Biomaterials for Bone Tissue Engineering Applications
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Module 5
Lecture No 22

Let us continue the discussion on the cell signaling. So after addressing the two points that I have mentioned in the last module that what is the critical distance and how this signaling molecule concentration on the target cell both are important in determining the cell cell communication.

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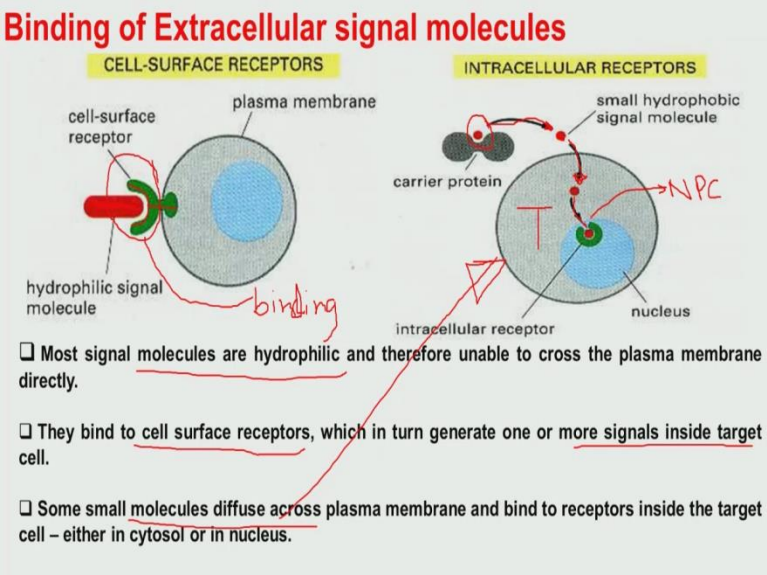

Now the next step, once these two questions are addressed, the next step of understanding would be how cell surface receptors would be linked or would be tagged or would be hooked to signaling molecules. Now there are two types of signaling molecules that has been shown in this particular slide. One is the cells are the hydrophilic signaling molecules and another is that small hydrophobic signaling molecule. If there is small hydrophobic signal molecule, fist of all because of their hydrophobic nature and secondly because of their small sizes they can be internalized directly into the target cell.

So this is your target cell here, so this is your target cell t, so this is your signaling molecule that we are talking about, this is small hydrophobic, so because of these two reasons they can be transported, they can be internalized into the target cell rather very easily. And again through the

nuclear pore complex if you remember here in the nuclear in the nuclear membrane, you have a very characteristic nuclear pore complex.

Through this nuclear pore complex the signaling molecule can be internalized well within the nucleus also. And therefore after it can cause some changes in the gene expression of the target cell. But the story is little different when we consider that the signaling molecule is hydrophilic in nature and it is a bit larger in size compared to the small hydrophobic signal molecules. Then the only option that is biologically possible that is it will be hooked to the cell surface receptors and therefore this binding efficiency comes into picture.

So this is your binding here, so binding essentially is a established between the the the hydrophilic signal molecule and cell surface receptors. So certain comments that is mentioned here that most signaling molecules are hydrophilic in nature and therefore they cannot cross physically to the plasma membrane directly. And in turn they are bound to the cell surface receptors which in turn generate one or more signals inside the target cell. The third point that has been described with reference to this particular scenario that when there is a small hydrophobic signal molecule then it can cross a plasma membrane, both of the cell membrane and also in the nuclear membrane and can directly be then internalized into the nucleus itself.

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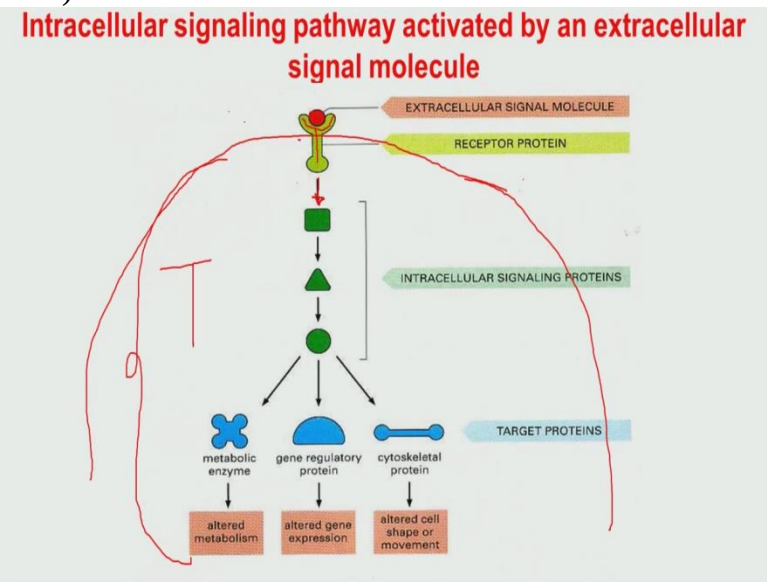

So that once it is once this hydrophilic signaling molecule is attached to the cell surface receptors here. So that is the cell surface receptions that is the kind of trans membrane protein, then what will happen? This attachment will further sense out the or will further activate the downstream intra cellular signaling proteins. So these intra cellular signaling proteins are shown here by different geometric separate objects like rectangle, triangle, or circle.

So essentially these different shapes indicates that these proteins, that intra cellular signaling proteins will have either different chemistry or different confirmations that is within the cytoplasm of the cell. So if I may show you the target cell, so this is your target cell, cell membrane and this is your cytoplasm of the target cell. So this entire thing what I have shown here, this entire thing is happening well within the cytoplasm of the target cell and this is your outside the cell that is the extra cellular space.

