

Interview with Venkatraman Ramakrishnan

Venkatraman Ramakrishnan, who won the 2009 Nobel Prize for Chemistry along with Ada Yonath and Thomas Steitz for his work on elucidating the high-resolution structure of the 30S ribosome, recently visited the Indian Institute of Science (IISc), Bangalore, where he has been a G. N. Ramachandran Professor since 2006. On 5 January 2009, Ramakrishnan delivered the IISc Centenary Lecture alluringly entitled 'From Baroda to Cambridge – a life in science'. The talk, at the J. N. Tata Auditorium, began on an autobiographical note; he traced his journey from the Convent of Jesus and Mary Girls High School, Baroda, to the MRC Laboratory for Molecular Biology, Cambridge, UK, where he is currently working. The following is from a conversation with the Nobel laureate, at the IISc guest house immediately after the talk.



Venki Ramakrishnan at the IISc guest house, with a poster announcing his talk in the background.

The scientist

What made you start working on ribosomes in your lab?

Because I'd done a post-doc on ribosomes. For many years, maybe 15 years, my lab worked on both chromatin and ribosomes. In 1998, I stopped the chromatin work because I realized that in order to work on the whole ribosome structure, it would take all of my attention. So I didn't want to be distracted by other problems because I felt this was too important a problem.

Why the 30S subunit?

That's interesting because when I started, Ada Yonath had already produced good crystals of the 50S subunit. And I thought, well, she ought to just carry on working on it. Then I learnt that the Yale group (Tom Steitz's group) was going to work on just that – 50S structure. So I didn't want to get into that whole situation. But, for the 30S, there were no good crystals at that time. And I also worked on the 30S in a different way in Peter Moore's lab – trying to understand where all the proteins were. And so I thought it was a natural thing for me to work on the 30S. And I also had this idea that I might be able to lock the 30S into a conformation (using the initiation factor, IF3).

... and get good crystals that way?

And get good crystals. Luckily I didn't have to wait around for that. Otherwise I still wouldn't have the structures today. But the point is that all of those things made me realize that there was an opening in the 30S.

*Your crystals were of the *Thermus thermophilus* ribosome. What made you choose that organism?*

That's because there were these early reports of *Thermus* crystals published by the Russian group (Marina Garber's group). You see, when you purify ribosomes, and they don't crystallize, there are many many possible reasons. One of which is that your ribosomes are not pure enough or not good and so on. So I thought that if we started with the *Thermus* system, we could at least try to get those crystals first – what the Russian group had done. And that would at least tell us if our ribosomes were reasonably good. And then we could go from there to the next step of getting well diffracting crystals. So I thought that was a good starting point. Whereas if we went with a different species, we wouldn't know what was happening.

What were the major obstacles that you faced before 2000?

Well, first, you know, we weren't sure that we'd get good crystals. And so that

was the first obstacle. And that, I think, we were amazingly fast in getting crystals that diffracted to about 3 Å.

How did that happen?

I think that happened because we were willing to try non-standard ways of purifying ribosomes and we got very very pure ribosomes that were homogenous and ended up giving us very good crystals. But I think there was an element of luck involved. You know, we could never be sure. But I think it was also careful biochemistry, not just luck. The other problem was, then, how would we go about solving it. So I had these ideas of using anomalous scattering, and that worked quite well up to 5.5 Å. But when we wanted to go to high resolution, we found that our crystals would diffract well, but each crystal was somewhat different from the other. There was non-isomorphism between crystals – there was variability between crystals. And so, that was something that really bothered us because we couldn't collect all our data on one crystal. You could do it if you wanted a low resolution data. But to get to where we could solve it to atomic resolution, we needed to collect data on lots of crystals.

So all of them had to be uniform?

So all of them had to be uniform. And until we solved that problem we couldn't actually make the breakthrough. And we were almost worried that we were going to be completely beaten by the other groups. In the end we were able to solve it.

So you got uniform crystals in the end?

We got it by a combination of things. One was brute force. We just froze and collected data snapshots on hundreds of crystals; then grouped them into uniform sets. And then another thing was a trick, which was to add a compound (osmium hexammine) that seemed to make all the crystals more uniform.

Were there any new developments that took place while you were working on the ribosome structure that kind of eased your path?

It did, because, I would say, even a year earlier, the synchrotron beam-lines were

not good enough to solve the 30S structure. I think they could have solved the 50S structure because they diffracted much better. The 30S diffraction was so weak that we really needed these high brilliance beam-lines at the APS (Advanced Photon Source, Chicago) and ESRF (European Synchrotron Radiation Facility, France). And even when we started, the ESRF beam-lines were not quite good enough, and our early usable data came from the APS. But the ESRF then, right around that time, developed their beam-lines to a point where we were getting very good data. So that helped us a lot.

