Cell Biology: Cellular Organization, Division and Processes

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Lecture 28

Cell Diversity and Properties of Specialized Cells: Budding Yeast as a Model System.

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Hello everyone, this is Debolina Dasgupta, Ph.D. student in Professor Shikha Laloraya's lab, Department of Biochemistry, IISc. And today I am here to discuss with you all about an interesting topic that is *Saccharomyces cerevisiae*, a model organism on which our entire lab works on and also a powerful and popular system to study the fundamental aspects of eukaryotic cell biology.

So right now on your screen, you can see different images of *Saccharomyces cerevisiae*. The first one is how is *S. cerevisiae* under light microscope. The second one is *Saccharomyces cerevisiae* on agar plate and the third one is a typical image of spot assay with *Saccharomyces cerevisiae*. So let us get started and get to know *S. cerevisiae* a little better.

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Saccharomyces cerevisiae is commonly known as Baker's yeast or Brewer's yeast. In fact, the scientific term *cerevisiae* is also derived from old word terminology for beer. It can ferment sugars to ethanol and carbon dioxide, an ability thoroughly appreciated by the Brewing industry. The carbon dioxide released by the yeast has been used in the baking process for centuries raise bread dough giving it a light texture.

Now coming to the scientific point of view towards cerevisiae, it is classified as fungus or Mold. *S. cerevisiae* is a single cell eukaryotic yeast cells are approximately 5 micrometre in diameter, which is between a bacterial cell and human cell. *S. cerevisiae* also has another common name that is budding yeast and it derives its name from its characteristic feature of bud formation during cell division.

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This is the cartoon representation of a *Saccharomyces cerevisiae* cell. This is the mother cell and this is a newly formed daughter cell, which during the process of budding has left a ring-shaped deposit on the mother cell wall. These are chitinous deposits and referred to as bud scars. A similar kind of structure can is also visible on the daughter cell; they are referred to as bud scars. Like any other eukaryotic cells, *S. cerevisiae* also has different membrane bound organelles like nucleus cytoplasm, mitochondria plasma, membrane and cell wall. In the previous lecture by Professor Shikha Laloraya, she has explained to you all in great details about the properties, structure and function of all these membrane bound organelles. Today, I will give you a brief idea about the cell wall.

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So what is cell wall? Cell wall is an extracellular matrix, which in case of *Saccharomyces cerevisiae,* is composed of a complex meshwork of beta-glucans, chitin and mannoproteins. You can roughly divide the cell wall in two different layers. The inner layer is composed of beta 1,3 glucan, beta 1,6 glucan and chitin. Beta 1,3 glucan and beta 1,6 glucan are glucose polymers linked by beta 1,3 linkage and beta 1,6 linkage, respectively.

To give you a more detailed idea about these linkages, let us focus on their molecular structure. This is a glucose molecule with its numerical nomenclature, this carbon right here is the C1 carbon. The next one is C2, then the next one is C3 and so on. In beta 1,3 glucan, two consecutive glucose molecules participate in the glycosidic linkage by their C1 and C3 carbons respectively. If this glycosidic linkage is established between the C1 of the first glucose and C6 of the next glucose then that is referred to as beta 1,6 linkage.

Now coming to chitin. Chitins are biopolymer of N-acetyl glucosamine. This is the molecular structure of an N-acetyl glucosamine and chitin two consecutive N-acetyl glucosamine are linked to each other by beta 1,4 glycosidic linkage.

The inner layer of the cell wall is responsible to provide strength to the entire structure. The outer layer of the cell wall is composed of mannoproteins, which are nothing but glycoproteins. They determine the surface property of the cell. The cell wall as a whole, is important to maintain the cellular integrity during growth, division, under stress condition, and also during the time of mating.

The cell wall is also a primary target for different antifungal drugs. Another important function of cell wall is to maintain the cellular morphology.

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The *S. cerevisiae* cellular morphology or cell shape changes as the cell progresses through the various phases of the cell cycle. The first one being the establishment of polarization for bud formation during the late G_1 phase. The activation of this mechanism is regulated by complex machinery

popularly known as the bud site selection machinery. The bud site selection machinery is composed of a number of integral membrane proteins, which are inherited by the newborn daughter cells from their mother at specific locations on the plasma membrane.

