Cellular Biophysics
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Energy and Thermodynamics of Life - Part- 01

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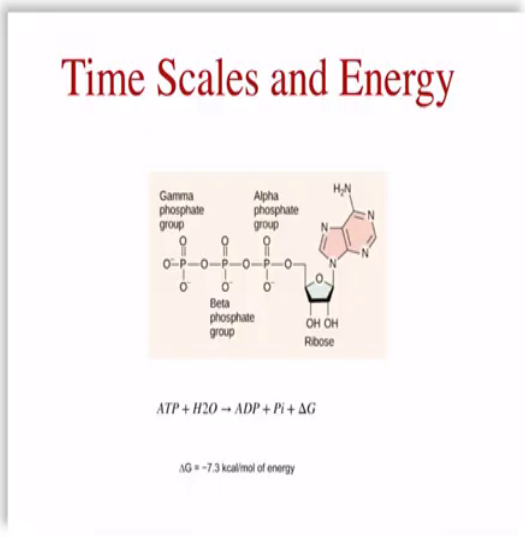

Hi, welcome back. So, so far we have spent a lot of time discussing the quantification in cell biology of spatial scales, meaning sizes. In this context we went over areas, volumes, mass, densities and other properties that can be considered to be the spatial aspect. But now we are going to consider time. And I am going to try and make the case as I hope you will see that time and energy are interconnected.

This is sort of a following section and this will take a little bit of effort, but I hope you will see the connection between the segments. Now, if I say energy to a biologist they always instantly will say ATP is the energy currency of life. Yes, and we are looking at an ATP molecule adenosine triphosphate.

Those of you who remember the DNA biochemistry also remember and realize, perhaps looking at this that the ribose sugar has its OH is intact there is an a bond to an n group that comes from H and then O phosphate one, two, three, triphosphate, therefore, adenosine, so this is the adenine, osine because of the ribose triphosphate, this is alpha, this is beta, this is gamma phosphate.

The chemical reaction that we are usually interested in considering with regard to ATP and its role of energy donor is ATP in the presence of water loses a phosphate Pi at the cost of release of 7.3 kilocalories per mole of energy. Now, how this affects time scales will come to?

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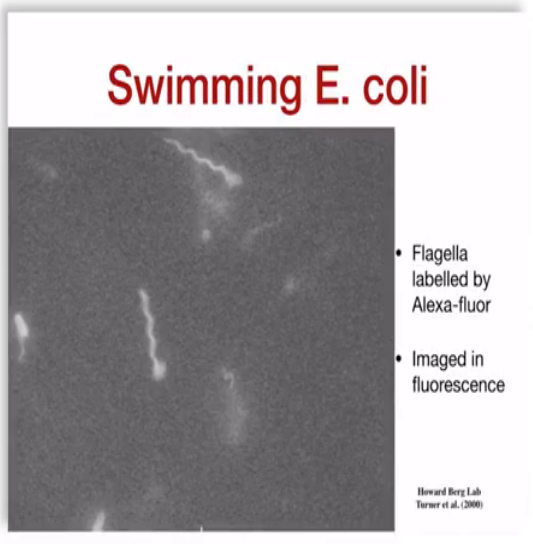

What you are looking at here are bacteria moving in a movie that is taken from Howard Berg's lab, Howard Berg is a pioneer in bacterial physics. He developed microscopy methods to image single *E. coli* cells. Again *E. coli* was chosen because this is considered that to be a model ideal simple cell and the flagella are labelled by Alexa. The whole microscope is done in fluorescence. What you should see is that these flagella vibrate vigorously and move and propel the cell forward.

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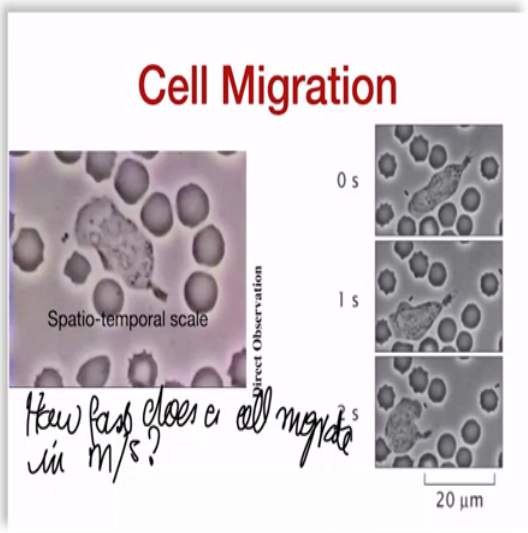

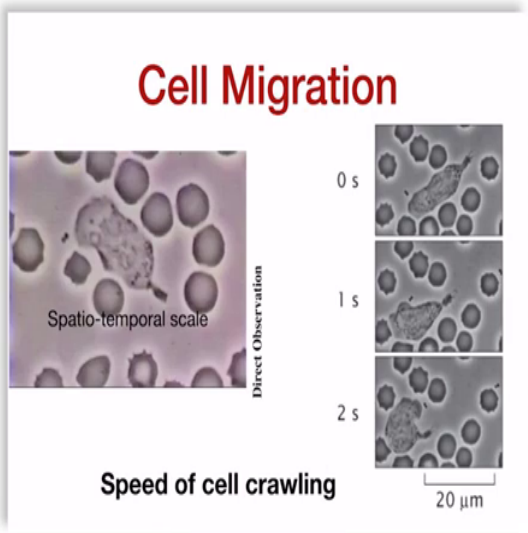

In contrast a movie of a single neutrophil demonstrates cell migration at its most apt, this movie is taken by David Rogers at Vanderbilt University and it involved adding *Staphylococcus aureus* bacteria which is this very small circle here and you see it here, here, here as it is being chased by a neutrophil, this is taken from whole blood, human whole blood and the neutrophil moves as it senses the direction of the bacterium turns and after some time actually captures it.

But we are interested in the rate the time the time scale, so we are interested in knowing how fast a cell migrates. So, in order to address that if we use direct observation as a method then using the scale bar of 20 microns knowing that it moved in one second by about 120^{th} of that

we know it is one moving one micron per second or 60 microns per minute and if we are to compare it to anything real world then we can consider how much it moves per meter.

And I want you to do the arithmetic and we will discuss this in class how fast does a cell migrate in meters per second.

