Cellular Biophysics
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Cytoskeleton

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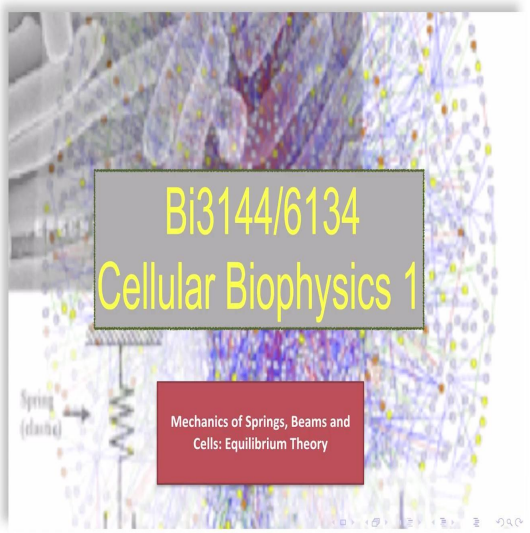

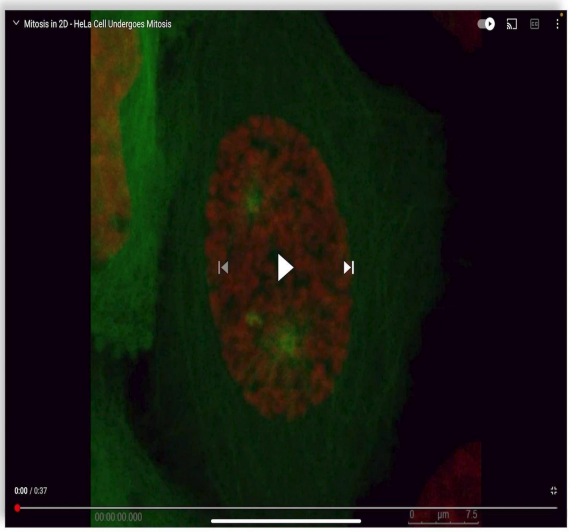

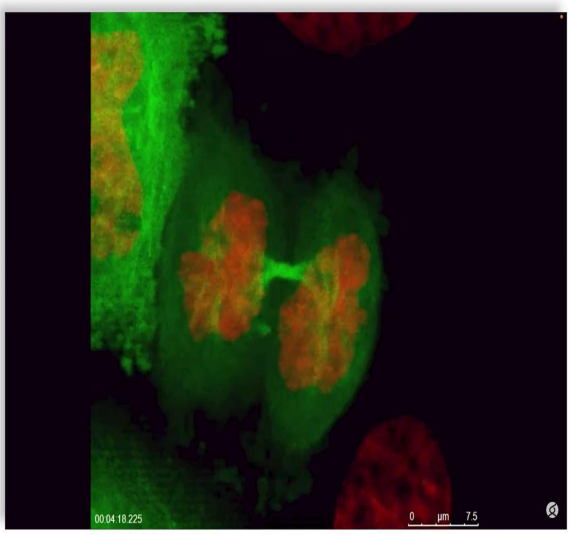

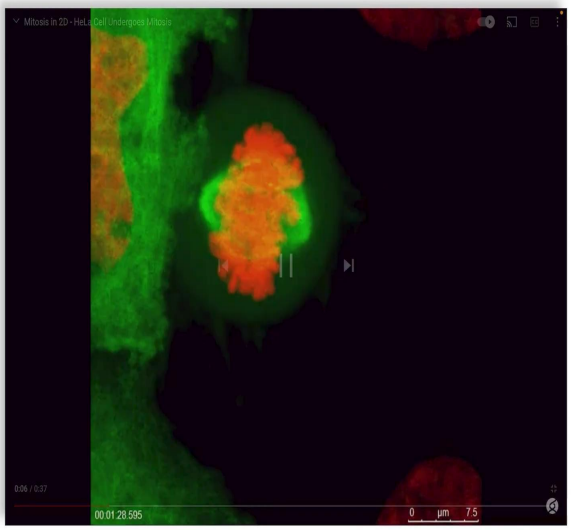

Hello, welcome back. So, there will bi 3144 and we are in Cellar Biophysics, I am going to start topic, which has mechanics of springs, beams, and cells, based on equilibrium theory. So, as a sort of precursor to that, I just want to remind you that if we look at a mitotic cell, basic process division, in a eukaryotic cell, in this particular case, this is a movie with hela cell, with the red chromosomes and green microtubules spindles and you see a lot of movement, you see this kind of pushing movement, which is the spindle segmentation machinery, you see the strand of DNA being the strand of the spindle being eventually truncated.

And if I go back to the beginning, you will also see the chromosomes being pushed into an arrangement. So, what all these stages, there is mechanics involved. And this is also

something we discussed in the early stages of this course, when I showed you the movies of crawling cells.

