Cellular Biophysics
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Model Gene

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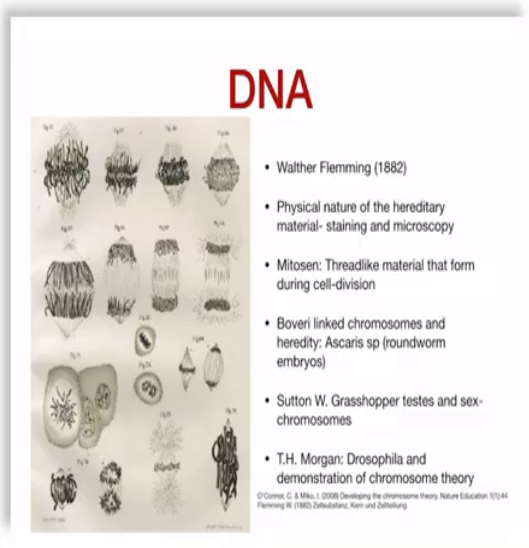

We have spoken about protein spacing so far. And now we move to DNA. Ever since the evidence that material inside the nucleus of a eukaryotic cell is responsible for heredity, meaning today transmitting characteristics from one generation to the next, people had been searching with great enthusiasm for the biochemical basis of this heredity or ability to transmit information, in a sense one of the foundational properties of life.

This search for the physical nature of hereditary material in the hands of Walther Flemming 1882 took the form of steaming with dyes that by trial and error seemed to highlight nucleic acids they did not by the way know the structure of DNA in 1882. And gave these drawings these are hand drawn from microscopy viewing of mitosis through the stages.

In fact, the fact that there were such canonical stages allowed Walther Flemming to identify the stages of mitosis. He coined the term Mitosen taken from the Greek to mean ‘threads’; to define these thread-like material that form during cell division. Today we call them chromosomes. Boveri who also did a lot of microscopy and really pushed the limits of what we understood demonstrated that these chromosomes when removed from embryos did not allow for the transmission of heredity.

He did these experiments using a round worm from the Ascaris species and in some senses furthered the understanding of the nature of the hereditary material. Eventually William Sutton used grasshopper testes and demonstrated, in fact, found by observing that in some of the testes sperms there were no chromosomal material of a specific kind while in some there were, this was the first identification of sex-chromosomes.

This is related to how sex determination happens in insects versus humans or vertebrates and indeed it is a topic for further discussion in a genetics class but I really encourage you to look into some of the textbook details, because these are beautiful experiments in terms of how they were conducted and the thought that led to their design.

Thomas Hunt Morgan who is considered the father of modern genetics used the fruit fly drosophila melanogaster and demonstrated more clearly the connection between chromosome theory, inheritance and cell division.

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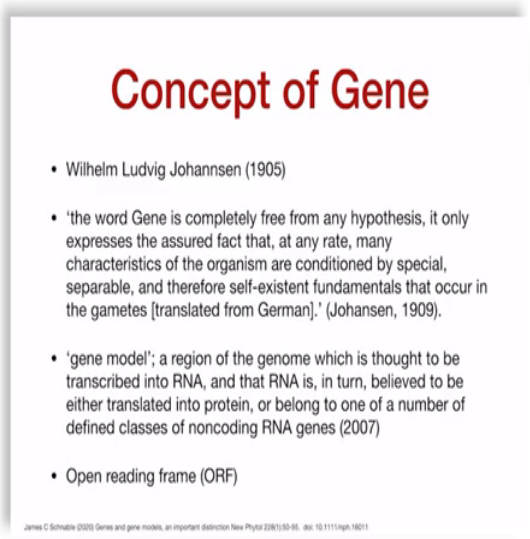

But the ability of this genetic material to do something, to perform action relates to its idea as a residence place or a location for a gene. But really this begs the question what is a gene. So, the first recorded mention of a gene is 1905 by Wilhelm Ludvig Johannsen who described in his German text that the gene in his opinion was free of hypothesis, it was a concept and they are transmitted through the gametes.

And this is very philosophical and not very concrete definition. At the same time a more modern gene model concept has emerged stating that a region of the genome which is

transcribed into RNA and that RNA is believed to be either translated into protein or belong to one of the number of classes of non-coding RNA genes can be called a gene.

In the bioinformatics sense we may even call something like this an open reading frame. The strange part is despite all the progress we have made from 1905 to 2022 a universal simple definition of a gene is not that easy.

