

**Regeneration Biology**  
**Rajesh Ramachandran**  
**Department of Biological Sciences**  
**IISER Mohali**  
**Week: 8**  
**Lecture: 36**

W8L36\_Organ regeneration: An overview

Hello everyone, welcome back to another class on regeneration biology. Today we will learn about organ regeneration. In an overview, we will dissect one by one organ regeneration. By now, you have a clear idea that we have seen different types of tissue regeneration, and organ regeneration in a simplistic sense means restoring a damaged appendage or a damaged organ in total in place of a missing part. So that is the simplistic sense of organ regeneration, and I am sure. All of you are familiar with this animal picture; this is an axolotl.

It's also called the 'axolot' by Americans. In either case, this is one of the fantastic animals used in regeneration biology studies, and it is native to Mexico, seen only in one lake. Xochimilco or something, you can Google it; you can find it only in one lake, where it has been seen. The most important thing is that, although we have plenty of relatives of axolotls across different parts of the world, the axolotl is seen only in one place, and that too is restricted to one lake, not even to one country, just restricted to one lake naturally.

But it is everywhere in the world in laboratories and households. In nature, it is nearly extinct. It is not there at all in nature in Mexico. Its scientific name is *Ambystoma mexicanum*, as you can see here, and it has been extensively grown in laboratories and also in pet shops. People use it as a pet animal, and an interesting thing about this animal is that it has gills and lungs, allowing it to breathe air as well as in water.

In other words, this animal shows a situation called neoteny. Basically means the larval stage, so what you are seeing in this picture is an adult, but it did not change from its larval stage at all, except that it increased in size; it retains every larval characteristic. You may wonder what larvae I am talking about; it simply means every salamander has this larval stage, but it metamorphoses into an adult. Land-dwelling forms can breed and come back to water for breeding purposes because the larvae are aquatic, but axolotls choose not to metamorphose by default. We will not get into the details of why they don't metamorphose; if necessary, we will discuss that at that time.

But understand that by default, the larvae become adults without undergoing metamorphosis, which is called neoteny. And this could probably be one of the main

reasons for its remarkable organ regeneration capacity. Because every animal, including other frog species and other salamander species, has exceptional regenerative capacity during the larval stage, even in mammals, birds, or any other animal. Non-regenerative species also, during the embryonic stage, have excellent regenerative capacity, so can it hold the clue that the neoteny feature is allowing axolotls to regenerate more efficiently than any other animal? We have to explore this, so this is one old issue in science; I think it was in 2005, when they discussed different questions—125 questions about what we don't know. So it's basically addressing what the challenges in science are and what challenges we should be exploring to make life better for humans.

So in that, they talk about one interesting topic, which is mentioned: those who are interested can read it. I am not going to read it out. One page has been put forth about what controls organ regeneration; that is one of the 125 questions. What controls organ regeneration means every animal has the capacity to regenerate a damaged organ. Like we regenerate the liver exceptionally well.

We can regenerate a nail, hair, or skin, etc. We regenerate a lot of tissues in our body. We constantly regenerate blood cells. Your RBC lifespan is only four months. WBC lasts only around 90 days.

So we regenerate. We constantly regenerate these blood cells. And they die, too. Otherwise, every human will live for only 4 months. Because if the blood you are not able to regenerate.

So we do regenerate. But when it comes to complex tissues, We lag behind, or we do not regenerate. So, organ regeneration is one classic field of study. If your kidney is damaged, why can't we have kidney regeneration? But when the liver is damaged, it can regenerate. So, intestine, if your intestinal epithelium is damaged, it can regenerate.

But if your intestine is chopped off, new intestine will not form. So these are all some questions that baffle scientists. So it is a widespread phenomenon in a group of animals. You can see Hydra here, a planarian here, a frog, an earthworm, a fish, and a mouse. Various crustaceans, Drosophila, and salamanders, such as axolotls, as well as the human hand, are being referred to.