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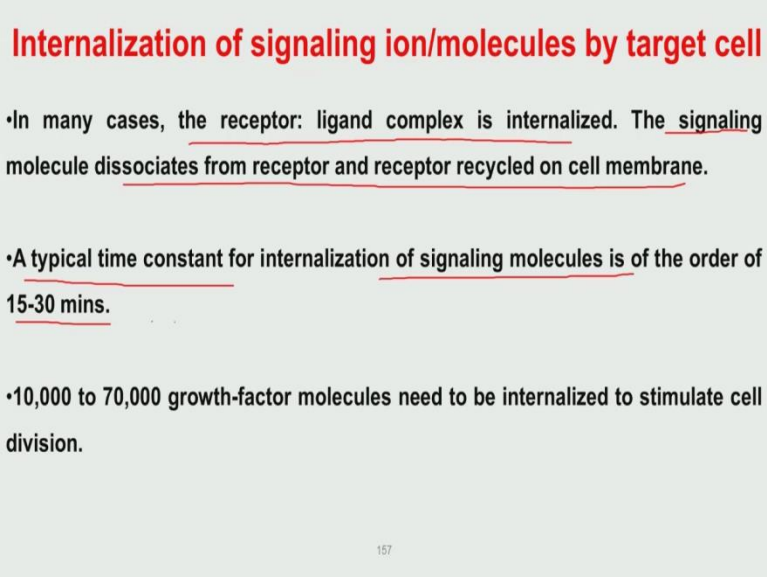

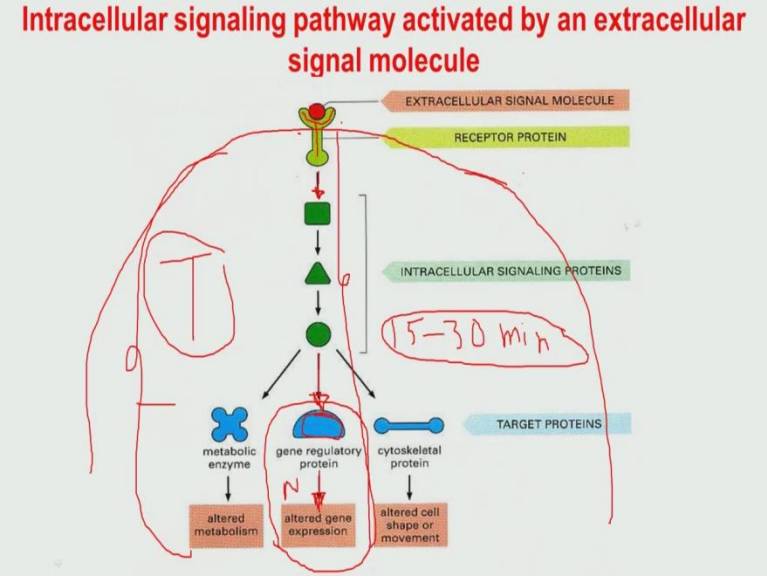

So some of the comments that I may like to make here that the receptor ligand complex, once it is internalized, so signaling molecules then dissociates from receptor and receptor recycled to cell membrane. A typical time constant for internalization of the signalling molecules is of the order of 15 to 30 minutes. What it means is that, so once this biological process gets activated, you have to know that what is the time scale over which this intercellular signaling proteins are activated and this time constant is somewhere between 15-30 minutes.

So within half an hour time period the inter cellular signaling proteins or downstream signaling proteins are activated within the target cell now. So this is your target cell. Now If the signalling signal transaction process had to activate or has to chase certain gene expression changes that means this has to enter the nucleus of the cell. So suppose this is your nucleus and this is your nuclear membrane, and there you have a nuclear pore complex is present, so that means this one of the signaling protein has to now travel through the nuclear pore complex to well within the nucleus so that this gene regulatory proteins are now activated, and the end result or the end point objective or the end point result is that this leads to altering of the gene expression.

So now from the basic description of this process you can see, now if gene expression has to take place then 15-30 minutes time scale is too small. So this entire process up to the inter cellular signaling proteins to activated and up to the gene expression will take few hours to few days. So therefore normally cell differentiation assess or cell differentiation process takes place from few days to few weeks time line, whereas simple metabolic activity or cytoskeletal reorganization

can take place from minutes to hours or maximum to few days in time. So certainly if any alterations of certain metabolic activity or to the cytoskeletal reorganization has to take place within the target cell, that takes place in a much shorter time frame than gene expression if the cell signaling has to activate or has to lead to the gene expression changes.

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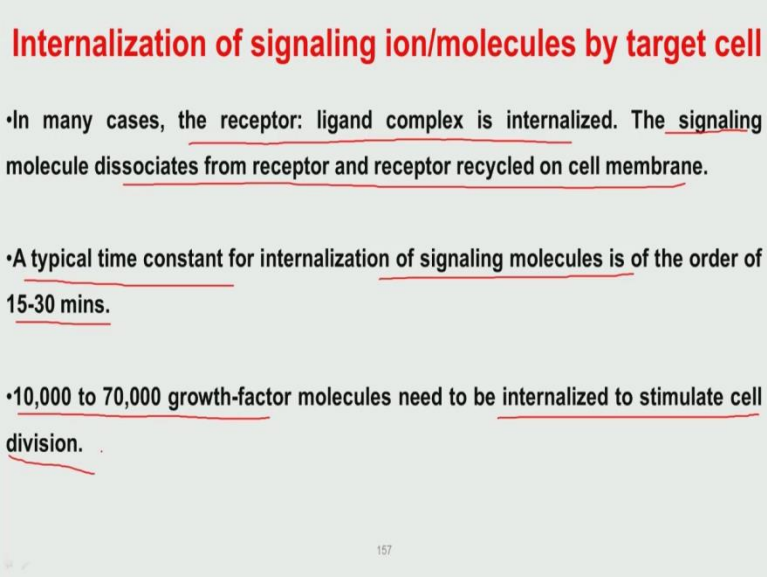

So this is mentioned here in this particular slide. Other things that has been mentioned here, 10000 to 70000 growth factor molecules need to be internalized to stimulate the cell division prices. So that means somewhere around 10 to the power 4 number if molecules are to be internalized so that cell division processes has to be activated in the target cell.

