Regeneration Biology
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Week: 3
Lecture: 13

W3L13-Position control genes and regeneration-Part A

Hello, everyone. Welcome back to another session of regenerative biology. As I mentioned in the previous class, we split the position control into one basic part, which is part A, and then advanced, which is part B. Okay, so the position control gene itself indicates that so far we have seen there is an anterior-posterior axis, medial-lateral axis, dorsal-ventral axis, etc. These are all gradients. We are talking about the gradient and how the neoblast responds to it.

or how and why a gradient determines or directs a particular gene expression in a given locus. That means you want me in the place where I am. You want the pharynx in the place where the pharynx is. So this is the simple logic behind regeneration.

And we all know that after an amputation, there is a blastema that is being formed, which is highly disorganized or highly rudimentary in terms of differentiation. They are basal cells. Specialized neoblasts are produced coarsely in a randomly distributed pattern below the eye, where their distribution is located posterior to the eye. And the most important thing you need to know is that an eye-forming cell, a neoblast, is capable of forming eyes present anywhere in the body. Or any segment you take that has an eye specialization, or any segment you take that has a nephron or a secretory cell producing, or any cell you take that has a neuron producing, so this is the logic: if there are 30 cell types, 30 different neoblasts are present.

everywhere in any fragment. Means they don't have any bias. Neoblast specialization can occur regionally. It is spatially coerced. Coerced means to be forced or compelled.

That is, specialized neoblasts are distributed much more broadly than in the final location. Say the final location is the eye; it is not located closer to the eye; it can be anywhere in the posterior-most portion where the eye is maximally distant. The eye can also have the logic; you probably already got it because you need to have an eye and head that are being formed even from segment number six, as we saw in the previous lecture. The extreme posterior-most portion should also have the ability to form the head and the eye. And then, the final location of their targeted differentiated tissue, where the intended target's final destination may be far away from the actual position.

This doesn't mean that it has to travel from this maximum distance to reach there because they are everywhere. It's not that every organ has only one cell type. It has many. If there are 100 eye progenitors, they are present in 100 different spots of the body rather than congregating in one zone. This attribute was first discovered in uninjured animals for eye-specialized neoblasts and has important implications for the logic of regeneration.

Eye-specialized neoblasts are specified between the centrally located pharynx and the eyes, which is the pre-pharyngeal area where they are abundantly located; however, they are not restricted to that area. This is what you should understand: these broadly distributed progenitors ultimately incorporate into uninjured eyes. Uninjured eyes are constantly being developed because they have to, so none of the eye progenitors are restricted. Jobless. They are waiting for their turn.

When will I get a chance to get into the eyes? So this is their way of life. To replace the cells lost due to natural cell or tissue turnover. So they keep getting incorporated. So during regeneration, two migratory eye progenitor trails emerge from the wound area. There is a wound; two trails will come.

Let us see what they are. I specialized in neoblasts to produce non-dividing progeny cells that progressively migrate to specific target locations. They are non-dividing, but their goal is to keep migrating to the target location in the head blastema, where they differentiate. The head is removed, and the blastema is being formed. The head structure has to come back, and in that newly formed head structure, the eye should form, not that in that blastema itself the eye is formed, so it is a constant maturation process; therefore, regulation of the migration of the specialized neoblasts from the coelomic pattern to their target destination appears to be an important aspect.

of specialized neoblast behavior. So specialized neoblasts are those neoblasts that can give rise only to that particular tissue, their masters, their specialized cell types. But they need to know from this coerce pattern what their target is for which the matrix and the position control genes come into the picture. We will see them one by one. So in this picture, as you can see, there are in the panel a picture we have seen before, where the neoblasts are the only mitotic cells, and they are proliferating.

You can see the pharyngeal region, where the neoblasts are minimal, and wherever you see the red-colored dots, they represent the neoblast population; they are the dividing ones. They are neoblasts, which are given in red, and they are mesenchymal cells distributed broadly. Mesenchymal means that it is the opposite of epithelium. In cancer, you would have studied epithelial to mesenchymal transition. Epithelium means adherent,

while mesenchymal means roaming around.

