

Keith Henry Stockman Campbell (1954-2012)

Keith Henry Stockman Campbell studied embryo growth and cell differentiation during the twentieth and twenty-first centuries in the UK. In 1995, Campbell and his scientific team used cells grown and differentiated in a laboratory to clone sheep for the first time. They named these two sheep Megan and Morag. Campbell and his team also cloned a sheep from adult cells in 1996, which they named Dolly. Dolly was the first mammal cloned from specialized adult (somatic) cells with the technique of somatic cell nuclear transfer (SCNT). Campbell helped develop cloning techniques that used a common form of connective tissue cells (fibroblasts). Besides working at the Roslin Institute, in Edinburgh, Scotland, for most of his career, Campbell also taught at the University of Nottingham in Nottingham, England.

Campbell was born 23 May 1954 in Birmingham, England, to Marjorie Regina Smith Campbell and Henry Stockman Campbell. Campbell had one younger sibling, a sister. At age three, Campbell and his family moved to Perth, Scotland, where Campbell began his formal education. His mother noted that he was a curious and adventurous boy who loved the outdoors and she often had to sweep frogs out of the family kitchen after Campbell had brought them home. Campbell returned to Birmingham with his family when he was eight, and he remained in Birmingham until he was twenty-one. Campbell attended school at the King Edward VI Grammar School for boys on a scholarship, but he received no outstanding grades.

Campbell said he did not like the atmosphere of that school, so he took a vocational exam and that qualified him as a medical laboratory technician at the age of nineteen, in 1973. Campbell worked at the Selly Oak Hospital in Birmingham, England. Campbell later said that the work as a technician did not intellectually satisfy him because he could not perform his own research. He enrolled at Queen Elizabeth College in London, England, to study microbiology. He quit his job at the hospital the same day he received his national certificate in medical technology. The certificate qualified Campbell as a medical technologist, but he later said that he did not want to continue with this career.

At Queen Elizabeth College Campbell studied the mechanisms of life cycles for cells. He graduated in 1978 with a Bachelor of Science in microbiology. After receiving his degree, Campbell worked as a medical technologist in southern Yemen until he returned to England a year later. He was in England from 1979 to 1980 as part of a program that aimed to eradicate a fungus that attacks trees, Dutch elm disease, from parts of southern England. Campbell said that he was also unsatisfied with this work.

Campbell began his PhD in 1980 at the Marie Curie Institute in Oxted, England. He joined a group led by Nutan Bishun that studied chromosome structures in cells. Bishun left due to illness shortly after Campbell joined, which left Campbell without a supervisor. Campbell worked alone on his PhD for six months until 1983 when the Marie Curie Institute awarded him a scholarship to study and finish his PhD at the University of Sussex in Sussex, England. There, Campbell studied the cell cycle with the direction of Chris Ford. Campbell learned of a technique to mature egg cells (oocyte) in vitro, a technique he later used at the Roslin Institute.

At the University of Sussex, Campbell studied the embryos and oocytes of South African clawed frogs (*Xenopus laevis*). He read the works of John Gurdon, at the University of Oxford in Oxford, UK. Gurdon researched cloning and nuclear transplantation, a technique of transplanting the nucleus of one cell to a host egg cell that had had its nucleus removed. Campbell met another biologist who studied nuclear transplantation, Karl Illmensee from the University of Geneva in Geneva, Switzerland, who further fueled Campbell's interest in cloning and the cell cycle. During his time at

Sussex, Campbell worked in the lab with Chris Hutchinson and collaborated with Ron Laskey and Julian Blow at Cambridge University in Cambridge, England. In 1986, Campbell received a PhD for his dissertation titled "Aspects of Cell Cycle Control in Yeast and *Xenopus*."

After receiving his PhD, Campbell returned to Scotland. Campbell said he enjoyed mountain biking, hiking, and the outdoors, and he felt that Scotland was where he could enjoy these activities and have a career of his choice. He became a postdoctoral research fellow in the zoology department at Edinburgh University in Edinburgh, Scotland. Campbell joined a group led by Peter Fantes. The group worked to control the cell cycle of *Schizosaccharomyces pombe*, a species of yeast that reproduces by fission. At Edinburgh, Campbell met Murdoch Mitchison, who worked in cellular biology and developed the fission yeast *S. pombe* as a model system for studying the cell cycle.

In 1989, Campbell left his fellowship in Edinburgh and began another post-doctoral fellowship at the University of Dundee in Dundee, Scotland, where he worked again with Hutchinson on frog embryonic development. In his studies of frog embryos, Campbell studied cell nuclei, and he began to hypothesize about the ability to reprogram an already determined cell. In 1991, Campbell saw a job posting for a post-doctoral position at the Roslin Institute. Later he said that he saw the opportunity as a better way to provide for his romantic partner, Angela, and for their first daughter Clair, and to pursue cloning. Campbell applied for and received the the job offer. Campbell and Angela later had another daughter named Lauren.

