Regeneration Biology
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W9L43_Animal   cloning: implications   in   regeneration

Hello, everyone. Welcome back to another class on regenerative biology. So in today's topic, we will discuss animal cloning and its implications in regeneration. What are the potential applications of animal cloning? But before going into the details, I will explain very briefly the developmental biology and also the Hox genes which we discussed in the previous class. So, the pattern formation in a developing organism is very important. In the early stages of pattern formation, two perpendicular axes need to be established.

One is anterior-posterior, which we basically refer to as head to tail. And then it's dorsal-ventral, back to chest, back to front, and head to tail. So that is the dorsal-ventral axis. And the polarity the term refers to is the acquisition of axial differences in the developing structures.

So differences where it is present are left to right and back to front or head to tail like that. Position information is very important for deciding this pattern formation, and it can influence the changes in gene activity. That is why certain genes are expressed only in my shoulder, so that the hand comes from here. Those genes are not expressed in my neck area. And if they express in my neck region, I'll get a hand formed from my neck also, so this is what you should keep in mind: gene activity helps to adopt a fate that is appropriate   for   their   physical   location.

This is what we discussed in the previous class; homeotic gene complexes, also known as home complexes, are well studied in Drosophila melanogaster as an example. And the main two complexes well studied in Drosophila are the antennapedia complex, which governs the anterior end of Drosophila. And then comes the bithorax complex, which governs the posterior end of the fly. So two of these home complexes decide the upper half of the body and the lower half of the body of the Drosophila. Interestingly, we should know the order of genes that mirror the order of body parts they can control.

So the order in which genes are expressed, each segment, each section, each locality of the body has a different body part coming from that segment. The homeotic gene complexes contain all of these genes, which have a conserved 180-base sequence that we often refer to as the homeobox; the fundamental role of this targeting protein is that it

encodes around 60 amino acids. Three bases encode for one amino acid, so 180 bases encode for 60 amino acids in the DNA binding domain. This conserved region allows the protein to bind to the DNA to cause a gene expression event, but the binding region is essentially a 60 amino acid region, which we call it. Homeo domain homeo domain containing proteins are called homeobox proteins, so homeobox containing genes are termed Hox genes.

Hox genes can have different names, such as Hox1, Hox2, Hox3, and so on. Vertebrates have four Hox gene clusters; vertebrates means all. Animals, starting from fishes to humans, fishes, frogs, reptiles, birds, and mammals, can be seen in this picture. The formation of a body plan shows a head, thorax, and abdomen, and you have got the antennopedia complex. You can see individual genes that are expressed; these are all homeobox genes.

Then you have got the bithorax complex that decides the abdominal region, and you can see how different types of color coding are represented, from green to green here. Blue to blue here. Orange, yellow, like that. See the orange region that makes the leg. And the yellow region.

And you can see different parts. The adult fly is retaining. And in the same way, the mouse does too. You have got Hawks 1, Hawks 2, Hawks 3, and Hawks 4. How differently they are expressed across the anterior-posterior axis of the whole animal.

And accordingly. It has a color coding that corresponds to individual body structures, which give rise to particular organs, both internal and external; that is why you don't see the heart formed somewhere in your belly region or your liver formed near the lungs. You don't see that because each positional information is taken care of accordingly. That is maintained, so although we don't have an externally visible segmentation pattern during the developmental stage, as you can see here, individual segments are seen in the body pattern, and these segmentations define how strong or how weak a given homeobox or Hox gene is expressed, which is detrimental in deciding why you have an eye in place of an eye or an ear in place of an ear, which you should get. So this logic you should keep in mind, and any alteration or perturbation you make will end up getting a Situation called homeosis, a homeotic transformation.

So I should have mentioned this in the previous class, but I thought this addition would help you know a little bit extra. So, coming back to cloning, that is today's class. We know that in 2012, the Nobel Prize in Physiology and Medicine was given to John Gurdon and Shinya Yamanaka. We all know the Yamanaka factors we discussed multiple times. That is OSKM: four transcription factors.

