Regeneration Biology
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W3L14_Position control genes and regeneration-Part B

Hello, everyone. Welcome back to another session of regenerative biology. So we will continue with the position control gene that is part B. So far, we have been learning how different gradients contribute to organogenesis. Now we will learn how different position-control genes contribute to the establishment of a given organ in a particular spot. So, as you can see in this picture, this is a planaria that is cut either at the head or tail, okay, as you like it, and there are conditions like you can see in one situation when Wnt is inhibited; you are preventing the Wnt, that is a Wnt downward arrow.

You end up getting two heads on both sides, and when you upregulate Wnt, you end up getting two tails on both ends, so this is the Typical morphology. So the position control genes are continually and regionally expressed in planarian muscle to control the maintenance of the adult body plan and regeneration. So there are two things here. One thing is having its expression.

Second is retaining its expression. So one part assures that the expression ensures a given organ, but on the other side, you need to have the prevention of the opposite end; you should have the prevention of any gene that is going to favor an unwanted structure. So it is just like if you want to become rich. You have to earn more money, and you also have to prevent others from getting more money. If everyone has as much money as you, you are not rich.

Of course you are rich, but you are not relatively wealthy. So this is the concept of various signaling. Not only is one signaling favored, it also has to make sure that the same signaling does not happen in unwanted places through inhibitors. So you can see here that a regeneration patterning phenotype left the trunk fragments that are regenerating the heads and tails depending on the situation. If you knock down wnt, you get ahead in both ends.

If you upregulate Wnt, you get tails on both ends. Incubation of the Wnt pathway results in a posterior-facing, anterior-facing head, which is normal and perfectly fine, but a posterior-facing head is not normal. So, the incubation of the negative Wnt pathway, which regulates and leads to the regeneration of the anterior-facing tail. A posterior-

facing tail is normal; an anterior-facing tail is abnormal. On the right side, what you are seeing is the BMP pathway, the inhibition of which leads to the dorsal appearance of ventral attributes.

In the previous class, we saw dorsalization and ventralization. This word we saw is like what you can see in this picture. So the method used is different RNAi, whether to downregulate Wnt or upregulate it. These are all done through RNAi, RNA interference. You can see RNAi of BMP; you end up getting the cilia, which are at the top.

So ventral cilia are what the planaria has for crawling on the surface. Now you have both ventral and dorsal cilia. And here in this B, it is the staining of the panel B; it is the PCG expression domains that are anterior up, posterior down, and different genes are there. SFRP is a negative regulator of Wnt signaling, and it's not important to get into the qualitative nature of these genes. But all you see from this picture is that different genes are differentially distributed, and they are all position control genes.

So now this picture, this cartoon, shows how different gradients of various molecules are distributed. The brightest spot shows the strongest, and the faintest shows the least. So many of these genes, if you see within this eye region itself, their gradient disappears, whereas some of them come, start faintly and then increase and then disappear like NDL1 and NDL3, one example. And there are several Wnts. Their expression and then disappearance.

In the same way, posteriorly, if you see, some of them are strong, and some of them are weak. You may wonder why some Wnts are present both anteriorly and posteriorly. But remember, the presence or absence of Wnt is not the stringent criterion; rather, it is whether that Wnt is inhibited by the proper inhibitor. So that is what the real hallmark is. So you have an activator as well as an inhibitor, so the abundance of one of them will decide which will get the upper hand.

As you can see here, this beta-catenin will be least in the anterior region because if beta-catenin is abundantly present, then it doesn't favor head formation, meaning you want low beta-catenin and to stabilize the beta-catenin, by the way, not the expression of beta-catenin but the stabilized beta-catenin. Towards the posterior side, you have abundant beta-catenin expression that is present; you can see here the same way in the D panel, like the cartoon map of the PCG transcription across the AP axis, and this beta-catenin is the only one which you should follow seriously, while other genes are basically contributing to the stabilization of beta-catenin, which is nothing but the effector of Wnt signaling that goes into the nucleus. If you see this dorsal-ventral axis on the right side, that is a BMP cartoon of PCG expression. PCG stands for position control genes. Domains on the

DV/ML axis.

