Cell biology: cellular organization, division and processes
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Lecture – 05
Regulation of the Cell Cycle – Part I

Hello everyone, today we will discuss how cells reproduce. This happens via complex series of events termed the cell cycle that we will discuss in this lecture.

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As you know, cells have a very complex organization. Cells have got DNA, the genetic material, that must be inherited accurately, and also several other components such as various organelles including some organelles such as mitochondria that also have their own genetic material. So, how do cells reproduce? Obviously, this would involve the duplication of all the constituents, their segregation, and the division of the cell into daughter cells.

So, shown here is the cell cycle. The process of cell division occurs when cells go through this cell cycle, it consists of 4 phases; shown here is the mitotic cell cycle that is the equational division where the daughter cells inherit one copy of the original genome of the mother cell. The phases of the cell cycle are shown here, there is G_1, S and G_2 and M and one of the important events occurs during S-phase and that is DNA synthesis, or DNA replication.

The process of segregation of the genetic material into 2 equal parts occurs during this process of mitosis or M-phase shown here; there are also 2 gap phases, G_1 and G_2, which are phases where the cell prepares for the next phase for the process of division and these also highly regulated phases. For example, G_1, it is usually the longest phase, and sometimes the cells they can enter into a non-dividing stage known as G_0 when the conditions are not favorable and when favorable conditions arise, those cells may again re-enter the cell cycle. Then there is another gap phase between S and M-phases known as the G_2 phase and this is also a growth phase, sometimes the cells grow in size and it prepares for the complex process of mitosis, coming up.

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Early biologists used simple light microscopy and they could observe chromosomes inside cells and with those simple tools they could also depict and draw the various stages of mitosis that were fairly accurate, which is shown here from the atlas of histology.

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In fact, such observations, coupled with the knowledge of genetics and the laws of inheritance that we discussed earlier, let to the germ-plasm theory of heredity, according to which the hereditary information resides in the germ-plasm or chromosomes, which are these thread like structures that

appeared in cells as they were about to divide and these thread like structures it was observed appeared to be more or less equally segregated between the 2 cells that were produced by cell division. So, it was thought that most likely the genetic information might be in these entities, the chromosomes, or it was this part of the cell that was referred to as the germplasm.

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So, mitosis was first observed by microscopists and it was appreciated as a very dramatic event that the cell goes through. I mean, a cell that was just lying there seemingly doing nothing, suddenly would get activated, lot of changes occurred within the cells. These magical thread-like structures, known as chromosomes, seemed to appear out of nowhere and they underwent division and one could also see the formation of a structure known as a spindle that helped in segregating these chromosomes.

So, this process of chromosome segregation was being studied even before the structure of DNA had been discovered, and of course, interphase was considered to be uneventful and somewhat uninteresting, because nothing seemed to be happening there. However, in science, technological advances lead to new discoveries, and labelling studies became possible when people started, biologists started using radioisotopes and by such studies, when labelling studies were done using tritiated thymidine and precise measurements of changes in the DNA content was done, S-phase was discovered. So, it was found that there is a period of DNA synthesis, which occurred only within a limited period of the cell cycle; this phase was within interphase and this led to what we now understand as the 4 phases of the cell cycle, $G_{1,}$ that is from birth to S, S or synthesis phase, where DNA synthesized, G_2 that is the end of S until M or gap 2, and mitosis.

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So, with the discovery of S-phase, we have become familiar with these 4 phases in the cell cycle and the image on the right or rather the diagram on the right shows a field of cells in which there are different stages and one of them is in S-phase as is depicted here by incorporation of BrdU that is bromodeoxyuridine, which is a thymidine analogue that is incorporated specifically into DNA, and this has been visualized by hybridizing with anti-BrdU antibodies that are labeled.

And a similar experiment can also be done using tritiated thymidine and incorporation of which also indicates that these cells are undergoing DNA synthesis and therefore, they are in S-phase. On the left is shown a panel where there are cells in different stages of mitotic division. At the top is interphase where there is not much structure, the cell is more or less flattened and DNA is somewhat amorphous to look at. And then you can see entry into prophase: chromosomes start forming these thread-like structures and this is followed by metaphase. When the chromosomes have congregated in one plane in the center, the metaphase to anaphase transition where they are separated, the replicated chromosomes or sister chromatids are segregated from each other, and finally, telophase when the DNA masses move away from each other towards the ends of the cells.