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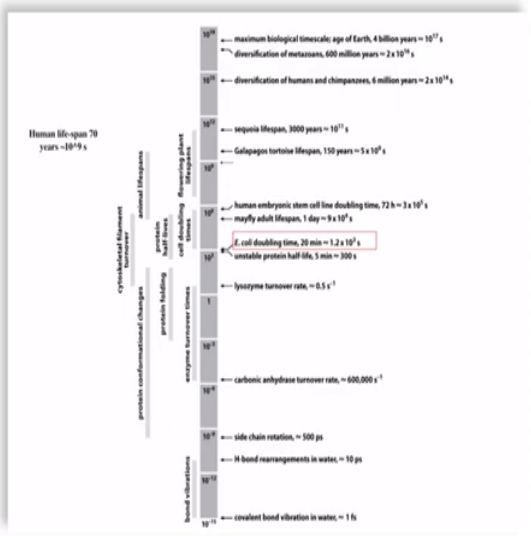

So, the speed of cell crawling is approximately micron per second, the speed of bacterial motion is about 20 microns per second and we can in fact put all the time scales the times taken by cellular systems to move onto one single scale. Just as a reference I have pointed out that human lifespan is this if we are healthy and live to our right age of 70 then 10^9 seconds, this is what we call order of magnitudes scale where we have used the 10 to the power something values to depict 10 fold jumps in seconds.

So, 10^{-9}, I am sorry 1000 fold jumps in time, 10^{-9} ,10^{-6} ,10^{-3} and 10^{0}, plus 3 plus 6 plus 9 plus 12 plus 15 plus 18, all this in seconds plus 18 at the upper limit is maximal biological time scale of earth 10^{17} seconds, as far as we know there is no life on any other planet other than earth until we find it, we are looking.

There are many exciting projects on extra-terrestrial life and search for extra-terrestrial life. Diversifications of humans and chimpanzees have been 6 million years ago that is 10^{14}. Sequoia lifespan is, sequoia is a giant tree found in western California in Northern America. It is 10^{11}. Galapagos tortoise has 10^9 human embryonic stem cell has doubling time of 10^5 seconds.

E. coli which we talked about earlier also has a doubling time or takes about 10^3 ~ few thousand seconds to divide. Unstable protein half time, half-life is 5 minutes, meaning to say the time it takes a protein to degrade is about 10^2 seconds, that is 5 minutes. Lysosomes turnover rate is 0.5 per second.

And then we go faster and faster and faster with the fastest speeds being times slowest, smallest times being covalent bond vibrations in water of one femtosecond 10^{-15}. So, knowing this at the cellular scale the time scales we are interested in are typically in the range of 10^3.

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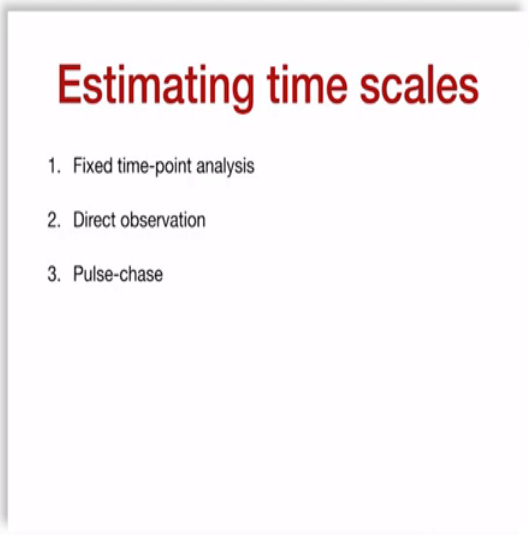

In order to estimate to measure the time it takes across cell, similar to this question that we asked earlier about measuring the number sensors, we need techniques and one of the techniques is called fixed time point. I showed you an example with the cell migration. The second is direct observation and third is pulse chase. These are the three typical methods that have been used vastly across biology to quantify.

Remember estimate time scales means actually measure time in seconds, not arbitrary units and I will get to this again about the difference between units, dimensions and the need for precise physical units in quantitative biology and biophysics.

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So, fixed time point analysis is based on the idea that you can look at a stage in let us say the development of an embryo of a drosophila fly from the stage of when it is born as an embryo to the first instar larva, to the second instar larva, third instar larva which is this creeping worm-like object to the pupa where it stops moving to the male adult.

Knowing the stages identifying them, measuring them hundreds of times now gives us a time scale or value for how long it takes to go from embryo to first instar, et cetera. Incidentally most of the work on establishing drosophila as a model organism was done by Thomas Hunt Morgan in 1910 and he selected for rapid generation time ease of breeding and clear delineation of times and stages. Meaning to say he could identify the specific stages and the times they took to go from one stage to another clearly.

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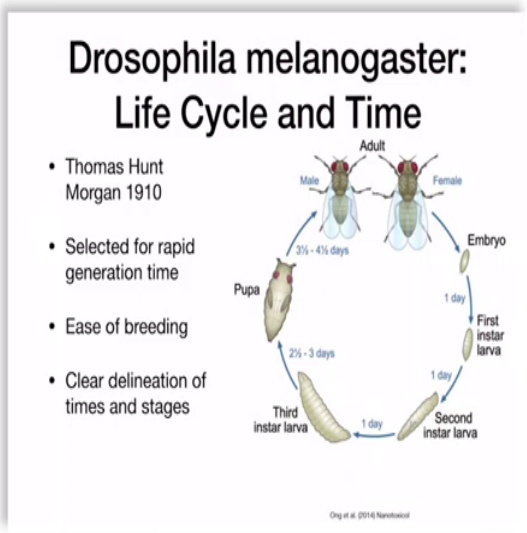

Early development is a little more involved and a lot of developmental biology and embryology involves identifying the exact stages of early development. Most of these images by the way are taken from single time points and then another fly is image after 5, 6 more hours and another flies image after 20 hours et cetera, which is what the idea of fixed time point analysis the same individual is not observed throughout.

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It is from this that we know that the process of fertilized egg in your one stage embryo to 9 nuclear divisions to nuclear peripheral migration to cellularization and then the entire development at 10 hours is critical for the growth and development over food flying.

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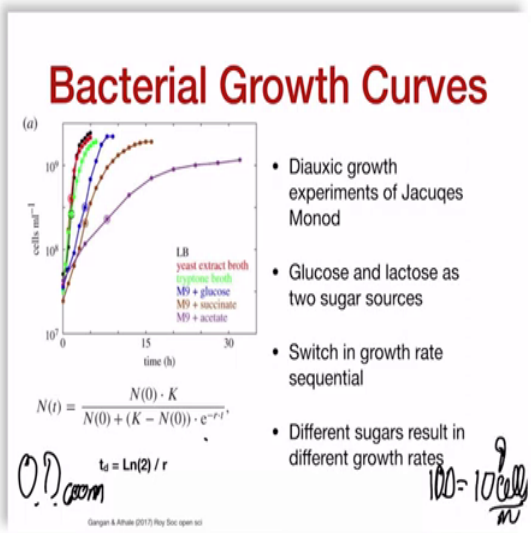

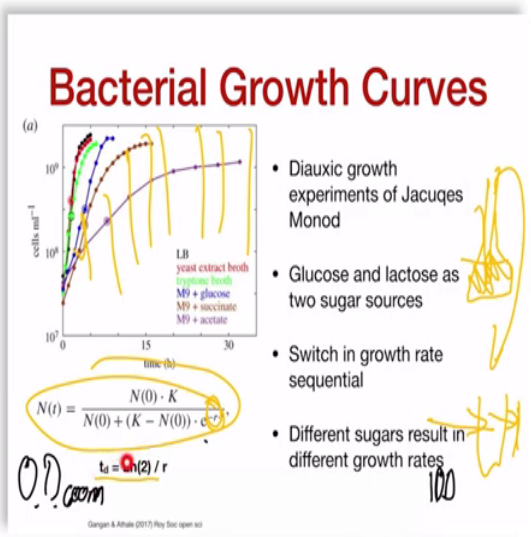

Another classic fixed time point analysis is bacterial growth curves. What you are looking at here is the translated cells per ml number for a growing bacterial culture. So, you will in fact find that the measurement is based on something that some of you may have heard of earlier optical density at 600 nanometers.

