AMMINISTRAZIONE CENTRALE
AREA RISORSE UMANE
UFFICIO PERSONALE TECNICO AMMINISTRATIVO

UNIVERSITÀ DEGLI STUDI DI PADOVA

SELEZIONE PUBBLICA N. 2023S44, PER ESAMI, PER LA STIPULA DI N. 1 CONTRATTO DI LAVORO A TERMINE, CATEGORIA C, POSIZIONE ECONOMICA C1, AREA TECNICA, TECNICO-SCIENTIFICA ED ELABORAZIONE DATI, TEMPO PIENO, PER 12 MESI, AI SENSI DEL D.LGS. 30.03.2001, N. 165 E S.M.I., DEL D.LGS. 15.06.2015, N. 81 IN QUANTO COMPATIBILE E DEL C.C.N.L. DEL 19.04.2018, PRESSO IL DIPARTIMENTO DI BIOLOGIA – DIBIO - TECNICO DI LABORATORIO AMBITO COLTURA E DIFFERENZIAMENTO DI CELLULE PLURIPOTENTI UMANE.

QUESITI COLLOQUIO

Elenco n. 1
Quanti cromosomi diversi ci sono nell'uomo e quanti nella donna?
A quale concentrazione di ossigeno e CO_2 vengono mantenute le iPSC e perché?
Elenca alcune funzioni statistiche di Excel.

Elenco n. 2
Descrivi la differenza tra aneuploidie ed aberrazioni cromosomiche
Come si generano le iPSC
Come si crea in Excel un grafico da una serie di valori.

Elenco n. 3
Descrivi le differenze tra cellule pluripotenti naive e primed
Come si individuano le contaminazioni da micoplasmi?
Come si introduce una animazione in PPT.

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mechanisms intrinsic to the individual chromosome. Elsewhere in the genome also, controls at the level of chromatin act in combination with other forms of regulation to govern the expression of each gene. Genes can be shut down completely, or switched on constitutively, or maintained in a labile state where they can be readily switched on or off according to changing circumstances.

The reprogramming of a nucleus transplanted into an oocyte involves dramatic changes in chromatin. The nucleus swells, increasing its volume 50-fold as the chromosomes decondense; there is a wholesale alteration in patterns of methylation of DNA and histones; the linker histone H1 (the histone that links adjacent nucleosomes) is replaced by a variant form that is peculiar to the oocyte and early embryo; and the preexisting type of histone H3 is also replaced at many sites by a distinct isoform. Evidently, the egg contains factors that reset the state of the chromatin in the nucleus, wiping out old histone modifications on chromatin and imposing new ones. Reprogrammed in this way, the genome becomes competent once again to initiate embryonic development and to give rise to the full range of differentiated cell types.

Embryonic Stem (ES) Cells Can Generate Any Part of the Body

A fertilized egg, or an equivalent cell produced by nuclear transplantation, is a remarkable thing: it can generate a whole new multicellular individual, which means that it can give rise to every normal type of specialized cell, including even egg or sperm cells for production of the next generation. A cell in such a state is said to be **totipotent**; a cell that can give rise to most cell types but not absolutely all is said to be **pluripotent**. Nevertheless, such a totipotent or pluripotent cell is not a stem cell as it is not self-renewing, but is instead dedicated to a program of progressive differentiation. If it were the only available starting point for study and exploitation of pluripotent cells, the enterprise would require a continual supply of fresh fertilized eggs or fresh nuclear transplantation procedures—an awkward requirement for studies in experimental animals, and unacceptable for practical applications in humans.