Apart from synchrotrons, any other development?

Well, I think computing became much better, and so even a few years earlier it could have been very very difficult to build very large structures on computers. But then, luckily, the speed and power of computing, computer memory, and disc space. . . all of those things helped us. I remember we were always right on the edge of what could be done with computers. And many of these programs also had to be modified because many of them were not written thinking that there would be a ribosome scale problem. And so we had to change some of the programs.

You had computer experts?

No, no. We could just do it.

What were the most important questions when you started working on the 30S?

First of all, nobody knew what it looked like. What is the tertiary structure of the RNA, where are the proteins located, where are the A, P and E sites, the m-RNA binding site, the antibiotic binding sites . . . so all of those were questions. When something is completely new, there are lots of questions. No shortage. And one advantage we had is that biochemists and geneticists have been studying ribosomes for 40 years. So they had accumulated a wealth of data. Now, if you saw the crystal structure of some new protein, often it is very hard to say anything from the crystal structure because there is not enough known about it. You look at the structure and you don't even know what to ask. But with the ribosome, it is very different. It has

huge, rich literature. And so once you have the structure, you can certainly start to answer a whole series of outstanding questions – this is where P site can bind, this is where the A site binds, what is the environment of the sites, where are the antibiotic sites, how they work, where is the decoding site and how does that look, and so on.

Now that the 30S and 50S subunits are known, what are the questions that are there?

It is basically, you want to get an understanding of how the ribosome works – we want to understand the mechanism of how the ribosome works. And one particular problem is initiation. There are no good structures of initiation, which is the complex of the 30S and to some extent the 70S make at the start site of the message. How does it all get started? That requires these factors and specialized t-RNA and there's a lot of work. But it is all very confusing because there's no structure. So you don't know how to interpret it.

The ribosome structure is very useful in antibiotic designing. Are there any other areas where ribosome structure would be important, any other applications?

I can tell you there are applications and they are going to be amazing, okay. But this is only a prediction. Now that people know what the ribosome structure looks like, they're able to mutate regions of the ribosome to make it do interesting things. For instance, read four base codes instead of three, terminate in a different way, and so on. My colleague, Jason Chin, is already doing amazing work. He's designed an orthogonal ribosome system where those ribosomes will only work on his message, because he has switched the Shine-Dalgarno and anti-Shine-Dalgarno. And he selected for it. He's not done it by design, but done selection. So there's no cross-talk between his ribosomes and the host ribosome. And now he's engineering his ribosomes because he can do that without affecting the rest of the cell and he's making it encode artificial amino acids like acetyl lysine. But eventually the hope is that it will make completely different kinds of polymers. And so I think we're at the beginning of a new chemical and synthetic biology. But I can't predict where it will

go because I don't know enough about it. But I just sense that. That's a long term process.

You're currently working on eukaryotic protein synthesis. . .

I'm trying. We've not made any real progress.

So what are the major questions, apart from the structure?

Well, the problem with eukaryotic translation is that initiation in eukaryotes works in a completely different way. There are about a dozen factors in eukaryotes. And eukaryotic RNA is also processed in a specific way so as to add a 5' cap and a poly-A tail and there are factors that are thought to circularize the m-RNA packed by proteins. And eukaryotic initiation is highly subject to regulation – it is regulated by phosphorylation and so on. It is also a target of a lot of viruses. Viruses can shut down the host initiation machinery by their proteases or by phosphorylation, and at the same time, they have their own way of avoiding eukaryotic initiation. So eukaryotic initiation is a whole new area of biology. It's also interesting because it may have some clinical applications on viral proteins. But I don't think the rest of eukaryotic translation will be different in the detail, but initiation is very different.

Was there any moment when you wanted to shout 'Eureka'?

No. But I think when we first saw the electron density maps where we could see clearly right-handed RNA structure, double helices, we could see proteins. . . then we knew we had cracked the phase problem, and that we were on the right track. I think that was perhaps a really defining moment in the work. And then we knew that it was just a question of working hard and getting to that high resolution. So perhaps that was the defining moment.

The person

In your talk you put up a slide saying you studied in a girls' school at Baroda. How did you happen to study in a girls' school?