But optical density per se does not have the units of cell density, cells per ml. So, how do we convert? We need a conversion factor, typically, for E. coli we consider 1 OD to be 10^9 cells per ml, this is a conversion, for every bacterium this has to be different and needs to be checked before you can use something like this.

This data has points which each individual point is sampled by taking a flask in which bacteria are growing, removing an aliquot, taking out some amount of the culture, taking it to the spectrometer and measuring the absorbance and so on and so forth for all the time points. And this is how we get our growth curves.

If we want to estimate general properties like growth rate we fit it to this equation which is a logistic equation and allow and use the standard definition of growth rate to identify the doubling time which is the ratio of the natural log of 2 to R.

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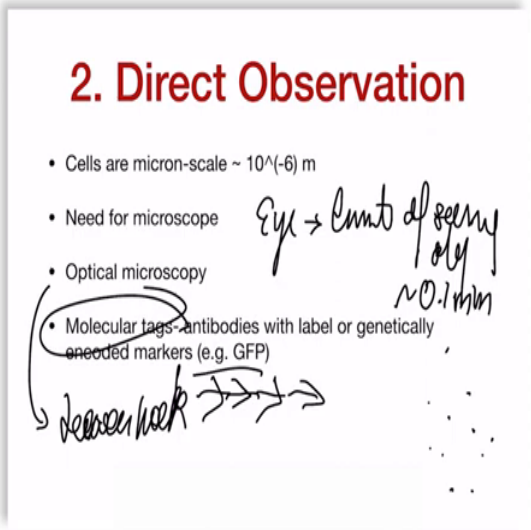

So, a second method of measuring time scales is direct observation. Cells are micron scale

10^{-6} and larger so we need a microscope because the human eye has a limit of seeing objects, this is approximately around 0.1 millimeter 100 microns, so anything smaller than that you need a microscope you in fact cannot see structure at 0.1 mm you just see a dot, tiny dot.

So, most of these dots I am making are around a millimeter if I make anything smaller than that you will not be able to see it. Optical microscopy is a very powerful tool and came out of the work of Leeuwenhoek and a whole series of biologists, inventors, physicists, opticians who went on to improve the methods.

In combination with the biochemistry that allowed us to get molecular tags for antibodies or genetically encoded markers like GFP microscopy has become almost the most favourite method of all cell biologists because it also allows you to directly observe.

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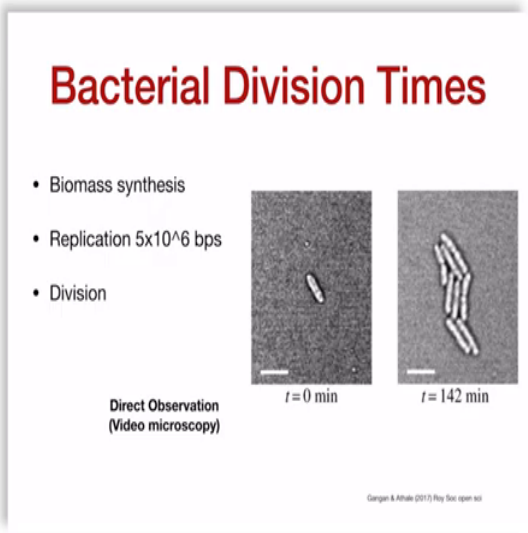

So, for example I can directly observe a bacterium growing and measure the time scale of its growth because I can see from time t_0 to time t_{50} how long it took for the which is how what I find typically in these experiments that it took the cell to grow this is taken from one of our papers and you can find more details in the manuscript. The idea is in other words to do microscopy while watching so that you catch the cell in the act and mark time.

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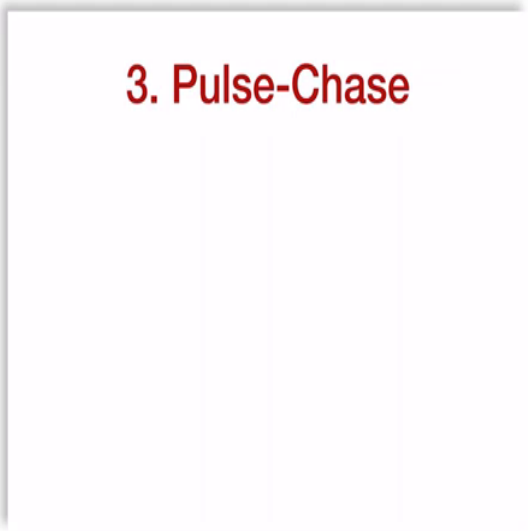

But there is more traditional approach called pulse chase.

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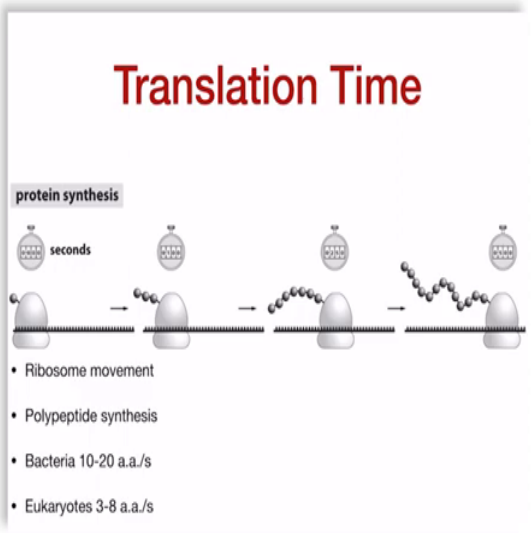

For the purpose of measuring translation time which is thought to happen in roughly half a second, one protein translates in about half a second, yes, we need to follow the ribosome movement, for that the polypeptide synthesis needs to be followed and for bacteria it turns out this number is about 10 to 20 amino acids per second being added.

For Eukaryotes it is 3 to 8 amino acids per second being added, which would mean that about 100 to 200 amino acids would be added, yeah amino acids per second is 10 to 20 which means about 10 seconds are required for full protein synthesis at least.