The bud site selection machinery then activates a downstream regulator protein called Cdc42. The activated Cdc42 then is recruited in a high concentration at the bud site highlighted here in the yellow colour. The Cdc42, then employs a number of effectors molecules, which then regulate a number of downstream events. They are, the recruitment of the septin ring, clustering of the actin patches, polarization of the actin cable, and glucagon synthesis. Together all these events lead to the formation of the bud.

The green colour here represents the septin ring. Septin ring is composed of septin proteins and is essential for the cytokinesis. They also regulate the positioning of the mitotic spindle during the cell division. The red dots here represent the actin patches. They are a complex of actin filaments. Once the bud is formed it keeps on growing in a polarized manner till the S phase of the cell cycle. This type of growth is often referred to as apical growth. During the G_2 phase a switch happens between the epical form of growth and isotropic form of growth. The unpolarized form of growth is often referred to as isotropic growth. After the switch though, the growth is still directed toward the bud but now it is more evenly distributed inside the bud and you can also see the regulator protein Cdc42 for polarization is evenly distributed throughout the bud cortex.

Now after the apical- isotropic switch, there is an asymmetry in the growth pattern of the bud and the mother cell. This is asymmetry is broken at the late M phase and the growth is now directed toward the bud neck leading to the cytokinesis. The changes in the cellular morphology during the cell cycle is also regulated by different cell cycle specific proteins. For example, the establishment of polarization and bud formation is redundantly regulated by the G_1 cyclin CDK Complex. The apical-isotropic switch is also controlled by the G_2 specific cyclin CDK complex.

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Now let us move on and get to know some key features of *Saccharomyces cerevisiae* genome. In 1996 Goffeau et al first published the whole genome sequence of *S. cerevisiae*, which is a landmark for the entire eukaryotic Biology. A typical haploid budding yeast cell genome is made of 16 chromosomes containing approximately 12,000 kb genomic DNA. And you can actually visualize these chromosomes in Pulse field gel electrophoresis. In budding yeast, the genome has a very high density of protein coding genes compared to humans, which directly implies the concentration of introns is very less in *S. cerevisiae*.

S. cerevisiae genome also harbours several dubious ORFs, which are unlikely to encode proteins. The non-coding genes mainly encode tRNA or transfer RNA, rRNA or ribosomal RNA, and RNAs that are involved in several stages of gene regulation. The origin of replication in budding yeast are termed as ARS or autonomously replicating regions that are located at every 20 to 40kb interval on each chromosome.

The centromeres on each chromosome are known as point centromeres because they are short, approximately 125 base pairs in length, and they are not surrounded by heterochromatin like other

eukaryotic centromeres in *S. cerevisiae* that is composed of specialised DNA sequences and associated proteins. The telomeres, rDNA locus that encodes for rRNA and silent mating cassettes are the only regions that form heterochromatin like environment in the budding yeast genome.

Now in this slide, I have introduced you all to a new term called silent mating cassettes. So, what is this silent mating cassette? To understand that, let us first understand what are the different mating types in *Saccharomyces cerevisiae*?

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The *S. cerevisiae* can exist in two different states, the haploid state and the diploid state. A haploid *S. cerevisiae* exists in two different mating types, MATa and MAT alpha; a and alpha are pheromones secreted by the specific mating type of *S. cerevisiae* cells meaning a MAT a cell secrete a-factors and MAT alpha cell will secrete alpha-factors. Each of these pheromones acts on the opposite mating type of cell and activates a signalling pathway, often referred to as the pheromone signalling pathway.

The pheromone signalling pathway in yeast is mainly composed of a 7 transmembrane G protein and Map kinase cascade. Activation of the pathway is initiated by binding of the pheromone to the extracellular receptor on the cells surface, this in turn catalyzes the dissociation of G protein subunit, G alpha from the rest of the protein components that is G beta gamma.

Now the G beta gamma is free and it can then in turn activate the downstream MAP Kinase pathway. This leads to the transcription of specific genes followed by morphological changes that is schmoo formation, cell division arrest in G_1 phase and ultimately fusion of two different cell types.

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This is the life cycle of the budding yeast. These two are haploid yeast cells of different matin types. The first one is the a cell, and the second one is the alpha cell. Each of these haploid cells can divide mitotically by the bud formation and can yield more haploid cells of the same mating type. Under suitable circumstances when these two haploid cells are in close proximity they can undergo mating.

