Introduction to Developmental Biology

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Lecture No-08

Genetic Basis

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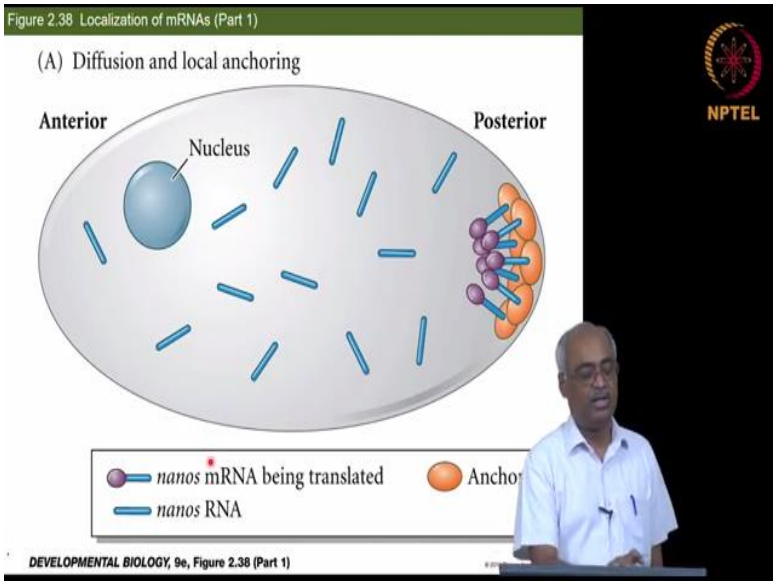

So yesterday, we did not complete our discussion on the RNA based differential regulation, so what did we discuss at length yesterday? What is the central theme? Yes, Alternative splicing and its impact. Then we discussed how micro RNA also contributes to regulation. So, I told you some of the mechanisms by which regulation happened, which we did not know till two decades ago. So, I was referring to micro RNA. So micro RNA is encoded by the genome, and its regulation in translation was not predicted until people discovered it through genetic mutant screens. Once discovered, we realized that it is there in all organisms, and its effect is a lot more widespread and general to the regulation than what originally was thought.

So that is why people called the non-protein-coding regions as junk DNA, so now we know probably there is no base in the genome without reason.

So today, we are going to look at how localization also plays a role; where an mRNA is present and when it is present also matters. So, here is one good example of Drosophila oocytes during its development. So even before fertilization, there is an asymmetric localization of molecules; a good example is the Nanos mRNA. So, it is deposited in the oocytes by the nurse cells. As the oocyte grows, the Nanos mRNA molecules move towards the posterior primarily by diffusion, but then at the posterior, they are bound by a protein complex primarily by another RNA binding protein called Oskar. This protects the Nanos RNA from degradation.

Oskar also activates Nanos translation, so RNA is being localized within the cell at the posterior region, and it gets translated there. So, the unlocalized Nanos RNA is not translationally active.

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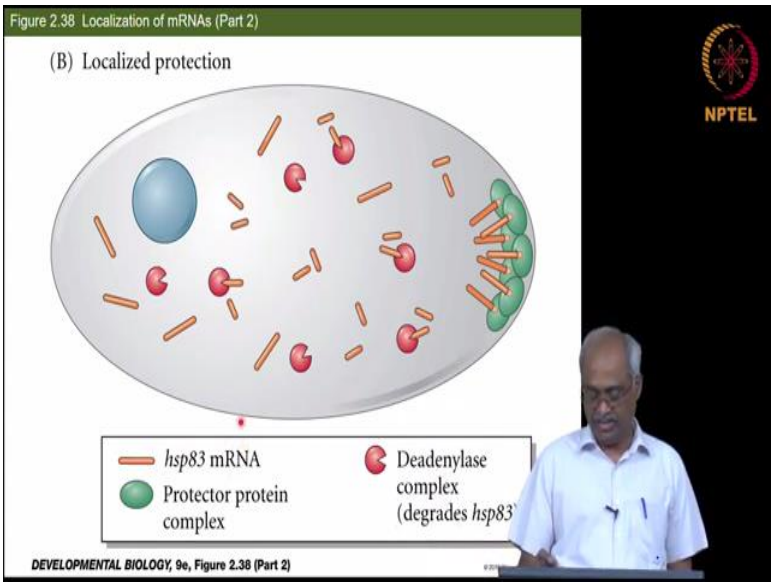

And another example we are going to see is again from Drosophila itself. The hsp83 mRNA is protected from degradation only when it is in the posterior. In other places, the Deadenylase complex is going to bind and degrade the RNA. So, therefore, localized protection.

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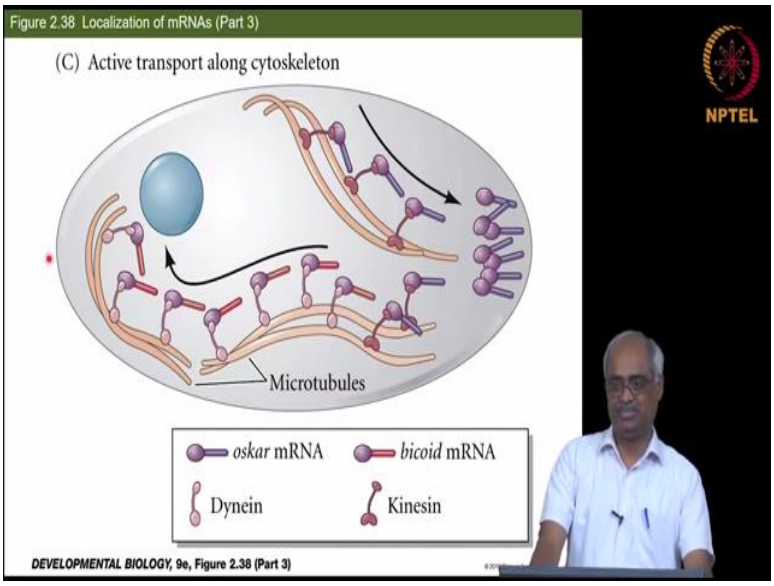

