BioMEMS and Microfluidics
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Lecture - 23

Hello and welcome to this 23'rd lecture on biomicroelectro mechanical systems, today I will be actually kind of after briefly reviewing what has been done earlier last time would delve into the process of translation. And then will also looking to some other aspects related to this beautiful codification process, where there would be a change of language from nucleotides into amino acid sequences.

I will also talk about essentially what would be useful for coding what terms of amino acids talk a little bit about the degeneracy component in coding and then compare this whole system of compression of information and translating of information as if it work floppy drive computer model. And then we will go on to study some other binding mechanisms between different molecules use for a mobilization and surfaces following which will talk a little bit about enzyme kinetics using Michaelis-Menten equations.

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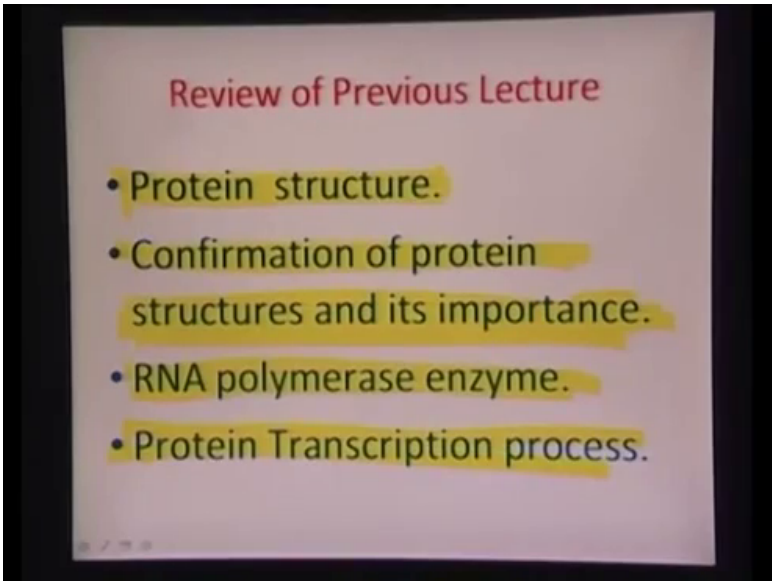

So, let us talk little bit of the review of previous lecture. So, last time we discussed details about the primary, secondary, tertiary and quaternary structure of proteins and also looked that how proteins can be formed as enzymes catalysis processes of condensation reactions essentially. Now, we talked about the various confirmatory stages of structures that proteins

have and it is importance and the importance of the R group and it is categories into polar, non polar, positive and negative charges for essentially the final structure formulation on the protein.

We then talked about essentially the RNA polymerase enzyme let us whole business of how it is used for compression of information from the DNA or the ribonucleic acid and convert that into a compressed mRNA and we look into the details of how this transcription can take place. So, let me just quickly review the transcription process once more.

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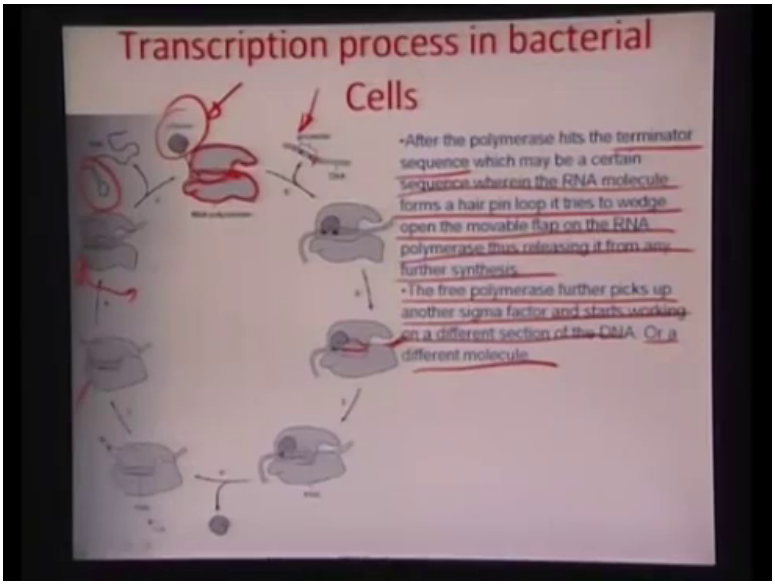

So, if you look at the transcription really it starts with this RNA polymerase, you call it a magician's hat. This structure here and essentially there is a sigma factor which comes as an information to find out what would be a region from where the binding and the opening of the DNA chain would starts. So, it essentially is use to identify the promoter region on the DNA. So, this is the DNA, the double stranded DNA and right here actually and you have this promoter region here on which the sigma factor goes and bites and starts opening the chain of the DNA into two.

So, the idea is that the chain passes to the other side in goes out of the RNA molecule, it again kind of zips back which unzips in this region and then the because of the binding of the sigma factor. And then essentially as it binds and opens there is in assembling of the nucleotides from the solution on to these structures here and these nucleotides essentially a sutured again

by the RNA polymerase enzyme. So, what it does is it changes the or it kind of sutures both the nucleotide of phosphate groups together by a sugar molecule in between.

Then, essentially after the 10 nucleotides are kind of operator, the sigma factor automatically loses it is grip on the DNA and then it is of course, a process down heal, down heal where the unzipping action on the movement of RNA polymerase automatically occurs, until it hits upon something which can give a kind of jerk to the RNA polymerase and it can ask the RNA polymerase to kind of decouple. And so once a decouples by a change in confirmation before that there is an automatic stoppage of the synthesis of the mRNA which is also kind of defined by something called a stop codon or a terminator sequence. And because of this certain change in conformation in the RNA polymer is, it also disengages the RNA molecule and sense it out of like you can see here the RNA molecule is been continually generated in this portion and whenever it hits of sequence where it can formulated here loop of something it changes conformation and detach itself. So, detach itself from the RNA polymerase and the polymerase actually finds out the other sigma factor to repeat this particular cycle. But, this whole beautiful process is about compressing in the information on the DNA in to this, this small strand here which is the ribonucleic acid essentially. So, this pretty much what it is.