When we talk about the human hand, we are referring to the bones. Basically, if a hand is chopped off, a new hand will not form, but if a bone is fractured, it heals—not perfectly, but it heals so that the continuity of the bone is restored. So all these species are blessed with the ability to regenerate, and they are extensively used as model organisms. So there is variability in the ways that the animals regenerate, suggesting that

regeneration has evolved multiple times in evolution. Therefore, there is a likelihood that there will be multiple strategies to consider, and each model system has something to teach us about how they are doing the regeneration.

This view begs the question of why humans have not evolved the ability to regenerate. There are so many reasons and arguments that can be made. There is also one theory that a group of humans migrated out of Africa, which you probably already know, suggesting that an Indian, an American, a Japanese, or a Chinese person is more similar to each other than a person living in one village in Africa is to someone in the neighboring village; the distance between those two villages may be only 10 kilometers. They are more diverse than you are to an American or to a Chinese or a Japanese person, so this may sound baffling. The reason is that multiple waves of migration have occurred out of Africa, but on an evolutionary time scale, it has happened that way.

At some stage, the human population outside Africa had come down to as small as around 1,200. Around 1200 people have come down. They colonized the rest of the population, so 1200 is a small cohort considering humans as a species, but what you get because of whatever features and qualities those 1200 people had was enough to make them fit and survive in that environment. That may not have had this so-called feature of regeneration. Hence, the rest of the population did not have it.

This is one theory that you can put forward. What do we need? Why, how, and what do we need to regenerate an organ like a limb? What is the stuff needed? It's just like asking, okay, I need a hand to regenerate. What are, like people say, your hand; your palm is the second most complex organ after your brain. I think it has around 27 different bone pieces. Just your palm, just your palm.

27 bones and around 30 different muscles. Around three unique nerves control two or three major arteries and two or three veins. These are all unique, so the 30 different muscles that have to be controlled by your brain make for a complex task. What I mean to say is that your hand regeneration is not just structural regeneration; its function is very complex. Although your fingertip is a simple organ when it comes to regeneration, it does not just make a copy of the hand; it is making a functional hand, which involves a lot of complexity.

Know trajectories that have to come into picture regeneration competent cells that can do what needs to be done; they have to migrate, they have to proliferate, and they have to differentiate. So this is very, very important, and the regeneration permissive environment—that is, the regeneration permissive niche—is a must for the regeneration. Do not happen properly; if the regeneration does not work effectively, then what can

happen is that it can influence the survival, or the selection of that cell at that spot can be negatively affected. So even though the ability is there, like you are a great singer, I have put tape on your mouth. So, how are you going to sing? So the same logic.

I have a singer doesn't mean that the song is a given or that the song is a fact. So, a regenerative instructive environment is a must. If regeneration is not possible in a given environment, the capability to regenerate is not going to work, even if you have the ability. So regeneration is the reactivation of development in post-embryonic life to restore the missing, damaged tissue. So during embryonic development, around two months of gestation, your hands, legs, and everything else is formed.

Your size may be very small. Maybe the size of two or three centimeters, but you have become a full-fledged organism. Even the hands and limbs, I think around two to three months of gestation, even your fingerprints are formed. Fingerprints are formed. You can imagine the complexity of development. Your thumb and all individual fingers and fingerprints are formed around that time of development.

But can we recapitulate it? If an organism is 20 years old, can we recapitulate? Can you reactivate that 20-year-old off pathway? That means if you are six months into your development as an embryo, say, or a newly born embryo. That is around nine months of development. But it stopped the limb formation around 7 months ago.

It is not having... The limb is growing, but no embryo is developing a new limb. So, if you are 20 years old, you can imagine it. Two months into gestation, the limb formation stopped. It is a very unhuman phenomenon that has led humans to wonder why we cannot regrow our arms and legs.

