Regeneration Biology
Rajesh Ramachandran
Department of Biological Sciences
IISER Mohali
Week: 2
Lecture: 7

W2L7_Regeneration: lessons from animal model Hydra

Hello everyone, welcome back to another session of regeneration biology. Today we will learn about a new model called Hydra, which has been well explored and well studied in the field of regeneration biology. It is quite an interesting animal. Humans, even before they started doing research on any subject, say hundreds of years ago, especially with regard to biology, have observed that the Hydra is a very interesting animal that has the capacity to regenerate; thus, there are many mythological connections for those who follow Hercules, though there is a monster animal whose name is also Hydra. However, Hydra, in general, is a small animal, but a lot of research interest has gone into Hydra in the past several years. So let us learn how Hydra is a good model in the coming few slides.

So, as you can see here, regeneration in Hydra takes place using two technical methods, or two technical terms are used to describe the regeneration of Hydra, which are called morphallaxis and epimorphosis. So we will see more in detail about these two terms, but I can explain a little bit now. Morphallaxis is the ability to form an entire organism from a group of cells. You may have heard about plant tissue culture.

What do people do with plant tissue culture? They take a small piece of the plant tissue, put it in a medium, and you end up getting a new plant. Not that it's a large quantity of tissue. You will end up with a new plant. In the same way, if you take a hydra's small tissue and just put it in a petri dish, you will end up getting a miniature hydra. So this is morphallaxis.

But what is epimorphosis? Epimorphosis, in the simplistic sense, is replacing the damaged portion. If you lose one finger, you don't want the whole animal to come out of that area. If you lost a finger, you want that finger back. So this is called Morphallaxis. Okay, we have seen this different category in one of the earlier classes, but we will revisit this terminology as and when it arises.

So Hydra is a genus of Cnidarians found in freshwater, and most are roughly around half a centimeter. That means around 5 mm in length. And it has a tubular body, a cylindrical body, and a fan-like structure, as you can see in this picture around its head. And there is a head and a distal end at the extreme end of the body. And it consists of a conical hypostome.

The hypostome is nothing but its mouth. So around the mouth, they have a ring of tentacles So in water, these tentacles will be turning around like a fan, and they will create a

whirlpool, and the food particles that are floating will directly come into its mouth. So it's a simple procedure that Hydra will use for catching its prey. And it's a very small animal. And there is also a foot that is located at the bottom.

As you can see here, this is the foot region. There is a foot, and it's also known as the basal disc. At the proximal end, it is also known as the basal disc, and it helps the animals stick to a substratum. It's a rock or a solid wall, etc. Otherwise, it will fly off or float away with the water current.

And they have a diploblastic body. Diploblastic body means it has ectoderm and endoderm. There is no mesoderm. That means normally every organism has got ectoderm and endoderm to start with, and the mesoderm is formed because of an induction event; it is an intermediate tissue that is in the middle of the body, but they don't have a dedicated mesoderm; instead, they have a mesoderm-like structure. And they are usually called diploblastic.

If it has all three layers, we call it a triploblastic animal. But Hydra is a diploblastic animal with two germ layers, ectoderm and endoderm. Though they do not have a true mesoderm, they have secretory cells and stinging cells that are nematocytes. That means it can create a small amount of toxins to evade unwanted intruders. And gametes and neurons that are not part of either ectoderm or endoderm, but are located in between, are often referred to as mesoglia, not mesoderm, and these cells are present, but it's not a true mesoderm.

So, if you look closely, you can see that the Hydra has a unique phylogenetic position. As you can see here, starting from Porifera. Protozoa are single-celled organisms, and the next level is Porifera. And then it is classified into several pieces. That is, Ctenophora is present.

Bilateria is there, and then there is a hydrozoa to which the hydra belongs, as well as scyphozoa, cubozoa, anthozoa, etc. Anthozoa normally includes all jellyfish, and in the group, cnidaria, all members have nematocysts, which are their stinging cells. The hydrozoa includes the hydra animal. And here, you can see a cartoon of Hydra. It has a foot region, and soon after the foot region, it has a budding zone.