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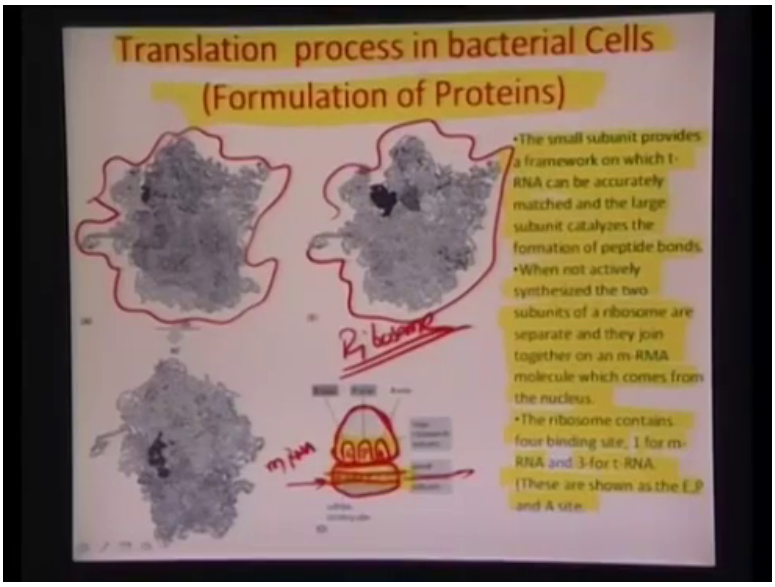

Let us now looking to a very different aspect of this poses of translation just as the prior process was discussed in the sense of the bacterial cells. This process also we will be considering as happens normally in the bacterial cells as, but essentially translation process as

a kind of applied to the mammalian cells also is similar to this translation process, where the few minor variance is there, but the basic mechanism is almost same as that is an DNA you know it is in the bacterial cell.

So, how does a translation process take place in a bacterial cell? So, you have this mRNA chain which has gotten out and you know from a certain region of the, as you know the bacterial cell does not have its a unicellular organism, which does not have a nucleus essentially. So, DNA is present always around that inside the membrane and there are organize dept within the cytoplasm and the cytoplasm essentially also contains all DNA.

So, all these mRNA synthesis work is inside there is no nucleus specifically for this purpose it is just inside the solution in the m RNA is actually then hits on to the endoplasmic reticulum where this ribosome's or protein assemblies kind of work on that RNA and try to sequence the amino acid based on the codons which are available let us look at it how. So, a small sub unit essentially this ribosome is one of the most intelligent nano machine is ever available in nature and given by mother in nature. So, it is a small sub unit which provides frame work on which t RNA can be accurately matched and the large sub unit catalysis the formation of the peptide bonds.

So, if you look at the basic RNA here you have, so if you look at the basic ribosome here, so this is the ribosome. So, we can classify the ribosome is an upper half and lower half and it is like a jaw, you must have seen this Pacman videos where there is a jaw which comes in start eating the balls or you know some kind of moving objects. So, here you can consider that these ribosome's are like a Pac man's which kind of move through the solution and the target the m RNA.

In the m RNA is essentially slipped through in the frame work on the lower portion of the ribosome and kind of goes there in attaches and the upper portion is ready to suture the transfer RNA which is their round solutions, essentially by matching this sequence of the transfer RNA on to the sequence of the m RNA. However, there is only a set of three sequence, three base pairs which are available to on to one transfer RNA.

So, therefore, the read outs of assembly of the t RNA on the m RNA is essentially a 3 by 3 by 3 combination. So, essentially you have three base pair at a time which is to be read and the corresponding tRNA has to be inducted which also has three active sides for binding on to

this three codans and then the next three groups are taken on then the next three groups are taken on and therefore, there is a set of transfer RNA which are sutured on to this change.

So, for the Pac man model the lower portion kind of engages the m RNA and the upper portion is able to suture the t RNA on the m RNA three at a time, three base pair at a time. So, that is how this kind of protocol works on, going to illustrate this details a little more. So, let us look at what these are really comprised of the ribosome essentially contain about 50 different kind of proteins and several different kind of m RNA structures, it is made up of a jaw like arrangement with an upper and lower part, this is how it really looks like the so many different proteins folded in different confirmations and also so many different RNA's with in this particular network.

You can look at it as a set of jaws this is the upper jaw, this is the lower jaw and essentially this jaws have three different binding sites here which is represented by let say E, P and A. So, these essentially are pockets is not very clear in the diagram here, but this E, P, A sides they are pockets on the upper portion of the RNA polymerase, the ribosome.

So, their pockets in which essentially the as we will see later the transfer RNA we will go and fit really. The lower portion as this rail here through which you can actually pass a small m RNA molecule. So, the m RNA can go through this rail and this is essentially the m RNA. So, you have these different base pairs on the m RNA which can pass through this rails it goes in here and out of this particular site. So, when not actively synthesized the two sub units of ribosome are separate and the join together on an m RNA molecule which comes from the nucleus.

In this case, because it is a bacterial m RNA it is produce within the solution and it comes to the ribosome. So, the jaw only closes whenever there is an m RNA, if there is no m RNA in the rails are open and the jaw is also in the open state the upper half is open like mouth. The ribosome contains four bindings' sites, one for mRNA as I already illustrated here, this essentially this area which is like a rail and three for the transfer RNS's as E P and A side as you can see here in this particular region on the upper flank of the ribosome.

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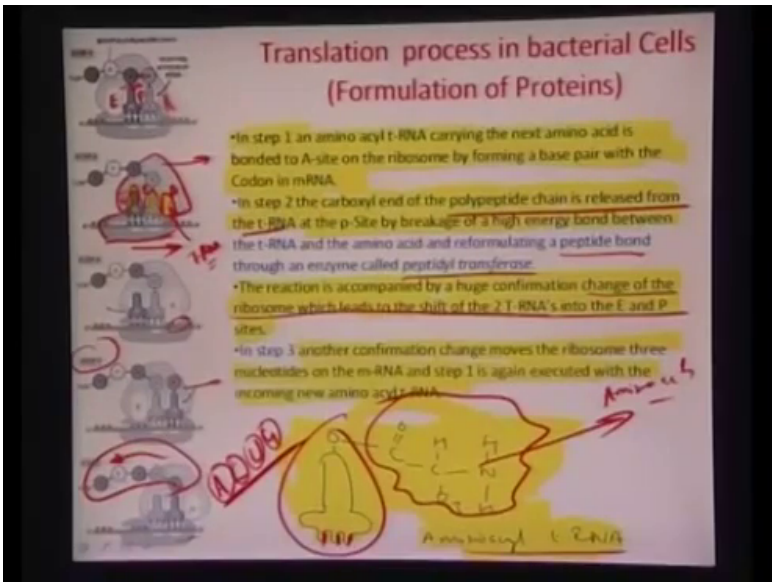

Now, a very interestingly there are these bunches of aminoacyl transfer RNA which are available in plenty within the environment of the cell. So, this is actually an aminoacyl t RNA. I will just like to illustrate here that there are about 20 different amino acids which have sequence somewhere of the other through this aminoacyl at tRNA. So, essentially this is the protein part of it aminoacyl group, you may just illustrate this through little more appropriately.

