For example, calcium imaging of astrocytes — the most abundant non-neuronal cell type in the brain — has revealed how neurotransmitters such as dopamine shape the response of astrocytes to neural activity[9,10].

Nonetheless, the latest GCaMP iterations have fallen short of reliably identifying individual action potentials under the 'noisy' conditions in living brains. Several research groups have been racing to identify GCaMP variants that can detect these changes — which occur over just a few milliseconds — without sacrificing brightness. Zhang and colleagues' work introduces a competitor to this race, and points to a bright future for calcium imaging.

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Yiyang Gong & Casey Baker
Time for the next generation

Single action potentials are the fundamental unit of neural communication. Zhang and colleagues' development is therefore a major advance. The authors' GCaMP8 sensors have significantly faster kinetics than do existing start-of-the-art GECIs (Fig. 1b), such as the GCaMP7 and XCaMP series[11,12]. The GCaMP8 sensors also avoid the typical trade-off between sensitivity and speed, whereby slower sensor kinetics produce larger optical responses.

Zhang and colleagues based their sensors on GCaMP6. The authors optimized this protein by engineering different versions of several of its modules, including the two linkers that connect GFP to the bound peptide and to the calmodulin domain that grabs the peptide when Ca^{2+} binds. They paid particular attention to the interface between the bound peptide (which in GCaMP8 is a fragment of endothelial nitric oxide synthase) and the calmodulin domain, because this region of the sensor has a crucial role in its response and kinetics[12,13]. The team then used an optimization process that involved replacing many amino acids and performing several rounds of testing to identify the best-performing sensors. They validated the performance of these indicators in flies and mice. The strong performance of the GCaMP8 sensors suggests that the way in which Ca^{2+} induces fluorescence in this series is different from that in other GCaMPs.

The fast kinetics and high accuracy of the GCaMP8 sensors will enable researchers to analyse phenomena in live animals that could previously have been studied only by using electrical measurements or genetically encoded voltage indicators (GEVIs), which respond to changes in voltage by fluorescing (much like how GECIs respond to calcium). Until now, it has been assumed that GECIs and GEVIs report different types of neural activity — GEVIs determine the timing of action potentials, and GECIs reveal key activity in neuronal compartments (for example, by measuring Ca^{2+} dynamics in processes called dendrites that receive signals from other neurons)[14].

However, with the development of GCaMP8, the information that can be gained from these tools has converged. Zhang and colleagues show, through computational analyses of the light bursts released by GCaMP8, that their sensors can detect action potentials nearly as accurately as can voltage imaging[15,16]. Similarly, GCaMP8 could reliably detect when fly neurons that normally fire frequently become transiently inactive, providing similar results to GEVI-based measurements[17,18].

When both voltage and calcium imaging can be used to record from many neurons simultaneously, practical experimental considerations might persuade future researchers to choose calcium imaging over voltage imaging. GCaMP8 is compatible with existing microscopy configurations and preparation methods. The series can be used to examine the activity of broad neuronal networks on a larger scale than is possible using voltage imaging. One might imagine that the GCaMP8 series will soon be used to interpret detailed, millisecond-by-millisecond sequences of activity that flow through many neurons in a specific area of the brain.

The fast kinetics associated with this generation of GCaMPs, along with existing GEVIs, will motivate the development of rapid optical-microscopy and imaging technologies. Such microscopes would be able to detect transient bursts of light emitted from many neurons simultaneously. But for now, the fact that calcium-sensor kinetics is no longer the bottleneck in interpreting rapid neural activity will be welcomed by many neuroscientists.

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Developmental biology

Eggs from male stem cells using error-prone culture

Jonathan Bayerl & Diana J. Laird

A screen of mouse stem cells that exploits their propensity to gain or lose chromosomes in cell culture has been used to convert male XY to female XX cells. Subsequent differentiation generates functional eggs and live offspring. **See p.900**

The ability of pluripotent stem cells to replenish themselves and to differentiate into any cell type of the body presents opportunities for studying diseases in culture and for producing personalized cells for therapy. But growing pluripotent stem cells *in vitro* comes with perils, too, because extended periods of culture can lead to abnormalities such as aneuploidy, in which chromosomes are duplicated or lost altogether[1]. Murakami *et al.*[2] report on page 900 that this tendency for aneuploidy can be exploited to convert a type of pluripotent cell — mouse embryonic stem cells (mESCs) — from male to female. The authors guide the sex-converted cells to form eggs that can be fertilized to yield offspring, marking a milestone in reproductive biology.

