**Transcript of Phil Sharp Interview**

**Tim**

In 1899, the great American cell biologist Eb Wilson wrote, to sum up the living protocol glass is a liquid or rather a mixture of liquids, consisting of a continuous substance in which are suspended drops. We can take up this statement today, with a new fascination, as dozens of researchers around the world are describing the biological activity inside of cells in terms of liquid separated emotions, which can be variously described as content sense or liquid liquid phase, separated organelles, or simply membrane lyst organelles. But overall, these set of observations seek to understand one main thing about cells. We all know that cells contain many different membrane bound compartments that kind of act like separate little rooms in the cells interior. But we also know that cells contain a whole lot of stuff that is not bound by these membranes. And it's sort of sloshing around back and forth as though it were all in the deep end of a big pool with everything kind of just bumping into everything else. But we know that it is a big, messy, Deep End pool of the cell, there emerged very finely orchestrated interactions between complexes that consist of hundreds or perhaps even thousands of different enzymes, nucleic acid, like DNA, or RNA, or lipids. So this for a long time, has left biologists with a kind of a conundrum, which is, how does everything kind of know how to find everything else? In order to carry out its specific function? This question hasn't really been answered, it's certainly not from the standpoint of the material property of the cell. And as we look back at EB Wilson's quote, it seems, a lot of scientists are coming back to this idea that there are liquid phase separated droplets inside of cells, much like oil and vinegar. So to investigate this question, here at MIT, we've interviewed three different researchers, and today is the first.

**Phil**

I'm Philip sharp, Institute professor at MIT. I'm a member of the Koch Institute, and the Department of Biology and I've been a professor at MIT for over 40 years now.

**Tim**

So the early 1990s, were going pretty well, for Phil sharp, his lab had found that the coding regions for genes are not continuous. But were split up into segments called Exxon's those the regions that code for the gene product. And He further found out that the axons had to be stitched together by a complex the proteins called the splices zone. For this discovery, Phil was awarded the Nobel Prize in 1993, which he shared with Richard Roberts, his path to discovery used in vitro biochemistry, which means proteins, DNA and RNA, or mixed together in a test tube. But he knew that these reactions were taking place seamlessly in the busy interior of living cells. And despite the great advances that he had made, that leap from the test tube into the living cell, posed a really big problem for him.

**Phil**

I didn't understand how that could happen, but just no physical models for, for putting all of these things together,

**Phil**

yeah, once in at once in a dynamic way where the system could read in seconds, right, a set of interactions collectively, collaboratively, and then facilitate the splicing reaction. But there was just no model, physical model that you could explain how these proteins could be bringing together Exxon's to then be spliced by the splices. I had worked out the spices Oh, I know what the machines were right. Right. I didn't know where the specificity was coming from.

**Tim**

He might have left this question behind at the right time. Because soon a new discovery was made, that would keep him really busy. A former graduate student of fills Andy fire, wrote to fill with some interesting results on RNA and gene expression. firewood later win the Nobel Prize with Craig mellow in 2006. Let's check out that story.

**Phil**

So I basically left the field and went on to at that time, we were just big. Andy Fire and Craig Melo had just discovered RNAi. Fire was a graduate student with me here at MIT. Okay, so we had a very good relationship, he sent me the paper. Then I went back and read his discovery paper. I instantly believed it and started reading the literature that Andy had, had cited. And that basically led him with the experiments he and Greg Miller dead to propose and in show that double strand RNA was the source of RNA,

**Tim**

postdocs with Phil and a fellow MIT professor David Martell, took up these observations from worms and demonstrated how the RNA is processed during RNA interference.

**Phil**

So they set up a cytoplasm make extract, added double strand RNA, and the first experiment worked. And they were seeing due to edition of double strand RNA in an in vitro reaction, that it was being converted in a process that would ultimately destroy messenger RNA that lead us off into RNA and micro RI.

**Tim**

And so you went really far from from spices, initial questions you're

**Phil**

having Yeah, I couldn't, couldn't do both. I didn't want to begin terribly big lab, I was also doing some things on transcription that were related to cancer. So that took me away from this whole splicing quest subject.