So, of course, there is also the G_1 and G_2 gap phases, which are shown in this diagram of the cell cycle again and this G_1 phase is very important. Its length can actually vary depending on the external conditions and when it is unfavorable as I mentioned, the cells they delay progression through this phase and they wait for the favorable conditions and sometimes they may even exit out of it and

enter a specialized resting stage known as G_0. And some cells in fact, in multicellular organisms remain in such a stage for days, weeks or even years before they will resume the process of cell division and in fact, many cells remain in G_0 permanently till they die or the organism completes its life and when the extracellular conditions become favorable, then the cells in early G_1, or G_0, they actually start progressing in the cell cycle, and they progress through a very critical point known as the commitment point, which is located here at the end of G_1, which is also referred to as start in budding yeast that we will be talking about, and it is also known as a restriction point in mammalian cells. So after the cells pass that particular point in late G_1, then the cells are committed to DNA replication, even if the signals that were stimulating their growth and division are removed, or they are no longer there or conditions become unfavorable, once they have crossed this point then the cells are committed to divide.

So, in these gap phases, basically the cells are preparing for division and the length of the cell cycle of course, varies the duration varies depending on the type of cell. For example, in a human cell in culture, the cells may take about 24 hours to divide, an interphase occupies most of that period and M-phase is relatively short, it may take about an hour and in contrast, microbes such as a budding yeast, *Saccharomyces cerevisiae*, that we will be discussing, the entire mitotic cell cycle is completed within 2 hours.

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So shown here are dividing fibroblasts: its an asynchronous culture of these fibroblasts in a culture dish and here actin is labeled in green and DNA is labeled in red and what you can see, in fact, in this field of cells is 2 of the cells are in mitosis and you can see the condensed masses, they have actually undergone anaphase and they are in 2 different stages of anaphase, and another interesting thing to note is that the cells they look different from the other cells. The other cells are somewhat flattened and well attached to the culture dish, whereas these cells are somewhat rounded up and that is a characteristic feature of cells, which are undergoing mitosis and in fact, sometimes by shaking the culture dish, one can get these cells to release more readily and thereby collect cells which might be in mitosis, as opposed to interphase.

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Here we can see different stages of cell division in human cells. The DNA is shown in red and tubulin is shown in green, which forms microtubules. So here is an interphase cell, which is reasonably flattened out and the DNA seems to be somewhat disorganized, and it progresses to early prophase where some structure appears in the DNA, you can still see a single centrosome, which has not yet separated into 2. This is followed by late prophase, now the 2 centrosomes have separated and you can start seeing more of DNA condensation happening. Here is prometaphase, which is a phase where the DNA has condensed and the chromosome and microtubules are forming associations, but this process of a chromosome attachment is still incomplete. In metaphase, all the chromosomes have aligned in 1 plane and they are all also attached to microtubules in a bipolar fashion, that is each pair of sister chromatids has attached to a microtubule coming from the opposite pole. When all the chromosomes are attached, the metaphase to anaphase transition occurs and in this step, all the pairs of sister chromatids split apart from each other and they move away from each other that is they start segregating. So you see chromosome separation happening in anaphase, which also has 2 phases anaphase A and B, the first where the chromosomes separate and the second, which involves

elongation of the spindle. This progresses to telophase where the chromosomes have completely segregated and reach the poles and now the cells, they start to flatten out, there is also decondensation of the chromatin happening, and here you see early cytokinesis, where the chromosomes are decondensed and the nuclear envelope has been reformed. And finally late cytokinesis where the cells not only have separated, but they have also moved apart.

So one important step which I mentioned, but it is not shown here is the process of nuclear envelope breakdown, which happens somewhere around here when the cell first enters mitosis and in this process, the nuclear envelope breaks down and therefore, the chromatin is released and it can actually spread out and the chromosomes can bind microtubules, microtubules and chromosomes find each other and make the associations which are required ultimately, for the process of segregation and of course, once again, the nuclear envelope is reformed at the end after the chromosomes are segregated to give rise to a normal cell which has a nucleus.

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So here is the time-lapse of the process of mitosis contributed by Drew Berry and Etsuko Uno and this was made by Jeremy Pickett-Heaps. In this video, you can see the process of chromosome condensation, you can see metaphase now here all the chromosomes at the equatorial plane, you can see the metaphase to anaphase transition that just happened, the chromosomes moving apart.

Now, the cells are in telophase and you can see decondensation of the chromosomes, cytokinesis has occurred, you have 2 cells now from the original cell. So this is a very fascinating event to watch and no wonder a lot of people were interested in studying this process and understanding how it is regulated.

