



Stem Cells:

Future Scientific and Medical
Opportunities

A Report of the ASCB Stem Cell Task Force



During the spring of 2012, the American Society for Cell Biology (ASCB) leadership organized a retreat with several thought leaders in biomedical research to discuss key opportunities for cell biology in the future. One of the themes that emerged was that of stem cells, in particular human embryonic stem cells.

Science is an ecosystem with many players and many incentives, and one of the major roles for a professional society is that of developing a particular field, cell biology and basic sciences in general, in the case of ASCB.

With these two concepts in mind – the strategic importance of human embryonic stem cells for the future of cell biology, and the role of ASCB in promoting science – we tasked a group of distinguished scientists in the field of stem cell biology to identify key opportunities in the field of stem cells. In addition, the group was charged with providing concrete recommendations on eventual initiatives that will be needed to capture the identified opportunities.

The work that we present in this white paper titled “Next Steps in the Stem Cell Revolution: The ASCB Taskforce Report” would not have been possible without the intellectual contribution of all the ASCB Stem Cell Task Force members and the indefatigable and able leadership of Larry Goldstein, who chaired this group. The ASCB leadership and all its members are very proud of this work and want to thank all the committee members and Larry in particular, for the work and the insights that they have been able to provide. We are convinced that this paper will serve as a road map for looking at the future of stem cell biology in the next few years.

This white paper contains the ideas of a group of leaders in the field. Every ASCB member and every stem cell biologist will have the possibility of contributing and adding their own ideas by commenting online on the ASCB website at www.ascb.org/stemcellrevolution.

Sincerely,

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ASCB Past-President

Don Cleveland, PhD
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Executive Summary

We are poised at the beginning of a scientific and medical revolution driven by the generation, study, and manipulation of cultured human embryonic stem cells (hESC), human induced pluripotent stem cells (hiPSC), adult human stem cells, and stem cells in model organisms. Taking advantage of this revolution to maximize discovery of new biology and develop new therapies will require capitalizing upon many new opportunities and solving several key problems and barriers to entry for the scientific community.

The American Society of Cell Biology (ASCB) tasked a group of distinguished ASCB members and stem cell scientists to identify new scientific and medical opportunities created by recent rapid progress in human stem cell science, and to provide recommendations for how to best reduce barriers to entry into this field and thus accelerate the creation of new understanding and novel medical therapies. The composition of the ASCB Human Stem Cell Task Force is listed in Appendix 1. The ASCB Human Stem Cell Task Force identified three key scientific opportunities in the human stem cell field that have the potential to substantially accelerate the creation of new understanding and novel medical therapies. These three opportunities are to:

1) Accelerate discovery of basic cellular and developmental mechanisms, and their relationships to development of disease, by focusing on euploid, developmentally manipulable models, especially human.

2) Probe mechanistic and phenotypic robustness by analyzing the impact of natural genetic variation.

3) Take advantage of a deep understanding of mechanisms to design and construct artificial, potentially enhanced cells, tissues, and organs.

These areas were highlighted by the ASCB Task Force because they have the potential to rapidly enable, accelerate, and enhance the value of normal human cells as a primary model experimental system, overlapping and mirroring their historically essential role in therapy development. In particular, the use of human stem cells as a pivotal cell system for basic research, will bring novel approaches and insights, just as *Escherichia coli* was used in the mid 20th century and other eukaryotic systems dominated the late 20th and early 21st centuries. We do not suggest replacement of other important model organism systems for biological studies, which continue to provide fundamental understanding from the cellular to the organismal level. Instead, we recommend enhanced focus on human stem cells, and the basic cell and developmental biology involving other model organisms that is necessary to achieve this focus. By capitalizing on the opportunity at hand, we have the potential to deliver rapid progress in the understanding of normal and disease biology.