When my parents moved there that was the only English medium school in

Baroda. And since they didn't know Gujarati, they were not comfortable with the idea of sending me to a Gujarati school because then they wouldn't be able to help me. They wouldn't be able to understand even my teachers, for instance. So they wanted me to go to an English medium school. So they sent me there because at that time it was for both girls and boys. Then what happened is that when I was in the 2nd or 3rd grade, another school called Roseway High School, which was a Jesuit school, opened up an English medium section. So my school decided to make their school strictly for girls because it was run by nuns. But they let all the old boys stay in the school. As I went through school, there were fewer and fewer boys. In the end it became mostly girls.

Did your physics background help you in your work?

I think it helped in the sense I had a good feeling for how much signal you will get from a diffraction experiment. I was not afraid of computers, I was not afraid of calculations. I think in those sorts of things having a background in physics gives you the confidence that you can figure things out. But on the other hand, people who've gone from physics into biology without becoming biologists have not done a very good job. They go in with some sort of arrogance that because they are physicists, they're going to help these 'stupid' biologists solve their problems. And if they do that, they end up doing second rate work, and they're useless. The best people who have gone from physics to biology have actually had a certain amount of humility. They learn biology, even if they don't go to classes like I did. They're bothered to learn what the questions of biology are. They've learnt how to do genetics, how to do biochemistry and so on. And once they do that, the physics background can sometimes help. And sometimes it doesn't help at all. I mean, not in a great way. Max Delbrück was a great phage geneticist. But before he became a phage geneticist, he was quite a good quantum physicist. Now, there is no quantum mechanics in phage genetics. But he learnt the whole problem of genetics and things like that. So I think you have to learn to do biology before you can do anything.

Did your physics background hinder you in any way?

No, it didn't hinder me. But except I wish I knew more chemistry. I don't have a good feel for chemical reactions. I would be happier if I knew more chemistry because it would help me understand things like transition states, chemical reactions and so on. I mean, it's odd considering this Nobel is supposed to be for Chemistry. But you know, they don't give it to you because you are a good chemist. They gave it because the ribosome does some very powerful chemistry, and our work has elucidated that. It doesn't mean we ourselves are great chemists.

Whom would you call the single most influential person in your life?

I think it's very very hard to say. I've learnt a lot from many different people. You know, I had very good colleagues at Brookhaven. And Peter Moore, my mentor, obviously was the person who got me into ribosomes and taught me about ribosomes. So I think those were the people who influenced my current work the most. But as I pointed out in my talk, you don't start doing high level research out of nothing. Somebody has to teach you when you are a school student; someone has to teach you at the undergraduate level. So, I think, all these people have contributed to my work. I don't think I can single out any person except Peter Moore.

Is there any question that you would like to be asked, but nobody has asked you so far?

(Laughing) I don't know. People have asked me so many different types of questions. I have no idea. I don't mind being asked serious questions. I can tell you the questions that I don't like – 'Would you consider working in India?' Now, most people know my children live in the US, that I'm actually far enough away from them. If I came to India, it will be even further away. My father, sister, my wife's family – all live in the US. They know that my wife is not Indian and has never been to India. So why are they asking me that question? I would prefer if people ask me, you know, what kind of decisions I have to make as a scientist, and things like that.

Some opinions on education and research

Any ideas on how science can be popularized in a country like India?

I don't know. . . I think, you know, first to have good teachers, obviously. Teaching cannot be the last resort profession, where we fail at everything and become a teacher. If that's the case then you won't have good teachers. My teacher was actually very good, and I was very lucky. But it's not always the case, right? Actually there's a terrible problem in India, which is, you have these elite institutions that everybody wants to get into and so the schools are teaching to that. But that's not enough. Now people take entrance exams to get into a coaching school which prepares you for the next entrance exam. Next you're going to have an entrance exam that prepares you for the entrance exam to get into the coaching school. It's kind of ridiculous. That mentality is counterproductive. I think, if that were stopped, that alone would actually help focus people's attention on where it finds interest. Instead, they are interested in exam problem solving. That's a real waste of time. I mean, these poor kids – they work all day in school, they come home, maybe they have a quick snack, and then they're off until 9 pm or something to this coaching school, you know. So when do they have time to think about science?

I said in an interview that I thought that people should have much less homework, that excess homework kills the imagination. Amartya Sen went a step further. He said we must have no homework. All the work should be done in the school. So when they go home, they're free to think, to read, to have hobbies, etc. And he said that coaching schools should be abolished. Because, he said, coaching schools also exaggerate the difference between rich children and poor children because they can't go to coaching school. I never went to a coaching school, and I'm perfectly happy.

Do you think entrance exams need to be done away with?

