Basic research must come first

An interview with Zuoyan Zhu, Vice President of the National Natural Science Foundation of China and member of the Chinese Academy of Sciences

EMBO reports (ER): Professor Zhu, you started working on transgenic fish as early as the 1970s, and you succeeded in creating transgenic carp that grow faster or are more cold resistant. When you started working on gene transfer in fish, was it basic research or did you originally have agricultural applications in mind?

Zuoyan Zhu (ZZ): When I studied biology in the 1950s and 1960s, our teachers taught us that we must not only learn about phenomena in nature, but also find ways to improve or alter existing things, including living things, for the benefit of human beings. I have been working in the Institute of Hydrobiology, Chinese Academy of Sciences, in Wuhan, Hubei province, where we breed fish. Traditional breeding uses wild and domesticated species for hybridization to increase their usefulness as farm species. In China, we have done this for freshwater fish for more than 2,000 years. But we have only used four species in those 2,000 years, so we have to find new ways to improve the quality of fish for farmers. Hybridization is good, but it's not enough. Since modern science has developed so quickly, we now have techniques and knowledge to find these new ways, such as gene cloning and gene transfer by microinjection. If we know about the function of a gene, the question then is how we can use it to improve the quality of fish. My supervisor at the Institute of Zoology, Chinese Academy of Sciences, Professor T.C. Tung, a famous embryologist in China,

initiated a technique of nuclear transfer in fish in the early 1960s. Of course, at the same time in the UK, Dr John Gurdon did similar nuclear transfer experiments on *Xenopus*, which are well known in the world. However, Professor Tung wanted to use this technique to improve the quality of fish for agriculture. He called them 'nuclear and cytoplasm hybrid' fish. Well, back then in the 1970s, we had just had the Cultural Revolution, and the available scientific literature and techniques were very limited in China. So we were not able to clone a gene at that time. Instead, we transferred total DNA, using DNA from a cold-resistant species, into the fertilized eggs of a warm-water species, *Cirrhinus molitorella*. To my great surprise, it seemed to work. About 6% of the fish survived in 3 °C lower than that of the control. I say "seemed to work" because we didn't get many details from the experiment. After that, I went abroad to learn how to clone individual genes.

ER: Where was that?

ZZ: That was in the UK in the early 1980s. In the spring of 1982, I also had a chance to come to Berlin in Germany to attend an EMBL course for microinjection of DNA into cultured cells. That was very interesting. So when I went back to China, I wanted to use these methods to do gene transfer to improve the quality of fish species.

ER: What importance does the Chinese government put on GM crops and animals for their food supply and agriculture?

ZZ: I don't think that most people in the world really worried about gene transfer or genetically modified organisms in the 1980s, but gradually people are getting concerned about this issue. But I also would like to make

a distinction between hybridization and gene transfer. If we hybridize two closely linked species, we combine one set of chromosomes from one species and the other from another species to create a new hybrid organism. In a way, that's a kind of gene transfer. But it is the transfer of whole chromosome sets from one species to another, so if the species has 30,000 genes or something in that range, that means that dozens of genes are transferred, but common people do not seem to care about this kind of gene transfer.

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But if we transfer only specific genes, suddenly people worry about the consequences. Of course, we can do very funny things with gene transfer, like transferring genes from very distant species, even from bacteria into animals. And that is where we have to worry about the potential consequences. But in our study in China, we just used what we call the "all-fish" gene construct that consists of all fish-gene fragments only. The transferred gene is from fish—of course another fish species—and the gene or the promoter may be modified, but the components of the construct come from fish itself. So in this case, I would say it's more like hybridization with a transferred gene from the same species. I don't think the consequences are so dramatic or so dangerous. Of course, we have to be concerned about ecological consequences.

ER: That was the main concern about similar experiments on salmon in the USA—that they might outgrow and outcompete wild relatives.

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Marietta Schupp, EMBL Photolab

ZZ: That's right. So even with the transgenic species that we use, we have to be concerned with the ecological consequences. In China, fish agriculture is only in inland water in a special region, but there have been accidental releases of fish. In my laboratory, we are therefore working on sterile transgenic fish, but a reversibly sterile fish. If we want the fish to reproduce, we can switch on a promoter or some key elements that we transferred, but in general they are shut down so the fish cannot reproduce and their gonads cannot develop properly.

ER: Is there public concern in China similar to that in Europe about the potential environmental problems of GM organisms?

ZZ: Yes. I see more and more Chinese people getting worried about this issue, especially government officials. Of course they worry about the environment, but they also worry about economic and international trade consequences if the Chinese release GM organisms into the wild that could cause problems. So the government and the Ministry of Agriculture have allowed only a few genetically modified organisms, and so

far they haven't allowed any transgenic animal to be put into agricultural use.

ER: How restrictive are these regulations?

