Cloning, clones and clonal disease

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ABSTRACT - In the past, cloning has been familiar to plant breeders because many plants can be easily reproduced in this way, bypassing the lengthy process of cross-fertilisation. Recently, the concept of cloning has become popular in human biology and medicine on two accounts. First, individual genes can be cloned from the enormous complexity of the DNA that makes up the human genetic material. It is expected that, within a few years, all the estimated 100,000 human genes will be isolated by this approach. This should make it possible to identify all the genes that determine the individual characteristics of human beings, including those responsible for causing human diseases or for making people more or less susceptible to pick up diseases from the environment. Cloned genes made into pharmaceutical products are already in use for treating a variety of diseases, from hormonal deficiencies to certain types of anaemia.

According to the Oxford English Dictionary, a clone is 'any group of cells or organisms produced asexually from a single sexually produced ancestor'. Since the notion of reproduction of like from like is at the core of the concept of life, we are dealing with clones all the time in biology and medicine, whether or not we realise it.

Cloning means making copies, so the term can be applied to different sorts of objects. In many cases, making artificial copies of a natural object may not produce much or any significant benefit over the naturally available original: for instance, why make copies of fireflies when there are already so many of them? If, however, we have identified a rare variety, for instance a firefly that can flash different colours depending on the longitude, the ability to make copies could be desirable. Thus, cloning does not just aim to make copies: often, the aim is to make copies of something that is rare.

Cloning molecules

Chemists have analysed and worked with chemical substances, including biological molecules and macro-molecules, for centuries. An obvious prerequisite for this type of work has been the purity of the molecules concerned: indeed, extraction, fractionation and purification have been, par excellence, the currency of chemistry. However, when it comes to a collection of a vast number of elements similar in structure but quite different in informational content, such as human DNA, their extraordinary complexity defies the power of conventional chemistry. What previously could not be achieved by conventional chemistry has been achieved since the early 1970s thanks to the astute use of microbial genetics and genetic engineering. In practice, cloning a DNA molecule has come to mean that the molecule is inserted into a vector, for instance a plasmid, capable of replicating inside a bacterial cell. In this way, it is possible to obtain in pure form any minute portion of the human genome, even though as little as one part per million may be present in the original material. In this respect, the phrase 'recombinant DNA revolution' is not an exaggeration. Cloning a gene has become synonymous with identifying a gene, because it is only through cloning that enough of that gene can be obtained for its full characterisation. In addition, in many cases the cloned gene can be transferred to an 'expression vector', so that large amounts of its protein product can also be produced. Thus, cloning has been at the core of the development of biotechnology.

Cloning cells

A vector and a host cell are always needed in order to clone a DNA molecule, so cloning of that molecule goes hand-in-hand with cloning of the host cell. However, a different concept is that of cloning a cell for its own sake. In the case of unicellular organisms, the difference between bulk growth and cloning is essentially a function of density. Whenever the density is low, cloning takes place: for instance, when microbial colonies are grown on ordinary culture plates for the purpose of identification or, more appealingly, when a single fungal hyphce produces a mushroom. By contrast, for multicellular organisms, in vitro cloning can again be a method for isolating a particular cell type from a heterogeneous population of cells. In this respect, it is not trivial to ask if and what properties of the original cell will be retained in the clone. In most cases, the genome of somatic cells in different tissues is not modified, so their differentiation must result from different patterns in gene expression that, when stable, is called 'epigenetic'. Cloning cells is one way to analyse epigenetic phenomena: perhaps the prototype of these is the inactivation of one of the two X chromosomes in somatic cells, a fundamental normal event in the development of female mammals. For instance, if fibroblasts are cloned from a woman who is heterozygous at the X-linked G6PD locus for two electrophoretically different variants
(G6PD B and G6PD A) of this enzyme, some clones will be obtained that make only G6PD B and others that make only G6PD A.

Thus, somatic cell clones can be used to study differentiation, and the behaviour of clones in vitro may reflect, at least in part, the way they behave in vivo. The one case in which cell differentiation is associated with true physical changes in DNA is that of lymphoid cells: functional immunoglobulin (Ig) genes are assembled from their component parts in developing B cells, and functional T cell receptor (TCR) genes are assembled from their component parts in developing T cells. This also applies in the case which has led to the most spectacular application of somatic cell cloning. When a B cell producing a specifically desirable type of Ig is grown up as a clone, the resulting 'monoclonal' antibody can be isolated from the culture and used as a reagent. If enough people have an interest in its use, the clone can eventually be marketed.

Cloning organisms

Cloning unicellular organisms means mimicking their normal growth; cloning mammalian somatic cells means exploring in the laboratory what those cells tend to do in the body. By contrast, cloning a higher animal such as a mammal differs from the natural process in an important way, in that it bypasses sexual reproduction. This was first shown to be possible by Dolly, the sheep with the highest record of publicity in history, and subsequently by similar reports concerning cows and mice. As a first approximation, the success of those experiments indicates that in an adult organism there are at least some cells whose nuclear remains totipotent, and that a diploid nucleus inside an egg cytoplasm can generate an embryo, whether produced by fusion of the gametes' pronuclei or preformed.

