Waiting for a Miracle

"Stem cells in reproduction and in the brain" was the theme of a workshop in Kobe, Japan. An international group of stem cell researchers met at the invitation of the **Max Planck Society,** *the* **Schering Research Foundation** *and the Japanese* **Riken Institute** *to exchange ideas about the current status of their field – a field where advances in stem cell knowledge and technical know-how often collide with ethical mores and restrictions. Various questions were brought up concerning the apparent collision between this field of science and the current ethics issues this research raises. For instance, will resulting conflicts of interest hinder the future advancement of human stem cell research? Can this conflict help motivate and foster new ways of thinking in order to stimulate the search for solutions to prevent the conflict? The current controversy surrounding human stem cell research seems to indicate that it can.*

Photo: Science Photo Library

A hotly debated number is currently circulating among stem cell researchers: the number of cellular components in a "magic" cocktail that could "reprogram" – that is, reverse – the potential of terminally differentiated adult body cells into embryonic stem cells. Embryonic stem cells are pluripotent, which means they have the capacity to differentiate into virtually all cell types of the human body. Some estimates range from "around three" to "seven to ten" or "perhaps even twenty." Such a cocktail would solve the fundamental problem of obtaining human stem cells. The classical approach for deriving human embryonic stem cells is through a process that results in the destruction of an embryo. This approach is not ethically acceptable for many people, and in many countries it is illegal.

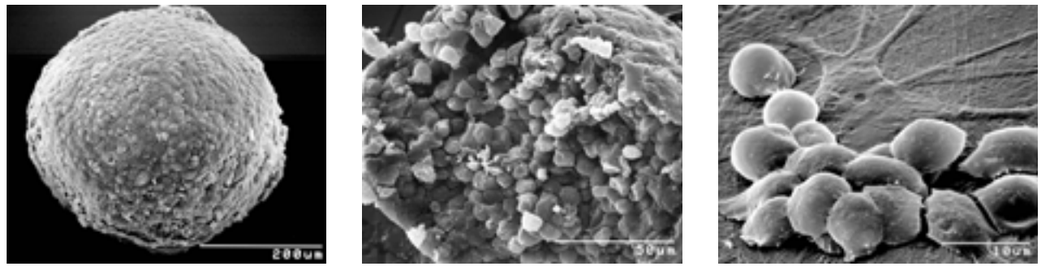
Nevertheless, there is a strong impetus to continue researching and to find better ways to derive human embryonic stem cells, as this field represents one of the greatest hopes in the treatment of diseases. It is hoped that stem cells can one day replace defective cells in tissues, such as pancreatic islet cells that produce too little or no insulin in diabetic patients, or nerve cells that have shut off dopamine production in patients with Parkinson's disease.

The central question raised at the workshop in Kobe was: How can pluripotent stem cells be obtained from differentiated adult cells – that is, without resorting to human egg cells and embryos – and healthy tissue developed from them? One goal would then be to replace diseased tissue from patients with the healthy tissue derived from pluripotent stem cells. These pluripotent stem cells cannot generate a living organism, but they can be used to form all tissues of the body, as well as egg and sperm cells. There are still many technical hurdles to overcome, but Hans Schöler, Director at the Max Planck Institute for Molecular Biomedicine in Münster, is confident that "Someday, stem cells will be used to heal diseases." Embryonic stem cells will probably be used primarily for diseases that arise from the malfunction of a single cell type, as is the case with diabetes and Parkinson's disease.

A Method under Discussion

To allow stem cells and new tissue derived from stem cells to be used for medical treatment, tailor-made embryonic stem cells must be made using a patient's own genetic material in order to prevent an immune response and rejection of the cells. At present, there is only one proven – and ethically controversial – method for obtaining human embryonic stem cells containing a patient's own genetic material: enucleated egg cells are injected with the nucleus obtained from a cell from the patient. Several as yet unknown molecules in the egg cell then "reprogram" the patient's cell nucleus back into its original pluripotent state. The egg is then cultured into an embryo and used to obtain pluripotent stem cells that are identical to the patient's own genetic makeup. The process results in the destruction of the embryo. A newer technology developed for obtaining mouse embryos uses only a single cell of an embryo, the blastomere, to derive a stem cell line. This procedure does not result in the destruction of the embryo, but so far, it has not been proven to work for obtaining human embryonic stem cells, and the ethical issue of what should be done with the remaining viable embryo still remains. "At the moment, this is the only method that leads to custom-made stem cells for patients," stresses Schöler. "All other options are still a long way off."