ZZ: They are quite restrictive. I think they are awaiting acceptance from other countries in the world. If, say, the United States or European countries said "This type of GMO is OK", I think the Chinese authorities would consider them to be OK too. They will not initiate this—they won't say "We have to do this", or "We can't do that". No, they are very careful at the moment.

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ER: Have you already applied for approval for your transgenic carp?

ZZ: I haven't done that because I don't think I would get a positive result. If transgenic fish have not been approved in the United States for very long, I don't think the

Chinese government will approve our transgenic carp.

ER: But in the United States you can now buy GloFish, a transgenic fish that glows in the dark. They are not subject to Food and Drug Administration regulation because they're not food or drugs. There's concern now that people that have these as pets could release them.

ZZ: These transgenic fish with a green or red fluorescent protein gene are not sterile. They could reproduce in a hybrid way with a wild type, so pet fish released in the wild could have consequences. Actually, it's not very difficult to create sterile transgenics, which could be switched on if you need them to reproduce. We are developing this technique, it's quite promising.

ER: Do the Chinese restrictions differentiate between transgenic animals as pets or as livestock?

ZZ: I think they're very careful. Even more careful than what I know of other countries. They don't want to get into trouble as the first country to release transgenic animals.

ER: China is increasingly and quite successfully investing in biotechnology as a new basis for its economy. Do you expect to catch up with the USA, Japan and Europe at some point, and become an equal player in the field of biotech research?

ZZ: If we just simply look at the speed of development, well, that has already progressed very quickly. But if we want to make Chinese biotechnology as developed as in the leading countries, I think this is very difficult at the moment. Even in the near future, it will be very difficult to do that because the basic research in the life sciences is still far behind that in the United States and the Western European countries. Any of the new techniques in biotechnology must be based on basic research and discovery. If China doesn't have its own specific discovery or innovation process, it can hardly create biotechnology-specific progress. At the moment, we just hope that we can catch up. So for the time being, we scientists should convince government officials that we have to place emphasis on basic research. Without basic research, without new discoveries, we cannot make any kind of advance. So China is now making a long-term plan for science and technology development for the next 15 years. During this period, many scientists in China must point out that we not only need to concentrate on technology itself but that we must also pay attention to issues concerning the development of basic research in China.

ER: China recently completed the rice genome. Are there any plans under way to sequence other genomes?

ZZ: Pig and, I think, chicken, in cooperation with other European countries and the United States. We also have the silk worm almost finished. I think the sequence projects run very well in China, but that is only sequencing, it doesn't mean anything for basic research.

ER: Many Chinese students still leave the country to go to universities and companies in the United States. How do you think you can attract those students back to the mainland?

ZZ: We already see more and more young Chinese scholars coming back from overseas. The main reason is that the situation both for living and for research or for technology development is improving in China. Many of these young scholars abroad cannot work or research as they want. They follow the instructions of their boss in the company, or they work on specific research projects, but they want to develop their own career and work on what they are interested in. For most of them, the best way is to come back to China because after a few years they could be very successful. If somebody asked them to go abroad again, I don't think they would

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because they are very well established and many of our facilities are even better than they used to have abroad.

ER: How is the National Natural Science Foundation involved in this?

ZZ: We have a specific programme, called the National Science Fund for Distinguished Young Scholars. Outstanding scientists under the age of 45 can apply and are selected by peer review. So far, we have supported more than 1,000 young investigators. Most of them develop very well and 10–20 of these have even become members of the Chinese Academy of Sciences. But the award is important not only for the funding itself but also for reputation. If they get the award, the

universities or research institutions pay specific attention to them. This programme has shown very good results. We also have funds for what we call Innovation Research Groups that are not given to a person but to a team for three years and then another three years, and even longer. We have funded so far more than 70 of these research groups in China.

ER: But this is support for research groups and primary researchers. What about attracting people who are at an earlier stage in their career, such as postdocs and graduate students?

ZZ: We have another programme called the Project for Young Scientists Fund. Scientists younger than 35 can apply for project-specific funding after their PhD.

ER: Do the recent restrictions in the USA against foreign visitors work to your advantage in keeping Chinese scientists at home?

ZZ: Many young scientists and young students applying to PhD or postdoc programmes in the United States now find it very difficult to get visas. I don't know if that's good or bad, but at least more and more excellent students stay in China. There's also a good chance for European countries to attract those students, as many people turn to the UK and Germany and other countries rather than the United States.

ER: Recently, South Korean scientists successfully created a human stem-cell line from cloned human cells. *The New York Times* and others expressed concerns that the United States could lose out in the field of therapeutic cloning and stem-cell research against Asian countries. Do you think that this research could be a potential strength of Asian societies?

