Back to the beginning

Epigenetic reprogramming

Efficient cloning will require the ability to reset the gene-expression programmes of specialized cells, in the same way that sperm and eggs form undifferentiated embryonic cells.

Wolf Reik and Wendy Dean

A newly fertilized egg has the ability to differentiate into all tissues of the embryo, a state known as totipotency. A process known as epigenetic reprogramming returns the highly differentiated sperm and egg nuclei to this nascent state in the early embryo. Somatic nuclei that are introduced into eggs in order to clone animals must also undergo epigenetic reprogramming, and recent work shows that the failure to do so may be the chief obstacle to cloning success.

The epigenetic view of development has long surpassed the theory of preformation (in which the gametes contain small but perfectly formed bodies waiting to grow). Epigenesis (or, in modern terms, lineage commitment) holds that differences in cells and tissues arise in development because gene-expression programmes change as cells differentiate. This view of development was most famously expressed by C. H. Waddington, who likened the path of a cell lineage towards terminal differentiation to a ball travelling downwards along branching valleys; once it has entered its final valley it cannot easily cross the mountain into the neighbouring one (transdifferentiation or plasticity) or return to the beginning (cloning or return to totipotency).

However, the differences in gene expression occur without any change in the sequence of DNA, which would render the process completely irreversible. Thus, although the model clearly envisaged difficulties with transdifferentiation or cloning, the reasons for these difficulties remained uncertain. The acid test of the prediction that changes in gene expression take place without underlying changes in the DNA sequence was to attempt to clone an animal by taking a differentiated nucleus (from the intestinal epithelium, say) and introducing it into an egg from which the nucleus had been removed. If the specialized transcriptional programme in a somatic nucleus is determined by the soup of transcription factors available to it, then by exposing the nucleus to the different soup that is present in the egg, reprogramming can occur. This was indeed demonstrated in the pioneering experiments by J. B. Gurdon, in which frogs were cloned from specialized cells, and more recently in several mammalian species (including Dolly the sheep). But despite these successes, the efficiency of cloning remains very low in all species in which it has been attempted. Has the right reprogramming soup not yet been found?

As well as changes in the availability of transcription factors and in gene expression, the genome undergoes physical epigenetic changes (the central topic of the science of epigenetics). Methyl groups are attached to C–G dinucleotide sites in the DNA, and the histone proteins around which the DNA is wrapped undergo chemical modifications such as acetylation, phosphorylation, methylation and ubiquitination. Specific combinations of these histone modifications can mark genes for activity or silencing, and histone methylation can combine with DNA methylation to reinforce its repressive effect on gene activity. The key property of these epigenetic marking systems is that they are thought to retain stability as cells divide. Thus cells rolling down Waddington's differentiation valleys accumulate epigenetic markings that keep some genes active and others silent. The problem of reprogramming has therefore become a matter of how to reset these markings.

The best place to look for reprogramming is where it takes place naturally, in the formation of gametes and in the zygote. There must be an epigenetic reprogramming mechanism by which cells in some of Waddington's valleys (those that result in sperm and eggs) can return to the beginning.

There is some evidence of epigenetic reprogramming in action in early embryos and in gametes. Sperm DNA experiences a drastic loss of DNA methylation only hours after fertilizing an egg, and before its DNA is replicated, but the egg-derived nucleus remains highly methylated. Further demethylation and reorganization of histone modifications takes place as cells in the early embryo divide, before the first differentiation steps begin. After that, and concomitant with differentiation, high levels and cell-type-specific patterns of DNA methylation and histone modifications are acquired. So do these create an epigenetic momentum that is difficult to reverse during cloning?

There is evidence that epigenetic reprogramming is aberrant in clones. Methylation levels remain high in the majority of early cloned embryos. Morphologically, the nuclei in cloned embryos resemble those of the somatic cells that were used for cloning, which probably indicates that epigenetic modifications have not been removed properly. The early signs are that epigenetic reprogramming is inefficient, which may explain many of the problems encountered by cloning attempts.

The idea of epigenetic reprogramming opens up exciting possibilities for the development of new medical avenues, such as therapeutic cloning and stem-cell therapies. First, it is important to determine whether monitoring reprogramming in individual cloned embryos will provide a diagnostic tool to select those embryos with the best chance of growing into a healthy organism. Second, the study of the mechanisms of reprogramming as it naturally occurs in the early embryo (particularly the processes of demethylation and remethylation) should provide a means of intervention in either the cells used for cloning, or in the early cloned embryos. Finally, the possibility of moving cells from one of Waddington's valleys into another (transdifferentiation) has recently received much attention, with several studies suggesting that cells are more plastic and pliable than we thought.

Transdifferentiation will also involve epigenetic reprogramming, although the mechanisms and changes in patterns may be less dramatic than those involved in returning to totipotency. Understanding how Waddington's cells travel down the valleys of differentiation, and how they can be returned to their starting point both naturally and by experiment, will help us to unlock many exciting possibilities in biology and medicine. ■

Wolf Reik and Wendy Dean are in the Developmental Genetics Programme, The Babraham Institute, Cambridge CB2 4AT, UK.

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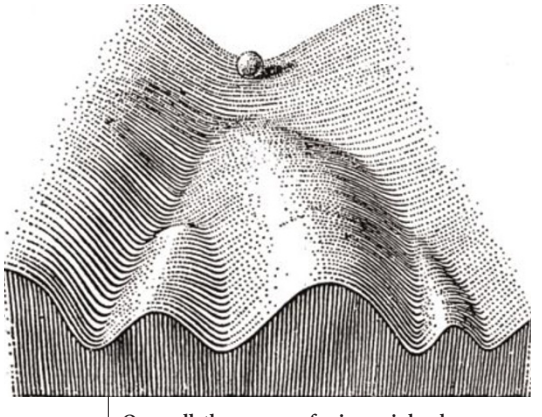

On a roll: the process of epigenesis has been likened to a ball falling into one of several valleys.

FURTHER READING

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