Take a cell, any cell...

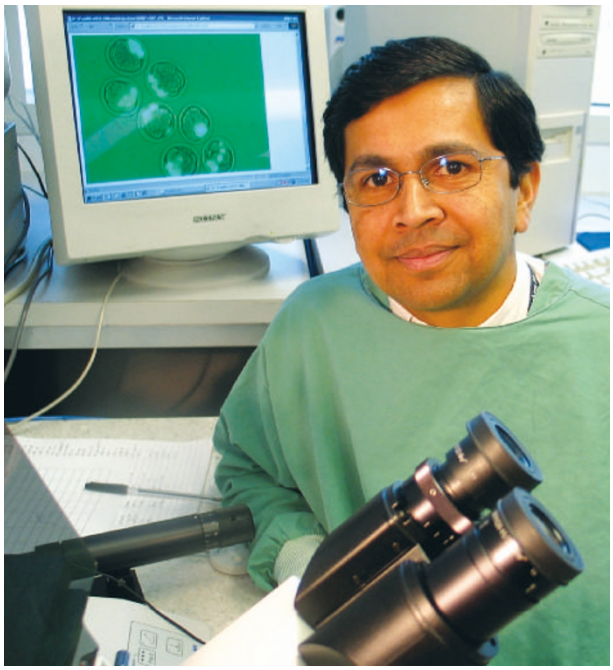
Can an adult human cell be turned back to an embryonic state without the need for cloning? If so, ethical objections to personalized regenerative medicine would be swept away. Carina Dennis reports.

It's the modern equivalent of alchemy: turning any nondescript human cell into biomedical gold. The treasure in this case is a cell that will resemble an embryonic stem (ES) cell, which can grow into any type of tissue. This cellular alchemy might one day provide the means to repair a failing body with grafts that are derived from the patient's own cells — and so won't be rejected by their immune system.

So far, there's only one proven method of turning back a cell's developmental clock: take an adult cell and fuse it with an unfertilized egg cell stripped of its own genetic material to create a cloned embryo. After growing the embryo for a few days in a culture dish, you can harvest its ES cells.

Huge technical and logistical challenges face those trying to turn this 'therapeutic cloning' into clinical practice. And even if these hurdles can be overcome, many people feel moral repulsion at the idea of creating a human embryo for the sole purpose of using its cells in a medical procedure. In some countries, such as France and Germany, the practice is already banned.

But if adult human cells could be reprogrammed into becoming ES cells, without creating an embryo, these objections would wither away. In any case, donated human eggs are in very short supply, and are in demand for *in vitro* fertilization. "We need to find an alternative," says Paul Verma of the Monash Institute of Reproduction and Development in Melbourne, Australia.



Fusion biology: Paul Verma is looking for more efficient ways of persuading embryonic stem cells to reprogramme adult cell nuclei.

The unknown factor

With this in mind, some researchers are experimenting with other cell types that seem to share eggs' capacity for developmental reprogramming. In the long run, however, the best solution will be to identify the biochemical factors sloshing around in an egg's voluminous cellular soup that can wipe an adult cell's slate clean and start afresh.

One place to look for these factors is in ES cells themselves. Fusing adult mammalian cells with ES cells, or with the embryonic cells that ultimately give rise to sperm and eggs, can make adult cells take on some of the characteristics of ES cells[1,2]. "Experiments suggest that ES cells and eggs have similar factors that can reprogramme adult cells," says Azim Surani of the Wellcome Trust/Cancer Research UK Institute of Cancer and Developmental Biology in Cambridge, whose team reported early work of this kind.

The problem is that ES cells are small and difficult to work with, and cannot readily be stripped of their genetic material in the same way that eggs can. So, reprogramming experiments involving ES cells typically end up with hybrids containing chromosomes from the ES cell as well as from the cell to be reprogrammed. This defeats the eventual purpose, which is to create stem cells that are an exact genetic match to the patient. It also raises fears that such genetically abnormal cells might become cancerous. "The next challenge is to eliminate the ES-cell genome," says Takashi Tada of the Institute for Frontier Medical Sciences at Kyoto University in Japan, a former member of Surani's team.

Verma and his Monash colleagues may have the answer. In work still in progress, they have fused pairs of ES cells to create large cells with nuclei that are double the normal volume. They then fuse one of these doubled-up cells with the cell to be reprogrammed, but prevent the nuclei from joining together by adding a chemical inhibitor that disrupts the hybrid cell's cytoskeleton. The heavier ES-cell nucleus can then be spun out of the hybrid cell using a centrifuge. Whether the remaining nucleus is effectively reprogrammed remains unclear, but Verma is optimistic. "It's early days, but we see gene expression changes that suggest rapid reprogramming in the hybrids," he says.

At the University of Oslo in Norway, Philippe Collas and his colleagues are trying to get round the problem by using ES-cell extracts, rather than the cells themselves. They punch holes in the outer membrane of adult cells using a bacterial toxin, then douse them with the cellular juice squeezed from embryonic cells and, after 30 minutes, allow the holes to reseal.

