Alternative to cloning technique does not yield pure clones, scientists report

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When is a clone not a clone? According to new research from Rockefeller University's Peter Mombaerts, creating mice by a two-step transfer of DNA does not reliably produce animals that are genetic duplicates of an original, and in some cases even creates "cloned" mice of the wrong sex.

Scientists typically clone mice (and other animals) using a single-step process in which a donor cell nucleus -- containing the animal's DNA -- is transferred into an egg, which has had its nucleus removed. The embryo is then activated, cultured and placed into the uterus of a female mouse.

Recently, scientists have developed an alternative, two-step cloning procedure that may be more efficient. In the first step, after nuclear transfer the embryo is allowed to develop to a blastocyst stage, an early form of an embryo that is essentially a hollow ball of cells. The embryo is not placed into the mouse uterus, but converted into an embryonic stem cell line in a culture dish. In the second step, the embryonic stem cells are injected into another blastocyst made up of tetraploid cells -- cells with twice the DNA of normal cells. The process was believed to help ensure that the resulting embryo contains only descendants of embryonic stem cells, while allowing tissues outside of the embryo, such as the placenta, to be formed from the tetraploid cells (descendants of embryonic stem cells do not contribute to the placenta).

Mombaerts and colleagues Jinsong Li, Tomohiro Ishii, and Duancheng

Wen report in the September 20 issue of Current Biology that the resulting embryo is not necessarily a clone of the original mouse. They supply two pieces of evidence.

In a first series of experiments the researchers applied the two-step method to 619 embryos. Their strategy was to mark the tetraploid cells with a "reporter" gene, called beta-galactosidase. They thus produced a total of 11 live born mice by injecting unmarked embryonic stem cells into marked tetraploid blastocysts. As expected, their placentas contained marked cells, but three mice also had marked cells within their bodies. In other words, some of their body cells were descendants of the tetraploid cells, meaning the mice were not pure clones but a mixture of two cell types.

In a second series of experiments, Mombaerts and colleagues applied the two-step procedure to 204 embryos, three of which survived to adulthood. The embryonic stem cells injected into the blastocysts were all derived from a male cell line, but two of the offspring were female. The scientists then mated a male and a female mouse and obtained offspring. Analysis of the embryonic stem cells showed that some had lost the Y chromosome, which is necessary for an organism to display male characteristics.

"Loss of the Y chromosome, a gross genetic alteration, indicates that the genome of the mouse donating the nucleus is not copied exactly in these mice," says Mombaerts. "If something as big as the Y chromosome can get lost along the way during the two-step cloning procedure, there may be many other genetic alterations that are not as dramatic, but do alter the genetic structure of the cells in those mice."

While the majority of the cells in these mice probably do come from the embryonic stem cells, the occasional contribution by the tetraploid cells and the genetic alterations that arise spontaneously during embryonic

stem cell culture are two reasons for which it is not appropriate to call the mice pure clones. Instead, Mombaerts now refers to these mice as “clonal” to differentiate them from true clones that are produced by the conventional, one-step cloning method.

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