At the Roslin Institute, Campbell met and became friends with Ian Wilmut, who led a research group that would eventually clone Dolly. Campbell studied the effects of cloning by transferring the nucleus of one cell to another (nuclear transplantation). According to Wilmut, Campbell felt that the key to cloning was to transplant the nucleus to the egg during the second stage of meiosis. Campbell also altered the amounts of Maturation Promoting Factor, or MPF, a protein activity modifier (kinase) that regulates the cell cycle in many animal cells. Campbell and Wilmut first experimented on cattle embryos for this research.

Using the techniques developed with the cattle embryo experiments, Campbell and Wilmut tried cloning sheep because they were cheaper than cattle and because the Roslin Institute already had grown sheep cells in the laboratory. Campbell first tried to clone sheep stem cells, but he was unsuccessful because sheep stem cells were difficult to grow in the laboratory.

Two years later, in 1995, Campbell successfully cloned two Welsh Mountain lambs using nuclei from sheep embryo cells grown in the laboratory. Campbell started with a multicelled sheep embryo. He used techniques of mechanical and enzymatic digestion to disassociate the cells apart, and he suspended the individual cells in a liquid. He then placed the cells into cell culture medium and incubated them at thirty-seven degrees centigrade. Those cells were called the primary culture. After several days Campbell removed a portion of the cells from the primary culture and placed them into fresh cell culture media, a process called the first passage. Campbell repeatedly passed cells to new cultures from six to thirteen times, and he used the nuclei from the transferred embryo cells, instead of nuclei from stem cells or primary culture embryo cells, to clone the two lambs.

The researchers transplanted nuclei from the transferred cells into oocytes, each of which had had its original nucleus removed, to form embryos, and then they implanted those embryos into surrogate ewes. When the time was near for the lambs to be born, Campbell, and other members of the team took turns watching the ewes from midnight to five in the morning each day to ensure that the pregnant ewes had no complications. The names of the lambs born in 1996 were Megan and Morag and they were the first cloned sheep. Campbell successfully transplanted nuclei from differentiated embryonic cells because he had discovered how to place these cells into a state called quiescence, which is a state when cell activity is limited. Quiescence more commonly called G0. This resting state is normally unattainable in stem cells, but is attainable in differentiated embryonic cells. Wilmut attributed the success of the experiment to Campbell's hypothesis that if you removed nuclei from differentiated embryonic cells at the G0 stage and transplanted them to oocytes with high MPF, then you could clone differentiated embryonic cells. With the success of Megan and Morag, Campbell insisted that researchers could clone sheep with nuclei from fully differentiated adult cells as well as from differentiated embryonic cells. This claim conflicted with the long-standing theory that once embryonic cells differentiated into specific kinds of adult cells, those adult cells

could not return to a totipotent state, or retain the ability to reinitiate the development of a complete organism.

Campbell continued his research with the techniques developed from the Megan and Morag experiment and funding from a new partnership with PPL Therapeutics in Roslin, Scotland. Campbell and his team extracted adult mammary cells from a six-year-old sheep and performed nuclear transplantation from these adult mammary cells into oocytes that had had their original nuclei removed. The team then implanted two hundred seventy-seven embryos, each of which had a nucleus and DNA from an adult mammary cell, into surrogate ewes. One of these attempts resulted in a pregnancy and successful birth of a female sheep in 1997. They named the sheep Dolly, after the singer Dolly Parton. They chose the name because Dolly was cloned from a mammary gland cell and, according to Wilmut, Parton offered an excellent example. Dolly the sheep's existence helped correct theories about the impossibility of cloning new organisms with differentiated adult cells. Wilmut later said that Campbell was the main contributor to the Dolly project. In a retrospective article, Wilmut claimed that Campbell deserves two thirds of the credit for cloning Dolly.

After Dolly was born, many people discussed the implications for human cloning as well as the authenticity of the experiment. However, genetic tests proved Dolly to be a clone, and accusations of fraud faded. Campbell defended his experiments, but opposed human cloning. At the same time as the Dolly experiment, Campbell cloned two more sheep named Taffy and Tweed from a the nuclei of fetal fibroblast cells. By using fetal fibroblasts, Campbell then helped genetically modify and create a cloned sheep named Polly. Campbell left the Roslin Institute in 1997 during the Polly experiment to become the head of the embryology department for PPL Therapeutics, and he finished the Polly experiment with PPL Therapeutics.

Polly was the first mammal that scientists made to have genes from another species (transgenic) through genetic engineering techniques. Researchers altered Polly's genes to express Factor IX, which is a human protein that doctors use to treat a type of hemophilia, a blood clotting disorder. Campbell's team incorporated the human Factor IX gene by transfection into embryonic fibroblast cells of sheep. Next they transplanted the fibroblast nuclei into unfertilized sheep oocytes to produce embryos, which they implanted in ewes, one of which yielded Polly. Campbell continued to work with and clone livestock, including sheep, pigs, and cattle. In 1999, Campbell helped clone the first gene-targeted mammal. These mammals were sheep named Cupid and Diana. Gene targeting is the process of placing genes on the chromosome exactly where they will be best expressed and controlled. Campbell's experiments with Cupid and Diana in 1999 led to the first piglets cloned from somatic cells, which were born in the year 2000. Campbell planned to use gene-targeted cloned pigs to transplant tissues from one species of animal to another.