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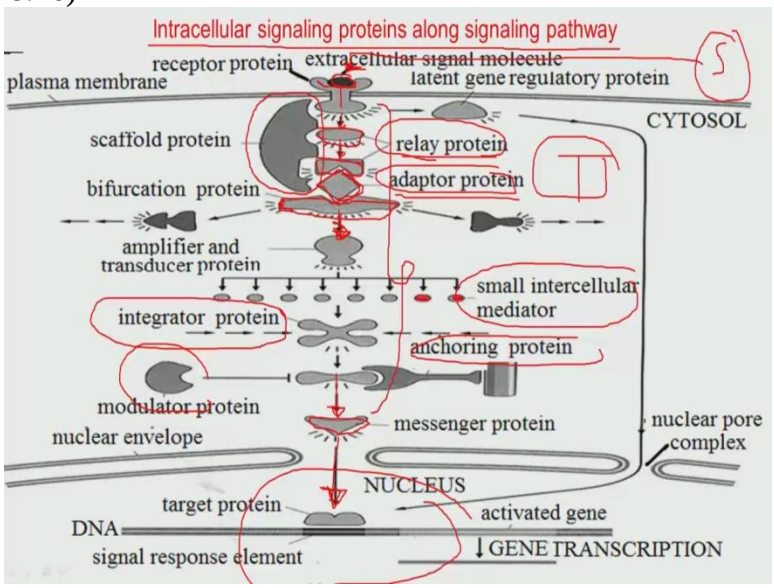

Ok, what are the more specific details of the cell signaling protein that has been summarized in this particular slide. So what you see here, it is very complex signaling processes that has been shown here, and in fact it is a busy slide, so let me spend some time to explain to you in a little bit briefly here. So what you see here that you have extra cellular signaling molecule and you have cell surface receptor proteins in a target cell. Ok? This is your target cell.

Now once this, so that moment that extra cellular signaling molecule get attached to the receptor protein, the switch is on. It is like you put your switch on in your home so that light is on. So similar analogy you can bring in here to understand the cell signaling process in a much more better manner. So I repeat that the moment the extra cellular signal molecule which is being transported from some far away from the source cell to the target cell here. Now if the moment the extra cellular signaling molecule gets hooked to the trans membrane receptor protein of a target cell, this target protein will be now switched on and not only this target protein will be activated but this entire signaling pathways would be slowly switched on or would slowly be activated.

And as a result the signal transaction processes will start taking place within the target cell. And as part of the signal transaction processes a number of downstream signaling proteins like relay protein, like adaptor proteins which are shown with different geometric objects here, that would be now activated, so the arrow essentially indicates, these kind of downstream arrows can surely

indicate that sequentially one by one these signaling proteins are now slowly gets activated. This slide also mentions something called scaffold protein. So scaffold protein essentially houses more than two three different types of proteins which are essentially really protein or adaptor protein.

And this relay protein, adaptor proteins take active part in the inter cellular, internalization of the signaling processes which essentially convey certain or certain communication from the source cell to the target cell. Now there is also certain other proteins which are present in the cell, within the cytoplasm also, it is like bifurcation protein. Bifurcation protein means the protein which will convey or which will channelize in two different ways from the adaptor protein itself. And the other types of proteins which have active role in the cell signaling process are called amplifier or transducer protein.

The name amplifier suggests definitely certain signaling molecule concentration will now be amplifies. What it means is that this large signaling molecule will activate very small inter cellular mediator proteins and you can see from the basic physical size of this inter cellular mediator proteins that this large number of inter cellular mediator proteins can be integrated to activate what is known as the integrator protein. So the role of integrator protein is to integrate all the information, all the signal intensity in the form of a anchoring protein now.

And this anchoring protein will be hooked to a modulator protein and if the gene gene expression is to be altered then finally the messenger protein will activate certain target protein well within the nucleus and this target protein essentially will activate what is known as the transcription process in the nucleus itself. So the transcription process is essentially defined is that transformation from DNA to RNA within the nucleus itself. And ultimately once this RNA is formed again the RNA is getting out of the cells through the nucleus pore complex and outside the cell, outside the cell nucleus in a eukaryotic cell, RNA can again transforms to protein.

So what is known as the translation process. So this entire transcription and translation process and many times reverse transcription process like it goes from RNA to DNA, all these processes are being activated once the appropriate signal transaction mechanism is activated well within the target cell.

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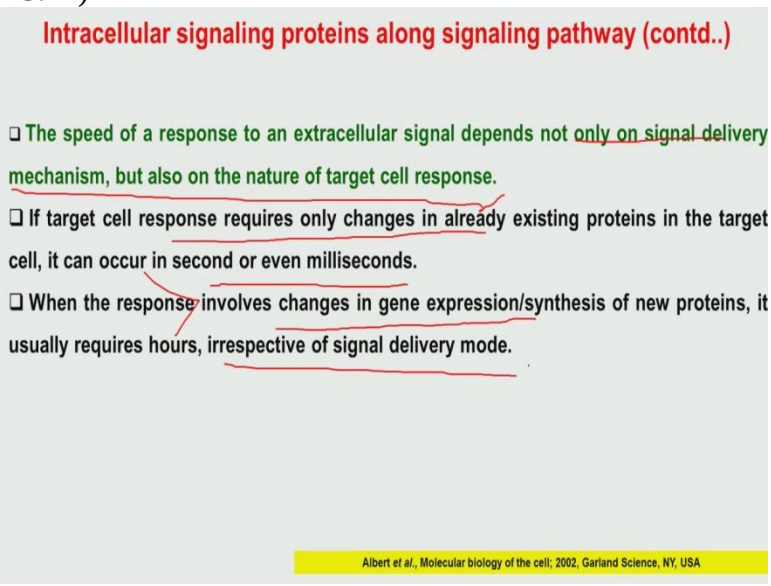