**Tim**

This is followed by many other fruitful endeavors by Phil, but he didn't revisit the earlier mystery until way later in 2016, when he spoke with Professor of biophysics, Mike Rosen, who described some new observations of certain sub cellular granules.

**Phil**

They were describing these face transitions in with the properties of surface tension. Yep. And free energies. And there's never been a cell biologist I knew who measured surface tension. Right. Right. And we just don't think in surface tensions sort of aspects. About I think it's 2008 Clift Brangwynne, one was a postdoc with Tony Hyman in Dresden. Yeah, they were at the time they made this recognition down, it was hope, summer course. And we're looking at these granules associated with a germline and C. elegans there are associated with the nucleus of the germ cells in in CL against another organisms and labeling the proteins in those bodies. They recognize that they were distorted, like liquids, yep. And they feel like liquids. And therefore, they said, This dense body of RNA and protein has the properties of a liquid. But it's a membraneless body that is, from a morphology point of view, reasonably stable. And that led to the recognition that this was a property of liquids, and then phase transitions.

**Tim**

Cliff Brangwynne and Tony Hyman published live imaging of a certain protein aggregate inside of the embryo of C. elegans, showing that these aggregates behave like a liquid inside the cell. They began describing these as membraneless organelles, because they are partition from the rest of the cell. But they still behave like a liquid. But this idea is not actually new. As the biophysicist Mike Rosen was aware.

**Phil**

Then Rosen understood the polymer chemistry of, of 75 years ago, and I was trained as a polymer physicist.

**Tim**

Oh, there you go.

**Phil**

Yeah. So it's, I mean, it has been around, it's been around and I actually read the books, but I didn't actually make the relationship. But when, when you looked at that, you could understand it from a polymer point of view. Arup Chakraborty, who's here at MIT came from Berkeley, he's a Chem engineer, I approached him as to whether he could deal with the modeling of a liquid phase transition type polymer system, where we could explain these concepts sites, which have liquid properties in in the terms of valence, the number of links between a polymer right, and the affinities. And those are two things by chemist measure, measure. And then the timescale varies and the properties of the condensate very, it's a very dynamic, some condensates are much more stable, and could be even described as a gel or fiber.

**Tim**

So this was all very nice for the granules from C elegans. But Phil was drawn to this idea for his own reasons. And that is the aggregates of proteins that coalesce at certain regions of DNA, called superenhancers. He was also drawn to the same idea to help explain his previous discoveries of the spliceosome.

**Phil**

Right. So we got together with Rick, we talked about us the theoretical model to illustrate properties of what a super enhancer would be like. And then we wrote this up for Cell. It was a perspective we originally submitted as a paper really, yeah. But that led us then to really move forward and in exploring if we could gain direct evidence that enhancers that drive genes, and particularly the subset of them, that drive genes at high levels called superenhancers do have the properties of these liquid phases in vivo. And so we Rick started a series of experiments that labeled proteins that were involved in the super enhancers, and he had previously shown they were in these complexes. And that was polymerase and mediator. Yep, and, and transcription factors. And we showed a correlation between a super enhancer with the properties of a liquid phase, it was dynamic infusion vision, and it was a super enhancer that was associated with a gene we knew was driven by super enhancers. So we add a super enhancer to gene relationship. And we have a super enhancer has the properties of a liquid phase. Yep. And that was a paper in science. In parallel with that paper. We knew a colleague here at MIT, Ibrahim Cisse, who was over in physics, he's an assistant professor. And in a paper that also appeared in the same issue in science. He has a paper that shows that you can tag in a single cell, a single molecule, mediator and polymerase. They are both in the same complex, which be consistent with it being a super enhancer that there are hundred of these, the polymerase molecules and mediator in such a cluster,

**Tim**

can we define what mediator does

**Phil**

mediator associates with polymerase. And it's that complex that lands at the promoter to give you an initiation of transcription. So mediator mediates polymerase engagement with the promoter site to transcribe. But it was very powerful set of experiments and papers that, that showed that these types of of clusters have the properties of a condensate with liquid properties, and that they are directly involved in the activity of polymerase on a gene. And so now, we know we can manipulate the properties of the mediator, and control or suppress or stimulate the levels of transcription of a gene that had been never been done before. We know we can manipulate the components of the enhancer and control the gene, the transcription, we know we can manipulate the DNA binding factors and control the transcription of GM. And here we have a condensate that explains how all of these are engaged with each other in a collective fashion to form a body that activates transcription.

