Stem cells: the new "model organism"

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ABSTRACT **Human tissue culture cells have long been a staple of molecular and cell biology research. However, although these cells are derived from humans, they have often lost considerable aspects of natural physiological function. Here we argue that combined advances in genome editing, stem cell production, and organoid derivation from stem cells represent a revolution in cell biology. These advances have important ramifications for the study of basic cell biology mechanisms, as well as for the ways in which discoveries in mechanisms are translated into understanding of disease.**

Monitoring Editor
Doug Kellogg
University of California, Santa Cruz

Received: Mar 21, 2017
Accepted: Apr 4, 2017

Tissue culture cells have long proven to be convenient vehicles for studies in molecular and cell biology and continue to yield fundamental discoveries in mechanism. However, the widespread use of most common tissue culture cell lines for such studies is problematic for several reasons. First, these lines have considerable aneuploidy, chromosome translocations, and genome instability. Such chromosomal abnormalities are expected, as they are hallmarks of the tumors from which most cell lines were derived. Chromosomal aberrations are a problem because they result in abnormal gene expression. Chromosome instability is a problem because cellular phenotypes are unstable. Exacerbating the chromosomal instability problem is the fact that common cell lines have been passaged extensively, and so, as an example, HeLa cells in one lab may bear very little resemblance to HeLa cells in another lab (Hyman and Simons, 2011). These sources of phenotypic perturbation and variation create nonphysiological conditions that change unpredictably over time. Detecting effects of experimental perturbations in a genetically unstable cellular background can be very difficult, and the nonphysiological state of the cells throws doubt on the biological relevance of the observations. Moreover, effects of the cancer mutations in these tumor-derived lines on cell structure and physiology cloud the ability to draw conclusions about mechanisms that function in the normal cell state.

Mechanistic studies of cellular processes in a natural physiological setting have been greatly enhanced by the availability of "model" organisms, which are selected in part because their genomes contain a normal, stable complement of genes and chromosomes (Rine, 2014). Moreover, these organisms are traceable to a common ancestral parent, with care being taken to minimize the number of generations separating each individual studied from the original parent. These model organisms include such lab staples as *Escherichia coli* bacteria, budding and fission yeast, nematodes, maize, the mouse-ear cress *Arabidopsis thaliana*, fruit flies, and mice. Because essentially all laboratory budding yeast strains can be traced to one of two parent lines, S288c and W303, variation caused by genetic differences is minimized, and both natural and experimentally introduced variation can be readily detected (Hall and Linder, 1993). In *Caenorhabditis elegans* research, the widespread use of the N2 line, for which the original isolate is still available, has been a crucial aspect of the success of this model organism (Brenner 1974).

Here we argue that stem cells, which include embryonic stem cells (ES), induced pluripotent stem cells (iPS), and adult stems cells (see Box 1), combine many of the advantages of tissue culture models with those of the traditionally employed model organisms. The following list includes what we see as the major advantages of using stem cells for the study of problems in molecular and cell biology.

1. Stem cells represent a normal physiological state because they are typically derived from healthy tissues.
2. Their genomes lack the abnormalities that characterize most tissue culture lines (Figure 1). Recent work has shown that the

Box 1. Definitions of stem cell types.
Adult stem cells (also called somatic stem cells): Stem cells found throughout the adult body to maintain the body's tissues.

iPS cells: Induced pluripotent stem cells. Stem cells derived from differentiated cells in adult tissues.

ES cells: Embryonic stem cells. Stem cells derived from embryos.

DOI:10.1091/mbc.E17-03-0183

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Abbreviations used: ES, embryonic stem cell; iPS, induced pluripotent stem cell.

