Reproductive Biology and Endocrinology

Editorial

Toward therapeutic cloning and regenerative biology research forum: To clone or not to clone

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Published: 13 November 2003

Reproductive Biology and Endocrinology 2003, **1**:97

Received: 21 October 2003
Accepted: 13 November 2003

This article is available from: http://www.rbej.com/content/1/1/97

Creating exact copies (clones) of an individual has long been a made-to-believe story in various cultures throughout mankind history. However, the report of successfully creating a genetic clone of an adult animal from a differentiated somatic cell [1] completely shocked the world. The cloning technology involves taking the nucleus from a somatic cell and then inserting that nucleus into an unfertilized egg that has had its own nucleus removed. The newly reconstructed embryo thus contains the entire genetic makeup of the donor somatic cell (except for mitochondria DNAs) and when transferred into the uterus of a surrogate mother can develop into an individual genetically identical to the nuclear donor. Cloning via nuclear transfer has succeeded in numerous mammalian species involving the use of various somatic cells as donors. These successes prove the principle that the nucleus of a differentiated somatic cell from a variety of adult tissues can be reprogrammed and converted to a pluripotent embryo cell capable of giving rise to all cell types, tissues and organs. While the technology of creating replacement cells, tissues or organs from our own body cells via nuclear transfer cloning (i.e., therapeutic cloning) is very promising, the possibility of cloning a human via this same technology (i.e., reproductive cloning) has created various ethical concerns and regulation debate. Little does the public realize that while the scientific community strongly supports cloning animals for purposes of agricultural improvement, biomedical research or saving endangered species, they are absolutely opposed to cloning a human being. The United Nations and all governments around the globe have called for a ban on reproductive cloning in humans.

Unfortunately, the concerns about reproductive cloning in humans have led many countries to ban any form of cloning research in humans including therapeutic cloning. Because of the great potential of the cloning technology for potentially converting any somatic cell in our body into a pluripotent embryonic cell capable of giving rise to any desired cells, tissues or organs for replacement therapy, the United Kingdom became the first country with a policy supporting therapeutic cloning and permitting government funding for this type of research. In contrast, the United States government is calling for a total ban to any form of cloning research in humans. This ban clearly will result in the loss for competition of the potential therapies and medical advances for the US market.

I firmly believe that the US government will lift this ban within a few years because the US government and the public will soon realize that the US risks losing its scientific leadership as well as enormous economic and human health benefits of this unprecedented revolution in human health care and medicine. In order to comply with the current US government policy and regulations, we are currently focusing for now our research on therapeutic cloning and regenerative research in animal models. A few years from now, when the US government removes the restrictions on therapeutic cloning research, we will be fully prepared.

I have been an active member of the cloning research community since 1983, initially studying embryonic cell cloning. In collaboration with Dr. Chikara Kubota, we cloned a 17-year-old prize breeding bull in Japan using ear skin cells in 1998, only just over a year after the report of Dolly's birth. This places us among the first groups to

successfully clone adult cattle in the world. This work was published in the Proceedings of the National Academy of Sciences [2], entitled "Six cloned calves produced from adult fibroblast cells after long-term culture". This significant publication was recognized with a commentary entitled "How close are we to implementing gene targeting in animals other than the mouse", by Mario Capecchi, a pioneer of gene targeting research, in the same issue of PNAS [3]. In June 1999, we reported the birth of Amy, the cloned calf, which marked as the first clone (cow) from an adult farm animal in the United States. Subsequently, among other studies, we reported that the telomere lengths in cloned calves are as normal as those from natural reproduction [4] and that abnormal expression of X-linked genes in cloned animals may contribute to their frequent abortions or neonatal death [5]. These achievements have caused a media sensation around the globe including coverage by the CNN Headline News, The New York Times, The Washington Post, The Wall Street Journal and US News & World Report. Our high-profile achievements have helped me to realize my dream for building a team for animal-model therapeutic cloning and stem cell research at the University of Connecticut.