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So scientists watched this dramatic event of mitosis for a long time, but they had no idea what was controlling these events, it was not even clear whether there was a system for controlling or whether all these processes they somehow control themselves and they were preprogrammed to occur in a particular order. So scientists wondered whether there is this inbuilt time-dependent program by which these events occur or could it be that this complex process is subjected to some kind of regulation?

And whether or not there is regulation was something everybody was wondering about, but evidence for regulation came from 2 classic experiments that I will be discussing in today's lecture. The first of them is shown here. This is an experiment done by Johnson and Rao, a long time ago, in fact, it is a very interesting experiment, where they carried out cell fusion. So, they fused cells, which were in S-phase with G_1 phase cells.

So, there are 3 sets of experiments here and to get a conclusion, you have to do all 3 of them. So, they observed that when S phase cells were fused with a G_1 phase cell then as you know, S phase cells are undergoing DNA replication, which can be assayed by the assays I described earlier. So, they observed that after the fusion, the G_1 nucleus entered S-phase immediately and of course, the S-phase nucleus continued its DNA replication, but the G_1 nucleus sort of prematurely, after the fusion, started replicating its DNA.

So, this indicated that the S-phase cell induced premature DNA synthesis in G_1. On the other hand, when they fused S-phase cells with the G_2 phase cells, the S-phase nucleus of course, kept on replicating its DNA, but the G_2 nucleus was unaffected that is, it did not enter S-phase, unlike the G_1 nucleus. When they fused G_1 cells with G_2 cells, the G_2 stayed in G_2 and the G_1 nucleus entered into S-phase as per its own timetable, as in it would have entered S-phase at some point anyway. So, it maintained that timetable, it did not prematurely enter S-phase. So, these experiments, although simple, they lead to some very interesting insights into regulation, 1, that the S-phase can induce premature DNA synthesis in G_1, indicating that there is some kind of replication promoting factor in S-phase cells.

However, the S-phase cell could not induce DNA synthesis in G_2 cells, even though it has this factor. So, this indicated that there is a re-replication block in G_2 nuclei. So, even if you provide the S-phase cell contents or the factor present in S-phase cells and that induces replication, this is resistant to it, it will not enter the process of DNA replication. So, this was very interesting; it indicated for the first time that there is some regulation going on.

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And also, at around the same time another evidence for regulation of the cell cycle I think came from experiments that were done with *Xenopus* oocytes. So, *Xenopus* oocytes, it is a type of a frog and these are very large cells and you can actually see them with the naked eye, the diameter might be 1 millimeters or so, and they are very enriched and they have stored up lots of macromolecules and they are sort of poised for entering into cell division immediately after fertilization. They provide a very rich source of various macromolecules, which may be required for cell division and also, they are very suitable for experiments on cell cycle.

So, shown here is a process of oocyte maturation and activation. Normally, an oocyte is arrested in the G_2 phase of meiosis I; meiosis is a type of reductional cell division and it is important in the formation of germ cells and we will be discussing that in detail later on. So, in this stage, the G_2 phase of meiosis, the cell awaits a hormonal trigger, such as progesterone to enter into the M-phase of meiosis 1. In the absence of such a signal, it remains arrested in the G_2 phase. When the appropriate hormonal trigger is available, then it enters into the M-phase of meiosis I and it undergoes meiosis I and then it is arrested in the M-phase of meiosis II until fertilization. During this first division there is a polar body, which is extruded, and after fertilization occurs, this of course, results in formation of the zygote and it results in a rapid sequence of cell divisions, which are known as cleavage divisions, because here the cell does not grow in size, the cell was already a large cell with a lot of content and without growing it undergoes periodic rapid divisions to produce an embryo, which has 1000s of smaller cells and then it undergoes of course, the process of embryogenesis and development into the adult-we are not concerned with that part here.

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Using this system, the scientists Masui and Markert, performed an experiment trying to test whether there are regulatory factors present in mature oocytes and one thing to remember is that because of the large size of this cell, it can be microinjected with various test substances by experimentalists and what they did is, they injected the cytoplasm from the M phase oocyte into the G_2 phase oocyte, you know, which was normally resting.

And what they observed is as soon as they did this, this G_2 phase oocyte could enter into M-phase that is it was driven into M-phase, which it normally does upon a hormonal trigger. So, now, it could enter M-phase without hormonal stimulation. So that was interesting. And at the same time, they also did some control experiments where they injected cytoplasm from interphase cells and when they did that, nothing really happened, the oocyte remained in G_2 phase. It was not driven into mitosis and this is an important control because it shows that it is not just the process of injection, you know, it is a big disturbance to a cell to poke a hole into it and inject some more liquid. So, this control experiments show that just going through this process is not something that signals and triggers entry into M-phase. So that is important to note.

