Regeneration Biology
Rajesh Ramachandran
Department of Biological Sciences
IISER Mohali
Week: 2
Lecture: 10

W2L10_Mechanisms of regeneration in Planaria

Hello, everyone. Welcome back to another session of regenerative biology. So this time we will start with a new model, which is planaria. Many of you may have heard about planaria since your school days. Planaria is a very interesting invertebrate that comes under the group flatworm, and it has numerous regenerative capabilities. So planarian regeneration is very old information for mankind, as old as Hydra.

Interestingly, planarian regeneration has caught the attention of many for decades or even centuries, to differing degrees and by different investigators. Many of you would have heard about Michael Faraday, who is the father of electricity and is known for his electrochemical research; he was interested in planaria research and published a paper. In 1833, Morgan, the father of all this genetics and the mutational studies, and T.H.

Morgan also contributed significantly to the studies of planaria using its regeneration; all you need is a blade, a magnifying glass, and the imagination to do an experiment. That's all that was needed: raw material, nothing more. These are all the papers that are cited here, published between 1833 and 1898. You can see the dates; you can imagine how old that research is. A few directives we need to know about planaria regeneration.

Planaria are blessed with the presence of numerous pluripotent stem cells known as c-neoblasts. "C" basically stands for clonogenic; that is the terminology for c-neoblast, which is the name of those stem cells. Roughly around 30 percent of the planarian cell mass is made up of these neoblast cells. There is another category called fate-specified stem cells, which are specialized neoblasts. Specialized neoblasts provide the cellular basis for regeneration in planaria, with positional information harbored in the muscle.

This is important information to know: if planaria lose a part of their body, they restore it purely because of the positional information. In a strict sense, you can say you know where your nose is, where your eye is, where your mouth is, and where your hands are; these are all positional information. You will not see a hand coming out of your forehead, nor will you see a nose coming out of your chest. Because these are all because of positional identity genes that are established right from embryonic development, that is why the eyes are in the place of the eye, the head is in the place of the head, and the tail is

in the place of the tail. Okay, so this information you should keep with you: the information that is gathered.

Different positions and their locations determine what part is formed. How the combination of positional information and its influence on this neoblast contributes to the regeneration. We will study this. And how progenitor targeting by extrinsic use and self-organization can combine to determine where the regenerative progenitors go. So these are all things that we need to address in future classes.

So, in a simplistic sense, you can see this as a picture. It's a cartoon of a planarian. You can see different types of amputations can be performed. What you see in this blue-colored animal is that it is irradiated. What you don't see is the blue color; as you can see here, this dotted pattern is normal.

So in a normal planaria, you chopped off the head and you can see that head is regenerated after some time. Whereas in an irradiated planaria, you chop off the head, new head doesn't form and eventually the leftover part of the body will disintegrate. That is what has been shown here. And now you take the planaria in the two halves, anterior half and posterior half. Anterior half you irradiate it nicely and then you chop off its head.

The anterior half also has the head. Okay. And then you chop off its head. Slowly, the head will regenerate as you can see here. So it tells you something if the posterior half has normal good cells; no radiation has been given.

All of you know it will cause DNA damage and cell death, so the posterior part did not receive any radiation; hence, the head could regenerate. That means something from the posterior healthy part moved into the dead area. Now comes the very interesting experiment with a normal planaria and a radiation-killed planaria. What you did was that radiation killed planaria, you know, they do not regenerate. I have seen it, and it will eventually die.

In the radiation-killed planaria, you took a small tissue piece from a normal donor planaria. and you implanted it and then you chopped off the head then it can restore the head and also it will restore the normal body what it tells you that something from this donor planaria which is nothing but did not receive a radiation that is the only difference it could contribute something so you can see here results in complete regeneration when you normal situation, it results in complete regeneration. But if you have heavy radiation, not only will the head not regenerate, but it will also disintegrate. On the other hand, that can be rescued by putting a small tissue from a donor. And this is something very interesting: this is an experiment done by Wolf and Lender in 1962.

