Thirty-five years of endless cell potential

Pluripotent cells have the potential to differentiate into any cell type in the body. Their isolation and propagation from mouse embryos was pivotal for advances in understanding human development and disease.

The human body consists of around 250 differentiated cell types, which all originate from about 15 cells in a structure called the inner cell mass that arises at the blastocyst stage of early embryonic development. These cells can each give rise to every cell type, but this pluripotent status is transient, with the cells soon differentiating into diverse types. This year marks the 35th anniversary of landmark studies by biologists Martin Evans and Matthew Kaufman[1], and Gail Martin[2], who independently captured and propagated these elusive pluripotent cells in a Petri dish for the first time, naming them embryonic stem (ES) cells. Studies of isolated ES cells have revolutionized our understanding of mammalian development, and enabled the simulation of human diseases *in vitro* (Fig. 1).

In the 1950s, it was reported[3] that mice, like humans, could develop testicular tumours called teratomas, which were derived from germ-cell precursors (precursors of eggs or, in this case, sperm) and contained various differentiated cell types not normally found in testes. The teratomas also contained embryonic carcinoma (EC) cells, which had key attributes of pluripotency.

However, EC cells could not efficiently differentiate into normal tissues if they were injected into blastocysts. In particular, their differentiation into sperm or eggs was not observed, meaning that EC cells could not be used to generate strains of mice that could pass experimentally induced mutations on to their offspring — a major goal of research into pluripotency. This shortcoming encouraged efforts to find other sources of pluripotent stem cells.

Evans and Kaufman established ES cells directly from mouse blastocysts that had been prevented from undergoing further development, for example by treating the mothers with steroids. Meanwhile, Martin grew inner cell masses from late-stage blastocysts using culture media in which EC cells had been grown previously, in the hope that the EC cells might have released factors conducive to the growth of ES cells. Both studies were successful, leading to the first isolations of blastocyst-derived ES cells that could be maintained indefinitely *in vitro*.

ES-cell isolation had an immediate and profound impact on the study of mammalian development. Unlike EC cells, ES cells could reliably give rise to germ cells[4], making it possible to introduce specific genetic mutations into mouse strains. To briefly outline the process, ES cells are genetically engineered to harbour a specific mutation, and are then transplanted into a host blastocyst. The resulting embryo is a chimaera — some of its cells will contain the mutation, others will not. If germ cells carry the mutation, it can be passed to the offspring. The consequences of the mutation can then be examined in detail.

A great many mutant mouse strains have been generated in this way, enabling detailed analyses of the functions of individual genes under physiological conditions. Genes vital for embryonic development, especially those that establish the body plan, were mutated to study their precise role[5]. Moreover, human diseases could be modelled in other mammals. For example, a gene that causes cystic fibrosis in humans was mutated in mouse ES cells[6], and mice carrying this mutant gene were then created to provide an animal model for the disease.

The development of large-scale mutation strategies such as gene traps (in which mutations are introduced into DNA at random through the insertion of a genetic construct that produces a 'reporter' protein) led to the production of mutation libraries — a useful resource of cells from which mice carrying particular mutations could be made. Before the isolation of ES cells, all such research was limited by the availability of naturally occurring mutations. But today, any gene can be investigated at will.

Following this impressive progress, attention shifted to humans. In 1998, human ES (hES) cells were eventually isolated[7]. Although not molecularly identical to mouse ES cells, hES cells nonetheless possess the key properties of pluripotency — self-renewal and an ability to differentiate into diverse lineages. Moreover, they provide opportunities for studying early human development.

Ethical considerations prevent hES cells from being used for germline therapy, in which genetic changes would be introduced into hES-derived germ cells with the aim of correcting faulty genes. However, two studies published last year[8,9] showed that germ-cell precursors can be derived from hES cells, paving the way for studies of human germ cells and indicating that it might one day be possible to make sperm and eggs in culture. Some key genes involved in the induction of human germ-cell precursors, however, are not involved in mouse germ cells[8,9]. Thus, these studies, with many others, have revealed key differences between mouse and human development.

Studies on ES cells also defined the four key regulatory genes that control pluripotency, which in 2006 led to a major advance — the conversion of differentiated cells into induced pluripotent stem (iPS) cells[10–12]. iPS cells have since been derived from patients and differentiated into specific cell types, to simulate the initiation and progression of disease in culture. Moreover, iPS cells are currently being used to develop *in vitro* models for testing potential therapeutic compounds to treat diseases such as Alzheimer's. Human ES cells and iPS cells might some day be used as part of cell-replacement therapies in diseased tissues, although the greatest advances in their use will initially probably be in basic research and in screens for therapeutic agents.

One useful feature of ES cells is their ability to self-organize into differentiated tissues and to assemble into structures that have recognizable attributes of organs. In the past decade, this quality has been exploited to produce synthetic organs called organoids in culture. So far, human organoids have been generated that model the brain, intestine, liver and kidney, among others[13–15]. More-advanced 3D models that approximate physiological settings are likely to be developed in the future using tissue-engineering methods. Attempts are now under way to generate human organs in animals, by incorporating hES cells into blastocysts from other species. This might alleviate the unmet demand for organs for transplantation.

Advances made following the isolation of pluripotent ES cells have coincided with progress in other technologies, such as live imaging, single-cell analysis and genome editing[16]. When combined to study human organoids, these approaches will allow analysis of the outcomes of increasingly sophisticated manipulations of genes and of the genetic elements that regulate their expression. Such studies will provide unprecedented understanding of how genes individually and collectively regulate human development, and how such regulation can go awry.

The history of ES cells shows how basic research has led to revolutionary advances. The future presents us with endless opportunities to explore how a single cell transforms into a multicellular adult organism. ▪_

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