However, Hans Schöler sees one way to avoid these ethical issues: "Using a cellular strategy where the egg cell could be changed in such a way that it can no longer form a viable embryo – even if it receives the nucleus of a body (somatic) cell." This method has been demonstrated using mouse eggs and RNA interference, but it is likely suitable for deriving human embryonic stem cells. In this relatively new technology, a short fragment of ribonucleic acid (better known as siRNA) is injected into the egg cell to silence specific genes. This RNA fragment is constructed in such a way that it specifically reacts only with the messenger RNA of the gene that carries infor-



Photos: Katherina Psathaki/MPI for Molecular Biomedicine

Images of an embryoid body comprised of numerous embryonic stem cells (left), the cell cluster broken up (center) and as individual cells (right). Embryonic stem cells are pluripotent – they can differentiate into each of the approximately 200 cell types in the body.

What's in a Stem Cell

Developmental biologists use the term 'potency' to describe the capability of particular cells and tissues to differentiate. The least predetermined potency is the fertilized egg cell (oocyte): it is able to develop into a complete human being. In contrast, a skin cell can produce only more skin cells when it divides – it has considerably less developmental potential. The capacity to differentiate decreases in the following order, although the transitions are fluid and not well defined.

1. TOTIPOTENCY (also called omnipotency): The capacity of a single cell to generate a complete, viable organism. An example is the fertilized egg cell. For Hans Schöler, stem cells that can potentially differentiate into all the cell types of an organism are also totipotent.

2. PLURIPOTENCY: Pluripotent cells can differentiate into nearly all cell types, but can no longer create a living organism. An example here is embryonic stem cells.

3. MULTIPOTENCY: Multipotent cells can produce multiple different descendent cell types. Classic examples are the hematopoetic stem cells of the blood-forming system – the source of all blood cells.

4. OLIGOPOTENCY: The capacity of a cell to differentiate into only a few descendant cell types. Again, an example is blood formation: hematopoetic stem cells produce so-called lymphoid or myeloid stem cells, which then produce blood cells.

5. UNIPOTENCY: Finally, we have unipotent cells, which are able to produce only cells of their own type. An example here is fibroblasts (skin cells).

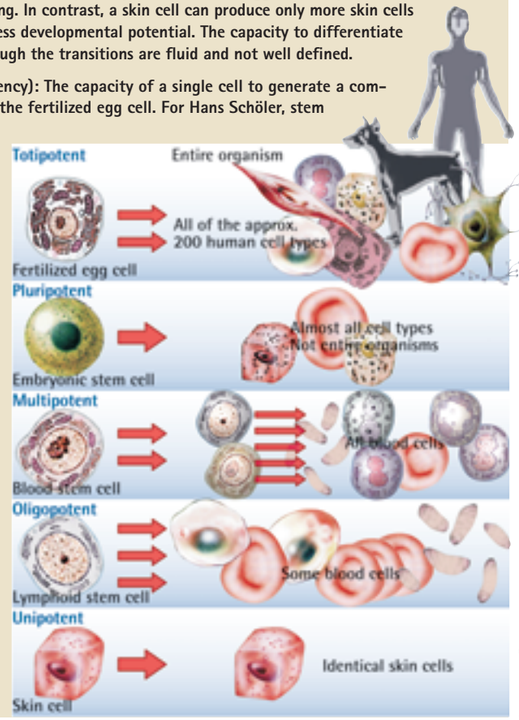


ILLUSTRATION: ROHRER

mation for forming the outer membrane of the embryo (the trophectodermal cells). The resulting RNA complex is recognized as foreign and faulty in the cell, and is broken down – so no membrane is formed. Outer membrane genes are targeted because the outer membrane cells are required for the embryo to embed itself and continue development as a fetus. The disruption of expression of membrane-specific genes results in "biological entities" that, unlike viable embryos, have no developmental future. "And if no embryo is formed, then none can be destroyed," says Schöler.

