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Cloning and Embryonic Stem Cells: a New Era in Human Biology and Medicine

Davor Solter
Department of Developmental Biology, Max-Planck Institute of Immunobiology, Freiburg, Germany

The cloning of mammals using adult cells as nuclear donors has been achieved and the same procedure can be, at least theoretically, used to clone humans. Another recent technological advance, the derivation of human embryonic stem cells, opens up new possibilities in cell and tissue replacement therapy and heralds significant improvements in gene therapy. Besides suggesting new and potentially valuable medical applications, the insights gained through the use of these techniques could significantly enrich our understanding of basic mechanisms regulating human development. On the other hand, these preliminary results are viewed by many as the opening of the Pandora's box and there are loud voices clamoring that research in these areas be forbidden in perpetuity. I suggest in the following article that at present we do not know enough to make anything but an entirely emotional decision about future applications of these techniques. I try to summarize the current state of the knowledge in the field and indicate how much further research is necessary if benefits and drawbacks are to be properly understood.

Key words: chimera; cloning, human; cloning, organism; DNA, recombinant; embryo; embryonic stem cells; genetic engineering; germ line; human cloning; molecular cloning; transfection

Recent technological advances encompassing whole genome sequencing, cloning of mammals using adult cells as nuclear donors, and establishment of human embryonic stem cell lines came so quickly that they vastly outpaced our biological understanding and our grasp of the ethical, sociological, and moral dilemmas involved. It is essential that we try to calmly assess what these technologies do and do not offer, what is necessary to be done if we are to use them profitably and wisely – in essence, where we stand and where exactly do we wish to go. It is obvious from much recent writing about these themes that single-issue views, be they economical, scientific or ethical, dominate and that balanced approaches seem to have been lost. In the following I will try to delineate what it is that we know for sure, how much we can reasonably infer and to point out how much we do not know and will not know without further research. Knowledge of the genomes (the whole sequence of the human genome will probably be known in a few years) is an essential prerequisite for any rational approach toward mapping our future goals. We will need to know the function of the genes and the role they play singly or in various combinations in causing or contributing to a multitude of human diseases. In designing strategies for preventing or alleviating errors in our genetic makeup various approaches and methods, which we are at this moment barely glimpsing, will have to be developed and understood. It is very likely that at least some of these strategies will involve cloning and embryonic stem cell technologies, and it is therefore necessary to clearly understand their potential and use.

Cloning – Past, Present, Future

Cloning, i.e., the derivation of several genetically identical entities from a single individual has been with us for most of our history and anybody who has ever taken a twig of a plant and grown a new plant from it was engaged in cloning. We have always been fascinated by the possibility to clone animals, vertebrates, and finally humans, and though there are numerous important scientific questions associated with this subject, large sociological implications have also become obvious. For our purposes we will restrict ourselves to the recent advances in cloning adult mammals, and for background information the reader is referred to the detailed and recently published book by Di Berardino (1).

The cloning of mammals (and obviously other species) essentially involves replacing the genetic material of the egg with the genetic material of somatic cells from an embryo or adult. Once the technical aspects of the procedure were solved in the early eighties, numerous attempts to clone various laboratory or farm animals, using embryonic cells as nuclear donors, ensued with variable and rather modest success. The logical assumption at the time was that the closer the nuclear donor is in developmental terms to the egg, the more successful nuclear transfer is likely to be. This assumption, derived from nuclear transfer experiments done in frogs, also suggested that, although cloning from embryonic cells may be possible, cloning from adult cells will be considerably harder if not

unattainable. This way of thinking persisted until 1996 when the report of cloned sheep derived from an established cell line (2) suggested for the first time that cloning from adult cells may indeed be possible (3), and this possibility was realized one year later with the birth of Dolly (4). Rarely has a single scientific report caused so much public attention and controversy, but implications that the existence of Dolly presages the cloning of humans released a veritable flood of books, articles, and opinions. Where do we stand now, three years later, as far as cloning from adult mammals is concerned? Dolly is as far as I know still the only sheep cloned from adult somatic cells, but she was followed by several cows (5) and recently by a number of mice (6-8). Several other farm and laboratory animals may have been cloned as well, but these were only announced in the popular press and not described in scientific articles.

One can see from the above that the number of cloned mammals (I am only talking of cloning using adult cell nuclei since, as it will be obvious later, this is the only relevant method of cloning for our present discussion) is relatively very small and the question is what we can learn from the available data. First it is obvious that cloning is a very inefficient procedure as the following figures indicate. Over 400 manipulated embryos resulted in one sheep (4), 250 in eight calves (5) and about 2,500 manipulated embryos developed into 31 newborn mice (6). The results of postnatal development are even more disturbing as 4 out of eight calves died soon after birth, as did 9 out of 31 mice. It is obvious that at the present time we know very little about what is going on with the somatic nucleus transferred into the enucleated oocyte. It has to undergo reprogramming, a mysterious process the molecular aspects of which we have but a very dim understanding, but we can certainly speculate. After fertilization both the sperm and egg genome, which are at this time transcriptionally silent, are very likely reprogrammed, resulting in the activation of the embryonic genome and regular development. The egg cytoplasm, the end product of a complex differentiation process during oogenesis, is probably exclusive in its macromolecular content and uniquely able to achieve reprogramming. The question is now if it can reprogram any other genome beside egg and sperm? Successful cloning experiments indicate that it can, but the low success rate may suggest that it can do so only very poorly.