To reduce barriers to entry, and to accelerate progress, we recommend four key programmatic initiatives at this time:

1) Supporting audacious goals for stem cell research: We suggest that human and other stem cell research is primed for an ‘audacious goals’ mechanism as it constitutes a new field that brings new technologies and intellectual paradigms to bear on old and previously intractable problems. Such a mechanism could fund investigators for projects that have risky, “over-the-top” yet feasible goals, that challenge the status quo technically or intellectually, or that investigate problems that were previously refractory to systematic inquiry.

2) Integrate differentiation pathway discovery in non-human and human systems: Non-human models continue to play

crucial roles in the elucidation of pathways that control cellular differentiation. In depth understanding of these pathways is critical to the ability to generate in vitro models of precisely controlled human cell types. The rate at which these pathways are coming into focus is increasing but this area needs continued support and well-supported collaborations could rationally and rapidly couple studies of non-human to human stem cell differentiation in vitro.

3) Human stem cell training courses and shared institutional core facilities: To encourage and facilitate wider access of investigators to human stem cell culture technologies, we suggest continued support for courses that provide a rigorous introduction to methods of stem cell line derivation, characterization, quality control, and directed differentiation. Shared core facilities for human stem cell derivation and culture will promote high standards of research practice and enable new investigators to access stem cell methodologies, and allows all affiliated laboratories to leverage common and oftentimes expensive and specialized equipment, thereby reducing the overall costs of research through economies of scale.

4) Human stem cell gene tagged libraries: We suggest that human stem cell lines with epitope or fluorescent tagged proteins or genes be developed, tested, banked, and then distributed as needed. Tagged libraries should be developed by investigators with domain-specific expertise using a grant supplement mechanism to ensure that investigators with specific relevant scientific expertise develop the actual tagged lines, which the NIH would then centralize, manage testing and quality control, and distribute to investigators who would use them in research. Strong data/material sharing policies should be put in place to require broad distribution.

Key opportunities in discovery and design using stem cell biology

There are many new scientific and medical opportunities presented by the ability to manipulate stem cells from humans and model organisms *in vivo* and *in vitro*. These range from the discovery of new biological control pathways to the *in vitro* generation of useful human cell types and organs for study and clinical treatment. Progress is being driven by the rapidly accelerating discovery of pathways that stem cells use to develop, maintain, and repair normal cells and organs during development and adult life. Medical need is driving the identification of new methods to understand and treat disease and to generate specific cell types and multi-cellular assemblies *in vitro*. Simultaneously, the ability to control these human cells in the lab is enabling analyses of biological pathways, the *de novo* generation and/or design of tissues, and is supporting the development of engineering approaches to the manipulation of transplanted materials.

Important contributions from various types of non-human model organisms will be essential to augment and synergize with studies in humans. Together these areas define a new and vital set of opportunities in science and medicine that should be vigorously pursued. However, as described below, there are a number of key barriers to entry for using human stem cells that can be surmounted by thoughtful programmatic investment. We delineate the scientific and medical opportunities, barriers to entry, and suggested solutions below.

1) Accelerate discovery of basic cellular and developmental mechanisms, and their relationships to development of disease, by focusing on euploid, developmentally manipulable models, especially human: Cell and developmental biology have for many years made many important discoveries using model organisms such as bacteria, yeast, worms, fish, mice, and flies. These systems are powerful and therefore important to continue to support, but cultured human stem cells offer new opportunities and important advantages and therefore should be brought into focus as experimental systems for a large and growing community of scientists to adopt for cultured cell studies. For example, many analyses of basic cellular processes such as endocytosis, cytoskeletal dynamics, mitochondrial function, nuclear structure, etc. are routinely pursued in cultures of simpler eukaryotes such as yeast, and in *in vivo* model organisms such as flies, worms and mice. The need for transitioning from *in vivo* to *in vitro* will always be complementary in yielding valuable and essential information. But, the cultured human stem cell system offers a genetically tractable, stable euploid system suitable for screening campaigns for genes that will be complementary to *in vivo* approaches and will be helpful for a variety of processes and identification and characterization of new functions. The value-added, however, is the ability to apply information gained by *in vivo* studies to differentiate human stem cells *in vitro* into key adult cell types. This opens the door to discovering how basic cellular processes are modified to the specialized needs of highly differentiated cell types, e.g., neurons, cardiac cells, etc. For example, simple sorting pathways in non-polarized cells will undergo substantial elaboration and expansion when non-polarized cells differentiate to polarized epithelia or neurons that have substantial segregation of cellular materials and functions into different compartments. Studies of cellular interactions and communication are similarly and favorably impacted.