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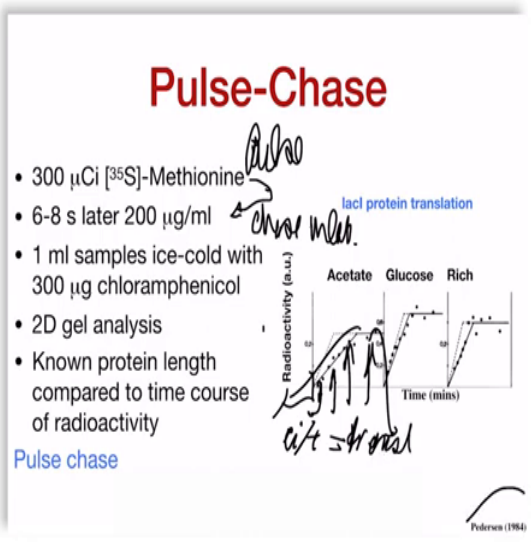

Pulse chase involves feeding the cells with a radioactive analog of an amino acid which has 32 micro curies of 35 S that is radioactive isotope of methionine in the methionine integrated in it. 6 to 8 seconds later the protein is translated 1 ml of samples are taken ice cold every few units and to the general analysis used to know the time course. Now, in this particular case you also need to detect radioactivity because it is only the labelled protein that you are interested in.

So, this part methionine labelled is followed by a chase of unlabelled methionine and this pulse and chase means that this methionine goes into the amino acids increasing and because it is chased it will reduce and knowing the rate that is to say the rate of increase of radioactivity per unit time the slope, we get the translation rate. This is work described in 1984 by Pedersen's classic method of pulse chase.

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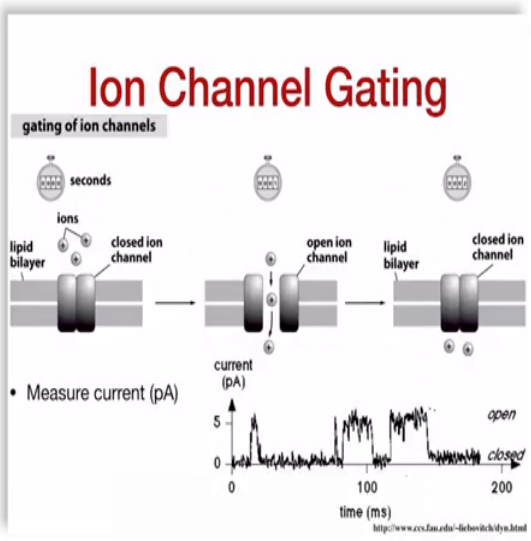

The second and sub choice processes things get a bit complicated and ion channel gating for example is in picoseconds, I am sorry in milliseconds. So, in order to do that you need faster measurements in the case of ion channel gating patch clamp method was invented as a method to try to catch the opening and closing of ion channels and this is a very powerful method, again awarded Nobel prizes for its invention.

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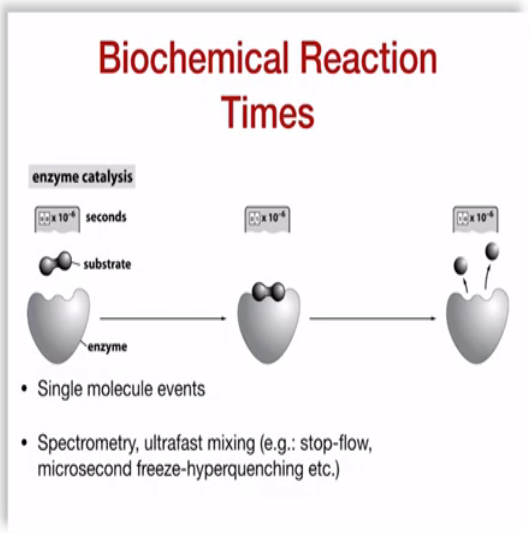

Biochemical reaction time scales can be even faster 10^{-6} seconds and you need stop flow methods, microsecond, freeze, hyper quenching and other approaches.

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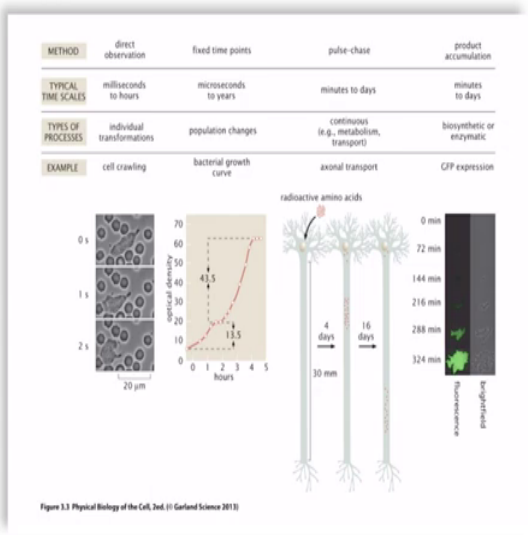

So, to summarize direct observation fixed time point and pulse chase and product accumulation are all methods to get time scales but they depend on the scales that you are observing on.

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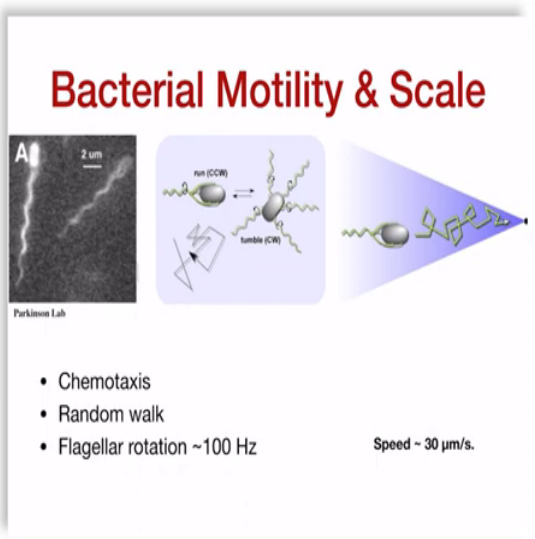

Just to give you an example the kind of outputs that we get tell us that chemotaxis, random walk and flagellar rotation are at different time scales themselves, the speed of directed motion is about 30 microns per second, flagellar rotation is 100 units per second or rotations per second which means 10 milliseconds is the time for one rotation, yeah, and the dynamics of the proteins are at an even faster time scale. So, scale of size determines the scale of time, things that are small do things faster, things that are big do things slower.

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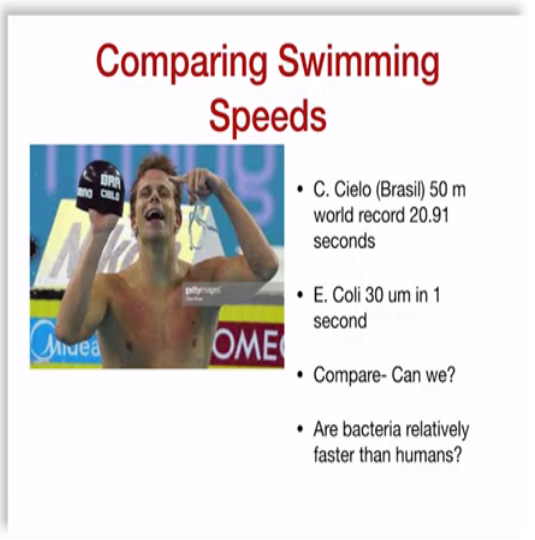

So, in a way we would like to when we compare time scales you remember I mentioned about how fast a bacterium of fibroblasts moves or neutrophil moves. You can of course take

the fact that it moves at about 1 micron per second and say 10^{-6} meters per second but you have to also perhaps consider that the time scale is dependent on the size.