The third aspect of this is the transport of mRNA, so in Drosophila oocytes, Oskar mRNA is transported on these microtubules by the motor proteins to the posterior side. At the same time, on microtubules, motor proteins transfer Bicoid mRNA to the anterior. So, this is how asymmetric localization happens in the very early embryo itself; in this case, it is oocyte; it is not even fertilized. So, therefore, when you keep following, like yesterday's question, the transcription cascade, like this one is specific to this tissue, then what made it specific to that, like if you keep going back, you will all the way go back to a one-cell embryo, the zygote. Therefore, the differential gene expression starts in the one-cell embryo by asymmetric localization of molecules in a single cell.

So symmetric cytoplasm is converted into an asymmetric cytoplasm meaning different parts of the cytoplasm have different components. So, people think this is the starting point, and they are excited about embryonic polarity, and they focus on that. So, a lot of labs focus on how polarity is established in the early embryo in different model systems.

So, this is, for now, our brief overview of how differential gene expression is brought about. First, we learned that it is primarily the nuclear material that seems to be controlling development. The early genetic evidence told the same and then other experiments like, for example, somatic cloning experiments told there is a genomic equivalence. This convinced us that development must be brought about by differential regulation of gene expression.

So, now we saw how that might be accomplished. So various aspects starting from chromatin structure to mRNA asymmetric localization. So now we are going to take a huge detour we are moving from developmental biology to genetics because the whole of development has been learned only through the methods of genetics.

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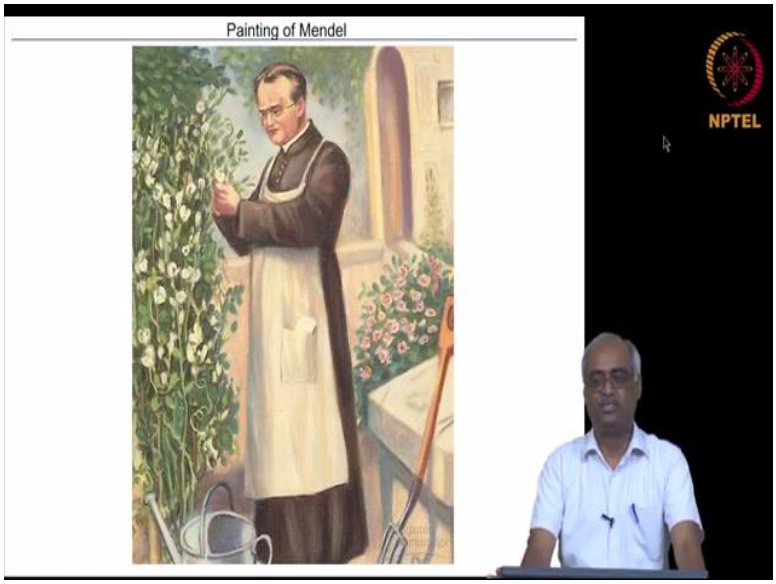

So, Gregor Mendel was an Austrian monk. He seriously tried to become a teacher and could not pass the exams and could never become a teacher. He failed multiple attempts and could not succeed; then, he realized the monastery is probably the place to spend the rest of the time. So before moving on to his experiments, I would like to tell the historical context in which his experiments were done. So, at that time, people knew genetic information is transmitted from one generation to another generation. Only a Homo sapiens can make another Homo sapiens similarly only a blackbuck makes a blackbuck so that already tells you that the blackbuck has the information to make another blackbuck, only humans have the information to make another human being. So obviously there is information, and that is being transmitted from generation to generation.

So the branch of science that tries to focus on how this information is transmitted? And what is this information? Are there general rules that govern this transmission process? That is genetics.

So people knew for transmission to happen information must exist, but they did not know how it happens. There are multiple theories, including the preformation theory, that is familiar to you.

But the predominant prevailing theory at that time was called a blending theory, meaning the information coming from the maternal and paternal source are blended to generate the offspring. But then there was a contradiction to this in everyday observation. For example, let us say your father and mother have straight hair, but your paternal grandmother had curly hair, and you have curly hair, so how blending will explain that? So, the curly hairiness has never been blended with straight hair and became intermediately curly in your parents, they both have straight hair. So, blending theory could not support this. In the F2 generation, that is in your generation; you get your grandmother's curliness in your hair. So certain features which resemble one of the grandparents are not there in your parents. So many features like that exist in nature.

For example, plant breeders quickly realized hybrid vigor happens in the hybrid, and then in F2 again, you get the grandparental qualities. So, like this, multiple pieces of the evidence contradicted blending theory. So, people were interested in finding out how exactly genetic information gets transmitted. So that is the context in which we need to look at Mendel's experiments.