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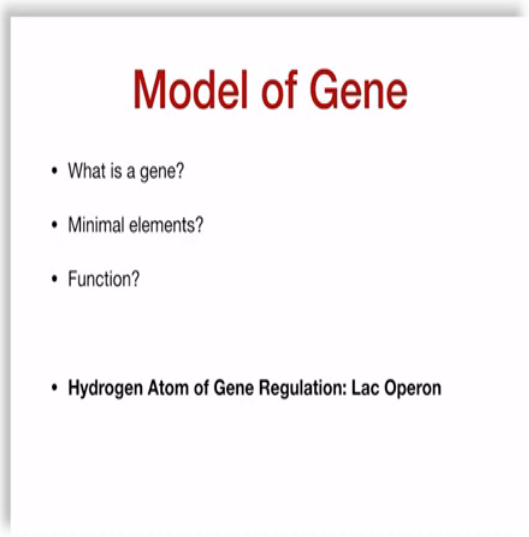

But in order to make our life a little bit easier we are going to take one example of a gene so that we do not spend a lot of time defining what is a gene in the first place, based on the same characteristics it expresses RNA, RNA expresses protein. We will look at its minimal elements and its function. And for that we need to consider a simple system.

So, like I have mentioned *E. coli* is a hydrogen atom for a cell, the Lactose Operon or Lac Operon as it is called is the hydrogen atom of gene regulation. Now, some of you may have taken biology courses and you may know a lot about it but I hope that some of the discussion we will have later on will show you that there is something different that comes at looking at from a biophysics perspective, not just a genetic and biochemical perspective.

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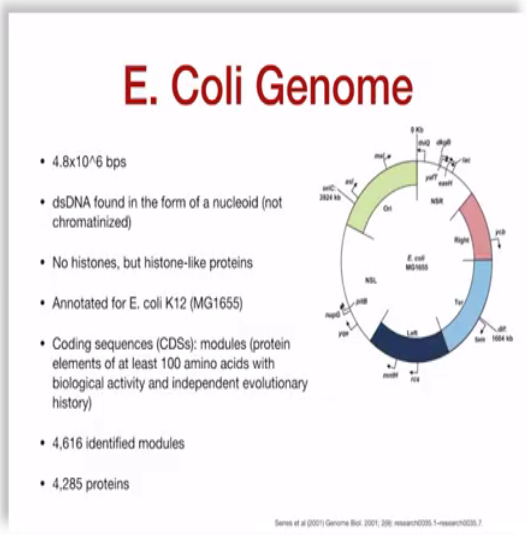

Just to remind ourselves how we got to this point the E. coli genome which we now know to be 4.8 into the 10^6 base pairs in length forms double-stranded DNA. It is called a nucleoid because it forms some kind of a cluster in the cell. It is not chromatinized, you remember we talked about histones. There are no histones in bacteria.

And the annotation of the genome of the standard E. coli strain K12 or MG1655 perform to some detail leading us to identify coding sequences that are modules, if we put the criterion must produce at least 100 amino acids or more length of polypeptide can be considered to be around 4616. The number of proteins produced measured from our spectrometry are 4285.

So there are some 4000, some approximately little less than 400 modules that are identified but do not make proteins.

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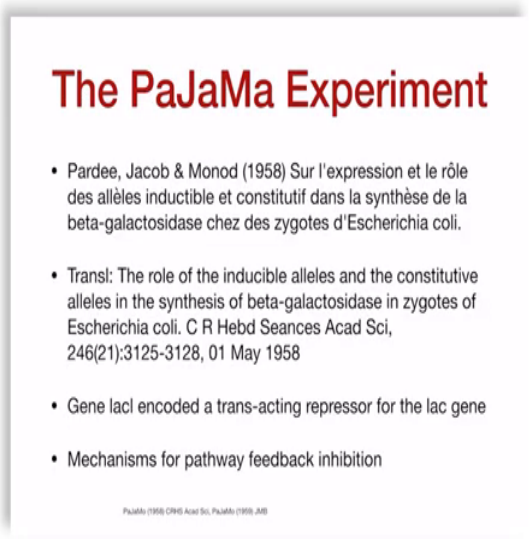

So, the discovery of this hydrogen atom of genes the Lac Operon is described in a 1958 paper Sur l'expression et le róle des allèles inductible et 'constitutif' dans la synthèse de la beta-galactosidase chez des zygotes d'Escherichia coli. The translation is "The role of the inducible alleles and the constituted alleles in the synthesis of beta galactosidase in zygotes of Escherichia Coli. So, in general called C R Hebd Seances Acad Sci 1958.

