Jacques Loeb Centre
for the History and Philosophy
of the Life Sciences
Prof. Ute Deichmann, Director

Ben-Gurion University of the Negev
P.O. Box 653, Beer-Sheva, 8410501, Israel
Building 39, Room-114 | Phone: +972-8-6472258
Email: jloebcentre@post.bgu.ac.il | Web: in.bgu.ac.il/en/loeb

Interview by Ute Deichmann with John Glass

J. Craig Venter Institute, La Jolla, 17 February 2015

John Glass is leader of the Synthetic Biology and Bioenergy Group of the J. Craig Venter Institute (JCVI), adjunct professor of the University of Maryland at College Park Cellular and Molecular Biology Program, and member of the Global Viral Network Scientific Leadership Board.

He studied biology and genetics at the University of North Carolina at Chapel Hill and did his Ph.D. on RNA virus genetics at the School of Medicine at the University of Virginia. Then, as post-doctoral fellow and faculty member at the Microbiology Department of the Univ. of Alabama, he studied polio virology and mycoplasma pathogenesis (until 1998). On a sabbatical leave (1995-7) he sequenced the genome of Ureaplasma and began to study Mycoplasma genomics. From 1998-2003 Glass was a researcher in the Infectious Diseases Research Division of the pharmaceutical company Eli Lilly, where he directed a hepatitis C virology group and a microbial genomics group. He joined the J. Craig Venter Institute in 2003.

The lure of simple systems

UD: Why did you, as a biologist, become attracted to research in medical microbiology and why did you choose mycoplasmal pathogenesis?

JG: It was a case of opportunity. I found the RNA virology where I started my academic career to be really interesting in that it was really small systems. So if our genomes have - if we have - 50,000 or 30,000 genes that can be expressed in 200,000 different ways to produce 200,000 different proteins, the viruses I started working with had 5

genes, 1 piece of RNA, and made 5 proteins. That's all they need, and I was enamored with these simple systems that are characteristic of RNA viruses. And they are still really complicated in the way that they interact with cells, but I like the idea that if one could understand for a small virus what each thing did, you might be able to predict the behavior of a larger system.

And after doing this with several different kinds of RNA viruses, it seemed to me that that era of being able to sustain a research lab in those fields was coming to an end. Because these problems were getting closer to be solved and they are a little bit outside of the mainstream of medical biology. And funding would be difficult to come by. And so I thought about moving into something that was more medically relevant, which led me to what I thought was a terrific position with a group that did medical mycoplasmology to understand these things because, again, this is still a really small organism and it might be easier to understand a small, simple organism than it would be to understand something like E. coli, or humans or plants. So that appealed to me.

UD: Were you convinced that understanding the small organism or the small virus would lead to an understanding of more complex ones?

JG: I think this was my training in the physical sciences that led me to think of really simple systems that are the equivalent of the hydrogen atom.

UD: So interesting. Going back to Max Delbrück?

JG: Yes, Max Delbrück and Niels Bohr. Max Delbrück, certainly.

In the 1960s and 70s there was a number of scientists who were writing about this, but the tools didn't exist to do anything about it. And I could see when I was having to make these choices about where to go in biology in 1991 to 1992, that this just seemed like a great place to go, that fit my interest, and there would be some interesting things to do here.

UD: In 1998 you moved to the pharmaceutical company Eli Lilly and you directed a Hepatitis C virology group and also microbial genomics. Weren't you afraid that your connection with industry would impair your academic career?

John. I was, but I was research-track faculty at the University of Alabama at Birmingham and my mentor there asked me if I would come with her and said, "We can run, in essence, an academic mycoplasma group inside Eli Lilly". That was her plan, and she wanted me to go with her there to continue doing this. And once I arrived, it was clear this this did not fit with Eli Lilly's plan, although I managed to do it for most of the time that I was there. And I've always found myself dividing my interests between things that I thought were interesting. I had been trained as a virologist, and I went and sat with the virology group just to listen. I offered some ideas, and they gave me a place to do this and some funding and a couple of people to work on the projects that I had suggested and so that's how the Hepatitis C things were going, and I was, at that time, one of the few people who had sequenced the microbial genome. Lilly had some projects involved in sequencing microbial genomes in order to identify new drug targets for antibiotics. So I got involved in that. But I still maintained, pretty much the entire time I was at Lilly, an interest in mycoplasmas and published a number of papers while I was there as part of that work. And I maintained my contacts in the mycoplasma community. And then when Lilly, in December of 2002, decided that they were getting out of the infectious diseases research business, because there was not enough money in it, I found myself needing a job and this position at the Venter Institute looked perfect.

Joining the J. Craig Venter Institute

UD: It is interesting that you didn't try to go back to the university.

JG: I thought about that. I had a number of possibilities, but this one just looked like more fun than anything else I might be able to do.

UD: And so you approached Venter or he approached you?

JG: I approached Venter. He knew who I was, because I had had collaborations with folks at the Venter Institute – then it was called TIGR, or the Institute for Genomic Research. I had collaborated with a number of people there and had known them for 5 or 6 years.

UD: To which project were you assigned at the Venter Institute?

JG: At that point the Venter Institute was not called the Venter Institute. There were a number of organizations under the umbrella of the J. Craig Venter Science Foundation. I was in an organization called the Institute for Biological Energy Alternatives. The mission of the organization – we had funding from the Department of Energy – was to build a minimal cell. Now, the minimal cell was something that Craig and other of my colleagues here at the Venter Institute had been thinking about since the early 90s. It is the idea that rather than trying to whittle down an organism like E. coli to make it a minimal organism that you would use as a platform to understand the first principles of cellular life, what their thinking was, is that you would never get there using that method. The way to do this was to take one of these near-minimal cells, which generally means mycoplasmas, although not always, and rebuild that because the technology didn't exist yet to gradually diminish the size of genomes, but to build a cell that was actually a minimal cell. And in order to do this, you would have to learn rules of genome design and a number of technologies.

This just looked like an amazing project. I remember reading an article in *Science* about this, which is how I heard about the Institute for Biological Energy Alternatives [an organization of the J. Craig Venter institute]. It said that Venter has the lofty goal of making this synthetic minimal cell, and it quoted various other scientists who said, "I don't think he's going to be able to do this." The article ended saying that this is the feeling of other scientists and we have noted that twice before in his career, Venter has set up lofty goals and

people have said that he wouldn't be able to do it. And both times he's gotten this done.