Figure 1. The hypothetical ways reprogramming may work. In one case we assume that reprogramming is extremely inefficient and that in the significant majority of nuclear transfer embryos none or hardly any reprogramming takes place. In the other case the assumption is that reprogramming is stochastic and that each gene has an equal chance of being reprogrammed or not. This results in a normal (Gaussian) distribution of the quantity of reprogramming of the entire genome. In either case we can suppose that a certain level of reprogramming must be reached for embryos to develop normally and grow into normal adults (right of dashed line). In the majority of embryos reprogramming is insufficient to support normal development and these embryos fail some time during development (left of dotted line). Between the dashed and dotted lines are embryos in which reprogramming is just sufficient enough to allow development till birth, but its failure results in a defective newborn or death after birth. This class of embryos is potentially the most troublesome and questions the safety of the cloning procedure.

There are two simple (there may be more) ways we can visualize reprogramming of somatic nuclei, both compatible with meager experimental data (Fig. 1). One is that the majority of reprogramming attempts fail very soon, resulting in the early loss of nuclear transfer embryos. This would imply that egg cytoplasm is almost totally unsuited for the reprogramming of the somatic nucleus. The other possibility is that reprogramming is essentially a stochastic event and that every gene which needs to be reprogrammed (either turned on or off) has an equal chance of being correctly reprogrammed or not. In this case the embryos will be distributed along the Gaussian curve in terms of the percentage of the genome being reprogrammed. Regardless which of these two scenarios is correct, the vast majority of embryos would fail due to insufficient or incomplete reprogramming and the consequence of the failure would be graver the later in development it occurs. When we discuss human cloning and if it should be permitted or forbidden, these data are highly relevant. Some failures in reprogramming will nevertheless be compatible with development to birth, but the high rate of newborns which died soon after birth (5,6) or even weeks later (9) indicate that the consequences of faulty reprogramming may be seen well after development has been completed. At the present time we do not know the extent of the necessary reprogramming, how many genes are involved, whether it depends on the kind of cell used as nuclear donor, if it is possible to assess the extent of reprogramming in early embryos, etc. This looming ignorance is quite sufficient to qualify human cloning for the time being as a medically unsafe and dangerous procedure which certainly should be discouraged until we know more.

Figure 2. Emphasis on specific goals to be achieved by cloning depends on the cloned subject. Cloning of laboratory mammals (mostly mice) will be done in order to explore basic biological questions like genome reprogramming, imprinting, and X inactivation. The cloning of farm animals will be predominantly done with practical applications in mind so that cloned animals can be used as bioreactors. For this purpose one will have to define a simple but effective cloning procedure which will allow the genetic modification of the nuclear donor cell in culture before it is used for cloning. The cloning of humans may have practical applications, but this possibility has raised numerous ethical and legal questions. Rules regulating the cloning of laboratory and farm animals will be essentially the same as those which currently ensure their safety and humane treatment. Many people believe that the possibility of cloning humans poses an entirely new set of ethical and legal dilemmas.

What then are the benefits to be derived from the application of cloning technology? This depends to a large extent on who or what is being cloned (7) (Fig. 2). Cloning of laboratory animals, notably mice, will provide us with many important answers to basic biological questions, some of which were alluded to above. Our understanding of the molecular biology of early mammalian development is very limited in comparison to what is known in non-mammalian species. However, we are beginning to identify and characterize genes expressed during mammalian preimplantation development and, as this knowledge accumulates, we will be able to ask whether these genes and how many of them are correctly expressed following nuclear transfer. If the panel of genes we are able to analyze is large enough, we may be able to predict which of the nuclear transfer embryos has the chance to develop normally.