In 2001, a visionary project to build a Center of Excellence to study into therapeutic cloning and stem cells was approved and instituted by the University of Connecticut and I was put in charge for recruiting five new faculty members with complementary expertise to add to our existing strong embryo development and biotechnology expertise. I am very pleased to announce that we have successfully recruited five outstanding scientists with the desired complementary expertise as our Center core faculty members. A recent scientific symposium entitled "First New England Symposium on Regenerative Biology and Medicine" was held in Connecticut to commemorate the founding of the University of Connecticut Center for Regenerative Biology http://www.crb.uconn.edu. The Associated Press (Donna Tommelleo, 2003-09-14) and the New York Times (Marc Santora 2003-09-17) both reported on this symposium along with other news media. US Senator Christopher Dodd (D-Connecticut) praised our research at our Center's dedication ceremony, "This is important, not only for the university, but the human condition". He further commented, "They are doing research here that will make the world a better place." (Robert Miller, The News Times 2003-09-16).

I am particularly pleased that the journal Reproductive Biology and Endocrinology has dedicated this special issue on Regenerative Biology to our new Center. I take great pride and pleasure in introducing our readers to our five talented new faculty members and briefly describing the complementary expertise they have brought to the University of Connecticut Center for Regenerative Biology.

One of the challenges we face, and a potential bottleneck for the successful application of the cloning technology is its low efficiency. This is due to a high rate of embryonic losses and abortions throughout the gestation period, high rates of neonatal death as well as other abnormalities after birth. Dr. **X. Cindy Tian** was recruited to the Center as a molecular embryologist and geneticist to address these issues by studying genetic programming using specific candidate genes as well as gene expression globally. Specifically, Dr. Tian studies the process of gene reprogramming by somatic nuclear transfer. Since the creation of Dolly, the first mammal cloned from an adult differentiated somatic cell, a long-held dogma in developmental biology, that the process of genes inactivation during development and differentiation was irreversible, was revolutionized. We now believe that inactivated genes can be reactivated by the somatic cloning technology and are capable to direct development of a new individual. The process of gene reactivation by somatic nuclear transfer is called nuclear or gene reprogramming. Nuclear reprogramming is the fundamental basis for tissue regeneration by the stem cell technology. The high rates of developmental abnormality of somatic clones, however, point to the fact that the nuclear reprogramming process is incomplete and imperfect at this time. Understanding the mechanisms regulating the nuclear/gene reprogramming events will lead to technologies for targeted re-differentiation of cells for cell/tissue regeneration. In this special issue, Tian et al [6] provide an overview on the state-of-the-art for cloning research and challenges. This article complements to a parallel paper by Tian [7] to review molecular approaches to address these challenges.

An important and essential component of therapeutic cloning is the capability to establish, characterize and differentiate stem cells from the somatic cell-derived cloned embryos. Dr. **Joanne Conover** was recruited to the Center as an expert in stem cell biology. Dr. Conover's laboratory offers expertise in mouse embryonic stem cell derivation and characterization, as well as various differentiation analyses. Specifically, Dr. Conover is interested in understanding two developmental pathways, one that instructs stem cells to form dopaminergic neurons (those lost in Parkinson's Disease) and another that instructs stem cells to generate pancreatic islet cells (those lost in Type I Diabetes). A better understanding of these pathways will allow researchers to reliably obtain specific cell types for therapeutic use. Dr. Conover also has extensive experience in studying adult neural stem cells and is interested in the potential application of these cells toward the understanding and treatment of neurodegenerative diseases. With expertise in both embryonic and adult stem cells, Dr.

Conover is interested in comparing derivation, differentiation, and signaling pathways of different stem cell populations. Dr. Conover's research further extends to the study of mechanisms involved in stem cell activation and differentiation during regeneration. In this special issue, Conover et al [8] discuss the fate of adult neural stem cells in the adult brain and the molecular mechanisms that regulate adult neurogenesis in mice.

