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An open letter to the American research community:

For 102 years Research Corporation for Science Advancement (RCSA) has supported unique, impactful basic research in the physical sciences at our nation’s universities and colleges. Today the level of real federal dollars devoted to basic research is projected to contract, even as the cost of research grows. Within this context RCSA, a private philanthropy, seeks to continue to make a difference. Therefore, during the decade ahead the Foundation and its partners will focus on expanding and improving RCSA’s two major programs:

**Cottrell Scholars** This program is expanded to include opportunities for early career Chemistry, Physics and Astronomy faculty at primarily undergraduate institutions (PUIs) as well as the traditional Cottrell Scholar beneficiaries from research universities. Cottrell Scholars are provided with unique opportunities to help them launch and establish truly outstanding careers. The size of the award is increased 33 percent to $100,000.

In addition to receiving financial support for research, Cottrell Scholars belong to a community whose members help each other to develop the skills and relationships necessary to become academic leaders. To strengthen this aspect of the program, RCSA announces two new awards:

- **Competitive Career Advancement Awards** of $25,000 will be presented at the Cottrell Scholars Conference held annually in Tucson, Arizona.
- **Cottrell Fellows Award**, $250,000. This is an annual competition for Cottrell Scholars who achieve tenure. It is meant to celebrate outstanding, forward-looking, creative ideas in basic research.

Since the inception of the program in 1994, RCSA has maintained its commitment to Cottrell Scholars throughout their careers. The Foundation continues to draw on senior members of the Cottrell Scholar family to serve as mentors for the next generation.

**Scialog** Supporting high-risk basic research on complex problems challenging society, Scialog seeks to stimulate real progress. Over the past five years, the Scialog (science+dialog) approach has proven highly effective at encouraging early career researchers to forge new collaborations across varied disciplines. Subsequently many of these collaborations, which have been focused on increasing efficiencies in solar energy conversion, have attracted federal support.

A second Scialog round, Molecules Come to Life, co-sponsored by the Gordon and Betty Moore Foundation, begins in March. It is projected to be a two-year initiative involving early career scientists from physics, biology and related disciplines interested in pursuing collaborative, high-risk discovery research on untested ideas in physical cell biology. This initiative aims to catalyze the development of a community in which theory informs experiment, with both working together to achieve understanding of fundamental cellular processes.

The challenge for Scialog is to identify timely and scientifically critical topics for future rounds. RCSA is asking all members of its 35,000-strong community of researchers, academic and science administrators to propose and discuss Scialog topics for the coming decade. Begin the conversation by contacting us at email@scialog.org.

As it has for the past century, Research Corporation for Science Advancement continues to fund innovative research as well as to nurture collaboration, community and superb leadership skills among America’s physical scientists.

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**Disease Modeling with Patient-Specific iPES Cells**

Whether in mice or macaques, disease modeling traditionally has been a tedious and expensive affair, not to mention unreliable, as mutations that are crippling in humans could have negligible impacts in animals, and vice versa. As drug development often requires preclinical animal studies, that disconnect has had real implications for the pharmaceuticals industry, both in money and missed opportunities. Today, researchers increasingly are migrating their models from the mouse house to the culture room. Armed with induced pluripotent stem cells made from patient cells, these investigators are probing everything from basic biology and disease etiology to drug discovery and cell therapeutics. **By Jeffrey M. Perkel**

In the nine years since Shinya Yamanaka first described induced pluripotent stem (iPES) cells, they have proven to be extraordinarily powerful research tools. Most obviously, they provide an ethically attractive alternative to embryonic stem (ES) cells, from which researchers theoretically can derive any cell type in the body. “An iPES cell and an ES cell are functionally equivalent,” says Emile Nuwaysir, chief operations officer at **Cellular Dynamics International** (CDI). But they are so much more than that.

For one thing, says Nuwaysir, they provide much-needed genetic diversity. Traditionally, researchers have little opportunity to test their models or therapeutics before initiating clinical trials, at which point projects all too often fall apart. Using iPES cells, however, researchers are limited only by their ability to acquire some noninvasive tissue samples, like a blood draw or skin punch, or the lines derived therefrom.

But it’s the iPES cells made from patient samples that truly expose the technology’s power. By recapitulating the growth and differentiation of those patient cells in a dish, researchers can investigate how mutations impact development, identify critical signaling pathways, and test potential pharmaceuticals—all without biopsy or animal models. “You can take a patient, … take their skin, [reprogram the cells in it], and then attempt to replay the disease,” says Clive Svendsen, director of the Regenerative Medicine Institute at **Cedars Sinai Medical Center** in Los Angeles. Researchers can even replay those diseases in cell types that otherwise are difficult if not impossible to obtain, simply by growing them from scratch. Svendsen notes, for instance, that “neurons are almost impossible to get from human tissues.” By using patient-specific iPES cells, “for the very first time, we’re able to interrogate neurons from Huntington’s disease … [and] Lou Gehrig’s disease, in the dish.”

**Columbia University Medical Center** ophthalmologist Stephen Tsang calls patient-specific iPES cells “a patient-in-a-dish.” In one study, Tsang generated them from two individuals with retinitis pigmentosa (RP), a form of inherited blindness affecting 1.5 million people worldwide. RP has multiple genetic sources, including a gene of unknown function called membrane frizzled-related protein (MFRP). Tsang’s study demonstrated that MFRP mutations lead to defects in actin organization, apical microvilli, and “leaky” cell-cell junctions—effects that could be reversed, both in patient cells and in mice, by delivering a wild-type copy of the gene via gene-therapy vectors. In effect, says Tsang, the study provided his team a budget-conscious way to test-drive its vectors. “It’s more cost-efficient to do testing of your viral vectors in culture than doing it in vivo,” he explains.