Several of the mammalian genes with important functions during development are controlled by the process called imprinting. Briefly, imprinted genes are expressed depending on the parent of origin, so that one allele is active and the other not (10-12). Since imprinting is indispensable for normal development, some of the somatic cell nuclei used for cloning must have retained the correct imprint. However, we do not know if some of the failures of cloning are due to the loss of or incorrect imprinting. This possibility is likely since numerous failed cloned conceptuses displayed abnormal growth of the fetus or placenta and several imprinted genes are involved in growth control. Examining the status and expression of imprinted genes following nuclear transfer will significantly advance our understanding of imprinting and its role in cloning. The related basic question to be addressed by cloning experiments is X chromosome inactivation. When a female cell is used as nuclear donor one of its X chromosomes is permanently inactivated. Will this X chromosome be activated following transfer, since both X chromosomes are normally active in the early embryo? The paternal X chromosome is preferentially inactivated in extraembryonic membranes, another aspect of imprinting. Does this imprint hold in somatic female cells and will this paternal X chromosome in the transferred nucleus be preferentially inactivated in the trophectoderm? As we age and our cells divide, the ends of chromosomes – telomeres become shorter and shorter until they reach a critical length and the cell dies. It has been suggested that the shortening of telomeres is the basic mechanism of aging and that the length of telomeres is repaired in the germ line. Thus the question arose will nuclear transfer result in the repair of telomeres or will the animals cloned from adult cells start with much shorter telomeres which should negatively affect their health or life span? Telomeres of Dolly and a few other sheep cloned from fetal cells were recently examined and found to be significantly shorter than the telomeres of age-matched controls (13). Is this an additional danger inherent in cloning? These sheep are apparently normal, but it is obviously too early to tell if they will suffer any future adverse effects.

The cloning of farm animals has always had very practical goals in mind. Numerous attempts to use farm animals as bioreactors started with the advent of transgenesis. However, injection of DNA into fertilized eggs of farm animals in order to have the desired gene integrated and expressed rarely resulted in satisfactory results. The yield of transgenic animals was very low and the expression of the transgene was either poor from the beginning or became poor after a few generations. Random and uncontrolled integration of transgenic DNA more often than not results in the silencing of the introduced gene. If one could introduce DNA into cells in culture and then select for those cells in which the gene is expressed well and use the nuclei of such cells for cloning, the possibility of success may increase. Several recent reports indicate that this approach may be possible (14-16) and we will probably see much more of such work in the future with an obvious benefit for human medicine. While these experiments relied on the selection of cells that abundantly express the transgene, it is very likely that in the future, using homologous recombination (see later), one could introduce the desired transgene into a previously selected site in the genome, placing it under the control of the appropriate endogenous promoter and thus further ensuring its adequate expression in the tissue of choice.

Finally, what about the cloning of humans? Presently and for the reasons mentioned above I think that any such attempt would be irresponsible and in conflict with good medical practice. Safety issues are quite sufficient to discourage the cloning of humans today and there is no real need to involve any others. However, what if we can deal with the biological problems and ensure safety? After all, many procedures of assisted reproduction (intracytoplasmic sperm injection, ICSI; injection of egg cytoplasm) have not been properly tested, their safety has certainly not been established, but they are nevertheless used in in vitro fertilization centers around the world. This situation is unfortunate and, instead of using it to support the permissibility of cloning, the safety of these procedures should be established before we face another possible medically induced tragedy. If in the end we can ensure that cloning is safe or at least not worse than normal reproduction, I see very little reason to forbid it. It is unclear if cloning, despite many opinions to the contrary, raises an entirely new set of moral questions and dilemmas. Viewed as another aspect of ever-increasing reproductive freedom, one is hard pressed to come up with a valid argument to forbid it. We do not prevent reproduction of people who by all biological and sociological standards should not reproduce, it is entirely right that we do not, and it is a lasting shame that we once did. Why then forbid somebody to reproduce by cloning? It is likely that the arguments will continue, at this stage cloning should be forbidden because it is dangerous and, once it becomes safe, we can hope we will have the collective wisdom to decide if it is right. However, one aspect of cloning, an entirely technical one, i.e., nuclear transfer into the enucleated oocyte without subsequent development and birth may become an essential part of cell therapy of the future, and it is important that this aspect is not confused with cloning leading to reproduction.

Embryonic Stem Cells – Past, Present, Future

In order to understand the potential benefits and limitations of applying embryonic stem cell technologies in human medicine, we have to, however briefly, examine what is known about mouse embryonic stem cells, as this represents essentially everything that is known about this fascinating subject.

Mouse embryonic stem cells were derived from mouse blastocyst in the early eighties (17,18), by now we know how to derive them from any mouse strain (19) and we know quite a lot about their biology. A similar type of cells, named embryonic germ cells were derived from primordial germ cells in genital ridges (20-22). Although we do not know quite as much about embryonic germ cells, they are certainly very similar to embryonic stem cells and the observed differences may not be biologically crucial for their function. Two properties of embryonic stem cells proved to be especially important for their use in biological research and one of them – the ability to contribute to the germ line in chimeric animals – has revolutionized the study of gene function in mammals.

Embryonic Stem Cells Can Differentiate in Vitro and in Vivo

This ability has always fascinated biologists working with these cells, but the process and controlling elements involved are not fully understood. The intensity of research in this area has somewhat slackened in recent years; however, as we now see the tremendous medical potential, interest is likely to increase. It is quite obvious that differentiation of embryonic stem cells in culture can be directed along desired pathways and that combined with some kind of selection, a reasonably pure population of differentiated cells can be isolated (23-26).