Here, however, nature has been unexpectedly kind to scientists. It is possible to take an early mouse embryo, at the blastocyst stage, and through cell culture to derive from it a class of stem cells called **embryonic stem cells**, or **ES cells**. ES cells originate from the inner cell mass of the early embryo (the cluster of cells that give rise to the body of the embryo proper, as opposed to extraembryonic structures), and they have an extraordinary property: given suitable culture conditions, they will continue proliferating indefinitely and yet retain an unrestricted developmental potential. Their only limitation is that they do not give rise to extraembryonic tissues such as those of the placenta. Thus they are classified as pluripotent, rather than totipotent. But this is a minor restriction. If ES cells are put back into a blastocyst, they become incorporated into the embryo and can give rise to all the tissues and cell types in the body, integrating perfectly into whatever site they may come to occupy, and adopting the character and behavior that normal cells would show at that site (Figure 22–25). They can even give rise to germ cells, from which a new generation of animals can be derived.

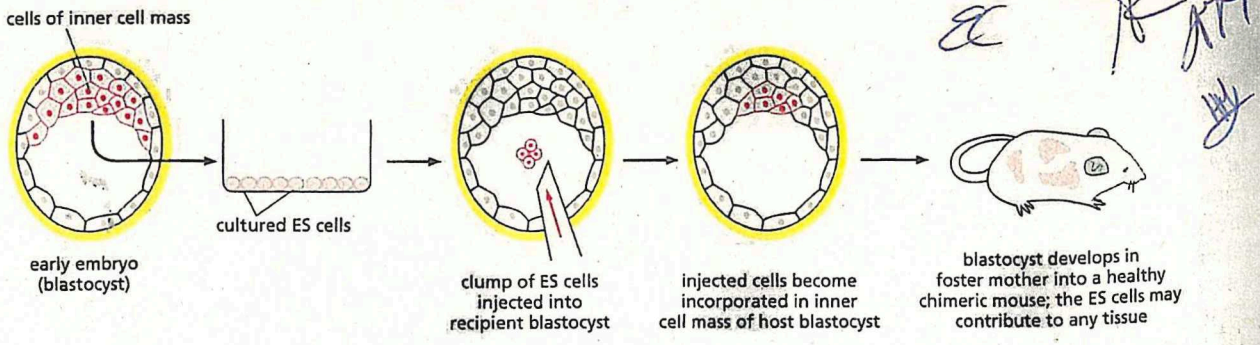

Figure 22–25 **Production and pluripotency of ES cells.** ES cells are derived from the inner cell mass (ICM) of the early embryo. The ICM cells are transferred to a culture dish containing an appropriate medium, where they become converted to ES cells and can be kept proliferating indefinitely without differentiating. The ES cells can be taken at any time—after genetic manipulation, if desired—and injected back into a developing blastocyst. There they incorporate into the inner cell mass and take part in formation of a well-formed chimeric animal that is a mixture of ordinary and ES-derived cells. The ES-derived cells can differentiate into any of the cell types in the body, including germ cells from which a new generation of mice can be produced, which are no longer chimeric, but consist of cells that all inherit half their genes from the cultured ES cell line.

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ES cells let us move between cell culture, where we can use powerful techniques for genetic transformation and selection, and the intact organism, where we can discover how such genetic manipulations affect development and physiology. Thus, ES cells opened the way to efficient genetic engineering in mammals, leading to a revolution in our understanding of mammalian molecular and developmental biology.

Cells with properties similar to those of mouse ES cells can also be derived from early human embryos and from human fetal germ cells, and even, as we explain shortly, from differentiated cells taken from adult mammalian tissues. In this way, one can obtain a potentially inexhaustible supply of pluripotent cells. Grown in culture, these cells can be manipulated, by suitable choice of culture conditions, to give rise to large quantities of almost any type of differentiated cell, opening the way to many practical applications. Before discussing them, however, we consider the underlying biology.

A Core Set of Transcription Regulators Defines and Maintains the ES-Cell State

What is it that gives ES cells and related types of pluripotent stem cells their extraordinary developmental potential? And what can they tell us about the fundamental mechanisms underlying stemness, commitment to differentiation, and the stability of the differentiated state?