So, if you now take the world record of 50 meter swimming of 20.91 seconds for a human being how can we compare this, can we even compare it, can we ask and answer the question of bacteria relatively faster than humans.

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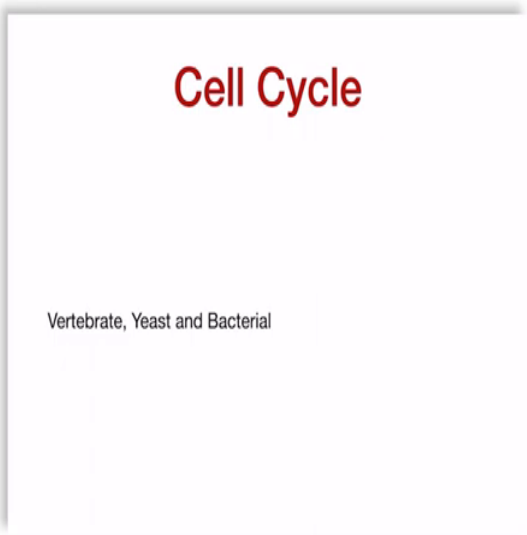

So, we say that if human swimming is 50 meters performed in 20.91 seconds it is 100 meters in 40 something seconds, so 41.8, we account for body size it is about a meter human body size is meter, scale is the distance travelled per second by body length, meaning to say how many body lengths does it cover, so it covers 50 meters in 20.91 seconds, how many bodies has it covered?

Assuming that the human body is about 2 meters in length which is a bit of an overestimate not everyone is two meters but Olympic swimmers can sometimes be very tall then the human is doing one body length per second, bacterium on the other hand is covering 30 microns per second which is 10 times actually exactly 15 times the body length in one second.

In that sense you could argue that the bacterium is swimming faster, yeah. And a similar set of analogies could be made for human running at hundred meters athletics which is 9.58 seconds I think that record is held by Usain Bolt, but I urge you to check your general knowledge.

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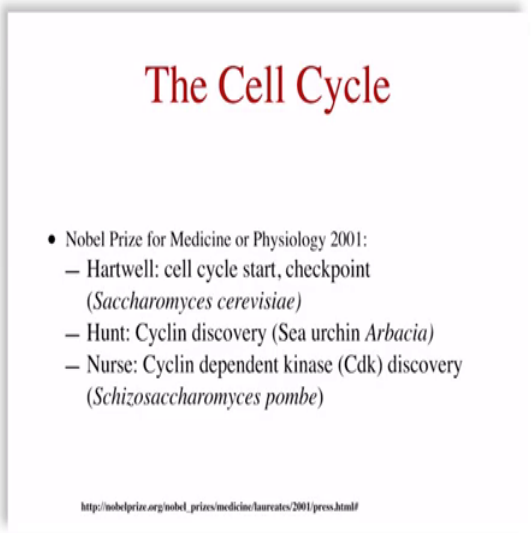

Another process in which time plays a very important role is the cell cycle, in fact, it is a cycle because it is clock-like and it is so important that the discovery of the molecules involved in it were awarded a Nobel Prize for physiology and medicine in 2001, to Hartwell for cell cycle start, checkpoint, saccharomyces cerevisiae.

And Paul Nurse as a third person. The Tim Hunt for the cyclin discovery. Cyclin discovery was in a not very typical organism called the Sea urchin Arbacia and Paul Nurse discovered cycling dependent kinase in schizosaccharomyces pombe, not the brewer's yeast but a yeast found in East Africa in locally made beer.

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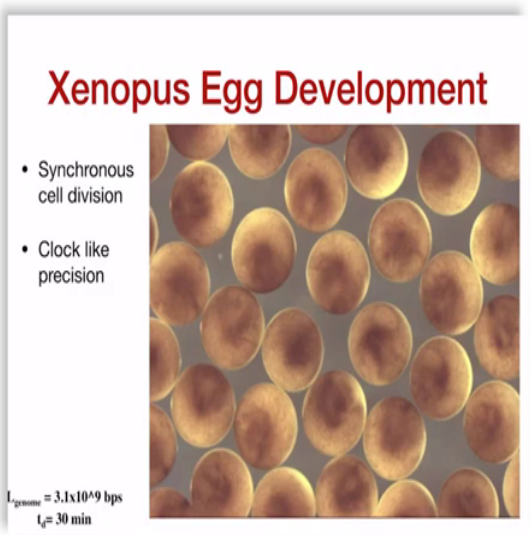

Now, what cell cycle really is stunning in terms of its synchronicity is observed in a Xenopus egg development where synchronous division appears to almost follow a clock-like precision, the genome by the way is 10^9 base pairs on 3.1 x 10^9 base pairs of *Xenopus* its distributable chromosomes.

But the doubling time is only 30 minutes this is amazing because you have to divide a billion bases more than a billion bases in 30 minutes, replicate, divide, segregate and so on. The precision with which this happens is in itself a fascinating question and not one of the very well understood problems.

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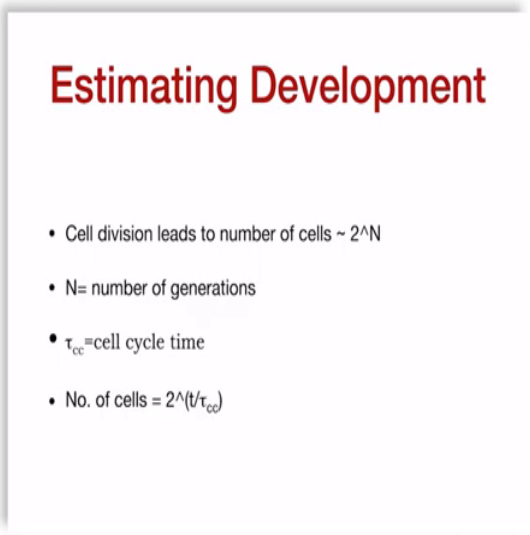

When we look at embryonic development itself the time scale of development is determined by the doubling time and that tells us that if there are two cells, if there is one cell then we have 2^N cells for N number of generations and the generations are given by the time divided by the cell cycle.