What is it that buds? New Hydra can reproduce asexually. And then it has a gastric region, and then it has a tentacular region. Right in the center of these tentacles, it has the hypostome. So this is a typical structure and morphology of the Hydra. If you take a cross-section, you can see it has a typical endoderm layer and a typical ectoderm layer, and it has tiny myofibrils, which are not dedicated muscles, but can make some tiny movements, and it has nothing but the mesoglea.

You have dedicated epithelial muscular cells that have sensory cells, and they do have some dedicated septate junctions; neurons are present, allowing them to sense the environment. They also have nematoblasts, interstitial cells, and nematocytes, which can produce toxins.

The glycocalyx also has cilia from different secretory gland cells, because whatever food it is eating inside has to be digested by the secretions coming from the endoderm cells. It is a very interesting and unique animal, as it has almost all the cell types required, and this is very important for us to understand when it comes to morphallaxis and epimorphic regeneration. If you look closely, you can see that the Hydra can reproduce sexually, but it does not usually reproduce sexually.

They always do the asexual reproduction that is called budding off. As you can see in this picture, this is a single Hydra, and a small miniature Hydra is coming from the surroundings. This is called budding. Hydra's body is not stable. As you can see in this picture, 8 days, 2 days, 20 days, and 4 days are written.

You may wonder what it means. It simply means that the cells of the body are constantly dividing and are displaced to the extremities of the column from which they are shed. So, extreme tip, the new cell is dumped off, and the new cell keeps proliferating and keeps replacing. Thus, the Hydra's body is always regenerating. You can tell in a realistic manner that Hydra's body is always regenerating.

Like our bodies, our skeletons also have a lifespan of around 7 years. If you are 7 years old, you are in the first version of your under 7 years, first version of skeleton. If you are 70 years old, you are on the 10th version of your skeleton. So your entire skeleton is revamped throughout the year. Hydra can produce every cell, so as you can see here, this is the hypostome area.

It takes 20 days to move a cell, which means the movement is limited. In this tentacle region, it takes four days from here to reach the extreme tip; it takes four days. In this area around the neck region, there is little or no cell movement because that is a place where the cells are confused: should I go upward or should I go downward? And here, somewhere in the middle region, say the chest region, if you compare it to that of a human, it takes around 8 days to reach all the way up to a budding region. Here, it takes around 2 days to reach all the way up here. And from this lower mid-region to the foot, it takes 20 days.

That means the rate, although the distance is short, is low. So this is on average because you need to know this is the movement of cells that are being formed here, and the cells formed here now take eight days to reach up to here, while the cells that are formed here take around four days, and then they fall off from the tip. Here, the movement is less; hence, it takes around 20 days in the neck region, but the hydra's body is always regenerating. So this cellular replacement happens from three types of cells. The ectodermal, the endodermal, and the constantly dividing cells constantly divide to produce more epithelial cells; the third cell type is a multipotent interstitial stem cell found in the ectodermal layer, which generates gametes, neurons, and secretory cells, so there is a dedicated stem cell that is helpful in constant replication, and it is called an interstitial stem cell.

So the three cell types are enough to form a new hydra, which means you need to have an ectoderm, an endoderm, and this interstitial cell. If you put them together, the new hydra will be formed. Why? Because the interstitial cell is good enough to give rise to gamete-producing cells, neurons, and other secretory cells. This way, if you disassemble the hydra into a mass of tissue, say 50 cells or 100 cells of hydra, it can give rise to a miniature hydra. Eventually, they grow bigger, so if you look closely, the regeneration capacities of Hydra, which is a Hydra vulgaris, a species with the ability to regenerate when dissected in various manners, and various morphological changes observed are shown here.

See, this is a Hydra, as you can see here, and one picture with scissors is given, which means... Normally, in a food packet, you will see a scissors symbol. That means that is a place where you should cut it.

Similarly, if you cut it through this plane, you get a left half and a right half. So what did the left half do? It produced the right half. What did this right half do? It produced the left half. But the important thing to note is that it simply did not produce the missing part. Eventually, it produced the missing part, but with the availability of this entire left half, it made a miniature whole animal.