The closest natural similarity with this kind of cloning is the production of identical twins, but there are important differences. First, in twinning, the situation is symmetrical. It is meaningless to argue whether twin 1 is a clone of twin 2 or vice versa. Identical twinning results from an accidental, very early splitting of the embryo; it might also be said that both twins are clones of the original unsplit embryo. Secondly, the age of identical twins is of course identical, but if a mammal is cloned from a somatic cell of an adult there will be a large age difference between the two organisms. Although this might suggest instinctively a parent-child relationship, the clone and its source would in fact be genetically identical, just like identical twins, thus creating an astonishing discrepancy between two basic components of human relationships. (In fact, there will be a genetic difference between the source and its clone: namely, the mitochondrial DNA (mtDNA), which is exclusively of maternal origin; the clone will have different mtDNA derived from the egg cytoplasm into which the source's nucleus was inserted. By contrast, identical twins have the same mother, and therefore the same mtDNA.)

Perhaps most important of all, if cloning was used extensively, it would reduce the amount of genetic diversity in the population. People with a different genetic make-up will have different fitness depending on environmental factors. It is a fundamental theorem in biology that genetic diversity is advantageous to the population because it provides the flexibility required for adapting to changes in the environment. Thus, cloning of humans could have far-reaching long-term consequences with respect to human evolution.

Diversity in somatic cells

Clones of cells grown in vitro are defined and identified by the procedure used in their culture, but how can clones in vivo be identified? In general, all the cells in the body have the same genetic material, differing only in the way they use it. Indeed, this is the fundamental role of the regulation of gene expression in the course of development and differentiation. However, there are important exceptions, whereby physical changes in the DNA of somatic cells take place. First, during the ontogenesis of lymphoid cells, functional genes encoding Ig and TCR chains are assembled in a modular fashion (in the precursors of B cells and T cells, respectively) from the inherited V, D, J and C gene portions. Secondly, given the large number of cells in the body, and the many rounds of DNA replication required to produce and maintain an adult organism, it is inevitable that some mutations will occur in spite of the high degree of accuracy of the replication machinery. Thus, genetic changes in the strict sense of the word (ie changes in the DNA sequence) do occur in somatic cells. (It is worth noting that, as a result of these changes, even identical twins are no longer identical; for instance, their immune systems might be substantially different, both because Ig and TCR rearrangements are inherently random and because of different exposure to antigens which exert selection on different B cell and T cell clones.) In addition, although epigenetic modifications of the chromatin do not alter the DNA sequence, they are faithfully inherited in somatic cells – the most spectacular physiological example is the phenomenon of X chromosome inactivation. Mutant cells can clone: sometimes they grow like the normal cells, sometimes less, sometimes more, while their growth is sometimes influenced by the environment, as in the case of antigen-driven selection of specific T cell and B cell clones.

Clonal disorders

The ultimate evidence that clones are important in human disease is derived from the study of patients. The example that immediately comes to mind is malignant tumours, but they are only one end of a spectrum, illustrating a situation whereby a cell that has accumulated several somatic mutations has a vast and absolute growth advantage over the normal cells. In many cases, a somatic mutation may not provide any growth advantage: for instance, freckles result from clones of melanocytes that produce more melanin.
than is normal for that person, but their growth does not differ significantly from that of the surrounding melanocytes.

A particularly interesting situation would be one where a clone is neither neutral nor has an absolute growth advantage, but has a conditional growth advantage (ie one that is a function of a specific environment). There is increasing evidence that this is true in the rare blood disorder, paroxysmal nocturnal haemoglobinuria (PNH). In this condition, a proportion of the patient’s red cells (referred to as PNH cells) have a marked tendency to intravascular haemolysis because they are deficient in the glycosyl phosphatidyl inositol (GPI)-anchored surface protein CD59 which protects normal red cells against the membrane attack complex produced by activated complement. This deficiency is secondary to a somatic mutation that partially or totally inactivates the product of the X-linked gene called PIG-A, which is required for an early step in the biosynthesis of the GPI anchor. Characteristically, in a patient with PNH a certain proportion of normal blood cells regularly coexists with the PNH cell population. Targeted inactivation of the PIG-A gene in mice has made it possible to obtain chimeric mice which produce a small proportion of blood cells with the PNH phenotype. However, as these animals grow older, the proportion of PNH cells decreases rather than increases. Thus, considering the human and the mouse data as a whole, we have a remarkable paradox. In the patients, the PNH clone grows up to the point where it supports a substantial proportion of haemopoiesis (although it never completely takes over the bone marrow, as in leukaemia), suggesting a growth advantage; in the mice, it fails to do this, suggesting that some other factor must come into play in the patients. It was previously suggested that this other factor may be a depression of haemopoiesis by the non-PNH stem cells: specifically, that an autoimmune process targets a GPI-linked molecule on the bone marrow stem cells. This would confer a selective growth advantage on the PNH stem cells that are deficient in the target molecule. One specific prediction of this model has now been verified by the finding that PIG-A mutations are commonly present in normal people. This provides formal proof that PIG-A mutations are necessary, but not sufficient, to produce the clinical picture of PNH (Table 1).

| PIG-A mutation | Bone marrow failure | Clinical picture |
|----------------|---------------------|------------------|
| %              | %                   | None             |
| -              | %                   | Aplastic anaemia |
| %              | %                   | PNH              |

It is therefore important to realise that, while we are actively seeking to pinpoint inherited factors and environmental factors that can cause cancer, there is always an element of chance — or bad luck — in the onset of this disease. This is a new challenge in our long-drawn effort to understand and control cancer.

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