DV means the dorsal-ventral axis. ML means mediolateral axis. As you can see in this picture, you can see the expression of the medial-lateral axis. Something very interesting is happening. The Wnt is expressed right in this boundary.

As you can see here, In the exact middle, you have a strong expression of Slit and BMP in the dorsal region, and also in the mid-dorsal area, you have the expression of Slit genes and the Wnt-Slit mutual inhibition, which is important for the establishment of medial-lateral patterning. So the importance of showing these pictures and patterns is to demonstrate that every region has an identity. Every part of the body has an identity. Credit goes to these position-control genes. As you can see here, BMP's gradient is highest dorsally and least ventrally.

And the medial and lateral are so medial to lateral that this is the portion to the lateral, and you can see the expression of slit is inhibiting the Wnt, and that makes sure that wherever there is a slit expression, it does not want Wnt signaling to turn on. Wnt may be there; it is capable of stabilizing beta-catenin, but it does not happen simply because the slit will antagonize the Wnt. The repressors are also as important as those of the activators. And there are a bunch of genes which are listed here. That is the different genes that are there.

NOTAM, which is an antagonist of Wnt signaling, is. And if you have an RNAi phenotype, this is the gene, and this is the RNAi. So if you and I get an anterior-facing tail. So that means if you prevent the Wnt signaling inhibitor. That means you are activating the Wnt signaling.

So if you activate Wnt signaling, you end up getting a tail instead of a head. In the same way, the Wnt itself, which is the ligand, has a posterior-facing head. So we don't need to go into each of these phenotypes. You can go through it one by one.

There are so many genes. And it will affect some of them, leading to, you know, two fairings, etc. So different phenotypes come up, and accordingly, you will know, oh, this gene is important in producing this particular phenotype. Like this list is continuing, and the name of the gene is not that important; however, you need to know that. Whether a given gene is an activator or an inhibitor of Wnt signaling or BMP signaling, you will have an anterior-posterior axis or a dorsal-ventral axis. So adult positional information is harbored in the planarian muscle.

So far we have been telling that PCGs are there, position control genes are there. But

where are they located? Who is holding? What is their address? So we know that the muscles are static cells. They are part of your body. Your kind of skeleton, your musculature, is fixed; they will, although they can move relatively based on their contraction, but they are not moving from one place to another. So, these muscle cells harbor or are deposited with position-controlled proteins.

The cells expressing PCGs are indeed muscle, so muscle is doing the muscle-specific role, but when a... Neoblasts are coming walking across, then the muscles that are expressing a position control gene will tell the neoblasts, "Okay, you begin; this area is meant for this, this area is meant for this, you become this." So, body wall muscles include longitudinal, circular, and diagonal fibers.

Across the anterior-posterior axis, dorsal-ventral, and lateral axis, there are the circular and then tangential angles, so they are the diagonal fibers. These are the main muscles; each of them has some position control gene expressed. So, MyoD is required for the regeneration and homeostatic maintenance of longitudinal muscle fibers. So another gene, NKX1, is required for the regeneration and homeostatic maintenance of circular fibers. And inhibition of both MyoD and NKX1 together leads to a substantial loss of body wall fibers and reduced PCG.

That is what we are interested in. Reduced PCG expression before the animal's death. That means. A muscle is a muscle only when it has myoD or NKX expression. It's just like your identity is on your face. I just completely take one slice of your face which covers your nose, eyes, mouth, everything and I made your face flat.

What is your identity? There is no identity. If you want to be recognized, you have to do DNA fingerprinting. In the same way, you get rid of the muscle's identity by knocking down MyoD or NKX1; automatically, the position control genes will also be affected. Means that when you make a muscle a non-muscle or reduce its identity, the position control gene will also be lost equally. So what you learn from here is that the quality of the muscle you are referring to decides what the quality of the PCG expressed in that muscle should be.

These animals displayed a patterned phenotype. That means they have difficulty in forming a proper pattern. Generating extra eyes because you don't know if there are positional control eyes. Position control genes specific to eyes should be expressed in a given muscle. Now you have gotten rid of the quality of the muscle.

