Regeneration Biology
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W3L15_Progenitor targeting and ectopic organs

Hello everyone, welcome back to another session of regeneration biology. We will continue with where we left off in the previous class, and today we will try to understand how different PCGs, that is, position control genes, contribute to the interaction with the neoblast and how they give rise to the lost or damaged tissue. And we also understand how ectopic organs can form. That means additional organ. Ectopic organ means, say, usually you have two eyes. If I make two more eyes, that is ectopic.

You have one nose. I make two more noises that are called ectopic. They are organs, but they are beyond the usual number and out of place. Okay, so progenitor targeting and ectopic organs are involved.

So we have seen so far that there will be a variation in the gradient of the anterior-posterior or dorsal-ventral gradient after amputation. Say, for example, let us assume a planaria has 10 segments, okay, segment 1 to segment 10. Let us assume the first three segments, the anterior most three segments, have one gene, and the posterior three segments have another gene. Now, if this planaria is cut in half, so 10 segments now become one segment, five segments per piece. Now, this anterior three segments are not in three segments; now it will be restricted to the anterior most one segment, meaning it is pushed anteriorly.

Why? Because this posterior part of this anterior segment will start expressing more of the posterior. genes so that the existing piece now readjust. Say you had a house that had five bedrooms, and now you have moved to a one-bedroom house. Then, with your five bedrooms and different allocations that you had, you have to do everything in that single bedroom. This is what the animal will do first.

In the same way as you can see here, there is a distribution of anterior in this picture; you can see blue color, and then it becomes white, and then it becomes yellow colored. Now, after a segmentation that happens, what you have seen here in this existing piece is that you have blue color, white color, and there is no dedicated yellow color yet, but after some time. The PCG pattern is re-established; you have one blue color, one light blue, one white, one light yellow, and either deep yellow, light brown, or deep brown. The

same thing happened in the other fragment as well. This is what repurposing, re-patterning, or re-establishing the gradient in that tiny fragment.

That is very important for pursuing further regeneration. So PCGs are expressed in the muscles. Left, you have the PCG RNA probe pool, which is red in color, and as you can see here, these are all different muscle-specific gene collagen probes, and SFRP2 is basically indicating one of the position controls. Gene expression and in this B pattern, the bottom also shows the topmost portion you are seeing, while the bottom portion is basically the cartoon where the reestablishment of PCG expression has happened. Now, the important thing you need to know is that the migrating.

Neoblasts will come and interact with these individual PCGs located in the muscle and differentiate. Like here, you can see normally this would have been a given tissue, and this would have been another given tissue. Now this has the anterior fragment continuing to do so, but eventually you see the posterior fragment giving; both of these tissues are formed as it happened here. The same thing happened here as well. Here, technically, only this piece of the posterior fragment will give rest to only the given set of tissue.

Now, the same posterior fragments start giving this anterior tissue also, so this is the logic behind this differentiation. Now, another gene that is very specifically controlled, like we have seen, soon after injury, Wnt 1 is expressed from every side, no matter whether it is anterior or posterior. We know that in the anterior region, head regeneration, Wnt signaling is not needed. But then the Wnt is expressed there. Who is controlling it? So we will be confused.

Wnt is expressed. Why not? Wnt signaling is not occurring. So NOTUM is preferentially wound-induced at the anterior-facing wounds. A NOTAM is a classic Wnt inhibitor. The induction of NOTUM is not very well known, but it is restricted to the anterior-most pole.

Hence, even if the Wnt is expressed, it doesn't matter. Wnt signaling is not going to kick start. Head can form. Credit goes to NOTUM. And the NOTUM inhibits Wnt signaling to promote head regeneration.

How NOTUM is selectively activated at the anterior-facing wounds remains unknown. Without the PCG pattern and regeneration in Follistatin, we remember that Follistatin is a responding molecule, like how someone will come and tell the plan area, "Oh, there is a body part that is lost." No one will come and tell the plan area; it is done through the induction of Follistatin. If you knock down Follistatin, it means you are not allowing Follistatin RNA to do its job or produce protein from the RNA using RNAi. If an

anterior-most portion fragment is present, that will remain as it is; it is not going to replace the lost posterior part.

