Gene expression

Silent clones speak up

Wolf Reik and Wendy Dean

An important category of genes — so-called pluripotency genes — are active in early embryos but silent in specialized cells. It seems that this silencing is difficult to reverse in cloned embryos.

Early on in the development of an embryo, the cells that are produced can contribute to a wide range of tissue types. But as development proceeds they become less and less 'pluripotent', as they are tailored towards specific functions. This restriction in developmental potential is associated with a relatively small number of genes being turned on at high levels, and a relatively large fraction of genes being switched off. A typical specialized cell thus expresses a minority of all the genes in the genome. An important question in both biology and medicine is whether and how these cells could be genetically 'reprogrammed' to adopt a different fate. The answers will provide insights into how cell specialization is maintained (which is of interest both fundamentally and in understanding cancer), and might open the way to personalized cell- and tissue-based treatments.

Reprogramming does occur during cloning: after the nucleus from a fetal or adult cell is inserted into an egg, the genetic information in the nucleus can direct the development of a whole organism. But studies in *Genes and Development*[1] and *Development*[2] now show that, in cloned mouse embryos, the reactivation of genes that are silent in most adult tissues but are needed for early development is defective — perhaps explaining why cloning is inefficient[3] (Fig. 1).

The two groups[1,2] approached the issue of gene reprogramming in cloned embryos in a logical way. The genes they chose to study are repressed in adult somatic (non-reproductive) tissues but expressed in pluripotent ones (such as early embryos and primordial germ cells, which give rise to eggs or sperm). Moreover, at least one of these genes (*Oct4*) is actually necessary[4] for the development of pluripotent cell lineages. Boiani *et al.*[1] looked specifically at *Oct4*, and found that a considerable proportion of cloned early embryos derived from cumulus cells (a somatic cell type frequently used for cloning) had reduced levels or aberrant spatial patterns of *Oct4* expression. By contrast, many more early embryos that were produced by cloning with *Oct4*-expressing primordial germ cells continued to express this gene in the appropriate pattern. So, the reactivation of a somatically silenced gene seems to be difficult.

Bortvin *et al.*[2] went further, by also looking at ten genes that are related to *Oct4* in the sense of being expressed in similar patterns in pluripotent cells and repressed in differentiated ones. (The protein products of these genes are not necessarily related to each other, and their functions need to be determined.) The authors found that, with one exception (*Dppa5*), all these genes showed failures to be expressed in cloned embryos derived from cumulus cells. Notably, there was no relationship between which genes were re-expressed and which remained repressed — it is as if each gene has a certain probability of being reactivated, and the combination of these stochastic events determines overall expression patterns in individual clones. It is also remarkable that each of the 11 genes was re-expressed in at least one of the embryos studied, suggesting that the ability to reprogramme is present, at least in principle, but is perhaps limited. (Of course, the possibility cannot be excluded that this finding reflects a bias of the experimental set-up: perhaps embryos that did not activate a certain number of these genes died before the

RNA interference

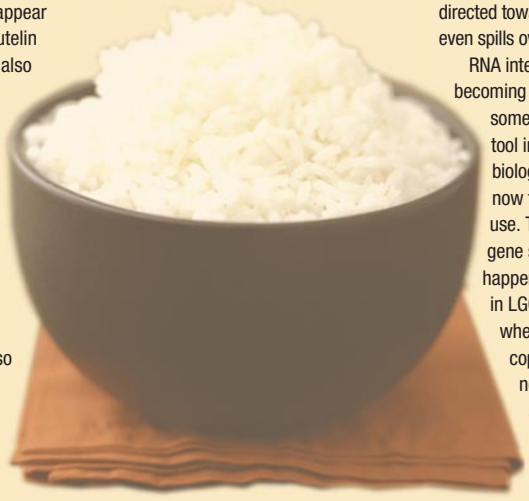
Cereal adultery

For people who must restrict their protein intake — such as patients with kidney failure — a mutant rice that is naturally low in proteins called glutelins is beginning to be used as a dietary therapy. Makoto Kusaba and colleagues have now discovered how this mutant achieves low glutelin levels (*Plant Cell* doi:10.1105/tpc.011452; 2003). The answer involves the increasingly well-known biological phenomenon of RNA interference.