What makes a stem cell a stem cell and how stem cells differentiate into various cell types remain mysteries in biology. Dr. **Theodore Rasmussen** was recruited to the Center as a cellular and molecular geneticist to study the mechanisms of stem cell maintenance and differentiation. Specifically, Dr. Rasmussen's research concerning the molecular mechanisms that govern differentiation processes will further the goal of achieving highly efficient and rationally-guided differentiation. Chromatin proteins are a diverse set of molecules that associate with DNA and regulate gene expression in a tissue-specific manner. Recent evidence suggests that chromatin proteins and chromatin remodeling activities play a substantial role in stem cell differentiation processes. This proposition is supported by research into the mechanisms of X chromosome inactivation (one of the earliest developmental changes in differentiating ES cells) and nuclear transfer (cloning) experiments that suggest that "reprogramming" may have its basis in chromatin remodeling. The Rasmussen laboratory studies chromatin dynamics in mouse embryonic stem (ES) cells as a model system to understand differentiation processes on a mechanistic level. Dr. Rasmussen is currently conducting extensive research on macroH2A1, a specialized histone variant involved in gene silencing and X inactivation. MacroH2A1 seems to be a general component of heterochromatin and is incorporated into the inactive X chromosome of female ES cells during the course of differentiation. In addition, Dr. Rasmussen is interested in the mechanisms that target the formation of specialized chromatin to particular genomic sites. In this special issue, Dr. Rasmussen [9] provides an excellent overview on the recent advances in the understanding of the mechanisms that govern epigenetic regulation of gene expression of stem cell maintenance and differentiation dynamics. As embryonic cells differentiate, certain genes are activated while others are silenced. These activation and silencing events, which are exquisitely coordinated with the allocation of cell lineages, are reviewed in this article.

Understanding gene expression and regulation during natural organogenesis and tissue differentiation obviously will provide the theoretical basis to study in vitro stem cell differentiation and tissue regeneration. Dr. **David Goldhamer** was recruited to the Center as a developmental biologist to study fundamental mechanisms of skeletal muscle development, growth and repair, with an emphasis on regulation of cell commitment and muscle-specific gene expression. One long-term goal of the Goldhamer laboratory is to define the genetic pathways that culminate in the activation of muscle regulatory factors in muscle precursor cells during development, an understanding of which will provide insight into how embryonic cells choose between alternative cell fates during development. A second major research area of his laboratory focuses on the biology of stem cells resident in muscle tissue. Following injury of adult skeletal muscle, or in diseases such as Duchenne muscular dystrophy, skeletal muscle undergoes a regenerative process that in many ways resembles muscle development in the embryo. Skeletal muscle's enormous regenerative capacity is mediated by muscle satellite cells, normally quiescent stem cells that are "activated" in response to injury or disease. Despite their essential function, key aspects of satellite cell biology remain unresolved, including their developmental origin, potential and regulation of commitment to myogenesis. Dr. Goldhamer's recent research focuses on the use of cell marking experiments and mouse genetics to investigate these fundamental aspects of satellite cell biology. Recent evidence indicates that additional types of stem cells also exist in skeletal muscle tissue, the identification of which is essential for understanding diseases of abnormal bone formation and for developing therapies for musculoskeletal diseases. Characterization of this stem cell population and its relationship to muscle satellite cells is also being pursued in the Goldhamer laboratory. In this special issue, Chen and Goldhamer [10] focus their review on the basic biology of the satellite cell with emphasis on its role in muscle repair and parallels between embryonic myogenesis and muscle regeneration. The relationship between the satellite cell and other newly discovered muscle stem cell populations is also discussed. The authors conclude with advances and prospects for cell-based therapies for muscular dystrophies.

Successful application of therapeutic cloning and regeneration requires critical expertise in cell/tissue engineering and transplantation biology. Dr. **William Fodor** was recruited to the Center to provide this critical expertise. Specifically, the goal of the Fodor laboratory is to develop genetically engineered cells and tissues to repair, restore and regenerate lost tissues due to disease and injury. Dr. Fodor's research efforts span many disciplines including, immunology, molecular biology, neurobiology, transgenic technology, enzymology, and transplantation. Additional research interests of his laboratory include developing molecules that modulate the immune system, cloning molecules involved in cytokine responses, and investigating growth factors and immune mechanisms responsible for cell survival and engraftment in various transplant models. In this special issue, the Fodor paper

[11] will provide an overview of recent advances in the development of cells, tissues and organs for the purpose of restoring function through transplantation. This review will also address some of the hurdles yet to be overcome as the technology and science improve the likelihood that Regenerative Medicine will become clinically routine.