Stem cells provide, above all, the chance "to bring the study of diseases to the culture dish." For example, the cell nucleus from a child suffering from a genetic nerve disease could be transferred to an enucleated egg cell. The stem cells derived from this egg will have the same genetic defect as the child. Subsequently, these defective cells, together with normal cells, can be differentiated simultaneously into nerve cells to investigate their genetic or even protein differences. "Gene chips can show which genetic material is activated during development of diseased and healthy stem cells," explains the Max Planck researcher. "We could then understand the disease process – and later perhaps even correct for it."

Models That Help Patients

Many researchers are trying hard to create made-to-order embryonic stem cells. The starting material could be cells from patients suffering from paralysis, type I diabetes or hypogammaglobulinemia, an immune defect. Such stem cell lines would then be available as models of these diseases. Until recently, derivation of embryonic stem cells containing a nucleus from a patient's cell was close to reality. In May 2005, in the US journal Science, Hwang Woo Suk from the National University of Seoul presented an approach that could potentially be used to obtain patient-derived embryonic stem cells. Unfortunately, it was later brought to light – but not until late 2005, after the Kobe meeting – that the data was falsified. After this disclosure, Hwang resigned all of his official duties, and the incident has called into question the feasibility of cloning human stem cells in the near future.

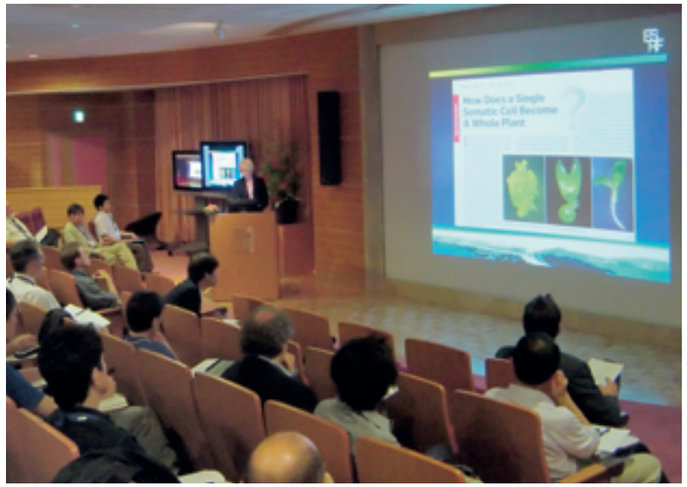
It was widely agreed in Kobe that advancements in technologies, such as the promise of therapeutic cloning, will open doors to novel effective therapies. For example, it is expected that robots could quickly pipette hundreds or thousands of substances onto the same stem cells and automatically check whether any could produce cells with desired effects, and if so which ones. Such high throughput screening could point to possible medications and strategies for treating disease. Suitable test robots and computer-compatible detection procedures are already available. "I am quite convinced that there will soon be important key publications here," says Hans Schöler.

In contrast, Shin-Ichi Nishikawa from the Riken Center for Developmental Biology in Kobe is primarily concerned with the question of what keeps a stem cell in the body in its particular and undifferentiated state over many years. He is using DNA chips to analyze which genes are active in quiescent and in differentiating stem cells. One of his results is that stem cells read their so-called housekeeping genes less frequently. These genes code for the cell's minimal requirements in that they take care of basic cellular functions, such as metabolism and cell structure development.