Embryonic Stem Cells Can Contribute to the Germ Line in Chimeric Mice

Soon after the isolation of embryonic stem cells their totipotency was tested by injecting them into normal blastocysts (27), and it was shown that they can contribute to all adult tissues, most importantly to functional germ cells. This finding, combined with the ability to manipulate embryonic stem cells in vitro by homologous recombination, has revolutionized mouse genetics. Homologous recombination allows elimination or alteration of any known cloned gene, and this mutation can then be bred through the germ line derived from embryonic stem cells and its phenotypic consequences analyzed (28-30). It is no wonder that in the last ten years our understanding of how genes function within the entire organism has progressed significantly (31). Despite the tremendous importance of our ability to manipulate at will the genome of embryonic stem cells, the imminent medical application of these cells is more predicated on their ability to differentiate. However (as described later), manipulation of the genome of embryonic stem cells will also have significant use in future therapeutic modalities.

Human Embryonic Stem Cells

Human stem cell lines with properties at least similar to mouse cells have recently been isolated from human blastocysts (32), human embryonic stem cells, or from human primordial germ cells (33), human embryonic germ cells. At this moment the possible uses of human stem cells are all in the future and many complex biological, medical, and ethical issues have to be resolved. However, this is a good time to determine what are the possible applications and which experiments need to be done

in order to make them reality. We are assuming that human embryonic stem cells are going to be similar in their biological properties to mouse embryonic stem cells. This is not certain and it is possible that cell lines derived so far are not optimal anyway. However, if the cells are similar, we can envision their significant use in cell and tissue replacement therapy (34-37). We can envision, yes, but there are quite a number of hurdles to be overcome and it is therefore crucial that current research on human embryonic stem cells does not get forbidden for many irrelevant reasons before we know for sure if the cells are really useful.
Mouse embryonic stem cells can differentiate into many cell types and we now know a great deal about factors and culture conditions which induce differentiation into a specific cell type. It is very likely, with strong inducement of possible health benefits, that this type of research involving human embryonic stem cells will attract more attention and funding, thus enabling it to proceed faster. We can reasonably expect that conditions for differentiation of embryonic stem cells into simple tissues will be established quickly and that hematopoietic cells, muscle cells, neurons or even various endocrine cells will soon be available (Fig. 3). It is not so clear that the formation of complex organs or anything requiring a precise structure will be so easy. Making kidney or heart out of embryonic stem cells is definitely some time ahead, if ever, but there are some encouraging signs that some simple structures can be built in culture. Using various polymers as scaffolding and colonizing such scaffoldings with endothelial and smooth muscle cells, investigators succeeded in producing in vitro functional arteries (38,39) which were then successfully implanted into pigs. Likewise, a functional urinary bladder was produced from biodegradable polymers and formed into bladder shape upon which smooth muscle and urothelial cells were cultured. These bladders were implanted into dogs, replacing their own bladders, and they functioned normally for up to 11 months (40,41). One could envision that, by using complex cell and tissue interactions and various extracellular matrices, it may be possible to build complex organs like the kidney in culture by trying to recapitulate its normal development.

Figure 3. Possible use of human embryonic stem (ES) cells in cell and tissue replacement. An adult cell of a given individual serves as nuclear donor and ES cells are derived from the resulting blastocyst. ES cells are induced to differentiate into various cell types depending on the need and these are implanted into the original nuclear donor. All problems of allotransplantation are thus avoided.

Embryonic Stem Cells and Gene Therapy

Beside cell and tissue replacement, differentiated embryonic stem cell derivatives will likely be used in the future in various forms of gene therapy. At present gene replacement therapy for single gene genetic deficiencies is working rather poorly for various reasons. It is difficult to have a very efficient and reliable gene delivery system and, once the desired DNA is delivered into the host cells and actually integrates into their genomes, the expression of the transgene is usually poor and short-lived. The reason is most likely that the majority of random integration sites is not suitable and this results in the integrated gene being rapidly turned off. Using embryonic stem cells, we can target the selected gene to its appropriate place thus securing its proper expression. Once the correct integration event has been identified and the cell selected, embryonic stem cells can be induced to differentiate and thus targeted differentiated derivatives introduced into the patient.
There are several problems which have to be resolved before even a simple cell and tissue replacement therapy using differentiated embryonic stem cells can be implemented. Based on our experience with mouse cells, differentiation along one desired pathway is never complete and several other differentiated cell types are usually present in culture. It will thus be necessary to develop vital markers for each desired cell type so that the sought after cells can be selected for. Alternatively we can imagine introducing into embryonic stem cells a selectable marker gene so that only differentiation into the desired cell type results in viable cells and all others are selectively eliminated (23). Having several differentiated cell types in culture would be a nuisance but the presence of undifferentiated embryonic stem cells could be disastrous. Embryonic stem cells in mice when injected are always tumorigenic resulting in the growth of teratocarcinoma, and it will thus be essential to eliminate all undifferentiated embryonic stem cells prior to cell replacement therapy. We will have to extensively explore in mice what is the tumorigenic potential of embryonic stem cells, i.e., how many cells are necessary to produce tumors. These experiments may not be sufficient as mice are very short-lived in comparison with humans, so they may not be the ideal model to assess the long-term danger of embryonic stem cell-based therapy. To guard against a possibility of inadvertently causing tumors while attempting cell replacement, it might be necessary to introduce into all the cells before injection some kind of ”suicide” gene, so that if some embryonic stem cells escape the selection