During the mating, this cell releases the pheromone, which causes the morphological changes in the cell that is the schmoo formation ultimately leading to the mating process. The mating yields a diploid cell. This diploid cell can again divide mitotically and yield more diploid cells or under different circumstances it can also undergo meiosis or sporulation leading to the formation of four haploid spores, which upon germination forms 2 alpha cells and 2 a cells.

Interesting factor is that these haploid cells can switch their mating type independently, meaning an a cell can get converted into alpha cell and an alpha cell can get converted into a cell. So how does this switching occur? To understand that let us focus on the genetics of this study.

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The mating type of the cell is determined by the *MAT* locus present on the right arm on chromosome 3. The *MAT* locus contains one of the two nonhomologous alleles responsible for determining the mating type of the cell that is *MAT* a and *MAT* alpha; in this case it is MAT a. Additionally chromosome 3 has two more loci HML alpha and HMR a. HML stands for homothalic mating left and HMR stands for homothalic mating right. These two loci contain additional copies of mating type alleles. HML alpha contains MAT alpha allele and HMR a contains MAT a allele. These two loci are transcriptionally silent because they are embedded in the heterochromatin region. During the mating type switching process an endonuclease comes to the action. This enzymes is called HO endonuclease enzyme. They have their recognition site right here indicated by the arrow.

The HO endonuclease introduces a double-strand break at the site, which is then repaired by homologous recombination, where one these two transcriptionally silent loci is used as DNA donor sequence. After the repair by homologous recombination, the MAT a allele is now replaced by MAT alpha allele. The purple coloured box here is referred to as recombination enhancer. This is a DNA sequence, which also regulates the homologous recombination.

So initially we had an a type of cell with us and now it has switched its mating type to the alpha type. Interesting fact is that most of the laboratory budding yeast strains have their HO endonuclease encoded gene mutated. So most of them have their mating type switching restricted.

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Till now we have discussed different cellular aspects of the *Saccharomyces cerevisiae*. Now let us try to understand why *Saccharomyces cerevisiae* is a popular and useful tool for geneticists. *Saccharomyces cerevisiae* has simple growth requirements. They grow rapidly in approximately 90 minutes under optimal laboratory environment. They can exist in both haploid and diploid states. They can be easily genetically manipulated and the large database is already available for *S. cerevisiae* genome referred to as a *S. cerevisiae* genome database or SGD. There are also many popular molecular biology techniques, which were initially standardized in *Saccharomyces cerevisiae*.

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The first one is the yeast two hybrid system. Here we use a gene referred to as Lac Z; this gene encodes a particular protein called beta-galactosidase, which in the presence of x-gal can turn the yeast colonies into blue colour. The transcription of this Lac z gene is regulated by a transcriptional activator. This transcriptional activator has two separate domains. The first one is the transcriptional activation domain and the second one is the DNA-binding domain.

The transcriptional activator binds the specific DNA sequence upstream of the lacZ gene by their DNA binding domain and then the transcriptional activation domain activates the transcription of this gene leading to the expression of beta galactosidase. So, this system is used to understand the interaction between two proteins. Let us consider two proteins first one as protein X or Bait protein

and the second one as protein Y or Prey protein. To understand whether these proteins are interacting with each other or not we first fuse one of these proteins, for example protein X with the DNA-binding domain of the transcriptional activator. And then we fuse the other protein that is the Prey with the transcriptional activation domain of the activator . Now if these two proteins are interacting with each other, then the transcriptional activation domain will be in close proximity of the lac Z, which will then activate the expression of the beta galactosidase ultimately turning the yeast colonies into blue colour in presence of a X-gal. If these two proteins are not interacting with each other then the transcriptional activation domain will not come in close proximity of the Lac Z gene. Thereby the transcription will not be initiated in Lac Z gene ultimately leading to the white coloration of the yeast colonies.

Just by observing the colour of the yeast colonies we can understand whether our two proteins of interest are interacting with each other or not.

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So the next up is 1 step PCR mediated tagging. Why tagging of a protein is important? If we can tag our protein of interest with the fluorescent tag then we can visualise them under the microscope. And get an idea about the subcellular localisation of our protein of interest, which in turn will also give us an idea about the function of the protein. During the Western blot analysis, when the antibody against our protein of interest is not available, we can actually tag the protein with different epitopes and then use antibodies commercially available against these epitopes and visualise the protein on the Western blot.

