Regeneration Biology
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W3L11_Planaria regeneration-polarity and gradient in regeneration

Welcome back to another session of regeneration biology, and today we will learn about how different polarities or different signaling events decide the gradient or polarity in the animal's body and how the gradient or polarity of a given chemical contributes to regeneration. So if you look, this is the plenary anatomy; as you can see here in this picture, it's very complex in that sense. Although it's a flatworm, it has eye spots, which are basically light sensors. It also has a cerebral ganglion and an extensive peripheral nervous system. Additionally, it has an intestine and a pharynx, which is hanging out; that is, it has a mouth and an anus. The mouth and anus are the same thing, so it doesn't have a dedicated mouth or a dedicated anus.

And so it also has transverse nerves, and it has dedicated secretory cells that are distributed throughout its body, which means nitrogenous waste material, normally called flame cells. This picture is mainly shown for you to understand that the animal has a very complex body structure; it is not as simple as hydra. And still it can regenerate by something called fragmentation, or you can cut it into as many pieces as you want; the animal regenerates from each piece. So this is the planaria anatomy.

Now the interesting question is, which cells formed the new head or tail? Although we have seen they have got neoblasts, we call them clonogenic neoblasts (c neoblasts), but the question is. Although they are there, they are circulating; which one is it? Throughout each of them, all of them have got equally potent capabilities. Any given cell can give rise to any given tissue type, or is there any predetermined distribution of function or labor? For many years, it was believed that the old cells de-differentiated at the cut ends of the planaria to form a regeneration blastema. De-differentiation, which is going back in time. Like I gave an example probably in the previous class, someone who is studying in plus two is now going and sitting in kindergarten.

So this is something about de-differentiation. That was the belief in olden times. A collection of relatively undifferentiated cells that can be organized into new structures by paracrine factors located at the wound site. This was the belief. At the injury site, there are a lot of differentiated cells.

They de-differentiate and give rise to a naive type of cells that can give rise to the lost structure. This was the belief. However, in 2011, a series of experiments by Wagner and colleagues provided evidence that dedifferentiation does not occur. There is nothing called de-differentiation that exists. Until then, that was the popular belief because there is a formation of a blastema, and how come the cells formed from where it is coming? The animal doesn't have that group of cells, so the belief was that it differentiated at that site, but later it was proven that it is not correct.

The regeneration blastema forms from pluripotent stem cells. They are called clonogenic neoblasts. So this is one of the earlier learnings of how regeneration occurred in planaria. A set of pluripotent stem cells in the platforms that serve as stem cells to replace the aging cells of the adult body. Any given body, like our skin, has a lot of shedding and replacement.

Whether you go in sunlight or whether you have wear and tear, no matter what, you are regenerating. Epidermal cells do die, and they grow back because of the skin stem cells. Adult stem cells, remember we studied, and the skin stem cells replace them. But the same process is happening for planaria, not just for skin, but for every tissue and every organ in its body, and this is done by C.

neoplasts. They have a cell surface marker. C-neoblasts have a cell surface marker called Tetraspanin 1. This is the name of the protein that acts as a marker for this C-neoblast. The picture shows what you saw. The previous slide also had this picture.

And this picture shows that the neoblast is in the planaria platform, and this is SMED. Smitia mediteranea, in short form called smed, is stained red by antibodies to phosphohistone 3. Phosphohistone 3 is a marker for labeling cells currently undergoing mitosis; phosphohistone 3 will mark only those cells that are currently in the mitotic phase, meaning the cell is dividing into two, not those cells that are in the cell cycle. Maybe if there are 100 cells that are in the cell cycle, some may be in the G1 phase, some may be in the S phase, and some may be in the G2 phase, but this will mark only those in the M phase. And if you stay in with phosphochristone-3, as you can see here in this picture, they are stained red; you can see how many of them are in the mitotic phase.