Say, for example, let us assume the Hydra is one kilo. Easy. So you cut it into two pieces, 500 gram and 500 gram. Now this 500-gram Hydra made a new Hydra, which is exactly 500 grams with a miniature Hydra. Now it will try to reach a size of 1 kilo.

So this is what you see. Say this central hydra is big, cut into a left half and a right half, and that this newly formed hydra is exactly half the size of the parent hydra. That is a longitudinal division. Now you see the transversion division. Head one piece, belly one piece, foot one piece.

Cross-cutting. You got, let us assume this 1 kilo hydra you made 330 grams, 330 grams, 330 grams. And now you end up with a tiny hydra. The head region made a full hydra, which is a miniature hydra. Body region made a hydra, which is again a miniature hydra. The foot region made a hydra, which is another type of miniature hydra.

So the newly formed three hydras are identical. They look like clones. but not that it is smaller than this hydra, which is made by two cuts. That means a 500 gram hydra is bigger than a 300 gram hydra. So this is technically what we refer to as morphallaxis, and ideally, in effect, it is epimorphosis because the left half was missing, the right half formed, but it's not identical in size; eventually, it will become the size of the parent.

So Hydra can regenerate missing parts during transfers and longitudinal dissection. Or you can do whichever angle you want to cut it. You can cut it. The Hydra gives rise to a proper full-fledged Hydra.

Experimental embryology. Or one might say the experimental biology of Hydra began with Abraham Tremblay's work on Hydra regeneration that was done in 1741. That means almost 300 years ago, people knew about Hydra regeneration in recorded form. Maybe people noticed that much earlier. If Hydra is cut into as many as 40 pieces, they are reborn as many complete animals similar to the first. Similar, not in size, but in appearance and morphology.

Each piece would regenerate a head at its original apical end and a foot at its original basal end. They'll be miniature hydras. Every portion of a hydra's body along the apical-basal axis has the potential to form a head and a foot. Every piece has the potential to form, whether it's a belly piece, neck piece, body piece, foot piece, or head piece; it doesn't matter. However, the polarity of the organism is coordinated by a series of morphogenetic gradients.

Gradients mean they are present in different concentrations. Say that if I say "head gradient," when I say "in head," it is maximum. As it moves away from the head, it is going to be lower, lower, lower, lower, and towards the food region, it is the least. So, that is the same way there can be a food gradient. When the food region has a given chemical, that given molecule will be highest in concentration, and it becomes lower, lower, lower, and lower.

This gives a positional identity. So, whenever you take a slice, you will find a place where both gradients are equal. You will see one gradient is 50, another is only 30, or in another place you will see one gradient is 80 and the other gradient is only 20. You will find that based on the gradient of different molecules, it's not just two molecules; there can be multiple molecules, and in the same way, it can have a left-to-right gradient, a right-to-left gradient, a back-to-front gradient, and a front-to-back gradient as well. They are called morphogenetic gradients that permit the head to form from only one place. When you take a body piece of Hydra, you won't get three heads all over the body.

One head in the above actual place, one on the left side, one on the right side. You don't act like that. You get only one head, no matter what is the slice size. This is something very interesting: one place of the basal disc, and it's also important to note that the head forms only one; in the head region, it will not produce the same tip region, say the anterior region.

It won't produce two heads; it will produce only one head. As you can see here, some experiments have been done to demonstrate that the regeneration capacities of Hydra vulgaris, a species used to study regeneration capability, are significant. This is a full hydra, and you macerate it, turning it into a cell suspension. Soon after, these cells are kept in a liquid medium. Medium means simple water.

They will assemble to form a circular shape. That means it forms like a football. It formed a sphere. And now this sphere is slowly changing its shape to that of a typical hydra. And if

you see in this cartoon what you are seeing here, you can make a cut anywhere.

Say you cut it near the neck region in this axis. You are left with this. It heals immediately. And somewhat like that of a blastema's structure is formed around it. Half an hour to three hours and around three to eight hours, the continuity is established; like here, there is no continuity. Here, the continuity of mesoglia or mesoderm is established, and by around 30 to 36 hours, it will start developing the tentacles slowly.