Therefore, this experiment implied that there must be something in the cytoplasm of M cells that was driving the G_2 cell to enter into M and hence, they concluded that there is a cytoplasmic regulator, MPF, which stands for maturation promoting factor, and is also now referred to as mitosis promoting factor, which controls the entry into mitosis. So, there is this unknown important regulator, MPF, present in the cytoplasm of M phase cells, which can induce G_2 cells to enter into mitosis.

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So, a major breakthrough in trying to understand the molecules that regulate the cell cycle came with the identification of a key regulator of the cell cycle control system. Now, this discovery was actually made by 3 groups led by the scientists Leland Hartwell, Paul Nurse and Tim Hunt and it is a very significant discovery, the discovery of this master regulator, the CDK-cyclin complex. CDK stands for cyclin-dependent kinase, and this discovery, was rewarded with the Nobel Prize in physiology and medicine, to the scientists mentioned here: Dr. Hartwell, Paul Nurse and Tim Hunt.

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So how was this done? Leland Hartwell was working with budding yeast. So, he is a yeast geneticist and he used budding yeast as a model system to sort of address or investigate the question of cell cycle regulation. Now, yeast are small single celled fungi and they are eukaryotes, as discussed in earlier lectures and the budding yeast, which was an experimental system in the Hartwell lab, is commonly known as baker's yeast and also used by brewers and it is this oval cell and it actually divides by budding, it gives off a bud. And therefore, it is known as budding . And the way it divides, it gives off a small bud which grows in size and this bud appears during G 1 and then it keeps on growing in size and then finally, it separates from the mother cell. So, the original cell is termed as the mother cell and the new cell, which, during the process of division of cell is still a little smaller than the mother cell, is referred to as the daughter cell. These organisms, they reproduce very rapidly. I already mentioned within 2 hours, you can have a complete doubling and their genome size of course is smaller than that of mammalian cells and they were also great favorites of molecular geneticists, because they were amenable to genetic manipulations. You could actually not only isolate mutants, but you could create mutations by deleting genes or replacing or altering them because the process of homologous recombination in this organism happened at a higher frequency than some of the other systems.

And another interesting thing about the budding yeast is that they have also haploid stage. So, they can exist in both haploid and diploid stages and in the haploid stage have only a single copy of each

gene. So, this is important because when you isolate mutations, that inactivate a gene, then you can even study the recessive ones because the problem of the second copy of the gene in the cell is not there and so, this was a good system to study cell cycle regulation.

And certainly Dr. Hartwell's lab decided to address this question and the way they went about it is that it is expected that you know, if there is a mutant which cannot complete the cell cycle, then it will not grow. So, they made use of what is known as conditional mutants, that is there are mutants whose mutant phenotype is evident only under certain conditions, but not other conditions, and one of the most popular types of conditional mutants are temperature sensitive mutants.

So, these are mutants, which at some normal temperature referred to as a permissive temperature, they grow just fine. But at a higher temperature known as the non-permissive or the restrictive temperature, the mutant phenotype is expressed and they would arrest. So, these types of mutants are more suitable for studying such important genes that are required for cell division or also genes which are essential for viability of a cell.

So, they took the cells and they did a screen for mutants and what they were looking for was some phenotype, which would indicate that the cell has some defect in the cell cycle. So, shown here is a field of budding yeast cells showing the actual morphologies of different types of cells. You may have an original single cell without a bud, which is in very early in G 1 and you start seeing small buds appear that grow in size and then they go bigger and ultimately, they get separated into mother and daughter cells as I already mentioned and it is also shown in this cell cycle over here, where you can see the shapes of the budding yeast cells, in relation to the stage of the cell cycle that they are in. So, this was an important feature of budding yeast by looking at the cells by light microscopy, you could actually have some indication what stage of the cell cycle they were in, and of course, what we will also be discussing is that these studies they lead to the definition of an important stage or point in late G_1 in the cell cycle, which is referred to as START and I already made a reference to it, but this is a point in late G_1, which is critical: if a cell crosses that then it is committed for DNA replication.