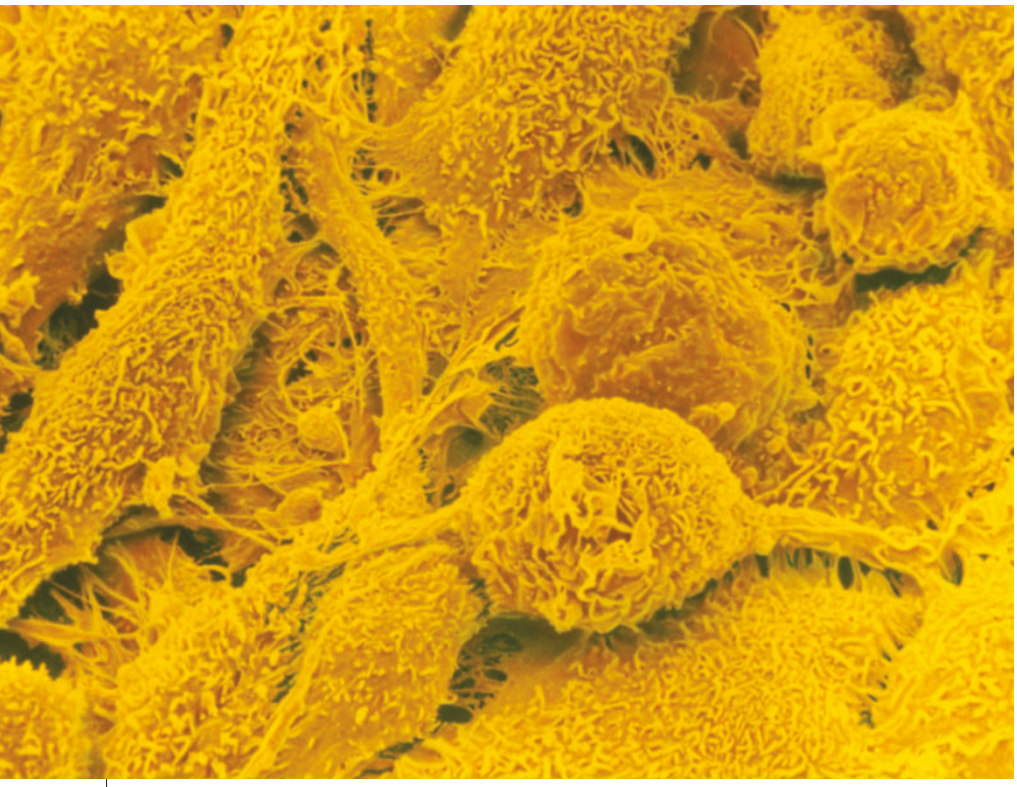
Collas's team originally used this approach to confer characteristics of immune T cells on fibroblasts — a common cell type in skin and other connective tissues[3]. The researchers are now treating mouse fibroblasts with ES-cell extracts. "We are seeing some ES-cell-specific properties," Collas claims.

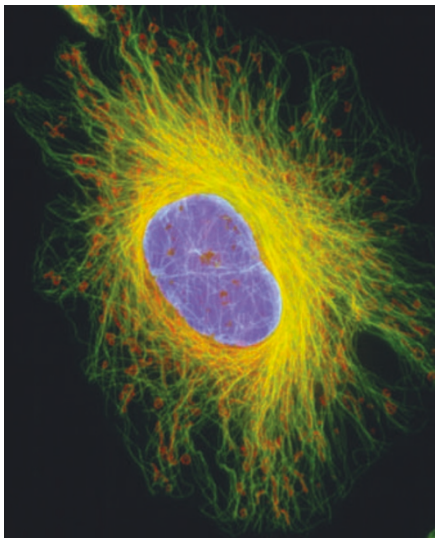
Not everyone is convinced. "I'm sceptical," says Rudolf Jaenisch of the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts. If an adult cell infused with an ES-cell extract subsequently shows some molecular characteristics of an ES cell, he says, it may simply reflect the material it has taken up — it doesn't necessarily mean that the adult cell's genes have been reprogrammed to an embryonic state.

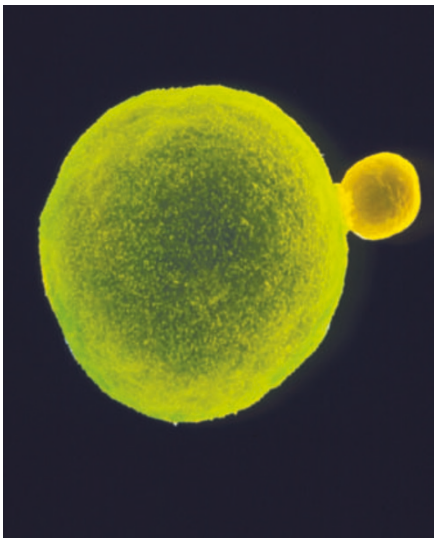
Back to the future

Another problem is that any solution that relies on ES cells to achieve developmental reprogramming does not remove the fundamental moral objection to therapeutic cloning, as the ES cells must originally come from a human embryo. It would be far more desirable to be able to turn back a cell's developmental clock by adding a defined cocktail of biochemical factors.