When you express in a pluripotent cell, it will become a stem cell. But before that, John Gurdon has done some very interesting experiments which have laid the foundation for doing this so-called animal cloning. So make a clone of an animal. I know all of you have heard about what animal cloning is. Like if I make a clone of myself, it looks exactly like an identical twin of mine.

So that is the whole concept of cloning. But when you do cloning, It automatically opens up the way for obtaining pluripotent stem cells. So, these fields overlap. That is why the Nobel Prize in Physiology or Medicine is given to these two eminent scientists for their discovery that immature cells can be reprogrammed to become pluripotent. Let us see what John Gurdon did.

It's a very simple experiment. He took an unfertilized frog egg. and then he also took an adult frog and adult frog he took the skin cells and separated them by enzymatic treatment on a petri dish so individual cells he isolated from those cells he took one nucleus from that cell and then what he did this unfertilized egg he took he irradiated with the UV radiation that will kill the haploid egg or haploid genome present in the unfertilized egg so it is killed because of UV radiation Now, what he did was take the nucleus from this adult frog, put it into the egg, and allow it to grow, becoming a normal blastula and then a tadpole. This tadpole will eventually resemble this frog; it will be a clone of this frog. So, John Gordon has done the first cloning of a vertebrate.

Okay, so what does this transplantation experiment done by John Gordon tell you? It tells us that localized cytoplasmic determinants are important. That means you cannot take any given cell; say you took a cell from the frog. Like here, you took a cell. Why doesn't this cell become a whole human? It won't because the cytoplasmic determinants from the oocyte are important for converting this nucleus into. Such a way that it can now give rise to the whole organism; the genome stays the same during cell differentiation; the genome cannot change, but the genome's expression pattern and expression nature can change.

Skin cells have the potential to generate all cell types you really need. Either you don't or you can't depend, or you really don't need the nuclei for cell differentiation; actually, any donor nuclei or the oocyte nuclei are not necessary for the formation of the entire organism. So, the oocyte without a nucleus is good enough, meaning the cytoplasm present in that oocyte is good enough to convert any donated nucleus into a full-fledged organism. So understanding the interaction between the oocyte cytoplasm and a recipient or received nucleus is capable of converting that nucleus. The nucleus has the genomic blueprint.

to convert into an entire organism. This is what a discovery is: very simple, but the impact is very large. So now we will quickly see more in detail. But before going into the details about cloning, we can see what the implications are in regenerative medicine. So the nucleus from the skin of a diabetic patient can be taken; for example, we are citing a diabetic patient from whom you took one cell.

Any cell from the patient is fine. Then the nucleus from the skin of a healthy patient can be taken, and the skin cell nucleus is inserted into the enucleated human egg cell. Another human's egg, which you can get from the ovary of any person, is used, and the nucleus from this diabetic patient is inserted into that oocyte. Cell cleavage occurs as the embryo begins to develop in vitro, and you place it in an appropriate environment. That oocyte now has a diploid nucleus, and the embryo reaches the blastocyst stage.

You can collect the inner cell mass and obtain the stem cells from this person because you have done exactly what John Gordon has done. Another way of doing that is to take the same method from a healthy patient instead of a diabetic patient; you can take from a healthy patient and do the same thing there. You can also obtain inner cell mass, so here you have inner cell mass from the patient and here you have inner cell mass from the donor. Two different scenarios now in the diabetic patient: what did you do? The embryonic stem cells from the inner cell mass are extracted and grown in culture, and then you can do something called therapeutic cloning. What did you do? The stem cells are developed into healthy pancreatic islet cells needed by the patient.

From this inner cell mass, you can make them into any cell type, like we have seen; it can be made into neurons, muscle, heart, or whatever you want, including kidney cells. So they made insulin-producing cells that are islets of Langerhans in the pancreas. They made it and the healthy tissues. Now what we made in the Petri dish is injected or transplanted into the diabetic patient who is unable to produce insulin.

This is a simple example. You may wonder, oh, an insulin injection is available in the market. Why should I do all these things? But if it is successful, it is easily done. You don't have to inject every day. You don't need to waste your money. Spend one time for the rest of your life; your body is producing insulin.