UD: But how was that connected to energy?

JG: The Department of Energy (DOE), at that point, was the first one who believed it was necessary to sequence genomes. It wasn't NIH doing this.

UD: How was this related to biological energy alternatives?

JG: The thinking was that if we can understand how cells work, then we will be able to build organisms that can produce bio-fuels efficiently. So the DOE funded the Human Genome Project and it funded the first microbial genome sequencing project.

UD: They funded the Human Genome Project?

JG: I think the reason is that the Department of Energy believed that this would – it took enormous computational capacity and that was something that the DOE had. And then there were visionary scientists in the DOE such as Ari Patrinos or Marvin Frazier who saw that this is where the world is going and that we are going to need biofuels. And they said that someone has to be pushing the envelope of what is possible in biology.

So it wasn't NIH, it was the DOE that really believed in this. Craig proposed to NIH to sequence genomes and they said that it was an unreasonable task – that it couldn't be done.

The minimal cell

So this is the history of our involvement in the idea of a minimal cell – that notion that if you assume that all cells have a certain critical core of functions that are common to everything. Protein synthesis, DNA replication, cell division, RNA synthesis, energy utilization – if we can really understand how those things work, we will be much better able

to design more complicated systems that might be used to solve human problems.

UD: You mentioned once that you wanted to create the minimal cell in order to understand the first principles of biology. I know first principles exist in physics, but I'm not sure that biology has first principles. What are these principles?

JG: What we're finding is that as we have been iteratively rebuilding a Mycoplasma mycoides genome with fewer and fewer genes, sometimes we make deletions of genes that prove to be lethal to that cell. Sometimes we make deletions that result in the cell growing so slowly that it ceases to be interesting. No one is really interested in working on a cell that divides once a week because it would take too long. So we arbitrarily said that our definition of a minimal cell will be a cell that contains only the genes necessary for the cell to divide every 2 hours. That number is somewhat arbitrary, but it means that every 2 days you can look at your cells and see what happened. And what we have found is that the minimal cell contains about 500 genes – a few less. And that of those 500 genes, at least 20% we have no idea what they do. None. And don't think of those genes as genes that express proteins. Think of them as there are 100 functions in this cell - things this cell does that we are totally unaware of.

UD: You talk about genes, but the cell is, of course, more than genes.

JG: We have always thought of a cell as – we make the analogy to a computer. The cell itself is the hardware - the box, the circuits. The genome of the cell is the software. And the software encodes genes. And those genes encode instructions to make proteins. So we know that in a cell that makes 500 proteins, about 100 of them – we don't know what they do. But we also know that those proteins are present in almost everything else. So think of it as one gene encodes one function. And sometimes we will run into instances where you will have several genes that essentially encode the same function. So you have genes A and B. At least one of them is necessary for life.

So you can have two proteins that perform the same function that don't look at all alike, but we know that there is nothing that we can take away without the cell growing much more slowly.

UD: But aren't there several possibilities to reach that state? So that many minimal cells -

JG: Yes, there could be many minimal cells, but there are probably only a limited number of functions that are necessary. So if A and B both encode an enzyme that is involved in producing energy, I only need to have one of them. But both of those genes, although they look different and they have different names, do the same operation inside the cell. So we have moved away from thinking about how many proteins or how many genes are inside a cell, but how many operations or functions does a cell need to do to survive?

UD: So the minimal cell would be the minimum function cell.

JG: Yes. And proteins are there to do a job. It can be DNA synthesis, it can be energy metabolism, it can be to transport nutrients into the cell. So the cell is the compilation of all those functions in a way that they all work together.

UD: If you succeed to have this minimal cell with minimum functions and minimum genes necessary to code for the proteins for those functions, why is such a cell not realized in nature?

JG: Nature is a very competitive place. What we are doing is making a cell that lives where there is no competition, where there is as little stress as possible, where we give it all the nutrients the cell needs. Now, in the natural world, the organism on which this cell is based, called the Mycoplasma mycoides, subspecies capri, is an opportunistic pathogen, not a severe pathogen, it is a caprinal goat pathogen. It can cause arthritis or mastitis (inflammation of the mammary glands) in goats. And it is a respiratory pathogen – at least that is the point of entry. I believe that this organism needs all 910 of its protein-coding genes to live in a goat. Because it has to avoid the immune system, it

has to be able to live in the upper respiratory system which certainly is different in many ways than the lower respiratory system – temperature, lots of things. But what we have done is make an organism that doesn't have to worry about any of these stresses; all it has to do is grow. And it doesn't have to compete with anything else. The important thing in it to us is that it is those core functions that are left in this organism that grows in a stress-free world that are also present – as least as far as we can tell – in all other organisms.

If we look at all 500 genes and we do a computational comparison of the genes in the minimal cell with the genes in – you name the organism – yourself, E. coli, Arabidopsis, mice, algae – what you see is that for the most part, in all these genes there is something that looks like them in everything. Even in the places that we see that there is not something that looks like them it may be just that that gene in the mycoplasma has diverged so far from the common ancestor; it may still do the same thing, it just doesn't look enough like it anymore for the recognition to be made.

UD: Because mycoplasma is also the result of evolution…

JG: And it turns out that as you look at graphs of how fast organisms are evolving, mycoplasmas seem to be evolving faster than any other cells in nature, probably because they have thrown away most of their capacity to repair damage.

Mycoplasmas started out as organisms that are more like Streptococcus pneumoniae that causes pneumococcus or strep throat or Staphylococcus aureus; they started out as organisms that were like other bacteria. They had cell walls, they had genomes of 2 million base pairs or more – a couple of thousand genes. But because they found their environmental niche in places that were very low stress, they were able to be more efficient by throwing away parts of their genome. So anything that the mycoplasmas don't need, they get rid of. That is one of the lessons we see from comparative genomics.

Changing genomes in prokaryotes and eukaryotes

UD: You say that though Mycoplasma is a very fast-evolving organism, these genes are ubiquitous in all other organisms. That means that they are highly conserved in evolution.

JG: Yes. These genes perform the core functions necessary to be alive.

UD: They don't change?

