identical daughter cells. For stem cells to proliferate in vitro, they must divide symmetrically.

Unlimited Expansion In Vitro Resident stem cells are often quiescent and divide infrequently. However, once the stem cells are successfully cultured in vitro, they often acquire the capacity to divide continuously and the ability to proliferate beyond the normal passage limit typical of primary cultured cells (sometimes called *immortality*). These features are primarily seen in ES cells but have also been demonstrated for tissue stem cells, such as NS cells and mesenchymal stem (MS) cells, thereby enhancing the potential of these cells for therapeutic use (Table 88-1).

Stability of Genotype and Phenotype The capacity to actively proliferate is often associated with the accumulation of chromosomal abnormalities and mutations. Mouse ES cells appear to be an exception to this rule and tend to maintain their euploid karyotype and genome integrity. By contrast, human ES cells appear to be more susceptible to mutations after long-term culture. However, it is also important to note that even euploid mouse ES cells can form teratomas when injected into immunosuppressed animals, raising concerns about the possible formation of tumors after transplanting actively dividing stem cells.

POTENCY AND DIFFERENTIATION OF STEM CELLS

Developmental Potency The term *potency* is used to indicate a cell's ability to differentiate into multiple specialized cell types. The current lack of knowledge about the molecular nature of potency requires the experimental manipulation of stem cells to demonstrate their potency. For example, in vivo testing can be done by injecting stem cells into mouse blastocysts or immunosuppressed adult mice and determining how many different cell types are formed from the injected cells. However, these in vivo assays are not applicable to human stem cells. In vitro testing can be performed by differentiating cells in various culture conditions to determine how many different cell types are formed from the cells. The formal test of self-renewal and potency is performed by demonstrating that a single cell possesses such abilities in vitro (*clonality*). Cultured stem cells are tentatively grouped according to their potency (Fig. 88-1). Only some examples are shown, because many cultured stem cells, especially human cells, lack definitive information about their developmental potency.

From Totipotency to Unipotency *Totipotent cells* can form an entire organism autonomously. Only a fertilized egg (zygote) possesses this feature. *Pluripotent cells* (e.g., ES cells) can form almost all of the body's cell lineages (endoderm, mesoderm, and ectoderm), including germ cells. *Multipotent cells* (e.g., HS cells) can form multiple cell lineages but cannot form all of the body's cell lineages. *Oligopotent cells* (e.g., NS cells) can form more than one cell lineage but are more restricted than multipotent cells. Oligopotent cells are sometimes called *progenitor cells* or *precursor cells*; however, these terms are often more strictly used to define partially differentiated or lineage-committed cells (e.g., myeloid progenitor cells) that can divide into different cell types but lack self-renewing capacity. *Unipotent cells* or *monopotent cells* (e.g., spermatogonial stem [SS] cells) can form a single differentiated cell lineage.

Source / Potency	Preimplantation	Embryonic, fetal	Postnatal	Adult
Totipotent	Zygote^{m,h}			
Pluripotent	ES^{m,h}	EG^{m,h} EpiSC^{m}	GS^{m} iPS^{m,h} USSC^{h}	EC^{m,h} iPS^{m,h} maGSC^{m}
Multipotent				MS^{m,h}
Oligopotent	TS^{m}			NS^{m,h}
Unipotent	XEN^{m}			SSC^{m}
Terminally differentiated cells				

FIGURE 88-1 Potency and source developmental stage of cultured stem cells. For abbreviations of stem cells, see Table 88-1. Note that stem cells are often abbreviated with or without "cells," e.g., ES cells or ESCs for embryonic stem cells. h, human; m, mouse.

Nuclear Reprogramming Development naturally progresses from totipotent fertilized eggs to pluripotent epiblast cells to multipotent cells and, finally, to terminally differentiated cells. According to Waddington's epigenetic landscape, this is analogous to a ball moving down a slope. The reversal of the terminally differentiated cells to totipotent or pluripotent cells (called *nuclear reprogramming*) can thus be seen as an uphill gradient. Nuclear reprogramming has been achieved using *nuclear transplantation*, or *nuclear transfer* (NT), procedures (often called "cloning"), where the nucleus of a differentiated cell is transferred into an enucleated oocyte. Although this is an error-prone procedure with a very low success rate, live animals have been produced using adult somatic cells as donors in sheep, mice, and other mammals. In mice, it has been demonstrated that ES cells derived from blastocysts made by somatic cell NT are indistinguishable from normal ES cells. NT can potentially be used to produce *patient-specific ES cells* carrying a genome identical to that of the patient, although such strategies have not been pursued due to ethical issues and technical challenges. Recent success in generating human ES cells by NT has rekindled an interest in this area; however, the limited supply of human oocytes will still be a major problem for clinical applications of NT.

An alternative approach that has become a method of choice is the direct conversion of terminally differentiated cells into ES-like cells (called induced pluripotent stem [iPS] cells) by overexpressing a combination of key transcription factors (TFs). The original method was to infect mouse embryonic fibroblast cells with retrovirus vectors carrying four TFs [*Pou5f1* (*Oct4*), *Sox2*, *Klf4*, and *Myc*] and to identify rare ES-like cells in culture. This approach was soon adapted to human cells, followed by a more refined procedure (e.g., the use of fewer TFs, different cell types, and different gene-delivery methods). Because a clinical trial using iPS cells is imminent, the safety of iPS-based therapy is a major concern and a variety of measures are being taken to ensure the safety. For example, it has now become a standard to use footprint-free methods such as an episomal vector, Sendai virus vector, and synthetic mRNAs to deliver reprogramming factors into cells, resulting in the production of patient-specific iPS cells with minimal alteration of their genetic makeup. In addition to cell replacement therapy, disease-specific iPS cells are expected to play a role in modeling human disease in vitro and in screening drugs for personalized medicine.

It has also become possible to convert one type of terminally differentiated cell (e.g., fibroblast cell) into another type of terminally differentiated cell (e.g., cardiac muscle, neuron, or hepatocyte) by overexpressing specific sets of TFs (called direct reprogramming). Direct reprogramming can bypass the step of making iPS cells, possibly providing the safer route to desired cell types for therapy; however, the technology is currently limited by its low efficiency.

Stem Cell Plasticity, Transdifferentiation, and Facultative Stem Cells The prevailing paradigm in developmental biology is that once cells are differentiated, their phenotypes are stable. However, more recent studies show that tissue stem cells, which have traditionally been thought to be lineage-committed multipotent cells, possess the capacity to differentiate into cell types outside their lineage restrictions (called *transdifferentiation*). For example, HS cells may be converted into neurons as well as germ cells. This feature may provide a means to use tissue stem cells derived directly from a patient for therapeutic purposes, thereby eliminating the need to use embryonic stem cells or elaborate procedures such as nuclear reprogramming of a patient's