In 1999, Campbell left PPL Therapeutics and became a professor of animal development at the University of Nottingham in Nottingham, England, where he continued his work on cloning. He also tried to make stem cells out of already differentiated cells. According to Wilmut, Campbell did not like bureaucracy and he enjoyed his time as a professor because he was better able to pursue his own research. During his time at Nottingham, Campbell served on scientific advisory boards for various academic organizations and companies. In 2008, Keith Campbell, Ian Wilmut, and Shinya Yamanaka in Japan received the Shaw Prize for Medicine and Life Sciences. Campbell died on 5 October 2012 at the age of fifty-eight.

Sources

1. Campbell, Keith. "Autobiography of Keith H S Campbell." The Shaw Prize Foundation 2008. <http://www.shawprize.org/en/shaw.php?tmp=3&twoid=49&threeid=56&fourid=72&fiveid=15>. (Accessed January 25, 2013).
2. Campbell, Keith Henry Stockman, "Aspects of Cell Cycle Control in Yeast and Xenopus." PhD diss., University of Sussex, 1988.
3. Campbell, Keith Henry Stockman, Ian Wilmut, William A. Ritchie, and Jim McWhir. "Sheep Cloned by Nuclear Transfer from a Cultured Cell Line." *Nature* 380 (1996): 64-6.

4. Cramb, Auslan. "I Didn't Clone Dolly the Sheep, Says Prof." Telegraph Media Group Limited, March 8 2006, UK News. <http://www.telegraph.co.uk/news/uknews/1512377/I-didnt-clone-Dolly-the-sheep-says-prof.html>. (Accessed January 26, 2013).
5. Creanor, James, and John Murdoch Mitchison. "Protein Synthesis and Its relation to the DNA-Division Cycles in the Fission Yeast *Schizosaccharomyces Pombe*." *Journal of Cell Science* 69 (1984): 199-200. <http://jcs.biologists.org/content/69/1/199.full.pdf+html> (Accessed April 3, 2014).
6. Gurdon, John B. "The Transplantation of Nuclei between Two Species of *Xenopus*." *Developmental Biology* 5 (1962): 68-83.
7. Hardie, David Grahame. "How I Became a Biochemist." *International Union of Biochemistry and Molecular Biology Life* 59 (2007): 793-6. <http://onlinelibrary.wiley.com/doi/10.1080/15216540701556873/abstract> (Accessed April 1, 2014).
8. Mitchison, John Murdoch. *Sequences, Pathways and Timers in the Cell Cycle*. Academic Press, New York, 1974.
9. Polejaeva, Irina A., Shu-Hung Chen, Todd D. Vaught, Raymond L. Page, June Mullins, Suyapa Ball, Yifan Dai, Jeremy Boone, Shawn Walker, David L. Ayares, Alan Colman and Keith H. S. Campbell. "Cloned pigs produced by nuclear transfer from adult somatic cells." *Nature* 407 (2000): 86-90. <http://www.nature.com/nature/journal/v407/n6800/full/407086a0.html> (Accessed April 3, 2014).
10. Schnieke, Angelika E., Alexander J. Kind, William A. Ritchie, Karen Mycock, Angela R. Scott, Marjorie Ritchie, Ian Wilmut, Alan Colman, and Keith Henry Stockman Campbell. "Human Factor IX Transgenic Sheep Produced by Transfer of Nuclei from Transfected Fetal Fibroblasts." *Science* 278 (1997): 2130-3.
11. Trounson, Alan. "Keith Henry Campbell (1954-2012)." *Nature* 491 (2012): 193.
12. Wilmut, Ian. "Retrospective Keith Campbell (1954-2012)". *Science* 338 (2012): 1553.
13. Wilmut, Ian, and Roger Highfield. *After Dolly: The Uses and Misuses of Human Cloning*. New York: W.W. Norton & Company Ltd., 2006.
14. Wilmut, Ian, Keith Campbell, and Colin Tudge. *The Second Creation: Dolly and the Age of Biological Control*. Cambridge, Massachusetts: The Harvard University Press, 2000.
15. Wilmut, Ian, William A. Ritchie, Keith Henry Stockman Campbell. "Nuclear-cytoplasmic Interactions during the First Cell Cycle of Nuclear Transfer Reconstructed Bovine Embryos: Implications for Deoxyribonucleic Acid Replication and Development." *Biology of Reproduction* 49 (1993): 933-42. <http://www.biolreprod.org/content/49/5/933.long> (Accessed April 21, 2014).
16. Wilmut, Ian, Angelika E. Schnieke, Jim Mcwhir, Alexander J. Kind, and Keith Henry Stockman Campbell. "Viable Offspring Derived from Fetal and Adult Mammalian Cells." *Nature* 385 (1997): 810-3.