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Cloning: A Definition

In this paper, cloning is defined as producing an individual or an embryo that is genetically identical to another individual or embryo, genetically identical in the sense of sharing the same nuclear gene set. Gene set, not genes, because genes may be changed or lost in the course of nuclear development; nuclear in order to exclude mitochondrial genes. Thus Dolly, the sheep produced in the Roslin Institute in Edinburgh by substitution of an adult nucleus for an oocyte nucleus, is regarded as a clone of her nuclear donor whether or not the two sheep share the same small set of mitochondrial genes.

Cloning can occur by embryo splitting or by nuclear substitution.

Cloning by Embryo Splitting

An embryo can split spontaneously, or can be split artificially, so that two or more individuals develop from a single fertilised egg.

Spontaneous embryo splitting occurs in some if not all animal species, including human monozygotic twinning (or higher multiples). Human monozygotic twins are very similar to one another in appearance and in other ways, more so than are dizygotic twins, but they also show marked differences from one another. These differences can be due to a different uterine environment (e.g. differential vascularization) or to different experiences after birth.

Artificial embryo splitting can be carried out at any preimplantation stage, from the two-cell to the blastocyst. In mice it is difficult to make monozygotic twins by splitting, and higher multiples have never been made. On the other hand in sheep and cattle, four or more genetically identical progeny can be made from a single embryo by splitting, and this procedure has been used in practice, to increase the numbers in economically valuable breeds.

Cloning by Nuclear Substitution

The genetic material can be removed from an oocyte, followed by the substitution of a nucleus taken either directly from an embryo, or from a cultured cell derived from an embryo, fetus or adult animal. The techniques involved in nuclear substitution will be described by Dr. Bulfield.

In sheep and cattle, successful development to term has been achieved with donor nuclei from embryos throughout the period of cleavage, but nuclei from post-implantation stages do not support development if transferred directly into enucleated oocytes. Since cleavage stage embryos derived by nuclear substitution can be used as donors for a further round of nuclear substitution, quite large groups of genetically identical animals can be produced. These can be used to propagate breeds of high genetic and economic worth ; they are also valuable as a means of minimising genetic variation in controlled trials.

When the donor nuclei are taken from cultured cells in a state of quiescence, cloning of sheep by nuclear substitution has been achieved not only from embryos but also from fetal fibroblasts, and in the case of Dolly from an adult mammary gland. Although the success rate is at present low, the technique is of great economic importance, since the cultured cells can be genetically manipulated, removing undesirable genes and adding genes that would be economically valuable, for example in the production of human pharmaceutical proteins.

If cloning of adult animals by nuclear substitution can be repeated and made more efficient, it will allow the multiplication of genetically desirable individuals in sheep (and perhaps cattle), not merely the propagation of genetically valuable breeds. If the technique could be extended to dogs and cats, it would permit pet owners to «copy» pets that they have loved and that are nearing the end of their natural lives. Wealthy people might be prepared to pay large sums for this facility, but it should not be expected that the «copy» (though genetically identical) would be an exact replica of the original. Original and «copy» would be gestated in different mothers, with perhaps very different postnatal upbringing, so they would be likely to differ more from one another than do monozygotic twins.

Basic Science Implications

The results reported by the Roslin Institute in Edinburgh raise many fascinating questions, of fundamental importance to our understanding of the biology of development. They will be hard to study unless the Roslin nuclear substitution technique can be extended to laboratory animals, such as mice or rabbits.

The fact that the nucleus of a differentiated cell, whether a fetal fibroblast or an adult mammary gland cell (either terminally differentiated or stem cell), can support the whole of development, from the egg to the adult, would not have been predicted five years ago. Extensive reprogramming of the genetic material must occur: genes expressed in the differentiated cell

must be switched off, and other genes switched on in the correct sequence to direct normal embryonic development. This reprogramming must be brought about by the cytoplasm of the recipient oocyte. What factors are involved? Is the genetic reprogramming complete, or is partial reprogramming sufficient? How does the quiescent state of the donor nucleus contribute to the reprogramming? What is the molecular basis of quiescence?

It is believed that the telomeres, DNA sequences at the ends of each chromosome, shorten at each mitotic division if the enzyme telomerase is absent, but its activity is restored in immortalised (eg cancer) cells and is thought to be expressed in the germ cell lineage. Is telomerase present in the sheep oocyte, and if so is it adequate to restore telomere length in the chromosomes of a nucleus taken from a differentiated cell? If not, will the chromosomes of a cloned sheep be shorter than normal? If telomere shortening contributes to the ageing process, will cloned animals have a shorter life span than normal? In mice the gene for telomerase has been removed by genetic manipulation, and no phenotypic effect was observed for several generations; but mice have unusually long telomeres.