The reason why it is called the PaJaMa Experiment is a name that Pardee, Jacob and Monod themselves came up with, it comes from their names Pardee PiPa, Jacob Ja, I think it should be Mo, not Ma, but they decided to call it PaJaMa. The fun part was that they did these experiments on a summer school in Paris where they all got together and they were looking at the ability to transfer genes using bacteriophages into E. coli the model organism for genetics.

They showed that when the lacI gene was introduced into a bacterium, it acted as a trans repressor for the lac gene it shut down the lac gene but it was not produced by the lac gene itself, not directly. This provided the first evidence for feedback inhibition.

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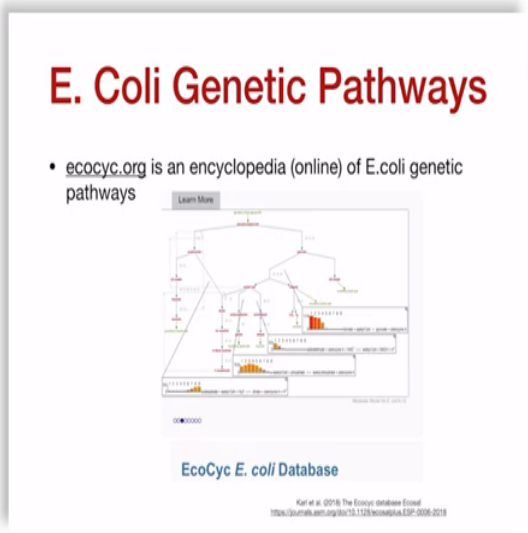

If you now go and look at E. coli genome regulatory databases you will find enormous numbers of genes and the regulatory pathways and complexity. So, in order to make sense of the complexity, of course, you can take a bioinformatics approach, a graph theoretical approach or numerical approach or a genetic approach, we are taking the small network approach.

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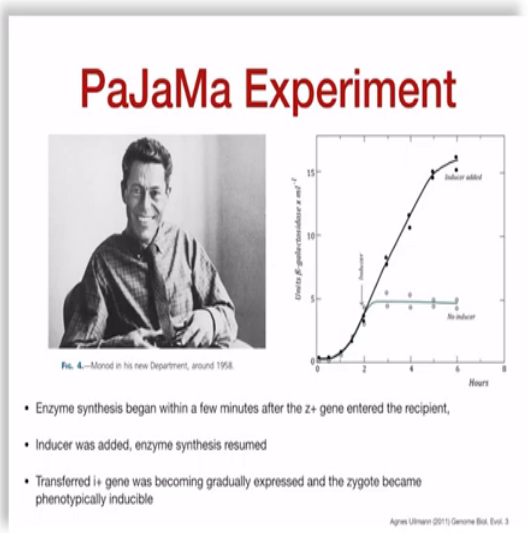

And in the PaJaMa experiment the key result of Jacob, Monod and Pardee was to show that the activity of beta galactosidase per ml, the presence of beta galactosidase per ml, if you

follow the green curve saturated at about 5 units per ml after 6 hours of adding the inducer. In their case they already found out that lactose could act as an inducer.

But in the presence of inducer the expression was higher. Enzyme synthesis begins a few minutes after Z gene has entered. When the inducer was added however synthesis resumed. In other words even without the inducer there was some expression of beta galactosidase, but not as high as with inducer. It appeared that the I gene had something to do with it.

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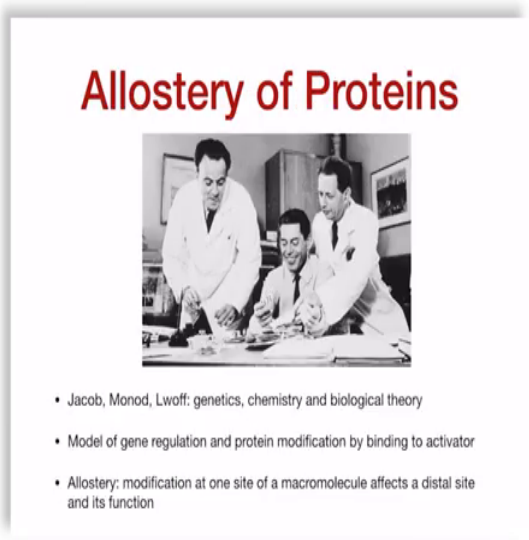

Amazingly this experiment led to so many different innovations because on the one hand it led to our better understanding of gene expression, gene regulation, but it also gave a hint to a property of proteins called Allostery. This image is one of Jacob, Monod and Lwoff, another contributor to the lac operon concept and experimental data.