Planaria did not know, "Yes, I am injured," so Follistatin is the one that helps without PCG pattern regeneration in polystatin in MyoD RNA animals. MyoD also, you should know, we know the muscles are the place where the, uh, where the, um, PCGs are expressed. If you knock down MyoD or NKX1, which are longitudinal or circular muscle markers, you will not be able to get any PCG expression because position control genes are expressed in the muscles. which will interact with the neoblast and give rise to a tissue. The motivation for the neoblast to differentiate into a given tissue type comes from the PCGs.

And PCG expresses only in muscle. But if you get rid of the identity of the muscle, then you cannot have PCGs. If you don't have PCG, there is no regeneration. So that is the logic. Regeneration fails to occur either with follistatin RNAi or myoD RNAi.

This requires longitudinal muscle fibers because myoD is expressed in them. Another example is a MEK inhibitor. Just as the folistatin is important, the ERK kinase, ERK MAP kinase signaling pathway is important for the persuasion of the regenerative response. Follistatin tells, yes, there is a damage we need to regenerate. But why? that doesn't cause regeneration.

To kickstart regeneration, you need to have the ERK signaling activated. So if you treat with an inhibitor, just like how folistatin and RNAi prevented regeneration, MEK inhibition also prevents the regeneration. However, the most important thing is that the removal of the inhibitor does not lead to regeneration because you prevented that initial urge. Say you were hungry during breakfast time. And no one gave you breakfast.

Now it's dinner time; I am bringing breakfast to you. And please have this breakfast. By that time, you may be hungry. But you are not in the mood to have breakfast. Or rather, you can't call it a maybe; some people have their stomachs full of gas, and if they eat something, they'll vomit.

They should eat because they are so hungry. They should be drinking some lime juice or perhaps having some orange juice. They cannot have idli if they will have severe stomach pain or vomiting, among other things. Because the stomach is full of acid. So you need to pacify your stomach.

In the same way, if you block follistatin or the MEK pathway, regeneration doesn't happen, and then you withdraw the drug. and telling okay now you regenerate i withdraw

the drug it doesn't happen but if you make a fresh injury, you cut again and then don't give any Follistatin RNAi don't give any MEK inhibitor then you will be able to get regeneration. So what does tell you ? It tells you that wound signaling or wounding is essential for kicking off the regenerative response. The coincidence of increased neoblast proliferation with positional information leads to the amplification of regional progenitor classes, even if the target tissue of the progenitor remains uninjured. Here, you can see that this is a normal planar area; here in this planar area below the eye region, there is damage that has been caused.

and this damage tells the animal that you know decapitation has happened actually nothing has happened there is a damage below the eye region so immediately the planaria thinks of making a new eye or new head because it is damaged well below so the positioning of the injury say if you do a tangential cut like across this plane You cut it; the lowermost cut is here; hence, the animal thinks the above part is whole, but the part is missing, although this side is perfectly fine. Since it's a tangential cut and this is the lowermost portion, the animal thinks the remaining portion is missing. Of course, the PCGS comes into the picture during regeneration, but that is how. As you can see in this picture, it immediately starts to amplify the eye progenitors. Mainly because it thinks the damage happened below the eye area.

Hence, the eye is damaged. Positional information and stem cells combine to bring about regeneration. PCGs can influence neoblast specification. PCG RNAi can impact regeneration. So the cellular output of neoblasts is, at some level, dependent on PCGs. PCGs are basically everything when it comes to giving meaning to the neoblast.

Zeta neoblasts produce progeny cells throughout the body that transit through transcriptionally distinct stages over six plus days as they migrate and enter the epidermis. So what we should understand here is that when there is any damage occurring to a given tissue, it has to come from the neoblast, and the gradient must be established in the form of neoblast. Without this, it is not going to work. Through single-cell RNA sequencing data, it was determined that the dorsal and ventral zeta neoblasts express different regional genes. And this time-dependent expression of PCGs decides or gives the message to the incoming neoblast about what tissue type it has to give rise to.