It is... Unhuman phenomenon, what it basically means is that we all want it, but we are not capable of it; that means it makes a person debilitated. What gives other animals the ability to regenerate tissues while we cannot? These are all some interesting questions that you can see in that science article that is published. So regeneration can occur in four major ways. We have seen them one by one. We are recapping that stem cell-mediated regeneration occurs; stem cells are the sources of regenerating the lost tissue or organ.

An example is hair shaft regrowth from stem cells in the hair bulge and the replacement of blood cells from the hematopoietic cells. So we know these are all formed because of the presence of... Unique cells, we can call them adult stem cells.

Another thing is epimorphosis. Adult cells in some species can de-differentiate into an undifferentiated mass, which then re-differentiates to form new structures, for example,

in regenerating amphibian limbs. So there are no stem cells available. They are formed based on the demand in that spot by the de-differentiation of the existing tissue. Then comes morphallaxis.

Morphallaxis, we have also seen it. What does it mean? Regeneration occurs through the repatterning of existing tissue, and there is little new growth. Example: regeneration in Hydra. Say you took 100 cells or 200 cells from a hydra, just a cell assembled in a petri dish. So it immediately forms a hydra, a miniature hydra. or you took a normal Hydra, chopped off its head.

So it has only the bottom portion. From the bottom portion, it will use the existing cells. Let us assume a Hydra had 1000 cells. Chopped off from the top, 500 cells have gone. Actually, there are 1000 cells in a Hydra. It's not a fixed number that I'm giving as an example.

500 cells were removed. So the remaining 500 cells, both pieces—the top piece and the bottom piece—rearrange and form a normal hydra, a miniature hydra. So repurposing. So some of the base cells or the stock cells are now assuming the tentacles, head, etc. The same way, the head portion now has to repurpose the cells into the stock, the base cell, the foot, etc.

It has to be repurposed. Then it will increase in size. So this is morphelaxis. And then comes compensatory regeneration. Here, the differentiated cells, such as those in liver regeneration, divide but maintain their differentiated function. That is what they normally observe in the mammalian liver.

Simple example. I have five fingers. Two of them are cut. And now these three fingers are growing and occupying this space. Only three. This becomes double the thickness.

This becomes double the thickness. This becomes double the thickness so that it occupies these two areas. Or if I chopped off all the four fingers, this thumb is now becoming like a paddle, becoming big so that it act like it. So that is compensatory regeneration. So if you look into the epimorphic regeneration in salamander limbs, it normally takes around two to two and a half months for complete regeneration. On average, you can tell 60 to 65 days, and this can go up to 72 days.

A picture has been shown. So two things are shown in this. One is distal amputation; another is proximal amputation. Proximal amputation means you are amputating close to the body.

For example, say you are cutting here. That is proximal. Cutting here is distal. Here is proximal. So proximal means closer to the body, and distal means away from the body. But no matter where you cut it, by around 72 days, both will have grown.

So not that if you cut here, it takes longer days. If you cut here, it takes only shorter days. So what you understand is that if the damage is here, the growth rate will be faster. Or if there is damage here, the growth rate will be slower. That is what you are seeing here.

If you cut here, it is growing at a rapid rate. Whereas you cut here, it is growing at a slower rate. So understand, no matter where you cut it, the time taken is going to be fixed. When a salamander's limb is amputated, the remaining cells regenerate. That is, once you remove these cells, what you are talking about, or these cells we are talking about, the remaining cells regenerate. They will de-differentiate and regenerate a new limb that is a complete copy or mirror image of the other limb on the other side, with all differentiated cells arranged in proper order, forming a functional limb.

Remarkably, the limb regenerates only the missing structures and no more. It cannot generate anything other than when one limb is cut; two limbs are not formed. In some ways, the salamander limb knows the point of the proximal-distal axis. This is what the proximal distal axis is. where it has been severed or amputated and regenerates from that point onwards.