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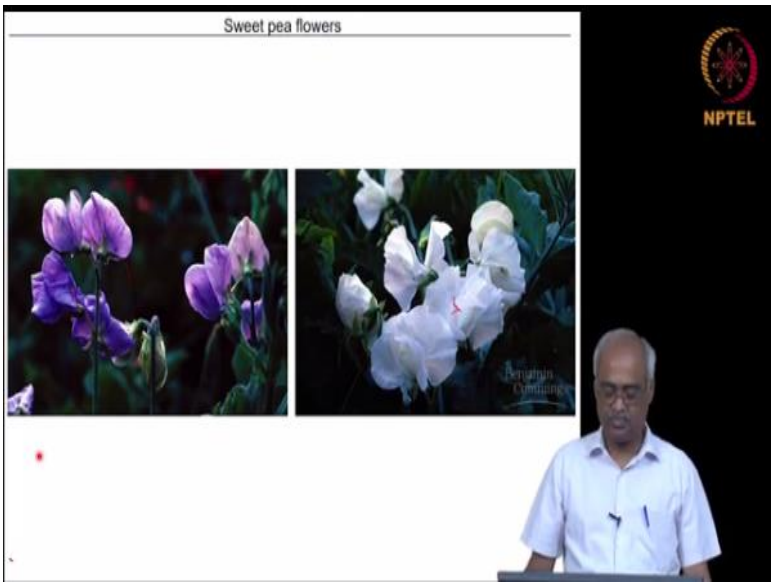

So first, let us look at his experimental strategy. So, garden pea plants were readily cultivatable earlier, and locally among the farmers. He could find seeds for garden pea having different phenotypes, for example, flower color variation, plant height, the position of the flowers, etc. Many variations were readily available, and therefore, he took them and cultivated and tried to do genetic crosses and observe what happens.

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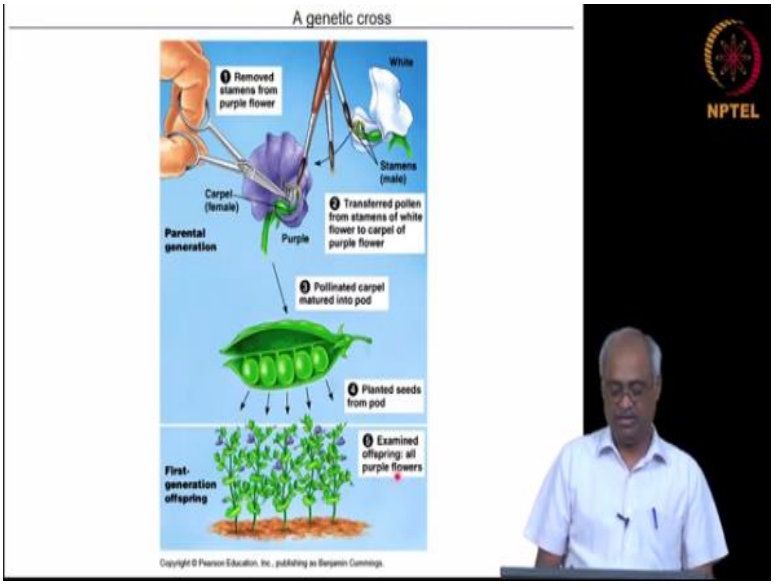

So, this is the experimental strategy, unlike us, humans, many flowering plants have both gametes in the same flowers, like pollen grains and ovum are present in the stigma and carpel. Since these flowers are bisexual, both the gametes come from the same genome.

So, if you take a garden pea variety, that is giving purple flower for several generations, then its genome contains the information to make only purple color. So, you cross a white one and purple one and follow the flower color to discover what rules govern genetic transmission.

Before that, we have to make sure gametes from the same flower are not fusing. So, for that, the flower is opened up even before it is ready for pollination, and the stamen is removed; this prevents the ovules in that flower from being fertilized by the pollen of the same flower. Also, it is covered with a paper bag that prevents fertilization by random pollen grain in the environment. Now you take a flower of a plant that you like, the white color one in this experiment, then with a paintbrush, you dab a little bit of the pollen grains from that, then open up the cover and coat it

on that carpel. So, this helps in controlling the pollen that will fertilize the oocytes of the plant that you have chosen as the female. So, this is how the genetic crosses are performed. So now the fertilized one is going to grow, so the ovary is going to form this pod; it makes more oocytes there, and therefore it is going to make several seeds. So, each seed is an embryo, it had one pollen grain's genome and one egg's genome fusing to create a diploid.

So, this is the experimental setup. When it grows now, if you are focusing on flower color, then you have to wait till the plant grows up to the flowering stage. And the flower color here is the phenotype, and the genetic information that is contributing to the color is called the genotype.

During performing these experiments, Mendel ensured that the seeds are pure breeding variety, which means for several generations in a row, that phenotype only was displayed by that particular set of seeds. Let us call it a strain, so that produce only that one variety, it was not giving rise to other phenotypes. So that is called pure breeding. So, as you see in the cartoon, all the flowers are like one of the two parents; in this case, purple color and he called this as the filial generation one, and therefore, this is F1, where this is the first generation.

