Regeneration Biology
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W3L12_Planaria regeneration: Neoblasts and organ formation & Species type and environment.

Hello everyone, welcome back to another session of regenerative biology. We will continue with planaria regeneration. However, I have combined this lecture and the next lecture together simply because both the contents are gelling well, and it is enough for one lecture. For this reason, there is one more reason: the position control genes require more elaboration, so that may not happen in one lecture; hence, that may be split into two parts. So the number of lectures will remain constant, so we will now get into planaria regeneration.

The topic is twofold: one is neoblasts in organ formation, which we have kind of seen, and we will get into some more refreshing ideas, and then the species type and the environment and how it is affecting the regenerative ability, because, like I told you in the beginning. Every planaria does not have the ability to regenerate, not just because it's a planaria; any given planaria species will regenerate. That does not happen, so different neoblasts we have seen, like the c neoblasts, mean clonogenic neoblasts are the only ones capable of restoring the whole animal. In the 1750 rads irradiation experiment, we have seen that sometimes if you transplant one neoblast, it does not restore the full animal, which means there is something wrong with that neoblast, so we will see what's wrong with it.

Is there any functional heterogeneity between neoblasts? Single-cell molecular analysis identified a functionally heterogeneous population of neoblasts, which is mainly done by single-cell RNA sequencing or absolute difference in neoblasts, to try to understand their transcriptome. From systematic single-cell multiplexed qPCR analysis, neoblasts were separated into two major categories with distinct gene expression, and they are categorized into Sigma and Zeta. So these are all the broad categories. Zeta neoblasts are specialized for the planarian epidermis and are the major specialized neoblast class. This includes Zeta neoblast and the Sigma class.

Sigma class is heterogeneous but capable of generating Zeta neoblasts as well. This means we can call Sigma a multipotent, whereas Zeta is more focused on the epidermis. So this is one kind of classification. And then there is another category called gamma

neoblasts. They represent another abundant neoblast class identified by the multiplex qPCR data, and they are the progenitors for the intestine.

They are focused more on generating the intestine, so these are all the broad categorizations of this neoblast. Thousands of neoblasts have been subjected to single-cell sequencing (SCS); SCS means single-cell sequencing. From this work, the transcriptomes of Many classes of specialized neoblasts were revealed. These are all broad classifications, like I told you: zeta, sigma, gamma, etc. However, if you look closely, you will realize that there is an abundance of transcripts that show variation among the neoblasts, which makes them common.

Categorizable into more than three, although we categorized into three, it can be more than three, and these include they were revealed including specialized in neoblast for proton nephridia, which are secretory cells, muscles, intestine, skin, nervous system, parenchymal cells, and other cell types; we know more than 30 cell types are there in planaria, so that means close to 30, you may have neoblasts because each tissue has to have its own stem cell, which is nothing but neoblasts in the case of planaria. The specialized neoblast classes are examined in depth, and they also exist in uninjured animals. We know that uninjured animals, in which every organ and every tissue is constantly regenerating without damage, mean that every organ has a fixed lifespan in the case of planaria. Hence, they need to be rejuvenated, and who will rejuvenate them? Naturally, they need to have a Tissue or a cell type-specific neoblast is available so that it will constantly keep pushing that organ. The emerging picture from this now large set of observations is that the consensus is that the neoblast includes pluripotent stem cells and numerous distinct specialized neoblasts, which means there are pluripotent cells that we call c neoblasts.

Along with them, there are plenty of tissue-committed or lineage-committed neoblasts; they are also neoblasts, but they cannot give rise to any cell type; rather, they can give rise to one or, at maximum, two tissue types. Those neoblasts are present; they are kind of lineage-committed, perhaps. One for every cell type of the body, so if there are 30 cell types, there are possibly 30 different types of neoblasts that exist in planaria. With this, we will move on to exploring different planaria types. Do the species decide their regenerative capacity? This is an interesting question and a very important question.

Planaria can be classified into Based on their habitat, some live in running water, some live in stagnant water, etc., and just like that, there are so many freshwater fishes; some are river fishes, and some are lake fishes, so there are differences. A lake fish may not live in a river; in the same way, planaria also have different statuses depending on the environment in which they are living. Like you can see here, there are plenty of planaria

in this picture of live images of a platform that were characterized and had been shown to be planarians. As you can see here in this panel B, the planarians belong to the order Tricladida and the suborder Continenticola.

That is not important; their classification, from left to right, shows that there are so many species that have been listed: first row, second row, third row, and fourth row; that information is given here. In the third row, Schmidtea nova, Schmidtea asexual strain, Dugesia—so many species are there. Remember, we were discussing Schmidtea, the Mediterranean one. and that we call it SMED and the other is Dugesia japonica. These are all widely used ones, but there are plenty of other planaria species also available in nature, and people do use them.