Think about it. And sometimes, every treatment and every lack of an enzyme are not available for purchase in the market. Insulin is fortunately available, but not some other genes. So the implication you should understand is rather than a case example. In a healthy person, once it is taken out, the blastocyst is kept intact and is implanted into the uterus of a surrogate mother. Instead of taking the inner cell mass, you put it into a

surrogate mother's womb, resulting in a baby that is a clone of the healthy patient.

So this baby, what you are seeing is an exact copy of that person. And you may have heard about this famous Dolly, which was done by Ian Wilmot. So now let us understand cloning. Human embryonic stem cells have enormous promise for treating a wide range of diseases, not just diabetes. However, stem cell research has raised profound ethical issues.

Very few countries have permissive policies towards human reproductive cloning. However, many permit embryonic stem cell research. So there comes the importance of Yamanaka, who, without touching the oocyte or anything, can simply take a cell and express Yamanaka factors; it will become a stem cell much faster and easier, making it a more adaptable technique. Now let us understand that different kinds of cloning exist. Cloning in nature does occur, and we can think of those scenarios as cloning through asexual   reproduction.

It is a very common form of multiplication. We have seen in planaria regeneration that when planaria are cut into multiple pieces, each piece develops into a full-fledged planaria, which is nothing but a clone. Like plants, you can do it. You take the branch of a plant, but you have a very beautiful and very tasty mango tree. And you cannot take the seeds   of   that   mango   tree   and   expect   the   same   quality.

You can't. It would have changed. But if you want the same quality, you have to take a branch of it, make a bud, and grow the roots, and then only will it become a clone of that tree. So keep that in mind. So asexual reproduction includes budding in jellyfish, corals, and tapeworms, fragmentation in worms, as seen in planaria, and parthenogenesis. Some fish, insects, frogs, and lizards do. Parthenogenesis basically means a female can lay eggs.

That can automatically give rise to a full-fledged animal without fertilization. So can the Komodo dragon; many lizards can do that. So there is an evolutionary advantage. That doesn't mean that they don't do sexual reproduction, but they can also do asexual reproduction via parthenogenesis, which will essentially be a clone of this mother. Though asexual reproduction in mammals is not a naturally occurring phenomenon, genetically identical individuals, such as identical twins and homozygotic twins, are known   as   monozygotic   twins.

So they do occur. These can, however, not be considered clones; they are basically clones of each other, technically considered clones in the strict sense, simply because they are not the result of asexual reproduction. They are a result of sexual reproduction,

but instead of one individual, we end up having two individuals. Sometimes, maybe even more. But they share the genetic material, whereas the artificially produced clones only share their core DNA. Why? Because you are taking the genotype or the nucleus of a fibroblast cell and putting it into the oocyte of another individual, the mitochondrial DNA in the oocyte is from someone else.

In the case of identical twins, mitochondrial DNA is also identical. So typically, when you say "clone," you should understand that the mitochondrial DNA is not donated from the donor; only the nucleus is taken from the donor, while the mitochondria belong to the oocyte. So that can have some slight variation, whereas the mitochondrial DNA differs in the case of an actual clone but not in the case of identical twins. Another option we have seen is sexual reproduction; another is embryo splitting.

The first cloning experiments were done on animals. Dates from the 19th century. In 1891, Hans Driesch mechanically separated the blastomeres and cells formed from the first stages of embryonic development of two-cell embryos of sea urchins by shaking them in seawater, just a mechanical shaking. The cells grow independently and form two whole sea urchins, which are nothing but clones, just like how monozygotic twins form. A few years later, 11 years later, the same experiment with similar results was performed by Hans Spamman, a famous developmental biologist in a vertebrate salamander.

He did earlier; Hans Dresch was done in sea urchin. Later, he did it in a salamander using a hair from his baby boy because kids' hairs will be very thin and he used it to make a knot and split it. However, the unavailability of an efficient handling system and, more importantly, the lack of recognition that mammalian oocytes and pre-implantation embryos require strictly controlled temperature and development hampered this research application from pushing into the mammal system. Whereas salamanders, sea urchins, etc., it is possible.