Nishikawa's institute is currently working on a comprehensive database in which the gene profiles of numerous stem cells are stored, as well as many of their forms. They hope to one day be able to use this to follow the path from the quiescent to the differentiating cell. The system is also available to other scientists so that, among other things, an attempt can be made to answer the question: Which substances are essential for a stem cell to remain a stem cell – and which factors nudge them in another direction?

The organism manages to store the body's stem cells over long periods of time by providing them with a range of niches. These "quiet zones" are the central focus of the work being done by Yann Barrandonn from the École Polytechnique Fédéral in Lausanne. Such a protective zone exists in the follicles of sensory hairs in rats. Some of the stem cells remain in this reservoir, where they divide slowly, while the rest migrate and contribute to the formation of the hairs.

In one of his experiments, Barrandonn transferred one of these stem cells to another, bare animal. It divided and multiplied there and, following deliberate injury, contributed to regeneration. The daughter cells could be transferred from the second animal to a third – and here, too, they retained their regenerative function. Barrandonn believes there is also another reservoir of self-maintaining stem cells beneath the cornea of the eye, in humans as well. It is also known that stem cell niches exist in bone marrow and in the intestines.

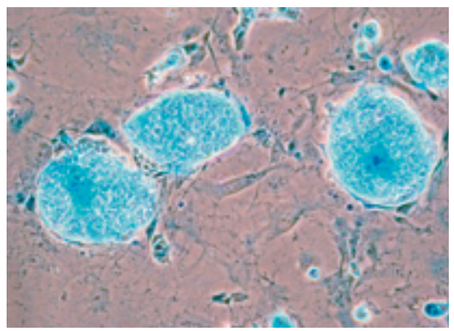


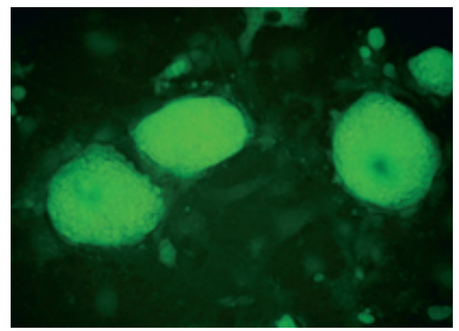
At the conference, researchers presented their latest results in stem cell research. The question of how many proteins are needed to reprogram somatic cells into stem cells was also passionately discussed.

The Chromosome Set – Doubled

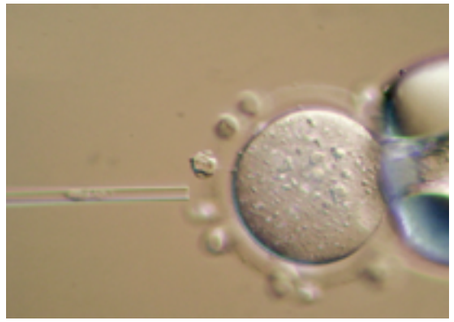
Another procedure to "developmentally reverse" or reprogram somatic cells uses existing embryonic stem cells. In an experiment that caused quite a stir, researchers from Kevin Eggan's team at Harvard University in Boston programmed skin cells from adults in such a way that they regained the characteristics of embryonic stem cells. Instead of the current method of transferring a nucleus into a mature egg cell, they fused the skin cells with embryonic stem cells. This created a stem cell chimera: a fusion product with a double set of chromosomes, or 92 instead of the normal human complement of 46. Despite this, in many respects, the construct behaved like an embryonic stem cell.

This high chromosome number is actually not as unusual as it seems at first glance, explains Max Planck researcher Tobias Cantz: "Many liver cells also have more than 46 chromosomes and manage to replicate prolifically." Hans Schöler already carried out experiments like Eggan's

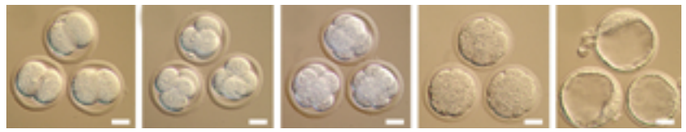




Fully differentiated cells were fused with embryonic stem cells (left). Here, the gene for the stem cell marker Oct 4 was linked to the reporter gene for green fluorescent protein (GFP). The reprogrammed body cell therefore fluoresces green and is an indication of pluripotency. The fusion hybrids are thus pluripotent stem cells that express the genes of both the somatic cell and the stem cell.