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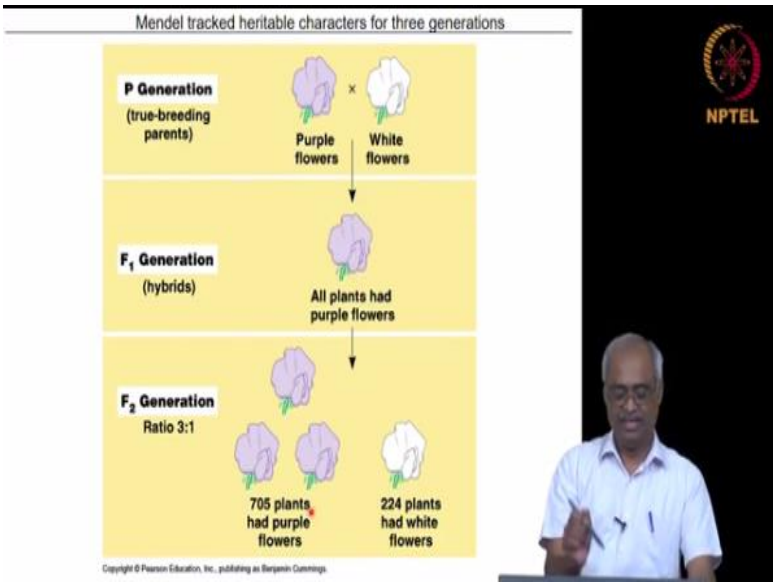

So, now let us look at what happens when you go further. So, this is the parental generation or P generation than filial generation one where all plants produced purple flowers. So, now what he does is; he does not open up and cover it and selectively brings stamen from somewhere; instead, he allows them to self-pollinate among themselves, and then collected the seeds and sowed it.

And when they grew, and new flowers came the F2 generation comes there, he ended up seeing roughly ¾ of the plants produced flowers that were purple color; the same color was there in the F1 generation, not the color that disappeared in F1. The color that was or the phenotype that was absent in F1 appeared in F2 in ¼ of the plants.

So, this is all the experiment and the result. So, the summary for one cross is there in this slide. So the parents are pure-breeding parents, one is always purple for generations, and the other was always white for generations. Then in F1, only purple is present, and white is absent, and in F2, when selfing takes place within its stamen and eggs, results in ¾ of the purple color and ¼ of the white color.

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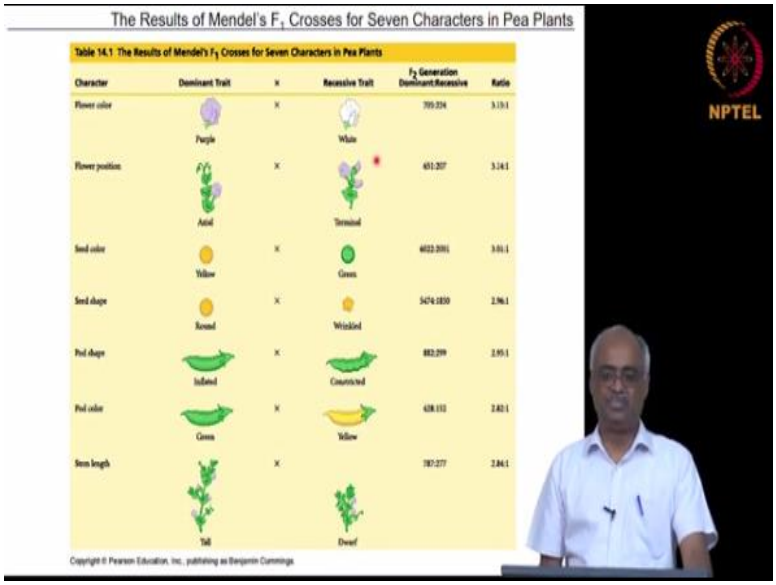

So the experiment was not done just with the flower color, it was done with various aspects of the pea plant as follows: axial bud versus terminal bud, yellow seed coat color versus green color, seed shape round or wrinkled, pod shape inflated or constricted, green or yellow pod, tall plant or short plant; like that he took multiple phenotypes. One phenotype at a time and did mating with the opposite or contrasting phenotype on the same thing like color or height or shape where you had a distinguishable another feature like purple color and white color.

So, he did multiple experiments, and in all of them, he got the same kind of numbers. He got 705:224, and therefore it is 3.15:1. So we never saw experimental data like this, so here if you see the numbers, these are the actual numbers that were counted. The color that was not there in F1 reappears in the F2 killed the blending theory.

If blending theory is wrong, then we have to come with another theory. You have to interpret the observed results right, so why in F1 one did not show up? Why 3:4 ratio, all the time, it is not random. So, the first thing is you need to assume that I am probably getting information from both parents. Parent 1 gave the information to make a purple color; parent 2 gave the information to make the white color. So, then you need to invoke this dominance and recessive to explain why one of them is absent in F1. So, to get to a 3:1 ratio, two sets of information are crucial. And only then this can be worked out.

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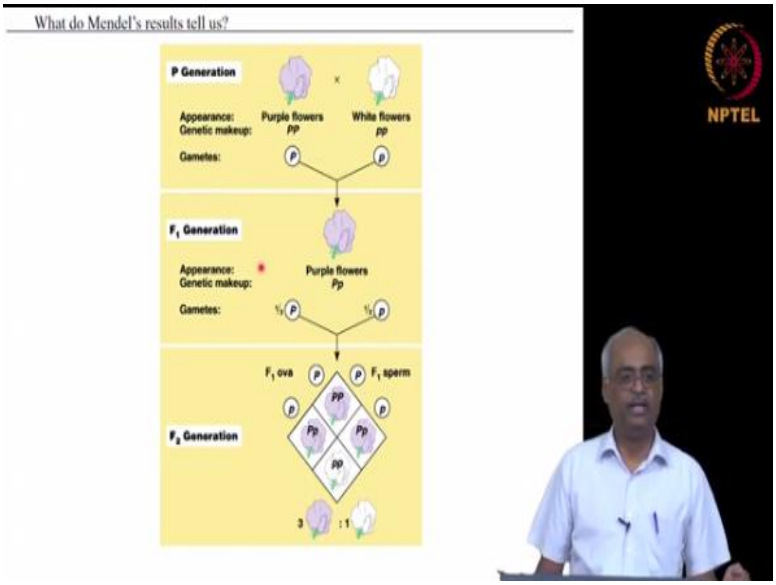

