



THE BRAINMAKER

BY DAVID CYRANOSKI

With his knack for knowing what stem cells want, Yoshiki Sasai has grown an eye and parts of a brain in a dish.

In December 2010, Robin Ali became suddenly excited by the usually mundane task of reviewing a scientific paper. “I was running around my room, waving the manuscript,” he recalls. The paper described how a clump of embryonic stem cells had grown into a rounded goblet of retinal tissue. The structure, called an optic cup, forms the back of the eye in a growing embryo. But this one was in a dish, and videos accompanying the paper showed the structure slowly sprouting and blossoming. For Ali, an ophthalmologist at University College London who has devoted two decades to repairing vision, the implications were immediate. “It was clear to me it was a landmark paper,” he says. “He has transformed the field.”

‘He’ is Yoshiki Sasai, a stem-cell biologist at the RIKEN Center for Developmental Biology in Kobe, Japan. Sasai has impressed many researchers with his green-fingered talent for

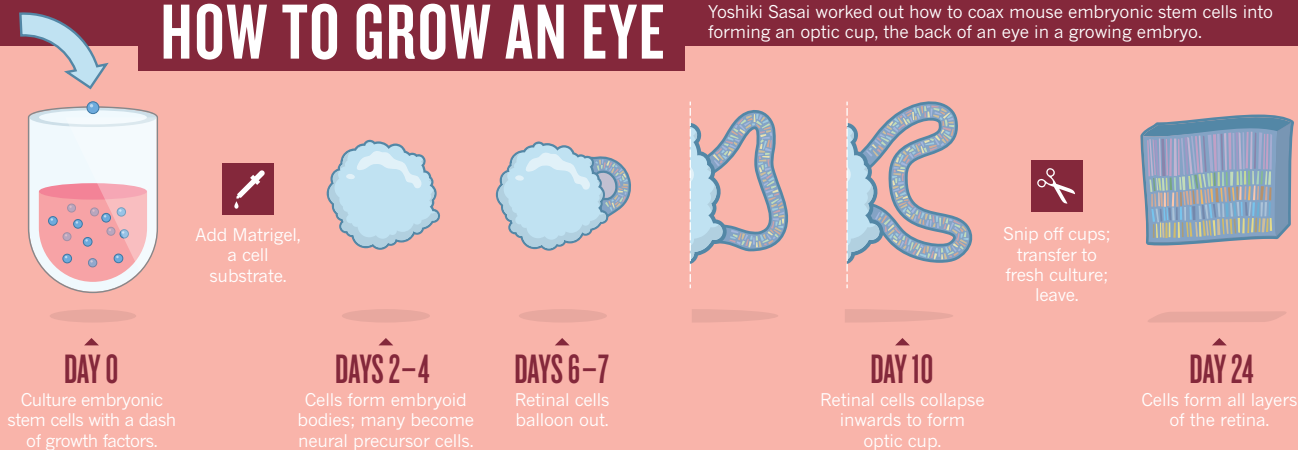
coaxing neural stem cells to grow into elaborate structures. As well as the optic cup¹, he has cultivated the delicate tissue layers of the cerebral cortex² and a rudimentary, hormone-making pituitary gland³. He is now well on the way to growing a cerebellum⁴ — the brain structure that coordinates movement and balance. “These papers make for the most addictive series of stem-cell papers in recent years,” says Luc Leyns, a stem-cell scientist at the Free University of Brussels.

Sasai’s work is more than tissue engineering: it tackles questions that have puzzled developmental biologists for decades. How do the proliferating stem cells of an embryo organize themselves seamlessly into the complex structures of the body and brain? And is tissue formation driven by a genetic program intrinsic to cells, or shaped by external cues from neighbouring tissues? By combining intuition with patient trial and error, Sasai has found that it takes a delicate

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HOW TO GROW AN EYE

Yoshiki Sasai worked out how to coax mouse embryonic stem cells into forming an optic cup, the back of an eye in a growing embryo.



balance of both: he concocts controlled environments that feed cells physical and chemical signals, but also gives them free rein to ‘do their thing’ and organize themselves into issues. He sometimes refers to himself as a Japanese matchmaker who knows that, having been brought together, two strangers need to be left alone. “They know what to do,” he says. “They interact in a delicate manner, and if the external cues are too strong, it will override the internal ones.”

Sasai’s work could find medical applications. Recapitulating embryonic development in three dimensions, it turns out, generates clinically useful cells such as photoreceptors more abundantly and efficiently than two-dimensional culture can, and houses them in an architecture that mirrors that of the human body. Sasai and his collaborators are now racing to implant lab-grown retinas into mice, monkeys and humans. The way Sasai sees it, maturing stem cells in two-dimensional culture may lead to ‘next generation’ therapy — but his methods will lead to ‘next, next generation’ therapy.

