disc cells. And you can see this is a stem cell, and this is a precursor cell. And here at the bottom, you can see four classes of differentiated somatic cells.

The nature of these cells is not important. It has been shown mainly to understand that the newly formed hydra, as part of regeneration, keeps producing every cell that is present in the organism: gland and mucous cells, ganglion and sensory neurons, nematocytes, germline cells, etc. And this is an infinite cell division that constantly keeps going as long as Hydra cannot die. It constantly keeps producing new ones, just like bacteria. Bacteria normally don't die because they keep producing one bacterium, then two bacteria, then four, and so on. Differentiation into the somatic cells happens, and eventually, the cells will die, following from the tip of the extremities of the body.

But it doesn't matter. It keeps pushing forward constantly. Let us look into a few other molecular mechanisms. Although we have seen the importance of TGF beta and Wnt signaling, regeneration starts with a wound. The healing may require some special actions, not essential during normal morphogenesis. Normal morphogenesis involves cells continuously dividing, stem cells replacing the newly formed cells, and their movement across the body of the animal.

That is normal morphogenesis. An evolutionarily conserved gene called the Kazal gene, which is also known as SPINK3 in humans, is expressed. Remember, this hydrogen is present in humans. That means how conserved this gene is.

That's why the Kazal gene is very important. It is basically a serine protease inhibitor. Kazal type, that is what the abbreviation of spink means. And in Hydra, it is called Kazal, which is expressed in the endodermal gland cells inside the body and upregulated during regeneration. Kazal1. In humans, the same gene is called spink 3. Kazal1 silencing by RNA interference resulted in dramatic tissue disorganization, followed by massive death of gland cells and the accumulation of autophagosomes.

Autophagosomes are bodies that are formed inside a cell when the cell is killing itself. That is called autophagy. That means eating yourself. Something like, if you are hungry, you will not eat your finger, right? If you are hungry, will you start eating the flesh in your hand? You don't. Normally, you don't. But the cells can eat, and when they are not finding a way forward, they will undergo autophagy due to various biological conditions, and they will recycle the nutrients.

So autophagosomes are required for autophagy within the cytoplasm of the digestive cells. So what you did was get rid of Kazal1. So that will trigger the self-destruction of the cells. Intact Hydra, Kazal1, which has serine protease inhibitor activity, is required to prevent excessive autophagy. If Kazal is not there, you will have an excess of autophagy.

So Kazal1, make sure that you don't have a serine protease. Protease means an enzyme that degrades various proteins. So that is kept in check. It is just like you keep petrol away from fire or fire away from petrol.

The same logic applies. Serine protease has to be kept in check, which is done by Kazal1. But once you block it, it goes unchecked, and various serine proteases will start acting. To prevent excessive autophagy and exert a cytoprotective function to survive the wounding stress. That is why the sudden increase in Kazal1 is necessary for the regeneration event to occur. And this gene is SPINK3 in the case of humans.

And that means it is present in all other animals as well. How do the cells in the regenerating tissue know? How do they know? That means it's aware of it. That their localization in the gastric region has changed to a position at the apical or basal end. Are they looking into the mirror to know that, oh, my head is missing? Or if someone is asking, oh, do they have any news channels that are reporting the head is missing? So this is what we should know: which part is damaged. Secreted peptides are responsible for this and have an impact on head and foot regeneration. Several peptides could be linked to epithelial differentiation along the apical-basal body axis, capable of inducing head or foot-specific differentiation.

A 12-amino acid peptide called HEDI is a novel gene. That is absent. In the genomes of.

Other animals. That is. 12-amino-acid peptide. HEADY. H E A D Y is a potent inducer. Of apical fate.

And also sufficient. For head induction. So, the HEADY peptide. If you deliver to a place. It can. Give a signal to that cell to start making a head, so disruption of head function by double-stranded RNA-mediated interference (RNAi), which is a technique that has received a Nobel Prize, resulted in severe defects in head formation. So, if you get rid of heady, that is a 12-amino-acid peptide, you will not have head formation. Treatment of regenerating heads with synthetic. HYM301 peptide that causes an increase in the number of tentacles formed. One gene, HYM301, is a synthetic gene; if you treat them, a greater number of tentacles are formed during the treatment with HYM301 double-stranded RNA, which means knocking down HYM301 leads to a reduction in the number of tentacles formed during bud formation or head regeneration.

At the opposite end of the body axis, two peptides, that is, pedibin and pedin (pedin's another name is HYM346), stimulate foot regeneration. HYM346 accelerates foot regeneration and also increases foot activation potential in the gastric tissue. So you can see in this picture the HEADY expression and HYM301 expression that are shown here.

Like you can see at different time points, this is the decapitation. And you can see soon after that at various time points—2 hours, 4 hours, 6 hours, 8 hours—you collect it; you can see the HEADY induction happens strongly at around six hours, and then it dwindles off.

This is the expression of HYM 301; this is the expression of HEADY, and the HEADY expression is very specific in the neck region and also in the newly budding region. So we will continue with some of the mechanisms in the next class, and we will study more about how Hydra is using this for morphallaxis and other sexual reproduction approaches. Thank you.