Not to study the regenerative ability but to understand why and how a given species does not have the regenerative ability. That is also equally important, right? so you can see this is basically a cartoon of the serial head regeneration assay so head regeneration assay means what every fragment in the body anterior to posterior if you cut it it should be able to regenerate the head right if it is head is removed head will come from that piece now the second fragment that is what the assay they have cut the planaria into six pieces piece number one is head itself piece number two is just below the maybe you can think as neck piece number three is somewhat its chest region piece number four is somewhat the belly region piece number five is somewhat lower belly region piece number six is towards its anal region there is no more piece like that Six pieces are being done, and the assay says that each piece can make the head. That means the sixth number, the bottom-most piece, should also be able to make the head; then only will it regenerate. So can the second piece make the head? Can the third piece make the head? Can the fourth piece make the head? Can even the sixth piece make the head? The sixth piece is also able to make the head. It is a perfect planarian.

So this is what you see here. In the Smed, which you know, Schmidtea mediterranea is one of the most widely used regenerating species. You can see the first piece here. Head is formed.

Second piece. First piece naturally head will form. So it's not a. In every species, it will form. So it's not a serious issue. So Smetia let us look.

First piece. Head is formed. And the second piece. 19 out of 19. The second piece also formed. Third segment 19 out of 19 formed the fourth piece 19 out of 19 formed the head, and so you can see all the way up to the sixth piece.

Each piece, no matter what, even the posterior most segment, also has the ability to form

the head, so this is called head regeneration. Now you see Cura pinguis, another species of planaria, if you see. First piece, naturally it forms; second piece, 14 out of 14 formed; third piece, 8 out of 9 only formed the head, which means one did not form. When you come to the fifth piece, fourth piece, 11 out of 12 it formed, then four out of five, then five out of five. So this species has some wobbling, but it is okay; it is forming.

Now, the interesting thing is this third species mentioned here, which is Sarsaira hastata, that is the species name. it head first piece if you remove the head head will be formed second piece 15 out of 19 only immediately after below the neck that second piece have the ability only 15 out of 19 third piece 17 out of 17 formed but fourth fifth sixth have no ability that means the anterior half can produce the head but below the belly region or below the pharynx region it cannot form the head at all that means Partial regenerative ability. Now, if you see another Dugesia species, you also know that Dugesia japonica has excellent regenerative ability: 15 out of 15, 3 out of 5, 17 out of 19, 11 out of 19, and 15 out of 19. They had the ability. Now, the most interesting species is the last one, which is Bdellura candida.

This first piece head if you remove that is not an assay at all that is why it is been given zero because they can regenerate head is removed but the second piece dead third piece dead fourth piece dead fifth piece dead sixth piece dead that means. It absolutely lacked the ability to regenerate. This is what you would say. It cannot regenerate at all. It's a cartoon of a serial section assay.

You can go through it as needed. I have already described. So this text could be read. The assay is simple. Any fragment, anterior-posterior fragment cut into six pieces.

Can they regenerate their head? So this is the question. And some species do not have the ability. Some have partial abilities. It makes the anterior half. Now, if you inhibit the Wnt, Wnt inhibition rescues all head regeneration defects.

The answer is clear: if you inhibit the Wnt signaling, if it is inhibited, the problem is solved. To experimentally modulate Wnt pathway activity, established RNA-mediated genetic interference feeding protocols were used to target the respective species-specific beta-catenin or APC, another gene like GSK3 beta we were discussing. Like that, there is beta-catenin RNA, Wnt RNA, and APC is another part of this destruction complex. What you call beta-catenin will be degraded, which is APC (adenomatous polyposis coli), and one of the mutations to this APC gene is one of the causes of colon cancer.

So it is a negative regulator. So normally, if APC is present, beta-catenin will be degraded in a normal scenario. But if APC is mutated, beta-catenin will be stabilized. It

will go to the nucleus. Wnt signaling will be on. So, the feeding protocol can easily be given RNAi RNA interference, which will target the RNA in the respective RNA because RNA is sequence-specific.

This protocol has been done, and you can knock down either the beta-catenin gene or the APC gene. Beta-catenin will do what it does; if you get rid of beta-catenin, you will not have Wnt signaling, and if you get rid of APC, you will be activating Wnt signaling. Interestingly, beta-catenin RNA rescued head regeneration or promoted the appearance of head-like structures in almost all planetary species, including the last one we saw in the previous slide. the last one, which absolutely lacks a regenerative ability. This observation is independent of the evolutionary history of the specific lineages.

In evolution, it does not have the ability, but you can make it or tweak it just by blocking the Wnt signaling or inhibiting Wnt signaling. Head regeneration rescue assay upon Wnt inhibition using beta-catenin RNAi in the indicated progeny B or C species. In any species, irrespective of whichever species. So you can see beta-catenin RNAi here.