JG: The functions don't change; the genes themselves change. A Uracil-N-glycosylase might need to be a little bit different if you live in a urogenital tract where it's 37 degrees all the time, than you would need if you live in the ocean, or in the dirt, or in a thermal vent in the ocean. So for 80% of the genes in our genome, they look sort of like some other gene. But another 20% really doesn't look like anything that we know what it does. But by computationally comparing the amino acid sequence of different proteins you can see that these proteins have, in essence, a common ancestor. And with common ancestors, you generally assume that they perform the same function – not always, but usually.

UD: When I read about your ongoing projects on producing cells with a minimal genome, I asked myself whether one can also conclude that during evolution, many unnecessary parts of DNA were accumulated. That the complexity of the genomes, especially in higher organisms –

JG: There are some organisms that do that – that are constantly accumulating new genes and they will hold on to them for a while on the chance that maybe they give them an advantage in some situations. There are organisms that are really good at taking up genes. They will find DNA floating in the environment, they will kill some other organism and consume its DNA – it varies. We have genes in us from bacteria; we have genes – we took in mitochondria, for instance. There are many examples of this in eukaryotes, prokaryotes, and for organisms that are able to live in lots of different

environments, this is important. Because if you look at bacteria, the ones with the smallest genomes are limited in the number of environmental niches that they can grow in. The ones with larger genomes, generally, can grow in all kinds of different niches under many, many different conditions; they are capable of adapting. And part of that is by accumulating different genes. In some cases you might have a dozen different variants of a gene whose function is the same, but one of them is better for low temperature, one is better for high acid, one is better for high temperature, for high salinity, etc.

UD: It sounds possible for prokaryotes, but not eukaryotes. And yet there is only a small part of the human genome which is coding for proteins.

JG: Only a very small fraction codes for proteins. But we are seeing that the informational content is much higher than we had thought, and what people used to think is junk DNA may not be that.

UD: Yes, but that is debated. People also think that the amount of not functional DNA has increased because it was accumulated during evolution and doesn't disturb. Of course, part of the non-coding DNA contains regulatory genes, which are highly important.

JG: Eukaryote genomes are vastly more complicated. Parts which don’t seem to have a function now, may have had a function at one point in ontogeny but no longer. I think that that's one of the lessons as we begin to learn about gene expression.

UD: Eric Davidson works on the hierarchical gene regulatory networks which you don't have in prokaryotes.

JG: We are seeing that prokaryote genomes are more complicated than we used to think they are. And we are finding that there are a lot of small RNAs in prokaryotic genomes that people didn't know about. Just as they're finding that there are a lot of small RNAs in human genes. Now in some cases these RNAs in prokaryotic genomes – I'm reviewing a paper right now where they argue that a lot of these are

just junk. But I'm not sure that they have convinced me. Anyway, there is more complexity in these genomes than we are aware of because they have been so honed – improved - by evolution.

UD: But still, it's possible that there were some virus RNAs that just remained in these genomes without functions.

JG: So you and I don't think that we do horizontal gene transfer any more.

UD: Yes, I would think so.

JG: But, in fact, we still can. Retroviruses insert into our genomes; we know that we are constantly exposed to our own microbiome and we do exchange genes with the organisms that we live with. In the intestine, on the skin. It is probably at a very low rate, but it happens. And we know that viruses will insert new genes into our genomes.

UD: Also in the germ cells?

JG: Possibly, yes. And we also know that humans are now thinking about genetically repairing germ cells.

UD: Yes, that I heard. Because there are transposons with detrimental effects and some of these small RNAs are involved in fighting them.

JG: In one of my current projects, there is a scientist at the University of Edinburgh in Scotland, Anura Rambukkana, who discovered something important about leprosy – the oldest known human disease. Leprosy grows in the skin, in the nose, in the periphery, because it cannot grow at 37 degrees; it has to be at 33 degrees. And the way that it spreads from the right hand to the left hand is not by the hands rubbing together. It is because the leprosy infects the sheaths of the nerves and the way it spreads is that it causes these Schwann cells to de-differentiate to become stem cells that float around the body, find another nerve at the right temperature somewhere, and that's how it spreads. The astonishing thing is that we've never heard of anything that will turn a fully differentiated cell back into a stem cell. So imagine that I can do genome therapy on a

cell, revert it back to this stem cell form, and that can be a germ line cell conceivably. We are certainly not there yet, but these are things that you could imagine someday.

I am stunned at what I see being done in the veterinary industry for instance there was a company at this meeting I was at in January called Recombinetics, where dairy cows – farmers don't want dairy cows to have horns because they hurt other cows. So they use just brutal methods to de-horn the cows – bolt cutters, torches etc, and this group has discovered the one gene that was necessary to eliminate horns. So they ended up using cas9 CRISPR technology which you may have heard about; it's called genome editing. And they went in and deleted that one gene and produced a cow without horns.

UD: By deleting one gene?

JG: By deleting one gene. And at least as far as the U.S. government is concerned – I don't think they do it in Europe - if you don't do anything to the genome, it's not even a genetically modified organism.

I much prefer the notion of getting my milk from a cow that hadn't had to suffer, having seen a movie of how they currently take off horns.

UD: And this gene is deleted permanently?

JG: Yes.

UD: Has it been used before?

JG: They have produced the cows and they are working at getting approval for this.

The way they knew this is they found a cow with a mutation that didn't have horns. They learned what the mutation was and they said that it would be possible by breeding to do this in 34 generations. But for a cow, that's a really long time. Whereas these people did this it in just

a few months and they have cows that don't have horns now. And the technology enables them to do this.

Recapitulating cellular evolution

There is a story that we have found in the work on the minimal cell. I have been in science for 30-something years and never have I had a finding that was so stunningly different than what you were expecting. I'm curious to see what's going to happen but I'm not expecting something that just astonished me and changed my whole thinking about how cells work:

We have collaborators across the street at the National Center for Imaging and Microscopy Research that do electron microscopy. And we sent some samples of the minimal cell at different stages of minimization to them to make pictures of. At first glance, all of the pictures came back looking sort of the same. But we realized that one of them looked a little different; the background didn't look the same on that one as it did on the other ones. And what we realized is that whereas in the wild-type organism that we work with, Mycoplasma mycoides, there are spheres that are about 400 nanometers (nm) in diameter. And for this variant – for this version of the minimal cell – they weren't 400 nm in diameter, they were 5000 nm in diameter. So that means they are 10 times larger. Now if you think about it, volume is the cube of diameter. So these cells have a thousand times the volume of the wild-type cell. And to our amazement, they still grow at the same rate.