An unexpected potential benefit suggested by recent studies of human disease, and which may be uniquely pursued in human cells being used for basic discovery, is that many of the same genes that are identified as key in a number of biological processes are also simultaneously key players in human disease. These clues can now be pursued beyond correlation and ultimately to mechanism by the use of new and efficient site directed mutation technologies that use TALEN or CRISPR technologies. Thus, disease-producing mutations can be placed into hiPSC with one or more defined genetic backgrounds for clarification of how that single mutation affects cellular phenotype of different types of differentiated cells. As an example in this context, a number of recent discoveries demonstrate that phenotypes generated by alteration of

key endocytic factors also exhibit considerable dose sensitivity or sensitivity to genetic variation in model organisms and humans. In fact, a number of serious diseases in humans can be caused by modest changes in dose of these same genes that play important roles in endocytic sorting. Specifically, Alzheimers disease and Parkinsons disease can both be caused by duplication of a single gene such as amyloid precursor protein or alpha-synuclein, i.e., an increase of two copies to three copies or a 50% increase in gene dose. Although these minor increases in dose are often present and ignored in past cell culture studies, careful analysis of phenotypes often reveals significant and measurable changes that may not be lethal to organisms in the short term, but do have significant impacts on viability or functional pathways over longer time scales. These and many other similar types of issues can be rigorously dissected using the technologies inherent to human stem cell research.

We also expect that analyses of stem cell pathways *in vivo* in model systems and *in vitro* in human cultured systems will stimulate the discovery of new biological pathways. Humans as a model system in particular, given the relative paucity of such studies in the past and given the lack of experimental tractability, provide the opportunity to bring a greatly improved *in vitro* model system to biological discovery that is naturally linked to therapy development. Finally, as is the case with all model organisms, humans have unique advantages, including a large population with substantial genetic and phenotypic variation, strong systematic phenotyping at clinics, and large size and longevity relative to other model systems. We anticipate that just as studies of any new model system yield new discoveries owing to unique environmental and evolutionary adaptations, a systematic increase in human studies, linked to rigorous model organisms, will provide new insights of both biological and medical significance. When coupled with the complementary *in vivo* studies with model organisms, cultured human stem cells offer a powerful new system to make major new advances on these fronts. In fact, the analysis of genomic sequences and gene dose that are routinely incorporated into many current human hPSC studies will be important drivers of new discovery in this domain.

2) Probe mechanistic and phenotypic robustness by analyzing the impact of natural genetic variation: An important and poorly understood basic biological, and disease-relevant issue is understanding how basic cellular and developmental mechanisms have evolved so that they can function even in the presence of substantial amounts of genetic variation within and between species. In the latter case, conserved cellular mechanisms function approximately equivalently across species, even when significant genetic variation in the sequences or amounts of multiple components exist across species borders. In the former case, many studies in current model systems strive to test and evaluate function in defined, constant genetic backgrounds, e.g., C57Bl/6 or Balb/c mice that have been inbred to genome-wide homozygosity. Yet, it is consistently observed that changing the genetic background around a given mutation can lead to substantial alterations in phenotype associated with that mutation. Thus, along with studies of wild type and mutant cells derived from hPSC with an isogenic background defining a future important direction, the effects of a varying genetic background on the phenotypic expression of the mutation of interest is also of great interest and importance. More broadly how a cellular process can simultaneously be very sensitive to genetic background in some cases, while functioning normally and robustly in other genetic backgrounds is poorly understood, but hints at unknown design features of these systems that normally ensure phenotypic robustness.