So, this is what Mendel worked out. He assumed that a sexually reproducing organism has two parents, and it probably has two copies of the biological information or genetic information. He assumed because this was before meiosis, cytogenetics is still not there, and microscopes are still not being used extensively. So, he believed that during gametogenesis, only one copy of the information goes to a gamete and not the other one, and therefore, two letters are used to denote a genotype. So, the reason we use two letters is based on the assumption that there were two

parents for that individual; therefore, two copies of the biological information and in gametogenesis each gamete gets only one copy. If you assume that I use P to denote a purple color and the gamete contributed by this, therefore, bring one copy of that. Now the other one similarly brings one copy of the white color, which is denoted by p. Now when these two fuses and make a diploid cell that is going to be Pp. Now, let us say the purple is dominant like purple is going to show up if it is present even in one copy, then this Pp will be purple.

Now let us look at gametogenesis in the F1 generation; half of the gametes will have P and half will have p that both eggs and sperm, now if there is a random fusion of P and p, then you will have this Punnett square representation. 50% will be P, and 50% will be p. Now you have a random fusion that I mean is during gametogenesis, there is no bias to have P or p, so therefore 50% of the gametes are P, 50% of the gametes are p. And similarly, when the two gametes fuse again, there is no bias, P is not looking for sperm with P. It fuses with either one of the two. Similarly, sperm does not have a preference for either one of the two types of oocytes. So, when you have total randomness, then you will end up getting this combination giving you 3:1, so this is what Mendel deduced from this experimental observation. And by considering that the parents chose had two parents, each he proposed the law of segregation. Meaning particular genetic information let us now use for convenience the word gene, probably exists in multiple variants; for example, one variant makes this purple color phenotype another variant makes this white color phenotype. And these two variants, how many ever variants exist they do not influence each other when present together as you see in the F1 generation, the white did not make purple to become something else or purple did not influence the other. They stayed together during which one does not alter the other one, and they independently segregate when gametes are made. One does not affect the other one, and then there is a random fusion of the gametes. So, that is what is the law of segregation.

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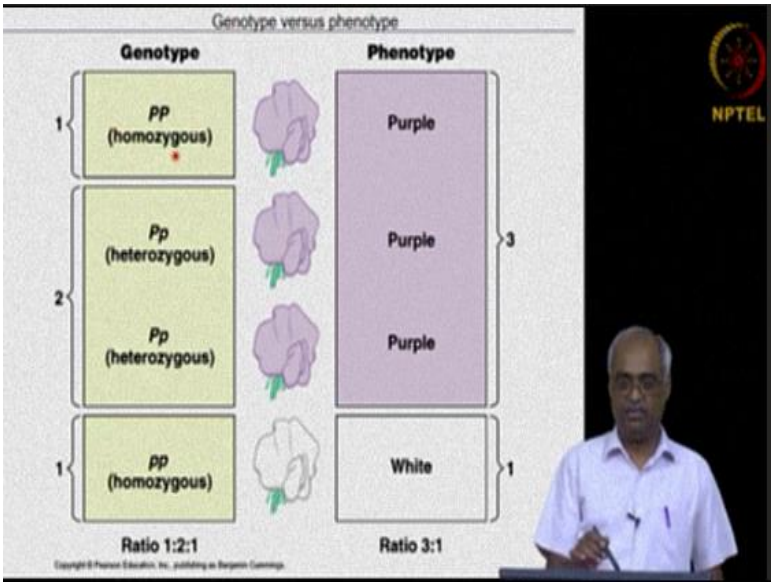

So now I should introduce these words, but you already know, you know what a genotype is? And what is phenotype? The genetic information or the informational constitution is what you call the genotype and phenotype is what is the visible biological outcome, biological function, in this case, the flower color. So, when you have the same type, you know both the variants are identical, so variants are alleles. When both the alleles are identical, it is homozygous, and when they are not, it is heterozygous. The genotypic ratio can be different from the phenotypic ratio; here, the genotypic ratio is 1:2, which means 1 is homozygous for the P, 2 is heterozygous, and 1 is homozygous for p, so 1:2:1. But the phenotypic ratio is 3:1, and this difference is explained by one of them being dominant over the other.

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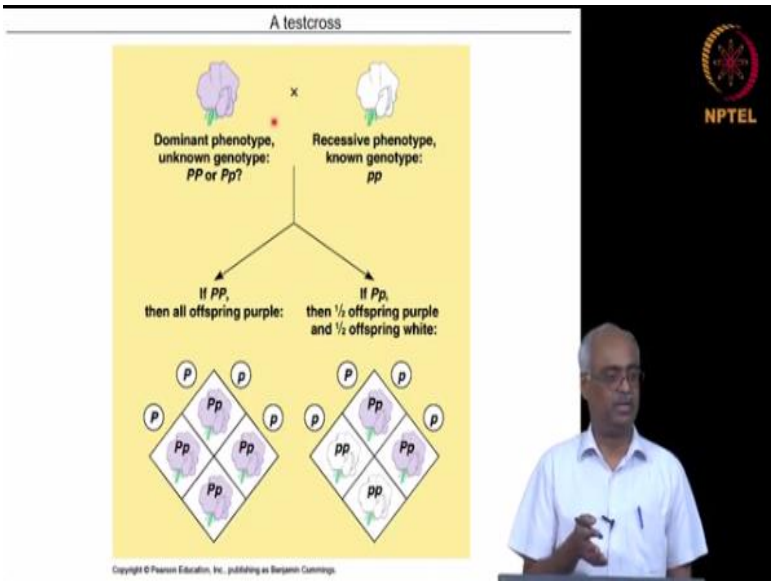