Normally, it would be 9 out of 9. No head will be formed. In here the head is formed. And here another species is also formed. Another species with at least a head-like structure is formed.

In another species, two heads are formed. And in another species, the head is formed again. Species are not important. The message is important because two groups have been completed here. One is group B and group C, in which they have followed different feeding regimes where they have given the RNAi to eat so they can have differential downregulation of genes of interest.

Here they have knocked down beta-catenin. And so what they found is that so post amputation they will feed them and then they cut it so when they feed it in the body the gene is knocked down and then you are cutting so chances of it appearing is minimal because once you cut the head it will not have the stimulus to eat so you have to feed beforehand and then cut it this is what is usually done and this will lead to This will lead to the beta-catenin RNA producing head. So in the indicated categories B and C, both classes, depending on the species, were able to restore the head that is formed back. The number pairs represent the observed frequency of the shown prototype, as it is mentioned how many out of 17; out of 17 means that many individuals, while 4 out of 7 means only 4 out of a total of 7 individuals. It could get, in any case, the ability to get a head formed from that species, which is something very remarkable. And the Wnt signaling is something that is made accountable for this lack of regeneration.

If you block the Wnt signaling, then the head appears, so there is now a difference in regeneration from an adaptation point of view. This is something that you should think about. Although it is a planaria, what advantage does this animal have, whether it is regenerating or not regenerating? What advantage this is something worth pursuing or thinking about because every regeneration scenario has an adaptive or evolutionary significance, like I told you when we studied liver regeneration. Also, if the liver is not able to regenerate, the organism is non-existent; there is no other organ that can take care of the function of the liver. Not only that, the liver automatically gets damaged; it is just like if you walk more, your.

.. The bottoms of your feet will have more wear and tear, just like your shoes, if you walk more; your shoes will tear more. So, if you are in a very rough environment, say if you are traveling by bike with your skin exposed, there is always more wear and tear on your skin than on a person who is walking because traveling creates a lot of air currents that rub against your skin. This logic applies to every organ, so the liver, being a detoxifying organ, can be damaged. Now the question in evolution is, if the liver was not able to regenerate, no organisms could restore their liver back. So after a few days, the animal will be liverless; hence, lifeless.

So every property, especially those connected with regeneration, has an evolutionary connection. So that is why mammals do not regenerate any complicated organ, but they do regenerate. Liver same the poor or restricted regeneration species tend to occur in stable habitats; stable habitats mean not running water; for example, lakes or the sea. The sea is stable in the sense that there is less movement of bulk water.

There is stagnation there. While robustly regenerating, the fissiparas strain. Fissiparas means something that propagates by fission. That is fissiparous. Strains or species tend to occur in less stable habitats.

Less stable means dynamic and unpredictable habitats. For example, fast-flowing streams or temporary water bodies. Temporary water bodies can dry up. So extreme conditions. Like there is water in a lake, it will be there throughout the year. In the sea or a larger body of water, there is water available.

But whereas temporary water bodies, it is vulnerable to desiccation. In running water, many rivers will have a lot of variation in water levels during the rainy season, or the water level will change. Accordingly, the animal has to get adjusted and not just adjusted; otherwise, the animal will be vulnerable to body damage. When the water or the environment is dynamic. So all fissiparous strains tend to occur in less stable habitats like fast-running water, etc.

These observations were consistent with the selection of robust whole-body regeneration as part of fissiparous reproduction because, if it is broken into pieces, nothing goes to waste. Each piece will become a new organism. So this is beauty. which may become dispensable in habitats that favor egg-laying species. So we should understand the egg-laying species; if they have the ability, then they don't have the ability to do Fisiparas regeneration.

It is okay. There is nothing serious about it. Fisiparas strain must have an exemption from egg-laying reproduction. But you think about a species that does not have the ability to do Fisiparas regeneration; it must have a strong egg-laying capacity. Then only will the species go. So let us see, do we have any evidence? In Smed planaria, unlike the more commonly studied Fisipa asexual strain, adults of the sexual strain reproduce by depositing large egg capsules and develop a hermaphroditic reproductive system including testes, ovaries, and yolk glands, which is seen in Smed because it has larger eggs. Interestingly, Wnt signaling levels were consistently higher in the sexual strain and also in the office RNAi, which is a GPCR (G protein-coupled receptor) essential for sperm and egg production.

So, if office RNAi is present, then it doesn't have the RNAi. Therefore, if you give office RNAi, it reduces the beta-catenin 1 levels to the asexual-like levels, which means if you block. If you have got office rni, the beta-catenin level Wnt signaling is compromised, which indicates substantial canonical Wnt signaling activity; in association with that, it means it is favoring a situation where the Wnt signaling is compromised, because of which regeneration can happen in them. Wnt signals activity in association with the Smed reproductive system. I'll go through it once again. In wnt signaling, levels were consistently higher in the sexual strain.