procedure, they can be killed by its activation.

The Future

Now that we have visualized at least some of the potential benefits and also problems in using embryonic stem cells in human medicine, what is to be done in the near future in order to make their use possible? Intensive work in studying all aspects of differentiation, determining the necessary culture conditions, identifying promoter factors, differentiation factor and selection markers has to be initiated. Most of such work can and will be done using mouse embryonic stem cells or human embryonic stem cells isolated so far. However, the real benefit from embryonic stem cells will be achieved only if the appropriate cells are derived from each individual who needs them. Alternatives would be to accept, as is the current practice in organ transplantation, the life-long use of immunosuppressive drugs or to try and isolate large panels of embryonic stem cells so that the majority of patients find at least an approximate if not perfect match. These alternatives, although possible, are certainly not desirable. It is now clear that allogeneic transplantation has many negative long-term consequences (42) and that the continuous use of immunosuppressive drugs may lead to cancer (43,44). It may be possible to produce a large panel of embryonic stem cells so that a match in at least the major histocompatibility antigens is always available. However, since at least some of the injected cells should eventually participate in complex tissue interactions, such an imperfect match may not be sufficient. It is one thing to accept something if there is no better alternative, but with embryonic stem cells this is definitely not necessary. It is very likely that we will be able to derive an autologous embryonic stem embryonic stem line for everybody who needs it; there are currently three possible ways this can be accomplished and there may be more (Fig. 4).

Figure 4. Three possible ways to make individualized embryonic stem (ES) cells. The nucleus from an adult cell is introduced into 1) a human egg, or 2) non-human, mammalian egg, or 3) into ES cell cytoplast. Each of these procedures could theoretically reprogram the adult nucleus, but the question remains as to which is the most efficient and if all three result in functional ES cells.

First and most likely to succeed is to take the nucleus from a somatic cell of a given individual and to introduce it into an enucleated human egg, let this embryo develop to blastocyst, and derive an embryonic stem cell line as described (32). Though there may be technical problems, these are likely to be minimal and a major obstacle to this approach will be of ethical nature. This procedure is identical to the first stages of cloning and the resulting blastocyst which will be destroyed in order to make the cells is a potential human being. The emphasis is on ”potential”, since the actual likelihood of its development to term, were it to be implanted into the uterus, is very small given the success rate of cloning. ”Very small” is nevertheless not ”zero” and in countries where the destruction of embryos is not permitted this approach will not be possible. One could argue that the benefit to the health of an adult necessitates the destruction of such embryos analogous to the argument which supports abortion on medical grounds. However, this embryo would have been made in order to be destroyed and, regardless of the benefits, some individuals and societies may find this unacceptable.

What are the alternatives? One could maybe use a non-human (mouse, cow, sheep) egg as the nuclear recipient, and one such attempt was purportedly made and reported in the popular press (45). It is not at all clear how successful this was, and our limited experience with cross-species nuclear transfer has been rather disappointing (46). In addition, even if it works, the nature of the resulting blastocyst is not clear and some may argue that it is also a human embryo with all the problems this entails. The third option, the least controversial but also the least likely to succeed (at least based on our present knowledge), is to use cytoplast of existing human embryonic stem cells as the nuclear recipient and to hope that it can reprogram the nucleus of the somatic cell. There is some evidence (rather old, since nobody was much interested in such experiments until recently) that partial gene reprogramming can occur in such somatic cell cybrids (47). It is very likely, given the need for autologous embryonic stem cell lines and the ethical and legal problems connected with most obvious approaches, that work on this third alternative will intensify significantly.