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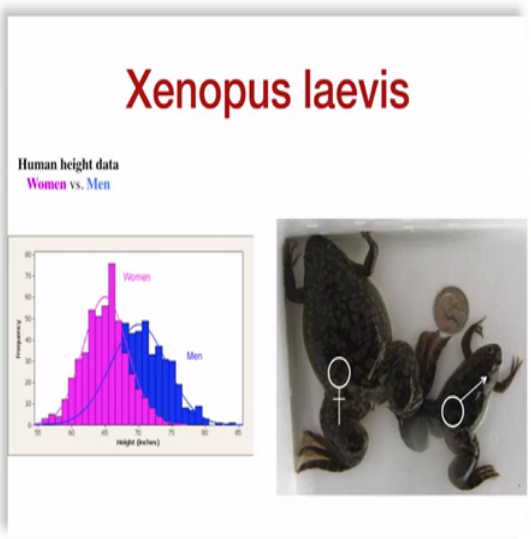

Xenopus laevis whose embryos we saw earlier are a classic model organism both for embryology as well as for developmental biology and cell biology and cell division research. The females of *Xenopus* are much bigger than the males, this is unlike the human height data which shows men to be bigger than females, human males are bigger than human females.

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The biochemistry of *Xenopus* oocytes is such that when you harvest them in unfertilized stage they might is one arrested. So, you can make a huge cytoplasmic volume with them of a few millilitres and use that for biochemistry.

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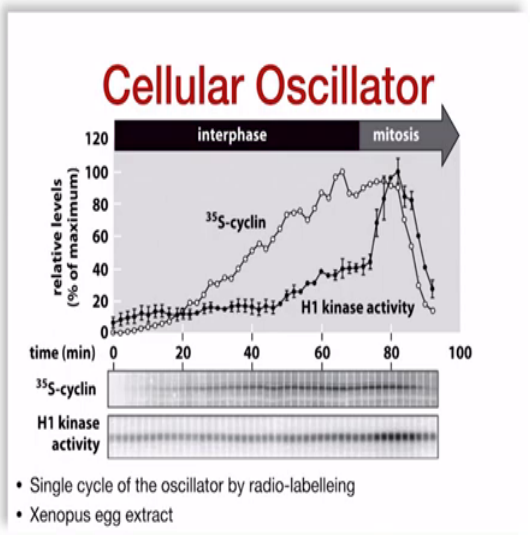

And it was in this that it was demonstrated that the transition from interface to mitosis is accompanied by an increase in drop in cyclin associated also with the H1 kinase activity, this was seen using western blotting and kinetics.

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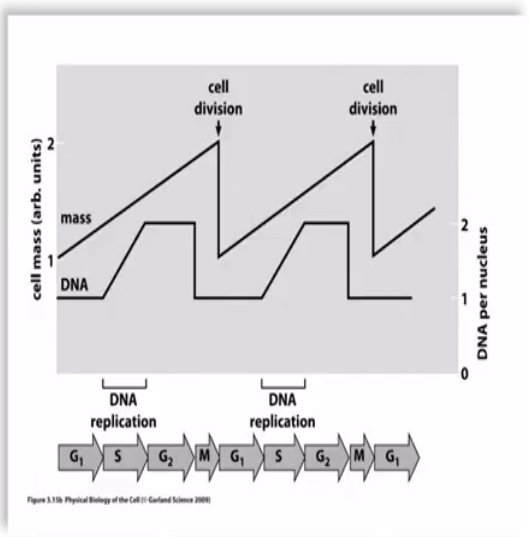

This led to the model that the cell division is triggered by an increase in mass and this mass is being monitored by the proteins that are driving this increase and that there is a concomitant increase in DNA replication which then leads to splitting at the diameter of segregation or anaphase. And this led to the discovery of the G1, S, G2, M famous stages of mitosis.

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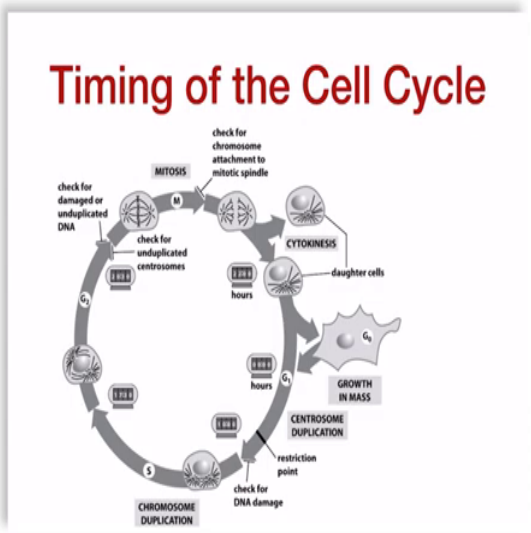

So, in a sense the timing of the cell cycle would be chaotic were it not for the fact that there are multiple checkpoints and these checkpoints for example from M2, G1 phase ensure that the chromosomes are attached otherwise you will have aneuploidy, check that centrosome duplication happens, ensure there is no DNA damage and there is no over duplicated DNA already present before division. So, the timing of the cell cycle is crucially not just regulated by the clock that drives it but also by the checkpoints.

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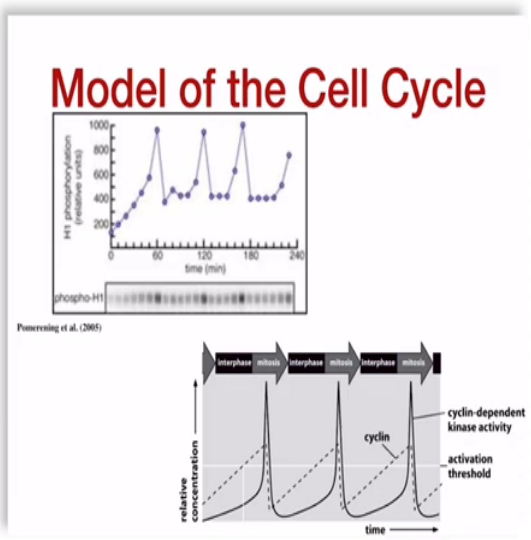

And this has led many people to speculate what mathematical model or biophysical model, can we construct in terms of equations for the model of the cell cycle. Something that goes up

and comes down, goes up and comes down suggests that the cycle rise and its threshold dependent triggering of cyclin-dependent kinase CDK which comes secondary, so cyclin rises and later on leads to spike in CDK which in turn degrades cyclin resetting the clock can be modulated.

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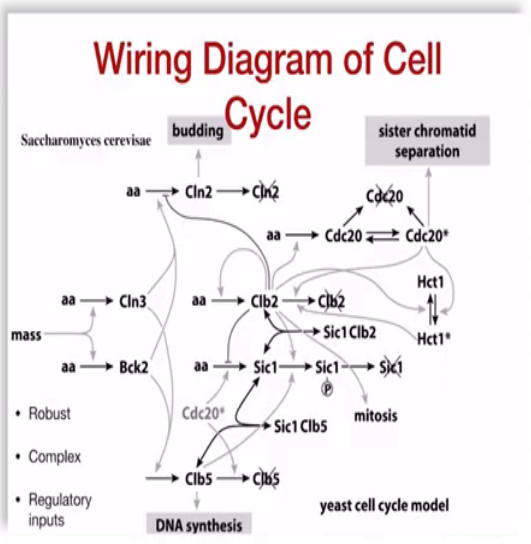

But the strange part is that the geneticists have found a lot of factors involved and it is all formed by knockouts, they found that this pathway while very complex is robust and involves multiple inter regulatory inputs. It always begs the question why is it so complex but I do not think we can answer this why question I do not know and the field is not very clear. So, largely the consensus is that it is likely to be a part of the robustness, the complexity is part of the robustness.