If it is cut here, it will not grow from there. If it is cut here, it won't grow from here. Not that I will get a palm formed here. It will make the whole thing. So the salamander accomplishes epimorphic regeneration by cell dedifferentiation to form a regeneration blastema. We have seen this terminology multiple times, which is an aggregation of relatively undifferentiated cells derived from the originally differentiated tissue that then proliferates and re-differentiates into the new limb structures.

So you can see here in its ultra structure, the source of regenerating competent cells, how it's being formed. The regeneration blastema is equivalent to a developing limb, but the apical epithelial cap, also known as AEC, is equivalent to the apical ectodermal ridge of the developmental stage where the limb is formed. The actual limb is formed from AER. Sonic hedgehog signaling comes into the picture.

That decides how you should have differently shaped fingers, etc. I will not go into the details of that. But AER is a major source; it also uses hedgehog signaling. So there are blastema mesenchymes. So this is a limb and this is a blastema picture.

And this is called a mesenchymal cell. We discussed epithelial mesenchymal transition

multiple times. And blastema mesenchymes. And it's equivalent to the limb bud mesenchyme. And then the zone of de-differentiation that is unique to regeneration is here.

The zone of dedifferentiation is unique to regeneration. And then there is also a mature, differentiated proximal tissue. There is a mature differentiated proximal tissue that is a source of the blastema. So there is a differentiated tissue that gives rise to the blastema cells, and they give rise to a cap. So we can also see the dermal fibroblasts; they migrate and proliferate to form an early regeneration blastema. So you have created amputation here and it forms a small lump and then it increases in number.

And up to 80%, which is nearly 50% on average, of the early blastema cells come from the dermal fibroblast. The dermis and epidermis make up the skin, with the dermis located below the epidermis. You can see here, this is the epidermis, this is the dermis, and this dermal fibroblast and epidermal cell are forming a cap. Apical ectodermal cap is being formed, and these dermal cells migrate into the wound site; they are the actual source. People have shown this by staining; now you can see these red-colored cells, and there are plenty, although other cells also come from here because there will also be fibroblasts in there.

However, the major source, on average around 80%, of the early blastema cells has to come from the dermal fibroblasts. So the formation of the apical ectodermal cap and the regeneration blastema has been seen here. So you can see this is a humerus bone and this is the epidermis. And this humerus bone also needs to be formed; you should understand. and when you block it you know there was an amputation site here and the blastema has formed and then there is a here also there is a muscle cells that is developing and epidermis and the humerus bone grows hand in hand and the apical ectodermal cap remain intact even when the blastema is growing further, the apical ectodermal cap also is growing forward And then you have the blastema here; it is the blastema that is at the extreme tip of it, and then you can see here these mesenchymal cells migrate, and the blastema becomes mature and mature and mature.

Then one more thing you should know is what is present near this bone: they continue to differentiate, or in other words, This zone is the place where active differentiation is occurring. And this zone is the place where active proliferation is going on. So here, although proliferation is occurring towards the proximal side, it is more a matter of differentiation because this blastema continues to grow further and further. So when a salamander limb is amputated, a plasma clot forms, which contains fibrinogen, etc.

, which allows these cells to migrate easily, fibronectin. So, within 6 to 12 hours,

epidermal cells from the remaining stump migrate to cover the wound surface, forming a wound epidermis. It forms a wound epidermis below that, the blastema forms. In contrast, wound healing in mammals does not form a scar, and in the dermis, it does not move with the epidermis to cover the wound. Thus, wound healing is seen in mammals, but in this animal, where regeneration occurs.

A scar is not formed. In fact, a scar is formed, and the scar is removed instantaneously so that regeneration is kickstarted. No scar forms, and the dermis does not move with the epidermis to cover the wound. So this is what you normally see in the case of a typical wound healing. If the dermis moves, then there is a chance it can produce more of the blastema cells. Two days after amputation, the skin and muscles have retracted from the bone surface.