A huge percentage is in the mitotic phase, and remember this is a normal plan area with no injury, and all nuclei are marked in blue. That means the rest of the cells in the body are marked in blue; the ones that are red are the ones that are. Marked with the phosphohistone 3. Neoblasts are scattered throughout the body, posterior to the eyes. That is where the eyes are; below the eye is the main location of the neoblast.

Assuming you think planaria are similar to humans, when you stand up, you have two eyes, and below the eyes is the major distribution site for neoblasts. And they are scattered throughout the body. But the preference is below the eye, posterior to the eye. But they are absent from the centrally located pharynx. The centrally hanging pharynx is there through which it acts as both a mouth and an anus.

There are no ceneoblasts in them. That is the only exceptional area. This doesn't mean that if the pharynx is amputated, it will not grow. Nothing like that.

It can grow. But they are not used as a reservoir of osteoblasts. So in this experiment, what Wagner and colleagues did was perform some very interesting experiments. So this is the picture that we have seen. Cineoblasts are present. So they did two different kinds of radiation regimens.

They took this normal SMED type of SMED planaria and irradiated it. And one set gave 1750 radians. 1750 radians only. What is the beauty of this radiation? It damages almost everything. It does not damage everyone, just almost all.

Maybe three cells will survive, or maybe five cells will survive. This is the idea. In another set, what they did was give 6000 radians. That means to forget about having any live cells in that animal. It's basically a skeleton or a dead body of planaria.

So these are the two sets. What they wanted to know is whether there are any clonogenic neoblasts. What we know now is that there is something called C-neoblasts, which we call clonogenic neoblasts. But how do we prove that? You cannot just name them and say that from today onwards you are clonogenic neoblasts. They need to have some evidence. Clonogenic means that they can create a clone of one individual.

Like if one cell from my body can give rise to a clone of myself, then I can tell, oh, that cell is a clonogenic cell. That means one cell can give rise to a clone of an organism. So Wagner and colleagues showed that if a planaria is irradiated with 1750 radiance, which kills nearly all dividing cells, that is, neoblasts, keeping a few or one alive. That is because of radiation. It is lethal, but not absolutely lethal for every cell.

Some cells do survive. This would be sufficient to produce the cells of all germ layers, indicating that they are indeed pluripotent stem cells. After radiation, 1750 radiation, you leave the plan as it is. Normally, it should die like we have seen in the previous class; also, we have seen that if the radiation is very severe, it will disintegrate, but with 1750 radiation, if you give it, it bounces back. However, it had only one cell, two cells, or maybe three cells of neoblasts, but that is good enough to repopulate the rest of the body.

Next, the same researchers, they irradiated the planaria with 6000 radians which killed all neoblasts.

Technically, that body will disintegrate. This planaria died because of a failed tissue replacement. No point in what you do. However, the introduction of a single clonogenic neoblast into such worms could in some cases restore all the cells of the organism; sometimes it will not be successful, but one is enough. Although you have treated them with 6000 radians, one is good enough to restore the entire organism, which is equivalent to the genotype of the donor C neoblast.

When this worm was split into pieces, each piece regenerated the missing parts, and the cells were all of the genotype of the donor neoblast. Naturally, the 6000 radiation units of radiation killed all the host cells, but the donor, when replaced, had no more radiation, yet it could propagate. However, that is a clone of. The donor neoblast-bearing planaria confirmed that regeneration takes place from pluripotent stem cells, so this was the first evidence to show that the entire organism can be produced; hence, the damaged part can also be produced by the neoblasts, which we call c neoblasts. Now the question is, what are the questions that remain? Very interesting questions may arise from this kind of set of results.

The first line of questions regarding the clonogenic neoblast. Are all neoblasts pluripotent? If the planaria has, say, 1 million neoblasts, are they equal? Or are only 100 of those 1 million so-called C neoblasts? Because we have evidence to say that sometimes in the 6000 radian irradiated planaria, when you put in one neoblast, it doesn't always work, while other times it does. So what this tells you is that not all c neoblasts, or not all neoblasts, are equal; there is some discrepancy and variation in the data we already know. But we can ask this question: Are all neoblasts pluripotent? What is the embryonic origin of this clonogenic neoblast? Are they produced right from the embryonic development, or do they have some other foreign source? What is the hierarchy by which these clonogenic neoblasts generate around 30 different cell types of the adult planaria? Planaria has around 30 different types of cells; are they capable of making all 30 cell types? These questions are currently being investigated, and some clues have already been obtained. Let us think about some second line of questions regarding the polarity: how does the flatworm cell function? The anterior blastema becomes the head and the posterior blastema becomes the tail.