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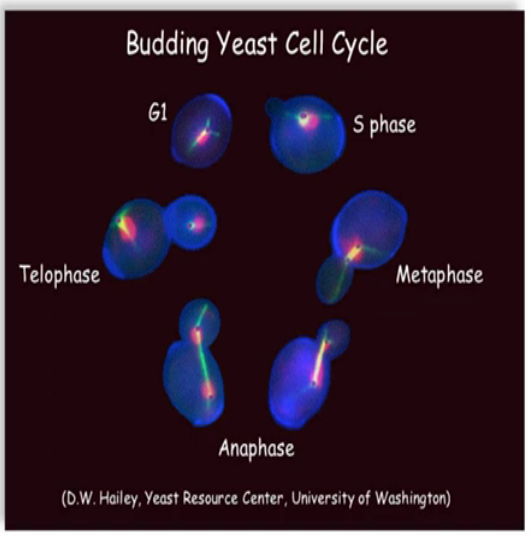

So, the budding yeast cycle looks like this beautiful image from Hailey's lab at the east resource centre, showing G1, S, G2, M transaction.

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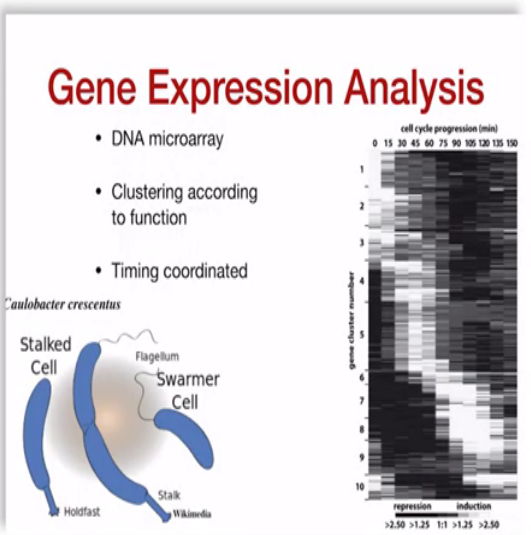

But not just yeasts show cell cycles even bacteria like the C*aulobacter crescentus* in a DNA microarray show that there is a timing of gene expression of certain genes early, intermediate and late, whose progression determines the cell cycle.

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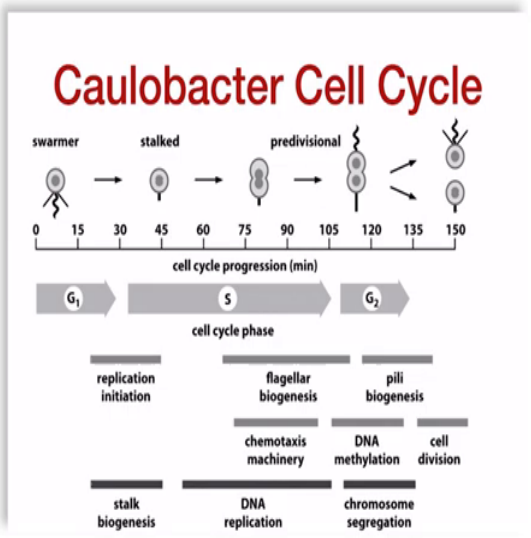

So, almost like eukaryotes caulobacter crescentus a bacterium prokaryote has a G1, S, and G2 stage, this stage involves going from swarmer to stocked to predivisional to then dividing and corresponds with DNA replication and chromosome segregation.

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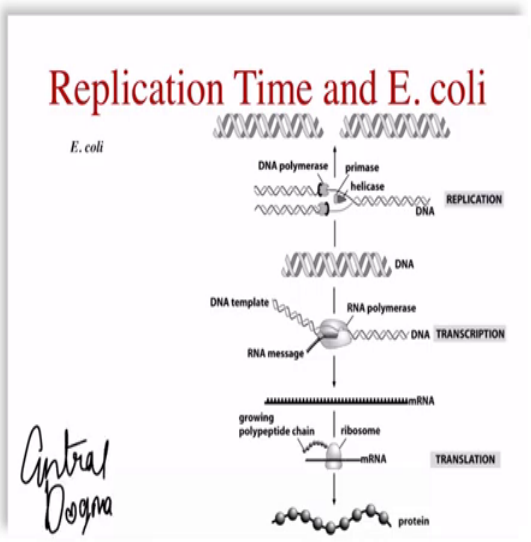

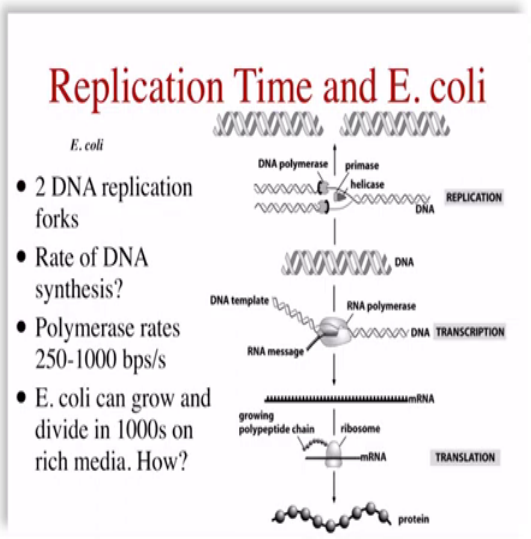

E. coli on the other hand are standard model has a slightly more complicated replication mechanism. So, while on the one hand replication can proceed in the semi-conservative fashion that the polymerase unwinds, the helicase unwinds the DNA, the polymerase is in the forward direction goes forward the okazaki fragments are made.

And you observe the replication the DNA is made the RNA polymerase transcribes and then translation happens, this is nothing but the central dogma that you are looking at of molecular biology. So, DNA polymerase polymerization rates are about 250 to 1000 base pairs per second that is very high by the way.

But *E. coli* can grow and divide in 1000 seconds which means that it takes more time for the DNA to replicate than it takes to divide. How can that happen? Turns out that *E. coli* fires more than one replication fork and in advance predicts its fate in the next generation and therefore takes care of the light.