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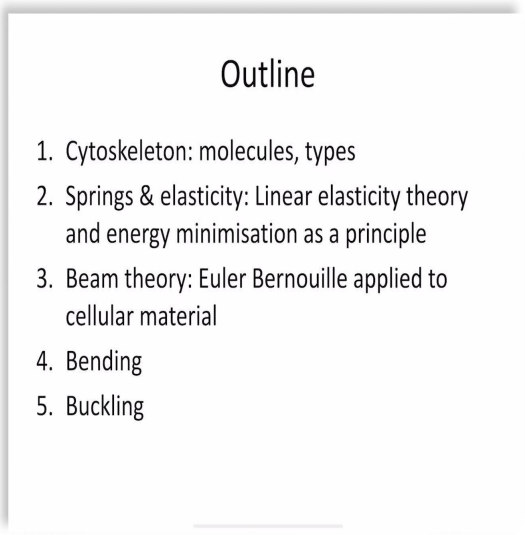

So, obviously, mechanics plays a role. But what are these molecules that drive it and so we going to talk a bit about the cytoskeleton, that molecules and types and then talk from that, dive into some theory, some mathematical derivations of springs, their elasticity. And linear elasticity theory and energy minimization as a principle. So, in essence, the math is really going to be about the question on energy minimization. And then I will talk a little bit more on the beam theory and other things. But for the immediate, this module, it is just going to be an introduction to cytoskeleton and molecular types. And some of these, for those of you who are taking cell biology is actually quite familiar.

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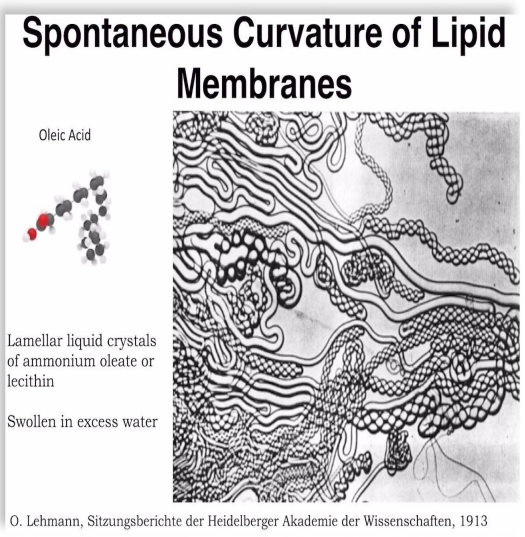

So, if you look at this image, it was generated in 1913, by swelling oleic acid with water in excess water and cause structures that look very much like cells, you see these tubes, convoluted structures.

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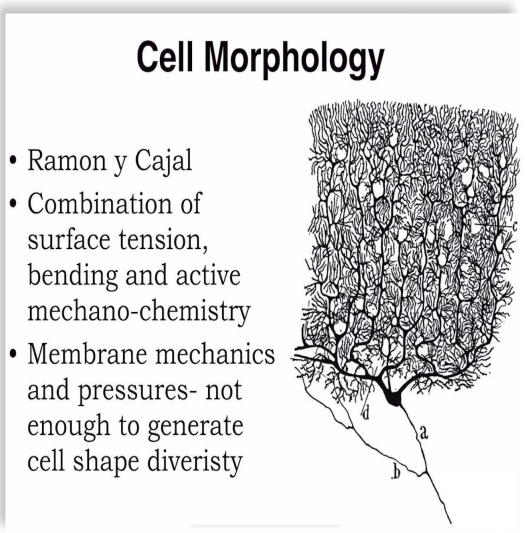