The dorsal regional identity of zeta neoblasts required for dorsal PCG is nothing but BMP4. BMP4, you know, the dorsalization or the dorsal meaning or message to the back of planaria is given by BMP4, and the ventral is by its inhibitor, which is noggin. Therefore, the PCG expression from muscle impacts the spatial identity of neoblasts, influencing their fate. So this is the interaction between the PCGs, neoblasts, and the gradient. Positional information and neoblast specialization together drive new tissue

production.

Every tissue damage sets in an alert leading to massive neoblast proliferation. Blastema production at the injury site and migration of neoblasts happen in situ. The interaction of neoblasts has to happen now with the PCGs. Morphogenesis ensues in a need-based pattern. What tissue is damaged? What tissue is missing? Accordingly, it will give rise to a new cell type or tissue type.

Research findings show that large wounds can amplify many progenitor types, even if their target organ remains as it is. As you saw in the previous picture, I can show it once again. Here there is damage; a big injury happened, but the target organ, the eye, is as it is. Nothing has happened to the eyes. So even when their target tissues remain, it suggests that positional information combines with the fate specification of stem cells to promote regeneration.

So the identity of an injury or the nature of an injury is understood by the animal based on which place and which part is damaged. Or in other words, if I got a wound in my chest, the organism thinks the head is missing because the chest is below the head region. Although the head is there, it will immediately start to make the head. You can see here wound architecture, migratory cues, and their extrinsic factors that are influencing the movement of the progenitors, especially how the cones or the poles of the tips are forming or how the newly formed blastema are integrating with the existing ones. The anterior-posterior axis and the dorsal-ventral axis, as you can see in this picture.

And this will lead to self-organization and produce a blastema pattern. Otherwise, what can happen? If you cut it at an angle, say you cut the planaria at this angle, you don't want a new head to form in this way. It looks like the planaria are bent like this. You want head like formed like this. You don't want the planaria head to form like this.

So unless it is aligned with this plane, which is the central midline, the anterior pole of this cone is determined by the induction occurring here, and of course it is connected with gene expression. Then there is an anterior-posterior pattern that is established. On top, what you can see is the anterior pole that promotes ML and AP, which stand for medial-lateral and anterior-posterior head blast MA pattern. Bottom wound architecture cues determine the point of anterior pole formation, connecting the blastema pattern to the pattern of the pre-existing tissue.

It has to gel well. It is just like making a tunnel. You may have seen many people; they will make a tunnel through the mountain. They start from one end to the other. If they don't touch each other, they will end up making two parallel tunnels. But the alignment

has to happen in such a way that they will meet at a central point, so that you save time.

So this fusion is very important. So if your planning of the tunnel or the blueprint of the tunnel was perfect, they are going to meet each other. So the pole progenitors decide how and why it should be formed. Let us think of an example.

This is NKX1 RNA. NKX is a marker of circular muscles. And if you knock down NKX, PCG expression gets affected. And this NKX RNAi, instead of having an anterior pole, has a spread-out movement of the neoblast, or this cone is not defined. This will eventually lead to the formation of two heads because there are two cones, and both of them are trying to align with the midline and the lateral line. This DV median plane and the median line, both together, are.

.. Confused, you end up getting two heads. That is the issue that you can create with NKX RNA. Self-organizing and extrinsic targeting cues govern the migratory behavior of progenitors. So progenitors are waiting for the migration, and they are just waiting for the proper PCG expression. But the PCG expression, in turn, depends on the muscles, and muscle quality depends on the nature of their housekeeping genes, such as MyoD or Nkx1, which decide whether a muscle remains a muscle. If a muscle has lost its identity, you can't expect PCG expression to happen; hence, you can't expect a proper response.

The formation of a newly formed tissue in this case, nkx rnai, happened and ended up getting two poles because circular muscles are contributing to the formation of the correct poles. That got disturbed, and you end up getting two heads, so the progenitors' final establishment at the destination depends on how the progenitors resolve. This dilemma of PCG discordance was demonstrated in a simple experiment. PCG discordance means that if PCGs are not properly expressed, confusion will occur. When a head was amputated, following a three-day delay, that is time for allowing PCG resetting.

