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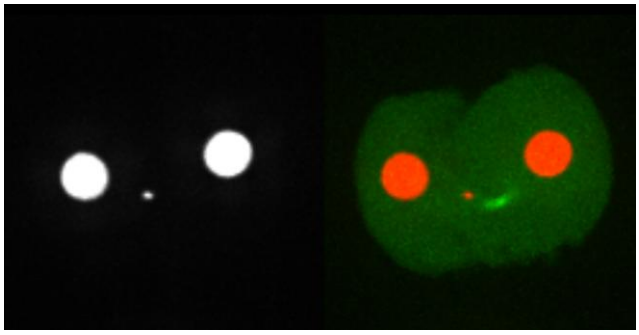
Chromosome segregation underlies cloning efficiency

February 6, 2012 – The announcement of the birth of Dolly the sheep in 1997 represented the first cloning by somatic cell nuclear transfer (SCNT) of a mammal, and sparked a global fascination with the possibilities of creating genetic copies of individual organisms. This technology has been adopted for use in animal husbandry and conservation, but in the 15 years since that first achievement, SCNT remains plagued by an extraordinarily low efficiency in giving rise to viable cloned offspring, which has never risen above a few percent of attempts. Many groups have tackled this challenge through studies of gene expression and epigenetic signatures in cloned embryos, but to date the problem of low efficiency remains unsolved.



Video showing chromosome segregation (image on right labeled for H2B (red) and tubulin (green).

A new report by Eiji Mizutani, Kazuo Yamagata and others in the Laboratory for Genomic Reprogramming (Teruhiko Wakayama, Team Leader) may shed new light on this murky question, showing that defects in chromosome segregation during early developmental stages may lie at its root. Published in Developmental Biology, this study takes advantage of live imaging technologies that enable the microscopic observation of living embryos. Mizutani has since moved to the RIKEN BioResources Center in Tsukuba, and Yamagata to Osaka University.

Yamagata and Mizutani began by focusing on the possibility that chromosomal abnormalities, rather than transcriptional or epigenetic differences, might account for the lack of progress in SCNT efficiency. To do so in live embryos, however, meant that they needed to find non-invasive approaches as an alternative to other traditional technologies such as immunolabeling and in situ hybridization, which require embryos to be fixed in advance. This led them to begin to develop a live imaging system used in this study, which allows users to observe chromosome distribution in very early embryos without disturbing their development.

They used mRNAs to introduce fluorescent probes for histone H2B and α-tubulin to label the nucleus and mitotic spindle, and tweaked the doses to optimal levels using their microscopy setup to minimize stress to the embryos. They next watched cloned embryos develop for around 60 hours to the morula-blastocyst stage using their 3D fluorescence imaging system, before returning the embryos to surrogate mothers, and confirming their developmental viability.

This meticulous observation yielded a surprising finding: of 330 embryos studied, around 80% showed some aberrant chromosomal segregation by the 8-cell stage. All such embryos failed to develop to term. Interestingly, however, the rate of chromosomal abnormality between the 8- and 16-cell stages was only 2.5%, and by the end of this early embryonic period, only 7.1% of the cloned embryos had not experienced a chromosome segregation error. Clearly, defects in chromosomal segregation in the first few embryonic cell divisions set the stage for developmental failure.

Mizutani and Yamagata next selected 72 cloned embryos at random and implanted them in surrogate mothers one-by-one to check their developmental potential. Of these, only three resulted in live births, and on reviewing the fluorescence imaging of their early development in vitro, they found that all three embryos had not experienced any chromosomal defects by the 8-cell stage.

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"We believe that the technology described in this study can be used to improve the screening of cloned embryos for the absence or presence of chromosomal segregation errors, which appear to strongly connected to their developmental viability," says Wakayama. "We also recognize however that there are likely to be other factors than epigenetics and chromosomal segregation behind SCNT efficiency rates, and we hope to continue in our search for those in future work."

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