EPIGENETICS

# Cloning senescence

Cloning by nuclear transfer (NT) of adult somatic nuclei into enucleated oocytes involves epigenetic reprogramming to allow the expression of gene programmes appropriate for early development. One of the key questions is whether cellular ageing is reset during reprogramming. It certainly seems to be, at least at the organismal level, given the reports of a relatively normal lifespan for many animals cloned by somatic cell NT. Previous reports have suggested that cattle fetuses cloned from cultured donor cells at, or near, senescence grow into healthy calves and yield fibroblasts with extended lifespans and telomere length relative to cells from age-matched control fetuses. Now, John Clark and colleagues revisit this crucial question by carrying out nuclear-transfer experiments in sheep, using cell lines with a range of proliferative capacities. Remarkably, primary cell lines derived from cloned progeny invariably had the same proliferative capacity as the donor cell lines. Furthermore, the actual age of the donor nucleus does not seem to alter the lifespan of the NT clone-derived cell lines.

To assess the rate with which the cloned cells age, the authors turned to the telomeres because they are eroded as the cell divides and, unless replenished, are thought to contribute to cellular senescence once they reach a crucial length. Based on the inverse correlation between telomere erosion rates and proliferative vigour of the cell lines the authors suggest that the rate of telomere shortening determines lifespan.

Importantly, the rates of telomere erosion of the lines derived after NT cloning closely matched those of the parental cultures. These data are consistent with previous reports from cattle, in that telomeres from donor cells near senescence are extended upon NT; however, they do not reproduce the claim that bovine telomeres are extended upon cloning relative to non-cloned controls. These data are also consistent

HUMAN GENETICS

# Detective work uncovers a new way

Most human genetic disorders arise from mutations that disrupt coding sequences or conserved local or distant (although this is much rarer) regulatory regions, including promoters and mRNA processing signals. Tufarelli and colleagues have now extended this list by showing that a genetic disorder — α-thalassemia, in this case — can be brought about by transcription of antisense RNA that leads to methylation and gene silencing.

The authors had previously faced a dilemma — they identified a patient with α-thalassemia but no corresponding mutation in the coding or regulatory regions of the adult α2-globin gene *(HBA2)*. This patient did, however, carry a deletion in the α-globin cluster. Despite the positively acting *cis*-elements being intact, *HBA2* was silenced and its CpG island was heavily methylated resulting in α-thalassemia.

It turned out that the deletion positions a truncated copy of a widely expressed component of the splicing machinery *(LUC7L)* next to *HBA2*. *LUC7L* is transcribed from the opposite strand to *HBA2*, and because antisense RNA has been implicated in maternal imprinting and X inactivation the authors used RT-PCR to look for *LUC7L* transcripts. The results showed that in the deleted globin cluster, transcripts that initiate from the *LUC7L* promoter fail to terminate (the termination site having been removed by the deletion) and extend into the CpG-island region of *HBA 2*.

To investigate the role of this antisense transcript on the silencing and methylation of *HBA2*, Tufarelli *et al.* made a mouse model to mimic the effects of this deletion. They found that, as in their patient, the expression of *Hba-a2* was abolished in the presence of the antisense transcripts. There was also a strong correlation between the methylation of the *Hba2* CpG island and the presence of the antisense transcript.

The authors also established a model of this deletion in embryonic stem (ES) cells. Working in tissue culture allowed them to determine that methylation was CpG-island-specific and that removing the minimal promoter of *HBA2* silenced the gene but did not induce methylation — further indicating that antisense transcription was necessary for the methylation and subsequent silencing.

Finally, the authors showed that other antisense RNAs that are complementary to the *HBA2* promoter also resulted in methylation and silencing, which indicated that the mechanism is not specific to the aberrant *LUC7L* transcript.

So, although the authors do not propose an exact mechanism by which antisense RNA brings about methylation and silencing, they point out that given their example and what we know about X inactivation and imprinting, regulation of gene expression by antisense RNA might be a general natural phenomenon. No doubt Tufarelli *et al.* will use their mouse model and the ES system that they have set up to look into the mechanism of this process more closely.