So from this previous description it should be clear to you that speed of a response to an extra cellular signal depends not only on the signal delivery mechanism but also on the nature of target cell response so that means if the target cell response to the external signal much faster then all these processes what I have mentioned like 15-30 minutes can be even little shorter also. So the two things that is important is how the target cell is receptive or is sensitive to a particular signaling molecule that it receives from a source cell and therefore what kind of downstream signaling proteins are activated and to what kind of complex communication network is also being activated downstream in the cytoplasm.

So that ultimately results in what is the time scale or what is the response time scale of the target cell itself. If a target cell response requires only changes in the already existing protein in the target cell, then it can occur seconds or mili seconds and when the response involves gene delivery or gene expression or synthesis of new proteins then it usually occurs hours irrespective of the signal delivery mode.

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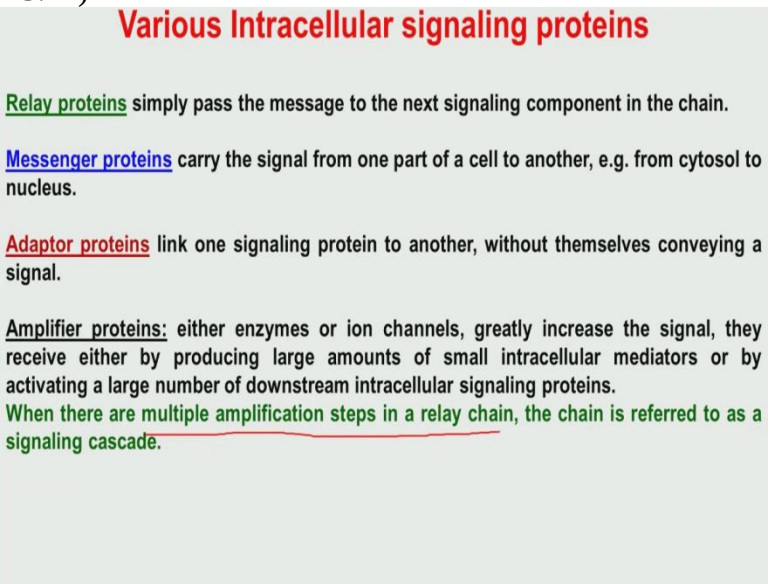

These are the different proteins I have mentioned in the last to last slide and in each proteins actually they have some specific role to play. When there are multiple amplification steps in a relay chain, the chain is referred to as a signaling cascade. So signaling cascade means when there are multiple proteins are involved then it is called a signaling cascade which is activated in the target cell itself.

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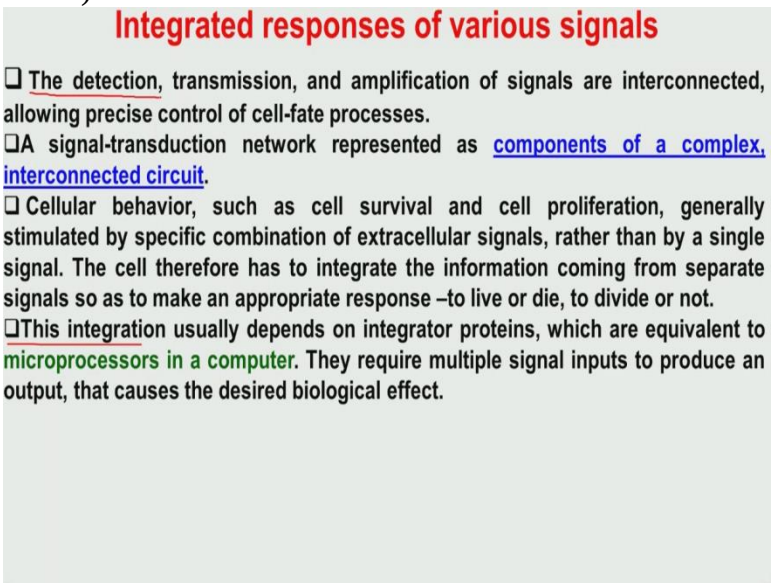

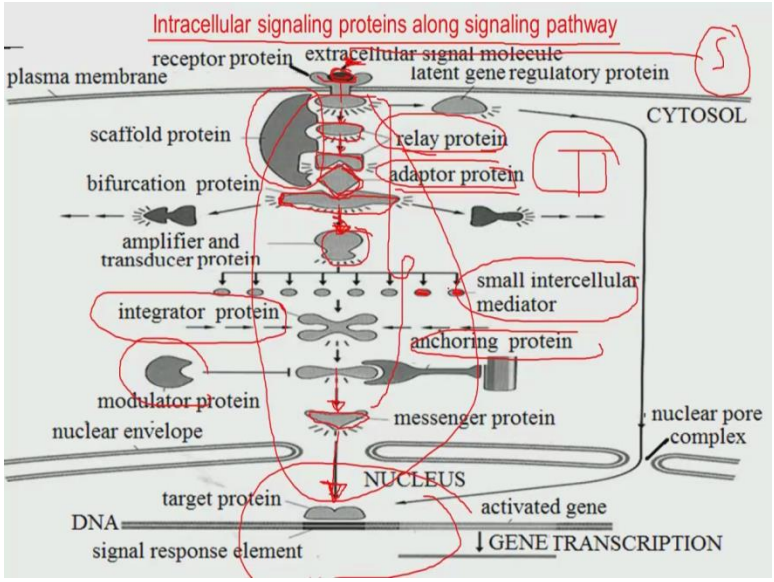

Ok so detection, transmission so detection means what is the so detection means that how what is the specificity or what is the sensitivity of a target cell to a specific signaling molecule. So what is the specificity of this target cell that it will sense this specific signal molecule which is transport, which has been diffused from the source cell at the target cell surface. The second thing is that transmission, now how the signal transmission takes place within the target cell once the cell surface receptor protein is activated or is switched on.