And we learned that a healthy planaria is able to restore normalcy. Now, another interesting feature about the planaria is that you can cut between the eyes of the planaria. You can see that planaria has two eyes, as you can see here. Depending on which species you are talking about, you just cut between, starting from the tip of the.

.. head until the middle of the two eyes. All you need to do is just to cut it. You will end up getting two-headed planaria. It will not become two planarians. It will become two-headed planaria.

Again, if you cut it, you will get four-headed. And like that, you can get one, two, three, four, five, six, seven, eight, nine, ten-headed planaria you can see here. So you can keep cutting. It will keep making more and more of two heads. But the planaria remain the same.

Again, you chop off the body into a small slice, very small slice took from somewhere in the belly region. You end up having a head on either side. In the actual head region, you will also get a head, and in the posterior region, you will also get a head. So double-headed planaria can be obtained. Planaria can also reproduce in a very unique way similar to how bacteria reproduce; bacteria will reproduce by binary fission, while planaria can reproduce by something called paratomy.

Paratomy is shown in Planaria fusipara, and different planaria have been used for various experiments. You will see different species in a while. Before the fission at the level indicated by the arrow, the posterior part of the planarian had already begun to transform into a new individual. As you can see here, it was a proper planarian. Then it started developing a fission, just like how binary fission happens in bacteria.

And then it started developing two eyes, among other things. Finally, this will pinch off, and you will end up getting a new planaria. So planaria use a stem cell-mediated regeneration approach. You can see here that this is a way of asexual reproduction where a planaria can be split into two, just like how if someone gives you a stick, you can start to cut it longitudinally, and it will become two pieces. In the same way, the planaria split into two, and each half will give rise to the last part, which will become two planaria.

Planarian flatworms reproduce by binary fission. So the previous picture you saw is cutting. It is cutting the top and bottom or anterior and posterior. Here you are cutting left and right. So splitting the body from top to bottom and regenerating the right and left halves.

The cells capable of doing this, pluripotent, are called pluripotent stem cells, known as neoblasts. And they are used for the repair and for the regeneration of the lost part. So from the year 1700, it is known that planarians cut in half can give rise to multiple pieces. It's one of the ancient pieces of knowledge.

Same way, T.H. Morgan and T.H. Morgan and Child, another scientist, realized that such polarity indicated an important principle in development. What does polarity mean? The head region has a head, and the tail region has a tail. This is polarity. You also have polarity in your body. You don't have a head in your base or near your leg.

You don't have a head. Your head is in the place where a head should be. Your legs are in the place where a leg is. That is polarity. So Morgan pointed out that when both the head and tail were cut off, the middle piece would always regenerate the head from the former anterior region and the tail from the former posterior region. It doesn't happen the other way around.

And never the reverse. If, however, the middle piece was too small, then the regenerating portions would be abnormal if they were too small. Based on these observations, they postulated that a gradient of anterior-producing material emanated from the head. The middle segment would be told what to generate, and then the two ends would be determined by the concentration gradient of these two materials. You can see here that this is cut into two pieces. The anterior piece made the posterior piece, and the posterior piece made the missing anterior piece.

And if you cut it into three pieces, you end up getting regeneration from this, which is understood. The middle portion created a new plan area. But if you make a small piece, you end up getting a head from both the sides. So if the piece is so small that the gradient is not worthy or powerful enough to distinguish which is anterior and which is posterior, you end up getting two heads. And you can see there are so many examples of planarian species.

You can see that there is no need to go through it, but the morphology shows their names are listed here, different morphologies are present, and some planaria do not have the ability to regenerate. Don't think that every planarian can regenerate. Some planarian species do not have the ability to regenerate. We'll see them shortly because this is an introductory class. A few more facts we need to know about planaria.

Planaria can grow and literally degrow. That means shrinking. It can shrink back due to the availability of food supply in the environment. Dependent on the adjustment of organismal turnover rates. Scaling the body plant proportions by as much as 50-fold. That

means a planaria that is 50 kilos in size.

I am giving an arbitrary number. No planaria is 50 kilos. It can be as small as 1 kilo. That is 50-fold shrinking. It is allowed based on availability, and it can bounce back just like the human liver; we discussed the liver, right? Its dynamic body architecture further allows astonishing regenerative abilities, including the regeneration of complete and preferably proportioned animals, even from tiny tissue remnants.