Human embryonic stem cells have been isolated only recently and nobody really knows if they will be as useful as we would like to think. Nevertheless, their appearance has generated another flurry of mindless publicity, the majority of it coming from individuals who are ignorant of the biology of the system and who have not taken the time to become informed and to reflect. Endless arguments as to whether embryonic stem cells are equivalent to embryos (they are not) and whether one should be allowed to work with them even if one is not allowed to isolate them has highlighted this controversy. Each country seems to be coming up with its own set of near-sighted and hasty decisions. It is refreshing to see that the National Bioethics Advisory Commission to the US President has finally realized the absurdity of allowing federal funds to be spent on the research of embryonic stem cells

but not on their derivation. Numerous opinions, for and against, a number of which are quoted here, can be found in any weekly issue of Science and Nature and in the popular press (48-54) for anybody interested in the moral and ethical questions related to the use of human embryonic stem cells.
Since the applications and further development of human embryonic stem cell technology raise so much controversy, are there any alternatives which can be used instead? It seems that our bodies contain a large number of various stem cells, more than it was thought up until now. We know that most of our epithelia are constantly being renewed and that stem cells are the basis for this process. Recent results indicate that, in addition to these, there are several multipotential stem cell populations which can conceivably be used in cell and tissue replacement (55). Neural crest stem cells were isolated from fetal peripheral nerve and they give rise to neurons and glia when transplanted into adults (56,57). If such cells can also be isolated from adult peripheral nerves, one can easily imagine their application in the therapy of various degenerative diseases of the nervous system.
Neural stem cells have been derived from adult ependymal cells and these cells can differentiate into neurons and astrocytes (58). It is interesting to note that neural stem cells are not only able to repopulate the central nervous system but can also give rise to hematopoietic cells and repopulate the bone marrow (57,59). These results indicate that some stem cells may have quite a broad differentiation potential and are not restricted to the tissues and organs in which they originate. Further examples of such multipotential stem cells are human adult mesenchymal stem cells which can differentiate into adipocytes, chondrocytes, and osteocytes (60) or bone marrow stem cells which gave rise to hepatic oval cells and were able to colonize the liver after hepatotoxic injury (61). It is evident from these few examples that many needs for cell and tissue replacement therapies could be satisfied by the stem cells present in every adult and that research along these lines should be intensified.
Why then bother with human embryonic stem cells, especially since they are so controversial and we know relatively little as to how to use them? Firstly, it may well be that some types of organo- typic stem cells cannot be isolated but only derived from embryonic stem cells. It could also be argued that derivation of embryonic stem cells from a given individual should satisfy all future needs without having to keep isolating different stem cells with restricted potency. Secondly and more importantly, we should consider not only the imminent use of human embryonic stem cells in cell and tissue replacement, but also what are the conceivable long-term future applications. At the present time we can envision several applications of this technology, some relatively simple and straightforward and some certainly requiring substantially more information (Fig. 5). As described before, embryonic stem cells could be used after in vitro differentiation and selection for cell and tissue therapy. The same cells could be used as vehicles for gene therapy and there are numerous levels on which this could be done. The most logical and, dare one say, ”natural” use can be illustrated by the following example. Different hemo- globinopathies like sickle cell syndrome or thalass- emia syndromes are caused by various mutations affecting the structure or synthesis of a- and b-globin. Deriving embryonic stem cells from an affected individual by any of the methods described above would give us the opportunity of replacing the affected gene using homologous recombination. Expression of the newly introduced gene could be assessed in vitro and the desired embryonic stem cell clone selected, induced to differentiate into hematopoietic stem cells and used to repopulate the patient's bone marrow. This would then represent the complete and permanent cure of the genetic defect.

Figure 5. Possible short- and long-term uses of embryonic stem (ES) cells. The simplest is the use of ES cells following differentiation and selection in vitro for cell and tissue replacement. The cells can also be used as vehicles for gene therapy in which the mutated endogenous gene is replaced by a correct copy. It is also conceivably possible to correct the genetic mutation present in the embryo from which EC cells are derived. Corrected ES cells serve as the nuclear donor to again produce a normal embryo, which after implantation into the uterus will give normal progeny to a couple in which both members are homozygous for the same mutation.

We can imagine the further, albeit rare, situation in which both parents are homozygous for a specific genetic disorder. There is no possibility today for them to have their own genetic but normal progeny. One can visualize that such a couple would in the future produce several blastocysts by in vitro fertilization. Derivation of embryonic stem cells and correction of the genetic defect would follow. Once the embryonic stem cell clone with a normal genotype is established, nuclei from these embryonic stem cells would be introduced into the enucleated egg and the resulting embryo implanted. In such a way the mutant embryo would essentially be recloned following the correction of the mutation and a normal child could be born to this couple. Most of these applications are obviously in the future and some of them may be difficult or impossible but there is no obvious biological reason

why they should not work. One should bear in mind that if DNA replacement using embryonic stem cell technology is possible in order to correct mutations, DNA addition with putative genetic ”enhancements” should also be possible. At the present time, the specter of genetic enhancement is always raised by the critics of this research and, although we do not know now what genes to add to improve our genetic heritage, it does not mean that we will never know. One has to keep this in mind and to hope that, as our technical abilities and biological knowledge advance, the wisdom to use them wisely and ethically will keep pace.

