Cell and Molecular Biology
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Week 12
Summary and Conclusions
Lecture - 45
Cell Factory: Concepts & Application

Hello everyone, this is Dr. Vishal Tevedi from the Department of Biosciences and Bioengineering, IIT Guwahati. In this course on cell and molecular biology, we are discussing the different aspects of how molecules interact with each other and what types of interactions are responsible for the various physiological processes. So in this context, so far we have discussed the evolution, the origin of life, the central dogma of molecular biology, signal transduction, the uptake of molecules, and so on. And in the previous module, we discussed immunology. And in the current module, we are discussing the different types of topics.

So if you recall from the previous lecture, we discussed gene therapy, and now in today's lecture, we are going to discuss the cell as a production factory. Now, the mammalian cells and the bacterial cells are capable of synthesizing different types of proteins, and they can be exploited for the production of various types of proteins. You can see that there is a clear-cut comparison, or there could be a similarity between the cell and the factory where you are actually going to produce the different types of products. So, you know you have the cell membrane, right? So, the cell membrane is just like the wall of a factory, right? So, if you see a cell, you have a cell where you have the cell membrane; you have the cytoplasm.

So the cytoplasm will behave like a floor on which you are actually going to build the different types of machines. You are going to put the machine away. So it is actually going to behave like a floor. And then we also have some of the, you know, the empty areas, and these are being called vacuoles in the case of cells, whereas the same is being used for storing, you know, the different types of products. So that is being a part of the reservoir.

Then we also have the different types of machines, right? So one of the machines is called a ribosome, which is actually the main machine for protein production. And then we also have that once the product is formed, it has to be packed. So, then for that, within the cell we have the Golgi bodies, whereas in the factory we have the packaging plant. Now, compared to here, once you are doing cellular

work in the factory, you also require the controlling machinery. So, you actually require the operations center.

So, that has been that the same job is being done by the nucleus within the cell. Then you also require a supervisor; you also require a person who is actually going to give the different types of instructions. So this job is done by the DNA within the cell, whereas it is done by a supervisor in the factory. Then you also require to know the energy source so that you can use it to run the machines. So that is the job that is being done by the mitochondria, whereas this is done by the powerhouse.

Then once you produce the product, you are also going to have the byproducts, and these byproducts are going to be taken care of by the lysosome within the cell, whereas the same is being done by the disposable system within the factory. And at the end, you are also going to have the distribution of this packed material. So you are going to have a manufacturing plant and a distribution center. So that is done by the endoplasmic reticulum within the cell. So what you see here is that the cell is doing all sorts of functions that are also available in a regular factory, and that is why people thought that we could utilize the cell for the cellular factory or use that cell for producing different types of products.

So the concept of the cell as the production factory comes from the fact that the cell is a building block for all existing creatures, from bacteria to humans. And it is so surprising that a cell can resemble an entire factory. Its main stations contain all the information like the nucleus. They encrypt their order in the form of DNA, which can easily be read by the messenger RNA, so that the DNA and RNA are the cellular code of conduct followed by the RNA in the process that is the main factory product. So in the cell, you are actually going to have the main product, which is the proteins, whereas in the factory, you are actually going to have the different types of products as per the requirements.

The protein is then going to be processed through the endoplasmic reticulum for the quality check, and if everything is processed correctly, it is going to be packed into different types of packaging material, which is done in the Golgi body, and then it is going to be ready for supply. Finally, all factories have their own source of energy, and the bacteria, mitochondria, or chloroplasts are going to do that function. Cell factory engineering is a process of rationally modifying cellular factories to produce the desired molecules of sustainable economic and scientific interest. The product can include native or foreign proteins, native metabolites, or molecules from a completely new biosynthetic pathway. So, the cell is capable of

producing proteins; the cell is capable of, you know, producing the metabolic products, right? So, it is actually going to produce different types of metabolic products; you can either take the product or modify it by utilizing or introducing different types of enzymes, and so on.

