Title: The Paul Ehrlich Prize 2005 has been awarded to Ian Wilmut

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Introduction The 14th March was the 151st anniversary of the birth of Paul Ehrlich - an appropriate day to award one of the most prestigious scientific awards in Germany. The prize has been won by many well know scientists. Since 1996, the winners have all been non-German. This trend continued this year when the prize was awarded to a British scientist, Ian Wilmut of the Roslin Institute. The award was given for his outstanding scientific achievements in the fields of immunology and medicine, specifically for using the technology of nuclear transfer (NT) to transfer a somatic nucleus into an unfertilized enucleated egg which led to the creation of the sheep known as "Dolly".

The award ceremony, held in Paulskirche in Frankfurt, began to the sounds of a string trio by Beethoven. The invited audience was first addressed by the representative of the mayor of Frankfurt, followed by Hilmar Kopper, the chairman of the scientific council of the Paul Ehrlich Foundation. Bernhard Fleckenstein of the University of Erlangen, who chaired the scientific jury, introduced the technology of nuclear transfer and the work of Ian Wilmut. The prize was then awarded by Klaus Theo Schroeder, the Federal Minister of Health, after which Ian Wilmut addressed the guests. All the speeches were understandable to the general public and were given without visual aids.

Under German law the technology of NT transfer into human germ line stem cells is prohibited so it is unusual that the subject of this award was this technology - especially as 42,500 EUR of the 100,000 EUR prize is given by the German National Health Ministry. Prior to the start of the ceremony the invited guests were confronted by a dozen demonstrators outside the church wearing plastic Dolly the sheep masks, three dozens policemen and security guards, and approximately six dozen journalists. The ceremony and demonstrations, together with opposition remarks by German religious and the National Ethics Committee were widely publicized in the German media that evening and the following day.

Bernhard Fleckenstein introduced the technology of NT and outlined how Dolly was generated. He also spoke about the career development of Ian Wilmut and the justification for the awarding of the 2005 Paul Ehrlich prize. Ian has spent his entire career in the UK - in Nottingham, Cambridge and Roslin. A distinctive feature of Ian´s scientific career is that he has spent 32 years in the same lab in the Roslin Institute working on sheep and pig related research. While working in Cambridge he performed the worlds first successful cryopreservation of a calf embryo which when thawed and was implanted became a living animal known as the "Wilmut Frostie". Ian’s father died from diabetes related complications in 1994 and his condition was a personal motivation for developing a career in regenerative medicine. Since 1973 he has worked on various aspects of transgenesis in sheep, including attempting to establish a sheep ES cell line, and also began to work on nuclear cloning technology.

"Tracy", the first transgenic sheep, was generated in 1991. A plasmid coding for alpha-1 anti-trypsin was injected into an oocyte, which, many steps later, produced Tracy, a live female animal that secreted the anti-trypsin protein in her milk. This protein is used to treat cystic fibrosis. Ian observed from this work that the efficiency of a successful transgene injection into a

sheep oocyte was very low and production of a sheep ES cell line that could be used for transgenesis was likely to be more efficient. As mouse ES cell lines had been established this technology seemed feasible and promising, however, after many years of experimentation without success he realized that it was technically impossible.

In the early 1980s Karl Ilmensee and Peter Hoppe claimed that they had transferred nuclei from the inner cell mass (ICM) of mouse blastocysts into enucleated mouse oocytes to generate a mouse by nuclear transfer. However, their results could not be reproduced by other groups and Ilmensee lost his faculty position at the University of Geneva in Switzerland. James McGrath and Davor Solter in Philadelphia formulated the generally accepted hypothesis that nuclei from very early embryonic cells are suitable for the generation of "cloned" embryos but not those from differentiated somatic cells. The belief was that the program of gene expression in the nucleus is pre-programmed to be in one direction and once differentiated the nucleus cannot return to the earliest totipotent embryonic stage.

Steen Willadsen, a Dane working in Calgary, was the first to clone sheep using ICM nuclei. Ian Wilmut brought this technology to Roslin in the late 1980s and from 1990 onwards the lab worked towards the goal of cloning sheep. Ian began to challenge the belief that the donor nucleus could only be from early embryonic cells. In 1995 the group showed that NT worked with early embryonic nuclei - the transferred nucleus differentiated. They also studied the relationship between cell cycle and the success of NT and found that cells in G_0 phase were the most successful donors of nuclei. It was also shown in 1995 that the embryonic NT cell could be grown in culture prior to transfer into the foster mother and this technique was used to generate the clones "Megan" and "Morag".