For some ES-cell attributes, the answer is simple. For example, an essential feature of ES cells is that they must avoid *replicative cell senescence*. As discussed in Chapter 17, this is the fate of fibroblasts and many other types of proliferating somatic cells: such cells are limited in the number of times they will divide, in part at least because they lack telomerase activity, with the result that their telomeres become shorter with each division cycle, leading eventually to a permanent cell-cycle arrest. ES cells, by contrast, express high levels of active telomerase, allowing them to escape replicative cell senescence and continue to divide indefinitely. This is a property shared with other, more developmentally restricted types of stem cells, such as those of the adult intestine, which similarly can carry on dividing for hundreds or thousands of cell cycles.

The deeper problem is to explain how the whole complex pattern of gene expression in an ES cell is organized and maintained. As a first step, one can look for genes expressed specifically in ES cells or in the corresponding pluripotent cells of the early embryo. This approach identifies a relatively small number of candidate *ES-critical genes*; that is, genes that seem to be essential in one way or another for the peculiar character of ES cells. A gene called *Oct4*, for example, is exclusively expressed in ES cells and in related classes of cells in the intact organism—specifically, in the germ-cell lineage and in the inner cell mass and its precursors. *Oct4* codes for a transcription regulator. When it is lost from ES cells, they lose their ES-cell character, and when it is missing in an embryo, the cells that should specialize as inner cell mass are diverted into an extraembryonic pathway of differentiation, and the embryo's development is aborted.

Fibroblasts Can Be Reprogrammed to Create Induced Pluripotent Stem (iPS) Cells

In Chapter 7, we saw that fibroblasts and some other cell types can be driven to switch their character and differentiate as muscle cells if the master muscle-specific transcription regulator MyoD is artificially expressed in them. Could the same technique be used to convert fibroblasts and other cell types into ES cells, through forced expression of ES-critical genes such as *Oct4*? This question was tackled by transfecting mouse fibroblasts with retroviral vectors carrying genes that one might hope to have such an effect. A total of 24 candidate ES-critical genes were tested in this way. None of them was able by itself to cause the conversion, but in certain combinations they could do so. In 2006, the first breakthrough experiments whittled down the requirement to a core set of four

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genes, all of them encoding transcription regulators—Oct4, Sox2, Klf4, and Myc, known as the OSKM factors for short. When co-expressed, these could reprogram mouse fibroblasts, permanently converting them into cells very similar to ES cells (Figure 22–26). ES-like cells created in this way are called **induced pluripotent stem cells**, or **iPS cells**. Like ES cells, iPS cells can continue dividing indefinitely in culture, and when incorporated into a mouse blastocyst they can participate in creation of a perfectly formed chimeric animal. In this animal, they can contribute to the development of any tissue and can turn into any differentiated cell type, including functional germ cells from which a new generation of mice can be raised (see Figure 22–25).

iPS cells can now be derived from adult human cells, including from various differentiated cell types besides fibroblasts. Numerous methods can be used to drive expression of the transforming OSKM factors, including methods that leave no trace of foreign DNA in the reprogrammed cells. Variations of the original cocktail of transcription regulators can drive the conversion, with different specialized cell types having somewhat different requirements. Myc overexpression, for example, turns out not to be absolutely necessary, although it enhances the efficiency of the process. And differentiated cell types may express some of the required factors as part of their normal phenotype. For example, certain cells of hair follicles already express Sox2, Klf4, and Myc; to convert them into iPS cells, it is enough to force them artificially to express Oct4.

