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Dolly *is* a clone — and no longer alone

Davor Solter

After the furore that surrounded the arrival of Dolly, the first mammal to be cloned from differentiated adult cells, doubts were raised that she really was a clone. Those doubts can now be set aside, and the technique has been further validated by the cloning of mice.

Two years ago, a report[1] that lambs had been successfully cloned from cultured, differentiated cells (that is, cells committed to be just one particular type) passed relatively unnoticed. Just one year later, the world sat up and took notice when the cloning of cells derived from an adult udder resulted in the birth of Dolly[2]. Subsequent discussions revealed the depth (or shallowness) of our homocentricity — very little was said about what we can learn from cloning experiments. This misplaced emphasis led to repeated statements, culminating in a letter by Sgaramella and Zinder[3], that a single case of cloning from an adult cell would have to be repeated before it could be taken seriously. Moreover, they suggested that Dolly might actually have been cloned from a fetal cell that had contaminated the udder-cell culture through a biological or laboratory accident.

Reports elsewhere in this issue now lay these doubts to rest. By throwing the full panoply of forensic DNA-testing methods at the problem of Dolly's origin (including the sworn, unbroken chain of custody in providing samples), Ashworth *et al.*[4] and Signer *et al.*[5] (page 329) have shown that Dolly is indeed the direct descendant of an udder cell derived from a nameless Finn Dorset ewe. They compared DNA isolated from frozen udder tissue, the cell culture derived from it and Dolly's blood, and found that the three DNA samples were identical. Further comparisons with DNA from control sheep indicate that the probability of contamination with fetal cells or mistakenly mixed cell cultures is vanishingly small.

But the fact of Dolly's uniqueness remains. Thus the significance of the report by Wakayama *et al.*[6] (page 369), who describe the existence (if my maths is correct) of 22 healthy, cloned female mice. In each case, the nuclear material was removed from a mouse egg cell and replaced by the nucleus from a granulosa cell (a differentiated type of cell that surrounds the egg), in a process known as nuclear transfer (see Fig. 5 on page 372). The importance of this report cannot be overemphasized — mice have a short gestation period, well-characterized genetics, and their embryos are much easier to manipulate than those from larger mammals, opening up the possibility of a broad experimental analysis of mammalian cloning and of the factors that determine its outcome.

Probably spurred on by the successes in cloning large farm animals, Wakayama *et al.* ignored the series of reports (my own included[7]) that stressed the difficulties — if not impossibilities — of cloning mice by simple nuclear transfer. One barrier to cloning was thought to be the early activation, during development, of the mouse embryonic genome, thus leaving too little time for the transferred nucleus to be reprogrammed. Normally, every type of differentiated cell expresses its own unique set of genes. When, during cloning, the nuclear material is transferred to the egg from a differentiated cell, it needs to be reprogrammed so that it can give rise to not just one type of cell, but to all of the different cells that make up a mouse. Reprogramming is thought to involve the shutting down of all (or most) of the genes, before the new sets are switched on. Wakayama and colleagues have now shown that there is, in fact, enough time for reprogramming to occur, and they deserve every praise for their skill and perseverance.

Given that so many of us failed, it is not immediately clear why Wakayama *et al.* have succeeded, although they list several technical reasons that need to be explored further. The point is, now that the cloning of mammals has become an accomplished fact and an accessible technique (and once the talking heads and presidential commissions have issued their reports and opinions), we can try to set some near and distant goals.

The purpose of a specific cloning experiment will largely be determined by the choice of cloned subject (Fig. 1), but Wakayama and colleagues' results indicate that some adult cells can be cloned whereas others cannot. Is this a biological or technical problem? If biological, what makes some cells reprogrammable and others not? And what is the nature of the reprogramming? Moreover, the success rate of cloning is still relatively low. Does this reflect the heterogeneous nature of the cells that are used to provide the donor nuclei, or is it due to the unpredictable nature of the reprogramming process? We have just started to characterize the genes that are expressed in a stage-specific manner during early mouse development[8,9], and these results could be used to determine whether the same set of genes is switched off and on in all of the cloned embryos, or whether each embryo is unique. This may allow us to work out which genes must be turned on and off for nuclear transfer to succeed.