Conclusions

The cloning of mammals using adult cells as nuclear donors and the establishment of human embryonic stem cells are two technologies with the potential to revolutionize human medicine and reproduction. Before their potential could be truly assessed, both technologies became mired in numerous relevant and irrelevant moral, ethical, and legal controversies. In these discussions it is often ignored that we actually do not know whether all or any of the putative uses will prove to be practical or even possible. Attempts to forbid a priori the use of these technologies will deprive us of essential biological knowledge and of potential beneficial therapeutic modalities. The cloning of humans should be discouraged for now on the grounds of safety, and considerably more research is necessary to establish which parameters determine the outcome of nuclear reprogramming and cloning. The biology of human embryonic stem cells is in its infancy and, while many experiments can be done using mouse embryonic stem cells, techniques aimed at practical applications in human medicine must be ultimately established using human cells. At the present time we have clear guidelines regulating tissue and organ transplantation, and work involving human embryonic stem cells can easily be controlled using the same existing regulations. Every country and ultimately every individual will have to decide for themselves how they feel about the moral aspects of the use of these technologies, but strident demands to forbid them before they are fully understood are short-sighted and potentially harmful. If we are asked to give up something, it seems logical that we should know exactly what it is that we are giving up.

References

1 Di Berardino MA. Genomic potential of differentiated cells. New York (NY): Columbia University Press; 1997.

2 Campbell KHS, McWhir J, Ritchle WA, Wilmut I. Sheep cloned by nuclear transfer from a cultured cell line. Nature 1996;380:64-6.

3 Solter D. Lambing by nuclear transfer. Nature 1996;380:24-5.

4 Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KHS. Viable offspring derived from fetal and adult mammalian cells. Nature 1997;385:810-3.

5 Kato Y, Tani T, Sotomaru Y, Kurokawa K, Kato J, Doguchi H, et al. Eight calves cloned from somatic cells of a single adult. Science 1998;282:2095-8.

6 Wakayama T, Perry ACF, Zuccotti M, Johnson KR, Yanagimachi R. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. Nature 1998;394:369-74.

7 Solter D. Dolly is a clone – and no longer alone. Nature 1998;394:315-6.

8 Wakayama T, Yanagimachi R. Cloning of male mice from adult tail-tip cells. Nat Genet 1999;22:127-8.

9 Renard J-P, Chastant S, ChesnÇ P, Richard C, Marchal J, Cordonnier N, et al. Lymphoid hypoplasia and somatic cloning. Lancet 1999;353:1489-91.

10 Solter D. Differential imprinting and expression of maternal and paternal genomes. Ann Rev Genet 1988;22:127-46.

11 Bartolomei MS, Tilghman SM. Genomic imprinting in mammals. Ann Rev Genet 1997;31:493-525.

12 Surani MA. Imprinting and the initiation of gene silencing in the germ line. Cell 1998;93:309-12.

13 Shiels PG, Kind AJ, Campbell KHS, Waddington D, Wilmut I, Colman A, et al. Analysis of telomere lengths in cloned sheep. Nature 1999;399:316-7.

14 Schnieke AE, Kind AJ, Ritchie WA, Mycock K, Scott AR, Ritchie M, et al. Human factor IX transgenic sheep produced by transfer of nuclei from transfected fetal fibroblasts. Science 1997;278:2130-3.

15 Cibelli JB, Stice SL, Golueke PJ, Kane JJ, Jerry J, Blackwell C, et al. Cloned transgenic calves produced from nonquiescent fetal fibroblasts. Science 1998;280:1256-8.

16 Anderson GB, Seidel GE. Cloning for profit. Science 1998;280:1400-1.

17 Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature 1981;292:154-6.

18 Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci USA 1981;78:7634-8.