So let us understand how one step PCR mediated tagging works. Here we have the plasmid with our tag of interest and the marker specific to the yeast cells. We amplify this entire sequence with a pair of oligos. The forward primer and the reverse primer have DNA sequences at their tail, which will be homologous to the region where we want to incorporate this tag. We amplify this tag by polymerase chain reaction and then transform the PCR product in the yeast cell. The blue DNA sequence here is homologous to this region and the red DNA sequence here is homologous to this region. After homologous recombination this entire cassette will be incorporated in the yeast genome and our total of interest will be tagged.

We can then screen for positive colonies by colony PCR and Western Blot. In colony PCR, we design a set of oligos, one specific to the yeast genome and the other specific to the cassette. And during Western Blot analysis, we can use antibodies against these tags.

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In the previous slide, I have mentioned that the plasmid contains a marker that will be specific to the yeast cells. Now in yeast cells, there are different categories of markers that can be used. First of all is the auxotrophic markers, genes that are necessary for the growth of the cell under an essential nutrient deprived condition example *LEU2, HIS3, URA3* etc. Importantly if you want to use these

auxotrophic markers our strain should have a genetic background with the mutated version of these genes.

The dominant markers are the genes that provide resistance to the cell against drugs to which the cell is sensitive, example Nourseothricin, Hygromycin B etcetera. Selectable markers, these are the genes whose expression select for the positive transformants. And then come the counter-selectable markers; these are the genes whose expression kills the positive transformants. Example counter selection for the *URA3* genes involves 5-fluoro orotic acid. Cells that express *URA3* accumulate a toxic metabolite like 5-fluorouracil in the presence of 5-fluoro orotic acid rendered in the cells viable.

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Counter selection is important during plasmid shuffling. Plasmid shuffling can be used for various purposes, one of them being to understand the function of an essential gene. Generally, when we want to understand the function of a gene, we eliminate it from the genomic background, but in case of an essential gene we cannot do that. So we try to introduce different mutations in the essential genes and try to understand the function. Plasmid shuffling is the way we can do that.

So, to begin with we have a yeast centromere plasmid with us. This is also referred to as a CEN plasmid. This plasmid contains a part of the sequence of ARS and centromere, thus they are autonomously replicating and single copy plasmids. This CEN plasmid contains a wild-type version of the essential gene and *URA3* marker, we transform these plasmids inside the cell.

Now the cell has two copies of the essential gene. The genomic copy and the plasmid copy. Next we go for the targeted deletion of the genomic copy of this essential gene. Further we take another CEN plasmid where we clone the mutated version of essential gene. This plasmid has a different marker that is *HIS3*. We again transform this plasmid inside the cell and plate these cells in 5-FOA containing agar plates. This will select for only those cells, which will have the plasmid that contain *HIS3* as a marker. Thus, it also contains the mutated version of the essential gene. We can then rescue the plasmid and go for DNA sequencing to understand the mutation. This mutation can be a conditional mutation or a reduced function mutation. If this is a null mutation, then the cell will be dead.

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Next is tagging with green fluorescent protein or GFP. GFP was first isolated from a jellyfish called Aequorea victoria. This is the crystalline structure of GFP and this is the protein data bank id. Now, if you excite GFP with UV light, the GFP will emit bright green fluorescence. So, if we can tag our protein of interest with GFP, we can actually visualise our protein by fluorescence microscopy.

So to give you an idea, how does it look under microscope? Let me show you some images. These are *Saccharomyces cerevisiae* cells where we have tagged the Net 1 protein with GFP. Now it is already known that Net 1 is a nuclear protein that remains associated with the rDNA locus. These green dots here are the Net 1 proteins tagged with GFP. Now there is an entire database called yeast

GFP fusion localisation database that will help you to study the global analysis of protein localisation in eukaryotes.

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This brings us to the end of today's short lecture. So far, we have learnt different cell morphology of *Saccharomyces cerevisiae*, their mating types, and how do they switch their mating types? We have also learnt some interesting features about *S. cerevisiae* genome and why *S. cerevisiae* is so useful as a genetic tool. We have also understood different Molecular Biology techniques with *Saccharomyces cerevisiae*. Hope you all learned something new today and enjoyed today's lecture. So, thank you and all the best.