Developmental biology

The mother of stem cells

Martin F. Pera

In the earliest stages of mammalian development, individual cells possess the unrestricted potential to form a new organism. Researchers are closing in on the goal of growing these cells in the laboratory. **See p.792**

The evanescent state of totipotency — the ability of a cell to form every other cell type required to generate a new organism — is difficult to study in developing embryos. Finding a way to propagate totipotent cells in culture would open up fresh avenues for research into early development. Cultured totipotent cells could also be powerful tools for deriving genetically modified animals for research or agricultural biotechnology, or for the conservation of endangered species, and could help to improve assisted-conception techniques in humans. A study[1] on page 792 by Hu *et al.*[1], together with two other studies, in *Cell Stem Cell*[2] and *Nature*[3], describes substantial progress towards the goal of generating renewable cultures of totipotent stem cells in the laboratory.

Totipotency is restricted to the first few days of embryonic development. Within a week of fertilization, totipotent mouse and human cells must begin to specialize, diverging into the lineages that will form the extra-embryonic tissues (placenta and yolk sac), which facilitate the exchange of nutrients and waste between the mother and developing offspring, and the 'pluripotent' lineage that will form the body tissues (Fig. 1). Cells from the extra-embryonic and pluripotent lineages rely on each other during development, and lack the capacity to develop into a new organism on their own. Pluripotent stem cells, as well as placenta and yolk-sac progenitors, can all be grown *in vitro*. But until the past few years, the goal of culturing totipotent cells in a dish had proved elusive.

Pioneering work (reviewed in ref. 4) showed that cultures of mouse embryonic stem cells — which are made up of pluripotent cells — also contain a minority population of cells that resemble the two-cell (2C) stage of mouse development, when embryonic cells are still totipotent. This discovery raised the intriguing possibility of propagating totipotent cells in pure form in culture. Subsequent work[5] attempted to define the conditions that could maintain cells in this state, but close examination revealed that several culture systems had failed to achieve sustained totipotency.

In the first of the three studies, Hu *et al.*[1] began with mouse embryonic stem cells, and carried out a chemical screen to identify small molecules that would enhance the expression of a fluorescent 'reporter' gene that is active in the 2C minority population. Remarkably, the resulting combination of molecules — comprising an activator of retinoid-pathway signalling, an inhibitor of the enzyme glycogen synthase kinase 3β, and an inhibitor of signalling through the transcription-factor protein NF-κB — enabled the induction and maintenance of cells resembling the 2C state *in vitro*. The authors named these cells chemically induced totipotent stem cells (ciTotiSCs).

The authors went on to analyse the genes expressed by ciTotiSCs (that is, their transcriptome), the packaging of DNA with histone proteins (their chromatin status), the patterns in which methyl groups are attached to DNA (their methylation status), and the metabolite molecules produced (their metabolome), providing validation of their similarity to 2C cells. As expected for totipotent cells, the ciTotiSCs could form placental precursor cells (trophoblast stem cells) *in vitro*. Moreover, when single ciTotiSCs were inoculated into host embryos, they contributed to the formation of the placenta and yolk sac, as well as to all lineages of the embryo proper, including those that form eggs and sperm (the germ line). The chimeric embryos could also develop into healthy live-born young — strong evidence that the cells had totipotent developmental capacity.

Similarly, in the second study, Yang and colleagues[2] used a 2C-state fluorescent reporter gene to identify culture conditions that support totipotency. But these authors adopted a targeted approach, aiming specifically to remodel the cells' chromatin status to resemble the unique configuration found in the nuclei of totipotent cells. More precisely, they used small-molecule inhibitors of histone-modifying enzymes that normally reconfigure chromatin status during the transition out of totipotency. These inhibitors — coupled with the intercellular messenger protein interleukin-6, which is expressed during early development — managed to shift embryonic stem cells into the 2C-like state and maintain them there.

This method also enabled the authors to derive cultures of totipotent-like stem cells directly from embryos. These cells met most of the molecular and developmental criteria for totipotency. Moreover, Yang *et al.* were able to turn their cells into blastoids — 3D structures that resemble the mammalian embryo just before implantation. When transferred to foster mothers, these blastoids could implant

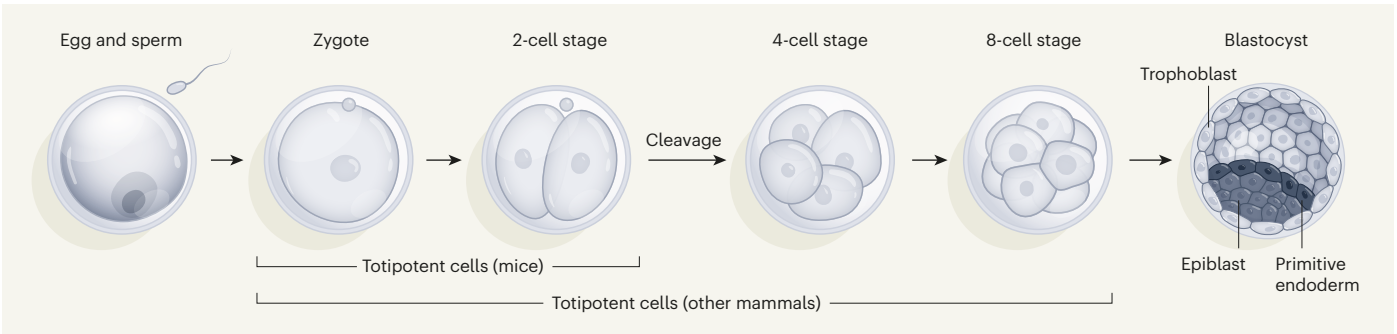

Figure 1 | Totipotency. After a mammalian egg is fertilized, the one-cell zygote acquires totipotency — the ability to produce a fertile adult individual. In mice, the two-cell stage is also totipotent; in other mammals, totipotency persists further (four to eight cells), but is lost as a structure called the blastocyst forms. The embryonic cells rapidly begin specializing to form trophoblasts (placental precursors), primitive endoderm cells (yolk-sac precursors) and epiblast cells (the pluripotent cells that give rise to all body tissues, including the germ line). Three groups[1–3] have developed cell-culture techniques that aim to derive totipotent mouse or human cell lines *in vitro*.