The blue color is the DAPI stain, which is the nuclear stain, and the pharynx is very clearly visible because it has the blue color but is devoid of red color simply because it is minimal in neoblasts; panel B, which shows the transplantation of a single neoblast, can regenerate colonies, as we have seen with broad differentiation potential. And the capacity to restore regeneration to a lethally irradiated host. This we saw in the previous class before the previous class that radiation can restore the animal only if you transplant one. And these resultant animals are, naturally, genetic clones of the donor. We have also seen this.

And they are capable of giving rise to all cell types. In panel C, if you see here, they are specialized neoblasts for the eye that produce progenitors that migrate into two trails, two different trails from the wound into the head blastema, from the wound region into the blastema because the blastema is the place where the head and the eye have to restore, where they coalesce into eyes. You can see here that this is a cut region, and you can see there are different types of specialized cells in the eye, the so-called eye-forming ones. They congregate into one eye; this is another eye. They will eventually congregate, and they will have two trails: trail number one for eye number one and trail number two for eye number two.

So you can see how they migrate through the blastema into their destined target. And. In the D, what you are seeing is the specialized neoblast model that involves the activation of distinct transcription factors in a neoblast-specific manner, which will seal their fate into the target and also enable tissue turnover and regeneration. So no matter whether the specialized neoblast migration is part of the steady state maintenance of that organ versus it being a newly regenerating structure. The job of the specialized neoblast is fixed no matter what situation the animal is going through, and it is governed by the transcriptional machinery.

So you can see neoblasts, they get into specialized neoblasts and give rise to neurons, muscle cells, or any other excretory cells. So neoblasts provide the cellular basis for new tissue production in planarian regeneration in general. So you can see here in this panel the E panel; eyes can be regenerated following a resection, which we know you can remove the eye alone—there's no need to chop off the whole head. You can simply pluck the eye, and it will restore back. This is the normal plan here: eyes are removed, and they can restore their eye.

So for this. Only eye progenitors need to come into the picture. You don't need to have any other progenitors. Eyes can be regenerated following resection. But this does not

trigger the amplification of eye progenitors. For reasons you could imagine.

Because you don't need to have an increase. In the previous class, we saw. Soon after an amputation. The rate of proliferation of cNeoblasts increases. But if you just remove the eye.

You don't see any accelerated proliferation. Because, the same specialized neoblast can give rise to an eye. You don't need to have any alarming injury responses, etc. You can see the eye progenitors that are magenta in color, and they are amplifying. They don't have any increase because you had an eye; you simply removed it.

They are a part and parcel of normal life for the planaria. If I give a simple example, you have 1 lakh rupees with you. If 10 rupees are lost from that, you won't even notice. Although it is a decline, with not too much effort, that will be restored.

Somehow, it will come back. You won't even know. The same logic applies. But if that 1 lakh rupees, if 50,000 is removed, restoring it back to 50,000 may need extra earnings, income, etc. Same logic. The existing specialized neoblast cells are good enough.

To restore the lost eye, you don't need help from other neoblasts, nor do you need to have an alarming increase in proliferation; that is the logic you should understand. On the right side, this can target whenever the eyes are removed; it becomes a blind area and the target, and this targeting. Can happen even without the eye. Earlier, we said the eye will constantly keep replacing, just like any other organ, but these cells know where the eye is. However, once you remove the eye, how do they know where they should form? Because there is no eye.

There are no eye-specific cells there. Whole eye is removed. So that thing happens, or that recognition happens, mainly because of position control, or position-specific, or the target-specific information. We will see where and how the information is located in a short while. But some information is available. Even though I am missing, someone or something tells me, oh, here is the eye.

So target blind regeneration can happen only because of this information. Regeneration occurs as an emergent property of constant progenitor production. During regeneration with no progenitor production rate change, if you remove just the eye, there are fewer cells in the forming eye accessible to undergo cell death, and they are young. Consequently, what happens? Fewer cell death events per eye passively enable regeneration. Normal eye, what happens? Let us assume one eye has 100 cells.