The scope of future basic biological research has been increased by the success of cloning. For example, mammals possess a set of so-called imprinted genes that are expressed depending on which parent they are inherited from. Both paternally and maternally imprinted genes are necessary for normal development. Now, using nuclear

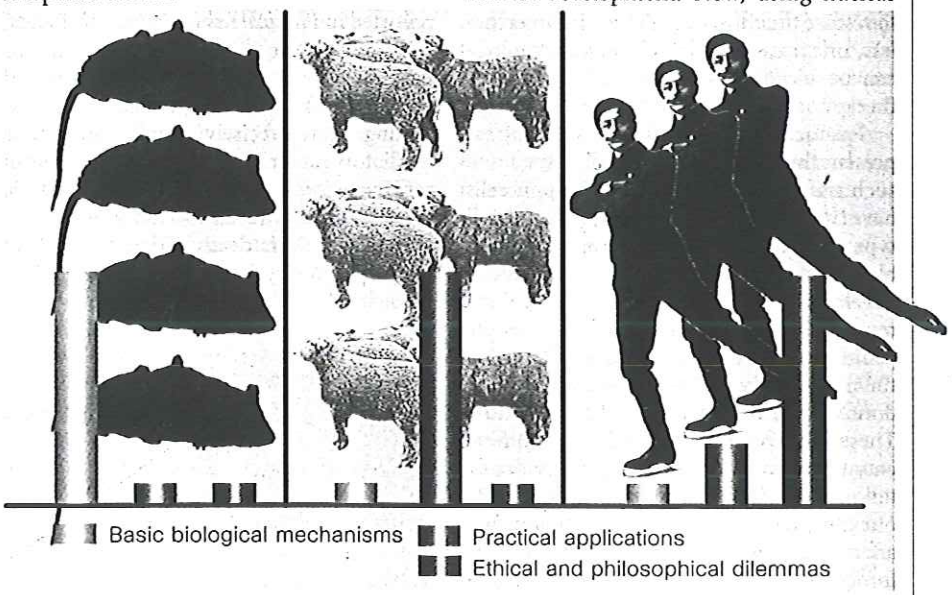

Figure 1 **Cloning strategies — the subject to be cloned determines the relative importance of various problems and questions related to cloning. Analysis of basic biological mechanisms will predominate in the cloning of small laboratory mammals; moral and philosophical questions will affect largely the cloning of humans; and practical uses and benefits will determine the extent of cloning of large farm animals and, to a degree, of humans as well.**

transfer, we can determine whether imprinting is preserved or erased in differentiated cells. The low success rate of cloning may turn out to be due to a random loss of correct imprinting. Moreover, differentiated female cells contain one inactive X chromosome, whereas in early female embryos both X chromosomes are active. So, we can learn much about inactivation of the X chromosome by monitoring what happens to the inactive X chromosome after nuclear transfer. Cloning by nuclear transfer from established, well-characterized cell lines will be much more informative than cloning from cells that have been freshly isolated from adult or fetal tissues.

With the cloning of large farm animals, the goals become economic[10]. The profit motive has, fortunately, kept cloning research alive, despite initial difficulties. Genetically altered fibroblasts (connective-tissue cells) can now be used to clone sheep[11] and cows[12] by nuclear transfer, and this should allow us to engineer the large-scale production of useful proteins by farm animals. Obviously, cultured cells must be used for precise targeting of the desired genetic manipulation, be it the addition or deletion of a specific gene. This again highlights the need to establish well-defined, cultured cell lines to be used for cloning.

Finally, what about cloning humans? At some point we will have to determine whether and when cloning — in the sense of taking somebody's cell nucleus, transferring it into an egg and raising the embryo to term and beyond — should be attempted. But we must remember that cloning is not an instant duplication, so mad dictators will not be able to expand themselves into huge armies of doppelgängers, nor will the bereaved be able to restore their lost ones. There is, nevertheless, one area in which cloning technology can be useful to humans: cell and tissue therapy.