One eye was removed. You take the animal and remove its eye. Remember? Even if the entire anterior half is removed, the newly formed blastema will have the eye spot and the head that is formed, and later the head will move forward. If you remove one of the eye, it just forms after three to four days, there will be an eye spot that will be formed and you are removing one eye, you're plucking it. And when this eye regenerated, once you remove the eye, naturally an eye will form. We have seen it. Even in adult, if you remove one eye, the eye will be formed right in this place.

But here, removing is slightly different. Why? The already formed eye is in a blastema. Blastema is dynamic. It keeps growing. In that, you removed one eye. Then what will happen? The newly formed eye is going to be shifted anteriorly.

What could the reason be? Because when the progenitors come, the existing eye will sequester. The other eye cannot sequester because the eye is missing. It has to make the eye and then sequester it. So then it is, keep looking, keep moving forward the minimum cells, and since the blastema is growing, the newly formed eye will be anteriorly shifted, so one eye will be here, another eye will be here, the normal eye is here, another eye is here. This is what happened: the progenitor targeted two different locations on the two sides of these head fragments.

The remaining eye on one side means the untouched eye. Acted as a self-organizing attractor that trapped incoming progenitors, making the progenitors unavailable, like you may have seen if you are putting food for two chickens, goats, or any animals in your house; when you put them together, they will eat together. But you are not allowing any goat to eat. The other goat is not going to spare the other chicken. It will eat all the food. And when you release whatever is left over, only that chicken or goat will get it.

So if this eats 1 kilo, the other will get only a quarter kilo. Rest ate 1.75 kilos, the other goat. This is what happened with the progenitors, too. So they trapped the remaining eye, and the progenitor failed to reach their TZ, which is the targeting zone. TZ stands for the targeting zone where the actual differentiation must happen.

Shifting the eye targeting zone medially and anteriorly by combining a parasagittal amputation, as you are doing in an angle cut. to the side of one eye with decapitation allowed the progenitors to bypass the self-organizing influence of the remaining eye resulting in the formation of a third eye. Say you cut it in an angle, existing eye is untouched, Then the newly formed blastemma assumes both are missing. You end up getting an extra two eyes. So if you remove only one eye through a parasagittal section, the existing eye will remain as it is.

Despite having one eye, since you made a plane below the eye region, you will end up getting new blastema, which will have two new eyes. And you will have one old eye and two new eyes. You will totally have three eyes. Further similar manipulations on the third three-eyed animal resulted in four- and five-eyed animals.

So you can, while new eye is being formed, you can continue to cut. Already the animal has got three eyes. You cut it at another angle. You will again end up getting another two more eye. Like you can make parasagittal sections by plucking or by cutting in sagittal sections. You can end up making three-eyed and four-eyed animals, as well as five-eyed animals.

And extra eyes were stably maintained and targeted by the progenitor. This is something important. Animals don't lose a single eye. It will retain all of its eyes, producing stable alternative anatomies in wild-type animals.

In the same way, you can also make an extra pharynx. Okay. So not just the eye, you can also make other organs extra. However, the beauty is that if you create a fresh amputation again, again, you decapitated, got rid of all the three head or five head bearing eye the newly formed head will have only two eyes you will not have five eyes restored back future of anomalous eyes why are ectopic eyes maintained will they regenerate after damage to them it was proposed that ectopic eyes were maintained simply because of their self-organizing properties combined with having access to eye progenitors Eyes are maintained in a specific region approximating the eye progenitor specification zone called the targetable zone. And defined as a region, the progenitors are capable of going to maintain an organ. That is in the newly formed blastema, two new eyes are formed. However, only the eyes in the target zone are capable of being regenerated following resection.

Spatially coerced progenitor specification is thus a central aspect of regeneration, allowing existing anatomy to access progenitors for their maintenance while positional coordinates are shifting. Similar experiments on PCG RNAi animals showed a patterning phenotype resulting in two pharynxes, but only one pharynx will be regenerated if damaged. If there are two pharynges, cutting one will prevent a new pharynx from forming because one pharynx is already there. Therefore, extra organs in incorrect locations, such as ectopic organs like the eye and pharynx, can be maintained but not regenerated upon their removal. These observations indicate that self-organization and extrinsic targeting cues combine with a broad progenitor specification zone, that is, TAZ, to determine the destination of migrating regenerative progenitors.