Three cells die every day. So, three cells need to be replaced. So it is a constant process. When an eye is missing, what happens? Three cells came in. They don't die because there are no cells to die.

Three cells just arrived. And one more three cells will come the next day. It became six cell. One more cell has arrived. It became nine cell. They are not dying because these are all young cells.

In the other case, some are the oldest ones and they are dying. So these are relatively new cells. Hence, death is not present. It's just like pouring water into a vessel that has a hole in the bottom versus one that has no hole. It is normal; if the rate of water flow into the pot is the same as the hole, at any given time, water will be filled.

This is a scenario in a normal eye in the absence of water and the absence of a hole; any water falling in will stay contained inside, and once it reaches a threshold, it will start overflowing. You can consider that of overflowing; if instead of a hole, it is best to think of it as overflowing. One liter pot, if water is falling and overflowing, will equalize. But if the pot is empty, just as an eye is removed, any water falling in will continue until the pot is filled before it can overflow. So same way, they'll keep migrating and they keep filling and the eye will be formed completely.

So this will consequently result in fewer cell death events per eye, passively enabling the regeneration. eye formation after amputation so decapitation also gets rid of the eye if you remove the head you do not do anything to the eye but the whole area is missing okay following decapitation eye progenitors are amplified in large numbers naturally because eye is missing head is missing naturally eye is missing But they can bring rapid eye regeneration in the new head. The new head is formed from the blastema, and then the new eye also has to form. And naturally, there is rapid proliferation of neoblasts along with the C neoblasts and the eye-specific neoblasts or specialized neoblasts. Would the absence of only the eye-following eye-specific resection lead to similar eye progenitor amplification? And it will not.

What about the removal of only a few cells? Would the amplification of only a few progenitors occur? You did not remove the whole eye, you removed maybe 25% of the eye. What will happen? The surprising answer is that the specific removal of the eye leads to no change in the number of eye progenitors, but the eye will regenerate nonetheless. Because of the reason I told you, maintaining an eye is a routine process. Maintaining, how is it maintaining? It is removing a cell and adding a new cell.

Removing a cell, adding a new cell. So, if you remove a little bit of cell, half of the eye,

or a full eye, getting a new eye does not require any active involvement or more progenitor proliferation. You don't need to increase the production of progenitors. Like a 100-member family, if 3-member visitors come, they don't need to cook extra. A 100-member family, with 3 extra people coming, doesn't need to cook extra. But if it's a three-member family, even if one person comes or two people come, it is a huge task.

This logic should be kept in mind because the eye-specific progenitors are present throughout the body. All you removed is one eye, and the progenitor number is so high that it can make multiple eyes and doesn't make multiple eyes everywhere, but it's constantly replaced. The same logic applies to the eye, which is a specialized organ that you can easily spot and is very easy to monitor under a microscope; unlike other cell types, that is why people work on the eye, but other cells and tissue types also follow a similar pattern. In animals with one uninjured eye and one regenerating eye, Think of it as two eyes.

You removed only one. Now that is an interesting scenario. Why did you remove the regenerating eye? Because you removed one eye. The two eyes incorporate progenitors at the same low rate. So let us assume three cells, five cells, or ten cells. Three cells I gave as an example.

So one eye intact, one eye you removed. So both eyes, not that the removed area gets 100 cells per day. No, both eyes are getting 10 eyes per day. But in the existing eye, 10 cells also die. Whereas in the missing eye, on the first day, there are 10 cells, and nothing is there to move out.

One more 10 makes 20 cells. No more cells are moving out. So whatever is coming in, the same logic applies as filling one mug of water. Until it is full, there is no way it can overflow. Yet one eye will grow. So this is the logic.

Incorporate the progenitor at the same low rate. Yet one eye will grow and the other eye will stay a constant size. How? I already told you the answer. Eye size is determined not only by the rate of new cells entering the eye, but also by the rate of cells leaving the eye through cell death. Leaving means they are not traveling.