Third thing is the amplification of the signals. Amplification means if you go back to this particular slide you can see that there is certainly amplifier protein or transducer protein is being activated and these will activate in turn the small intra cellular mediator proteins.

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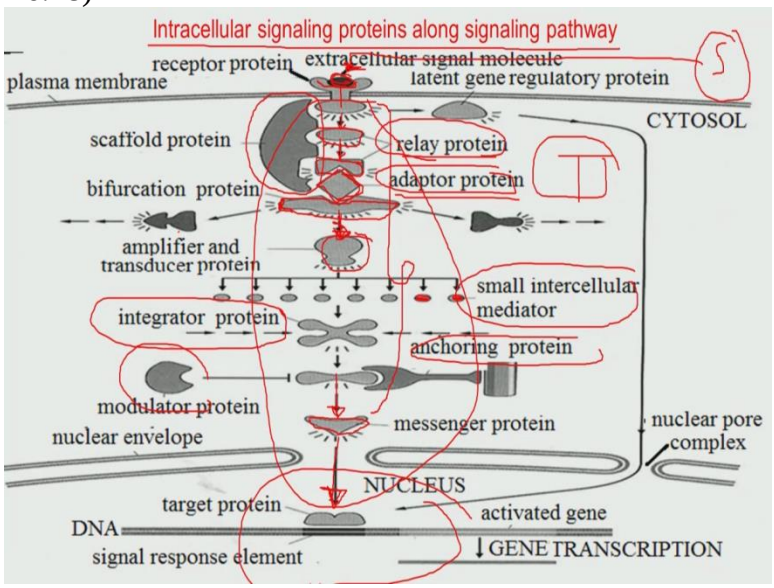

Integrated responses of various signals

- The detection, transmission, and amplification of signals are interconnected, allowing precise control of cell-fate processes.
- A signal-transduction network represented as components of a complex, interconnected circuit.
- Cellular behavior, such as cell survival and cell proliferation, generally stimulated by specific combination of extracellular signals, rather than by a single signal. The cell therefore has to integrate the information coming from separate signals so as to make an appropriate response –to live or die, to divide or not.
- This integration usually depends on integrator proteins, which are equivalent to microprocessors in a computer. They require multiple signal inputs to produce an output, that causes the desired biological effect.

So this signal transduction network therefore can be represented as the components of a complex inter-connected circuit. And what is the elements of the circuit like if you are electrical engineering you know that there is resistance, capacitance, inductance, these are like different components of electrical circuit. So therefore equivalent to the electrical circuit description you can also see that whether this relay protein, adaptor protein or amplifier protein, what kind of role it plays whether it will resist the way the signal transduction process takes place or whether it activates or whether it more acts like a capacitors or amplifier. So all these kind of things, that can be described by a complex inter connected circuit.

The other things that is important that I will explain in the next slide is that, cellular behavior like cell survival, proliferation, generally stimulated by a specific combination of extra cellular signals rather than by a single signal. Let me substantiate this statement in the next slide.

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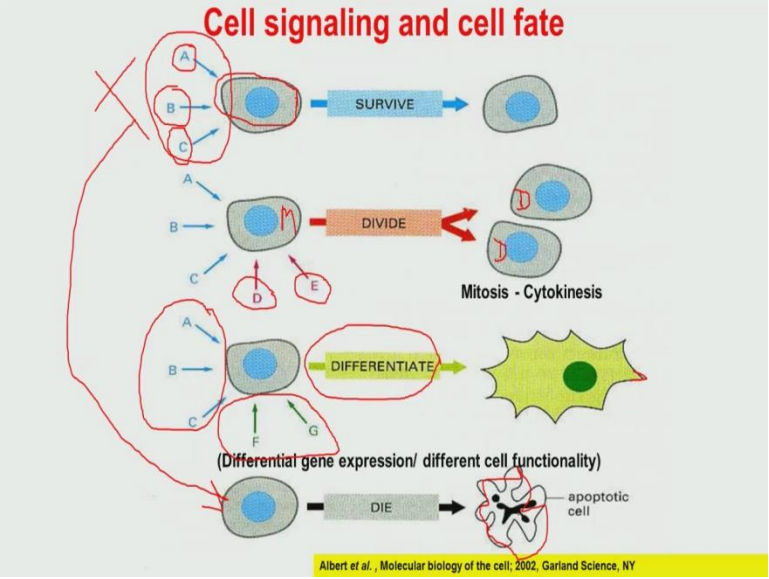

What it means is that, suppose this is one specific cell and this specific cell is receiving three type of signaling molecules from its cellular micro environment. That is the A, B, C these are three type of different signaling molecules and under the receipt of these three different signal cell molecules this cell now survives. So essentially cell survival depends on not only one type of signal molecule that is A but on three different types of signal molecules that is A, B, C. Now once the cell survives then cell has a tendency to divide into two daughters cells by process called cytokinesis mitosis.

And this is your mother cell and this is your two daughters cells. And this mother cell to two daughter cells that means cell division process has to take place. And cell division means you need the some extra signaling molecules here that is D and E. So the rationale behind this one is that first of all the hell has to survive and therefore you need A, B, C, three different types of cell molecules. Now once the cell has to divide in to two daughter cells that means cell needs some additional signaling molecules so that it receives the instruction for its division to two daughter cells.