They are expressed everywhere, here and there. And then the eye will also form here and there. So there is no specificity. Further supporting the model that muscle has an

instructive role, it is telling in regulating the adult planarian body plan. Since the muscles are static in nature, they are the best candidates to harbor this position control gene, which means position control protein.

They are the ones that provide guidance to the migrating neoblast cells. So the muscle rapidly regenerates the pattern of positional information. So whenever there is damage, muscle should restore immediately and start expressing the PCG for the migrating neoblast to form a lost organ in that spot. How positional information regenerates its pattern is a central problem in regeneration. Now if a part is disappeared like head is chopped off, now you have is a trunk.

Now, where is the muscle? There are no muscles. It has to grow from the blastema. So the first line of formation has to be the muscle, not the organ, and this muscle will start expressing the PCGs, and these PCGs will guide the migrating neoblasts, which are coming from the rest of the body or from the remaining trunk. The pattern of PCG expression in the muscle rapidly regenerates after an injury. So the PCG expression is very critical in every muscle that is formed in the newly forming blastema or the regenerating tissue structure.

Beginning between 24 and 48 hours after amputation, a tail fragment expresses numerous anterior PCG. Remember, the fragment is the tail. So, since the anterior portion has been removed, the leftover, that is, the posterior part, now starts expressing PCGs responsible for anterior identity. Why? Because these cells will now move into the anterior region, the future anterior region. And it has got the posterior expresses, and it will start expressing numerous anterior PCGs at the anterior-facing wound because they are just waiting to jump into the lost region or lost part, just waiting for, and the posterior PCGs restrict their expression most posteriorly.

So, from the existing, say for example, a planaria, let us think that one planaria was 10 centimeters—I'm exaggerating—10 centimeters, and you cut off right in the middle; one half is removed. So now, the remaining posterior body fragment has got only five centimeters now. This 5-centimeter section, which is only midway and posterior to anterior, is lost until the midway point. Now, this anteriormost part of the 5-centimeter remaining fragment will start expressing the maximum of the anterior-specific position control gene, and the posterior-specific position control gene will be extremely posterior. Otherwise, they can be somewhere in the midway, maybe in the upper two-thirds; it can be there.

Because when you consider a 10-centimeter worm, maybe some posterior, most some three centimeters also posterior only, but now you have only a five-centimeter fragment,

then the posterior-specific region or posterior-specific position control gene will be expressed more posteriorly than it was in the full-fledged animal when it was there. So then there is a relative. It is just like saying that if there is a lion and a hyena, they will fight. That will be a very serious fight.

But there are no lions. Then hyenas will fight among themselves. Right? So to know who the boss is, something like that is also happening in the case of these positive control genes. When a fragment is small, they start distributing there. You know, this is my place; this is your place. So accordingly, the polarity is restored with respect to PCG in the existing fragment, not in the regenerating fragment.

Opposite process happens in an amputated head fragment. So now you are talking about the posterior fragment. What happened about the anterior fragment? The posterior end will start expressing the maximum posterior-specific gene, and the anterior-specific gene will be at the anteriormost end of the anterior half. Like I told you 10 centimeter you cut into two pieces. The rapid regeneration of PCG expression domains as an upstream regulatory step is a central pillar of planarian regeneration.

So the PCG expression is the backbone of the actual regenerative event. Wound signaling, as we have seen in the previous class, promotes the regeneration of positional information in the muscle. Two key molecules are Wnt and Notum; they are, you know, opposites. Wnt is a pro-Wnt signaling molecule, while Notum is the anti-Wnt signaling molecule. Both are activated by wound signaling and contribute to the resetting of positional information, one of the early steps of regeneration.

position control gene expression. Wnt is activated generically at all wounds by six hours post-injury. Wnt is a sudden release, like we have seen; sometimes these cells will rupture, as in the case of Hydra, and they will release a burst of Wnt ligand on the side. Wnt RNA can cause posterior-facing wounds to regenerate ectopic heads or tails, indicating that Wnt promotes posterior and inhibits anterior identity, which means head formation is not welcome, but wherever there is a wound, Wnt is expressed, so someone has to control it; thus, irradiated amputated head fragments lacking neoblasts cannot make. Posterior pole, and yet they are capable of initiating the expression of some of the PCGs, such as WntP2. This is one of the Wnt-related molecules; WntP2 expression starts.