SELF-DETERMINED

A bit stiff in movement and reserved in manner, Sasai nevertheless puts on a theatrical show with a cocktail shaker at parties held by his institute after international symposia. “My second job is bartender,” he says, without a trace of a smile. It is, however, the cocktails he mixes in 96-well culture plates that have earned him scientific acclaim.

Like many members of his family, Sasai studied medicine. But he soon became frustrated by the lack of basic understanding in the field, especially when it came to neurological conditions. “Without knowing the brain, a doctor cannot do much for the patient and therapeutics will always be superficial,” he recalls thinking. There seemed no better way to know the brain than to study how it emerges and folds in the embryo. “It’s complex and usually complex systems are messy,” says Sasai. “But it’s one of the most ordered.” He wanted to know how this elaborate system was controlled.

One piece of the puzzle was well known: the Spemann organizer, a node in vertebrate embryos that induces surrounding cells to become neural tissue. How the organizer works had been a mystery since its discovery in 1924; to find out, Sasai accepted a postdoctoral position at the University of California, Los Angeles. The post got off to a difficult start when Sasai was robbed of his money and passports at the airport on his way to California. But his scientific efforts were soon rewarded. “He replaced the passports

and within a month produced the clones that gave us the famous gene *chordin*,” says his supervisor, developmental biologist Eddy De Robertis.

Sasai and his colleagues discovered that the chordin protein is a key developmental signal released by the Spemann organizer⁵. Rather than pushing nearby cells to become neurons, they found, chordin blocks signals that would turn them into other cell types^{6,7}. The work helped to establish the default model of neural induction: the idea that, without other signals, embryonic cells will follow an internal program to become neural cells.

By the late 1990s, embryonic-stem-cell scientists were also looking at these signals. They wanted to turn stem cells into mature cell types — particularly neurons — that might lead to therapies. The problem, says Sasai, is that scientists generally “push too hard and perturb the system”. Sasai knew that in the embryo, subtracting signals from the system is what counts, not perturbing it. “We tried to minimize external cues,” he says.

Sasai built an experimental system around that philosophy. He threw out the serum usually added to growing embryonic stem cells, which contains a brew of uncharacterized growth factors and other signalling molecules. He also removed physical cues, such as contact with the plastic surfaces of a culture dish, by allowing embryonic stem cells to spontaneously form floating aggregates known as embryoid bodies. “If they’re attached, they’re like prisoners and can’t act out their own desires,” he says. Keeping the cells alive without these support systems was a challenge but, five years of careful experimentation later, Sasai published⁸ and later patented his serum-free embryoid body culture — a pared-down life-support system with just the right mixture of ingredients for cells to survive. It would go on to form the heart of his brain-tissue factory.

TAILOR-MADE

Embryoid bodies in Sasai’s system quickly become what he calls “brain balls” — populated with neural precursor cells. Sasai found that balls left entirely alone give rise to cells like those in the developing brain region called the hypothalamus⁹, but those given just a whiff of growth factors start differentiating into cerebral-cortex cells². And when Sasai cultured the cells for about two weeks, he got a surprise: the cortical cells spontaneously started to form a layered structure that ended up strikingly similar to the cortex of a 15-day-old mouse. When transplanted into the brain of a newborn mouse, the structure survived. “That’s what we do,” says Sasai. “We set up the permissive conditions, selecting

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“WE SET UP THE PERMISSIVE CONDITIONS. BUT AFTER THAT WE DON'T DO ANYTHING. KEEP THEM GROWING AND LET THEM DO THEIR JOB.”

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The lab-grown cortex wasn't perfect, however: it had only four of the cerebral cortex's six cell layers, for example. Sasai thought that a retina — a layered tissue that sprouts from the embryonic brain and contains light-sensing photoreceptors — might be easier to grow. The retina is thinner than the cortex, forms earlier in embryo development and doesn't require a complex vascular system.

To adapt his system to a different type of tissue, Sasai makes minute changes to the culture conditions that nudge the cells down a developmental path. He genetically engineers fluorescent 'reporter' genes into the stem cells so that they are expressed when the cells differentiate into the desired type — in this case, retinal precursor cells — and reveal whether the system is working. “Our success depends on knowing how slight modifications can lead to dramatic change,” he says.