**Tim**

Okay, did you guys all that. So in one paper, Phil and his colleagues show that the machinery that associated with DNA to form a super enhancer behaves like a liquid while it was doing its job. In the second paper, Ibrahim Cisse observed the same protein complexes land on DNA inside of living cells using live superresolution microscopy.

**Phil**

This is just scratching the surface of a much deeper and richer set of experiments where we now have to figure out why or how a given promoter is forming a transplant condensate to activate transcription from that promoter. And that's really the point where I've been for many decades trying to get

**Tim**

you mean, like why this promoter?

**Phil**

Why this promoter…now? Yeah. So that's the real, the real essence of gene regulation. Yeah. We need to know how to decode why this promoter is active now? Yeah, I think that is extraordinarily exciting. We're moving forward on all of that. Yeah, we know, there's another condensate type process almost certainly involved in the splicing. Yeah, you know, now we can begin to turn to understanding that. It's very clear transcription has these properties. Very clear DNA replication, the initial DNA replication is based on these types of processes. It's very clear the synapse, the association of the vesicles that contain the stimulatory molecules are almost certainly associated with the synaptic region through condensates. Similar properties are clearly involved in stress granules in the cytoplasm. Yep. So we're looking at probably, at a universal process in cells that organize a lot of the components in cells. And I think if you begin to, to picture what has to happen at these processes, where many complexes have to come together to have a reaction occur? Yeah, then using condensate type processes to concentrate them to bring many components together in a local high concentration to form a super complex that then initiate transcription. Or other processes assemble a microtube or give you initiation of replication. It it makes a lot of physical sense. It's very exciting principles of biology. Students are still puzzled by it, people don't believe it. Yeah, yeah. There's lots of lots of chaos out there.

**Tim**

we have been a bit we I mean, you know cell biologists have been given amazing tools to understand genetics and genomics now, by sequencing. Yeah, right. You can run a set of reactions, and then you can look at a sequence and your answer will be a DNA sequence, or RNA sequence. And that, you know, that led to genomics, all of these computational approaches that we are now more and more used to looking at genome wide at everything. And it's so it's a kind of data that is very, very powerful. And yet it seems now at this point where you can't really answer these questions, just by sequencing, it seems like, and certainly people are trying, but it seems like it's been such a huge shift in the tools that are being used to explore these things. I mean, like, it's a real stark difference in the tools and the kind of thinking.

**Phil**

And that's what makes it so interesting to be part of the science now. Yeah, that you have what you're saying with genomics and high throughput nucleic acid, one high level view of what has to be happening in a cell, right. And then you got, you know, decades of biochemistry, where you have a sort of micro view, a single protein view of what has to have, but the real important regulation is occurring in that middle ground between a high level integrated view and the low level, protein chemistry view, right? Yes, you can maybe see now super complexes super. So we can put these proteins in complexes that are then clearly important in making their chemical reaction go and that's where the regulation is. And so that's where we can sink in a new type of, of science, not new, it's all science being focused in a new place. Yeah. But either understand and then manipulate genetically to understand and intervene with a therapeutic drug or protein. Uh huh. You have to understand at this level of protein complexes, mechanistically What's going on? Right? Are you are you get results you have no way of weeding one from another and it's just an endless circle of chaos. So now we've got tools and thought processes and concepts that allow us to look at that middle ground with large numbers of complexes and integration of a lot of signals is giving us new biology we've known about for a long time Yeah, but a new way of looking at the biology. So that's where we're at and we're just gaining those insights and those will come over a decade. It takes a decade to do this. new ways of controlling and manipulating and, and that that will be exciting. But it will happen.

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