Interview Transcript – Dr Ferenc Mueller

Hello, I'm Dr Sharon Ann Holgate and I'm here at the centre for rare diseases and personalised medicine at the University of Birmingham to talk to Dr Ferenc Mueller who's been doing research on BHD syndrome, which has been funded by the Myrovlytis Trust.

So you're using zebrafish in your work at the moment, what are the advantages of using those as an animal model?

So we use zebrafish for two reasons. One is because zebrafish, like us, are vertebrates and have all the main organ systems that we have. Of course there are obvious differences – they don't have limbs, they don't have lungs etc. But on the molecular and genetic level, in particular, the mechanism of how the organism develops and its physiology are very well conserved. Therefore when we study the genes in a fish embryo, for example, and we obtain conclusions on gene function- it's highly likely to be relevant also for humans. On the other hand, to study fish and fish embryos is far easier than looking at, for example, mouse embryos or other animal systems which require intervention to obtain those embryos in the first place. Because fish lay their eggs in the water and we can study them in a petri dish- the full development of an embryo from a single cell to a fully functional young larva can be studied without any interference or invasive techniques. Most importantly the embryos are transparent, for at least 2 days of their development, which is long enough in a fish embryo's life to develop all the major organ systems. It has full blood circulation for example, and a beating heart, even moves, and this can be visualised fully by microscopy. This also allows us to see on a cellular level, we can see the individual cells in these transparent embryos without any intervention.

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So what we're looking at here is a zebrafish embryo at one day of development. This was fertilised just over 24 hours ago. What you see is the fish embryo already has some of the main organ systems developing, at least the rudiments of organ systems. You can see the retina and the lens evolving. You can also see that this embryo is already able to move, which means its muscle has developed and its primitive nervous system has developed to such a degree that it can twitch its muscles and even respond to touch.

The other advantage of the fish system of course is that it produces a lot of offspring, a lot of embryos, so it is easy access for genetic studies. Thirdly, the generation time is only 3 months and this allows us to carry out genetic analysis over generations. It is quite easy to produce genetic mutants which model disease and mimic disease in humans and therefore, again this system provides easy access and a cost efficient way of studying the genetic basis of disease.

So what research are you currently doing?

My lab is split between two research areas. On one hand we carry on with work that I brought over from Germany, where I was working for 6 years as a junior group leader, which is to study gene regulation, in particularly transcription regulation in the embryo. Our focus is on so-called developmental genes. These are the genes that define the embryo pattern and which define the morphology of the animal and have key roles in the early few days of embryo development. We ask how these genes are switched on and off in a specific time and space in the embryo to contribute to the development of different organs and tissues. We look for the genetic elements, the gene elements, the specific DNA pieces in the genome which are regulating the activation of genes. These are called promoters, enhancers and other additional noncoding regulatory elements and we study their function through the embryo model, where we address gene regulatory elements by looking at reporter gene activity. So what we're doing is that we link the putative regulatory element to a reporter construct, a reporter gene, for example the green fluorescence protein gene, and visualise the activity of the reporter by looking at the live embryos by fluorescence microscopy. This work involves genetic analysis of transcription factors, which are the proteins that regulate the gene activation and bind to the DNA elements which are the regulatory switches.

On the other hand, several people in the lab work on developing models for human disease by using the zebrafish system. This work has been mostly motivated by my environment, which is a set of scientists who are mainly clinicians and paediatricians and human geneticists who identified a number of genes which cause rare syndromes in human. There is very little knowledge about the function of these genes. Using the fish model we can address the biochemical and biological functions in the whole organism by manipulating the gene function in the zebrafish, particularly in the fish embryos. We can detect specific defects in organ development which give us hints about the functions of these genes.

So has this work aided the understanding of the role of folliculin within BHD syndrome?

This is very early days. We have been working on the BHD gene folliculin for over a year now and this is the first time I believe someone is trying to establish a zebrafish model for this disease. What we have managed to do so far is to show that this gene, the folliculin homolog of the human, is indeed expressed in the zebrafish. It is a gene which is functional in the zebrafish and which is required for the normal development of the zebrafish. We have carried out so-called gene knockdown experiments by which we block the gene activity, which means the production of the protein, which is encoded by this gene, is blocked in the embryo. As a result, specific defects arise in the embryos and these defects indicate to us that folliculin is required for multiple functions in embryo development.

So what's the ultimate aim of your research?

Well, the aim is to develop a disease model. That is, to use these knockdown or mutant embryos to screen for potential pharmaceutical drugs which may revert phenotypes and may eventually help in identifying drugs which may be used in the future for therapies. Here we would like to utilise another advantage of the fish system which I haven't elaborated on so far- that we can quickly go through a very large number of embryos by automated screening technologies and automated imaging technologies and look for phenotypes. So this allows us to screen for drugs in a large scale and when observing the embryo, we can screen for multiple phenotypes and changes in phenotypes in the whole organism context.

So where have you presented your research so far and have you any indication of how well it's been received?

This is early days, we're in an early phase of this research and as I said we've only spent about a year and a half altogether studying BHD. But we have presented our results in London at a symposium on BHD in front of a dozen or so laboratories and indeed there was interest. There was great interest in using the zebrafish model system and we were very happy about that and would like to collaborate with these groups. Indeed, several collaborations have been initiated on the basis of that meeting.

So what's your feeling about how BHD research is going to look as a field in 5 to 10 years' time?

I'm pretty sure we'll know a lot more about the biological basis of BHD and we'll be a lot closer to providing drugs which may help in treating or controlling the disease. I would imagine that other research fields will open up and BHD will be studied not just by animal models or tissue culture systems that have been applied so far. Of course there is structural work going on. I think there'll be a lot more going on about imaging, looking at the interactions between molecules *in vivo* as microscopy techniques evolve. We'll have a much better understanding of interactions between proteins, and folliculin-interacting proteins, how they localise in the cell, how they behave in the cell and how they interact in the cell. I'm expecting a lot of development in that area. Also I'm sure we'll have a better understanding of what drug treatments might be useful for BHD specifically.

Well thank you ever so much for talking to me today Ferenc and best of luck for your research for the future.