So therefore cell division, signaling molecules or signaling network of the cell for the cell to divide essentially needs the molecules which are essentially required for survival plus the molecules which are required for cell division. Now third thing that I have mentioned here is the differentiation. So differentiation means it not only needs to survive but also it needs to change its gene expression profile. Therefore it goes to a more matured cell mature cell type which will have a altered, which will have a different cellular functionality.

So therefore differentiation needs two again additional two signal types that is F and G which is different from that of the signaling molecules which is required for a cell to divide. So A, B, C that is 3 for the cell survival and F, G these are like specific signal molecules which will stimulate the cell to differentiate to a mature cell type. So now you have 5 different types signaling molecules. One for survival and one for differentiation.

Now under certain circumstances in any cellular micro environment if you kind of remove all these three signaling molecules A, B, C then what will happen? Now cell is not receiving any signaling molecule and therefore it will activate its own suicidal mechanism which is known as apoptosis or programmed cell death. And that will be reflected in a very characteristic morphological changes. As you can see here it will be shrinking, blabbing and this is apoptotic cell.

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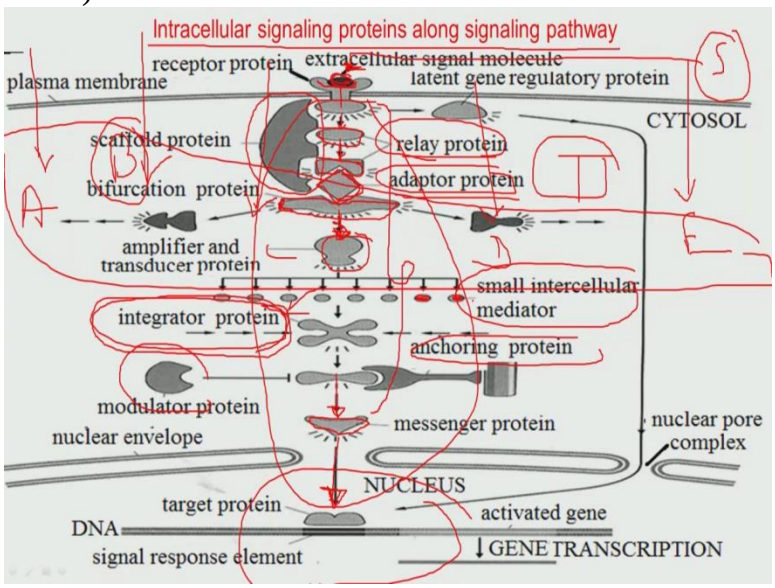

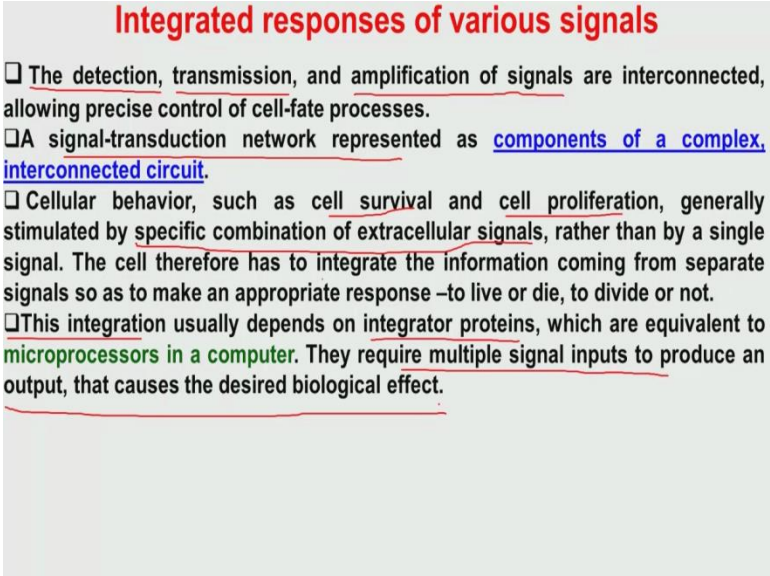

So the take on message from this slide essentially is that all the cell behavior is stimulated by specific combination of extra cellular signals rather than a single cell and a cell therefore has to integrate all these signaling molecules. And then integrate and therefore and there after it can it can survive. Now for a cell to divide cell has to integrate a, b, c, d ,e all the five types of signaling molecules and then final end point result is the two daughter cells.

Ok, so the integration usually depends on this integrator proteins. And this integrator proteins I have shown you before that integrator proteins are also present in the cytoplasm. So from different places the cell is receiving a to e let is say, three different, five different proteins here signaling proteins. So essentially that all these different proteins once it receives, so all five different signaling pathways now activated.

Now a, b, c, d, e all five signaling pathways now finally should be linked to a integrated protein which will integrate all the information and then finally will give the cell the required instructions for its division process to activate. And the required multiple signal inputs to produce an output and that causes the desired biological effect.