And in fact, when we go back and look at the classic images, the first few images of complex eukaryotic cells, like the neurons by Ramón Cajal, then it becomes apparent that these structures are even more complex with an interplay of both external and internal processes happening, that are driving this formation. And we can say a combination surface tension, bending of the membrane and active mechano-chemistry. But just memory mechanics are not enough to generate this actual diversity that we see.

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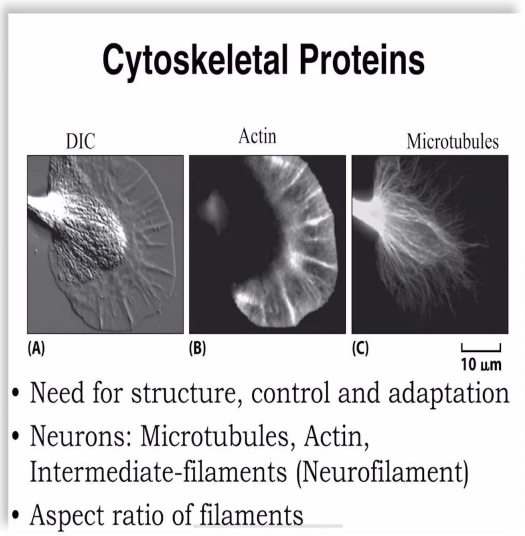

And for this, we are going to today talk about the so called endoskeleton of the cells or cytoskeleton. This is the skeleton that is inside the cells, we are not going to talk about the extracellular matrix and some other structures that also give structure because they are not always as ubiquitous. So, you will see this, these cytoskeletal proteins almost all across the evolutionary tree and actin on microtubules are very commonly described ones.

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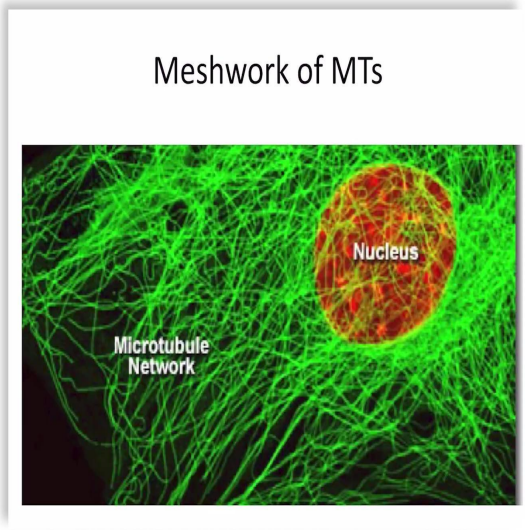

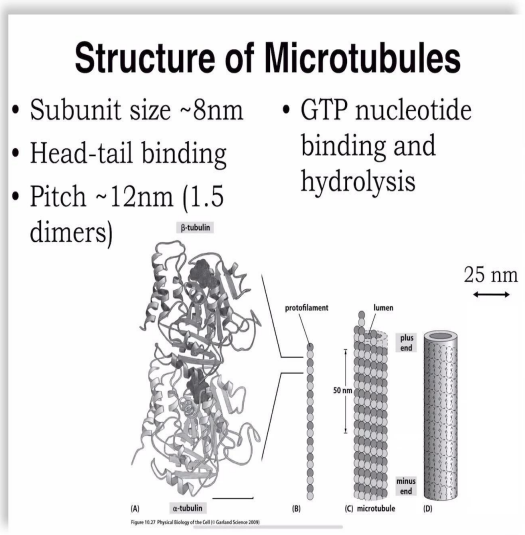

So, we are not going to talk about actin microtubules and intermediate filaments property, the third of these so called canonical set of skeletal limits, what you see is a meshwork of microtubules with a nucleus in the centre, and it is made up of an attribute of tubulin subunit, which forms a proto-filament in a sort of head tail fashion, these proto-filaments form circle, a cylinder, a wrap around each themselves with a plus end indicating the going in and the minus end indicating the shrinking end, this does not have to do with electric polarity, this is just simply a kinetic polarity. And these cylindrical structures have a diameter of 25 nanometers.

