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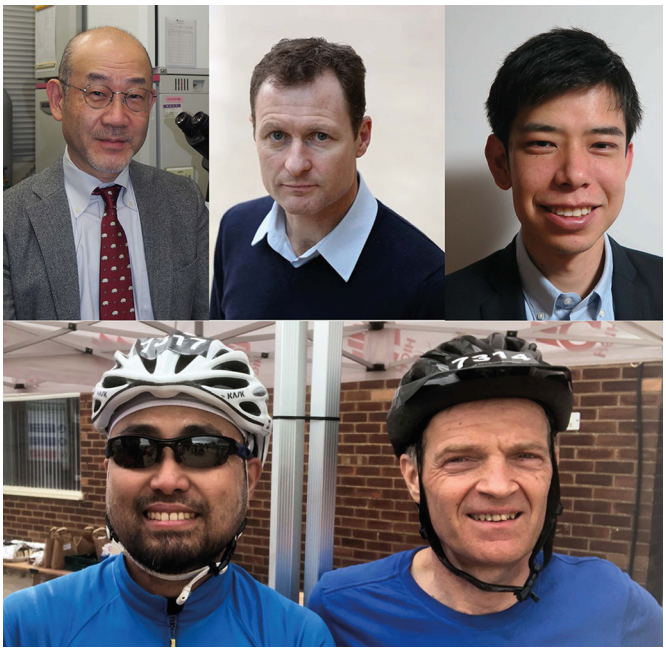
INTERVIEW

The people behind the papers – Masaki Kinoshita, Toshihiro Kobayashi, Hiroshi Nagashima, Ramiro Alberio and Austin Smith

The ability to derive and maintain pluripotent stem cells (PSCs) from livestock species in defined media conditions will contribute to many new research avenues, including comparative embryology and xenotransplantation. In a new paper in Development, Masaki Kinoshita, Toshihiro Kobayashi, Hiroshi Nagashima, Ramiro Alberio, Austin Smith and colleagues describe their three-component medium, which supports long-term propagation of PSCs in the absence of feeders or serum factors. We caught up with the authors to find out more about their research and their future plans.



Clockwise from top left: Hiroshi Nagashima, Ramiro Alberio, Toshihiro Kobayashi, Austin Smith and Masaki Kinoshita

Hiroshi, Ramiro and Austin, can you give us your scientific biographies and the questions your labs are trying to answer?

HN: I started my career, after earning a PhD from the University of Tokyo (1984), working on embryology of large domestic animals, mainly pigs and cattle, at the Research Center of Nisshin Flour Milling, Japan. In 1991, I moved to Australia to work as a visiting research fellow at the Department of Obstetrics and Gynaecology, Medical School, University of Adelaide, and then took a position as a senior research scientist at BresaGen, a South Australia-based biotechnology enterprise. I returned to Japan in 1997 to work on the generation of genetically engineered pigs for xenotransplantation at the Biomedical Research Center, Osaka University Medical School. I then took a position as associate professor at Meiji University in 1999. My recent research focus includes *in vivo* organ regeneration using genetically engineered pigs, creation of disease models using genome editing and somatic cell cloning technology, production of genetically modified pigs as potential organ donor for xenotransplantation, and cryopreservation of embryos and artificial tissues. I'm currently trying to reveal the developmental mechanism in porcine blastocysts that limits interspecies chimerism.

RA: I gained my BSc in veterinary medicine from La Plata University in Argentina (1996). After a short spell as a clinical practitioner, I became interested in reproductive biotechnologies and decided to do a PhD with Professor Eckhard Wolf at the University of Munich (1996-2001). During my PhD, I worked in somatic cell nuclear transfer in livestock and developed new approaches to oocyte activation. After graduating, I moved to the University of Nottingham to work with cloning pioneer Professor Keith Campbell (2001-2003), where my focus was to gain a better understanding of the process of nuclear reprogramming using amphibian oocytes. During this time, I met Professor Andrew Johnson, with whom I cultivated an appreciation for the significance of phylogenetics in developmental biology, and this led me to use axolotls oocytes as a model. In 2002 I was awarded a Marie Curie Fellowship (2002-2004) followed by a RCUK Fellowship (2005-2010) to start my independent lab. Since then, I have focussed on gaining an understanding of the development mechanisms in bilaminar disc embryos, such as humans and pigs. This work led to the identification of SOX17 as a key regulator of primordial germ cell development in the pig, similar to humans. We are now extending these investigations to other lineages using these novel cell lines by creating 'gastruloid' models of development and validating our findings in *ex vivo* pig embryos. This resource will be valuable for comparative developmental biology in the coming years.

AS: I graduated from the University of Oxford in 1982 and pursued PhD research in Edinburgh. After a post-doctoral period back in Oxford, I returned to Edinburgh in 1990 as a Group Leader.

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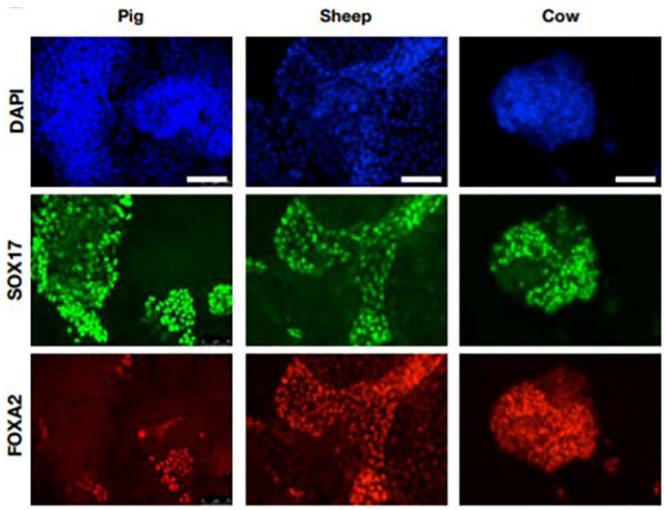

AFX cells, from pig, sheep and cow, differentiated into definitive endoderm and stained for SOX17 (green) and FOXA2 (red). DAPI is in blue. Scale bars: 100 µm.