If you have a small tissue, it will assume. A shape of a small planaria, and then it grows back. Scientists use two species: Schmidtea mediterranea, which is called SMED planaria, and Dugesia japonica, which is called DJ. Both have excellent regenerative capabilities, and clonal strains originating from single animals are used in labs. They are clones because making a clone is very easy; just like animal tissue culture, take a planaria, cut it into three pieces, and you end up getting three. If you want more planaria, cut it into 10 pieces, and you'll get 10 animals.

It's like a meat piece that keeps growing, so you don't have any difficulty maintaining them in a clonal manner. Vegetative propagation is very easy. Planaria have an abundance of adult stem cells known as neoblasts. They are collectively referred to as neoblasts. Transplantation of a single neoblast into a lethally radiated planaria, as we saw in the previous slide, involves thoroughly irradiating the organism.

You end up getting disorganized or completely damaged planarians. It will not grow. It will not survive. But into that planaria, irradiated planaria, you transplant one cell from a healthy donor. The entire animal will come back, but that will be a clone of this donor, somewhat like you may have heard about how animal cloning is done.

They take the nucleus from a donor and put it in the recipient zygote; you end up getting a clone of that nucleus owner, something like that. In a stem cell depleted, irradiated planaria, you can put one neoblast from a donor. It will, the recipient, give rise to perfectly healthy animals of the donor genotype. Donor genotype means whose c-neoblast you took. This paper was published in Cell Stem Cell a few years ago, suggesting its totipotent status.

What does totipotency mean? If you can make one animal from one cell, that is called totipotency. However, understand that if you take a C neoblast and put it in a medium, it will not give rise to an animal. So what it tells you is that C neoblasts, although they are totipotent, need guidance from the extracellular matrix or positional information. Because of irradiation, nothing will happen to the proteins. They will stay there, and nothing will happen to the matrix.

DNA gets damaged and the cell will eventually die. So this is what you should keep in mind. A further unusual feature of planarian neoblasts is their high basal mitotic activity. They keep growing and keep dividing. So planaria tissue keeps on replacing.

If planaria has an eye, last month is a different eye. This month is a different experience. It has been completely revamped. It's just like, you know, you keep buying furniture for your home. Your furniture is staying in your house for only one month.

You threw it away and bought new furniture. Like that, planaria keep constantly doing their cells. So none of its organs are old. Every time it is new, credit goes to the senior blast. Remember, this happens at a steady state level.

No regeneration is needed. It constantly regenerates every organ. The cellular and molecular basis of planarian regeneration, if you look closely, involves the production of something called blastema; unlike in the liver, you don't see it in hydra, but in planaria, you see the formation of a group of cells. Disorganized, they don't have any tissue specificity, and that is called a blastema cell. We will see more about blastema in future classes because a small planarian body fragment cannot eat until suitable anatomy has been regenerated, including the pharynx and brain. What if the broken fragment of planaria doesn't have a mouth? It cannot eat, right? So there is no food supply, so until.

.. The mouth is developed; it has to continue to survive on the available resources in the form of glycogen, proteins, lipids, etc. In its body. So missing tissues cannot be regrown at their original scale. Missing tissues cannot be regrown to their original scale. Thus, it cannot be regrown at its original scale, but it will form in a miniature form.

The blastema thus typically only regenerates some of the missing tissues, such as the head, to start with. if a body is cut the anterior piece and posterior piece and posterior piece is having only half of the planaria body it will not create the rest of the anterior portion it will just create a head right in the tip of that posterior portion and then it will keep growing building the bulk this is the way in which they grow and some tissues will initially be over abundant in this case of repurposing and in the amputated fragment so sometimes when this repurposing is going on the amputated fragment will have somewhat more than Adequate or more than required tissue will be there, so they will undergo apoptosis, and sometimes a required tissue will be missing; then the existing tissue will take over the function of that tissue, and it will keep changing slightly for the time being, but it's okay simply because it has got c-neoblasts, and it will keep revamping it, and the proper cell will be replaced back. The position and relative position of the organs have to be maintained, and it will be taken care of by the position control proteins,

which we will see in future classes. The changes that are leading to mini planaria formation have been referred to as morphallaxis because it is done by repurposing, like we saw in the case of Hydra. Morphallaxis is largely the result of new cell production and cell loss.