So, this part is essentially the aminoacyl group on to this moiety here which is a transfer RNA. The beauty about transfer RNA, transfer RNA is this, this three group here for binding they are essentially three among the four different groups that normally and RNA would have that is adenine, cytosine, euro cell and guanine there is no thymine on the RNA as we told before. So, these three groups essentially are bonding sides compressing of one or three of this molecule, three of these four molecules.

So, based on the sequence here you can find out a sequence on the mRNA and try to bind this particular aminoacyl tRNA, but what is important is that this sequence matching essentially leads to development of another sequence here of all these different amino acids and that is what essentially a protein all is about. So, in step one as you can illustrate or as you can see here in this particular figure the aminoacyl tRNA caring the next amino acid is bonded to the A side for.

So, this is the A side here you can consider this is a sight let me just illustrate this little more clearly this the A side this particular transfer RNA is essentially with the three groups here and it bonds over three different base pairs on the m RNA. So, incidentally this three base pairs on the m RNA is also known as codon sequence, it is called a codon.

So, therefore, the synthesis of these amino acids one by one on the mRNA is essentially codon by codon. So, you read three base pairs at a time and try to match it with the three base pairs complimentary to the codon sequence present on the transfer RNA and it has an aminoacyl group at the end of the transfer RNA which kind of get synthesized, because of the binding of the t RNA with the m RNA at a particular sight of interest. So, this is the sight A in which the first transfer RNA is kind of assembled.

Now, note here that based on the sequence of 3 the RNA this ribosome would pick up a molecule the transfer RNA molecule which is exactly the complement three base pair, it will not do something where there is mismatch. And so the very fact that you know certain a sequence is only determined by the sequence of the m RNA comes very, very clear. So, there is a matching between complimentary bases forming a bases of binding of the t RNA with the mRNA.

So, and sight A this binds first and then leave and amino group here, as you can see in this particular illustration, the amino group is this circle here which is been left. So, let say you have an amino acid 4 which you are trying to at this chain. And step 2, the carboxylic and the polypeptide chain this released from the transfer RNA and at the P side the breaking of a high energy bond between the same. So, essentially there was a previous transfer RNA which was already immobilized as you can see here in this particular example to another that is the P side here, so this is the P side, this is the A side and this is the E side as I talked about in my last slide.

So, there is already you know at transfer RNA which has been bonded to this amino acid ef 3 which is already formed a bond with earlier produced amino acid 2. So, this chain is kind of how exiting out of the ribosome in this particular manner. So, you have a amino acid 4 here. So, after the suturement of this or filling up of this active side A with the transfer RNA of a particular kinds. So, as this brings up the particular aminoacyl group or the amino group here close by the polypeptide chain is released from the t RNA.

So, essentially we are talking about releasing this particular portion of the molecule, let us just close all this. So, we are talking about breaking this particular bond here of the molecule, so this polypeptide chain with tRNA. So, essentially what does engaging this portion of the molecule by breakage of this high energy bond, this is the very high energy bond between the transfer RNA and the aminoacyl group. And simultaneously what this enzyme does is this ribosome does is that and kind of creates and other bond or another peptide linkage between the 3 and the 4.

So, it is not only breaking this high energy bond, but also developing a bond between the 3 and 4 this is essentially again a polycondensation reaction you need and enzyme catalyzed polycondensation reaction here if you remember from your first slide how a peptide linkages may between two groups of a chain which as OH on the group and NH 3 essentially NH 2 on the other hand. So, you have H_2O coming out, so it is condensation reaction in the present of enzyme.

So, you are making this bond and you are breaking this particular bond. So, that you are disengaging the transfer RNA which is there in the sight P and engaging the amino acid on the bound transfer RNA on the sight A. So, the enzyme that does this activities also known as the polypeptide transferase this is an enzyme which would be able to break the high energy polypeptide chain from the transfer RNA and at the same time suture the peptide linkage between the two amino acids.

So, somehow this reaction, because there is a sudden liberation of the amino acid from the transfer RNA and a certain bonding of this particular amino acid with the another amino acid group on the last transfer RNA, there is a huge change in conformation or huge change in size of the particular ribosome. So, there is a confirmatory change in the ribosome suddenly due to which there is a change leading to shift of the 2 tRNA is into the E N P sights respectively.

So, what happens is that from, so this is the E sight, this is the P sight and this is the A sight. So, there is a confirmatory change suddenly, because of this additional this bond break it here in this bond formulation here, the certain change which will Leeds this upper charge you are seem to move one unit on the right. So, essentially what happens is that if you see, if you look at these different pocket C, P AND A, the two transfer RNA shift from pocket p and A to sight e and sight p, A is left open and what a does is essentially it takes in and other t RNA aminoacyl t RNA from the solution.

So, therefore, the reaction is a combine by huge conformation change of the ribosome which leads to the shift of the 2 tRNA from into the E N P sides. And that is step 2 here, so there is a confirmatory change leading to the opening of this a side and closing respectively of the E N P side, transfer RNA change the position. And in the following step there is another conformation change which moves the ribosome, three nucleotides on the m RNA.

Essentially,, what you are doing is, this lower group if you see here the lower part of the jaw is kind of shifting in this direction and it changes by three groups and three next groups are inducted inside here. So, essentially it is a biting a moving process, so you have this chain of m RNA, so it has bit in let say on this my three fingers and so there are this three fingers on the upper jaw of the ribosome and this lower one has a track like this. So, you have bitten here and then there is certain conformation state change of this three and then following it there is a conformation change state of the lower jaw and it keeps on crawling like this over the whole m RNA.

So, there is a continuous rail of this mRNA passing through the lower jaw as there is a slow pick up of the three side to the next due to conformation change on the upper half and then the lower half coming back, then again the next three sides for three new transfer in his and the lower half again coming back. So, it keeps on reading on the whole RNA change like this. So, the same process is again repeated after step 3, where a new transfer RNA is inducted and mind you all these coding is happening, because there is a match between this codon, the three new induced groups on the m RNA and the three on the aminoacyl tRNA.