And later it gives rise to a head, a proper head with a hypostome that is being formed. And this is the normal method: roughly around one to one and a half days, the new hydra, the miniature hydra, is formed from a mass of cells. So regeneration from dissociated and re-aggregated cells is shown in the top panel. And this bottom panel is the kinetics of gross morphological changes during hydra's head regeneration. Now you can see there is some evidence to show the importance of the gradient.

We said that you know there is a morphogenetic gradient. Can we prove it? For such gradients were obtained from the grafting experiments done by Ethel Brown in the early 1900s. That means more than 100 years ago, these grafting experiments were done. As you can see here, this is the graph. Head region, like hypostome. You take a portion of hypostome and attach it onto the trunk region of another hydra.

So the donor hypostome is taken and attached to the trunk region. That is you are sticking something onto your belly. And you see a new head is being formed from that region. So you only put in the hypostome. There is no injury; keep that in mind. You just transplanted the hypostome region onto the belly of another recipient.

When the hypostome tissue from one hydra is transplanted to the middle of another hydra, this transplanted tissue forms a new epicobasal axis. Because you put in a hypostome, the hypostome has a strong concentration of the head gradient, which is dissolving into the belly region. And you created an epico-basal axis with the hypostome extending outward. And that will be established by the movement of the hypostome away from this body.

So how will it move? It cannot fly. It will slowly continue synthesizing cells and keep walking out of the body. And you end up getting a new hydra just like a budding one, as you saw in this case. In the same way, let us take a basal disc. Took another donor hydra, took the foot and attached it to the same belly region. And you see, what do you see? Now, instead of a head coming out, you see a foot that is coming out.

That means there is a basal disc. The hypostome has a basal disc that has another gradient, like the hypostome, which has one head-to-tail or head-to-feet gradient, feet to head; also, there is another gradient when a basal disc is transplanted to the middle of another hydra, where a new epico-basal axis forms but with the opposite polarity. The basal disc is now extending. The head is not coming because you did not put the hypostome; you instead put

a base or a foot. And now the feet started to move out. In both the head and both feet, it moves away from the donor's body while attaching, holding on to its body.

That means the strong gradient is trying to establish the actual gradient by moving away from that point. And you can see here the new feet is formed from the belly. Now you can see, now if you take from a donor, you took a head, half head, and half base together, you put both together onto a donor in the same place. That means if you're taking a portion of hypostome and a portion of basaltase, both are put in one implantation; together, you put them. Then what do you end up getting? You will get either a weak apical induction or a strong one.

It doesn't grow very well. A weak apical induction or a weak basal induction. Or if it is absolute, why do you have a weak apical induction? Because although you took 50-50, maybe a little bit by one cell or two cells was a little more in the hypostome. Hence, it gets a weak basal. Head induction. And why do you get a weak basal disc induction? Because maybe the basal cell was a little high. But if it is an absolutely precise number, 100 hypostome cells, 100 basal disc cells precisely, then you get no, zero induction.

There is nothing coming out of that body. That means it got neutralized, it got nullified. So that is called a non-induction. So when tissues from both ends are transplanted simultaneously into the middle of another hydra, either no new axis forms or the new axis has less polarity. That means some canceling out is happening. That means one gradient wants to take over, while the other gradient is suppressing it.

That is why you feel weak or have nothing. These experiments have been interpreted as follows: There is a head activation gradient that means favoring the head, highest at the hypostome. Head activation gradient is highest at the hypostome. And there is a foot activation gradient that is highest at the basal disc. This gradient can be found anywhere in the body, but there is the highest head activation near the hypostome and the highest foot activation in the basal disc.

That is the inference you can draw from this. The head activation gradient can be measured by implanting tissue rings from various levels of donor hydra into particular regions, wherever you want, of the host trunk. The higher the level of the head activator in the donor tissue, the greater the percentage of implants that will induce the formation of new heads. Instead of going into the proper hypostome region or instead of going from the proper foot region, you can go closer around the neck region, say considering humans, so your head, you know, and your neck is closer to the head; the chest is a little bit more closer to the head, and the belly is somewhere in the midway, right? In the same way, you take a tissue disc from closer to the head, and you implant it anywhere into a donor hydra; then there is a higher chance it will form a head, not the foot. In the same way, if you go to the lower half, closer to the foot region and away from the head, there is a higher chance that the foot will come. Not that you need to take donor tissue precisely from the hypostome or precisely

from the basal disc.