When the donor nucleus comes from an adult animal, additional questions arise. During the course of the donor's life, the genetic material in its somatic nuclei will become progressively changed by mutation. Will the clone show malformations, or an increased tendency to develop cancer? Will its expectation of life be shortened in respect of the age the donor had already achieved, by ageing processes unconnected with telomere shortening?

Mitochondrial Diseases

Successful cloning by nuclear substitution in laboratory animals will also facilitate the study of mitochondrial stability. The clone will initially contain almost all the mitochondria in the enucleated oocyte, plus some donor mitochondria (the number will depend on the technique by which the donor nucleus is transferred). Will the initial proportion remain stable? Or will the majority component take over? Or will it be the mitochondria corresponding to the donor nucleus that win out?

These questions are not just of academic interest. Mitochondria are mainly concerned with energy metabolism. Mutations in mitochondrial genes are responsible for a growing number of serious diseases which, since mitochondria are transmitted only from the mother, will be passed on by an affected woman to all her children. If the genetic material from the oocyte of such a woman could be transferred to an enucleated oocyte from an unaffected woman, which would then be fertilised by the father's sperm, the resulting child could grow up free of the mitochondrial disease - but only if the normal mitochondria persisted in sufficient numbers relative to any defective mitochondria that were transferred.

Such mitochondrial gene therapy would be a possible application of the nuclear substitution technique, but it would not involve cloning. Only one embryo would develop, which would have

the same mix of its mother's and father's nuclear genes as any normal child. Since the donor nucleus would be from an oocyte and not from a somatic cell, the abnormalities and developmental problems surrounding the reprogramming of a somatic nucleus would not arise: indeed, nuclear transfer from one mouse egg to another has been carried out on many occasions, with a high success rate in terms of liveborn young.

Human Reproductive Cloning

Cloning which involves implantation in the uterus, and hence production of a cloned fetus or baby, is known as reproductive cloning. Concerns about human reproduction cloning have focused on the possibility of «copying» some existing individual by nuclear replacement from a somatic cell. Such a procedure could not be contemplated at the present time, on account of the very high failure rate and the high incidence of abnormal development that accompanies nuclear replacement cloning in sheep.

If the techniques were ever to be developed to a point where they were both safe and efficient, there would still be major ethical issues to be confronted. These are addressed in the papers by Dr. Puigpelat and Dr. Shenfield. The ethical objections to «copying» a human being would be least where no fully formed personality had yet developed, for example cloning of a dead or dying fetus or newborn baby conceived by IVF after many years of infertility and many unsuccessful attempts at assisted reproduction, then fatally injured through some tragic accident.

Homosexual couples might one day wish to make use of the somatic nuclear replacement technique, using a nucleus from each partner to produce two embryos which could be aggregated together before transfer to the uterus. The resulting baby would be a chimera, not a clone: such babies would not be genetically identical to either partner, but would show the same range of variation, the same random and unpredictable mix of characteristics from the two partners, as would a normally conceived baby. The same approach could not be used for a heterosexual couple, since aggregation of a female and a male embryo would be likely to lead to abnormal sexual development.

Non-reproductive Cloning

The reprogramming of a somatic nucleus that is involved in cloning by nuclear transfer could be exploited for therapeutic purposes. Attempts could be made to treat cells from cloned embryos *in vitro*, without any transfer to a uterus, in such a way as to generate stem cells which would of course be fully compatible immunologically with the donor of the nucleus. By this means it might be possible to grow, for example, muscle tissue for the repair of a damaged heart, neural tissue for the treatment of degenerative diseases of the nervous system like

Parkinson's disease, skin for a badly burned patient, or bone marrow for blood disorders, all generated by transfer of the patient's own nucleus.

Such therapeutic measures, even if they prove feasible, are far in the future. They will require a great deal of animal research on embryos cloned by nuclear substitution, to identify the appropriate signals required to generate various types of stem cell. If successful, this would need to be followed by some equivalent research on human embryos cloned by nuclear substitution, before the methods could be applied clinically. If laws are passed prohibiting human cloning, it would therefore be desirable to distinguish between human reproductive cloning on the one hand, and on the other the use of cloning by nuclear substitution for *in vitro* research purposes, that could ultimately lead to a therapeutic approach of considerable value.