UD: With fewer genes than the others?

JG: No, it looks like they have maybe thousands of genomes. But what we have done is: We have uncoupled cell division from genome replication and we have taken away probably the genes that made cells go from – when cells first evolved, they probably didn't divide; they blebbed off or budded off little cells. Because their membranes

become unstable as the size grew. And sometimes those have a genome. And then that cell grows, and grows, and grows as it takes in stuff and there's lots of genomes in it and it buds off. And then at some point in the evolution of life on earth this cell division machinery evolved, which is in everything – everything. You, me - in bacteria it's FtsZ, in us it's tubulin - but this is the way that cells divide. And what we've done is we've taken away that machinery and now what we're seeing is what we think is somehow we have recapitulated what cell division looked like, or cell proliferation looked like before cell division occurred. Because we can put certain genes back and we restore cell division. This was just so cool, because we just didn't expect this.

UD: So you think you reversed evolution?

JG: Well, in a sense. What we have done is we have recapitulated an earlier stage of cell biology before cell division evolved, when instead cells proliferated or budded off new cells as a result of membrane instability.

So there are yeast that bud, but those cells also divide. This is just something that those yeast can't do in addition. So this is, I think, letting us take a look at something that it turns out evolutionary biologists had thought about but no-one had ever seen.

UD: This really looks like experimental evolution - I mean you are experimentally approaching questions of evolution.

JG: I think what a lot of this will be about is to understand how cells came to be because the first cells had to be just this minimum set of functions.

UD: But we cannot conclude that the first cells looked like your minimum cells.

JG: No I don't think so. Well, they may, in fact, have looked something like this. But the first cells – we know that our cell will divide in two hours with these 500 genes. But we also know that a third of the genes that are in our cell can be taken out with the consequence that the cell

grows slower. Now I doubt that you can take all of that third out, but any individual gene, when you take it out, slows growth. So probably the first cells that evolved could have taken decades to make new cells.

So it doesn't look exactly like the original cells but it gives us an idea – this is what it might have looked like; something like this. And what we're seeing fits nicely with what people had theorized might happen, but not exactly. We have movies of these cells growing and it turns out to be considerably more complicated than we expected. But just really exciting.

UD: Where did these additional genes for this cell division machinery come from in the beginning? It's quite complicated.

JG: It is, but where did any firsts come from?

On this project, I am working with Petra Schwille, who was at your meeting on Synthetic Life [the 2012 workshop of the Jacques Loeb Centre in Beer Sheva] - I had known her from previous meetings.

UD: So are you addressing the question of where the genes might have come from?

JG: It's not something we've even thought about. And what we're limiting ourselves to at this point is: We know that the cell division genes aren't necessary for life.

UD: Not for life, but for the life which we - the higher organisms- have.

JG: Because once cell division evolved, it worked so much better than blebbing did, that those organisms that didn't have it got lost.

UD: Is it possible that those genes had a different function in another organism and that they just combined?

JG: Well, possibly. Odds are that there was a stretch of DNA that there might have been two copies. So originally it did something critical. And then it made another copy of itself and one copy kept changing

and kept changing and kept changing and at one point it did all these things and then as the mechanism kept getting better for cell division only cells that divided were left at some point.

UD: They divided faithfully and remained the same, right? The others are different.

JG: Because these are processes about taking a cell and making a septum that more or less divides the cell equally into two parts. It's a complicated mechanism because not only do you have to do that, but you have to have something that will segregate chromosomes into each daughter. So it's a complicated system that had to evolve. And how it evolved, I've wondered about but we haven't done anything to address that. As we get a better sense of what all the genes are that may be involved in cell division, it may be that we'll get a better idea. Because these are genes that we have intentionally put back, and we have a cell that divides and a cell that proliferates. And the stunning thing is that they do it at about the same rate – at least in our organism. One of them will divide in an hour with cell division, and 90 minutes without cell division. Which still meets our criteria. That's just a little vignette.

From genome sequencing to synthesis - the underlying philosophy

UD: How did Craig Venter and his collaborators (and I think you were already one of them) get from genome sequencing to genome synthesis?

JG: We have always been tool makers at the Venter Institute - developing new technologies that enabled us to gain greater understanding of biology. And the technology for synthesizing DNA got better and better. In the mid-1970s, Gobind Khorana got a Nobel Prize for sequencing a tRNA. But as the technology for synthesizing pieces of DNA got better, it became obvious to a lot of biologists that it will become possible to build organisms to solve problems. And also to

use the technology of making things in order to understand how things work. We are often brought back to - and I'm sure I'm not getting this exactly right – the mantra of synthetic biology based on the teachings of – I can't remember his name – what I can't build, I can't understand.

UD: In your lecture in 2012 you said it came from Feynman.

JG: Yes, Richard Feynman. And we didn't get it right when we wrote it in the Cell.

UD: Right, but then you fixed it. I'm smiling because I know it as the mantra of the structural organic chemists around 100 years ago. They said something like ‘before we synthesize a molecule, we don't understand it structure.’ Feynman may have taken it from them

JG: Makes perfect sense.

Anyway, by being able to build things you really experimentally – if you're just sequencing, what you're doing is observing what is. But because we can rebuild things, we can make mutations in a way that we've never been able to before. So we can understand structure and function. The problem with mutations is even when they are directed, they tend to be one at a time. Whereas now, I can imagine the way an organism works and then test it and see.

What Craig gave us, was that he wanted to be able to understand. His goal has always been to understand life, to understand how cells evolved. And so our mission at the early stages of this synthetic biology organization was to develop the tools to enable us to synthesize large genomes or microbial genomes and to enliven them. So really up until the last couple of years we have been exclusively in the tool-making business. Developing the methods that enable us to do what we do now – which is to get in, and we have built this minimal cell, and we are now starting to play with it and see how it works.

Another thing that we are doing: I'm sure that genomes appear to be organized, or there is certainly organization to the location of some of the genes in the genome. There are some genes that are pretty much always in the same place relative to the origin or terminus of replication in most any cell you look at. But other genes, not so much. For instance, the 10 or so genes involved in glycolysis are strewn around the genome, and they are not in the same place in any organism. And so what we have begun doing, is we have taken our minimal cell and we have been reorganizing it so that the genes are organized in functional modules. All of the aminoacyl tRNA synthetases are in one unit, and all of the glycolytic genes are in another unit, etc. So that the genome becomes, in some ways, like a computer breadboard.