So, to find whether a given genotype is PP or Pp, a test cross is done. A test cross is when you have an organism displaying the dominant phenotype and if you want to find out whether its genotype is homozygous or heterozygous for the dominant allele. To address that, you do a genetic cross with a parent that is homozygous for the recessive phenotype. It is also called a back cross; because crossing the selected one with the parent with a recessive phenotype. Since you are crossing back with the parent many times, it is called a back cross, but a good way of saying it would be a test cross because it is a cross to test. So, this cross will result as follows: PP will make only one type of gamete, whereas Pp will make two types of gametes. If the unknown genotype is homozygous for P, it will produce all purple flowers, and if it is heterozygous, it will produce ¼ homozygous for the white flowers, which is the recessive phenotype.

So, if it is heterozygous, you will get a 1:1 ratio; if it is homozygous, you will only see the dominant phenotype. So, this is how you will determine the genotype whether it is homozygous or heterozygous for the dominant allele.

So, this is a back cross or test cross, so you remember crossing with the homozygous recessive parental phenotype.

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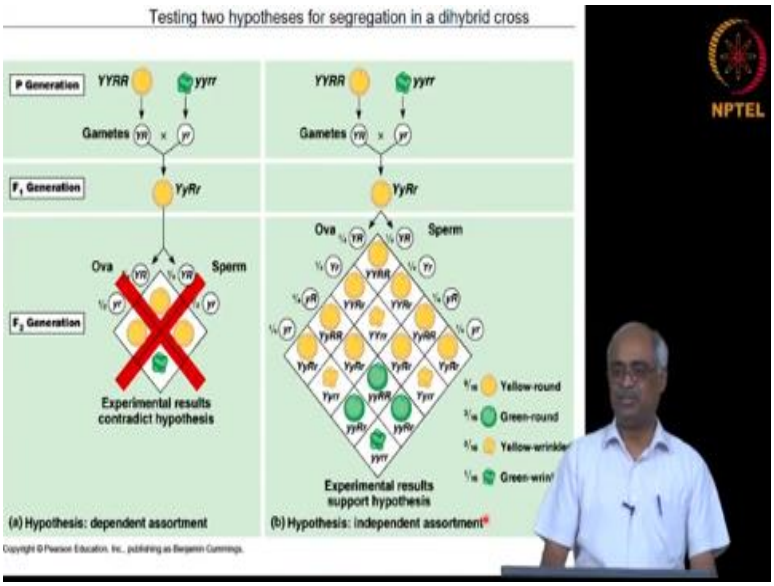

So, the next experiment is relatively easy to follow, so in the previous one, it is only looking at one particular phenotype, flower color alone, not two phenotypes at the same time. Now if we consider two phenotypes like color and height of the plant, will one influence the other?

Let us take a tall plant with a purple flower and a short plant with a white flower. Now, will the inheritance of white demand going to the short plant, or it can be on a tall plant too and vice versa. So, does the genetic information for one phenotype influence the transmission pattern of the genetic information for another phenotype? So that is what is this second experiment, the dihybrid cross.

Let us assume it will influence, then the tall and purple will always go together, then it will boil down to 3:1 ratio, it will be like a monohybrid cross, and that is what is illustrated in this cartoon. Here we are taking round and a yellow seed; the seed shape is one phenotype, and seed color is another phenotype. So, you assume there are two copies for each of those two phenotypes. It is also called a trait. Now the yellow and round seed plant is crossed with the green and wrinkled seed plant. During gametogenesis, you will find one copy for each, and when they fuse, you will get the combination in the slide. F1 is heterozygous for both; it is round, and yellow, meaning yellow is dominant over green round shape is dominant over the wrinkled shape and when you go to F2, if the round and yellow always were going together, then you will only get this 3:1

ratio of round yellow to wrinkled green. But when Mendel experimented, he found that they do not influence each other. So, he found 9:3:3:1 ratio, 9, 3, 3, 1 ratio of all possible four combinations.

So yellow and round did happen like one of the two parents, and you got green and wrinkled like the other parent, but you got the mix like green and round, yellow and wrinkled. This Punnett square explains how that might happen.

To explain this, one should assume that there is no bias in which allele goes into the gamete with respect to both of the phenotypes. So, a gamete that inherits the Y does not worry whether it is going to have R or r for the other information. So during fertilization, gamete fusion happens randomly, only then you will get 9:3:3:1 ratio, so when the inheritance pattern of alleles of one gene does not influence alleles of another gene, that is called independent assortment, and when alleles of a given gene do not influence each other, and they go separately; we call that as segregation, that is the law of segregation.

So, the independent assortment is the second law of Mendel. So, these are the basic principles like rules of addition and subtraction in math. The entire set of genetics is derived from these two laws. If you know this then you know genetics, now everything else is derivative of this. So that is why it is worth taking a lot of time to comprehend this completely.