Means of sexual strain do not bother to reproduce asexually. Same species. Smet species can have a hermaphroditic system, some of which favor sexual reproduction and some of which favor asexual reproduction. I am talking about the strain which has higher levels of reproductive capacity or sexual reproductive strain. Then another condition is where you knock down the office, office RNAi, if it is happening. Then, because office is a GPCR essential for sperm and egg production, if you get rid of office, then naturally it is not a sexual strain, but by default it is a sexual strain reduced beta-catenin level to that of the asexual strain.

Asexual strain naturally will not go for sexual reproduction, although it's the same species; the levels go lower, because of which now it doesn't care. To do egg and sperm and who do a sexual reproduction, rather it will go for a fissiparous reproductive cycle

with wnt signaling activity in association with the reproductive system; the expression of both yolk and shell gland markers was practically undetectable under beta-catenin RNA where the Wnt signaling is low, which means the animal has no plan of doing any. Sexual reproduction occurs because the wnt signaling is low, and it is fine for the animal because it can use a fissiparous reproductive system; however, it is denser in the case of APC RNAi in sexually reproducing smeds. For example, if a smed that was sexually reproducing had a proper thickness.

.. Yolk, etc. But the APC RNAi is a negative regulator of Wnt signaling; if you have it, then it will favor the wnt signaling. What will happen is the opposite of beta-catenin RNA will occur. So, if you tweak the wnt signaling with beta-catenin RNAi, which will negatively regulate wnt signaling, or accelerate the wnt signaling with APC RNAi, you get an extreme result if you remove the beta-catenin signaling. Then the animal can automatically reproduce asexually. But if you favor it, accelerate the wnt signaling, because APC is one of the strong negative regulators of wnt signaling.

But if you block that one, then that degradation do not happen. Beta-catenin degradation does not happen. And then it will favor the Wnt signaling very actively. Now, if you look closely at the wnt signaling and regeneration in reproduction, this cartoon should be seen closely because you can see three phases. One is molecular mechanisms, reproductive strategy, and the environment. So, in terms of molecular mechanisms, we can see how beta-catenin is influencing head regeneration.

We know that if Wnt signaling is blocked, then head regeneration can happen perfectly if there is no Wnt signaling actively occurring; now, what happens becausenaling, where sexual reproduction is favored, whereas active Wnt of this Wnt sig signaling favors sexual reproduction. It favors the same Wnt signaling, which favors better production of sex organs and better production of gametes, because the same Wnt signaling, when it is absent, favors asexual reproduction or head regeneration. Same Wnt signaling, if it is present, favors sexual reproduction or gamete production. So in either case, if Wnt signaling is low, asexual reproduction is favored.

If Wnt signaling is high, sexual reproduction is favored. Now, when you look into the reproductive strategy, It can either go into a fissiparous condition or an egg-laying condition. Now, what is the influence that is happening? In fissiparous, as I told you, it can decide based on the Wnt signaling level. In the same way as egg laying, based on the Wnt signaling, it can decide whether it should go for a sexual or asexual mode of reproduction. Now, the environment and the creeks. If it is a creek, the fissiparous animal should retain its fissiparous lifestyle.

It cannot afford to switch to sexual reproduction. Another complexity is sexual reproduction. It also needs to find a breeding partner. In turbulent water, it won't be able to find them. Hence, sexual reproduction is the only way that the fissiparous condition can be maintained. Whereas in egg-laying species, it can have either stagnant water or larger rivers where it can find local spots, or another option is that it can have a marine environment.

In this condition, what will happen is based on the availability of species, which means both genders of the species should be available or a hermaphrodite. Hermaphrodites can make both gametes, eggs and sperm they can produce. But sexual reproduction is not favored. In rare cases, it will self-fertilize. But usually when egg laying has to be favored, it must have stagnant water and the marine environment must be present.

Now this extra arrow indicates what the other conditions are in which it is going towards further adaptation, so you can see the same strain of SMED that can have both asexual and sexual reproduction. If you tweak the Wnt signaling, it can think about whether it should stick with an asexual mode or a sexual mode. All we need is to reduce the Wnt signaling; then it can form a proper head, even in those species that do not have the ability to regenerate, but they have the ability to reproduce sexually. The same way in the same strain, this strain is important because if you use another strain, it will be like comparing apples to oranges; the same strain has an affinity for sexual reproduction, but the activated Wnt signaling, like by APC knockdown, allows them to have a better gamut, thickened eggshells, and bulky sperm that will be formed so that they will have a better chance of survival.

Through sexual reproduction, but they lack their asexual reproductive ability. We will study more about planaria regeneration in the next class. Thank you.