Indeed, the tubulin dynamics are the growth and shrinkage is driven by GTP nucleotide binding and hydrolysis to GTP. Tubulin has a GTPase domain an active enzymatic domain for GTPase.

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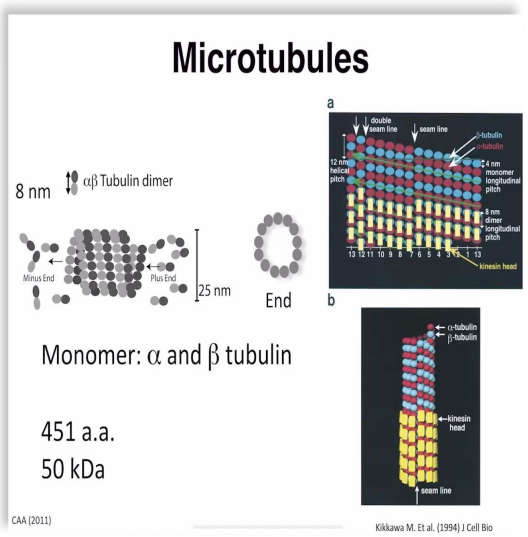

These microtubules with this particular 25 nanometer diameter canonically, also have an unusual structure in the sense that they have a seam which is to say that when you go around the cylinder, there is a slight spiral and there is an offset. So, at some point, if you follow any one set of tubulin dimers, along or across the proto-filaments, you come to a structure where there is an offset and that offset is called the seam and these seams have been indicated to play a role in dynamic instability. There is a lot more literature on this. We will not be talking about the biochemistry or structure of it as much as we will talk about the dynamics in the coming lectures for the moment I only want you to remember that this is a cylinder and it has a thickness which is determined by the wall of the cylinder is determined by the alpha-beta tubulin dimer.

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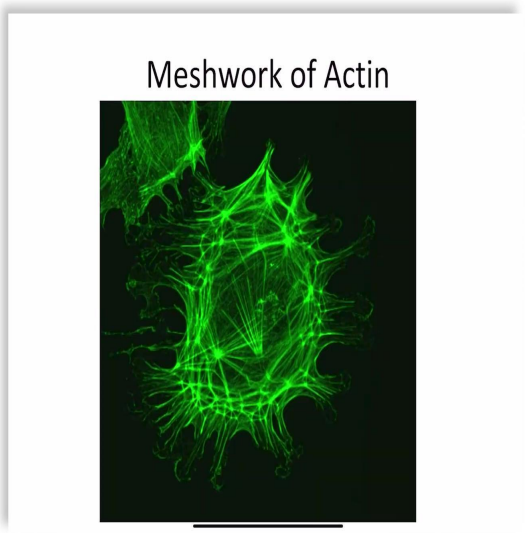

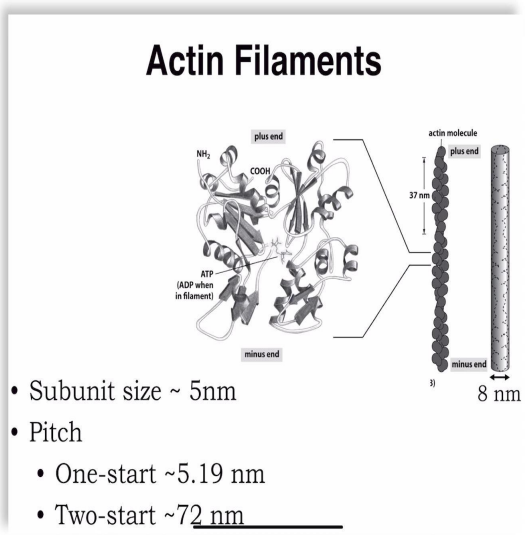

Actin on the other hand forms these kind of meshwork like structures, which consist of just one kind of protein in that typically global acting or G acting with a subunit size of 5 nanometers and a pitch which either if you consider a 1 or 2 proto-filament system is 5.19 nanometers or 72 nanometers pitch if you remember is the height travelled for one circle to be completed. So, in an angular sense along the length of the lattice, how much time how many what linear distances travelled before it returns to the same angular position.