All it took to grow a retina, it turned out, were a few tweaks, such as a reduction in the concentration of growth factors and the addition of a standard cell-culture ingredient called Matrigel. The result closely mimics eye development in the embryo (see ‘How to grow an eye’). By the sixth day in culture, the brain balls start sprouting balloon-like growths of retinal cells, which then collapse in on themselves to make the double-walled optic cups. Sasai's team snip them off — “like taking an apple from a tree,” says Sasai — transfer them to a different culture and let them be. Two weeks later, the cups have formed all six layers of the retina, an architecture that resembles the eye of an 8-day-old mouse (which, at that age, is still blind). That the cells could drive themselves through this dramatic biomechanical process without surrounding tissues to support them¹ stunned Sasai as much as anyone else. “When I saw it, I thought, ‘Oh my god.’ Shape, topology and size are all recapitulated,” he says. Carefully explaining the pun to come, he adds: “In English, when you are surprised, you say ‘eye-popping’ — so we really thought this was eye-popping.”

Reproducing the results with human cells was the obvious, but not simple, next step. Peter Coffey, an ophthalmologist and neuroscientist at University College London, tried following Sasai's recipe to grow optic cups with human cells, and, as Coffey puts it, “failed catastrophically”. Sasai, who reported¹⁰ this year that he had accomplished the feat, says that it took careful tweaks to accommodate the sensitivities of human embryonic stem cells. Because these cells grow three times slower than those from mice, for example, Sasai had to start with of 9,000 cells instead of 3,000. Coffey says that his experience made him realize how much expertise has been built up in Sasai's lab. “They've been doing it a long time. Good on 'em,” he says, with an air of good-natured jealousy.

ALL EYES

All this will not create eyes that can be plugged into an eye socket like a light bulb into a lamp. Even if Sasai could get his optic cups to develop into mature retinas, researchers have little idea about how to wire a transplanted retina up to the brain.

What the work does offer is a potentially abundant source of pure, dense, well-organized photoreceptors, the developmental stage of which can be precisely selected — something that has been difficult to achieve in standard two-dimensional culture. Eventually, Sasai hopes, his optic cups will provide sheets of photoreceptors that can be inserted into a retina damaged by conditions such as retinitis pigmentosa or macular degeneration. Sasai demonstrates the procedure by grabbing a stack of papers to stand in for the retinal layers and then slipping one sheet between the others.

But linking the transplanted photoreceptors with the rest of the retina and with the brain will not be easy, as researchers working on eye stem-cell technologies have found. Robert Lanza, chief scientific officer of the stem-cell therapy company Advanced Cell Technology in Santa Monica, California, is sceptical. “I don't think we're anywhere near when we get those cells to connect up in any meaningful way,” he says.

Ali is more hopeful. In April, his team reported¹¹ that it had improved the vision of partially blind mice using transplants of photoreceptor precursor cells taken from mice a few days old. Ali and another of Sasai's collaborators, Masayo Takahashi at the RIKEN Center for Developmental Biology, are starting to extract sheets of photoreceptors that have been grown using Sasai's methods and transplant them into mice; Takahashi plans to transplant them into monkeys by the end of the year. Both are cagey about their early results, but Takahashi says that in mice, the transplanted photoreceptors “survived well”.

HORMONAL CHALLENGE

Sasai has set his sights on more complex neural tissues. Last November, he reported³ the formation of a part of the pituitary gland — his “most complicated” tissue yet. In the embryo, the pituitary gland arises when two different tissues integrate to form a pouchlike structure. Sasai managed to recapitulate this *in vitro* partly by starting out with more than three times more embryonic stem cells than he had used to grow a mouse retina; the adjustment seems to increase the levels of signals that the cells exchange. When transplanted into mice in which the pituitary glands had been knocked out, the rudimentary organs restored the endocrine system and saved the mice. This work, too, might eventually provide a supply of pure, specialized cells, which could be used to treat endocrine disorders.

Sasai hopes to improve on his early efforts by growing a better pituitary gland, equipped with a blood supply; a cerebral cortex with all six layers of tissue; and photoreceptors mature enough to detect light. But his next major task is to culture a cerebellum, which will involve growing and integrating three tissues of different embryonic origins. The matchmaker is already at work, trying to conjure up the right atmosphere. “When a boy meets a girl, they start their own story — but not in a large auditorium full of people,” he says. “You need to put them on a beach or in a disco. Our system is simply going to create this environment.”

What Sasai plans to take on after the cerebellum is a secret, but he hopes eventually to encompass the whole brain. He does not mean building one — that would be enormously difficult and ethically fraught. Instead, he wants to work out how brain parts, with their remarkable capacity for autonomous growth and organization, combine and fold into a structure of such tremendous complexity.

“I don't want to be a parts-maker, making more and more tissues,” says Sasai. “I always want something conceptually different.” ■

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