Several research teams are looking hard at the biochemical soup inside eggs and embryonic cells in the hope of pinning down these factors. The exact nature and number of the







YORGOS NIKAS/SPL

GOPAL MURTI/SPL; P. MOTTA/SPL

Going for gold: currently, deriving embryonic stem cells (top) from an adult mammalian cell, such as this fibroblast (above left), requires a cloning step. But a handful of research groups are now trying to achieve the same trick using reprogramming factors isolated from egg cells (above right).

factors is unknown. "But we can make some educated guesses," says Surani. The chief suspects are molecules that have been shown to erase the previous memory of a cell's state and to reconfigure the genome to reactivate long-silenced genes.

A cell arranges its genome like some of us organize our wardrobe. Active genes, like this season's fashion, are kept handy and are readily accessed by the cell's biochemical machinery. But genes that are rarely used are tucked away in the genome's attic like last winter's overcoat.

DNA inside each cell is wrapped snugly around histone proteins and packed up into a structure called chromatin. In regions of the chromosomes where genes are active, the chromatin is loosely packed so that genes can be accessed; dormant genes tend to be buried in dense chromatin. Reprogramming a cell's nucleus involves dusting off mothballed genes by unwinding chromatin and modifying histones. Methyl groups, which can alter a gene's activity, are also added or removed from the DNA during reprogramming.

Surani is looking for changes in histone modification and DNA methylation in adult cells that have been reprogrammed by fusing with embryonic cells. Although he says it is too early to name names, "we expect to see patterns of modification that suggest specific candidates".

Other researchers are looking for reprogramming factors in egg cells — a search spurred on by experiments conducted by John Gurdon, also at the Wellcome Trust/Cancer Research UK institute, which have shown that human and mouse nuclei can be reprogrammed when injected into frog eggs[4]. "Reprogramming factors found in frogs are likely to be conserved in other species, including humans," says Gurdon.

Inside the nucleus

Developmental biologists have carried out cloning experiments with frog eggs for decades. They are huge, compared to mammalian eggs, and abundant. "You can get a litre of eggs from 20 frogs every day," says Nobuaki Kikyo of the Stem Cell Institute of the University of Minnesota in Minneapolis. Kikyo has already fished out several factors from frog eggs that may be involved in reprogramming, including a complex of proteins that is involved in loosening up chromatin[5].

Most recently, Kikyo's team discovered a factor that induces dramatic structural changes in the nucleus during reprogramming. Inside the nucleus is a tiny compartment called the nucleolus, which contains a reservoir of enzymes that repair and maintain the genome. During reprogramming, the nucleolus breaks down and then reassembles. By trawling through frog-egg extracts, Kikyo's team has pinned down a complex of proteins that causes the nucleolus to disassemble[6]. "We imagine that some factors in the nucleolus need to be released in order for the nucleus to be reprogrammed," he says.

The key to identifying reprogramming factors is knowing how to look for them. "It's important to have a good assay system," says Gurdon, who is using the human gene *Oct-4*, which is switched on in embryonic cells, as a 'bait' to fish out reprogramming factors. If it becomes active when treated with a particular extract, that's a strong sign that the extract contains a reprogramming factor.

Researchers hope that the number of essential reprogramming factors will be manageably small — otherwise it may never be possible to create personalized ES cells without resorting to therapeutic cloning. But if the cellular alchemists can find a handful of factors that does the trick, we all stand to be enriched by a biomedical gold rush. ■

Carina Dennis is *Nature*'s Australasian correspondent.

1. Tada, M., Tada, T., Lefebvre, L., Barton, S. C. & Surani, M. A. *EMBO J.* **16,** 6510–6520 (1997).
2. Tada, M., Takahama, Y., Abe, K., Nakatsuji, N. & Tada T. *et al. Curr. Biol.* **11,** 1553–1558 (2001).
3. Håkelien, A.-M., Landsverk, H. B., Robl, J. M., Skålhegg, B. S. & Collas, P. *Nature Biotechnol.* **20,** 460–466 (2002).
4. Byrne, J. A., Simonsson, S., Western, P. S. & Gurdon, J. B. *Curr. Biol.* **13,** 1206–1213 (2003).
5. Kikyo, N., Wade, P. A., Guschin, D., Ge, H. & Wolffe, A. *Science* **289,** 2360–2362 (2000).
6. Gonda, K. *et al. Nature Cell Biol.* **5,** 205–210 (2003).