rather than changes in the differentiated state of the cell. So they are, like I told you, repurposing, and that is forming a miniature planaria because the miniature planaria, if it has a mouth that is developed because of the repurposing of the tissue, can start eating. So this is the overall idea of restoring a miniature planarian to start with. So planaria can also regenerate missing tissues on their mediolateral angle. Medial-lateral angle means, for example, that you have a chest, you have a sternum right in the center of your body, and your right hand is at the medial-lateral angle. So your chest to your right hand is one medial-lateral angle, and your chest to your left hand is another medial-lateral angle.

So it can make organs that side also, not that it has to be cut horizontally or longitudinally. And it means it can have sagittal and parasagittal sections. So partial amputations can result in duplicated structures, like we saw in the ten-headed planaria. Partially cutting it results in getting two heads. A sagittal incision between the eyes, for instance, can result in the regeneration of two heads and you can keep repeating it.

But there is something interesting that will come. We'll discuss the removal of irregularly shaped tissue fragments. Say it ended up getting ten heads. Now what do you do? You did one amputation and got rid of all the ten heads. Can you get ten heads back? The answer is no, because the animal's blueprint says it has to have only one head.

So you ended up making ten head. You cut with one cut. You got rid of all ten heads. You end up getting only one head back. So irregularly shaped tissue fragments can result in regenerative intercalation of the missing anatomy. That is called intercalary regeneration. That means that regeneration happened because of the way of living, a time ago.

It is trying to adjust to the stress, and that results in regeneration. Sometimes you can make multiple eyes also just by amputating eye alone. But we will also see that in the coming classes. But what I'm saying is that you can create an abnormal development or regeneration in planaria. But once you get rid of all the abnormal regions, normalcy comes back.

Between the fused wound faces, you can end up with complicated structures. Similarly, one region of the body can be transplanted next to another, and regenerative responses can be triggered, including the formation of outgrowths that appear to intercalate missing

coordinates between juxtaposed tissues. We have some pictures so that you will know it's easy to understand. In this picture, you can see that the head is cut and the tail is cut. So naturally, from the fragment of piece one, the rest of the body is formed. The brown color brown color is the actual and the ash color is the newly formed part from the middle from the side part from the posterior part the remaining part will be the fragment to the head is formed back but most interesting thing is this third piece where it is taken a small piece and you can see here from this small piece it got head and left side of the body and also the bottom so this is something very interesting you can see what is mentioned blastema here that is the inner disorganized tissue that is formed immediately And it's panel B and regeneration from a fragment that results in a small animal that can eat but grow towards the original size.

You can see a small fragment, and it gave rise to a miniature organism, and a big fragment; it also gave rise to a miniature organism. Eventually, both of them will give rise to a proper organism. This is the most interesting part here: if you cut it, one small piece is removed. And then you have a big brown piece.

So the big brown piece made the small missing piece. Whereas this missing small piece created an entire normal organism. Proper-looking organism. It did not even have a pharynx.

You should remember. So the center portion is the pharynx for eating. And the mouth is not here. The head and eyes are here. But the pharynx is in the middle. The same way you cut it into two, you end up getting two-headed.

If you cut again here, you will end up getting four-headed. Both sides you cut it. Now another exciting part is that you have an anterior portion and a posterior portion, and if you got rid of this fairing spot, then it will selectively fuse; you took this anterior portion and the posterior portion and fused them together. This middle portion you removed, but you will end up getting this middle portion to grow back. So in this experiment, what they have done is take this head piece and this tail piece and fuse them; this much portion has been thrown out. But that portion grows back.