ZZ: I don't know the details of this news on human cloning, and how real this situation is. Even if it is true, I still don't think the Asian societies are in a leading place for this research. The reason is, just as I said, that basic research is not very good. There is still a lot of research required to do cloning. I'm not even speaking about human cloning but also animal cloning. We still have a lot of

problems to solve. For example, if we clone fish, the success ratio is only around 5%. Of 100 nuclear transfers into individuals, about five succeed, get larger and become adult fish. Why the other 95% do not work, we don't know. So if we choose a blastula cell nucleus as a donor, maybe only 5% of the cells have the potential for further development or reprogramming. At the moment, people are still very naive when it comes to cell differentiation or even cell commitment, and that means we have to study carefully the basic aspects of development or embryology rather than taking the risk and trying to clone human beings.

ER: But there are also different political and societal realities. The US government has blocked further production of stem cells and therapeutic cloning, and in Europe only the United Kingdom has so far set guidelines and regulations to allow it. So what is that status of the whole political, ethical and moral debate in China?

ZZ: Personally I haven't studied this topic in China very carefully, but I have a feeling that if the well-developed countries say "No, we shouldn't do that", I don't think the Chinese authorities would say "We must do it, we could do it", just as for transgenic animals. If the United States or most of the European countries come to the conclusion that there's a big political or social problem, we have to take care of that too. I heard that the Chinese government was about to make official regulations and that they were absolutely against human cloning. I think they allow cloning for therapeutic studies but would completely stop reproductive cloning.

"We shouldn't think we have found a way to amplify stem cells by creating chimaeras."

ER: What is the public's perception of cloning in your country?

ZZ: I don't think the common people are interested in this issue like people here [Europe] or in the United States. We have a different background. First, if you go to the countryside and you ask the peasants "Do you know about cloning?", they probably don't have any idea. There are 1.3 billion people in China and if you take this number as a base, only a very small percentage of

people are concerned with this issue. Even in the cities, people who are not studying biology, even important figures in physics or other fields, when they hear 'transgenic', they think "Whoa, transgenic, that's terrible!" They get such an impression from the media, so the public perception of cloning is the same situation as in other countries.

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ER: How do they get this impression from the Chinese media? Or is this the influence of international media?

ZZ: The newspapers, the TV, they all reproduce the information in such fields of cloning and transgenic organisms from the Western public media. They get the news from them and then they transfer it to the Chinese. So the Chinese people know about this issue and its importance but I don't think they really know what GM or cloning means.

ER: Would the government ban you mentioned earlier also apply to the creation of chimaeras? *Nature* last year made comments on a paper from a Chinese scientist who transferred a human nucleus into a rabbit oocyte.

ZZ: It's a very controversial topic, even among Chinese scientists. Of course, some people are working on chimaeras, because the purpose of this technology is to produce more human stem cells. But they still have problems. If they create a chimaera by transferring a human cell nucleus into a rabbit oocyte, at the blastula stage they get stem cells—but are they real human stem cells or not? The components of the rabbit egg in the cytoplasm would contribute something to the cells. So we're still not very sure whether those influences will disappear or still exist from one generation to the next. Nobody knows, and we have to study very carefully, for example, what happens to the mitochondria or other organelles or other components of the cytoplasm. We shouldn't think we have found a way to amplify stem cells by creating chimaeras.

ER: So there should be no medical applications of such cells?

ZZ: Sure, no medical applications, of course. I heard a Chinese investigator a few years ago saying that he injected cultured human cells into a goat embryo. I cannot remember exactly at what stage, it was not in the fertilization stage but later in the embryonic stage. And they found some cells in the goat liver that came from these cultured human cells. He believed this would be great progress. But I thought this kind of research should stop and won't be supported by our foundation. Why would people like a goat to have a liver with human cells? This was not an acceptable way to use the chimaera liver for transplantation. In addition, it is very dangerous if somehow the human cells get into the gonad and switch to germ cells. This kind of research should stop in China.

ER: Do you still have time to do research?

ZZ: Yes, I still do cloning studies of fish, mainly so-called 'cross-species' cloning. Fish is a very different animal; the nucleus and the cytoplasm from quite distant species can be joined for development. This is not the case for other higher vertebrates or even for frogs. We are interested in these mechanisms, and why cross-species cloning works in fish. As I said, we also work on sterile or reproduction-controlled transgenic fish and do a lot of work on ecological assessment and evaluation. We have special isolated fishponds of more than six hectares, specifically to study long-term ecological consequences.

ER: So do you raise the transgenic fish together with wild species to see how they interact?

ZZ: We do modelling to see how the transgenics would dominate or not over the wild type—we will have to follow that for maybe at least ten years. I am really getting a bit tired of public opinion—they want us to show everything that could happen but won't wait for scientific evidence. Whatever the situation, you have to use the scientific way to prove it.

ER: Professor Zhu, many thanks for the interview.

The interview was conducted by Holger Breithaupt and Caroline Hadley.

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