From the archive

An observation of aggressive anemones, and the overzealous celebration of Louis Pasteur's 100th birthday.

50 years ago

Aggressiveness is not a trait very obviously associated with sea anemones. Yet Francis ... has ... found overt aggression in the Californian anemone *Anthopleura elegantissima* ... A most striking feature of the spatial distribution of the anemones is that they live in clumps ... of the same colour pattern and of the same sex. The anemones are known to reproduce asexually ... so that the different clumps are almost certainly groups of genetically identical individuals (clones). When taken into the laboratory and mixed together, the anemones segregate back into their clones. Contact between genetically different individuals was found to lead to ... aggressive behaviours, one or both animals being damaged in the process ... *Anthopleura* shows this behaviour neither to species upon which it preys (for example, small mussels) nor to its predators (for example, sea slugs) and the curious clumped distribution of these anemones ... would seem to be attributable to their own aggressive behaviour.
From *Nature* 25 May 1973

100 years ago

France is occupied this week with the celebration of the centenary of Pasteur's birth. We, in Great Britain ... are very proud of Shakespeare ... yet our national gratitude toward Pasteur ... ought to be even more certain than our gratitude toward Shakespeare ... Things have been done better in France. It is possible that the worship of Pasteur has gone too far, in the "filming" of him ... Men and women of science may or may not stand the test of acting; but they are not intended for "filming." Take some names at random — Newton, Darwin, Lister, Kelvin: films "featuring" them would be nightmares. Besides, the whole meaning and beauty of their work would be left out. Their work began in them, but did not stop there ... So with Pasteur's work: he founded his kingdom in every country of the world.
From *Nature* 26 May 1923

into the uterus, but did not develop further.

In the third paper, Mazid and colleagues[3] sought to derive human totipotent cells, beginning with cultures of human pluripotent stem cells, which represent a later stage of embryonic development than do mouse embryonic stem cells. Through a series of manipulations of the culture conditions, the authors gradually wound back the developmental clock *in vitro* to obtain cultures that were enriched in cells similar to those of the eight-cell human embryo (thought to represent a totipotent stage).

These cells — which could be purified from mixed cultures by using a fluorescent reporter specific to the eight-cell stage — had molecular characteristics consistent with the totipotent state in human embryos. Like their mouse counterparts, these cells could also give rise to extra-embryonic lineages as well as to the tissues of the embryo proper in interspecies chimaeras formed after injection into host mouse embryos. The cells differentiated into trophoblast cells and blastoids *in vitro*, and, when injected into immunologically deficient adult mice, produced benign growths called teratomas that contained trophoblast tissue.

All the culture systems described here contain a mix of cell types, and it will be important to define clearly the key biological properties of their main subpopulations. Moreover, the cells have not yet passed the most rigorous test of totipotency: the ability to generate a new organism independently of other cell types. And although careful examination of the molecular profiles of the cultured totipotent cells found them to be a close replica of their embryonic counterparts, undiscovered 'epigenetic' abnormalities might compromise their developmental capacity. For example, it could be that the absence of maternal-effect gene products (transcripts that come from the egg and are required for normal development[6]) somehow interferes with the development of the cultured totipotent cells.

Given the progress now reported, however, it seems likely that such limitations will eventually be overcome. Will it then be possible, using *in vitro* technology alone, to produce viable embryos directly from induced pluripotent stem cells derived from living animals, including humans? If embryo-like structures or blastoids produced from cultured totipotent cells are eventually endowed with the capacity to undergo normal development to term, broad new horizons in embryological research — along with new ethical challenges — will lie ahead.

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1. Hu, Y. *et al. Nature* **617**, 792–797 (2023).
2. Yang, M. *et al. Cell Stem Cell* **29**, 400–418 (2022).
3. Mazid, M. A. *et al. Nature* **605**, 315–324 (2022).
4. Baker, C. L. & Pera, M. F. *Cell Stem Cell* **22**, 25–34 (2018).
5. Posfai, E. *et al. Nature Cell Biol.* **23**, 49–60 (2021).
6. Mitchell, L. E. *HGG Adv.* **3**, 100067 (2022).

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Cancer

Molecular basis for muscle loss that causes cachexia

Laura Antonio-Herrera & Andreas Bergthaler

Muscle loss during chronic disease is a life-threatening condition for which there is no effective treatment. The identification of an underlying molecular mechanism might offer new therapeutic targets. **See p.827**

Loss of body weight is common in people who have chronic inflammatory conditions such as cancer, cardiovascular disease, infections and metabolic disorders. Such individuals show a decrease in appetite and loss of skeletal muscle and fat mass — a manifestation of a multi-organ syndrome called cachexia[1]. People with cancer and cachexia are at higher risk of a poor response to treatment and death than are those without cachexia[2]. Efforts to alleviate cachexia include nutritional interventions, exercise regimes and pharmacological targeting of suspected mediating molecules[2]. However, interventions against current molecular targets are insufficient to reverse this muscle loss[3–5]. Bilgic *et al.*[6] describe on page 827 a molecular mechanism involved in muscle degradation that offers new therapeutic targets for tackling cachexia.

Muscle mass is maintained by a balance between protein synthesis and degradation. Cachexia-related muscle loss, or atrophy, is