So, what the RNA what the ribosome really does is in matching of between the transfer RNA three groups, three n groups and the mRNA. So; that means, there is a complimentary next which it kind of defines for attaching the right transfer RNA which would also transport aminoacyl group by aminoacyl group and then this enzymes peptide transfer, etcetera they would just cleave off the polypeptide chain and make a peptide between the two amino acid sequences.

So, that way as this think is sliding there is continues production of amino acids, as you can see here this chain grow with time in this particular slide. You can see this chain here growing from this, you know this is continuously you can see then it is keeping on adding these this different groups, one, two then this is the third group, this is the forth group, fifth group and this chain is kind of getting liberated as the RNA slowly moving by upper half moving, then

lower half following again upper half moving to the right and then the lower half again following.

So, the steps are repeated again and again, so there is a continues production of this protein change and these are primary interest. Because, the sequence that you are bringing to MRN from all the way from nuclear and the normal sell or in a bacterial cell in the solution general is really dependent on the portion of the DNA that you are binding that is number 1, number 2 is that sequence is translated in to another sequence of molecule which are so called proteins of the particular reaction.

So, this really brings us to very, very interesting aspect of cell biology in an a sense this is probably one of the most fantastic nano machinery that mother nature has provided as, the rate at which the synthesis can be done is tremendous there are thousands of proteins which are generated in a single second by the several different ribosome’s which are floating around in the cell. And at the same time there are lot of these mRNA's which are being produced on the nucleus and they are based on the signaling which the sigma factor or some other chemical signals which the RNA polymerase gets and it gets an information where to start binding and start the production.

So, there is a rapid production of these all these, you know different groups matching each other and thousands of mRNA's getting generated every second which leads to thousands of proteins and this proteins sometimes, you know get to the surface, express on the surface and try to get back the physiological state of the cell back in to equilibrium every time there is some kind of different equilibrium created by process it is essentially a chemical signal which would give you more proteins that the process can be brought back in to the equilibrium state of the body.

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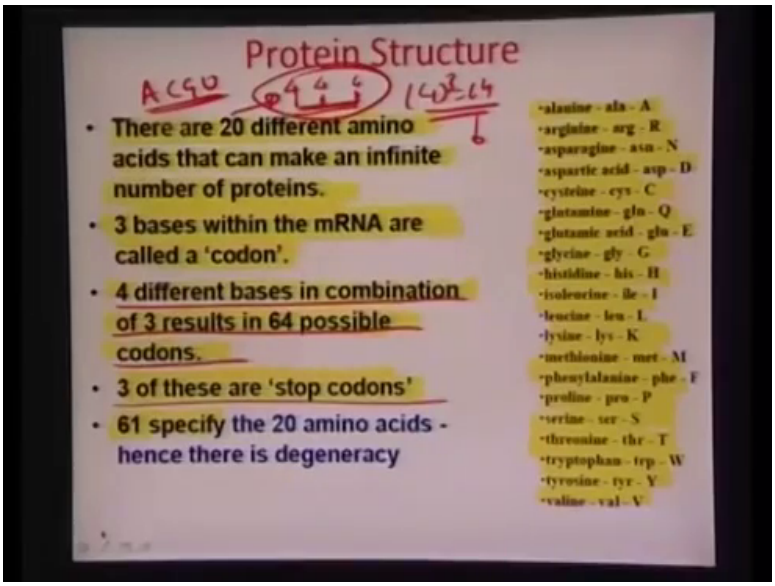

So, looking in to summary of whatever we have done so for and then trying to do some facts and figures, we know there about 20 different amino acids that can make a infinite number of proteins, you already know what happens if there is a 25 amino acid sequence there about 20 to the power 25 different combinations of proteins which are available. So, this big a number only for about 25 amino acids let alone the other proteins which are probably thousands of amino acids nit together.

So, there is really, really very high and you can produce many combinations of these different base pairs together. So, three bases within the mRNA which can code an amino acid is essentially call the codon, the word codon actually comes from the word coding. So, you are using three base pairs successfully placed on mRNA molecule to essentially code a certain amino acid and then there are four different bases in combination of 3.

So, if you look at the three sides let say you have three sides here and you have four bases A, C, G and U. So, if you put the different combinations that make exist in the sequence there are four that can come and one with another four on and other and other four assuming that there is repetition of the particular group. So, the really about 4 to the power 3 or 64 different such combinations of base pairs which can exist in one codon.

So, there are different there about 64 different codons that are possible based on this arrangements. So, four different bases in combinations of three results in 64 possible codons. Now, as we know there are some terminating sequences or stop codons essentially on you

know the on the RNA where essentially there is a disjointing action, because of a conformation change of the RNA polymerase from over from sliding over the DNA.

So, it kind of stops the process of m RNA production and the m RNA from the RNA polymerase and also folds backs the DNA or zip backs the DNA into a normal double standard structure. So, these three codons are also known as stop codons or terminating sequences. So, let say there are three codons, so how many are really left with you have about 64 possible combinations sort of which three are always use for termination of the process so this termination sequences.

So, therefore, there about 61 combinations then maybe use for specify amino acids, but then unfortunate adders that you know there only about 20 amino acids as a explain here and this particular illustrations. So, there about 20 different amino acids as you can see in this particular illustration and there are about close to 61 different combinations of this sequences or codons which are possible to code this 20 amino acids. So, definitely that is going to be degeneracy.

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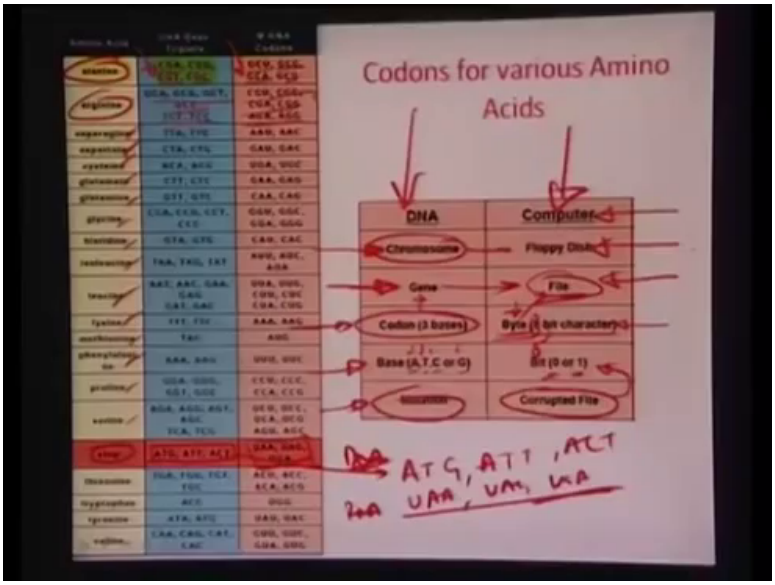

So, let us look into some fraction figures this right here is illustration of what really happen. So, this is the particular amino acid and this is being coded by these DNA base triplets, CGA, CGG, CGT and CGC and correspondingly the mRNA code on that are produced GCU a mind you, if you look at the little more closely that is complimentary in this therefore, if there is C and there is G and the mRNA that is the G and the DNA based apply, so there is a C and the

mRNA that is a 99 here in the DNA based reflect is a U uracil on m RNA mind I mean again I would just like to remind you that uracil is the substitution for thymine group on the RNA and the RNA molecule.