Ian and his group continued to develop the NT technology using differentiated adult somatic nuclei as donors and the successful generation of the sheep "Dolly" was published in *Nature* in 1996. Post-transfer of an adult nucleus from a mammary gland cell into an enucleated unfertilized egg the NT cell was cultured through 13 passages before transfer into a foster mother. The entire experimental process used 267 pre-implantation eggs to produce 29 NT-blastocysts that were implanted into 13 foster mothers and resulted in one Dolly. A micro-satellite assay was used to follow the progeny of the NT-cell. They clearly showed a somatic nucleus can be reprogrammed, i.e., the cytoplasm of the enucleated egg can re-program quiescent somatic differentiated nuclei to restore totipotency and thus initiated the field of epigenetics. Twelve months later a transgenic clone, "Polly", that secreted Factor IX in her milk was generated (frequency of 19%).

The applications of NT from adult somatic cells in the fields of cell biology, cancer research, biotechnology, and animal breeding are enormous. Ian's contribution was acknowledged by the Royal Society when he was elected a member in 2002. However, it is also important to reflect on how Ian developed such an important application. Fleckenstein acknowledged that luck played its part. If Dolly had not been born in 1996, many scientists would have concluded that the cloning of adult cells was not possible. Ian worked for ten years on the basic technology of cloning without producing any publishable data. It was only after his success with Dolly that he submitted two patent applications that protect the essence of the cloning technology
- firstly, that the donor adult somatic nucleus to be transferred and reprogrammed be in G0 phase and secondly, that the enucleated oocytes used as the host cytoplasm be in M2 phase.

There were many other factors in Ian's success including his gifted co-workers, Keith Campbell

amongst others, the research infrastructure of the Roslin Institute, but perhaps most importantly, his courage in persisting in working on adult cell cloning even though published opinions opposed the direction of his work and stated that there was no possibility of these experiments being successful.

With the birth of Dolly it was immediately clear that the technology of NT could also be applied to human beings. Ian has on many occasions expressed his opposition to such experiments. He outlined his position in great detail in his 2000 book, entitled "Dolly", where he discussed the reasons why he opposed human reproductive cloning referring to the complications of spreading diseases, possible misconduct in the procedure, the incalculable psychological burden on a cloned human being and a movement towards science fiction.

On the other hand, there are merits in exploiting this technology for so-called scientific or therapeutic cloning where applications in regenerative medicine are the aim. The goal is for patient cells to be reprogrammed in order to produce healthy NT-tissue that can be transplanted to replace a damaged or malfunctional organ. As the NT-tissue would be autologous to the patient no immunological rejection would occur - this potential application was acknowledged by awarding the PE prize in the field of immunology. The long-term goal of NT is to provide therapies for patients with diseases such as diabetes, Parkinson's, and Amyotrophic Lateralsclerosis (ALS). NT is not yet clinically applicable, but in the future it will, without any doubt, be an important application in biomedicine. Ian's predictions for therapeutic cloning in humans took a giant leap forward last year when Woo-Suk Hwang, a Korean scientist, reported* somatic nuclear transfer to an unfertilized enucleated egg to generate a human embryonic stem cell line (NT-hES). (*Note: The report in Science by Hwang was withdraw).

Ian's Nature paper has triggered more discussions by international groups of all kinds, including the UNO, than any other recent scientific article. The debate relates to questions of ethics, the origin of human life, when life begins, etc. Fleckenstein spoke at length on the ethics of this technology, with arguments and quotations from theologians and church leaders attempting to define which day after fertilization human life begins.

He concluded by discussing the current state of affairs in the scientific and political communities in Germany. Fleckenstein believes that the pharmaceutical industry in Germany has been ruined by lack of support for new developments from a society that is influenced by intellectually gray arguments. He observed that the Green Party is dogmatic in its views on the right to life of the unborn and when 'life' begins, and that the new Green law opposes any and all genetic technologies. These traditional beliefs are not being reviewed in the face of progress and development. An opinion change in German people who do not accept the benefits of modern biotechnology and the potential of regenerative medicine is necessary to prevent all future research in these fields being conducted outside of Germany.

Ian Wilmut, speaking in English, began by acknowledging the teamwork of fifteen years by experts in many different fields that led to these discoveries and accepted the prize, with honor, on behalf of all these people. His group is moving from its home at Roslin Institute to the medical school of University of Edinburgh and the prize money will help settle the group in the new laboratories and begin research in new directions. He had forgotten his glasses so could not read his lecture notes and spoke instead from memory, giving a vivid and powerful account of the development of the nuclear transfer technology that was understood by all.