And at five days, a thin accumulation of blastema cells is seen under the apical ectodermal cap. And at seven days, a large number of mitotically active blastema cells are seen distal to the humerus. So what you see here now is, in the four days following the amputation, the extracellular matrices of the tissue lying beneath the wound epidermis are degraded by proteases, liberating single cells. So the proteases allow cells to become free from one another.

That means it becomes more of a meson kind and undergoes dramatic de-differentiation. Soon after cutting, proteases come into the picture. Single cells are formed, and they reprogram. and then start proliferating. Bone, muscle, cartilage cells, and fibroblasts lose their differentiated cell characteristics. Differentiated cell markers are downregulated, and increased expression of genes such as MSX1, associated with the proliferating progress zone seen in mesenchyme in the embryonic limb, is observed.

So MSX1 is a marker of Blastema, and increased expression of MSX1 is seen associated with the proliferating process that is nothing but Blastema. And here, you can also see they start becoming D1 and D2 digits that are forming. Okay. Different radii, ulnae, and parts of the humerus bone are formed in all zones after a few days. This cell mass is the regeneration blastema, and the cells will continue to proliferate and re-differentiate to form new limb structures.

The wound epidermis thickens during this process to form the apical epidermal cap. which is similar to the AER during limb development. This is an 8-day blastema that elongates by mitotic activity, and much de-differentiation has occurred; at day 9, early re-differentiation can be seen. A marks typical mesenchymal blastema as it is mentioned here, and H marks the proximal part of the regenerating humerus bone. U and R are cartilaginous structures of the radius and ulna.

And P represents the stump where the amputation was performed. This is the plane of amputation. And these are all the structures that are formed from the blastema. At day 10, C marks the precartilaginous condensation of the carpal bones, ankle, and the D1 and D2 marks, which are the digits being formed; D1 and D2 refer to the first two digits, as you can see, D1 and D2. So what are the contributing tissues of the blastema cells? Epidermis gives rise to wound epithelium, which gives rise to the epical ectodermal cap.

Stem cells give rise to blastema. And the contribution of blastema, if you look at the total blastema you are taking, if there are 1000 cells in the blastema, you are observing. Dermal fibroblast, around 19... 43% can range in muscle myofibers; actually, in the stump, there are 19% dermis, which is now increasing to 43%, and muscle cells in myofibers are at 55%, which will reduce their quality and become 17% in the blastema.

And in the skeleton, the cartilaginous bone, around 6%, will become 2%. So in general, the dermis contributes a large chunk of fibroblasts. But remember, once they are all reprogrammed, they are all going to become typical, normal cells. Tissues in the stump all contribute to the blastema. Tissues in the stump do not contribute in proportion to their availability.

Progenitor cells are lineage-restricted except for fibroblasts. They are lineage-restricted when it comes to their fibroblast qualities, and even if they are present, they do not have what you call the ability to give rise to any tissue. Although the blastema is a mixed population, they retain some identity that allows them to give rise to specific tissues, even though they are undifferentiated. However, not every cell can give rise to bone, and not every cell can give rise to muscle. So there are many challenges that are facing regeneration.

One non-regenerating model is like playing in a lotto. That means you can heal a wound; you heal it. Or if you get it, you understand. Sometimes you get it, especially if you are in the early stage of development. Amputation studies in the regenerating models are inherently loss-of-function analyses. So you study the loss of the limb, and amputations create noise that are extraneous signals that obscure the regeneration signals, and many gain-of-function studies in the non-regenerating species are often incomplete. And this is a picture where amputated man and there is a confusing road direction that is given to a cell that is trying to regenerate.

The goal is to reduce the complexity of regeneration and design gain-of-function experiments for the identification of the signals that are necessary and sufficient for

regeneration. So this is the overall theme of regeneration. And we will study regeneration biology more in the next class. Thank you.