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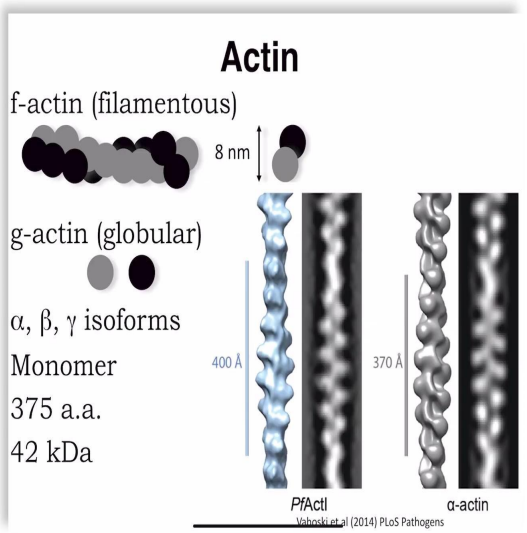

In fact, there are multiple isoforms acting Alpha, Beta, Gamma and these are usually developmentally expressed the protein itself even across species. So, right hand side is alpha actin from a mammalian system and compared to *Plasmodium falciparum,* malaria parasite actin and you will see that the structure slightly more elongated in the contract comparison that is made here in from prior electron microscopy data.

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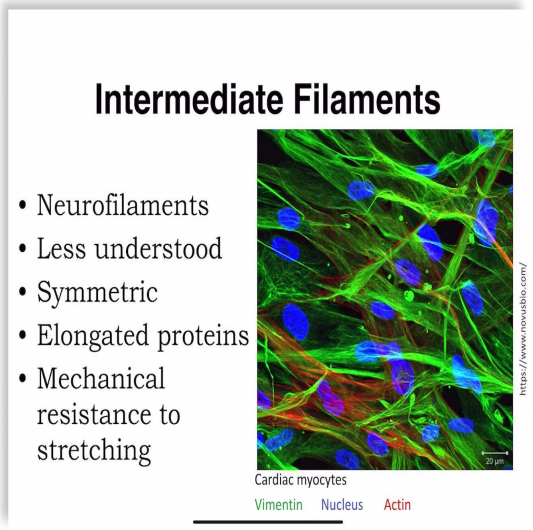

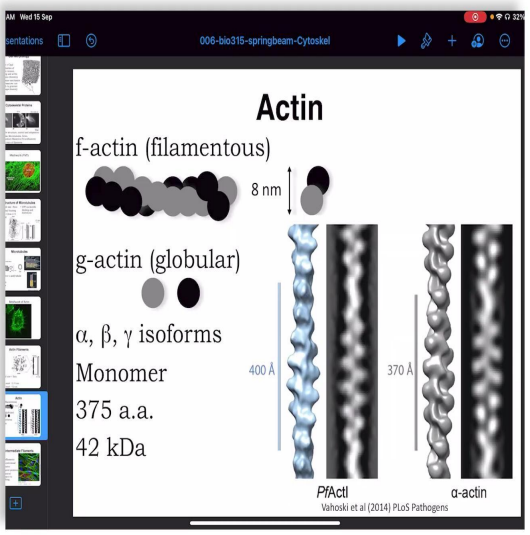

Coming to the so called third system it is intermediate filaments and intermediate filaments are less understood, they also form more symmetric that is to say not like plus and minus any structures. So, I may have missed that, but barbed ends of acting are like the blessings of materials and pointed ends are like the minor sense of microtubules, these terminologies become relevant to come back to polymer dynamics and polymerization kinetics at the very end. But suffice to say that, for the moment, we will focus on the mechanics and assume that the structures are static.