19 Brook FA, Gardner RL. The origin and efficient derivation of embryonic stem cells in the mouse. Proc Natl Acad Sci USA 1997;94:5709-12.
20 Matsui Y, Zsebo K, Hogan BLM. Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture. Cell 1992;70:841-7.
21 Resnick JL, Bixler LS, Cheng L, Donovan PJ. Long-term proliferation of mouse primordial germ cells in culture. Nature 1992;359:550-1.
22 Rossant J. Immortal germ cells? Curr Biol 1993;3:47-9.
23 Li M, Pevny L, Lovell-Badge R, Smith A. Generation of purified neural precursors from embryonic stem cells by lineage selection. Curr Biol 1998;8:971-4.
24 Palacios R, Golunski E, Samaridis J. In vitro generation of hematopoietic stem cells from an embryonic stem cell line. Proc Natl Acad Sci USA 1995;92:7530-4.
25 Nakano T, Kodama H, Honjo T. Generation of lymphohematopoietic cells from embryonic stem cells in culture. Science 1994;265:1098-101.
26 Chen U, Kosco M, Staerz U. Establishment and characterization of lymphoid and myeloid mixed-cell populations from mouse late embryoid bodies, "embryonic-stem-cell fetuses". Proc Natl Acad Sci USA 1992;89:2541-5.
27 Bradley A, Evans M, Kaufman MH, Robertson E. Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. Nature 1984;309:255-6.
28 Thomas KR, Capecchi MR. Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. Cell 1987;51:503-12.
29 Doetschman T, Gregg RG, Maeda N, Hooper ML, Melton DW, Thompson S, et al. Targetted correction of a mutant HPRT gene in mouse embryonic stem cells. Nature 1987;330:576-8.
30 Capecchi MR. Altering the genome by homologous recombination. Science 1989;244:1288-92.
31 Muller U. Ten years of gene targeting: targeted mouse mutants, from vector design to phenotype analysis. Mech Dev 1999;82:3-21.
32 Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science 1998;282:1145-7.
33 Shamblott MJ, Axelman J, Wang S, Bugg EM, Littlefield JW, Donovan PJ, et al. Derivation of pluripotent stem cells from cultured human primordial germ cells. Proc Natl Acad Sci USA 1998;95:13726-31.
34 Gearhart J. New potential for human embryonic stem cells. Science 1998;282:1061-2.
35 Rossant J, Nagy A. In search of the tabula rasa of human cells. Nat Biotechnol 1999;17:23-4.
36 Smith A. Cell therapy: in search of pluripotency. Curr Biol 1998;8:R802-4.
37 Solter D, Gearhart J. Putting stem cells to work. Science 1999;283:1468-70.
38 Ferber D. Lab-grown organs begin to take shape. Science 1999;284:422-5.
39 Niklason LE, Gao J, Abbott WM, Hirschi KK, Houser S, Marini R, et al. Functional arteries grown in vitro. Science 1999;284:489-93.
40 Hubbell JA. A new-for-old urinary bladder. Nature 1999;398:198-9.
41 Oberpenning F, Meng J, Yoo JJ, Atala A. De novo reconstitution of a functional mammalian urinary bladder by tissue engineering. Nat Biotechnol 1999;17:149-55.
42 Leiper AD. What is in store after stem-cell transplantation? Lancet 1999;353:1544-5.
43 Hojo M, Morimoto T, Maluccio M, Asano T, Morimoto K, Lagman M, et al. Cyclosporine induces cancer progression by a cell-autonomous mechanism. Nature 1999;397:530-4.
44 Nabel GJ. A transformed view of cyclosporine. Nature 1999;397:471-2.
45 Marshall E. Claim of human-cow embryo greeted with skepticism. Science 1998;282:1390-1.
46 Solter D, Aronson J, Gilbert SF, McGrath J. Nuclear transfer in mouse embryos: activation of the embryonic genome. Cold Spring Harbor Symposia on Quantitative Biology 1985;L:45-50.
47 Lipsich LA, Kates JR, Lucas JJ. Expression of a liver-specific function by mouse fibroblast nuclei transplanted into rat hepatoma cytoplasts. Nature 1979;281:74-6.
48 Lanza RP, Arrow KJ, Axelrod J, Baltimore D, Benacerraf B, Bloch KE, et al. Science over politics. Science 1999;283:1849-50.
49 Wolpert L. Is science dangerous? Nature 1999;398:281-2.
50 Abbott A. 'Don't try to change embryo research law'. Nature 1999;398:275.
51 Gordon JW. Genetic enhancement in humans. Science 1999;283:2023-4.
52 Wadman M. NIH stem-cell guidelines face stormy ride. Nature 1999;398:551.
53 Marshall E. NIH plans ethics review of proposals. Science 1999;284:413-5.
54 Wadman M. Ethicists urge funding for extraction of embryo cells. Nature 1999;399:292.
55 Vogel G. Harnessing the power of stem cells. Science 1999;283:1432-4.
56 Morrison SJ, White PM, Zock C, Anderson DJ. Prospective identification, isolation by flow cytometry, and in vivo self-renewal of multipotent mammalian neural crest stem cells. Cell

1999;96:737-49.
57 Sikorski R, Peters R. Some nerve! Science 1999;284:453.
58 Johansson CB, Momma S, Clarke DL, Risling M, Lendahl U, Frisén J. Identification of a neural stem cell in the adult mammalian central nervous system. Cell 1999;96:25-34.
59 Bjornson CRR, Rietze RL, Reynolds BA, Magli MC, Vescovi AL. Turning brain into blood: a hemato- poietic fate adopted by adult neural stem cells in vivo. Science 1999;283:534-7.
60 Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143-7.
61 Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, et al. Bone marrow as a potential source of hepatic oval cells. Science 1999;284:1168-70.

Correspondence to:
Davor Solter
Department of Developmental Biology
Max-Planck Institute of Immunobiology
Stubeweg 51
79108 Freiburg, Germany
solter@immunbio.mpg.de