So, therefore, if you see more there are this CGG and the DNA based upon corresponding to GCC and so on so forth. So, this alanine amino acid is been coded with these many based triplets and these many m RNA codons. So, definitely that is going to be a degenerates as I told you before there about 61 codons which can codon 20 different amino acids; that means, the more than one codons which could be responsibly coding one amino acid, this is essentially called a degeneracy component of the coding.

So, there is a region here, amino acid and you can see the number base sequence on the DNA as well as in the RNA m RNA which would be use for coding this. And similarly there is a these things go through for asparagine aspartate system glutamate, glutamine, glycine ((Refer Time: 30:54)) isoleucine, leucine, lysine, methionine so on so forth.

What I am also interested in looking it this stop codons here, if you look at essentially this particular portion you know of the molecule, it is actually a stop codon let me just do well little bit of. So, this is essentially a stop codon and there are the sequences here, which we will I will just illustrate here a little bit. So, the base triplets for the DNA, ATT ATG, ACT are essentially stop codon this correspond to a normal sequence UAA, UAG, UGA. So, these are essentially the RNA triplets, this is the DNA triplet.

So, wherever the RNA polymerase hits one or more of these sequences that is almost always are detachment and that is almost always a separation of the RNA and stopping of the process, so that is why the termination sequence of stop codons. So, these are the stop codons and the other 64 combinations which are there essentially a coding this different amino acids, this 20 different amino acids. So, it is really like a mini computers systems.

So, I would like to jaw very strong analogy between what happens in a computer as to what happens on this DNA as you do this transcription and translation. So, on a computer if you look at you know you have something like a floppy disk and you can consider that in this DNA codification and translation process, the chromosomes are like floppy disk they are essentially read outs of data points, where this compressed DNA, compressed all this information is present.

So, on a floppy disk you have several files to store, whereas in the chromosomes we have this different genes of this DNA which are super coil and placed inside the chromosomes. The chromosomes is kind of equivalent to the floppy disk here and the gene is kind of equivalent to the file on the floppy disk here. And so therefore, if you consider of a file really it is made up of this an electronic information call bytes and one a byte is basically 8 bit character, a bit could be either 0 or 1.

So, you have like an 8 bit character which comprise a byte and then there are several kilo bytes, mega bytes you know those kind of things which can be the size of the total information on a single file as you can see here. So, the bit here is essentially either is 0 or a 1 correspondingly, if you look into a gene really it is the codon of these three base pairs on the mRNA which is comprising of the same information as is there on a byte which can correlate to a file.

In this case the codon is the information a three base pair on the DNA or a base step on the DNA which is a codon which actually comprises of the gene several of this codons all together comprising of the gene just as several of this bytes, kilo bits or mega bytes comprised of the file here. And just as in a byte you have about 0 or 8 bit characters where one the characters could be a one bit could be a either a 0 or a 1. So, you have 8 such numbers or 8 such combinations of 0's and 1's for representing one byte.

In this case there are this four different combinations A T C and G, Adenine, Thymine, Cytosine, Guanine which are the four base pairs on DNA which comprises of this codon. So, these three bases are essentially made up of 4 rather than 2, so do you see the complication here in a computer what is done with two characters in the real life in DNA it is done with four different characters and the computation essentially just increases 4 times I mean 2 to the power 2 essentially times.

So, you are considering here instead of 0's and 1's four different characters to write a bit of information and then from that 8 byte, 8 bits are taking to gather to a byte and here from the several of this bases and taken in to gather to make a codon which is three base pair law. And so what is important here for need to tell use that corrupted file in this kind of a case maybe something which has an information or a link missing.

In case of the DNA this corrupted file mutation, where there is a certain sequence mismatch or there is a certain sequence redefining which may modify all together the way that a DNA

performs in all it is you know processing including codification including everything, there it particularly does. So, there is definitely correlation between the DNA a between what the DNA does not what a computed as.

So, this is really, really very interesting model which source how the human machine can behave in the preprogram in to manner. And in fact, in the way that as cell dies also is pre programmed or prior programmed inside the cell using this information and that is the most amazing part of what we are really and what probably mother nature has made us of, so that we have predefine time lines of existence.

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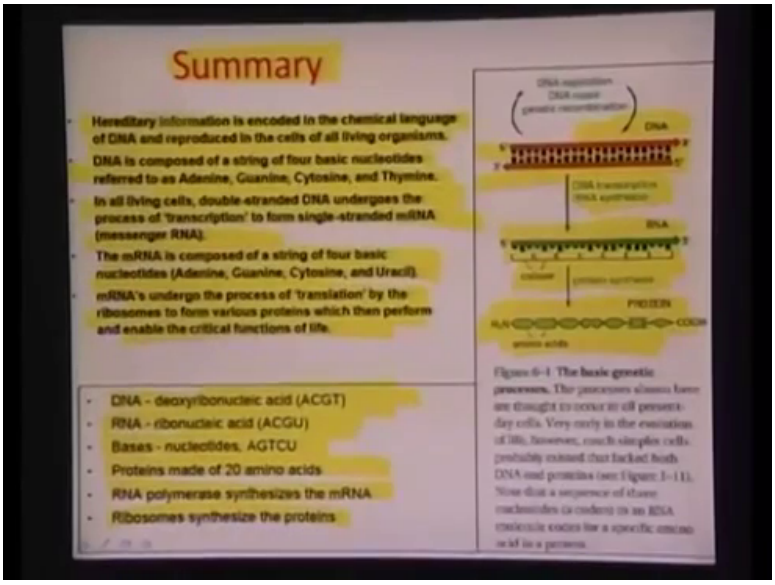

So, some philosophical thoughts and that and then let us looking into another kind of summary of the whole transcription translation process. So, in summary what we would say here is that you know essentially the hereditary information, hereditary information is encoded in the chemical language of DNA. And this is also a bases of the production and cells an all living organisms.