So there is a combination of multiple events: gradient, PCG, progenitors; everything has to come together for the generation of a new organ. As you can see in this picture, the amputations led to PCG shifting. But the self-organizing nature of the eye prevents the progenitors from reaching their target zone, regardless of what we discussed.

This is one eye has been plucked. It was shifted anteriorly. And here you have created a parasagittal section. And the newly formed blastema will always have the ability to.

.. So you can continue to cut. Like if you cut it again, it will have extra eyes. This is the real-time picture of animals that have three... All of them have three extra eyes, but you can create additional eyes if you wish, and enough medially to miss the eye during their migration, as you can see in this bottom D panel.

E panel is what the wild-type animals, as I already mentioned, have: extra eyes. F shows that WntP2 and PTX7 RNA animals develop a second pharynx; the original anterior pharynx is maintained but not regenerated, so this is maintained, but if you cut it... The extra ectopic organ does not come back, even for these animals; if it has three eyes, if you cut it here or get rid of the head, the newly formed head will have only two eyes. So, a model for planarian regeneration you can see here; whatever we have studied has been described as involving stem cells, and stem cell fate specification and the missing tissue response are essential.

The target of the blind stem cell specification happens along with the positional information. I'm not going through each of these sentences because you can read them, as each step is very important to understand and they have to happen in a sequential manner. And then the positional information outside the progenitor is also important because the muscles contain the positional information, and the instructions must be stored in order to maintain it, as the organs are revamped regularly. So would the signaling initiate the pattern of regeneration? Of course, the wound signaling contributes to the reestablishment of the pattern. As we have seen, if you block the ERK signaling or block follistatin, you will not get a regenerative response.

You can make them regenerate again if you make one more fresh cut; if you make it, then it will continue to a further regenerative angle. It will switch into the regeneration angle, and positional information impacts the fate of the stem cell depending upon where you are. Dealing with, say, you took, like we have seen it, if you block the, if you feed the animal with the Wnt, one RNA, you will end up getting ectopic heads that are formed all around the animal; we have seen it. So at that time, what happens is the priority goes to the absence of Wnt signaling; that is the priority that goes. It doesn't look at which is the gradient you are talking about or which pieces you are talking about because Wnt signaling is anti-head.

Absence of Wnt signaling becomes pro-head, so these logics should be put into the picture because it can also affect the anterior-posterior gradient, and organizers promote the blastema pattern. Organizers are very important; we have seen that one of the classic organizers is the notum, which gives the anterior-most positional identity. So, progenitor migratory targeting, which is basically the specialized neoblast, contributes to this progenitor's migratory targeting and then the self-organization of the progenitors, because sometimes what happens is that these progenitors must organize themselves. It encounters a differentiated target on its migratory path; it will immediately get into that organ, as that is the cause. Once you pluck the eye from a blastema, the newly formed blastema is moving anteriorly shifted because these progenitors are encountering an

already existing eye; hence, it will get migrated into that existing eye as part of the establishment or maintenance of the existing organ.

So this enables progenitor targeting of the remaining or partially injured body anatomy during regeneration. So these points should be kept in mind: it's not just regeneration that happens because of the congregation of all these factors. It also looks at what is there in the immediate neighborhood.

If it finds an organ, it will become trapped. So, this picture shows the model. That's the model for planarian regeneration involving positional information and stem cells like A, B, C, and D. All this pattern, whatever we have seen, has been discussed clearly. That means PCGs are important, and the gradients are also important, which in turn decides the PCG. And also, what happened 0 to 6 hours after? What happened in 48 hours? 70 to 96 hours: what happened two to four weeks? What happens? What are the changes that are occurring in a step-by-step manner? How is anterior identity maintained, how is posterior identity maintained, and how is dorsal-ventral identity maintained? Everything contributes to the formation of the organ, like you can see here in this cartoon of this fragment. This fragment made a miniature head and a miniature tail within 70 to 96 hours, but it is not a full animal; it is slowly differentiating with the help of the gradient established in the animal, so we will study another topic of regeneration in the next class. Thank you.