*Magdalena Skipper*

## References and links

**ORIGINAL RESEARCH PAPER** Tufarelli, C. *et al.* Transcription of antisense RNA leading to gene silencing and methylation as a novel cause of human genetic disease. *Nature Genet.* **34**, 157–165 (2003)

with a previous claim that the first cloned sheep, Dolly, had shorter telomeres than control animals of the same age. Evidently, cellular senescence also correlates with telomere shortening in animals cloned by NT. Indeed, the rate of telomere erosion might determine the lifespan of both donor and cloned sheep fibroblasts.

The authors conclude that proliferative capacity and rates of telomere erosion are conserved during nuclear transfer, and are therefore likely to be a genetically determined property in sheep. A crucial question that remains unanswered is whether there is any effect on overall organismal ageing or pathologies such as cancer. How and if replicative senescence and telomere biology are related to ageing remains an important frontier for future investigation.

*Bernd Pulverer, Editor,*
Nature Cell Biology

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**ORIGINAL RESEARCH PAPER** Clark, A. J. *et al.* Proliferative lifespan is conserved after nuclear transfer. *Nature Cell Biol.* **5**, 535–538 (2003)

GENE REGULATION

# Join the club

The worm is no longer the 'odd one out' when it comes to the involvement of Polycomb-like proteins in the maintenance of Hox gene repression, thanks to the work of two groups who report their findings in the June issue of *Developmental Cell*. Although this regulation has been known for years in *Drosophila* and mammals, up until now there has been no evidence for it in *Caenorhabditis elegans*. Previously, worm genes that encode the ESC-E(Z) Polycomb group (PcG) complex were thought to function solely in germline development.

In *C. elegans*, as in other animals, Hox genes pattern the anterior–posterior (A/P) axis. The male worm tail produces sensory rays that adopt characteristic morphologies along the A/P axis, and so rays provide a useful readout of defects in Hox gene expression. Both groups carried out suppressor mutagenesis screens on ray-defective mutants to identify potential repressors of Hox gene expression.

In the process of looking for suppressors of ray defects, Ross and Zarkower isolated *mes-3*, which is known to function in a complex with the worm PcG proteins, *mes-2* (the ESC orthologue) and *mes-6* (the E(Z) orthologue), during germline development. When the authors investigated single mutants of all three genes they found weak defects in ray patterning and ectopic Hox gene expression along the whole body axis. So, in the worm soma, as in mammals and the fly, PcG proteins negatively regulate Hox gene expression.

How do these *mes* genes control Hox expression? A clue comes from the fact that RNAi targeted against certain histone deacetylases mildly phenocopies the *mes* mutants. Therefore, PcG proteins in worms might affect gene expression through histone modification, as they do in other species.

Zhang and colleagues give a different insight into Hox regulation in worms, from their study of the *sop-2* mutant. This mutant was previously isolated in a suppressor screen on *pal-1* mutants, which have incorrectly patterned sensory rays. Similar to the *mes* worms, *sop-2* mutants ectopically express Hox genes and produce extra rays. SOP-2 is a novel protein, but it does contain a self-associating SAM domain that is also found in certain PcG proteins. So, this provides another link between PcG-like genes and Hox regulation. In flies and mammals there are two distinct Polycomb group complexes, ESC-E(Z) and PRC. Worms do not have genes encoding the latter complex, and therefore it is possible that SOP-2 fulfils its functions.

These studies provide convincing evidence that *C. elegans* and all other metazoan phyla maintain correct Hox gene expression patterns through PcG-like regulation, but both papers raise questions. For instance, why are the somatic phenotypes of the worm *mes* mutants so subtle, when PcG mutations in *Drosophila* are severe? Another intriguing finding is that at the sequence level, *sop-2* is more related to the ETS family of transcription factors than Polycomb proteins. This indicates that *sop-2* is not merely a highly diverged PcG protein. But it remains to be seen how, when and why this gene evolved a function in chromatin regulation and to what extent SOP-2 replaced the functions of the PRC complex.

*Catherine Baxter*

**References and links**

**ORIGINAL RESEARCH PAPERS** Ross, J. M. & Zarkower, D. Polycomb group regulation of Hox gene expression in *C. elegans*. *Dev. Cell* **4**, 891–901 (2003) | Zhang, H. *et al.* Global regulation of Hox gene expression in *C. elegans* by a SAM domain protein. *Dev. Cell* **4**, 903–915 (2003)

**WEB SITES**

**Zarkower's laboratory:** http://www.cbc.umn.edu/~zarkoweb
**Emmon's laboratory:** http://worms.aecom.yu.edu/index.html