Well, it's very hard. Look, if you have a very large country, you have a limited number of seats, and there's a lot of variation between schools and states, then there's no alternative to an entrance

exam. I don't think you can ever avoid it. But coaching classes... I think it's very hard to legislate against coaching classes, but maybe, if people develop a culture where they sneer at people who go to coaching classes, where parents and educators realize... And maybe the questions could be changed so that going to a coaching class won't really help you. I think the US tries hard in its entrance exams, so that the coaching classes don't have a huge effect. They do have an effect even in the US, but not as dramatically. Maybe the exams could be re-designed. I don't know how to do that. But maybe they can be re-designed so the coaching class actually has no use.

What do you think about interdisciplinary research?

I don't think you can ever force interdisciplinary research. I think what you have to do is not have barriers towards interdisciplinary research. Ultimately, interdisciplinary research comes when two people who have complementary skills but a common interest come together. For example, a geneticist and a biophysicist may come together. Or it is happening in bioinformatics. You know, people who understand metabolism, or microbiologists who understand microbes, are coming together with mathematicians, because all these genomes have been sequenced and there is all this information about microbial colonies and so on. So they come together and they can collaborate. But I don't think you can ever force collaboration between people. I think if you try to artificially force it, it's not going to happen. But you can foster it. You can make sure that there are no barriers.

Do you see any difference in the way research is done in developing countries and developed countries?

I think, well, if you had asked this question 20 years ago, I would've said there's a big difference because the amount of resources people had were very very different. They were even at different scales. I know that in my father's department, there was only one spectrophotometer in the entire department, and

everybody had to use that. That was a big deal to have a UV spectrophotometer. Whereas in a Western lab, every lab, or maybe surrounding labs, had spectrophotometers. It was a big deal to have a pH meter. But that's all changed. So I think part of it is psychological. One thing that taught me at the LMB – when I went to LMB, I found that it was not that different in terms of its equipment. In fact, it was very crowded; it almost looked like a rundown place. There are all these centrifuges in the hallways, freezers in the hallways, and so on. It didn't look like a posh place at all. Of course, it had almost every equipment you would need, but it was shared. It didn't have hundreds of different kinds of equipment. It's not that every group owned its own equipment. Expensive equipment is shared throughout the lab. So why did the LMB do so well? Why does it continue to do well? I think it's a psychological problem. You have to say I'm not going to do boring derivative problems where I'm doing a second or third example of something that's already been done, and I'm not going to learn that much new from it. I see a lot of that going on in India where something is done in one system and they'll do it in another system. And I don't think that's going to lead to really important breakthroughs. Actually, if people wanted to, they could do particularly Indian problems. They could study specifically Indian plant diseases or even Indian biology. They could look at ecosystems and molecular biology related to it. Or they could compete on worldwide problems where all the molecular biologists are interested in it. They could go either way. And I think the worst thing is to do something where someone has established something in one, say *E. coli*, and somebody does it in some other bacteria. In general, it's not going to be helpful.

As you said in your lecture, the first few proteins of the ribosome were published in Nature, the next in lower impact journals...

Exactly. Exactly. So that's an example. You know, I could have made a career just going on doing that. And as long as I kept publishing papers, I would've got

ten grants. And that's the kind of mentality that we see more of here. But in good labs in the West, they would see immediately – okay, this is not getting so interesting. We need to move on. Even with the 30S – I could keep on doing a bunch of antibiotics. There are dozens of antibiotics, right? We just did six and we stopped. But if I wanted to publish a paper in say *JMB* or *Acta Crystallogr.*, I could just do one antibiotic, one paper. And I could just make a career out of it. That would be the kind of thing I see more in India. But that's a psychological problem. It's not a problem of resources or infrastructure. I think if people had good ideas, at least my colleagues tell me, there's plenty of funding.

Do you see any difference in the way science is regarded in developed and developing countries? Maybe by the general public...

I have to say I cannot count what my visit this year has been like. India has somehow gone slightly overboard about this Nobel Prize. And so, that's not a fair comparison. I think India has a genuine respect for scholarship. I don't know how to compare it. I think in the US there's a respect for science, but there's also a lot of respect for being successful with money. I think that's universal. If you make a lot of money, people look up to you. They think, you know, you're a great guy because you've made billions of dollars. I think with science the bar is higher. You know, any millionaire is considered great. But if you're just a good scientist, most people don't think much of it. You have to do something really, really visible, like a Nobel Prize, whereas, if you're just a very good scientist, people don't pay much attention. On the other hand, I think they do respect that that you're a scholar and doing something interesting. I think human nature is the same everywhere. In that, I don't think there's a difference.

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