Ian stated that the principles of NT technology are very simple - only two cells are needed: an unfertilized egg from a donor female sheep and a donor somatic cell for obtaining a nucleus. As ooctyes can now be obtained from slaughterhouses the number of animals that need to be sacrificed has been drastically reduced. The unfertilized egg is the size of a grain of sand, and to remove the genetic material from such a cell requires technical experience, skill and patience. The group used mammary gland cells from pregnant sheep as a source of donor nucleus. An electric current was used to fuse the enucleated oocyte and donor nuclei. This also stimulates cells to divide, a signal normally provided by the fusion of sperm with the egg. The NT cells were then cultured in vitro before implantation into a surrogate mother. Though simple, it is amazing that this process works at all. Unknown activities in the cytoplasm of the unfertilized egg have the ability to rewind the reading of the genetic information in the adult cell. He then went into a lengthy discussion of why NT is useful. Giving two practical examples of the technology, firstly, the genetic manipulation of farm animals and secondly, the in vitro differentiation of cells from NT cloned human embryos.

Farm animals can be genetically modified to produce therapeutic proteins in their milk. Ian gave the example of a clinical trial currently being conducted in the USA that is investigating a clotting factor production in milk. He also mentioned Jim Robl, a US based scientist supported by Japanese funding, who is producing human antibodies in bovine blood and milk. This group constructed an artificial chromosome carrying human genes coding for antibodies and some other genes. The nucleus of a cell containing this artificial chromosome was fused with an enucleated egg and transferred into a foster mother. The resulting fetuses were collected before full term and cultured in vitro to generate larger number of fetal cells. These were then subjected to drug selection to identify those cells carrying artificial chromosomes. From these cells more fetuses were generated that produced the appropriate human antibodies. However, there have been downstream difficulties with this process as separating human antibodies from animal antibodies has been problematic. The group are attempting a third cloning to remove the bovine antibody genes but it will be critical to monitor the health of such animals as they may be susceptible to infection.

Ian also spoke of the efforts to produce farm animals that are more resistant to certain types of bacterial infection, e.g. mastitis. His own group will work on treating foot and mouth disease using iRNA technology instead of a vaccine based approach. They plan to produce a new gene to direct iRNA to disrupt the viral RNA-protein that causes foot and mouth disease. With regard to the use of NT-farm animals for food he said that there may be safety concerns but as yet there is no information on this subject.

On moving to their new laboratories the group will begin to work on human genetic diseases and hope to eventually obtain hES cells from NT technology. hES cells can differentiate in vitro to bone, neuron, liver, etc. Liver failure is the 5th most common cause of death in the USA, and liver tissue generated via NT could be used to develop effective treatments and new drugs. As liver transplantation requires two donors to obtain enough tissue hES cells may provide a source of tissue in the future. Ian also spoke of applications for Parkinson’s disease, spinal cord injury, heart failure, and diabetes. Treatment of inherited diseases would require donated cells and/or tissues. Donated embryos for NT can be used for inherited diseases, hepatocytes of immunologically inert cells.

His group plans to work specifically on ALS. The onset of the disease is in mid 50 year olds with death 4-5 years later. The condition affects motor neuron function - the patients cannot swallow and eventually asphyxiate. The cause of the disease is unclear - about 2% of cases have a known mutation, the rest have not yet been identified. The group has recently obtained a license to work on the genetics of ALS using NT. ALS hES cell lines already exist and these cells can differentiate into motor neurons. It will also be possible to generate hES cell lines from the 98% of cases where the mutation has not yet been identified. This will facilitate further genetic analysis and the development of drug therapies. Screening of new drugs using animal models is very costly but high throughput technologies based on NT hES cell lines and their derivatives will drastically reduce costs.

This technology will be applicable to many other genetic diseases including those with unidentified mutations such as cardiomyopathies. Ian believes that treatment of degenerative diseases using cells that have been immunologically matched to patients will be an alternative to long-term immuno-suppression. He illustrated the point with the example of Rudi Jaenisch's study of SCID repair in mice using NT technology.

Ian then briefly addressed the controversial questions of when the life stops and when life and a functional human being begin.

He concluded that the success of nuclear transfer is dependent on unknown activities within the unfertilized egg and to fully understand the technology further research in this area is necessary. It is also not known whether skin or blood makes better somatic donor cells. Dolly was produced against all predictions - showing how difficult prediction is! Ian is fully supportive of ambitious research projects and believes it is healthier to create the maximum possible opportunities for progress. However, mistakes are unavoidable and we should be cautious when applying research results to human medicine in the next century.