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So shown here is the life cycle of budding yeast; yeast exists in 2 mating types, a and alpha, that is 2 different genders if you will, and these are the haploid forms and they can grow happily undergoing mitosis, when conditions are optimal for their growth or conditions are suitable for their growth and each of these, they actually secrete a pheromone. So an a cell secretes an a pheromone, and an alpha cell secretes an alpha pheromone, which can actually attract the cell of the opposite mating type and cause fusion. So when an a cell an alpha cell are in proximity, they can sense the direction from where the pheromone signal is coming and they form this protuberance known as a schmoo and eventually they would fuse with each other by a process also referred to as mating or conjugation and they form a diploid and the diploid now can also survive.

So, there is cell fusion as well as nuclear fusion and it can also undergo mitotic divisions under suitable conditions. However, if it encounters unfavorable conditions or starvation, for example (and you can induce this in the lab also), then the diploid can undergo meiosis or sporulation and give rise to tetrads of spores. Each tetrad has 2 a and 2 alpha spores and these can germinate to produce the respective haploid forms a or alpha haploid strains.

So, one can take advantage of this lifecycle and normally when people do a screen, it is advantageous to do the screen for mutants in both an a and alpha populations. Because eventually when you get a collection of mutants, then you can mate them and assign the mutants into different complementation groups.

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To perform a mutant screen first cells are mutagenized, using a mutagen, such as EMS that results in base substitution mutations in DNA, and one usually mutagenizes both a and alpha cell populations and then you screen or select for your desired phenotype and this is known as a primary screen. And this is followed by screening for the specific phenotype affecting the process you are interested in using an assay, which is designed for this purpose and this is known as a secondary screen. The various mutants that are obtained are then crossed, that is a mutants are crossed with the alpha mutants to assign complementation groups. And this actually indicates the number of genes that might have got mutated that are affecting the process that you are studying. And finally, you can identify the genes defective in those mutants by complementation from a gene expression library and in case of budding yeast because very few of the genes have introns, we can use a genomic DNA library, but usually it is good to use a cDNA expression library. So in this case, for the cell division cycle mutant screen, the primary screen was temperature sensitivity, which is basically making a collection of a large number of mutants, which were temperature sensitive, were collected and then they were screened by microscopy, which is a secondary screen, to look for cell cycle arrest phenotype with the expectation that the mutants which are arrested showing a uniform cell cycle arrest phenotype that is an arrest in a particular phase of the cell cycle, those may be defective in factors which are important for cell cycle control or cell cycle regulation.

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So, by doing this, the Hartwell lab, they identified a large number of cell division cycle mutants, which are abbreviated as *cdc* mutants. So shown in this panel is a field of asynchronous wild type cells, this is a diagram and you can see different types of cells. Here is a cell with a very tiny bud, a larger bud. Here is a cell, which has duplicated its DNA, but the DNA has not yet been divided and here is a cell which is just entered the process of division, you can see a bilobed nucleus, that is, it is about to divide, it is in the process, and here is another cell that is in telophase, the two DNA masses have segregated away from each other yet they are still connected by little strand of chromatin so the process of division is not yet complete. Here the nuclear division is complete, but the cells are still attached. So, in an asynchronous population, you can see all of this.

In the *cdc* mutants, of course, I told you there are a large number of mutants that were discovered. I am just showing the phenotypes of a few of them. So, the first one is a very important mutant that we will be talking about a lot in today's lecture, termed *cdc28* and the phenotype it shows is sort of like early G_1 phenotype with a single cell which has got a single DNA mass that is haploid, one end it has one copy of the DNA. Two other mutants are also shown: *cdc14*, which looks like it has got a telophase like arrest, but the process of segregation is not fully completed, there is still some strand connecting the 2 DNA masses and of course, cytokinesis has not occurred, and *cdc15*, which is more or less similar to *cdc14*, but it is a little further along in that the DNA masses in most of the cells appear to be completely separated while in few of them, you may see a strand connecting the 2 and it has not yet undergone the cell division or cytokinesis.

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When the screen was envisaged, the scientists wondered what might they find from such a study. It is possible to predict you know, what you might find from such type of experiments and they thought most likely that they would find genes falling into various functional categories, such as most importantly of course, controls which are responsible for progress of the cell cycle, processes that are monitored by checkpoints, which are regulatory pathways in cells, which check whether everything is proper for the cell to progress forward in the cell cycle and we will discuss more about these pathways later on in the course. They also thought that maybe they might find genes involved in signaling pathway that cause arrest in response to external signals and also those which are involved in morphogenetic steps of the cell cycle, that is even though these genes are not regulatory they are important for certain morphogenetic step and if it does not occur, then you might get a cell cycle arrest.