Glutelins are the major proteins in cereal grains such as wheat and rice. They are produced from two families of genes, the *GluA* and *GluB* families, which occur on at least three different chromosomes. Several mutations in rice disrupt one or another of these genes, but do not significantly reduce the total amount of glutelin produced. But the mutation studied by Kusaba *et al.* — 'low glutelin content-1', or LGC-1 — has a much broader effect, completely abolishing production of one GluB protein, radically reducing the levels of other GluBs, and even limiting the GluA content.

Kusaba *et al.* have now found that LGC-1 plants lack a large stretch of DNA between two of the *GluB* genes, *GluB4* and *GluB5*. This region may encode a very short protein of about 25 amino acids, but loss of this protein does not appear to be the cause of the low glutelin levels. Crucially, the deletion also removes the 'stop' signal from the end of *GluB5*, so that the messenger RNA produced from this gene runs on into the *GluB4* mRNA. As it happens, these two genes have opposite orientations on the chromosome, and their sequences are almost completely complementary, so the mRNA folds over and the two sequences can bind to each other, forming double-stranded RNA.

Double-stranded RNA is involved in sequence-specific suppression of gene expression in organisms from plants to fungi to animals. The first step in this RNA-interference process is the production of smaller fragments, called small inhibitory RNAs, from a double-stranded RNA. These fragments act as templates to guide suppression of specific genes by mechanisms including methylation. Kusaba *et al.* found potential small inhibitory RNAs, as well as increased methylation of glutelin genes, in LGC-1 plants. The similarity between glutelin gene sequences means that suppression is directed towards all *GluB* genes, and even spills over onto *GluA* genes.



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RNA interference is fast becoming an invaluable, if sometimes unpredictable, tool in the molecular biologist's armoury. It has now found yet another use. The promiscuous gene silencer that happenstance has produced in LGC-1 plants might, when inserted as multiple copies into otherwise normal rice, result in still lower glutelin levels — a 'super-low-protein' rice.

Christopher Surridge

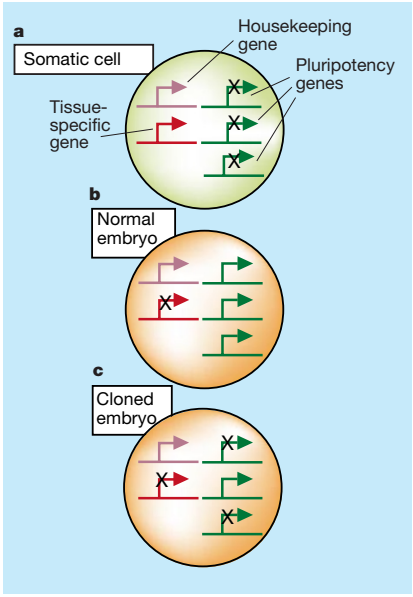

Figure 1 **Why is cloning inefficient? The figure shows gene expression in specialized (somatic) tissues, normal embryos and cloned embryos. Housekeeping genes are needed for activities common to all cells; tissue-specific genes are activated only in particular somatic cells; and 'pluripotency' genes are expressed in embryonic cells that generate a wide range of tissue types.** a, **A somatic cell (such as a cumulus cell), with an active housekeeping gene and tissue-specific gene and three inactive pluripotency genes.** b, **In normal embryos, the housekeeping and pluripotency genes are expressed; the tissue-specific gene is repressed.** c, **As shown in the new studies[1,2], after cloning with the nucleus from a cumulus cell, gene activity is not reset correctly in many cloned embryos; in this example, whereas the housekeeping gene is expressed and the tissue-specific gene is silenced, only one of the pluripotency genes is reactivated.**

stage at which the authors studied them.)