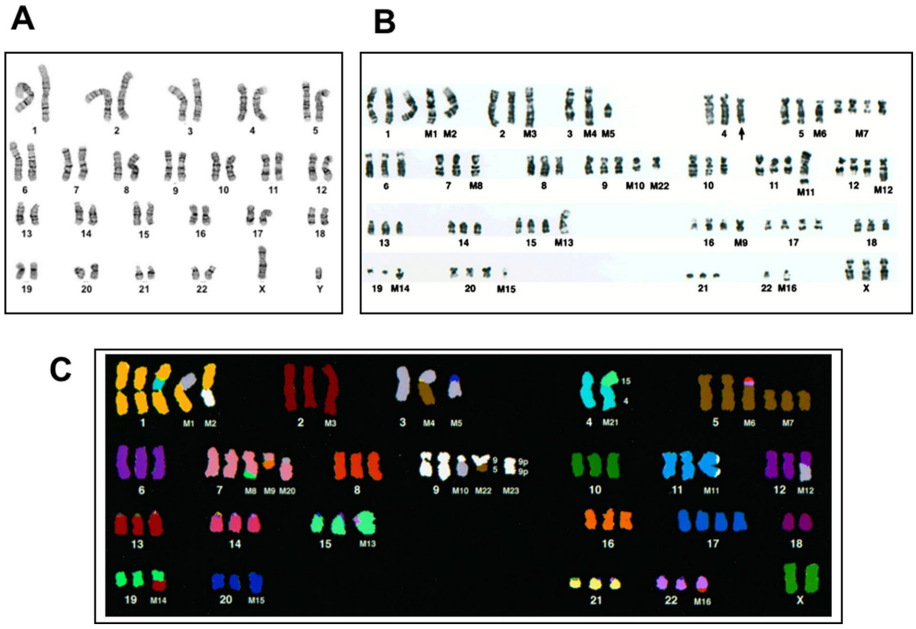

FIGURE 1: Karyotypes of WTC iPS cells (A) and HeLa cells (B, C). Note that the WTC cells have a normal chromosomal complement, whereas the HeLa cells are characterized by many irregularities in chromosome number (B) and by massive translocations revealed by a spectral karyotype (C). Reproduced with permission from Mandegar *et al.*, 2016 (A) and Macville *et al.*, 1999 (B, C).

genomes of adult stem cells passaged in culture are remarkably stable (Martins-Taylor and Xu, 2012), although it is always advisable to minimize passages.

3. Stem cells can be differentiated into many different cell types whose phenotypic differences can be reliably detected and studied in isogenic cells that were all derived from a common parent cell line (Noggle *et al.*, 2005).

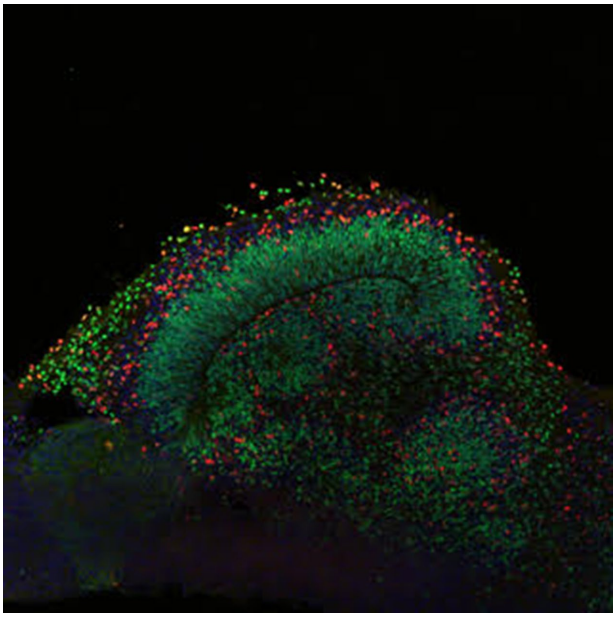

FIGURE 2: Cross-section of a human brain organoid grown for 42 d. Forebrain cells (progenitors and neurons) are labeled in green and a subpopulation of forebrain progenitors in red. All nuclei are labeled in blue. Image provided by Veronica Krenn and Juergen Knoblich.