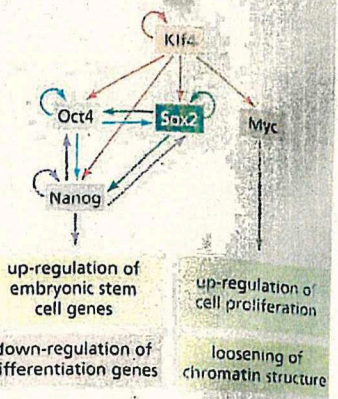

Figure 22–26 **Reprogramming fibroblasts to iPS cells with the OSKM factors.** As indicated, the transcription regulatory proteins Oct4, Sox2, and Klf4 (OSK factors) induce both their own and each other's synthesis *(gray shading)*. This generates a self-sustaining feedback loop that helps to maintain cells in an ES cell–like state, even after all of the experimentally added OSKM initiators have been removed. Myc overexpression speeds up early stages of the reprogramming process through the mechanisms shown (see Figure 17–59). Stable reprogramming also involves the permanently induced expression of the *Nanog* gene, which encodes an additional transcription regulator (see Figure 7–10). (Adapted from J. Kim et al., *Cell* 132: 1049–1061, 2008.)

Reprogramming Involves a Massive Upheaval of the Gene Control System

Converting a differentiated cell into an iPS cell is not like flicking a switch on some predictable, precisely engineered piece of machinery. Only a few of the cells that receive the OSKM factors will actually become iPS cells—one in several thousand in the original experiments, and still only a small minority with more recent, improved techniques. In fact, the success of the original experiments depended on clever selection strategies to pick out those few cells where the conversion had occurred (Figure 22–27).

Conversion to an iPS state by the OSKM factors is not only inefficient but also slow: fibroblasts take 10 days or more from introduction of the conversion factors before they begin to express markers characteristic of iPS cells. This suggests the transformation involves a long cascade of changes. These changes have been extensively studied, and they affect both the expression of individual genes and the state of the chromatin. The time course is outlined in Figure 22–28. The process begins with a Myc-induced cell proliferation and loosening of chromatin structure that promotes the binding of the other three transcription regulators to many hundreds of different sites in the genome. At a large proportion of these sites, Oct4, Sox2, and Klf4 all bind in concert. The binding sites include the endogenous *Oct4*, *Sox2*, and *Klf4* genes themselves, which eventually creates positive feedback loops like those just described, making expression of these genes self-sustaining (see Figure 22–26). But self-induction

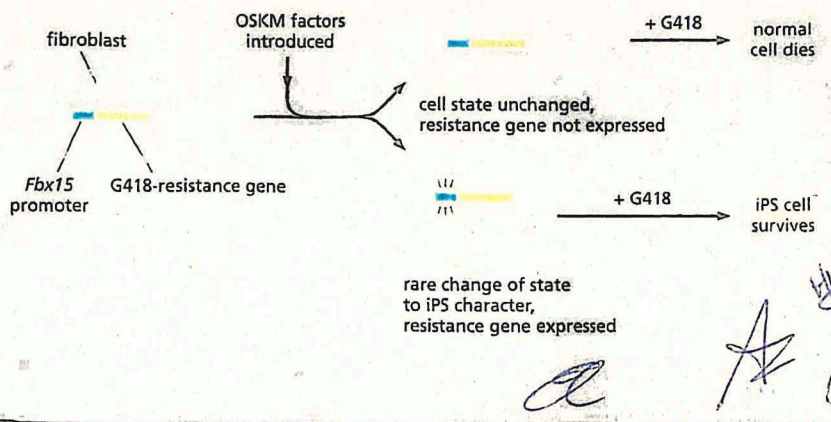

Figure 22–27 **A strategy used to select cells that have converted into iPS cells.** The experiment makes use of a gene (*Fbx15*) that is present in all cells but is normally expressed only in ES and early embryonic cells (although not required for their survival). G418 is an aminoglycoside antibiotic that blocks protein synthesis in both bacteria and eukaryotic cells. A fibroblast cell line is genetically engineered to contain a gene that produces an enzyme that degrades G418 under the control of the *Fbx15* regulatory sequence. When the OSKM factors are artificially expressed in this cell line, a small proportion of the cells undergo a change of state and activate the *Fbx15* regulatory sequence, driving expression of the G418-resistance gene. When G418 is added to the culture medium, these are the only cells that survive and proliferate. When tested, they turn out to have iPS-cell characteristics.