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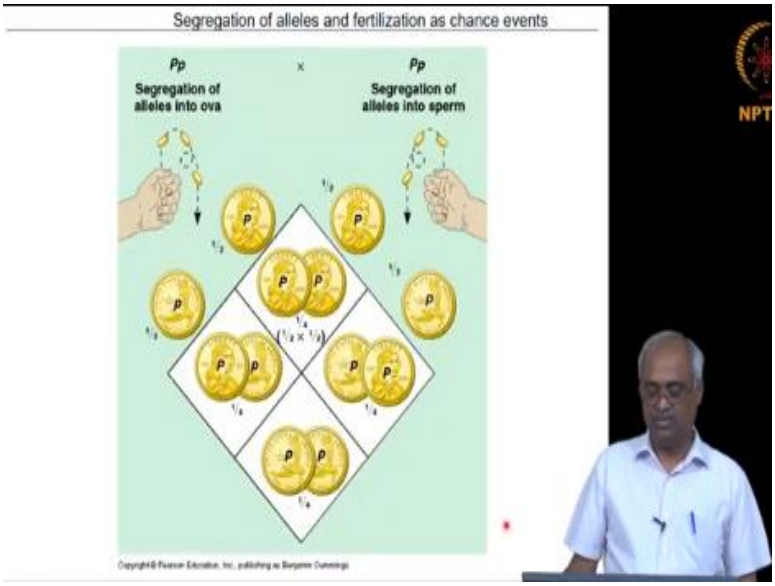

So whatever I already highlighted is shown here, so it is random; there are no biases.

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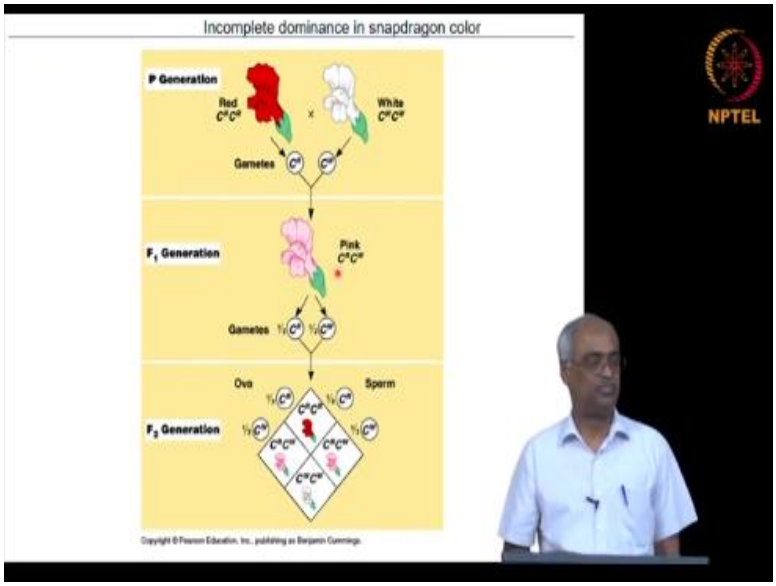

So now, we are going to consider the situation that appears as if they do not follow Mendel's laws, and we will try to understand how Mendel's rules are followed there. One case is what we call as incomplete dominant. The F1 generation phenotype does not resemble the phenotypes of either one of the two parents; instead, it is like an intermediate phenotype. When you go to F2, the phenotype accurately reflects the genotype 1:2:1.

To explain this, we need to get into biochemistry a little bit; then, it is easy to understand. So let us assume a particular enzyme that was catalyzing the reaction to make the pigment to make the

red color. When you had double the dose of it, remember we have already learned dosage compensation, and when you have double the amount, then it makes enough pigment to make the flower red. But if you have only one copy that means half the quantity of the enzyme, now the pigment produced is let us say only half of what would be made normally, and if that is visible, the diluted state is readily recognizable then that is called Incomplete dominance. But Mendel's laws are strictly followed with respect to the genotype here, only in the phenotype, you see a variation, and this is called incomplete.

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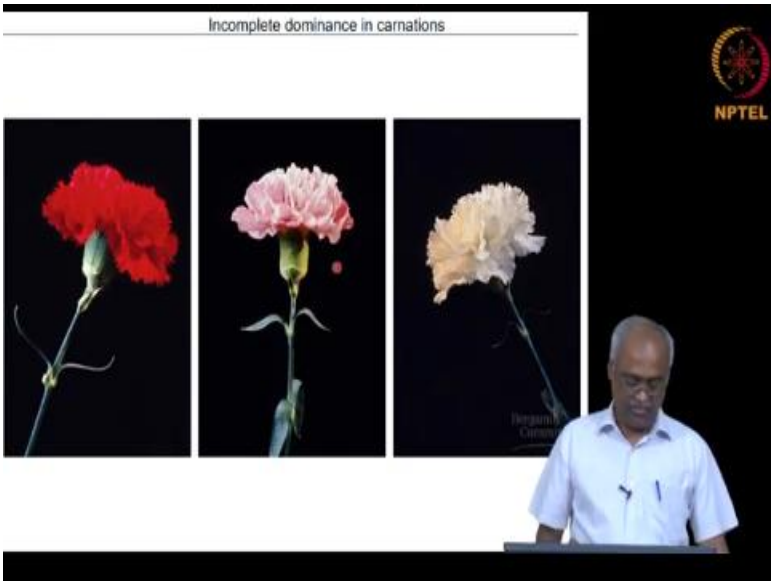

And so this is carnation, so that is Snapdragon you know Darwin's favourite plant, then this is carnation having a very similar thing.