Bortvin *et al.*[2] also used embryonic stem (ES) cells, which express the 11 'pluripotency genes', for cloning, and found that expression continued in the cloned embryos. They argue that this might explain why ES-cell-derived clones are more likely to develop to term than cumulus-derived clones. What about genes that are normally expressed in somatic tissues and silent in early embryos? Bortvin *et al.* examined three such genes, and found that all were silenced in cloned embryos — perhaps implying that silencing is more efficient than reactivation. However, another study[5] suggests that cloned embryos do retain some memory of the differentiated cells from which they were derived, in that a tissue-specific gene remained active, so this issue probably needs further investigation.

Several studies[6–8] have now shown altered gene-expression profiles in cloned embryos, including amphibians. But these recent experiments[1,2] that look at pluripotency genes have taken our understanding further. They link gene-expression defects to defects in early development, and they provide good gene candidates with which to examine the precise mechanisms of reprogramming.

Why is reprogramming so difficult? The answer is probably that, once cells have differentiated into specific types, the silencing of unwanted gene expression is very tightly controlled, involving many reinforcing mechanisms. For instance, the modification of DNA with methyl groups (methylation) is commonly associated with gene silencing, as is the methylation of the histone proteins that bundle DNA into a compact form (chromatin) in the nucleus. These 'marking' mechanisms are likely to be connected in a way that makes the silent state very stable. Conversely, gene expression is often associated with histone acetylation.

It would be interesting to find out whether and how such marks can be reprogrammed, particularly on the genes studied by Boiani *et al.*[1] and Bortvin *et al.*[2]. The early embryo can certainly reset the chromatin modifications characteristic of the male and female gametes from which it was formed[9,10]. In terms of cloned embryos, so far only genome-wide chromatin reprogramming has been studied[10]. But it seems that, for the most part, the somatic patterns of histone methylation and acetylation are reset very inefficiently (although in a few embryos these marks look relatively normal, and are associated with a higher rate of successful development to a crucial stage, the blastocyst stage). So chromatin modifications might indeed provide a mechanistic explanation for the difficulties in reactivating silent genes, and silencing active ones. Efficient reprogramming might require enzymes that remove acetyl groups from histones and methyl groups from DNA or histones (although DNA and histone 'demethylases' — if they exist at all — are still elusive[11]).

Whatever the arguments for and against cloning, its study is already providing insight into the biology of cell differentiation, the extent to which cells can have many different fates, and the factors involved in reprogramming. With patience, this line of research should lead to more efficient and safer applications of reprogramming technologies in medicine. ■

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Condensed-matter physics

Thermopower to the people

Cronin B. Vining

The larger-than-expected thermally generated voltage seen in a layered-oxide material — which may prove useful in power generation or cooling — is now attributed to the spins of moving charges.

Thermocouples generate a voltage in a temperature gradient. This is known as 'thermopower', or the Seebeck effect, after its discoverer Thomas Johann Seebeck. These devices have found a range of applications, from cooling devices for seats in luxury automobiles to power supplies for spacecraft (including the Voyager missions; Fig. 1, overleaf). Metallic thermocouples generate relatively small voltages, but semiconductor thermocouples produce much larger voltages and can convert heat directly to electricity or generate cooling from an electrical input. Two different groups have reported semiconductor thermoelectric materials that are about twice as efficient as any previously known[1,2], achieved by carefully controlling the composition and structure of the materials on the atomic scale. But an entirely different approach to high thermopower uses magnetic cobalt oxides — layered materials that combine the thermopower of semiconductors with the electrical conductivity of metals. On page 425 of this issue, Wang *et al.*[3] account for their extraordinarily high thermopower.

These cobalt oxides ($Na_xCo_2O_4$) were first considered as thermoelectric materials by Terasaki *et al.*[4] and have a variety of unusual properties. They are ionically bonded (unlike the classic semiconductor thermoelectric materials, which are covalently bonded), and can be doped with varying numbers of sodium atoms to achieve the desired properties. At room temperature, their thermopower is as much as ten times larger than might be expected, much larger than is typical of metals.

At the same time, $Na_xCo_2O_4$ has some unusual magnetic properties. At low