Practical problems notwithstanding, at present there are no theoretical obstacles to such tissue therapy. Embryonic stem cells have the ability to differentiate into any cell type and could be produced from human blastocysts (embryos at a very early stage of development). Indeed, this has been done repeatedly in mice. This means that people could provide their own cells and, by using them to replace the nuclei of their own or donor eggs, obtain stem cells in culture. These cells could then be induced to differentiate in culture, providing individually tailored cell and tissue replacements without the current problems of rejection that affect transplantation from the same or foreign species. But, as far as I know, it would be illegal in most countries to sacrifice such blastocysts by turning them into a cell line in culture, because they represent potential human beings. Moreover, many technical problems will have to be overcome before this approach can become reality, but its potential value for human medicine is enormous.

There are many other beneficial aspects of cloning — easy preservation of genetically important strains and mutants of laboratory and farm animals, preservation and propagation of endangered species (provided that interspecies nuclear transfer is biologically possible) and genetic improvements, among others. There are undoubtedly many obvious and hidden dangers as well, although these do not include the groundless fears and blanket bans that seem to be the knee-jerk reaction to any cloning news. The work of Wakayama and colleagues[6] may be the last report (or at least the penultimate, as the first cloning of humans is bound to raise a few eyebrows) to induce a media frenzy. After this, I hope that we can move towards times when cloning will become a standard and useful technique to address numerous problems in basic and applied science. □

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Nonlinear dynamics

Death by delay

Steven H. Strogatz

Anyone who has ever taken a shower in an old dormitory knows that time delays can cause oscillatory instabilities. First the water is too cold, so you turn up the heat. But as you stand there shivering, the water does not get any hotter, because of delays in the antiquated heating system. If you impatiently turn up the heat even more, you are in trouble — by now, your original request for heat has registered and the water is scalding. Furiously reversing the setting, you are about to trigger a series of wild oscillations.

This much is familiar. The new twist, reported in *Physical Review Letters* by Reddy, Sen and Johnston[1], is that delays can also have the opposite effect: they can damp out oscillations that would otherwise be self-sustaining. More precisely, coupled limit-cycle oscillators can drive one another to a state of zero amplitude — often called amplitude death — if their mutual interactions are suitably delayed. This 'death by delay' may affect coupled limit-cycle oscillators in physics, medicine, biology and chemistry.

In the new models, each oscillator is assumed to have a stable limit cycle (Fig. 1). This assumption is appropriate for any system that will oscillate on its own, that is, in the absence of external periodic forcing. Examples include the heart's own pacemaker cells, rhythmically chirping crickets, flashing fireflies, a pendulum clock with an escapement, aeroelastic flutter in airplane wings, oscillating chemical reactions, and Josephson junctions driven by a constant-bias current[2].

In the earliest research on limit-cycle oscillators, between about 1920 and 1970, most theorists concentrated on the behaviour of a single, periodically forced oscillator, or two coupled oscillators. The questions were prompted in part by such emerging nonlinear technologies as vacuum tubes, phase-locked loops, radar and lasers, as well as by their intrinsic mathematical interest. Nowadays, the important theoretical problems concern the collective behaviour of large arrays of oscillators[3]. Synchronization, wave propagation, spatial patterns and self-organized criticality have

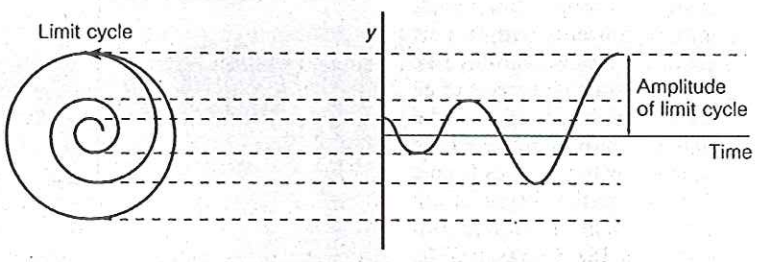

Figure 1 A self-sustained oscillation in voltage, concentration or some other physical variable is represented geometrically by a limit cycle. Here the *y*-axis records a measurable variable, and the other coordinate represents the remaining variable (such as current, or rate of change of concentration) needed to characterize the state of the system completely. If a disturbance suddenly reduces the amplitude, the oscillation spontaneously builds back up to its standard size.