The DNA essentially as we knows comprised of four basic groups, adenine, guanine, cytosine, thymine and in all living cells essentially these double stranded DNA under goes the process of transcription and to form single standard messenger RNA. And it is a much more compressed code of information then that on the DNA itself. The m RNA of course, is comprised of adenine, guanine, cytosine and uracil, uracil is the new group which is added on

to essentially all RNA's and which kind of replaces thymine on the DNA, so that is how they are belt.

Then, the m RNA's which are generated this way and it goes this translation mechanism in the ribosome where in a group of amino acids just is on the basis of three base pairs at a time are a codon in the m RNA and that way you can have a sequence of amino acid from a sequence of m RNA essentially. So, there in a nutshell what this all this process is comprising of you can look at whether the nice cartoon here in the right which talks about this DNA structure is you can see with the help of RNA polymerase you can generate this RNA and from the RNA again you can produce a protein using ribosome's is the enzyme.

So, some facts and figures and some take homes at the DNA essentially comprise of ACGT RNA ribonucleic acids comprise of ACGU and nucleotides are bases essentially in ACGTU all of them, proteins are made of 20 different amino acids in different combinations and variety of combinations. The RNA polymerase in this is the m RNA from the DNA and the ribosome's synthesis with the protein from the m RNA, these are in general take homes.

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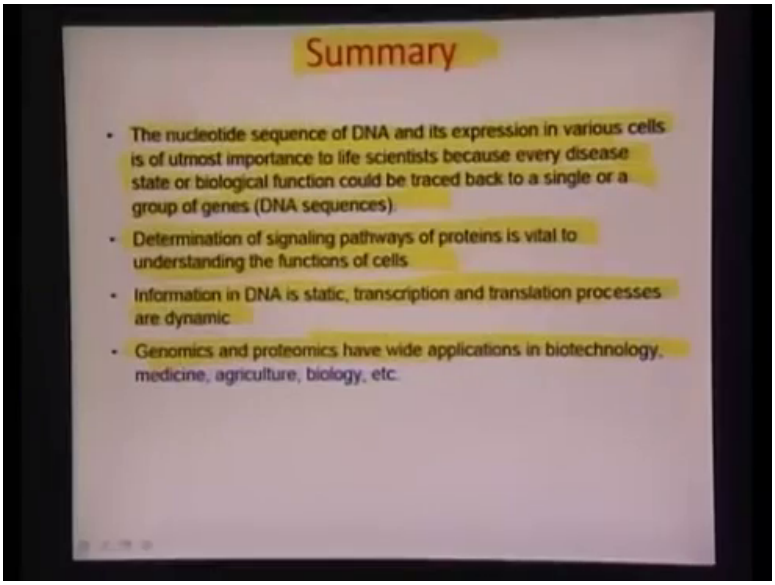

In summary I would like to say the whole area that we have a discussing so for of this translation and transcription is essentially based on or evolving around the fact that the nucleotides sequence of DNA and it is expression in various cells is at most importance. Because, every disease state, essentially or biological function of a cell could be traced back to a single of a group of genes, in the DNA sequences.

So, it is very, very important to find out how a cell can be able to arrive itself from a disease into another equilibrium static where they do not perform any malfunction and this whole root of a conversion from DNA to RNA to a protein is essentially helping it to do that. So, the determination of the signaling pathway of proteins is very, very vital to the understanding functional of cell, this is essentially what the signaling pathway would mean.

So, information in DNA is pretty static and it does not change much with time except when there are notations. However, the m RNA which is transcribed on to the DNA is a continuously changing, continuously generating form of molecule. So, several copies of this would be produce time again and the transcription process are very, very dynamic along with the transcription processes. So, it is not that stable as the DNA it is a very, very Meta stable molecule.

Therefore, sometimes in a detection is also it is a better idea to look at the messenger RNA instead of the DNA as a bases of detection of the particular entity, a logical entity. Of course, this two fields of a time trying to study what is on the gene and trying to go particular protein trying to study what is on the amino acid in the protein they do make up this area genomics and proteomics and these two standalone have very, very wide application is on did in the field biotechnology medicine agriculture biology etcetera.

So, therefore, in a nutshell, this is what the life process all the reverse around for kind of getting back into another wise health is state from the disease state. So, if you could work out in a better way on this whole, this principle of transcription and translation and could have identify the individual path ways which would cross the signaling to happen to produce proteins or not to produce protein or produce at a certain rate you could control disease is essentially in human body.

Therefore, this whole area systems biology now is actually more concerned with working and biology from systems point of view, where in all these information pieces that you get a kind of stored up in an organized you know bases, where you could have a proper control on the signaling process on the way that the RNA would be essentially produce from the DNA in the way that the protein would be produce from the RNA and any of this mechanisms if disbalanced or changed or altered can produce all together different set of processes for the cell.

So, looking from that angle the system biology this kind of derives his theory and data base accordingly. So, we are kind of towards the end of this transcription translation process, another very interest thing factor which I would like to discuss before moving on to the next topic is that how proteins can be used as linker molecules and how they can be use immobilize different moieties is biological and this on to surface. And for that we need to study the protein complex is as bio links.

So, we have just few pieces of information put together here, which talks about and this paper this essentially as we taken up from variety of literature, you know which is available worldwide there is certain references which have also quoted here.

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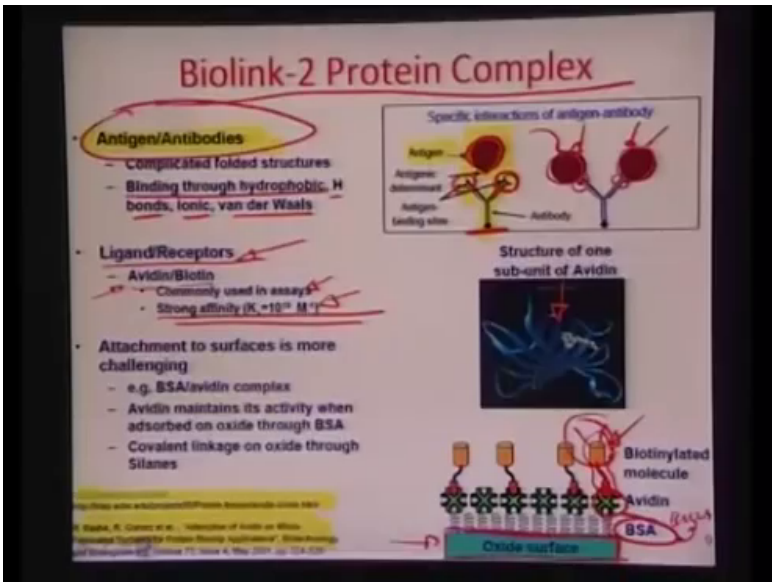

Essentially this paper on absorption of avidin on the micro fabricate surface protein by applications has been used for sighting this particular slide. So, there are several bio links that when uses for doing or capturing cells or different biological entities in the surface. Why is capture needed of course, because we have been talking about the whole area of electro chemistry of sensing essentially you have mobilize something in order to find out more details about the information contain in that thing.