This feature of genomic and species evolution is very apparent in analyses of humans where considerable inter-individual genetic variation of unknown impact is present, and where relatively minor changes in sequence can alter phenotype to generate disease. These issues can now be probed, with an enhanced focus on humans, by the union of contemporary complete genomic analyses with studies of cellular and

developmental mechanisms of stem cells differentiated in vitro. Some examples of scientific and medical problems that can be probed for the first time include a) how biological mechanisms are constructed to be functional and “resistant” to natural genetic variation; b) how basic cellular mechanisms are controlled so they can function in the context of different cell types with different overall identities, organization, and expression patterns across evolution; c) how basic mechanisms have evolved so that they function comparably in multiple species with different phenotypic requirements and different genomic sequences in key pathway components; and d) how germ-line and somatic genetic variation drives evolution of different types of misbehavior including malignancy and the evolution of pathways that generate malignancy.

3) Take advantage of a deep understanding of mechanisms to design and construct artificial, potentially enhanced organs and their components: A key goal in biology and bioengineering is to take insights from basic analyses of mechanisms and apply them to the design and construction of biological materials and devices. The ability to achieve these goals in “synthetic” biology will be markedly enhanced by methods developed to generate well-differentiated and stable cells needed for cell therapy in disease; better understanding of pathways and how they respond to different combinations of genetic variants from analyses of in vivo and in vitro differentiation; and the development of methods for supporting in vitro differentiation in 3-D complexes. Some examples of the in vitro development of complex subsystems include motor units, brain regions and circuits, retino-tectal projections, and kidney filtration systems. Successfully achieving the design and construction of these types of systems will confirm our understanding of their mechanistic underpinnings, provide enhanced materials for additional mechanistic analyses, provide materials for drug testing, test predictions from small molecule design efforts, and support experimental disease treatment by transplantation of in vitro generated organs or their components.

Suggested initiatives to accelerate discovery

Stem cell research is poised to revolutionize biology and medicine. We are just at the beginning to comprehend the possibilities for research and translational medicine offered by the ability to manipulate stem cells in vivo and in vitro. Scientists from different fields are moving their research focus toward stem cell research. This diversity of talent offers unique opportunity for scientific collaboration and unexpected breakthroughs.

1) Supporting audacious goals for stem cell research: Stem cell research has the potential to radically change the course of biological and medical research and disease therapy. But, current funding systems tend towards the conservative, and breaking with established systems, approaches, and/or research paradigms is more difficult than ever in a time of highly constrained funding. We suggest that stem cell research, viewed broadly, is primed for an audacious goals mechanism as it constitutes a new field that brings new technologies and intellectual paradigms to bear on old and previously intractable problems. Such a mechanism could fund investigators for projects that have risky, “over-the-top” yet feasible goals, that challenge the status quo technically or intellectually, or that investigate problems that were previously refractory to systematic inquiry.

2) Integrating differentiation pathway discovery in non-human and human systems: Non-human models will continue to play a crucial role in the elucidation of pathways that control cellular differentiation, all of which are likely to be critical for the generation of human cells in vitro with precisely controlled cell types, and for testing the physiological relevance of paradigms derived from cultured human stem cell research. The rate at which these pathways are coming into focus is increasing, and this type of investigation with non-human systems needs continued support. Importantly, owing to the substantial evolutionary conservation of pathways, insights into model systems become even more significant and impactful when rationally and rapidly coupled to studies with human stem cell differentiation in vitro. There will be great value in supporting individuals or teams of investigators to rapidly translate discoveries about developmental pathways in model organisms discovered via rigorous genetic or other analyses. For example, many signaling, transcription and secreted factors are known and available in forms that are readily usable in human stem cell differentiation in vitro. As in vivo analyses with non-human systems identify the order, timing, or identity of factors that drive the determination of specific cell types, these findings could be quickly applied in the human system. At the same time, distinctly new pathways may be discovered in the human cell system or important modifications of non-human pathways.