The production of eggs and sperm in mouse embryos begins from precursors known as primordial germ cells (PGCs). The

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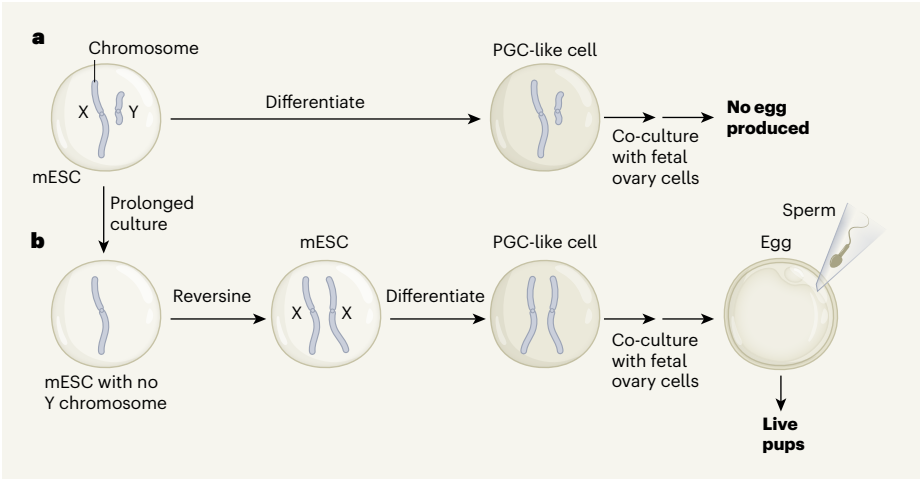

Figure 1 | A protocol to make eggs from male cells. a, XY mouse embryonic stem cells (mESCs) grown *in vitro* can be differentiated into primordial-germ-cell-like (PGC-like) cells, which are precursors of eggs and sperm. But the XY PGC-like cells cannot become mature eggs, even if co-cultured with cells from the fetal mouse ovary. **b**, Murakami *et al.*[2] subjected male mESCs to culture for prolonged periods, which led around 6% of them to lose their Y chromosome. The authors cultured cells that had lost their Y with a chemical called reversine, which disrupts quality control during cell division, making duplication of the X chromosome more likely. The authors took the resulting XX mESCs and differentiated them into PGC-like cells. Placing them in culture with fetal ovary cells promoted their maturation into functional eggs (chromosomes not shown), which could be fertilized and transplanted into the uterus of a female mouse to produce live pups.

initial differentiation of PGCs towards an egg or sperm does not depend on intrinsic sex chromosomes, but on sex-specific chemical cues that come from the surrounding gonad. However, the final stages of differentiation to mature eggs or sperm do require XX or XY sex chromosomes, respectively[3].

Mouse eggs and sperm can also be produced from mESCs *in vitro*[4,5]. First, a PGC-like cell is derived. Culture of the cell together with cells from the fetal ovary or testis (or equivalents generated *in vitro*)[6] is then required to initiate differentiation, and intrinsic sex chromosomes are needed to complete differentiation into a mature egg or sperm[3].

Murakami *et al.* set out to generate eggs from male cells, an achievement that would greatly expand the possibilities for future fertility treatments. Because the production of mature eggs requires XX chromosomes, the authors devised a way to find rare male stem cells that jettison their Y chromosome and then duplicate their X (Fig. 1).

The researchers engineered mESCs to carry a gene that encodes a red fluorescent protein (DsRed) on their X chromosomes, allowing the number of such chromosomes in each cell to be monitored. They then cultured male mESCs for eight rounds of growth and replating into fresh culture dishes — conditions under which the cells are prone to sporadically losing their Y chromosome[1]. This loss can be readily detected through a DNA-amplification process known as PCR. The authors found that 6% of cells lost their Y chromosome, forming mESCs with just one sex chromosome, X (denoted as XO cells).