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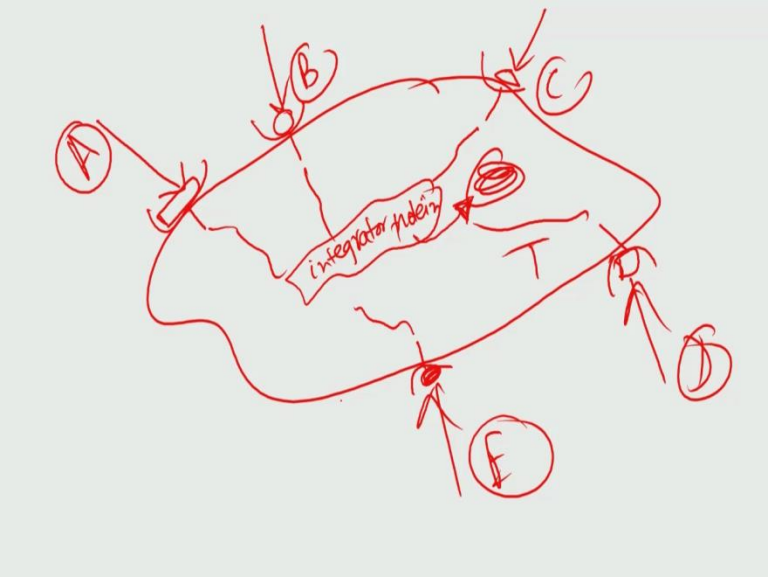

So what I mentioned here, so suppose you have a eukaryotic cell and this is your target cell. Now target cell is receiving different signaling molecule of different type let is say a, b, then c, then d, and then e all different signaling molecules. So all these signaling molecules will now get attached to a different type of trans membrane protein and then for simplicity or just to show the distinction between different signaling, to show the different type of signaling molecule, I'm showing you the different signaling molecule by different geometric object.

And so one is a circle, one is rectangle and all. Now in the target cell, now each time so all the all the five different signaling molecules once they are hooked to the cell surface receptors, then they will essentially activate or five different signaling pathways will now be switched on. So all these five different signaling pathways is to be now integrated by a called protein called integrator protein and this integrator protein is also present within the cytoplasm of the target cell.

So the role of integrator protein it is to integrate all the signaling information into a single information and that single channel now will be activated and the net result can be either cell differentiation or can be cell division. So this is what has been, this is what can be summarized from the cell signaling and cell transaction process.

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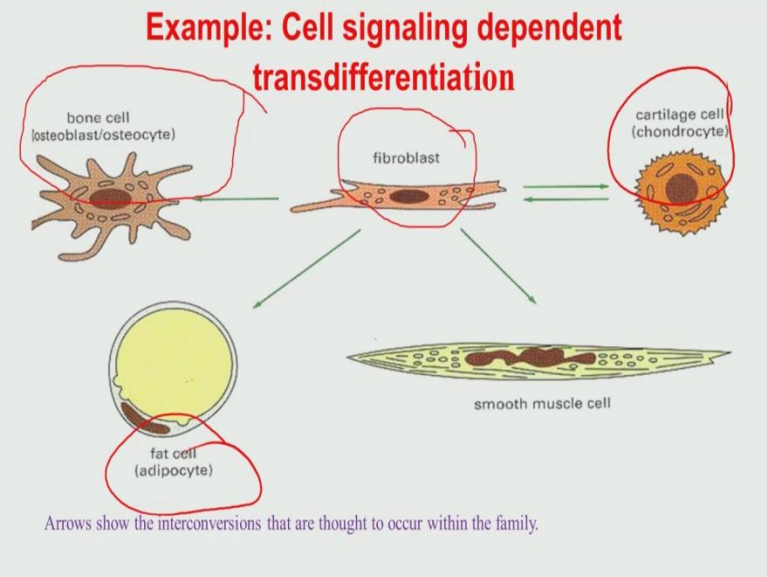

Some examples of the influence of the cell signaling , so you have a fibroblast cells, fibroblasts is the connected tissue cells and you have a nucleus and then it can either go trans differentiation to smooth muscle cells. Now smooth muscle cells are present in the typical muscle tissue as well as the cardiac tissue also. Or fibroblasts can go can undergo trans differentiation to osteoblast that is the bone forming cells. I think I have mentioned in some of the other modules that osteo means bone, osteoblast means home formation cells, osteocytes means that is the matured osteoblast.

So when a stem cells will undergo ontogenesis or will undergo differentiation through osteoblast lineage then the end member of ontogenesis is the osteocytes which are the more mature osteoblast cells. The stem cells also sorry the fibroblasts also can undergo trans differentiation to cartilage cells that is the chondrocytes or the fat cells that is adipocytes so this is the inter conversions that are thought to occur that can take place within the family.

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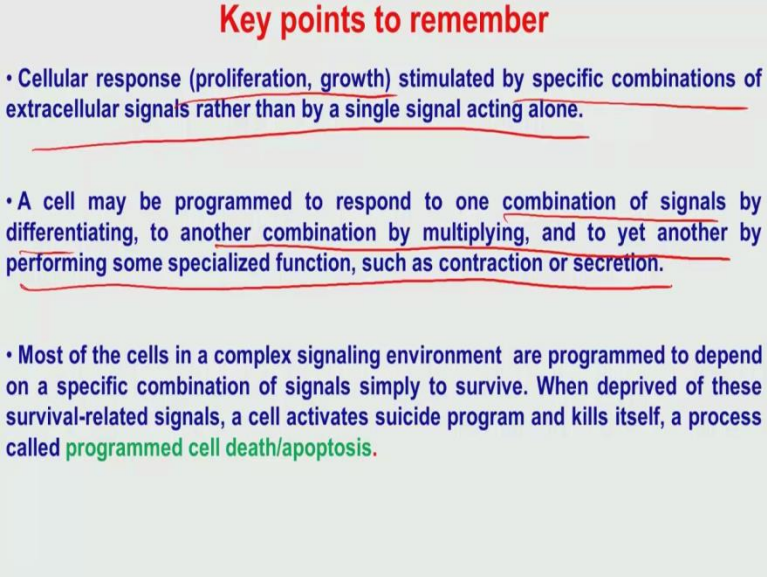

So these key points from this so much discussion that I had in this last two modules can be summarized here that cellular response like proliferation growth can be stimulated by specific combinations of extra cellular signals rather than a single signal acting alone. A cell maybe programmed to respond to one combination of signals by differentiating or to another combination of multiplying or to another performing to specialized function that is contraction or secretion.