Suggested initiatives to reduce or remove barriers to entry and enhance availability of shared community resources

Many investigators would like to transform or augment their research directions by making the transition to incorporating stem cells, and in particular hiPSC into their basic and translational research. One of the most important barriers to making these transitions, however, is the relatively specialized knowledge base needed to work with stem cells. Although the information and methods are straightforward for most skilled scientists to acquire, there are many important methods that require specialized technical expertise, such as the derivation and culture of stem cells, or specialized and expensive equipment, like flow cytometry, that one or a few scientists working on their own will have a hard time fully incorporating into their work.

3) Stem cell training courses and institutional core facilities: A key barrier to entry for scientists seeking to begin

working with stem cells, particularly hESC and iPSC, are the idiosyncrasies and special features of culture conditions and manipulation, culminating in analysis and in vitro differentiation. Similarly, the rapid evolution of methods, culture reagents, and support equipment makes it very difficult for a single lab or investigator to keep pace with the field and to develop and maintain a consistent and novel research program using iPSC lines and adult stem cells. Thus there is a significant need for training courses offered by faculty and staff with proven expertise, performed in a manner that is free of commercial bias. The NIH has a history of supporting courses offered at WiCell, Pittsburgh, Children's Hospital of Cincinnati and others, but the need persists and will likely grow with further validation of the utility of stem cell systems in basic research.

Beyond foundational introductory training, there is a need for support of shared institutional core facilities. Core facilities supervised by expert investigators and staffed by professional scientists and technicians promote high standards of research practice and enable new investigators to access stem cell methodologies and oftentimes expensive and specialized equipment, thereby reducing the overall costs of research through economies of scale. First, institutional core facilities can test and QC reagents that are then supplied to research labs at reasonable cost. Second, they can develop and test new methods for cell line derivation, propagation and differentiation. Third, they provide local expertise and training, including institution-specific policies in ethics and human subjects research. Fourth, core facilities can serve as a repository of a number of valuable (e.g., well-characterized or otherwise desirable) hESC, iPSC or other stem cell lines, and keep a registry of stem cell lines available in their community. Even with current budgetary constraints, funding agencies can play a critical catalytic role by providing support to high quality institutional core facilities, akin to support for critical infrastructure for sequencing, imaging, proteomics and animal transgenesis.

Without access to good training and competent core facilities, researchers may never venture into the field, or worse, may initiate stem cell projects while poorly informed, leading to wasted effort and resources and publication of poor quality research that adds confusion to the literature. Funding agencies can catalyze stem cell research by providing support for shared core facilities, which would then recoup costs via charge-back to academic researchers and private sector contracts for a variety of specific services or training of corporate personnel. In fact, drawing academics and private sector personnel together into shared core lab facilities has value added for the whole enterprise.

4) Stem cell gene tagged libraries: The past several decades of research in model organisms have demonstrated the exceptional and far-reaching value of libraries of "tagged" genes and proteins. These tags have historically included small epitopes or fluorescent proteins such as GFP and can be used to report on transcription, RNA state, fate and location, and protein localization and stability. When introduced into endogenous loci at normal gene dose such that function is not perturbed, these libraries have been of exceptional value in analysis of biological processes, genetic screens for additional functions in complex pathways, and observational studies of cell, organ, or organismal behavior. We suggest that rather than creating a large top-down, centralized facility, tagged libraries should be developed using a grant supplement mechanism to ensure that investigators with specific relevant scientific expertise develop the actual tagged lines, which the NIH would then centralize, subject to establishment of quality standards, and then bank prior to distribution to investigators who would use them in research. Establishment of tagged libraries might serve as the precursor to establishment of libraries of gene knockouts or knockdowns, pending need and technical developments.

Appendix 1: ASCB Human Stem Cell Task Force

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