Murakami *et al.* continued to grow the XO cells, screening for chromosome duplications. These duplications arise owing to faulty cell division: after DNA replication, identical sets of chromosomes are normally segregated into two identical daughter cells, but mis-segregation of chromosomes can result in both copies of the replicated X from XO mESCs being partitioned into a single daughter, forming an XX cell. The researchers used the intensity of DsRed fluorescence to distinguish between XO and XX cells. They found that treating cells with a drug called reversine, which inhibits a key checkpoint in cell division, increased the number of X duplications.

"The authors' protocol opens up new avenues in reproductive biology and fertility research."

Next, the authors differentiated the resulting XX mESCs into PGC-like cells using a previously developed protocol. They added cells from the mouse fetal ovary, which provided signals for the PGC-like cells to differentiate into eggs, with the XX chromosomes enabling maturation. The final step was *in vitro* fertilization by wild-type sperm, resulting in embryos that could be transferred into a mouse uterus and that gave rise to viable offspring. The researchers showed that similar results could be achieved by triggering *in vitro* sex conversion in an induced pluripotent stem cell (iPSC) — a type of pluripotent cell that is derived from a differentiated cell (in this case, an XY skin cell) by reprogramming its cellular identity to an embryonic-like state using transcription factors.

Not all aneuploidy involves sex chromosomes. For instance, an extra copy of chromosome 21 (known as trisomy 21) leads to Down's syndrome in humans, and trisomy 16 in mice replicates some aspects of this condition. In a final experiment, Murakami and colleagues showed that mature eggs could not be grown from trisomy 16 mESCs. However, treating trisomy 16 mESCs with reversine could promote the loss of the extra chromosome 16 without affecting other chromosomes. This restored the cells' ability to mature into eggs.

Murakami and co-workers' protocol opens up new avenues in reproductive biology and fertility research. The capacity to generate both sperm and eggs — and, ultimately, offspring — from an XY skin cell could be a means of rapidly producing inbred strains of mice, which, like identical twins, provide a consistent genetic background for laboratory experiments. Furthermore, the authors' strategy for screening for cells that have lost a chromosome could be used to investigate why some chromosomes have lower thresholds for mis-segregation than do others, both in ESCs and in cancer cells. Certain aspects of chromosomal structure (such as the position of structures called centromeres) can affect the propensity for mis-segregation[7], but whether and how this depends on cell type remains unknown.

The work could offer a way to propagate endangered mammals from a single male, provided that a female surrogate of the same or a related species can carry the embryos to full term. And it might even provide a template for enabling more people — including male same-sex couples, and transgender and XXY individuals — to have biological children, while circumventing the ethical and legal issues of donor eggs.

However, the protocol has limitations, most notably, low efficiency. Although about 30% of converted mESCs matured into eggs, and 40% of those could be fertilized, only 1% of the embryos transferred to a surrogate were born alive. It remains unclear whether the limitations that led to this inefficiency are technical or biological.

Importantly, it is not yet known whether the protocol would work in human stem cells. Despite successful differentiation of human pluripotent stem cells to PGC-like cells, subsequent sex differentiation towards eggs remains incomplete and inefficient[8], and will require the use of human fetal ovary cells — the derivation of which has only just been established from pluripotent stem cells *in vitro*[9].

Moreover, despite valiant efforts, the authors could not produce viable embryos from sperm and eggs derived *in vitro* from the same male iPSCs, which could prove a major obstacle to many of the prospects outlined

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above. One explanation for this might lie in imprinting marks — sex-specific chemical modifications to DNA that are inherited from an embryo's parents. Imprinting ensures that only copies of certain genes inherited specifically from the mother or father are expressed in the embryo, preventing the genes' overexpression. A mismatch in imprinting marks could lead to some genes being erroneously under- or overexpressed, which can cause developmental defects[10,11].