So I can take out one computer module and replace it with another module that we synthesize from a different cell to see how the glycolytic genes in the cell sol is – what's different about those.

UD: Did you find a difference?

JG: We're just getting to this, so we haven't found that much yet.

UD: That would be really interesting – whether gene location impacts its performance.

JG: I think it will in some cases and in others it won't. The young man that I spoke to in the lab earlier is a visiting student from France – an undergraduate who will be here for six months – and his assignment is to exchange a group of genes just to see how it works. We're still building this modularized genome but I think that will be done within the year.

An eighth of the genome was modularized and it worked really well. Based on the success there, we just have now to go back and redo the rest of it.

UD: Why did this apparent mess in the genome come about?

JG: Because genomes get damaged and there are repair mechanisms to fix genomes when they get broken and things move around. Or it might need to be that some genes get expressed better closer to the origin of replication so they move to where they need to be. When you've got millennia to find just your right spot, the dynamics in the genome enables you to do that. But from our perspective, as opposed to being 20 operations to compare to see if I can exchange modules from one genome to another, this becomes a single operation. It enables you to a lot of things. I think in this sense the minimal cell will become a chassis for industrial use to think about a basic cell, that is, what are the consequences of making different kinds of changes?

UD: And it might also be interesting to find out, once you have your modularized cell, what happens after a certain number of replications.

JG: That's certainly something that we have talked about, that we will just have cultures that we continually pass, and every 6 months or so we will take a culture and see what happened.

UD: What I found interesting, reading your paper [in Perspectives in Biology and Medicine 55] again, is, first, that the synthetic genome project, which filled the headlines of all the newspapers, was basically part of the project on minimum genomes, like a side-effect, and not the other way around. Second, people have commented that your synthesized genome was not designed from scratch but used the sequence of a natural genome and that you didn't synthesize a cell, only a genome. Here I would like to talk a bit about the philosophy behind. When Craig Venter and his team - I think you were the head of the synthetic genome project - called this bacterium the first artificial cell or the first synthesized cell, they equated the cell or cellular life with the genome. The philosophy here seems to be that cellular life is causally determined by the DNA sequence. And that

other cellular factors, which are of course important, are secondary and follow the order of the genome. Is this right?

JG: Now we know a great deal more about modifications to the genome – epigenetic modifications – than we knew in 2010. And I get beaten up by a lot of people about the minimal cell or about the synthetic cell saying that, "you didn't synthesize a cell". I begin seminars by saying that "synthetic cell" is a euphemism for a cell with a chemically synthesized genome.

UD: I remember the discussions we had in Beer Sheva [in 2012].

JG: And some people are really offended by at least the idea of a synthetic cell. Just that we are boasting about something we didn't actually do. It is not like we took membranes and ribosomes – it's not a fully synthetic cell – and certainly the synthetic cell is almost completely based on a design of Mycoplasma mycoides. We have changed very little from the natural genome. But this was a step in the process. So the first thing you make, is something that you know works.

UD: But the idea that cellular life is causally determined by the genome remains the philosophy, right? If you change the genome, the whole cell will change.

JG: Yes. And what we have seen is that we can build a genome in yeast where none of the epigenetic modifications done by yeast should be the same as the modifications that would be done in a natural bacteria. And that none of the natural modifications in that bacteria would be in any way carried forward because every base that we put into the genome originally was from a synthetic piece of DNA.

UD: Let me see if I've got it right. You said you built the genome in yeast where none of the epigenetic modifications are the same as in the bacterium.

JG: Correct.

UD: But that means that these modifications don't impact the –

JG: So apparently for bacteria – at least this bacterium – you can park that genome or dry-dock that genome in a eukaryotic cell, which will not modify the genome in the same way, because it has different mechanisms. And you can move that genome into a bacterial cell and it produces a bacterial cell that is indistinguishable from the one from which we derived that genome.

UD: What happens to the modifications?

JG: If the yeast modified it, they are lost. And then the instructions for how to modify the genome still remain in that bacterium because they are encoded in the software. But they are not necessary. And it looks like you can do the same thing for a yeast cell, because the group led by Jef Boeke that made a single synthetic yeast chromosome have shown that you can make a synthetic yeast chromosome and redesign that chromosome and it still functions normally.

UD: What do you mean by "redesign"?

JG: They took yeast chromosome 3 and they took out some of the DNA, rather than build an exact copy. So I guess this doesn't address that since it's not an exact copy. But it still seems to make a cell that is indistinguishable. By changing the order of some things and eliminating some of the genes they took out all that was necessary, then minimalized it and modularized it to some extent.

UD: What is the importance of epigenetic modifications?

JG: I think that these factors are important for higher eukaryotes - less so for the lower eukaryotes. We know that in our genomes wherever you have a C followed by a G in most cases the cytosine gets methylated. In bacteria this doesn't happen very often. In yeast it doesn't happen. So in the lower eukaryotes it doesn't happen but in the higher eukaryotes it does.

The genome is the primary factor, but the other factors are still very important. I don't think I could synthesize a human genome and make it human doing it that way. But I think we will develop better and better methods for modifying genomes in the same vein as what we had been doing for bacteria. I have another project in collaboration with Pam Silver from Harvard where she and I are trying to develop technology to make human artificial chromosomes. HACs is the abbreviation. And this has been around for some time. The way people have always done this in the past is they would take an existing chromosome and they would whittle it away and then try to add new things. So they would take out most of, say, chromosome 14 and replace it with a small amount of other DNA but they would leave the centromere and telomere and regulatory regions. And what we're trying to do is to synthesize these chromosomes – build them purely synthetically in yeast. And then also develop some methods as we work on this to methylate or acetylate the chromosome while it's in yeast with specific enzymes and then see if this affects the function of these chromosomes. This is early stage in the process, but we are not the only people who are looking at this.

UD: You do it with human chromosomes?

JG: Human or mouse. Whichever seems more relevant at the time. So right now we are doing this in a human-derived cell line.

We are going to see if we can actually make sequence-specific changes and see to what extent it pans out in terms of the function of what we build. So again, as opposed to just observing it, we're going to make a hypothesis and test the hypothesis. We can be completely wrong, but why not try? And again, the goal here is not to make new humans, but say I wanted to make cells that produce humanized antibodies. Or I wanted to make human cells in tissue culture that produce something. At least initially.