They are just being killed off or shedding off. Thus, if the rate of new cell incorporation into a regenerating eye is not changing, then the rate of cell loss in the regenerating eye per eye should be less than the loss rate of an uninjured eye. An uninjured eye has many old cells; a newly forming eye has every cell that is young. Only their difference is by one day, whereas the existing eye may be a few months old. The instructions for tissue turnover and regeneration explain how it works. So tissue turnover is one thing where it

has to constantly supply new cells.

Constitutive positional information. Positional information is very important. Constitutive means at any given time, this information knows, yes, this area is for the eye, this area is for the neuron, this area is for the brain, this area is for the pharynx, like that. Beta-catenin RNAi; again, we are going back to beta-catenin because a lot of research has been done, and a lot of this anterior-posterior axis is maintained via Wnt/beta-catenin signaling. Beta-catenin RNAi led to dramatic regeneration outcomes; heads were regenerated in place of tails.

You can make them if you block. We have seen it: if you make a cut, a head will normally form, but if you block... Wnt signaling beta catenin RNAi, the other end, you will also end up getting a head, resulting in two-headed animals with both heads fighting to pull the same body in different directions. That is a very funny situation where every animal moves where its head is, so it will move.

toward the direction where the animal's head is. You can look into any worm or caterpillar, any worms, tiny to small, or a snake; anything, wherever its head is, no animal will keep its head in the extreme backside and then travel. No one moves backward. Of course, some animals will transiently do that, but default movement is forward. But if planaria has two heads on either side, it will be stretching its head in either direction.

At any given time, it will be moving around and rotating. That means it's not a simple thing that shows both heads have got equal weightage. That means fully functional. It's not a dummy head. That's what this logic says. This observation suggested a role for Wnt signaling in the decision-making process at the transfer amputation planes to regenerate a head or tail.

So you can add value to the Wnt signaling that head formation is important for the decision-making process of the newly formed animal. So you can tell Wnt signaling was important for deciding which direction the animal would go. It's a decision, isn't it? So can you, you want to make a, just like you are designing a bike, you are thinking about whether I should, where should I keep the handle? Should I keep the handle in the front or the back? It's purely based on the engineers. So here, the Wnt signaling decides where the head should form. Accordingly, Wnt signaling determines where the animal should form because the head dictates that.

Following beta-catenin RNAi in uninjured animals, a slow and steady transformation of the body plan occurs during tissue turnover. Many heads appear around the animal's perimeter. If you block, there is no injury, nothing. Took a planaria, blocking Wnt

signaling.

Wnt signal, you just gave shRNA against beta-catenin. What will happen? Heads will start forming everywhere.

Means any area is vulnerable to forming a head. That means the position... The overall position and location of a structure called the head is decided by one signaling event, or the positional information is so important that usually you know that we saw in the previous class; also, in a non-regenerating species, after decapitation, if You block Wnt signaling; you can end up getting a head. So now, in a normal animal, if you block Wnt signaling, you can end up with multiple heads. You can imagine how plastic the system is. So Wnt signals upregulation through APC or NOTUM. NOTUM is a carboxyl esterase that removes an essential palmitoyl moiety from Wnts to deliberate.

It's basically a functional role, and it forms the first non-extracellular protein deacylase. It's not important to know the mechanism written down here, but APC is a negative regulator of beta-catenin, which means beta-catenin is degraded if APC is active. If APC is functionless or jobless, beta-catenin gets stabilized, so Wnt signalling can be upregulated. Can be upregulated if APC is removed, can result in the regeneration of tails in place of heads; the opposite occurs when Wnt signaling is inhibited. You get heads, but if you favor Wnt signaling by getting rid of the negative regulators of Wnt signaling, you will end up getting the opposite—that is, the tail—that is the polarities maintained by.