Even closer is good enough. That means there is a gradient that is in action. That is the inference. Now, if you see the formation of a secondary axis following the transplantation of head regions into the trunk region. We saw different transplantations if you are doing. Can we create a secondary axis? One axis we know of. Make it sound like you know about a parallel government or parallel economy; something like that.

So many such research studies are done in embryology and regeneration biology. One experiment involved grafting hypostome tissue into the trunk, which induces the host trunk tissue to become tentacles and a head. Now, what do you understand from here? You are tweaking the system. So this is a reference you can refer to. Hypostome tissue is now grafted onto the trunk. The hypostome can be the same animal, or it can be another animal of your liking; it induces the host's trunk tissue to become tentacles, which means new cells are recruited, and this gradient has the potential to influence the neighborhood just like.

If I put a fragrant material in a room, it can be a normal room, a dirty room, or an already fragrant room. Wherever I put it, it influences the neighboring molecule. Sometimes it can be mixed. But in this gradient, what happens? The gradient is powerful enough to influence the neighborhood.

Human company, if you are with good company, you can be influenced by that good company. Or if you are with bad company, you can get influenced by bad company. Similarly, the trunk region, which is weaker for both the head gradient and the tail gradient, is somewhat like being in the midway. That time you suddenly put a head gradient, all the cells will start responding to it and they will start making heads, hypostomes, tentacles, etc. So subhypostomal donor tissue, hypostome is one tissue; subhypostome, you went a little bit below, grafted onto a trunk; the cell differentiates into head and upper trunk. So what does it tell you? Below the hypostome, it also has a gradient that favors the formation of a head.

So what you can see here is that these experiments are done by Ethel Brown. He noted that the hypostome acted like an organizer or the director of a film. Organizer means something that can influence the neighborhood. That is the organizer, a leader, just like how several freedom fighters influence the masses, like a leader, a global leader influencing the public, something like that. That's called the organizer, the hydra. And Brown and Body in 2002 confirmed this by demonstrating that when transplanted, the hypostome can induce host tissues to form a secondary body axis.

If a primary body axis already exists, it creates a secondary body axis, and that secondary body axis is formed by a small tissue that is what is more important. And the hypostome produces both head activation and inhibition signals. This is maybe a little sound conundrum. We'll come to that because when you put in a new tissue, it helps in the

formation of one head. But you also know why it matters who counts? Why can't it form one more head if it is favoring? You can have five heads, can't you? But it doesn't.

So it allows once one head starts forming, it does not allow one more head to start forming. So this is something very interesting. You should know that too. Head favoring signal allows the head to form, but it does not allow heads to form.

Multiple heads do not form. So the hypostome produces both head activation and inhibition signals. The hypostome is the only self-differentiating region of the hydra; it can self-establish or self-influence the neighboring cells, and the self-differentiating region just in the hypostome is good enough. It contains a head-inhibiting signal that suppresses the formation of a new organizing center, so head activation is one thing, and head inhibition is another. So later, people figured out what these molecules are, etc. But these experiments told the researchers that the signal can be activating and must also be inhibiting so that only one structure will be formed.

The scientist also found that even transient contact with the hypostome region was sufficient. You take a hypostome; no need to transplant. Take a hypostome from a donor, go and touch the belly of another donor just to touch it and come back. No need to keep it there. It was sufficient to induce new access from the host hydra. How interesting it is! If you think about the regeneration, how exciting and interesting it is! You are not transplanting.

You are not doing anything. Just physical touching. Take a tissue, go, and touch it. Just like smelling it. If you want to smell a fragrance, you don't need to pour fragrant material into your nose. Even agarbatti if you stick it. If you pass through, you get the sampling.

So this system is so powerful that one time touching, it is done for the rest of the life. So this is why regenerative biology becomes so exciting and interesting. And we will study more about hydra regeneration and its mechanisms in the next class. Thank you.