And their idea of a model of gene regulation and protein modification was based on the binding of activator to the inhibitor. Allostery is a phenomenon by which a modification at one site of a macromolecule affects its function at a different site. When this first came about this was very counter-intuitive. Along with lacI many of you will be aware that another classic example of Allostery is Haemoglobin, oxy haemoglobin versus haemoglobin.

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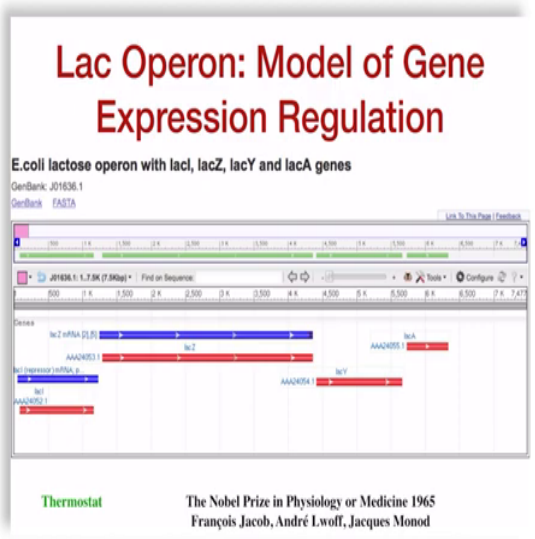

But returning to the hydrogen atom of the lac operon you are looking at an output from the gene bank, all of you can if you have internet you can search on gene bank and look for the lactose operon of E. Coli and you will find like going from left to right lac I the repressor mRNA producing gene, lac Z, lac Y and lac A.

We also argue that there are intervening binding sequences, very short, much smaller than the kilo bases that this represents to which lacI binds called the promoter and the operator. Incidentally Jacob, Lwoff and Monod were awarded the Nobel Prize for physiology of medicine in 1965 for their work. The idea of the lac operon is to almost function like a thermostat. So, some of you have maybe an air conditioner in your office, your classroom or your home, some of you may have seen it, some of you may have seen the refrigerator.

So, refrigerator I hope many of you have the possibility of using a refrigerator, fridge has a thermostat, it has a set point you put a temperature on it, if the temperature increases above it, it turns on the compressor and cools down the fridge, if the temperature goes below it, it stops pumping till it warms up, in fact you cannot help conventional compressor based refrigerators cannot warm, they only cool.

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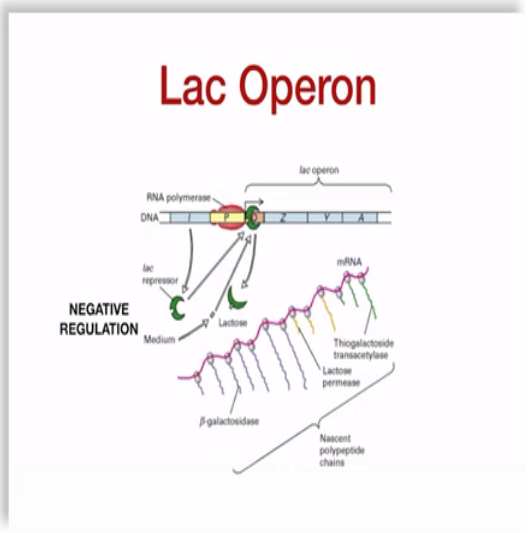

The lac operon has a set point that depending on the environment and the lactose present when there is no lactose for example, the RNA polymerase binds to the promoter, the red circle, but cannot proceed with transcription because the operator IPO, Inhibitor Promoter Operator oocyte is bound by a broad protein that is the gene product of the I gene, the inhibitor or repressor. So, the I gene is constitutively expressed made all the time.

It is capable of binding to the operator and only and only when the medium or the environment has lactose does it bind to the inhibitor, modify its conformation, allostery and fall off the operator side. Thus, the RNA polymerase is now free. It can go down and synthesize mRNA that corresponds to Z, Y and A.

This is polycystronic mRNA as we call it, because it produces the information for the protein that is going to become beta galactosidase. All of this is translated by the way by the ribosome, permease and thio galactosidase trans-acetylene. Permease is the channel and thio galactosidase trans-acetylease is a metabolic intermediate.