Wnt RNAi head fragments, however, could not activate WntP2. Okay, head fragments are unable to make it now after amputation at the posterior facing wounds. Demonstrating the role of wound-induced Wnt 1 in the process of resetting the posterior positional information or identity. If Wnt 1 is generically activated, generically activated means irrespective of the place or the location of the wound; irrespective of that, Wnt 1 is

activated by wounding and promotes tail regeneration. How do anterior-facing wounds regenerate heads? Because anterior-facing wounds should not have Wnt, as Wnt is not welcome for head formation. But how the head is being formed is a very interesting question, right? The answer is, at least in part, that it involves the expression of a gene called NOTUM, N-O-T-U-M.

Whereas Wnt1 is expressed in the posterior pole in uninjured animals, NOTUM is expressed oppositely. That means its expression is greater in the anterior end, the anterior pole at the head tip, which antagonizes the Wnt signal. After amputation, unlike Wnt 1, NOTAM displays preferential expression. Wnt, I told you, wherever there is a wound, Wnt is expressed.

But NOTAMs are not like that. NOTAM has some positional identity. Preferential expression at the anterior-facing wounds. Anterior-facing wounds, when expressed in a specific manner, will antagonize the Wnt. So, it doesn't matter whether the Wnt is expressed somewhere or not; notum will make sure that the Wnt is not going to do its job.

Hence, a head can form. NOTAM RNAi. If you get rid of NOTAM RNAi, animals regenerate tails instead of heads. That means Wnt is activated. Wnt is activated for no reason. Wherever there is a wound.

It means it is waiting for Wnt signaling to kickstart. But if NOTAM is not there to antagonize it, you end up getting a default tail instead of a head. Because for the head to form, you don't want Wnt signaling. For a tail to form, you want Wnt signaling. And the absence of NOTAM ensures there is Wnt signaling.

Why? Wnt is induced everywhere shortly after injury. Indicating that NOTAM promotes head identity and inhibits tail identity. That is why you have to knock down NOTAMs because NOTAMs are antagonistic to Wnt signaling. So Wnt signaling at the wound site, Wnt, and NOTAM are part of a generic wound response. Induction of Wnt 1 and NOTAM results in a high-Wnt signaling environment at the posterior-facing wound. Not in the anterior-facing wound because in the anterior-facing wound, you have got a NOTAM.

And Wnt inhibits the environment at the anterior-facing wounds because of the presence of NOTAM. Not because of the absence of Wnt. and that this promotes the initiation of the changes in the PCG expression that ensue. So the Wnt signaling is contributing because the Wnt, along with the TCF, binds to the DNA and turns on some gene expression, and these genes are nothing but PCGs that control tail formation or head formation.

Beta-catenin is required for maintaining the posterior PCG pattern. Beta-catenin has to be stabilized to maintain the posterior identity with the help of PCGs, respective PCGs, and to prevent the anterior PCG pattern from being expressed in the tail. You don't want your head in the tail region. So that is taken care of by the maintenance of PCGs, which is facilitated by active Wnt signaling at the posterior end. An estimated 200 or more genes are transcriptionally activated within the first 12 hours following injury in planarians, regardless of the wound type.

It doesn't matter what kind of wound you give. Soon after the injury, around 200 or more genes are turned on. That shows how important the position control genes are. The induced genes fall into specific categories, such as those activated within one hour of injury, which include stress response genes and are expressed in most of the tissues, and genes in a second category. are preferentially upregulated in specific tissues.

Some are generic gene expression events and stress response events. Some of them are upregulated only in certain tissues. Muscles can have the PCG expression in them. Muscle epidermis and neoblasts are some of these specialized tissues that specifically express some genes. Wound-induced genes, which means genes that are expressed because of the presence of a wound within 12 hours post-amputation with asymmetric activation at anterior versus posterior facing wounds, yielded only one dominant gene, which is NOTUM. So NOTUM plays a very pivotal role in determining the anterior identity of a planarian.