What is the goal? To turn a cell like a microbe into a high-performance production unit for various types of applications. So a cellular factory is a concept where you actually use the cell as a factory so that the cell produces your desirable product. Why is there a need to have the engineered cell factory? Because you are going to have mass production, and it is going to be more sustainable. Natural sources of variable compounds are often scarce, or production is too low for the commercial uses that engineering a cell factory allows for large-scale controlled production. So, one of the major issues with the natural product is that its quantities are very low, and it is also present with the other molecules, right? So, the purification and the other problems have always been there, right? So, as an alternative, what you can do is produce the product in a cellular factory where you will actually give the instruction to the cell so that it will produce that particular product in very large quantities.

And the other point is sustainability, so we can actually create renewable petroleum-based chemicals and non-renewable sources. And sustainability is also one of the important components because if you are going to utilize the cellular factories, they are actually going to be more sustainable since they are going to use natural products or they are actually going to use natural sources. And for these purposes, you can actually use these cellular factories for producing vaccines; you can use them for producing monoclonal antibodies; you can use them for producing bioplastics and biofuels; and you can also use this for different types of tissue engineering applications. And the other third point is the precision, so we can design the cells to produce the molecule with the specific properties, such as a therapeutic protein with enhanced specificity, and then instead of harvesting a rare plant for the drug, we can engineer a common microbe to produce the same compound efficiently in a lab. So, in the cellular factory toolbox strategies, what you are going to do is group the cellular strategies into three main categories based on the type of product being modified.

You can have the native metabolites, the heterogeneous pathways, or actually have the protein expression. So, depending on the type of metabolites you are trying to produce in these cellular factories, you can have different types of toolboxes. So, boosting the native metabolites. So the goal is to make the cell produce a compound it naturally synthesizes. How is it going to work? So you're

going to do the overexpression, increasing the number of key enzymes in the biosynthetic pathway of that particular metabolite.

Then you need to remove the bottlenecks. So you delete the competitive pathway that uses the same starting material. Say, for example, if you are trying to produce pyruvate, right? If you are trying to generate a large quantity of pyruvate, right? And you know that pyruvate will come from glucose by a process called glycolysis, right? So, if you are trying to generate pyruvate, the first thing you have to do is increase the concentration of the glycolysis enzyme so that you produce more and more pyruvate. But once the pyruvate is produced, it is actually going to enter the Krebs cycle, and that is how the Krebs cycle will use it. Whatever pyruvate you produce will enter the Krebs cycle, and eventually, it will be used.

So, you have to do something so that it is not going to be the case; it is actually going to remove it, and it is going to accumulate, right? And then you are also going to ensure the degradation prevention. So, you are also going to ensure that the pyruvate remains stable and should not get degraded, right? So, for example, engineering the function Aspergillus niger to increase the metabolic flux through its glycolytic pathway to boost citric acid production. Then you also have the new assembly line. So you can actually use the heterogeneous pathways. So the goal is to enable a cell to produce a compound it has never made before.

So this is going to be the exogenous molecule that it is going to produce, right? So in the previous examples, we have seen where the cell is capable of making a particular product. So we have enhanced the production of that particular product simply by increasing the pathways enzyme, and we have also done the blocking so that the biometabolites produced should not be utilized in the other pathway. We also reduce the degradation in the other one in this particular strategy, where you are actually going to have the heterogeneous pathway, and you are going to introduce a particular gene. So, what you are going to do is introduce a gene cluster. So, identify and transfer the entire gene cluster to that code all the necessary enzymes for the new pathway, right? So, in this case, the cell is intrinsically not producing that particular molecule.

So, what you are doing is putting the whole pathway into that particular cell, and then you are doing the host optimization. So, ensuring that the new pathway is compatible with the host cell's internal environment, such as providing the necessary cofactors or substrates. An example is introducing a gene for the anti-cancer drug Taxol from a rare plant into endophytic phosphate to allow for large-

scale production, or another example is engineering the yeast Pichia pastoris with a gene from a fungus to produce gamma-linolenic acid, a fatty acid used in nutrition. So, these are the 2 examples where you are actually putting the complete pathway into the cell and then asking the cell to produce particular biomolecules. Then you also have strategy number 3, where you are going to express the specific proteins.

The goal is to produce a single specific protein for a particular use. How is it going to work? So, you are going to do the promoter engineering, right? So, using a strong on-switch promoter to drive a high level of gene expression. You can also make the secretion. So, engineering the cells to efficiently secrete the protein makes it easier to purify, or you can do the post-translational modifications. So, modifying the protein after it is made, for example, by adding the sugar molecule or the glycosylations, phosphorylations, or acetylations.