How is it possible? How do the blastema-derived cells retain their dorsal-ventral polarity? The dorsal side has the dorsal structure. The ventral side has the ventral structure. How is it possible? Who is guiding them? So this is an interesting question. Polarity establishment in flatworms. How has it been done? This is something very interesting to follow up on.

Flatworms are capable of establishing anterior-posterior and dorsal-ventral polarity. Anterior-posterior means, you know, head to tail. That is anterior-posterior. Dorsal-ventral polarity means from the backside to the chest. So worms also have got this, you know, backside, front side, etc.

So two polarities are predominant: anterior-posterior and dorsal-ventral polarity, using the same factors and the processes employed by the vertebrates. Although the planaria is an invertebrate, it is one of the primitive lines of individuals, but it follows the same strategy and logic with which a vertebrate follows its anterior-posterior polarity and also ventral polarity; this is something very interesting. What is their dorsal-ventral polarity? Dorsal-ventral polarity is mainly maintained by the BMP signaling. BMP is a bone morphogenetic protein.

So BMP is superabundant on the dorsal side. So we can say BMP signaling maintains the back as it is. The absence of BMP signaling and BMP signaling are turned off on the ventral side. First of all, the BMP distribution is high on the dorsal side, and the concentration fades toward the ventral side. And not just that, on the ventral side, you have the expression of noggins superabundant. That means even if there are traces of BMPs expressed in the ventral region, the noggin makes sure that BMPs are not going to do any biological function.

So noggin, which is an antagonist of BMP signaling, is expressed abundantly in the ventral region, and its concentration depletes as it moves towards the dorsal side. You can see in this picture that BMP is very high at the top and it fades off, and the noggin is very high at the bottom and it fades off. The BMP expression defines the dorsal region in flat forms; the BMP inhibitors such as noggin are produced on the ventral side, so in a stricter sense, you can say that the presence of BMP signaling and the absence of BMP signaling decide the dorsal and ventral. The presence of BMP signaling, because noggin is an antagonist, does not cause any signaling. So the presence of BMP signaling and the absence of BMP signaling are enough.

Pure presence and pure absence will decide the dorsal and ventral. And there is something we discussed about the medial-lateral, medial-lateral angle. That means from your chest region to your right hand and from your chest region to your left hand. So this is your medial lateral angle.

That is also decided by noggins. So noggins are expressed right in the center of your median line and dorsal. Right hand or median line and the left hand, that is what you are seeing here. So the noggin, make sure which is towards the ventral side, that abundance is between the central portion and the left side central portion and the right side. So that gradient is also taken care of by the noggin; that is the medial-lateral gradient.

Now, if you see the anterior-posterior polarity. Anterior. Anteriormost. That is towards your head. Like in this plenary, you can see this is the anterior-most portion.

Where its eyes are, there. And this is the posterior-most portion. Where its tail is, there. For you also.

Your head or neck. That is the anterior-most portion. And your buttocks. Not your feet. Because feet are two structures that are projected from you. You are basically.

Your body extends from your mouth to your anus. That is your body. Legs are an extension of that body. So from your mouth to your buttocks or your anus. Anus is your posterior-most portion.

The same logic also applies in this planaria body. Anterior-posterior polarity is determined by wnt signaling. We have also seen the same wnt signaling happening in Hydra. And the same wnt signaling is involved. The gradient of wnt signaling is involved in the anterior-posterior activity, and the anterior-posterior gradient also.