So, let us discuss *cdc28*. They found this mutant *cdc28*, which has the arrest phenotype shown here, it has an early G_1 like arrest phenotype with an unreplicated genome and they found using certain experiments involving cell cycle synchronization using alpha factor (alpha factor can arrest cells in a G_1 like state), that in this case, G_1 arrested *cdc28* cells would block progress at start , that is a certain point in the cell cycle, when they were released from the G_1 arrest at the restrictive temperature. So, when they were blocked at this stage, they could still conjugate or mate with each other and also these cells, they did not initiate DNA synthesis. So now we know that *CDC28* encodes a cyclin dependent kinase.

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Another group led by Paul Nurse was also interested in this question and they used the fission yeast, which is another type of yeast, different from budding yeast to also obtain a collection of *cdc* mutants. Now, the difference between budding yeast and fission yeast of course is that fission yeast divides by fission, and not by budding, and there is a septum formation when the cells are about to divide and the division is equal, that is the 2 cells which are formed after the process of cell division are equal in size and also an important distinction is that the G_2 phase in the fission yeast lifecycle is much longer. Whereas in budding yeast the G 2 phases somewhat indistinct, actually it is not well defined. But in this case, after S-phase, the cell actually elongates in size, until it reaches almost the size which is required at the time of cell division to give rise to 2 equal sized cells, which are of the same size as the original mother cell. So Paul Nurse's lab made collections of *cdc* mutants of the fission yeast, *Schizosaccharomyces pombe*.

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And here are examples of some of the phenotypes that they saw. So again, in this case, by looking at the length of the cell and whether or not it has a septum, you can get a pretty good idea what stage of the cell cycle it is in and, for example, here is a cell which has duplicated its DNA and it has elongated but it has not yet divided and here is a cell which has just divided into the 2 daughter cells and so on, in a wild-type or normal cell population.

So they found some interesting classes of mutations. One class was this one, where one of the main mutants we will be discussing in this category is *cdc2* and *cdc25*. So, this particular mutant

phenotype was that the cell was highly elongated, but division did not happen, duplication of DNA had happened, but it was not undergoing the mitotic division. So there is some sort of block in mitosis. And another very interesting category that they observed were these tiny cells - and one of these mutants was referred to as *wee1*, which is a Scottish term for tiny or small. So the *wee1* mutant undergoes division, perhaps more rapidly than it should, it divides before the cell has actually attained the length that it should have and here you can see, such a divided cell where the cells are smaller than a normal cell would be at this stage.

So, the *cdc2* mutant is one that we will be talking about a lot. The *cdc2* mutant was studied and the gene defective in it was cloned by complementation and of course, to study this further, once the gene has been cloned, you can predict its protein sequence and develop antibodies and using these tools, it was found that the *cdc2* gene encodes a 34 kilodalton phosphoprotein, that is a protein which is phosphorylated, and it was also observed in experiments where cells that were exponentially growing, ultimately entered stationary phase, and it was observed that the protein levels and phosphorylation do not change during mitosis. So, in the exponential phase lot of cells are in mitosis and as the cells entered stationary phase, the p34 protein seemed to become de-phosphorylated. And, again, the state of phosphorylation of p34 was correlating with a high mitotic index, that is when you score the septated cells, that is an indicator of mitotic index and when there were more cells in mitosis, that is when you saw more of the phosphorylation of p34 and when the cell cease to divide, the phosphorylation was not seen. It was also observed by biochemical experiments that the p34 protein had kinase activity, that is it could itself phosphorylate other proteins and the kinase activity was thermolabile in a $cdc2^{ts}$ mutant, that is this mutant was defective in the kinase activity of the protein, which is encoded by this defective mutant gene.

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To summarize the first part of the lectures on cell cycle regulation, the mitotic cell cycle has G_1, S, G_2 and M phases, which occur in a sequential order. There is evidence for regulation of the cell cycle coming from cell fusion experiments and the experiments related to maturation of *Xenopus* oocytes that were carried out that led to the discovery of MPF. It was found that S-phase cell contents can induce G_1 cells to enter S-phase, but cannot induce G_2 cells. M phase extract can induce G_2 cells to enter into M phase. In addition, mutants that were showing cell cycle arrest phenotypes that is the, cell division cycle mutants or *cdc* mutants could be isolated from budding and fission yeasts. And these mutants are potentially defective in genes encoding key cell cycle regulators. Stay tuned for part 2 of the lecture on cell cycle regulation.