You can do all these kinds of things so that you are going to produce a functional protein. Example is producing therapeutic proteins like insulin or monoclonal antibodies using engineered yeast or fungal cells. So in insulin production, how are things going to work? You have the bacteria you are going to use, so what you are going to do is make a recombinant plasmid, right? Where you are actually going to put the insulin gene, and you are actually going to produce the insulin, you know, so you are taking out the insulin gene from the beta cell, right? You are putting that into this particular plasmid using this particular multiple cloning site, and that is why you have produced the recombinant clone. And then you put that into the bacteria, and the bacteria is going to express a large quantity of insulin, and then you can actually do the purification extraction using different types of chromatography techniques, and that is how you are going to have the insulin. What are the key players in cellular factory or cellular factory engineering? So you can actually have different types of hosts.

You can have a bacterial host. You can have the yeast stored. So often used for its fast growth and simple genetics, E. coli is a common workhorse. Then you can also use the yeast. So fungi, such as Saccharomyces cerevisiae, are great for expressing proteins in well-induced genetic systems.

Then you can use filamentous fungi, such as multicellular fungi like Aspergillus and check Trichoderma, as they are hyper-producers and excellent at secreting enzymes, making them ideal for industrial applications. And then you can also use mammalian cells; they are used for insulin production, hormones, erythropoietin, and so on, right? And then they are used for growth factors and blood clotting

factors. So, depending on the type of product you want to use, the first thing you have to do is choose a particular host where you are actually going to do the production part. Then the second thing is the application side. So, it is these cellular factories that are actually going to have extensive applications in different types of areas.

So, in industrial applications, you can know when you will be able to use this for producing pharmaceuticals, different types of enzymes, biofuels, biochemicals, and also for producing natural products. So, you can use this for producing therapeutic proteins like insulin or monoclonal antibodies, or you can use this for producing different types of enzymes. For example, you can use this to produce enzymes like lipase, horseradish peroxidase, catalase, and other similar substances, and they are used in different types of industries, such as food processing and biofuel production. Then you can also use this for biofuels and biochemicals. So, engineered microbes produce ethanol, biodiesel, and bulk chemicals like adipic acid.

And then you can also use this for the biosynthesis of antibiotics as well as vitamins. Then we also have applications in the research field. So you can actually use functional genomics; you can do the discovery, and you can use this for medical and therapeutic applications, such as producing prebiotics or therapeutic proteins. So in the research application, you can study how the genes and the proteins work by engineering them in a controlled environment. For drug discovery, you can use engineered cells to screen for potential new drug candidates; for example, you can use them to produce random molecules, and then these random molecules can be extracted from the particular host and tested for different types of applications.

Then it can also be used in medical and therapeutic applications where you can use engineering prebiotics to produce the beneficial product directly inside the body. So basically, what you can do is, instead of giving the prebiotics that have already been purified, you can give them a bacterium, or I would say recombinant bacteria, which will actually produce different types of prebiotics. Then you can also produce the protein with enhanced quality for pharmaceutical applications. So one of the examples is recombinant protein production with filamentous bacteria, and the challenge is that filamentous bacteria are excellent at secreting protein. But they also produce protease that degrades the desirable protein; additionally, their glycosylation patterns are different from the male, which is a problem for the human proteins, right? Because the glycosylation proton is actually going to work, they are actually going to generate the antigenic response,

and because of that, these proteins will probably cause inflammation or other kinds of immune reactions.

What is the solution? The solution is that you can use the protease-deficient host. So scientists have created a mutant strain of fungi like Aspergillus niger that is missing the gene for degrading the protease. So it is actually a minus protease strain that you can generate, and because of that, it's not going to degrade the proteins. Then you can also have the fusion proteins, also called the gene fusion strategy, where the target protein is fused to a highly secreted fungal protein, which improves secretion and stability. And then you can also have humanized glycosylation.