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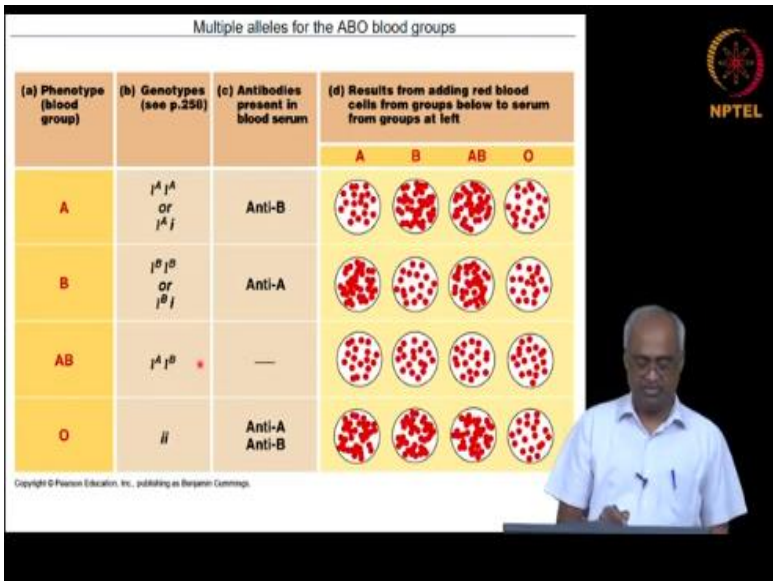

And then there is an opposite situation where both the alleles show their presence. For example, cell surface markers you can have two markers at the same time. Let us say there are 100 receptors on the surface of a cell, now 50 receptors could come from one allele, and 50 receptors could come from the other allele. Both are there on the same cell, and if one receptor bind one particular variant of a ligand of another one bind another variant of the ligand now, this cell will bind both of them. So, in such a situation, both can show their presence, and we call that as co-dominance. So, blood group antigen reaction is one such thing, so that is shown in the slide. I am not going to go into detail because you will readily understand this.

So for example, if you have blood group A, the RBC surface will have particular glycosylation, so you call that A, and that could be homozygous or heterozygous, similarly, blood group B has the B variant, a different sugar attached and that could be homozygous or heterozygous. As I just explained when you have multiple receptors, some could come from one allele; some could come from the other, and both may not be present, you probably lack both the modifications. So now, the way it reacts with the corresponding antibodies is where you find that it is co-dominant.

So when it is AB, both the modifications are present, so they have antigens for both, and lack antiserum for both. Therefore, you do not get any reaction. So that is co-dominance.

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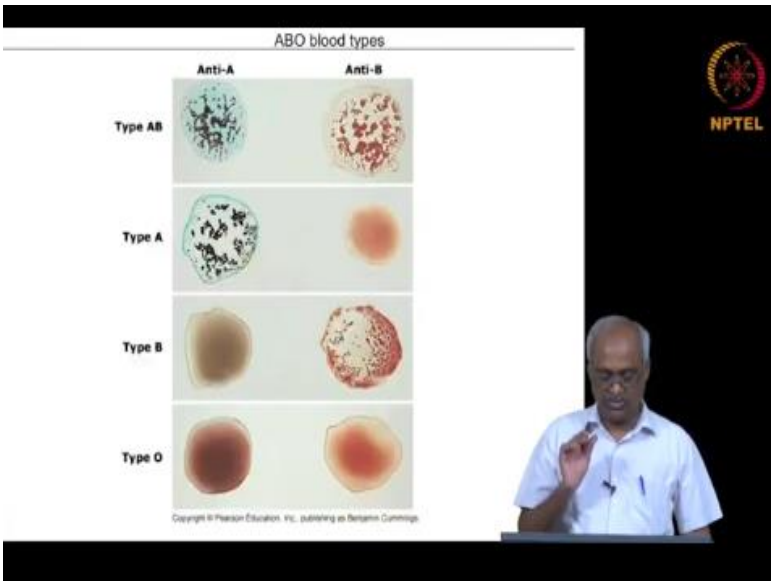

You understood how co-dominance is possible, so in both cases, we are getting to biochemistry to explain this, so this is the actual blood test I will ignore that.

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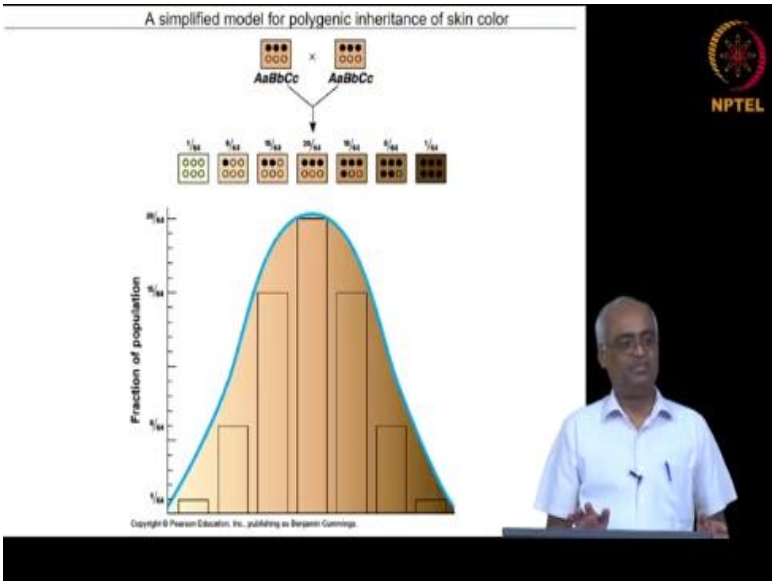

We are going to consider multiple genes at the same time, so that will be in the next class.