So, immobilization is a primary goal of any bio sensor how you can mobilize a recognition element, how you can immobilize the particular biological entity that is the first primary goal of a bio cells. So, on the first such bio link of courses, this chemistry between antigens and antibodies. So, as we already know that antibodies are essentially proteins which has a

complicated Y shape, so there are two light change side of the chain which are essentially arms of the Y and that there is a heaver chain which is at the bottoms as term of the Y.

This kind of linkage and the lighter change you have this epitopes sides or chemicals sides which should be able to recognize sometimes selectively in case of monoclonal and sometimes nonselective as in case of polyclonal, the surface proteins of this antigenes, antigens could be some infecting cells etcetera. So, here as you look at this is balloon like a feature here is essentially the antigen and you have these two moiety is which are the lighter chain moiety is and this is the heaver a chain on the Y as Stem of the Y and light long this particular region here and the top you have so called the epitopes size.

So, let me just quickly change the ink color here, so that it causes better visibility. So, this is essentially what is there one an epitopes side and similarly have another side here in this region, which is also and an epitopes side. So, the way it works is that the proteins which are on the surface of this particular antigen here would be able to bind specifically to this epitopes sides. So, therefore, whenever there is an antibody which is some immobilized some where you could bind the two or more of those cells on the bases of the surface proteins using the epitopes which are there on the antibodies.

So, the binding could be through hydrogen bonds, it could be through ionic, van der Waals or even covalent sometimes among the proteins on the surface and the epitopes sides. So, that is a one mechanism where you could actually use this molecular hook from trapping of a flow samples. Now, how you put the immobilize or mobilized antibody on a surfaces another kind of backward integrated process which I am just going to stop in the next few minutes.

Then we have this ligand receptor binding, what this essentially means and this is one of the very, very famous lock in biochemistry is called the avidin biotin lock. So, you can see how the structure of one sub unit of avidins really looks like it is a very complicated essentially molecule as you can see here and biotin is essentially again organic molecule it is a vitamin. So, you have a bonding between the avidin and biotin, where the affinity constants as high as above 10 to the power of 15 per molar.

So, they are commonly uses molecular locks, this is something that biochemistry has to offer. Also very important is the binding of some kind of antibody using this concept of biotin avidin chemistry. So, here what you do is let say you have a surface which you want to bind something on and you create a protein in it is you know positive stated as certain PH. And so

once the protein is protonated you can actually create this charge surface of the oxide to have the exact opposite charge. So, that there is a binding of the protein in this case the protein is BSA on to the surface of the oxide as you can see here.

What you also do is that these BSA essentially is somehow biotinylated. So, you have it biotinylation done over the BSA, you put a biotin moiety one the BSA. On the other hand let say this is the some kind of biotinylated molecule maybe it is an antibody, you biotinylated intentionally the n group here. So, you have a biotin on both ends one in the BSA, another in the molecule that you are capturing in that in and you are using and avidin linker between these two biotin groups, one on the BSA and one on the antibody.

You are actually trapping the antibody in this manner on to the surface. So, whatever you have done here, which is concern with probably the upper portion of this molecule in trapping the cells, the lower portion is a fixed using the biotin avidin chemistry using a some kind of a species which can adsorbed electrostatically bond itself to the surface oppositely charged surface any surface. In fact, silicon dioxide, you know any metal surface, so on, so forth.

So, this is a very interesting approach of how you could actually immobilize protein complexes to a surface and further immobilize the different biological entities by using links like antibodies to identify or select. So, common mechanisms like ELISA have looked before also consider this antibody antigen kinetics in order to determine change in coloration from where we can figure out, what is the immune response of the patient or whether has been a reflected by HIV or not.

So, now, after doing this we would like to do a little bit more work in understanding how enzyme is behave and for that we need to find out some mathematical formulation related to the rate constants of various reaction as the enzyme catalyze this reactions.

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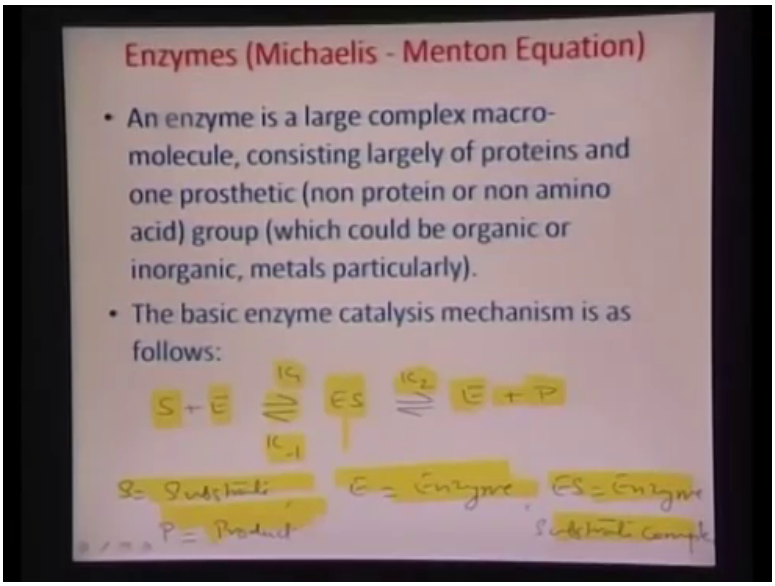

As I have discussed many times earlier, the enzymes by themselves do not react permanently to substrates, but they do participate in the reaction and create in intermediate complex would themselves as a part of that complex and the later on the dissociate the molecule making a product and associate themselves completely as a free enzyme which are all have the next cycle. So, really if you see at definition look at the definition then enzyme is a large complex macro molecule consisting of proteins.