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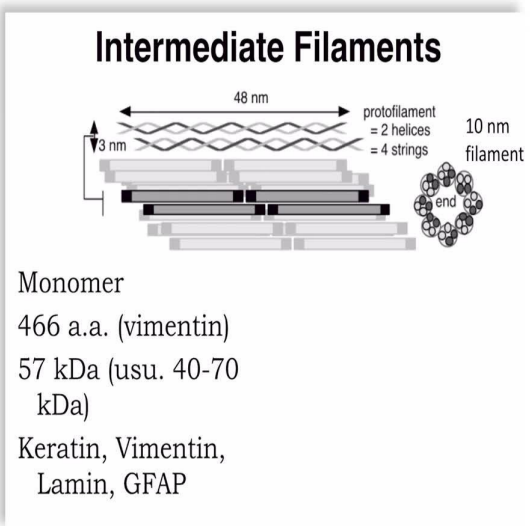

So, it turns out that intermediate filaments are more likely to be static, they form these kinds of higher order structures of proto-filaments of 2 helices grouped together into 4 strings, and

so on and so forth. And, Vimentin, Keratin, that is higher Lamin, all these examples of intermediate filaments.

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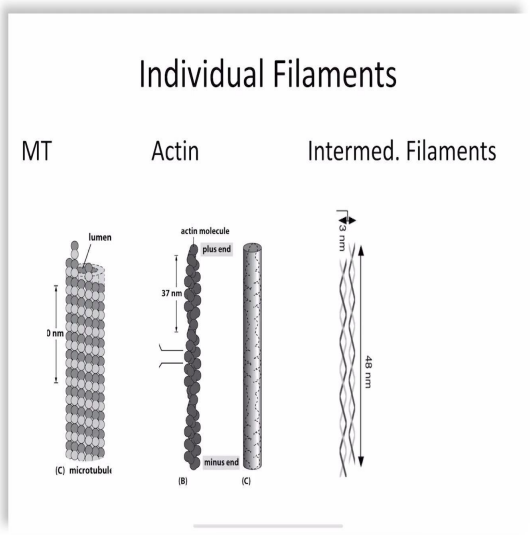

And part of the reason for calling them intimate filaments is historical because the diameter of the actin filaments is the narrowest, the thinnest 5 nanometers, 13 proto-filaments, 27, 25 nanometers, the diameter of the microtubules. And intermediate filaments are intermediate to these two.

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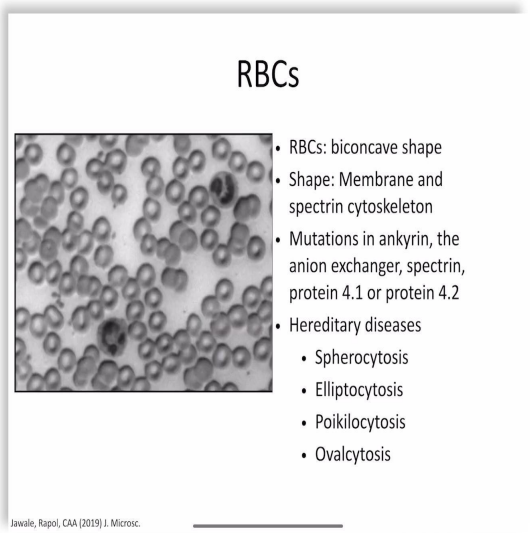