Wound-induced positional information and reestablishment are required for its regeneration. The positional information or PCG expression must be restored as early as possible for the reestablishment of the lost tissue structure. One muscle wound-induced gene, called Follistatin, is required for the missing tissue response. The previous class, we saw that whenever there is a wound, the neoblast will start proliferating; that doesn't happen when you just pluck off one eye; it will run the show with the existing neoblast. So how is this regulated? Who goes and tells that a tissue is damaged? So, folistatin is required for.

eliciting the missing tissue response. Follistatin RNAi can result in failed regeneration. That means there are no monitors. It's like a supervisor. If there is no supervisor, you can't expect anyone to do the job properly. So, supervisor, although not doing the work, ensures that it is done the way it should be done.

So, Follistatin RNAi results in failed regeneration. Follistatin RNAi tail fragments fail to reestablish the AP pattern. That means if a tail fragment is already there, the head should

be formed, but the anterior-posterior pattern is lost because Follistatin is not present. So, the supervisor is not there. You will not get this anteroposterior pattern. The AP pattern of PCG expression accordingly never regenerates missing cell types if Follistatin is knocked down.

Instead, amputated fragments endure maintaining with turnover of tissues they do have for some time because animal has to eat. If the pharynx is gone and the brain is gone, it will not be able to make a living. But the existing pattern is restored, which means holistatin is not needed for running the show or maintaining it as it is, but it is needed for regeneration to happen in planaria. Extracellular signal-regulated kinase, also known as ERK.

Phosphorylation is triggered within minutes of the injury. Soon after a cut, within minutes it will be turned on, and pharmacological inhibition of ERK activation blocks regeneration and multiple aspects of the missing tissue response. Missing tissue response is present in Follistatin. Follistatin governs. It tells the animals what has happened. If Follistatin is blocked and the supervisor is missing, you will not get a proper PCG expression.

But in the same way, if ERK activation is also inhibited, you will not get either PCG or regeneration. So, ERK signaling is required for wound-induced expression of many genes, such as Runt1, Wnt1, and Notum. So, ERK signaling decides where Wnt should be expressed, where Notum should be expressed, and where genes like Runt should be expressed, indicating that ERK acts upstream of wound-induced gene expression essential for regeneration. So these are all wound-induced gene expressions, but they are in turn regulated via ERK signaling. A failed regeneration in the presence of an inhibitor of ERK activation; alleviation of ERK inhibition does not allow regeneration to occur.

So what you get if you prevent ERK activation is that you don't get regeneration, but the alleviation of ERK inhibition. First you inhibited, then you de-inhibited. Inhibition is removed; the inhibition is withdrawn.

That is called alleviation. Does not allow regeneration to occur. So ERK inhibition. or ERK activation. These are controlled in a timely manner. So, failed regeneration is the result of the presence of an ERK inhibitor, or ERK activation is prevented. You will not get proper regeneration.

However, the reentry of such animals induces a generic wound response. Say a wound was there; you block the ERK signaling, and the wound is healed somehow. you have no regeneration. But if you want them to regenerate, you have to make a fresh wound. That

means wound response is must.

Induces regeneration, supporting the view that the generic wound response is important. For example, say you have a finger; it is cut, and a fragment is lost. It is healed now. Now, if you want that finger to grow back, whatever cocktail of gene expression you know, you need to create a fresh wound.

In planaria, a healed wound cannot regenerate. You need a fresh wound. Only then can it think of regeneration. In that situation, if you allow ERK signaling to continue, then you will achieve proper regeneration. So muscles are essential to memory. Wound-induced Notum and Follistatin expression occurs specifically in the longitudinal muscle fibers.

MyoD RNAi led to a progressive specific loss of longitudinal fibers. Consequently, Notum and Follistatin were not induced at wounds in myoD RNAi animals because myoD is not there; muscle lost its identity, and hence you will lose the expression of Notum and Follistatin, and these animals fail to regenerate. MyoD RNAi animals, like Follistatin RNAi animals, were incapable of PCG re-establishment. after amputation and it was therefore proposed that these amputated animals could maintain whatever tissue they did have but failed to regenerate any missing tissue because they didn't know anything was missing. No one was there to monitor the missing parts.

These findings support the model that wound-induced PCG reestablishment is required for regeneration. So we will study more about this gene expression in the next class. Thank you. Thank you.