And it was stagnant for almost 80 years. For around 8 decades, it was frozen. Means not much progress. Eventually, the first successful embryo splitting was performed in domestic animals to rapidly multiply valuable individuals. It has huge implications. For example, think about an animal that gives birth to only one baby in one delivery.

So if you can artificially split it into two and implant them, you end up getting two embryos. So if it is a business firm, instead of getting one baby, if they get two babies at the same time and with the same expenditure, it's a profitable business for producing quality farm animals. Embryonic cell cloning, which is another method of cloning and a completely different approach to asexual reproduction, was discovered by accident in an experiment conducted by Jacques Loeb in 1894. What Loeb discovered was a primitive

model for all future nuclear transfer experiments. We have seen what John Gordon has done with nuclear transfer, and we also saw that in therapeutic cloning there is nuclear transfer.

But nuclear transfer became one of the standard procedures of animal cloning. This was the pioneering experiment proving that embryos could be created by moving the nucleus from one cell to another. That means a differentiated adult skin cell nucleus was pushed into the oocyte using his more consistent hair loop technique; what he did was further matured to create the first transient and later complete separation. Initially started with the formation of a dent by making some knots. And later, after complete separation, he repeated the same experiment in a salamander embryo and found that you can consistently do this embryonic cell cloning in salamanders.

At Cambridge University, Steen Malte Willadsen, the scientist, has repeated these experiments, which were previously demonstrated by Loeb. Experiments conducted in sheep, from salamander to sheep, after improving the technology in much greater detail, but without any significant success, led him to decide to use oocytes, as transferring them from one cell to another and implanting them to create embryos can be challenging at times. Using oocytes, such as egg cells, is preferable to using zygotes; although an oocyte is a cell that contains all the cytoplasmic determinants, using a zygote is much better. A zygote is nothing but a diploid, so after fertilization, the cortical reaction and other events of sperm entry have occurred. Better-developed scenarios persist in a zygote than in an oocyte.

Because oocytes are not fertilized, They are just unfertilized eggs. So oocytes are recipients. Since Wlladsen's experiment, pioneering initial work, the same principles have resulted in the births of embryonic cell-cloned offspring in other domestic species, including cattle and pigs. Ian Wilmut has worked with sheep and has used a more advanced technique called somatic cell nuclear transfer. What did he do? He took the principle of Hans Spamman, who suggested that one could transfer the nuclei of more or less stage embryos approximately four days old and/or older nuclei from any cells—a skin cell, heart cell, liver cell, or any cell—into an enucleated egg.

Enucleated egg means one whose genome has been removed, and you are replacing it with a new egg. Using frogs, researchers removed the nuclei of the recipient eggs and inserted a donor nucleus. Initially, the donor nucleus was obtained from more or less stage embryos. To start with, they took it from a morula of one or a few cell stages from a donor embryo and put it into the. Eggs of another embryo later produced the same experiment from a tadpole, which is not a morula but a free-swimming larva, and also from the intestinal epithelium of an organ.

Any cell's nuclei are good enough; the procedure resulted in considerable success in the early development of the embryos. But at that time, people also did not know much about it because these are all externally developing organisms. But you are talking about mammals. What about that? Scientists did not attempt to use adult somatic cells as the nuclear donor in mammalian experiments performed between 1986 and 1997. Until that time, people did not conduct any such experiments even though there were some earlier publications that showed that the cultured cells of the embryos at an advanced stage of development could be used as donors for nuclear transfer.

That means if you have a cell line made from a person who died several years ago, we can bring that person back to life. He or she is living in a Petri dish. You may have heard about HeLa cells. Of course, HeLa cell is a cancer cell that is from a lady, Henrietta Lacks. But if it was a normal, non-cancerous cell, and that cell line is there, you can bring that person back to normal.