Stem cell researchers from Hans Schöler's department at the Max Planck Institute for Molecular Biomedicine work with mouse embryonic stem cells. The scientists inject the nucleus of a somatic cell into an egg cell (left) – a process called somatic nuclear transfer. The images show (from left) the development of cloned mouse embryos from the two-, four- and eight-cell stage through to the morula and blastocyst (150 to 200 cells). The white bars represent 10 micrometers.



a year ago using mouse cells. Since the procedure circumvents producing an embryo, it was lauded with much preliminary praise – also in the media – and awakened great hopes. But several hurdles must still be cleared before such new fusion cells can be put to therapeutic use.

Many hopes are directed at tracking down the substance or substances in embryonic stem cells that are capable of back-programming an adult cell nucleus. The cytoplasm of the stem cell alone is not enough. The proteins being sought, and perhaps other unknown cellular components, are presumably located within the cell nucleus of the embryonic stem cell, or adhere to its exterior. Based on this, Jeong Tae Do, like Cantz a colleague of Schöler, proposed in Kobe that the chromosomes of the embryonic stem cell should be removed or destroyed shortly before fusion – since the reprogramming requires only the proteins of the nucleus and not its DNA. This may prevent the fusion product from having double the number of chromosomes.

Philippe Collas from the University of Oslo is working in a similar area. He, however, wants to transform adult cells into embryonic stem cells through "selected extracts," that is, not by fusion with other cells. His approach, should it work, would be one step closer to creating a "stem cell cocktail." Indeed, he and his colleagues succeeded in altering skin cells using cell extracts: under the influence of their cocktail, three cell lines developed that showed considerable similarity to pancreas, heart muscle and immune cells.

Collas is now trying to isolate those components from his extract that have the power to reprogram – no small task with the very rich mixture of shredded cells. Collas is still not sure whether he has produced stem cells or only transformed one cell type into another, but believes that: "in any case, we have managed to program something." He adds that his work is rather like that of an alchemist, since much depends on chance – and not only in his lab: "At the moment, reprogramming a somatic cell is still like poking around in a black box."

Speakers at the workshop in Kobe, with Hans Schöler from the Max Planck Institute for Molecular Biomedicine in Münster at front left.

Is a Handful of Proteins Enough?

Nevertheless, Philippe Collas has an idea about what is happening in the cell nucleus when a somatic cell becomes a stem cell. And why this may require only a handful of proteins: the chromosomes in the cell nucleus are presumably packed so close together that only a few regions are accessible – namely only those genes the adult cell requires. An initial signal molecule could then be responsible for rearranging the DNA to condense previously active regions and expose, instead, those DNA regions that start the embryonic program: "And then perhaps another two or three factors will be sufficient to trigger the transformation."

Collas' work is being met from another, much more transparent direction by Peter Schultz and Sheng Ding, who work at the Scripps Research Institute in La Jolla, California. They are searching for "suspect" molecules, which they add to their cell cultures and then check to see if the cells transform into stem cells. They thus know precisely what is in their experimental mix – unlike colleagues who work with cell extracts or fusions and have to narrow down their experimental mix step by step. Among other things, the US researchers are searching for substances that turn muscle cells into their precursors.

The developmental biologist and Max Planck Vice President Herbert Jäckle is also certain that, one day, the much-desired spare parts box of stem cells will be a reality. "I am optimistic and convinced that it will work. But I am completely pessimistic in terms of the time line – that it can happen in five or ten years," says the Director at the Max Planck Institute for Biophysical Chemistry in Göttingen.