Applications

UD: You are now working, among other things, on producing bacteria which produce biofuel, is this correct?

JG: Part of our group is, yes. Actually we're working on getting yeast that would be better at converting. We have a project that is aimed at getting algae to produce hydrogen, and that's not one of my projects but one of my colleague's projects. And there's another project where we are making yeast that are better able to convert biological material, like the part of the corn that is left after you harvest what you want, to ethanol. It turns out that yeast can handle a higher level of alcohol than most any bacteria.

UD: It can tolerate it or –

JG: It can tolerate it.

UD: It should, right? Yeast produces alcohol.

JG: Yes.

UD: So they are protected against the damaging factors. And in order to do so, do you use artificial genomes?

JG: Pieces of artificial genomes – parts of them. An example: we have known for maybe 100 years that there are bacteria in the ocean that can, in the presence of oxygen, take sunlight and water and produce hydrogen. We have never found those bacteria that do it well. Now, some of my colleagues have taken a sailboat around the world, about one and a half, maybe two times; I don't know exactly how many times they've been around. And they are taking samples from the ocean every couple of hundred miles. They filter out the bacteria, send them back to us, and we sequence just *en masse*. And in doing this, we have identified thousands of hydrogenases – the enzymes that convert water to hydrogen. We found thousands of these.

UD: A thousand different ones?

JG: Thousands of them, yes. Because different bacteria have different versions of the same – subtly different. And based on computational

predictions, we have seen that some of them are likely to function in the presence of oxygen. Even though we don't know what organism they came from, we can predict what the genes might look like. So we have synthesized these hydrogenases; synthesized the genes, put those into either E. coli or into algae to see if they can produce hydrogen in the presence of oxygen. And in fact we found that this works to some extent.

Now, it isn't efficient enough with oil at 50 dollars a barrel. No one has much interest in this. And it turns out that hydrogen is a terrible greenhouse gas, it's hard to contain, it maybe isn't the best biofuel to make, but still this is an example of how genomics may be used at some point when it is economically practical to do so for more biofuels.

J. Craig Venter

UD: I would like to talk a bit about Craig Venter and his institute. He came from NIH, I understand; he left it.

JG: In frustration with the absence in risk-taking and the bureaucracy.

UD: From all the years you have been working with him, what do you think is the impact of him as a person and as a scientist, on the research and the outlook and spirit of the research at the J. Craig Venter Institute (JCVI)?

JG: It's huge. He has a huge impact on how we view the world and what is important. He has just a nimble mind and can listen to what we talk about and he can see several steps beyond where we are, it seems. And he is very good at listening to things and saying, "You guys do this." And generally he doesn't ask us to do things that we can't do. We may have no idea how we're going to do it, but he's got a vague idea how he thinks we're going to get there and we seem to solve problems. But also when you go in with specific questions he's really

good at making good suggestions and helping steer the research.

He now has three organizations that he is shepherding. The J. Craig Venter Institute – this place (La Jolla), Synthetic Genomics Inc. which he founded about 9 years ago, and a new company that was founded last year, the Human Longevity Institute aimed at trying to help us live longer. The other two organizations are for-profit organizations and he is dividing his time between these three things. But he keeps looking at draft versions of our papers describing the minimal cell and suggests changes and things that we need to do, and what isn't clear, and he's involved really in many, many things going on here at the Institute. And he brings us exciting opportunities also. Because of his presence people give us credit that we can do amazing things. You only get to do the science that you can get someone to pay you to do.

Pure and applied research

UD: From what you told me here you are quite free to decide yourself what to do.

JG: We are, so long as you can get someone to ultimately pay for it.

UD: I was surprised by what you told me before, namely that the institute in Rockville and here in La Jolla are largely functioning on grants and donations.

JG: Yes, mostly on grants. Or grants and contracts, I suppose.

UD: And not on patents or any kind of profit?

JG: No. There is almost none of that. Only in the last year or so have we had any of that. And that was like $700,000 that came as a result of the work we did with Novartis to make an H7N9 influenza virus vaccine which was just stockpiled in case it was needed by the government. And we may be getting more royalties from Novartis for work that we did a number of years ago to help them with a

meningococcus B vaccine which will be for sale later this year in the U.S.

I think the time may come when this is different; but right now this is the way things are. We have been more focused on producing patents and so, for instance, Synthetic Genomics has funded a great deal of the synthetic biology research. So while I suppose we are the inventors for this, Synthetic Genomics owns the rights to the patents. And so if the minimal cell is eventually commercialized, this could be a source of revenue for JCVI.

UD: I see. But it was not the main idea to get the money from profits. And what you also told me is that you succeed to do things with less people in a shorter amount of time than other researchers. So do you have better people, or are you better organized?

JG: It is, I think, a just different approach to science. It is a coordinated team as opposed to a lot of individuals working on independent projects. In that sense it's a little more like industry.

UD: Because you are doing, on the one hand, very clearly application-oriented research. But on the other hand there is a lot of work that contributes to basic science. You are also cooperating with other academic institutions; for example, Harvard University. So you are recognized as an institution that is doing research in basic research, right?

JG: Yes. As well as very applied things as well.

UD: I think that at our workshop in 2012 somebody suggested that applied research may take the lead and brings basic insights; not only the other way around. What do you think?

JG: I think most of our research is not applied. Most of the research that is done by the JCVI is funded by the National Institutes of Health and it is aimed at understanding aspects of medical microbiology, for instance patterns of influenza virus infection, or the human

microbiome. And then there is National Science Foundation work that is aimed at understanding microbes in the environment. I am just starting a new project aimed at developing a vaccine for a pathogen of poultry; it's a mycoplasma. And that will be funded by the Department of Agriculture. And for a closely related organism to the Mycoplasma mycoides that is the minimal cell. We have a project going on that is funded by the Gates foundation. This closely related organism is arguably the veterinary pathogen most in need of a vaccine in the world. So in Sub-Saharan Africa, a disease called Mycoplasma mycoides which causes Contagious Bovine Pleural Pneumonia or Lung Plague which is a disease of cattle that is a terrible problem in that part of the world and there is no good vaccine. Actually there are vaccines but they are not very good. You can tell if a cow has been vaccinated because it doesn't have a tail usually; the vaccination makes the tail fall off.