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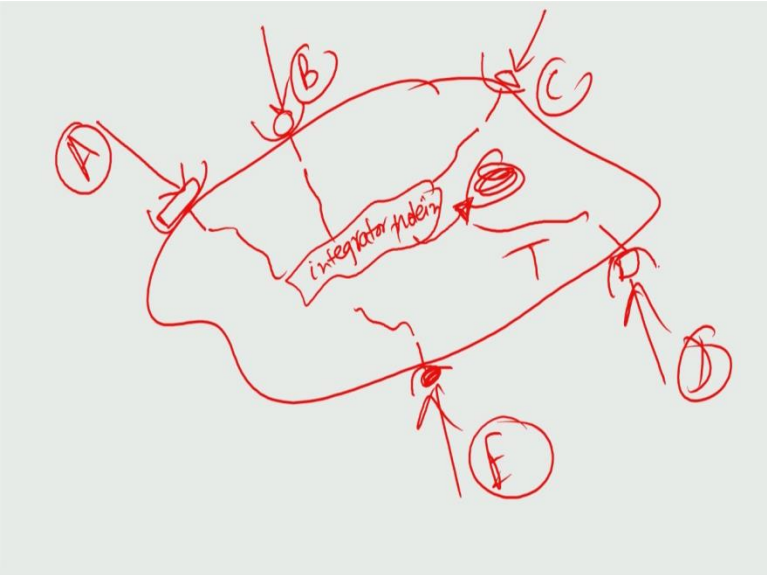

Let me go back to this schematics that I have drawn for your understanding a few slides back. This schematic essentially shows that a target cell is receiving five different types of signaling molecules a to e. Now this a to c is required maybe only for survival itself. Now this d e can be the two different kind of cell two different kind of signaling molecules. So d, e can be different type of signaling proteins and the d, e the integrator protein contained within target cell will now integrate all the information or all the different signaling pathways or downstream signaling proteins which are activated as a result of receiving five different signal molecules and therefore will instruct the cells to either divide or differentiate.

But my point was that for division this d, e can be two different signaling molecules. For differentiation this d, e can be two different signal molecules. So this point needs to be borne in mind that different types of signal molecules are needed for cells to perform different types of functionality as a result of the cell signaling process.

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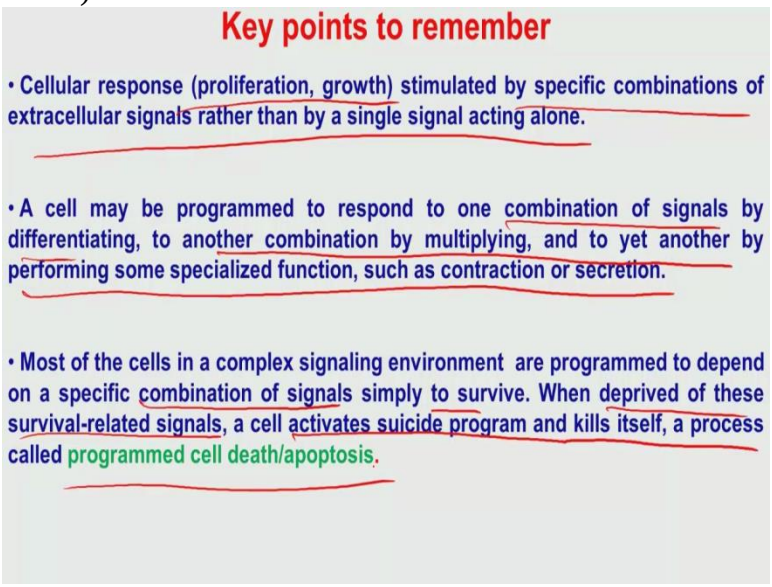

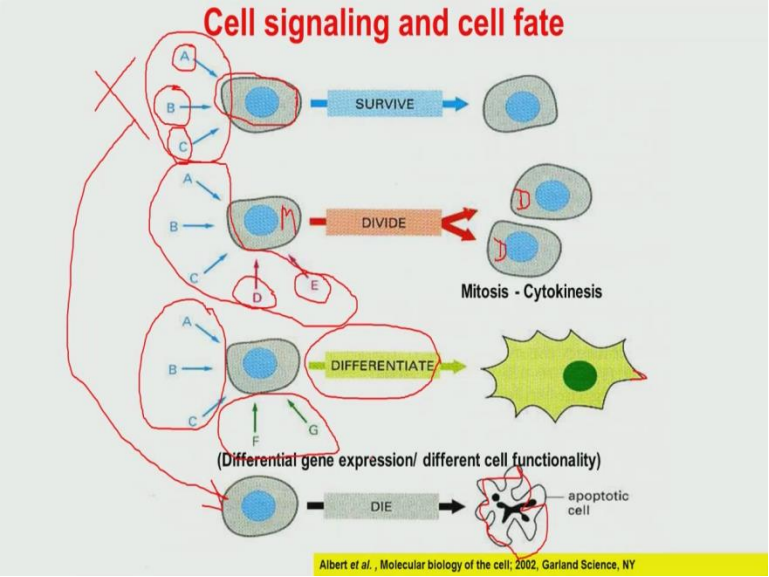

Ok, most of the cells in a complex signaling environment are programmed to depend on a specific combination of signals, simply to survive, and when deprived of the survival related signals a cell activates suicidal program and kills itself, a process called as a programmed cell death or apoptosis. So this has been mentioned also here in this particular slide. So I redirect my earlier statement that a given cell has to receive the three different signals a, b, c.

Now under certain circumstances, in a cellular micro environment, if a cell does not receive any of the signaling molecule either a or b or c then what will happen? Cell would activate it is own suicidal mechanism and therefore cell would undergo programmed cell death which is known as apoptosis in cell biology. So I think I will stop here and in the next module I think I will start with the cell fate processes.