A Journey to an Unknown Land

Jäckle stresses that, at the moment, nobody really knows what happens to stem cells when they are kept in culture for weeks or months: "Even if we were to succeed in converting embryonic stem cells into insulin-producing cells, how can we be sure that these cells will do exactly what we want? How many of the hundreds of genes in such a cell do we want to test to see if they are functioning correctly? Five? Ten? Or perhaps fifteen? At some point, the decision as to when clinicians have done enough testing becomes purely arbitrary."

"And that's not all," continues Herbert Jäckle "How can we know how long the reprogrammed cell will continue to do its job?" And nobody knows yet what really takes place during the reprogramming of a somatic cell back to an embryonic cell. That it works at all is amazing enough – after all, this never occurs naturally. On the contrary, a finely tuned system ensures that each fertilized egg develops into a well-organized collection of billions of somatic cells that form an organism capable of reproducing itself.

Like all other stem cell researchers, Jäckle stresses that – should it come this far – only differentiated cells should be injected into a patient, never undifferentiated cells. Otherwise, there is a great danger that teratomas could form: a cancer-like proliferation of stem cells that suddenly grow uncontrollably into all kinds of tissue types.

However, the scientist expects that stem cells will one day be suitable for use in medical treatments: "The first contraceptive pill and the first heart transplants also had catastrophic consequences." At the same time, he warns against raising the public's hopes too much and too soon: "Many individuals are working in this field because they are convinced it will work one day, but we still have a long road ahead of us – the end of which many may never see." At present, stem cell therapy is a professed intention of science. "And some forget to make this clear," criticizes Jäckle. THILO RESENHOEFT

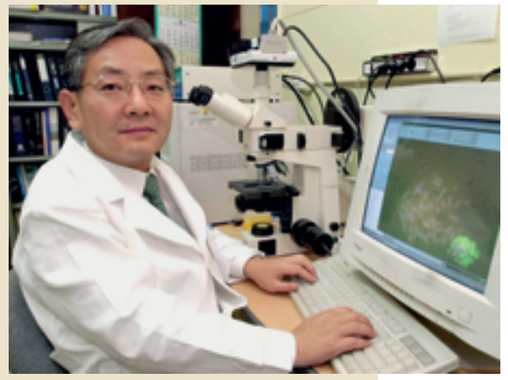
"It Is Impossible to Create Cloned Babies"

According to the South Korean stem cell pioneer Shin Yong Moon, there is no threat – neither at the moment nor in the foreseeable future – that a cloned human will be born. "It is impossible to create babies by reproductive cloning," the researcher from the Korean National University in Seoul said at the meeting in Kobe, Japan. Moon is one of the researchers who claimed, in 2004, to have cloned a human embryo for the first time in order to extract embryonic stem cells. It is now clear that human cloning is far more difficult than previously thought.

The scientist indicated that at least 100 cloned human embryos would have to be implanted in 100 uteruses in order to obtain one cloned human. That's how meager the success rate is – as yet derived only from animal experiments. "It is crystal clear that this is impossible," says Moon. In addition, there are not enough egg cell donors for such a project, which is the second reason why it would not work. And finally, reproductive cloning is prohibited in South Korea.

Moon advocates experimenting with those surplus embryos that accumulate in fertility clinics. His question: "Which is more ethical: discarding the embryos before they are used for research, or after?" However, the best cloning results were obtained with freshly donated egg cells from women under the age of 30, which is why his group prefers to work with these cells. At the same time, he stated that one of his colleagues had succeeded in transforming stem cells into insulin-producing cells. However, these cells show only a distant resemblance to pancreatic cells.

The Korean researcher expects that the Parkinson's nerve disease will someday be the first ailment for which stem cells will bring relief. "I am a physician. Before any application of stem cells, we need clear evidence that such cells are absolutely safe. But it is still nowhere near the time to be thinking about clinical trials," stressed Moon. First, human stem cells have to be established in the lab and grown without defects: "Tiny mistakes could happen in the culture dish, since embryonic stem cells are much like my son: unfortunately, neither of them always does what I tell them to." Currently, Moon sees the main application for embryonic stem cells in gaining a better understanding of diseases. Only then can we turn to the question of whether and how patients can be helped.



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