So research is ongoing to engineer human-like glycosylation pathways in fungi to make them more suitable for producing human therapeutic proteins. Then we also have the connections between trichoderma and biofuel products. So trichoderma is an exceptional producer of cellulases and hemicellulases, which are key enzymes for the breakdown of plant biomass. And the industrial IPTAC can have the hyper producer of over 100 grams per liter of the extracellular protein, which matches cellulose. This makes it a principal target for creating cellular-derived ethanol as a biofuel.

And then you can use this for the biocontrol agent. So some Trichoderma species are also used in agriculture to protect plants from pathogens by attacking other fungi. What are the challenges and future directions? So what is the challenge? You can actually have a low transformation rate. So, it can be difficult to get the new genes into some of the fungi.

Then you also have protein degradation. So, as mentioned earlier, the host protease can still degrade the target proteins. You can also have the glycosylation. So, the mismatching glycosylation pattern for the human therapeutic protein remains a challenge, and people are already working on introducing new glycolytic pathway enzymes so that the glycosylation pattern found in the fungi could be as good as the glycosylation pattern in the mammalian system. And what is the future direction? So you can actually use better genome editing tools; for example, you can use CRISPR-Cas or synthetic biology. So gene editing tools like CRISPR are making it easier to precisely modify the genome.

And you can do the system level design. So instead of modifying one gene, scientists are now using the computer model to design and optimize the entire metabolic system. And then you can also work on the microbial consortia. The

future may involve engineering multiple different microbes to work together in a complex system for a specific task. And then you can also use the engineered fungal pathogens, so you know you can actually use them, as fungi cause significant diseases in humans, animals, and plants, leading to major economic and social costs. And what is the opportunity? The opportunity is that you can use recombinant technology to understand the molecular mechanisms behind fungal pathogenesis.

For example, the scientists are using the engineered Rhizopus to understand how its secreted protease contributes to its virulence as a pathogen. And this knowledge can lead to a new drug that targets its specific virulence factor without harming the host. And then you can also study the biosynthetic pathway in the other host, right? So, the fungal fungi are an enormous source of the novel biosynthetic pathway, with an estimated 1.5 million species, most of which have not yet been studied. Many natural fungi that host these pathways are not suitable for large-scale manufacturing.

And the solution is transferring these valuable biocentric capabilities to other, more manageable hosts like fungi, bacteria, or even plants. There is an example of transferring the gene for the highly unsaturated fatty acid from fungi into canola plants to create new oilseed crops. Then you can also do the engineering for the environmental solutions; for example, lignin is a very important metabolite that is present in plants, and that makes the grass a little hard to digest. So this is a bottleneck for biofuel production.

So what you can do is generate a fungal strain. So you can actually generate white rot fungi, which are the only microbes capable of efficiently degrading lignin without attacking cellulose. What scientists can do is study the unique enzyme from these fungi to transfer these capabilities to more conventional industrial hosts. Examples of Lacose enzyme from the white rot fungi have been expressed as a recombinant enzyme in Aspergillus oryzae and are active against the pollutants. Cell factory engineering is a paradigm shift, right? So, from a single product to a complex system, the field is moving beyond just making a single protein to engineering an entire metabolic pathway and multi-protein complexes.

From one host to many hosts. We are not just using one host organism. We are transferring valuable capabilities from one species to another to find the best production platform. From manufacturing and beyond. So applications are expanding beyond manufacturing to include understanding and solving complex

biological problems like pathogenesis. So, this is all about the cell, as you know, the production factory.

We have discussed the different strategies on how you can use that to produce the different types of products. So, we can actually take the product that has been a part of the metabolic pathway. So, we can actually enhance the production by increasing the pathway enzymes, or we can actually reduce the degradation. The alternate strategy could be that we introduce a complete pathway into a particular organism and produce the product we need. And the third strategy would be to exogenously express the particular protein so that it actually provides a large quantity of that product.

So, these are some of the strategies that you can use to exploit the cell as a production factory, and the advantage is that since it is biological in nature, it is natural; you will probably have a better product without even having to put in much effort to purify the particular product from different types of indigenous products and so on. And then, at the end, we also have the flexibility to modify the product as per your needs, actually. So, with this, I would like to conclude my lecture here. In a subsequent lecture, we will discuss some more aspects related to cell and molecular biology. Thank you.