Wnt is produced at the posterior end. So Wnt activity is extremely high at the posterior end. And the Wnt inhibitor is extremely high at the anterior end. So now you have a gradient. Same as what we saw about the BMP signaling. You have a superabundant expression of wnt ligand and also the stabilization of beta-catenin in the posterior end.

And it is fading as you move towards the anterior end. So, what do you understand? If you want head, you don't want to have wnt signaling in the head region, or the maintenance of head requires the absence of Wnt signaling, and in the anterior most portion, you have got many Wnt inhibitors; many Wnt inhibitors are there, like where DKK is, there SFRP is, there WIF is; many, many molecules are there which are active inhibitors of Wnt signaling, indicates that they are super active in the anterior-most portion. Just as we saw in the case of BMP signaling with the presence and absence of BMP signaling, you have the presence of wnt signaling in the posterior and the absence of wnt signaling in the anterior. Wnt inhibitors do not create a new signaling; rather, Wnt inhibitors ensure that Wnt signaling does not occur here, which is sufficient for developing a head region. So wnt antagonists are produced abundantly in the anterior region so that even if there are traces of wnt signaling or wnt ligands or stabilization of beta-catenin happening in the anterior end, that is unwelcome.

That shouldn't happen. So, that is the logic of maintaining the anterior-posterior axis. Now, can we think about tweaking this AP polarity during the regeneration of the flatworm? So we have seen it. You can take a flatworm and cut it. You want to take DJ, you want to take SMED, whichever species you want, and you cut it; it grows back.

A blastema will be formed. Normally, Wnts are produced in the posterior blastema. Say I took a planaria, cut off its head, like you can see here. It's a normal planaria; I cut off its head here, then the head has to form. I cut off the tail region; the tail has to form.

This is the normalcy, but... Say, if I cut the tail, what happens to the rest of the body? I cut here and this piece is gone. And what happens to the rest of the body, the remaining portion? What happened? Wnts are produced in the posterior blastema, which then forms a tail bud. That will eventually lead to the formation of the proper tail. But if RNAi, or RNA interference, is a technique used to block gene expression.

Normally, people use siRNA; people use shRNA. Basically, it means that if you are making any given mRNA bind with this siRNA or shRNA, it will be marked for degradation. That RNA will be marked for degradation. You will not get that protein. So it is as good as that gene knockout. So siRNA, RNA is used to create a gene knockdown, not a knockout because the genome has that gene.

RNA is being produced, but it will never get a chance to make the protein. So RNA is used to target either Wnt or β-catenin. So Wnt is the ligand. So if you use an RNA against Wnt, it won't be able to go and bind to the free cell receptor. But if you get rid of beta-catenin, what happens? Whether the wnt is there or not doesn't matter.

The beta-catenin will not be stabilized. Beta-catenin will not get a chance to turn on wnt signaling-specific genes. So you can use either one of them. What happens if you use RNA against the wnt or beta-catenin in the posterior blastema that forms a head? This is something very interesting. If you cut it, the tail; you cut the tail here.

And you blocked the wnt signaling. And instead of getting a tail, you end up with a head. As you can see here, the wnt is blocked.

So, this was a normal planarian. Its back is cut. Tail was there. All I did was block the Wnt, wnt signaling. Now I ended up getting double-headed planaria. So sometimes, if beta-catenin is blocked, in this case, Wnt is blocked, and both are producing the same phenotype because Wnt is blocked. There is no stabilizer, no beta-catenin stabilization. In the second case, beta-catenin never gets a chance to go to the nucleus, even if there is

Wnt, so that is the only difference.

There also you end up getting a head that is being formed, but it is slightly different. If you note it carefully, this looks like a proper head. This does not look like a proper head. If you think a little, you'll know the answer because beta-catenin is an essential gene.

Beta-catenin is needed to maintain the cell's integrity. By knocking it down, it has compromised cellular integrity. That is why you don't see this much beautiful head being formed from here. This planaria is fine, but the head doesn't seem that beautiful.

