Tissue_Specific_Gene_Expression

Hey, I was told you were in the Animal Room.
I'm glad you came.
They're so small and pink. How old are they?
Actually, I think these ones are a couple of days old. And they're pink because they're hairless. They should be growing hair within the next week to look just like their mother.
It looks like they're feeding. They must be hungry.
They sure are. This is why we keep them next to the mother.
Does the mother always produce milk?
No, she doesn't. Actually, only after giving birth, the breast cells receive certain cues to start milk production. For example, casein, a protein found in milk, is only expressed in these cells at this specific stage. On another note, insulin, which is an unrelated protein, expressed in the pancreas, is never produced in breast cells, not at any stage.
Hmm. Can you explain a bit more?

Hi, Elia.
Hi, Rabih.
[INAUDIBLE] what are you up to?
I'm actually feeding the cells.
Ah good. These are the breast cells?
Yes, the mouse breast cells.
The mouse breast cells.
Yes.
Can I have a look at them? How do they look?
Actually, they're great. I'm sure-- they're for next week's experiment.
OK. This is what day in culture? This is day two in culture? Yes.
OK. So can I have a look at them?
Sure.
OK.
The color looks good.

Hello to all. To understand why milk proteins are expressed in breast cells or what we also call the mammary gland, and not in other cells and other tissues, I'm going to have to take you through a trip into the cell and all the way to the nucleus. The nucleus is a fascinating organelle

that I'm sure you all know houses the DNA, which contains our 25,000 genes.
But that's not all there is in the nucleus. In addition to the DNA in the nucleus, there's a whole array of proteins. And these proteins interact with the DNA and dictate a specific architectural design of the DNA in the nucleus.
And this plays a major role in dictating tissue-specific gene expression. For example, the architectural design of DNA in the breast cell is in such a way that the milk proteins are expressed, but this is not the same design that is found in other other cells-- for example, in pancreatic cells and the other.
Now before we start this trip into the nucleus, I'm going to state one fact and then ask you one question after that.
All somatic cells have the same 2n DNA content but express different genes in different tissues and across development within the same tissue. Since all cells have the same genes, then how do they acquire different functions and even look different?
Going back to our question, since all cells have the same genes, then how do they acquire different functions and even look different? The answer to this is simple. Since the different cells express different genes and therefore make different proteins, the way the cells appear will look different depending on the tissue that they are coming from.
For example, if we look at the four panels here, on the top left hand side, you will see cells at different magnifications. But the one on the top left hand side are the breast epithelial cells. And you can see they look quite differently from the cells of the inner ear, with the stereocilia on top, projecting from these cells. The panel right next to it shows you these same stereocilia at larger magnification.
The other panel at the lower left hand corner of the slide, you will see a neuronal cell, with all its branching. And then the one to the right, you will see the large egg interacting with many sperm cells. From this, you can see that the cells in different issues do look different and behave different. Now, before we explore this any further and take you into the cell and then into the nucleus, where the DNA is housed, I will leave you again with few questions and then we'll come back to you, later.

Now that you had the chance to go over the questions and analyzed your answers, let's have a look at what the answers really are. You will note that you cannot see a cell with the naked eye. It's about 20 microns

in size, that is, 0.0002 centimeters. And the nucleus is about half the size of the cell.
In your body, you have about 50 trillion cells, that is, 50 million-million cells, not counting the micro-flora or the bacterial cells on your skin or the normal micro-flora in your digestive tract. Having said all of this, let's look really at how big the cell is. Let's try to have an analogy to things that we are familiar with.
So this series of slides, you will note that someone is holding a pin in between his fingers. And you can clearly see the pinhead. Now as you start zooming down, you will see that there is a human hair on the pinhead that you didn't see before.
As you zoom down further, you will see a dust mite. You roll down further, you will see a ragweed pollen. And by the time, you will have assumed about 10,000-fold and enlarged the pinhead, you will note that you will start seeing the cell that we are interested in.
This is what 20 micrometers are all about. It's something that you cannot see with the naked eye. And the nucleus is half the size of that, and it is planted within the cell. I will now ask you to draw on a blank sheet of paper a cell with about the size of a 20-centimeter diameter, assuming that this is equivalent to 20 microns.
After that, I will ask you to draw the organelles in that cell that you are familiar with. That is the nucleus, the Golgi apparatus, the ER, the mitochondria. Draw them to scale, and then from that try to guess the approximate size of each of these organelles.

By this time of the year, you should have studied DNA replication, DNA folding and structure, gene structure and function, and protein synthesis. I'm sure you also recall that in every human cell, you have 23 pairs of chromosomes in the nucleus and that the DNA is folded around histone proteins and packaged into the nucleus.
Well, here is the problem. Inside the nucleus of each cell, you have 3 billion nucleotides that make up the entire length of your chromosomes. These 3 billion nucleotides, if you stack them right next to each other, they would extend to be two meters in length. Of these two meters, you have your 25,000 genes scattered across the entire length of the DNA. And this only constitutes about 5% of the entire genome.
The remaining nucleotides are what we call as noncoding sequences. Now keeping in mind that every cell expresses different genes-- for example, the breast cell expresses milk proteins, pancreatic cells

expresses insulin, so on and so forth-- how does the cell manage to package the DNA inside the nucleus to ensure that every cell expresses a different set of genes that is specific to the tissue that it is in.
I will leave you now with two questions that would help you understand the organization of the DNA inside the nucleus. After you attempt to answer these questions, I will come back again and try to explore this issue further.