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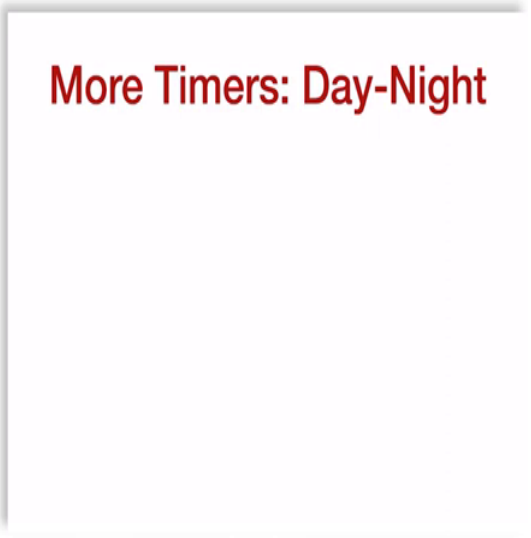

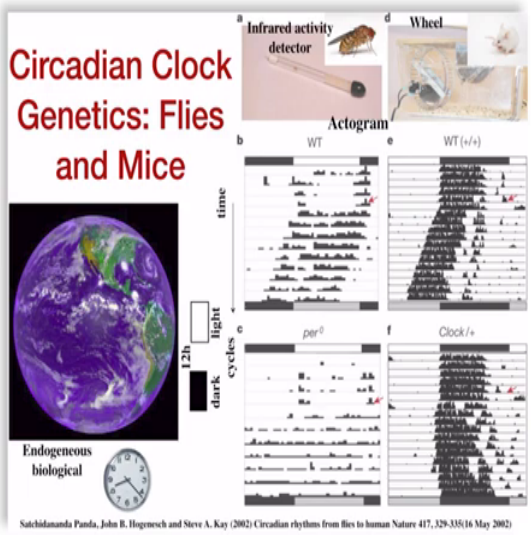

One more example of time keeping mechanics or clock mechanisms is the most obvious, most of you unless you are now working in a call centre working for United States companies wake up with the sun and go to sleep at night after you maybe had some discussions with your friends or meeting them and hanging out if you are lucky, if you are a farmer you have to wake up with the sun and go to sleep with the sun because the next morning is very hard.

This day night cycle is conserved across most animals. In fact it looks like even single-celled animals have single-celled organisms have daylight centres and it is endogenous and it is amazing because it is coupling something that is planetary that is movement of the earth

around its own axis pointing away from the sun and slowly gradually moving away towards it again.

And it is seen in this actogram that is an activity monitor of drosophila movement showing that circadian rhythms of flies are modified in mutants for *per* and *clock* genes, these *per* and *clock* genes were identified by these groups panda and company to be essential for the process of circadian rhythm.

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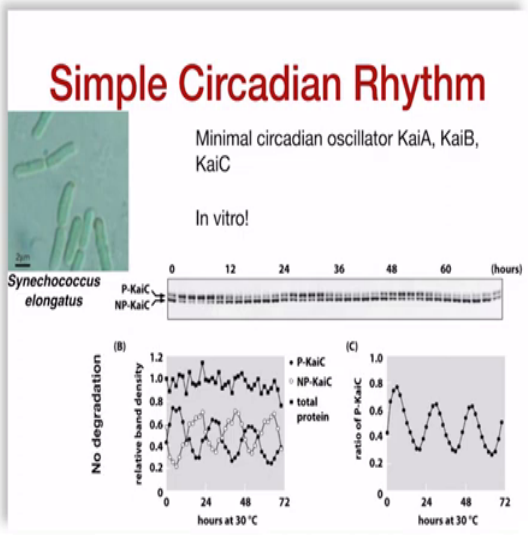

But in order to find a simpler organism in which this happens people searched and found a blue green alga called *Synechococcus elongatus* in which a minimum circadian, minimal circadian oscillator KaiA and B and C function and even in vitro will give you a beautiful clock like pattern over 24 hours that is if you notice here NP-KaiC is dark, becomes light and returns to dark all in the space of approximately 24 hours.

So, one cycle is completed in 24 hours. This is reaction done at 30 degree Celsius there are temperature dependent processes that affect the dynamics of the *Synechococcus* circadian cycle but there is no degradation involved it is only phosphorylation.

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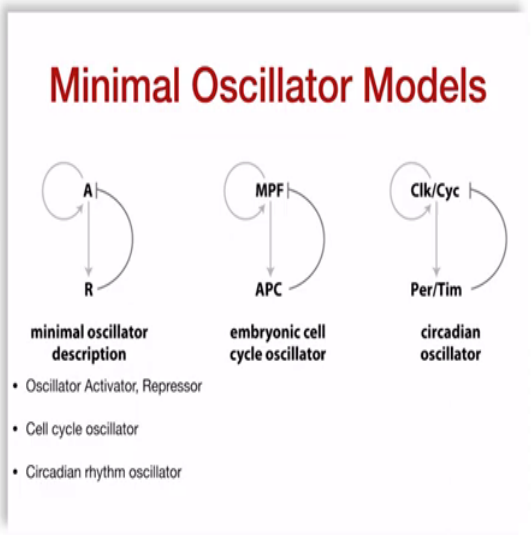

So, in order to understand something we try to model it, we try to make a mathematical model an equation and try to compare it to reality. In the case of cell cycles the minimal oscillator cell cycles and circadian rhythms minimal oscillator description is this. There is an activator that is A, there is a repressor that is R, activate activates the repressor and itself self-activates and repressor inhibits the activator.

This is a feedback loop, negative feedback loop and auto activation. This can be used to describe either the embryonic cell cycle oscillator or the circadian oscillator, this is really surprising because these are very different kind of phenomena but the time scales of biological clocks can be described by these simple feedback models, fairly well.

Of course that raises the second question which I had asked earlier which I think is hard to answer and has not been answered which is why does the complexity exist we say robustness we do not know exactly in the case of each of the systems what it is.

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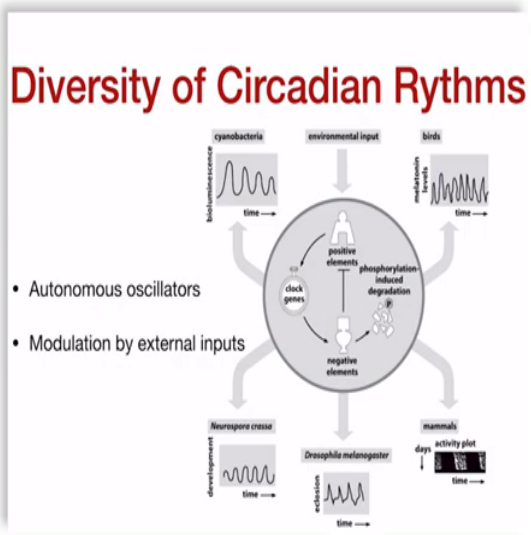

Indeed there is a diversity of circadian rhythms from cyanobacteria to birds to animals to mammals to *Drosophila* to *Neurospora* and the time and the nature of the cycles and the mechanisms are different but they all seem to have a positive component, a negative component and either degradation of phosphorylation and there is a connection with clock chains. So, it would seem that time scales in cellular life are set by very similar conceptual maps for the last part I am going to talk about energy scales.