From 1995, I brought together embryonic and adult stem cell biologists to form the Institute for Stem Cell Research. In 2006, I relocated to Cambridge and founded the Cambridge Stem Cell Institute, where I was Director until 2016. In 2019, I took up the position of Director of the Living Systems Institute at the University of Exeter. My research interest is stem cell biology and, in particular, pluripotent stem cells that harbour the capacity to generate all cell types of the mammalian organism. I hope to derive universal principles underlying the establishment and progression of pluripotency in diverse mammalian embryos. In my research group, we seek to expose and control network properties that enable the long-term self-renewal *in vitro* of transient *in vivo* cell states, and to recapitulate, in culture, the developmental trajectory from an emergent naïve population into lineage-specified progenitors. We use approaches ranging from computational modelling to *in vivo* chimaera studies.

Masaki and Toshihiro, how did you come to work on this project and what drives your research today?

MK: My research interest is pluripotency in the early embryo and capturing it *in vitro*. I was thinking of expanding my research from rodents to other species to help understand human development. I met Ramiro at the BSDB spring meeting in 2018 and we discussed establishing pluripotent stem cells (PSCs) from pigs. Later that same summer, Ramiro came down to Cambridge with pig embryos and we succeeded with our first attempt! Toshi and I became friends when he worked with Professor Azim Surani in Cambridge. He is a very open-minded person and we often talked to each other about our science, so we decided to collaborate when we found we were doing similar things.

TK: One of my interests is how pluripotent cells determine germline or soma fate in a variety of mammals. As a model, I have been working on rabbit embryos, which develop as a bilaminar disc, like most non-rodent mammals, including human and livestock. I derived rabbit PSCs from disc-epiblast to investigate the mechanisms of germline specification, which was recently published (Kobayashi et al., 2021). Then, I applied the same culture system to pig and successfully derived pig disc-epiblast-derived stem cells with the support of Hiroshi.

This collaboration started after a long chat at the bar between Masaki, Ramiro and Austin during the BSDB 2018 conference in Warwick

Can you explain how you came together to collaborate on this research?

This collaboration started after a long chat at the bar between Masaki, Ramiro and Austin during the BSDB 2018 conference in Warwick. Later, Austin and Masaki met with Toshi, and learnt that he was also deriving pig PSCs. We agreed to collaborate immediately and Toshi switched to the feeder-free system developed by Masaki.

Can you give us the key results of the paper in a paragraph?

We show that embryo-derived stem cells can be isolated from the three major livestock domestic species, using the same minimal set of factors under serum- and feeder-free conditions. These stem cells have an identity related to the pre-gastrulation epiblast of the bilaminar disc. They can be propagated long term, are amenable to genetic modification, can differentiate efficiently and can be used as donors for nuclear transfer.

How do your results differ from previous attempts to derive and culture livestock pluripotent stem cells?

Over the years, there have been many efforts to derive livestock stem cells, including by one of us (R.A.). The advance here is that the three component medium we use (activin, FGF and the tankyrase inhibitor XAV939 – collectively AFX) supports stable long-term propagation, is equally effective in all three species and does not require use of either feeder layers or serum factors.

When doing the research, did you have any particular result or eureka moment that has stuck with you?

MK: Yes, I had a few special moments observing cells down the fluorescent microscope.

And what about the flipside: any moments of frustration or despair?

MK: Actually, the same as above! Sometimes my observations under the fluorescent microscope disappointed me a lot.

Masaki, what's next for you after this paper?

I am preparing to start my own group and research programme. I will keep working on mouse, human and livestock PSCs. I am interested in changing the production of chimaeric livestock with PSCs.

What is the next step for studying/using livestock PSCs, in your labs and for the community as a whole?

HN: Pig PSCs and syngeneic cloned pigs will surely be useful as a model for cell transplantation therapy.

RA: It will be interesting to continue to characterise the different features of epiblast cells during the continuum of development, particularly in species that have a long time-window between segregation of the inner cell mass and onset of gastrulation. It will also be important to develop robust differentiation protocols for generating specific cell types from these species that can be used for the creation of novel food products, such as meat.

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AS: My obsession is naïve pluripotency, so we will continue to chase and try to capture stem cells in the naïve state from livestock and other mammals.

TK: I plan to use the PSCs as a tool to study early embryo development, including the germline. Making genetically modified foetuses/animals from PSCs, as we show in the article, will be a very useful approach for this purpose.

Finally, let's move outside the lab – what do you like to do in your spare time?

MK: I like long bike rides. Before the pandemic, I often cycled with Austin outside Cambridge. I have more solo rides these days, and while sometimes I think about experiments, there is also plenty of time to simply focus on pedalling, which is very refreshing!

TK: I enjoy making crafts with my 6-year-old son. One of the desk drawers in my office is full of crafts he made and gave me.

HN: My pet frog (*Hyla japonica*) named Lieutenant Shirase (1999-), who survived hibernation over two winters in my small garden, is about to go for the third winter. I wish to see her (maybe) baby frogs.

RA: I spend a lot of time with my son doing sports, as a football coach and as team captain at a tennis club. When we are not training, we love going to gastro pubs to enjoy local cuisine after long walks in the Derbyshire dales.

AS: Cycling, cooking, gardening, being by (or in) the sea, and supporting Everton FC.

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