4. Organoids can be produced from stem cells. Because they represent many tissue types, organoids allow cellular processes to be studied in the context of a differentiated tissue (Figure 2; e.g., Lancaster and Knoblich, 2014; Clevers, 2016; Fatehullah *et al.*, 2016). Moreover, tissues made from stem cells can also be used for drug screening. For example, organoids made from gut adult stem cells are already used in the clinic in certain situations (van de Wetering *et al.*, 2015). Organoid production is still in its infancy. For example, organoids are often missing key aspects of normal tissue physiology, such as vascularization. However, improvement in organoids is essentially a problem in engineering, and we can be confident that as greater resources are poured into their production, especially in the commercial world, they will, over the next decades, approach the physiological state of organs formed during normal development.
5. Genome editing first used zinc finger nucleases, then transcription activator–like effector nucleases (TALENS), and now clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9. It is now routine to integrate fluorescent protein coding sequences (e.g., green fluorescent protein and red fluorescent protein) in-frame at the chromosomal locus of any protein-coding gene so the fluorescent protein fusion is expressed from the native promoter of the gene of interest without ectopic overexpression (Doyon *et al.*, 2011) and to construct mutants at any genomic locus. Genetically encoded tags inserted at the genomic locus avoid fixation artifacts and overexpression artifacts (Doyon *et al.*, 2011; Gibson *et al.*, 2013) and make possible real-time analysis of protein dynamics in live cells. Introduction of these tags at the endogenous locus by genome editing also makes it possible to count molecules within macromolecular structures in living cells. One only needs to calibrate the fluorescence produced from known numbers of reference fluorescent proteins (Dambournet *et al.*, 2014; Grassart *et al.*, 2014). Genome editing can of course be applied to all tissue culture systems. Because stem cells can be differentiated into many cell types and tissues, however, genome editing also allows essentially any mutation engineered in any gene in the stem cells to be expressed in different cell types and organoids derived from the parent stem cell line carrying the engineered mutant. This powerful approach provides a sensitive isogenic system for detecting effects of the mutations and determining how they affect different cell types and tissues.
6. As cell biologists, we have often had an uneasy relationship with translational research. Many of our studies are in model organisms, and considerable time is needed before discoveries in these models can be translated to address human disease. Working in stems cells allows cell biologists to investigate fundamental mechanisms, secure in the knowledge that their discoveries can be rapidly translated into understanding disease.

Considering these advantages, it seems clear that cell biology is entering a new era in which mechanistic studies can be conducted in systems that are close in physiology to human biology and that cell biologists should enthusiastically embrace this shift in emphasis. Most cell biologists are unfamiliar with the techniques and procedures necessary to work with stem cells. Therefore the community must make resources and training available for such an endeavor. Such resources will be particularly important for stem cells in part because of the current costs involved and partly because of the care needed to maintain the stem cell state while modifying their genome. As stem cells become more widely adopted, costs should be expected to decline and more robust protocols to manipulate and maintain the cells developed.

A particularly promising development in this direction is the efforts of the Allen Institute for Cell Science to use genome editing in stem cells to construct a collection of isogenic cell lines endogenously expressing fluorescent protein fusions. The idea for the focus of this effort came in part from discussions among the leadership of the American Society for Cell Biology on how to make such modified stem cells as widely available as possible (www.ascb.org/files/ASCB-Position-Paper-Stem-Cell-Report.pdf; www.ascb.org/newsletter/novemberdecember-2016).

The Allen Institute is engineering, into one parental stem cell line, in-frame gene fusions whose hybrid protein products will mark most major cellular structures and signaling pathways. The genetically encoded fluorescent tags are being inserted at the natural chromosomal locus for each gene to minimize perturbation of expression. Each of these gene fusions is being constructed in the same well-characterized, parental iPS cell line, WTC, which was created in the Conklin lab at the University of California, San Francisco, and is a true pluripotent diploid with a stable genome (Kreitzer *et al.*, 2013). Of importance, there are no licensing restrictions on distribution of the parent WTC line, which it turns out is somewhat unusual. The genome-engineered stem cell lines constructed at the Allen Institute will be available to all researchers, promising to provide for studies of human cells in culture many of the advantages of the commonly used model organisms. Of importance, although it is routine in model organisms to backcross an engineered locus to the wild-type parent several times to ensure that no other changes picked up during editing are being carried in the genome, this is not possible with engineered stem cells. To address this deficiency, the Allen Institute is implementing rigorous quality controls and keeping multiple independent clones (Roberts *et al.*, 2017).

If these genome-engineered cell lines and the protocols for culturing and differentiating them are widely adopted, as is anticipated, progress in many studies of molecular and cell biology and the translation of the results to address human health issues will be substantially enhanced. In addition, comparisons of results obtained in different labs using these isogenic diploid, pluripotent sibling cell lines will be considerably more meaningful than comparisons between results from studies on different tissue culture lines.

In conclusion, the past half-century has witnessed a remarkable increase in understanding of basic cellular mechanisms. The introduction of stem cells, which can be differentiated into multiple different cell types and tissues, will herald similar advances in our understanding of how basic cellular mechanisms are modulated during the healthy process of differentiation and the pathological consequences of disease.

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