In this special issue, two additional reviews were included. Xu and Yang [12] provide a comprehensive review of the controversial issues surrounding the genetic age of clones with a focus on the telomere debate and how telomeres are related to the ageing problems of clones. Wang and Zhou [13] review the possibility and reality of gene targeting of somatic cells followed by cloning in farm animals. In this review, the principles of gene targeting in somatic cells and the challenges of nuclear transfer using gene-targeted cells, are discussed. The relevance and potential impact of gene targeting in domestic animals for applications in bio-medicine and agriculture are also examined.

Finally, it has been a privilege to edit this special issue of "Regenerative Biology" on behalf of Reproductive Biology and Endocrinology. I would like to thank the Editor-in-Chief, Professor Antonin Bukovsky for the kind invitation, advice and guidance throughout the preparation of this special issue. I would also like to thank President Philip Austin, Provost and Executive Vice President John Petersen and the other senior administrators at the University of Connecticut for helping to make my dream come true for building this Center of excellence. A special note of thanks goes to Dr. Ian Hart, Professor, Associate Dean for Research of the College of Agriculture and Natural Resources, for recruiting me to UConn and for his persistent support to my career development at the University. Let me take this opportunity to thank all guest speakers at our Center's recent dedication symposium: Drs. James Battey of NIH, Charles Jennings, Gerald Schatten, Mark Mirando, Harris Lewin, Jean-Paul Renard, Robert Wall, Marc Lalande, Gerry Berkowitz, Tim Hla, Eugene Chen, Alex Lichtler and David Reisner. Susan Ayers, Rafaela Rivera, Wendy Zhou, May Liu and the many hard-working graduate and undergraduate students and research associates for doing an excellent job to ensure the success of our dedication symposium. Finally, a special thank you to Marina Julian who has served me very well as my assistant guest editor for this very special issue of Regenerative Biology.

References

1. Wilmut I, Schnieke AE, McWhir J, Kind AJ and Campbell KH: **Viable offspring derived form fetal and adult mammalian cells.** *Nature* 1997, **385:**810-813.
2. Kubota C, Yamakuchi H, Todoroki J, Mizoshita K, Tabara N, Barber M and Yang X: **Six cloned calves produced from adult fibroblast cells after long-term culture.** *Proc Natl Acad Sci U S A* 2000, **97:**990-995.
3. Capecchi MR: **How close are we to implementing gene targeting in animals other than the mouse.** *Proc Natl Acad Sci USA* 2000, **97:**956-957.
4. Tian X-C, Xu J and Yang X: **Normal telomere lengths found in cloned cattle.** *Nat Genet* 2000, **26:**272-273.
5. Xue F, Tian X-C, Kubota C, Du F, Taneja M, Dinnyes A, Dai Y, Lewin H, Pereira L-V and Yang X: **Aberrant patterns of X-chromosome inactivation in bovine clones.** *Nat Genet* 2002, **31:**216-220.
6. Tian X, Kubota C, Enright B and Yang X: **Cloning animals by somatic cell nuclear transfer – biological factors.** *Reprod Biol Endocrinol* 2003 in press.
7. Tian X: **Reprogramming of epigenetic inheritance by somatic cell nuclear transfer.** *Reprod Biomed Online* 2003 in press.
8. Lennington JB, Yang Z and Conover JC: **Neural stem cells and the regulation of adult neurogenesis.** *Reprod Biol Endocrinol* 2003 in press.
9. Rasmussen T: **Embryonic stem cell differentiation: a chromatin perspective.** *Reprod Biol Endocrinol* 2003 in press.
10. Chen CJ and Goldhamer DJ: **Skeletal muscle stem cells.** *Reprod Biol Endocrinol* 2003 in press.
11. Fodor WL: **Engineering and cell based therapies, from the bench to the clinic: The potential to replace, repair and regenerate tissue.** *Reprod Biol Endocrinol* 2003 in press.
12. Xu J and Yang X: **Will cloned animals suffer premature aging – The story at the end of clones' chromosomes.** *Reprod Biol Endocrinol* 2003 in press.
13. Wang B and Zhou J: **Specific genetic modifications of domestic animals by gene targeting and animal cloning.** *Reprod Biol Endocrinol* 2003 in press.