Going back where we left off with the questions that I posted before, there are two facts that you need to be aware of. The first one is that DNA packaging in the nucleus is not random. And the second one is that DNA packaging and organization differs between cells of different tissues, meaning that organization and packaging of the DNA in the breast cell differs from the organization and packaging of DNA in a pancreatic cell that expresses insulin.
Why is that? Before I attempt to answer this or you attempt to answer this, let's try to go back to the chromosomes, the 23 pairs of chromosomes, that are found in every human cell. The basic question we were asking is that why is it that a specific gene, for example, a milk protein gene-- is expressed in the breast cell, but not in the pancreatic cell?
For all practical purposes, let's assume that the gene of interest is found somewhere on chromosome number eight. And I just picked this up at random. And we want to try and understand why is it that this gene is expressed in the breast cell but not in the pancreatic cell. To attempt and answer this, I'm going to have to move to the other end of the table and lay out the three factors that regulate gene expression in a cell.
What we have here are playdough models, basically, of the RNA polymerase, which is a housekeeping gene and it's expressed in every cell. And it is the one that binds to the promoter of the genes that are to be expressed. We also have here playdough models of the transcription factors.
The transcription factors, they vary from one cell type to the other. For example, in the breast cell, you may have orange, green, and red transcription factors, whereas in the pancreatic cell, you may have different color transcription factors. So the transcription factors vary between the tissues, but the RNA polymerase doesn't.
The third element that is needed for gene expression, obviously, is the gene itself. Now for all practical purposes, let's assume that what I'm

holding here is actually chromosome eight that I had just talked to you about. So if now this is chromosome eight and the gene of interest you can see here is highlighted for you, what you need for this gene to be expressed is that the RNA polymerase must bind to the promoter sequence of the gene. In addition, the right combination of transcription factors should also bind to the polymerase and/or to the DNA.

Once this complex assembles at the level of the promoter of the gene, then the gene will be expressed into a messenger RNA and then translated into a protein. So if this is one of the milk proteins, casein, for example, once this transcription complex assembles at the promoter level, the mRNA for casein will be made. And then the protein casein will be made after that, leading to a breast cell expressing the casein milk protein.

Now the question is why is it that one cell expresses it but not another? Is it only dependent on the presence of these transcription factors? We should keep one other thing in mind, and that is the complexity of the organization and the space limitations inside the nucleus for the organization of the DNA, and whether it is exposed or not.

Now, if you would take this chromosome and you would wrap it in such a way that the polymerases can no longer access the gene of interest, then this gene will no longer be expressed. So how does the cell regulate this, and how does it expose the needed genes in the right cell at the right time and how does it hide them? That's why I need to introduce you to a new player in the game of gene expression in the nucleus. And that is the nuclear scaffold, or what we also call as the nucleoskeleton. This is a structure that has been recently, in the past 20 years or so, that has been defined inside the nucleus. It spreads throughout the nucleus. And it's a scaffold, basically, but the interesting thing about it is that it is flexible and it is dynamic, that is, it is capable of changing, depending on the cell's needs.

And may I remind you that the DNA is wrapped around this scaffold with its histones and all. It wraps itself around this scaffold, and then the wrapping of the DNA itself becomes dynamic and flexible as well. Having said this, I'm going to go back and leave you with the original question that we started with. And that is how do cells regulate which genes are expressed in what cell and at what time?

I'm back to our model. Let's try and answer the question that I left you with. And that is, basically, why is it that this gene-- the milk protein

gene-- is expressed in the breast cells, but not in the pancreatic cells .
We have introduced a new player, the nuclear skeleton, in addition to the transcription factor and the RNA polymerase
Now as I mentioned before, the chromatin wraps itself around the nuclear matrix, in such a way that certain areas of the chromosomes are exposed, such as this one now, it is quite exposed. Or alternatively, it could be tucked away and not available for binding by the RNA polymerase or the transcription factors.
Now let's assume that this is actually the nuclear matrix of a breast epithelial cell that is actually producing milk. Most likely, this gene would be exposed in such a way that the RNA polymerase can bind to it, and so can the transcription factors bind at the promoter site. This, as I explained before, will then trigger the expression of this gene.
The other scenario would be that if the gene needs to be shut down, then the nuclear matrix can undergo a different type of folding, the gene can be tucked inside in such a way that the RNA polymerase can no longer access it, nor can the transcription factors. So this will shut the expression of the gene down. And alternatively, if it is exposed like I have just showed you, it can be expressed again.
So this is basically how genes are regulated in tissues. The gene needs to be exposed. The right transcription factors need to be present. And RNA polymerase is also there.
Now there is one other factor that we did not talk about, which is not the topic of this presentation. And that is, the gene itself can also be chemically modified by methylation or other. And this will also affect the binding of the transcription factors. But this is not something that we talked about at this point. We are now concerned with introducing the structure of the nuclear matrix.
In conclusion, what regulates tissue-specific gene expression, then, are two factors. Namely, the first is the presence of the right combination of transcription factors, and then the other one is the gene being exposed and available for binding of the transcription factors. This is mainly regulated by the nuclear matrix which can either expose the gene or tuck it away and block its expression. I hope you enjoyed this module and you've gained some new insights into tissue-specific gene expression.