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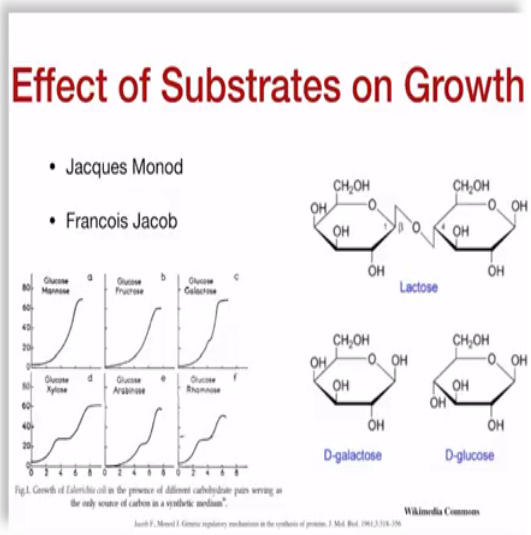

What Jacob and Monod showed in their experiment is that in the presence of different sugars, lactose, d galactose, d glucose, arabinose, rhamnose, galactose, fructose, mannose, xylose, the absorbance at used to measure the growth of bacterial cultures these are now cell densities show either a single phase or a dual phase.

This suggested that there is some regulation which is sensing which sugar is present and in fact that there is a preferred sequence of usage of sugars. So, not just does lactose turn on and off it is the expression of the lac operon, other operons are also regulated in a similar manner by the presence of the sugars.

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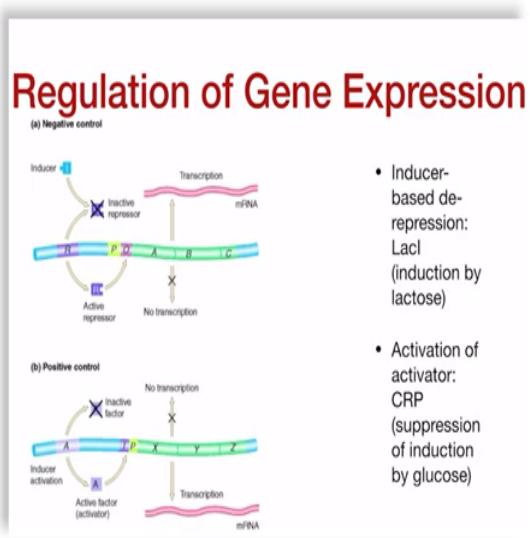

Indeed the regulation that we mentioned about inhibitor can be considered to be a negative regulation. Meaning to say, the gene is switched off in the null case, in the normal case, because the repressor is bound to the operator and when the inducer is added it de-represses or switches on the gene turning on the transcription which in turn turns leads to translation.

Positive control on the other hand implies that the gene transcription is induced by some active factor A which can either come from the environment or from some internal process and turns on the transcription in the absence of the activator there is no transcription that is the meaning of positive regulation.

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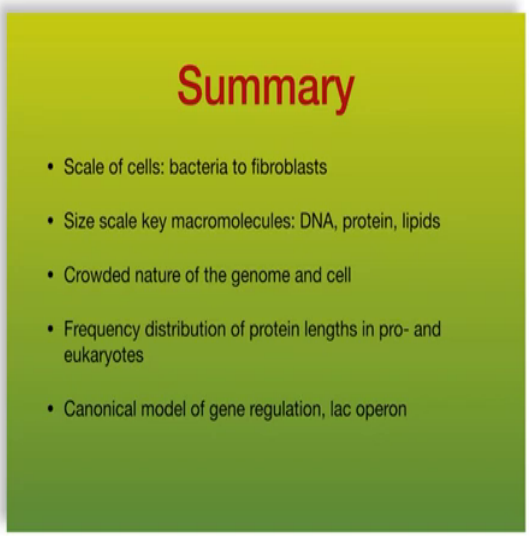

So, in summary so far we have looked at relative sizes of scales from bacteria to fibroblasts, 1 micron to 50 microns. Micron as you remember is 10^{-6} meters. The size scale of macromolecules, DNA, proteins, lipids. The crowded nature of the genome and cell, this is relating to the spacing that we discussed of proteins and the relative packing frequency, packing density of chromatin in the nucleus.

It is a problem I did not solve completely and I expect you to try to do it and I will ask you this during the live sessions. Frequency distributions of protein lengths in prokaryotes and eukaryotes which allow us incidentally to calculate the abundance of proteins on an average. And a canonical model, the standard model, the hydrogen atom model of gene regulation namely the lac operon.