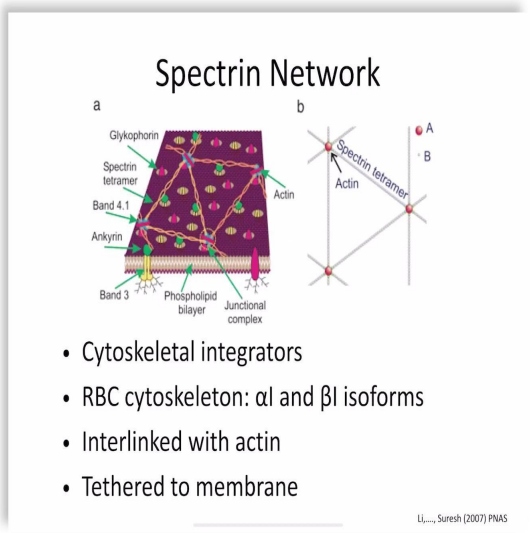

So, in a living cell in an intact cell, all these proteins play a role. And I am just going to take an example of the fact that hereditary diseases that are involved in mutations in ankyrin, spectrin, protein 4.1 or protein 4.2 lead to cell shape defects in a disease which can lead to diseases. So, where are all these proteins localized? So in the case of red blood cells, which is the simplest cell you can think of, because it does not have a nucleus, in its mature form, in most mammalian systems, at least, consists of an actin pinning point and spectrum fibers spectrum ropes, you may say, between these pinning points and these are anchored in the membrane as you can see, through these band proteins, this entire pinning point is then called the junctional complex, which is quite complex consisting of band 4.1 ankyrin so on and so forth.

So, the point is that the membrane has or I am sorry, the cell structure is determined by the cytoskeleton. And mutations in it sequence mutations, and it cause mechanical differences, which in turn cause diseases and we are going to try and understand the basic mechanics so that we can come back to answer the more complex questions.

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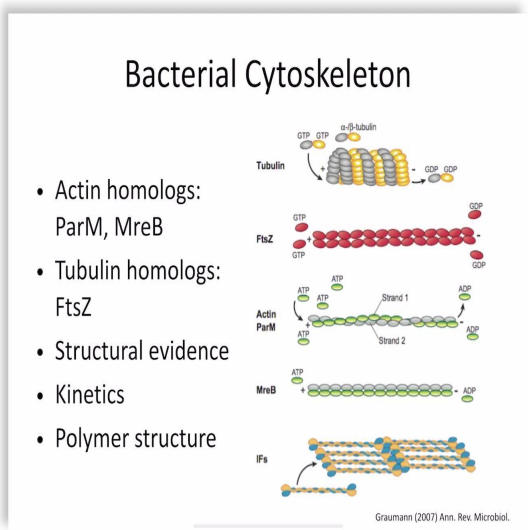

But as I said, at the beginning, we are only looking at the endogenous cytoskeleton because that is in fact, apparently even conserved for bacteria, as we can see over here with the bacterial cytoskeleton, where MreB is an actin homolog, which you see in the green lower panel. As ParM, which segregates plasmid, FtsZ which is responsible for the Division of cells, is a tubulin homologs at a structural level, but none of them form the kind of elaborate tubulin like structures that we see in eukaryotes. So, you could argue it is a primitive tubulin in some sense.

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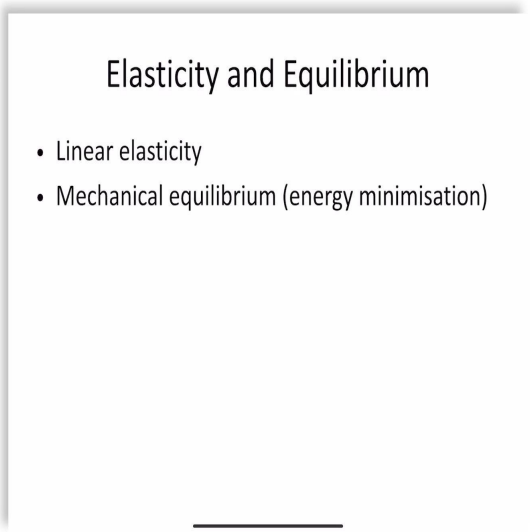

So, for the next step, we are going to talk a little bit about the theory that helps us understand what the nature of these properties that govern the details of cytoskeleton image and lead to

the properties at cellular level. And we are going to talk a little bit about mechanical equilibrium with the idea of asking the question, can we find the optimum and what is a system optimizing a mechanical system and can we then apply that to cellular protein molecular systems.