Teacher's Guide. How is it all cells in our bodies have the same genes, yet cells in different tissues express different genes? Throughout this video, students are provided with five exercises aimed to demonstrate, towards the conclusion of the video, the role of the nuclear matrix in exposing a gene or hiding it, and how that contributes to regulating tissue-specific gene expression.

The teacher is expected after each exercise to spend time with the students, analyzing their answers, and discussing with them the outcome of their answers before moving on with the video towards the next exercise. In the upcoming slides, recommendations are made on how best to analyze the outcome of each exercise, but individual teachers may find better ways and are encouraged to use what they see best fits their students.

Exercise I. Since all cells have the same genes, then how do they acquire different functions and even look different? To answer this, students can break into groups or discuss the possible answers with a teacher. Here the students ought to become aware, if not already aware, of the difference between housekeeping genes and tissue-specific regulated genes.

The expression of the latter imparts on a cell specific functions and shapes. This is illustrated in the example of the four cells that are shown in the video. Namely, breast cells, inner ear cell, neuron, sperm and ova. Note-- the images for these cells are colored due to the fluourescence-based imaging technology, as in breast and neuron, or visualized via scanning electron microscopy-- as in inner ear cell, ova and sperm. The latter allows for a greater degree of magnification.

A basic notion in biology that most high school students fail to conceptualize is the fact that all cells in the animal or human body contain the same DNA yet different cells in different tissues express different genes. Genes can be broadly divided into two classes. One, housekeeping genes. These are needed to sustain basic functions of a cell, such as nutrient uptake, protein synthesis, DNA synthesis, and other basic metabolic functions.

Two, tissue-specific genes. The expansion of these genes vary depending on the type of tissue and the stage of development. These genes are responsible for the synthesis of proteins, specifically produced by one cell or tissue type, but not by the other, and at a certain stage in development. Milk proteins in breast cells, insulin in pancreatic cells, et cetera.

Exercises II and II. How big is the cell? In the second exercise, students are asked four questions that basically alerts them to the fact that the cell cannot be seen with the naked eye, and that it's about 20 micrometers in size. In the third exercise, students will be able to guess the size of all cellular organelles by sketching to scale the cell and benchmarking the size of the organelles with the actual size of the 20 micrometer cell.

The intent of these two exercises is to allow the teacher to guide students into the nucleus, while appreciating the small size of this organelle. Nevertheless, and despite its size-- 10 micrometers or so-- the nucleus can efficiently package the entire DNA in a manner that is highly organized, as will become evident to the students as they proceed through them the video.

Note, for Exercise II, it may be a good idea to tabulate the student responses and show them how many got the answers right vs. those that go it wrong. This can be followed by discussion of the results.

In Exercise III, the teacher should be after approximate values for the size of organelles, rather than specifics. For example, a nucleus is about 10 micrometers. A mitochondria is about 1 micrometer. The membrane thickness is few nanometers, so on and so forth. These measures will alarm the students as they learn that the DNA a is 2 meters in length and has to be packaged in an orderly manner inside the nucleus.

Exercise IV. DNA packaging and organization in the nucleus. In this exercise, students will most likely answer the questions posed correctly, i.e,., that the DNA packaging is not random and that its packaging differs between tissues. At this point, it is a good idea to discuss with the students how such an organization can be achieved, maintained, and modulated.

The teacher can at this point suggest that the existence of a tiny scaffold in the nucleus, later to be identified to the students as a nuclear matrix, also known as a nuclear skeleton, will allow the cell to achieve the packaging and organization it needs.

Exercise V. How does the cell expose and/our hide the genes that it needs to express or shut down? In this exercise, it is important that students understand that the nuclear matrix helps in the organization and packaging of the DNA. In addition, and most importantly, students should be aware that the nuclear matrix is also a dynamic structure. As it changes its architecture, the DNA also changes its mode of packaging, thus exposing or hiding genes of interest.

Note, as a teacher, please remember that other factors also affect DNA packaging and gene expression. Examples are histone and non-histone

proteins and DNA methylation-- epigenetics. But these topics were not discussed in this presentation. This was mentioned in passing at the closing of the video, prior to the concluding remarks.
Next slide demonstrates, in a schematic diagram, what the nuclear matrix looks like. A transmission electron microscopy image of the matrix is also shown.