The risk of aneuploidy in routine cell culture is a double-edged sword. It calls for the development of improved culture conditions, particularly for cell-based therapies. But it also provides insights into fundamental cellular processes. The relative rarity of chromosome loss observed by Murakami and colleagues implies that pluripotent stem cells have active DNA-repair and genome-surveillance mechanisms to protect them from harmful mutations. These as-yet-unknown mechanisms are much more robust *in vivo* — in mouse and human embryos, aneuploid cells are diverted to extra-embryonic supportive lineages such as the placenta to safeguard the embryo proper[1,12–14]. This contrast in behaviour between cells *in vitro* and *in vivo* indicates that we have much to learn before we use cultured stem cells to make human eggs in a dish.

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Particle physics

A glimpse at the inner structure of the proton

Anna M. Stasto

The size of the space taken up by a proton's mass has been measured, and it's much smaller than previously thought. The result is a key step towards understanding the complex structure of this fundamental building block. **See p.813**

Most of the visible mass of the Universe is contained in atomic nuclei, which are made up of protons and neutrons. Protons consist of tiny particles known as quarks and gluons, but the mass of the quarks adds up to only a fraction of a per cent of the total proton mass. The gluons and their interactions with the quarks are responsible for making up the rest of the proton's mass[1], but gluons have no mass and no electric charge, so probing them is an experimental challenge. On page 813, Duran *et al.*[2] report a measurement of the proton's 'mass radius', a quantity that can reveal how its mass is distributed and thus inform the understanding of the structure of matter.

Much is already known about the proton's electric charge; it arises because of the electrically charged quarks whizzing around inside the proton. This motion defines the proton's 'electric charge radius', by analogy with its mass radius.

The electric charge radius of the proton was first measured in 1955, in experiments that involved high-speed electrons being shot at a target of hydrogen atoms[3]. The way in which the electrons were deflected off the target was used to deduce the electric charge radius of hydrogen's proton. The constituents of the proton were revealed 14 years later, in an experiment that relied again on the scattering of electrons, albeit with higher energies[4]. The results of this experiment suggested that the proton must be made of particles that were more fundamental than itself, and researchers concluded that those particles must be quarks.

Quarks carry another type of charge, known as colour charge, which is responsible for the strong interaction — the fundamental force that confines quarks within a proton. Gluons are the elementary particles that mediate this interaction, and because they have no electric charge, they cannot be probed directly with electrons. They do, however, have colour charge, so the challenge is to perform measurements that are sensitive enough to reveal the gluons' dynamics through their colour charge.

A suitable process from which one can extract information about the mass

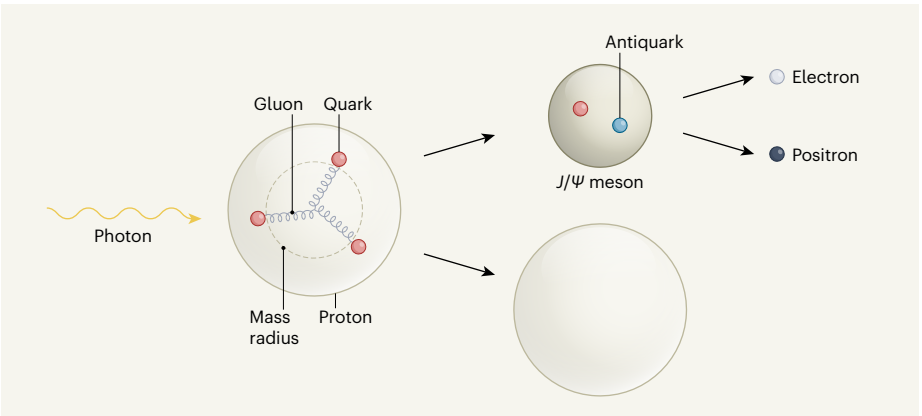

Figure 1 | A process for measuring the mass radius of the proton. The mass of a proton is determined by interactions between tiny particles in the proton, called quarks and gluons. The region in which this mass is confined is defined by a distance known as its mass radius. Duran *et al.*[2] undertook an experiment to estimate the proton's mass radius by measuring the production of a particle called the J/ψ meson, which can be generated when a photon strikes a proton. The J/ψ meson comprises a quark and its antiparticle, an antiquark, and quickly decays into an electron and a positron (the antiparticle of an electron), both of which Duran *et al.* measured. They then used theoretical models to extract the value of the mass radius.

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