At one kind of prosthetic group which could be either non protein or non amino acid or even some times organic or in organic group may be metal and times. So, the other part by and large is made up of protein except one which is a non protein, non amino acid kind of moiety that is what enzyme essentially is. So, if you look at the basic enzyme catalysis mechanism, let suppose we start with the substrate here and by substrate what I mean again is some molecule which you need to modifying to a product.

You have an enzyme E and that is a binding kinetics going on between S and E at a certain rate k 1 and this is an equilibration with the intermediate enzyme substrate formulation which has been formulated here and then there is a equilibrium setup. So, there is a reverse rate constant, which is also same as the forward rate constant and there is an equilibrium between the enzyme a substrate in dependently and the enzyme substrate complex.

Now, if you look at what happens to this complex, this complex is essentially again converted into an enzyme in a product and there is a rate constant k 2 in the forward direction in the

case. We are not concern with k minus 2 here for reasons which have in this closing little bit later. So, we have from substrate to enzyme, an enzyme to product, the enzyme essentially is retain as it is and substrate is converted into the product or catalyzed by this enzyme. So, that is have in this whole enzyme complex formation process is define.

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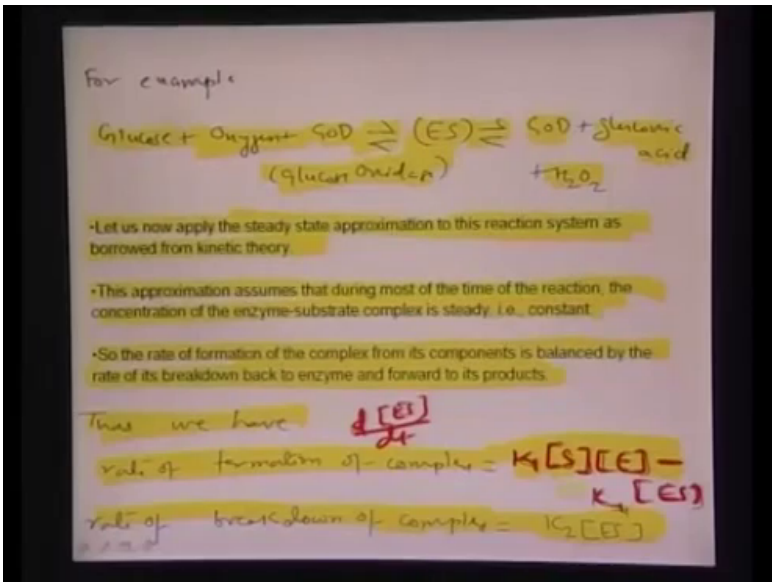

Now, let us look into some examples like for example, we have been taking about this enzyme glucose oxidize. So, when you convert this when you mix this with glucose and oxygen and you break it down there is an the enzymes of substrate complex which would be formulated and which would generate the back into the god the gluconic acid and the H2O2 which are products of this particular reaction. So, the enzyme get separated as it is.

Now, we want to apply the steady state approximation of this reaction system as borrowed from, you know the kinetic theory. So, we want to find out what in terms of concentration of various substrate enzyme or enzyme substrate complex can we really model the rate constants as. So, some approximations we assume that during most of the time of the reaction, the concentration of the complex, the enzyme of substrate complex is very, very steady that is constant is essentially. So, that is one approximation that we make here.

Then also from this we can derive the fact that the rate of formation of the complex from it is components is balance by rate of break down. So, there is a equilibrium between formulation of enzyme substrate and formulation of the enzyme product and also that is an equilibration between the formulation of enzyme substrate and the kind of back you know formation of the

enzyme is substrate individually. So, there is an equilibrium in the forward and the reverse direction as you forming the complex and then there is a equilibrium in the forward direction as you are forming the enzyme product from the enzyme substrate.

So, the rate of formation of the complex from it is components is balanced by the rate of break down back to enzyme, forward to it is product. And thus we have the rate of formation of the complex as kind of represented in this kind of a manner. So, k 1 is the equilibrium constant, so you can have the rate of formation of the complex; that means, d [E S] by dt as a positive rate. So, you are formulating essentially this k1 which is the rate constant times of the concentration of the substrate times concentration of the enzyme minus the reverse rate which is k minus 1 times of concentration of E S which also shows the breakdown of E S into individual enzyme and substrates.

So, on the other hand the rate of breakdown of the complex essentially into the enzyme and product. So, this is call the breakdown of the complex into the enzyme itself on the product that is dependent on the forward rate at which E S complex is converted into the enzyme on the product, so that is k 2 times of e s this k 1 s e minus k minus 1 e s.

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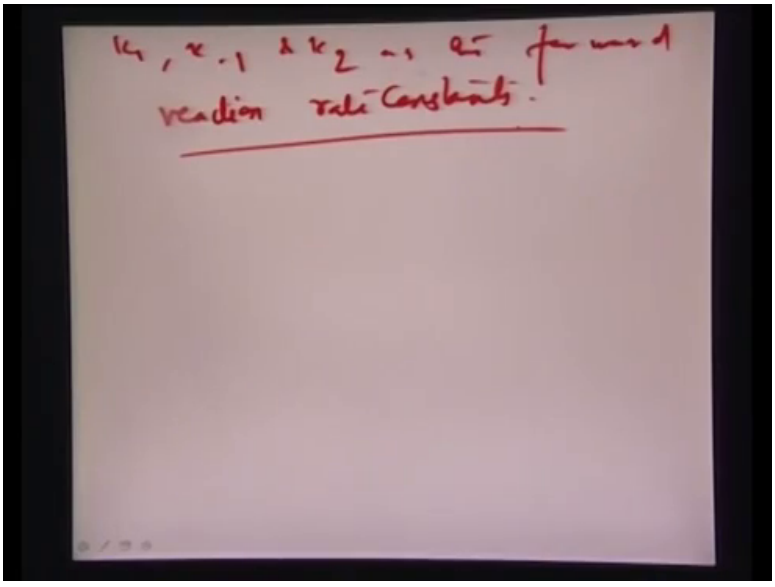

So, let us look at some of these formulations here let me just write it down once again. So, we have k 1, k minus 1 and k 2 as the forward reaction rate constants, so I think because we are just more or less towards the end of this lecture, but I am going to do is to pick it up in this

particular topic in the next lecture and try to derive what you call the Michaelis Menten equation. So, I would like to close the lecture with this.

Thank you.