They have no genetic tools to build a vaccine, so we are trying to use our synthetic biology tools to take genes out of this organism that caused the pathogenesis but leave enough genes in the organism so that it would be immunogenic, and it would still be amenable to cheap vaccine production techniques that would be needed for the developing world.

So those are examples of really applied things - the minimal cell is the least applied thing you can imagine. (Maybe not to our funders; they try to imagine it as applied so they can sell it to people, but I have a hard time believing that a lot of people are going to buy it).

UD: It is interesting that the NIH funds the Venter Institute, though he left them.

JG: Yes, but you write research proposals and you compete to see if you can win awards.
Years ago we were one of the large DNA sequencing centers. And that's no longer the case at all. We are a tiny genome sequencing center. But we are still a large center for the analysis of DNA

sequences. Given that now sequencing is such a commodity, what we have now are our computational biologists, who never go into the lab. Instead they sit at their computers and analyze human genomes or microbial genomes or microbiomes in order to make inferences about biology.

Ethical issues

UD: This huge capacity of analyzing genomes or also synthesizing them raises ethical questions. Here I cite a colleague of yours, Neil Gershenfeld, who at a conference at MIT envisaged major ethical problems "when the ability to convert biology to data and data to biology becomes very cheap and very easy to do." Do you agree with that, and if, which problems do you perceive?

JG: In his book 'Life at the Speed of Light' Craig Venter envisioned a coming age of digital biology, in which a genome can be designed on computer and transmitted over long distances by electromagnetic waves. Then it can be reconstituted in a synthesizer to produce a new form of life.

UD: You want us to make new humans in other places?

JG: The idea may be impractical for humans to travel hundreds of light-years away in starships that don't exist yet. But imagine that you just sent the information to make new humans -

I'm sort of paraphrasing from his book. It certainly means if it was cheap enough and easy enough, people might be doing bioterrorism without having to have enormous research investments and skills. And there will be the parts of the world that are capable of this and the parts of the world that aren't. And is that fair?
How long do we really need to live?
It opens up so many possibilities that may seem like science fiction now but who's to know what's going to be science fact soon? I believe that there will be a day, before long, when I can design a

genome today on my computer and have it synthesized and delivered to me a few days, a week or two later, from a commercial DNA foundry that I would then insert that genome by genome transplantation into a suitable recipient cell and make that cell. Or I do thousands of these in order to find the cell that does what I want. And that's a different world from now, but maybe not that far away.

I think it's going to be great!

I was asked, "Do you worry that this is going to lead to people doing nefarious things?" Probably. But I also think that this will be the technology that will make my children's lives better.

UD: Enough bad things are happening without it; that we already know.

JG: With language you get Shakespeare and you get libel. Slander, libel.

UD: It seems to me that in several ethical statements these possibilities are not really perceived. They are pointing to the danger of genetic engineering or something like that. They are not worried about the speed with which organisms can be changed.

JG: Scientists fear climate change; they don't fear genetically modified crops.

UD: No, but the general population does.

JG: Because it is easier to imagine bad things – I suppose they give us more credit than we deserve. I don't think we're going to make dinosaurs or monster humans or incredible pathogens. I think that there's so much thought into this and making organisms is still really hard. At best we can mimic things that already exist.

UD: I think this is very important. You can make genomes and you can transplant them but that doesn't mean you can create a new species which doesn't exist.

JG: Or even this minimal cell. In a sense, Craig wants to think of it as a new species, but it's not really. The whole species concept gets vague at a certain point in that it still has almost all its parts from the original species; it just doesn't have all of the parts.

UD: How do you define a bacterial species? In contrast to a strain, or in contrast to a genome.

JG: That is probably the most difficult question you've asked me today. There are rules for this. In the modern world we look at the sequence of the 16S ribosomal RNA gene because that is the easiest gene to compare among different organisms. Just as for eukaryotes you would look at the sequence of the 18S ribosomal RNA gene, which is the corresponding gene. But by the official rules governing bacterial taxonomy you would look at immunological characteristics in terms of surface proteins. There is rehybridization kinetics where you melt the DNA by making the two strands come apart and then having it go back together – the rates of that.

No one does these things anymore. The way it is done now is exclusively by 16S but officially that's not correct. And then to make a new species you have to have it recognized by this organization that recognizes new species. Now, how different do two genomes have to be in order to be different species? It's like a continuum in some ways, so this is where it gets hard but generally people think of it as the 16S ribosomal RNA. Now Craig has asked us to change the 16S ribosomal RNA of our organism. And we thought that this would be relatively straightforward, but it turns out that you can't change it very much.

UD: Probably that's why it became the mark of the species.

JG: Well, but no one knew that, and so what we can do is: We have changed it enough so that the computer models that will look at a 16S sequence and tell you what species it is just say, "unknown mycoplasma". That's as far as we can get.

Now we might be able to exchange the whole protein synthesis system, where you took out the ribosomes, all of the ribosomal RNAs, the ribosomal proteins, the tRNAs, the release factors, the tRNA synthetases – maybe 200 genes. Now you might be able to change that gene from another species and make it not a mycoplasma any more, but I don't know that you're going to be able to take that gene out; that may be a change that we can't make. But we're testing now to see how far we can go.

UD: What I find most peculiar is that there are bacterial species which have obviously remained constant over a long time. I mean, we still speak of the tuberculosis pathogen for more than a hundred years.

JG: But it is not just one sequence. Every strain that we sequence is just a little bit different.

UD: Yes, but they have enough characteristics in common to call them tubercle bacteria and to cause the symptoms of tuberculosis in humans, despite horizontal gene transfer and spontaneous mutations…

JG: They have been evolving for a long time to get that way.

UD: Yes, but they have also stayed for a long time.

JG: I think that one might be able to design experiments where you try to direct an organism to be a different species, and you might be able to do this in a few years of steady effort – to turn one organism into something that is really different than what it evolved from, where it would be so different that the algorithms would say that it's not the same species.

UD: You mean in prokaryotes.

JG: Yes. I think you could do it with yeast also. Or some of the other lower eukaryotes, just because it takes many, many generations to do this. Because we can, using directed evolution, push organisms pretty far, pretty fast to do new things.

Genome design, synthesizing life, origin of life

UD: How do you think the work you are doing, supervising or directing, will contribute to basic science? For example, to the theory of the genome, the question of the origin of life, or evolution?

JG: Origin of life is hard. That's really not where we're going. We are getting to the idea of how you design a genome – what are the rules for building genomes? We really are working on that. So, for instance, we're listing the things that a genome has to have. And we are developing sets of rules about how these genomes are organized.