Not all the eyes are there. It looks like it shrunk. It looks like it was born in a... famine periods or their undernourished appearance are due to beta-catenin, which is not only necessary for entering the nucleus but also for housekeeping functions; that is why the head looks smaller. Now, if you look at some more facts on the neoblast. In planarians, the neoblasts are the only cells that divide. If an eye is there, if an intestine is there, if there is a flame cell, there is a gamete, neuron, whatnot; there are 30 different cell types, but they do not divide.

Once a cell is formed, it is like our neuron. Our neurons normally don't divide, especially in the central nervous system. It stays for the rest of your lifetime. Many of your muscles don't divide. They are terminally differentiated.

So planaria, every cell in its body is terminally differentiated except for the neoblast. They are the only cells that divide. The planarian PV, a special type of planaria PV homologue, is a well-known neoblast marker. So PV homologues are a marker for this neoblast, and they have a specific marker that is expressed in them. They are used to detect them because you can use the markers of C neoblasts, and you can track them.

You can stay in for it. And then look at whether they are incorporating a BrdU or have any proliferation markers, etc. So PV is used as one of the target molecules for detection. So PV and the closely related organodes are other proteins. So organodes are another group of proteins.

They are highly conserved mediators of gene expression. Like I told you about siRNA. I also told you about, you know, shRNA. It goes and binds to the mRNA, and it degrades, right? For this, it has to form an RNA-induced silencing complex, RISC. And this is formed with the help of proteins such as organelles, okay? So, PV and closely related organoids are highly conserved mediators of gene regulation in general and especially in planaria. Argonauts are widely expressed and mediate gene silencing via small RNAs like miRNA and siRNA.

This is something important. So we should understand that sometimes some animals or some cells do not have an adequate amount of Argonaut, etc. Because they do not want any gene silencing to occur in those cells. But there are other animals that rely a lot on this siRNA and miRNA-mediated gene regulation. So this example of this PV and Argonaut indicates that they are widely expressed in planarian neoblasts.

One essential function of PV proteins is transposon silencing via PV-associated small RNAs. They are also known as piRNAs. So what we understand from now is that the expression or the quality of genes that are expressed in a differentiated planaria versus the quality of gene expression in a neoblast. They are extremely important for maintaining their pluripotency status and for maintaining their differentiated status. So that is why they rely heavily on this piRNA because it is mediated by this risk complex of proteins.

So that is why their gene regulation is very stringent. One more thing we need to know is. About those animals that follow, this RNA-mediated gene regulation is that they can handle the situation. One example I will tell you is that many neurons need to have abundant expression of let-7 microRNA. Let-7 microRNA targets a lot of mitotic mRNA genes that are capable of driving. The neuron enters a proliferative phase.

And you don't want that because neurons are our storehouses of information. A lot of vital biological functions exist. So let's have a microRNA make sure that even by accident, a given pro-mitotic gene is turned on. The cell will never think of dividing its DNA because let's ensure that the newly formed mRNA will never get a chance to be translated. In other words, micro RNAs can act as guardians of their status.

The same way a CNEO blast needs a particular status. It should allow that if the gene expression events occur, then another set of gene regulation events will kick start. Understanding all the planaria is a simple structure; the gene expression events in a given cell decide whether it is terminally differentiated or if the senior blasts need to be constantly under their proliferative phase. So, this is what we should keep in mind: that piRNA is very important in regulating their gene expression. Transposons have been estimated to account for 31 percent of the Smed Smedtea mediterranea genome; 31 percent of their genome, which is one-third of their genome, consists of transposons.

What does it tell you? It has the vulnerability to reshuffle and rearrange its genome. Transposons are jumping genes, which means they have a transposase coded in them. They can use the transposes and cut off from the region where they are. They can insert it into a new place.

That means the genome is vulnerable to alteration. These are all additional facts. What we learned is some additional facts about the neoblast because, having said that they have the great capacity to regenerate lost tissue parts, we should also understand that the underlying gene expression or the underlying gene regulation events are pivotal in controlling these remarkable properties. We will study more about regeneration in planaria in the next class. Thank you.