And the birth of Dolly, the cloned sheep, was achieved by Ian Wilmut for the first time using cells taken from the udder of a donor. So it also has huge biomedical applications. The most significant potential of farm animal cloning is the biomedical application. A lot of things can be done, such as producing vaccines from milk or having specific proteins produced in the milk, etc.

Can be done. Since the 1980s, genetically modifying mammals by injecting copies of desired genes into one of the two pronuclei of the zygote has been possible. Any genes can be inserted, despite various alternative attempts. SCNT, or somatic cell nuclear transfer, is currently the most efficient way to produce genetically modified farm animals and other mammals. By introducing the genetic changes into cells and then choosing the ones with the desired changes for the cloning procedure, other and more precise modifications can be introduced into the genomes of farm animal species.

In the US, people clone their pet animals. If the pet animal is dead, they will be sad. They want exactly the same thing. So they take the tissue of the dead animal and they do make the clone of that pet and be happy that, you know, my animal came back. On the other hand, from a strictly economic perspective in biomedical applications, the inefficiency of the technology is a minor problem that is lurking in the system, since the animals that are created will have relatively high commercial value, so these are points one has to consider when venturing into a. Cloning applications, as I already told you, have huge implications in regenerative medicine, such as we discussed in the case of diabetes.

Additionally, the SCNT can be used for the rapid multiplication of individual transgenic animals known to carry a desired gene. You want 10 animals in no time; by doing embryo splitting, you can make 10 animals and then grow them happily outside. So two applications that seem to be most realistic within the next three to five years are the creation of various disease models. So a third application, which you can see, is xenotransplantation. That means it's often mentioned when discussing the perspective of farm animal cloning, say a pig; you have a pig on the farm, but you are introducing some genes that are humanized.

We call it a humanized pig, so you introduce some of the MHCs from humans so that when you bring that organ from that pig into a human for regenerative medicine purposes, it will not reject; that pig's kidney or heart will not be rejected in the human. So, they use it; it's often referred to as humanized. It's often done for the so-called xenotransplantation. For this, the cloning approach is usually used. When you look into the disease models, they are animals designed to express either at the genotypic or phenotypic level for a particular human disease.

They can be used to further understand the disease and to conduct initial tests as possible treatments. Sometimes when humans have a disease, you don't know which organs will be affected. You have no idea because you cannot do experiments on humans. So the best is if you can model an exact copy of that disease in a cloned animal, and you can study how this disease is progressing.

Since the 1980s, many mouse models of human diseases have been developed. However, these models have limitations due to the physiological differences between mice and their limited lifespan. Mice don't live as long as humans. So if a disease lasts up to 60 years, the trouble starts; mice cannot live up to 60 years. Genetically modified farm animals may offer a solution to this problem.

Many farm animals can live much longer than mice. An example of this is a sheep that expresses cystic fibrosis, which is studied or envisaged. Sheep, especially pigs, are ideal animals because of the similarities in physiology and size of their organs to those of humans. Moreover, we should understand that farm animals are relatively inexpensive; breeding and maintenance are well established, and their relatively long lifespan allows us to study many human diseases that manifest exclusively in the later stages of life.

Furthermore, the pigs... They are traditionally used as models for xenotransplantation experiments, so we should keep those points in mind. There is an increasing number of candidate genes available for establishing possible models of human diseases. Examples include neurodegenerative diseases such as Parkinson's and Alzheimer's, skin alterations,

and   psoriasis.

Epidermolysis bullosa is another example. There are many such experiments. We can study extensively that will help in designing strategies in regenerative medicine, as they are suspected to be related to a given genetic background, similar to that of diabetes mellitus. Nobody knows why a person gets type 2 diabetes, although we know what type 2 diabetes is and what the genetic background lying behind it is. These things need to be addressed, including atherosclerosis, breast cancer, and other cancers, as we know many genes involved. Given the huge biomedical potential, the considerable economic value, and the relatively positive public acceptance of such disease models, the area is undoubtedly among the fastest growing fields of somatic cell nuclear transfer technology. So these have importance in fixing the troubles in the human context, and that will contribute to regenerative medicine, so we can study more in detail about other aspects of regenerative biology in the next class. Thank you.