Wnt signaling. These findings indicate that constitutive regional expression of Wnt ligands is usually posterior and Wnt inhibitors anterior. Near the head region, Wnt inhibitors prevail. The tail region governs the Wnt activators. Control the regionalization of the planarian AP-axis. This positional information decides how different tissue structures are formed and how they function.

And now comes the dorsal-ventral axis. The dorsal-ventral axis is something that is very interesting. And dorsal means your back side. Ventral refers to your belly and chest region. That is a ventricle.

Another prominent model of planarian adult positional information involves BMP signaling. We kind of discussed it in the previous class, which regulates the dorsal-ventral axis. If you inhibit the BMP, there are various methods available. You can use RNAi, etc. Because the good thing about planaria is that you can feed the animal with an RNAi.

Normally, it goes in. Since it's a small RNA, it gets into the circulation and inhibits. So it's a very easy system to work with. So BMP inhibition results in progressive ventralization. What is ventralization? Let us assume you know your chest. Normally, you have your umbilical cord; your back and your front are different.

They are not the same. Now, if you block the BMP signal, you know that in planaria, the ventral side also has a pharynx that is coming from the ventral side. Now, if you block the BMP signal, you end up getting the ventral side on either side, just like you saw the head on both sides, just like you can make a tail on both sides through tweaking the Wnt signaling. If you block BMP signaling, you will get the ventral side on either side. And which includes, there are real solid structures with ventral tissue types that are predominant, such as nerve cords and ciliated epidermis appearing dorsally, which are supposed to be in the ventral region. Now it has gone to the dorsal region; it is just like we all say, what is east? The sun rises in the east if.

.. The sun rises from the west or from the north; will you call the sun rising from the north, or will you call north as east? This is a conundrum. If you inhibit BMP signaling, you end up getting these ventral structures appearing in the dorsal. If your legs are coming from your shoulder, you can say that you got, Posteriorized means the leg is a posterior structure.

The hand is an anterior structure. Hand came on your leg. We can call the person who got anteriorized. The anterior structure went into the posterior. The same way, vice versa.

The posterior structure came into the anterior region. We can call you got posteriorized. In the same way, it got ventralized. Because of the so-called dorsal region, you have a ventral structure. For example, BMP4 RNA is mentioned.

BMP is a ligand of BMP signaling. RNA animals can flip over and glide on their backs. They're gliding on their backs naturally because... They think the animal thinks their back is their belly. So instead of moving with the belly down and the back up, it will go with the back down and the belly up.

So this is decided by BMP signaling. The DV axis is also governed by roles for other pathways regulating the dorsal-ventral axis, that is, the DV axis, including ADAM. That is a variety of noggin that is also known as nog and noggin, like genes that are antagonistic to BMP signaling, just like we mentioned Wnt activators and Wnt inhibitors, similar to BMP signaling activators and BMP inhibitors. BMP4 is expressed dorsally, naturally, because you want BMP signaling in a medial to lateral (ML) gradient, so dorsally, the backside, in a medial to lateral manner.

Your chest is to the right-hand side. Chest to the left-hand side. So the chest is your central portion on the right-hand side. Central portion on the left-hand side.

That side of the dorsal. That is a BMP force plate. Place where it is expressed. Now on the ML axis, the Wnt5 is expressed laterally and inhibits the medially expressed gene called slit. Wnt5 RNAi and slit RNAi result in medial-lateral patterning abnormalities. So these are all interplayers like SLIT and NOGGIN. These are all influencers of BMP signaling. And what is important to understand is that Wnt5 RNAi and SLIT RNAi result in medial-lateral patterning abnormalities; in other words, it is difficult to explain.

Say your right hand now comes to the left-hand side. You know how your right palm looks. What if it came on the left side? Your thumb, instead of looking towards your right hand thumb, is looking towards your chest region; now your right hand thumb will look away from your body. Isn't it? Usually, this is the variation you will get. That is the medial-lateral patterning that is to be followed, not just the dorsal-ventral patterning. Genes with constitutive and regional expression and their association with patterning are called position control genes.

In short form, we call them PCGs. We will study more about these genes and positional control in the next class. Thank you.