So the blueprint of the organism knows which portion to restore. So initially, it will do the tissue rearrangement and perform the morphallaxis, which is further favored by blastema formation and the growth of the proper head, tail, or pharynx, whichever part is missing. So the clonogenic neoblasts are basically the pluripotent stem cells that produce the missing cell types. So the planarian regeneration can involve new tissue production in the blastema. So the blastema will always have new tissue that will be produced and that has to differentiate as expected or as required. Because a small planarian body fragment

cannot eat until suitable anatomy, including the pharynx and brain, has been regenerated.

Regeneration must occur with existing resources. Otherwise, it will not happen. Blastema formation typically only regenerates some of the missing tissues, such as the head, and is coupled with changes in the pre-existing body regions for the regeneration of other missing tissues. I will explain once again that blastema formation typically only regenerates some of the missing tissue; although blastema has all the tissues capable of producing all the cells of the organism, it will not make all the tissues in the head region; it will make from that blastema, although it is a Totipotent cell mass will make only the head; it is coupled with changes in the pre-existing body regions, which will rearrange so that you will end up getting only the missing part. These changes have been referred to as morpheallaxis. The missing tissue can also respond with proliferative and cell death responses, so neoplasts respond to injuries by increasing their rate of proliferation.

Whenever there is an amputation, say, let us assume the... A given plan area has 1000 neoblasts, and they are dividing once every day, let us assume. So if there is an amputation now, these 1000 neoblasts, of course, would have come down because neoblasts are distributed throughout the body. They don't have any reservoirs. So if a 30% of the body is cut, this 1000 neoblasts will now come down to around 600+.

So instead of dividing once a day, they will divide once every hour. So it's the rate of proliferation that increases. Though the body, c-neoblasts, know whether an amputation occurred or not. So the neoblasts respond to injuries by increasing the rate of proliferation.

And that's not it. It should also reduce the apoptosis rate. An initial peak of the proliferative response is widespread. Occurs approximately 6 hours after the injury. And it is followed by the second phase of sustained proliferation near the wound. Occurring approximately 48 hours after the injury.

So, within 6 hours, it will respond with adequate proliferation. It is followed by a second phase of sustained proliferation near the wound site. Which led to the production of blastema. Blastema occurs only at the wound site.

And this happens in about 48 hours. This increased proliferation is occurring throughout the body. Now they will all kind of filter out, just like, you know, they will all move towards the tip, and they'll form a mass lump of cells that is called blastema by around 48 hours. The initial proliferative response occurs after any type of injury. Injury can involve amputation, removal of one eye, or removal of the pharynx, etc. Including injuries such as incisions that do not require substantial production of new tissues, sometimes when

you are injuring, like when you cut across, it knows that since you cut across two eyes, it will make the missing part; that is why you end up getting two-headed animals.

So here, the logic of the animal, maybe I will go back to that picture once again after completing this. By contrast, the second phase of proliferation only occurs at wounds that can result in substantial tissue loss and is referred to as missing tissue or a regenerative response. Keep this phrase in mind: missing tissue response or regenerative response. They are slightly different. And this response is associated with neoblast accumulation at the wound, which leads to the blastema.

Let us see this picture once more. If you cut the animal into two, as you can see here in this panel D, If you cut into two, the immediate response of this fragment, like you saw here, this piece, this tiny piece, what is this tiny piece was trying to make the entire organism like that this is cut only up to here you did not chop it off this piece immediate goal is to make the remaining missing piece it do not know it is there in the neighborhood it will not try to fuse it back so this left hand piece it made a proper head and the right hand piece made a proper head this can be continued for a prolonged amount of time that is what You see that morphallaxis ensures a rearrangement of the cells. However, in this kind of cutting, where you did not bring about a morphallaxis response because it has the proper head, you did not remove the head. But that local disruption was powerful enough to replace the missing part.

So, in this way, we kind of confused the animal. Whether it's a proper amputation. Actually, there was only a wound that needed healing. But the animal is so plastic or cNeoblasts are so determined that they made a proper head. So that is what we should understand. The response, by contrast, the proliferation can occur specifically in the missing part.

This will eventually lead to the proper formation of the structure when there is an amputation. Otherwise, it will simply create the missing part. We'll continue to learn about planarian regeneration in the next class. Thank you.