UD: But you are not asking whether this was the way life originated.

JG: I don't think that that's where we're really going now. Even if we eventually go so far as to rebuild a living organism purely from chemicals off the shelf, that would be our vision of how an organism is, and probably something fairly far from the original organism because I bet those organisms lived so slowly that it's just not practical to think of doing that now.

But could we take a genome – we have shown that we can build ribosomes. Imagine that I extracted ribosomes from another organism, just because it's easier than building all of those. So you have ribosomes and you have in-vitro transcribed proteins. And you make this mix and you somehow squeeze it all together and put it in a membrane and then change the exterior milieu around that membrane so that you create a set of chemical imbalances. That could be enough to start a cell. I could see doing that, which gets you to the idea of AN origin of life – how you might build a cell. And it gets you to understanding even more about the different functions that you have to recapitulate. And maybe as you start looking at these things, you get a better idea of how these things evolved. So, for instance, seeing how cells function in the absence of cell division; this is a

recapitulation of a stage in early evolution of life on earth that we are seeing what it looks like – or what it might have looked like, probably looked like.

UD: How cell division started – what the genes are for that. And what about the theory of the genome?

JG: Well the theory of the genome is, I think– what are the rules for genome design? Why do bacteria have one chromosome, generally, instead of multiple chromosomes? So I think that as we get a better handle on how genomes are put together and we see on an experimental basis what you can do and can't do, it will be much more reasonable to think about this theory of a genome.

UD: What does it mean: how genomes are put together?

JG: How are the genes organized, what is necessary or what advantages do you get by different orientations of genes and genomes.

UD: Are there overlapping genes in prokaryotes?

JG: There are. And so when we find an overlapping gene that's naturally overlapping, we separate them. Because we know the sequence, and so if you have a gene that has 10 or 20 nucleotides overlapping another gene, I just pull it apart and put some stuffer DNA in between them and we empirically test to see if it works.

UD: They don't need for example additional promoters?

JG: So here, what we've got – the ability to do this – is that we have unused promoters and terminators from the genes that we have taken out of the genome. And so we start dividing things – if you've got two genes that are adjacent to each other and the same function, you don't do anything. But if they are different functions then you divide them, resynthesize new promoters and new terminators and move on. And so it worked very nicely the first time.

UD: It seems to me that this means, "the whole is exactly the sum of its parts", reversing an ancient statement.

JG: In one sense it is, although I'm not sure that if you just had the parts that you'd have a living cell.

UD: You mean the part of the chromosome?

JG: The chromosome IS the sum of its parts. Well clearly, in one sense the chromosome is 25% each base. It only is what it is when all of those bases are properly sequenced. Then it is something. But if it is broken down into individual parts, unless they're in one piece, then it's the sum of their parts. If they are separated into smaller pieces, it really is nothing.

So in the sense that you can put new parts in the chromosome and the chromosome is now bigger and capable of more, yes. And so that is how we think of bacteria genomes from our work and we are coming to that conclusion about more eukaryotes as well.

UD: So really this does not fit the idea of the whole being bigger than the sum of its parts.

JG: I think that this minimal cell will be a blow against that. And if you think that I can put a genome in dry-dock and manipulate it any way I want so that it's going to lose all of its epigenetic modifications which it may have had going in if I didn't synthesize it, but I can make modifications and I can put it back into a cell and it makes a cell. Or if I don't change anything, it will make a cell that is, for everything that we can characterize, exactly the same.

UD: The determination of cell properties - or of early eukaryotic development by DNA is difficult to accept for many people.

JG: Is it that we don't like the idea of – people want to be more than just their DNA.

UD: DNA may be a metaphor for chemistry and physics. Life is something different than chemistry and physics.

JG: We say life is something different than chemistry and physics, but maybe it's not.

UD: Chemistry is already different from physics and properties of chemical compounds cannot be explained on the level of particle physics.

JG: But it's still – this is the thing about the hydrogen atom – we still cannot model higher atoms but the things that we learned about matter through the hydrogen atom have proven to be true for everything else. And in the end, cells and organisms are still, for the most part, just five different kinds of atoms – carbon, nitrogen, oxygen, phosphorous and sulfur. A few other things too, but these are atoms that are governed by the same laws of physics as this bottle, that pen, etc. So I think that life is governed by the same rules, it's just more complicated.

UD: And there are different properties. Jacques Loeb, who was one of the arch-mechanists 100 years ago, did not accept the notion that the whole was bigger than the sum of its parts, which he thought, is a vitalistic idea. I think Craig Venter, too, is fighting neovitalism, which he perceives in attitudes that turn the focus away from DNA to an ‘emergent’ property of the cell that is greater than the sum of its molecular parts.
Jacques Loeb already envisaged synthesizing life, and he attributed a crucial role to the nucleic acid of the nucleus. Now you are on the way to carry this out. I consider the current attempts to relativize the importance of genes dangerous, because they evade the problems related to genetic injustice by rendering them irrelevant.

JG: We have politicians here who don't want to be associated with the idea of evolution. The governor of Louisiana was asked if he believes in evolution. And he said, "Well, I'm not a biologist so I don't know." What he leaves out is that he was a biology major and graduated with honors from Dartmouth. It just doesn't play well in much of the country.

UD: But would they also go against synthetic biology?

JG: Oh, yes. Unless they thought it could bring jobs to their district! Maybe 3% of the U.S. economy is biotechnology now. That's huge. I wonder what it is in Israel, probably bigger.

UD: It might be. I have no idea.

JG: Because the detergent industry, for instance, is the biggest part of the biotechnology industry.

UD: Detergent?

JG: Yes. Because there are enzymes in modern detergents.

UD: Not pharmaceuticals?

JG: Apparently not. Because so much of modern pharmaceuticals is still, for the most part, organic chemistry.

UD: But the big chemical companies have large…

JG: They have biotechnology divisions, but most drugs are still not made using biotechnology.

UD: Except antibiotics?

JG: Well, antibiotics might be an exception. Most drugs started as biological molecules. In biotechnology, I think, you have organisms doing recombinant molecular biology to do this. And so most drugs may be started as natural products and then are chemically combined, even if you start as glucose. But there is no recombinant DNA technology involved.

UD: Thank you very much for sharing with me these fascinating results and insights into your research.