**Cell therapeutics**

Researchers use patient-specific iPES cells to drive three primary applications: basic biology, drug discovery, and cellular therapeutics. The former two applications are most widespread to date, but cell therapeutics are advancing, too. In 2013, Japanese researchers launched the world’s first clinical trial of a cellular therapeutic derived from iPES cells. Led by Masayo Takahashi at the **RIKEN Center for Developmental Biology**, the trial targets age-related macular degeneration (AMD), differentiating iPES cells from patients into retinal pigment epithelium (RPE), the cell layer underneath the retina that dies in the disease. Six patients are enrolled in the trial, the first of which underwent transplantation in September 2014.

The advantage of iPES cells for cell therapeutics is immune compatibility. Because the transplanted cells are derived from the patient’s own cells, no immunosuppression should be required—at least theoretically. “We don’t know that for sure yet,” says Dennis Clegg, codirector of the Center for Stem Cell Biology and Engineering at the **University of California, Santa Barbara**, “but there are at least a couple of experiments in mouse model systems that suggest that that’s the case.”

Clegg is working with David Gamm, director of the **McPherson Eye Research Institute** at the University of...
Wisconsin to develop strategies to differentiate iPS cells into RPE and photoreceptor cells for transplantation into patients with AMD.

The former cells are relatively simple: RPE proliferate easily, grow as a sheet, and “are used to taking a beating,” Gamm says. Photoreceptors, in contrast, “are kind of the divas of the retina.”

Photoreceptors have a required physical orientation, and they must form synaptic connections to function. They also are post-mitotic, Gamm notes, and fragile and difficult to purify to homogeneity. Once transplanted—and it isn’t yet clear which developmental stage would be best—the cells must integrate into preformed retinal neural circuitry, something cells never normally do. As Gamm puts it, the cells are “being asked to mature on their own, without the normal cues that would normally be there, and then make connections with cells that may not be too happy to begin with.”

Despite these difficulties, researchers are advancing such programs for eye and other disorders. Yet some say it is neither practical nor cost-effective to create, differentiate, and quality control iPS cells on demand. Instead, they advocate assembling banks of HLA-matched iPS cells—at least for some applications. It depends on the speed of disease progression and age of onset, says Joseph Wu, director of the Stanford Cardiovascular Institute. For a child with juvenile diabetes and a long life ahead, for instance, autologous cells could represent a sound investment, Wu says. “On the other hand, if you have an 80-year-old patient who has a heart attack, it probably makes less sense to create a line just for him or her.” Besides, “the cells are needed more urgently in this situation,” he adds.

The required size of such banks varies with a population’s genetic variation. By one estimate, 150 lines could match 93% of the U.K. population, whereas 50 lines would match 90.7% of Japanese (scim.ag/15LyY8). In the United States, CDI has recruited five donors for its bank, and two lines have been stored, Nuwaysir says. “Between those two donors, you have a match to 18% or more of the U.S. population.”

Technical considerations
Yamanaka’s original iPS cell study used lentiviral expression vectors to initiate cellular reprogramming. But as lentiviruses integrate their genetic material into the host genome, they can complicate downstream clinical applications.

Today, researchers have several alternatives. For a recent study into the pathophysiology of Niemann–Pick type C, a lysosomal storage disease, Rudolf Jaenisch, professor of biology at the Massachusetts Institute of Technology, used a Cre- excisable, doxycycline-inducible lentiviral expression system. More recently, his team favors mRNA-based reprogramming, a method that avoids genomic integration. Other RNA-based strategies include a self-replicating RNA developed by Steven Dowdy at the University of California, San Diego (and commercialized by both EMD Millipore and Stemgent) and Sendai virus, available from Life Technologies/Thermo Fisher Scientific.

iPS cells can also be made without nucleic acid initiators.

Lisa Ellerby at the Buck Institute for Research on Aging reprograms her cells by delivering purified transcription factors, a strategy that, like mRNA-based reprogramming, has been commercialized by Stemgent. In 2013, researchers in China demonstrated the ability to make iPS cells using just seven small molecules, several of which are available from STEMCELL Technologies.

At CDI, which has been awarded some $16 million from the California Institute for Regenerative Medicine to create 9,000 iPS cell lines representing 11 disease areas, iPS cells are made using episomal vectors, basically DNA plasmids that behave like eukaryotic chromosomes, Nuwaysir explains. “They have an origin of replication, so they replicate once per cell cycle like a mini-chromosome, but they’re relatively inefficient and lost over time,” he says, meaning by the time the cells are reprogrammed, the plasmids are long gone, leaving no genetic footprint.

Those cells will be banked at the Corell Institute for Medical Research. But CDI, Nuwaysir says, “is focused on using iPS cells as a platform for the manufacture of terminally differentiated cells, and the use of those cells from research to preclinical applications.” The company has developed protocols to reliably differentiate human iPS cells into 10 cell types, he says, including cardiomyocytes, dopaminergic neurons, and skeletal myoblasts, all of which are available under the company’s iCell brand.

Researchers who wish to differentiate their own cells can purchase preformulated differentiation media for select cell types from companies such as STEMCELL Technologies and Thermo Fisher Scientific. For most cell types, though, researchers are on their own.

Rohit Kulkarni, senior investigator at the Joslin Diabetes Center in Boston, for instance, uses patient-specific iPS cells to study the pathophysiology of diabetes and screen for therapeutics. But first, he’ll have to figure out how to turn those cells into insulin-producing beta cells, something that nobody has yet gotten exactly right, he says.

A true beta cell has distinctive anatomic, signaling, and secretory characteristics, Kulkarni explains, and iPS-derived cells often fall short of the mark. Thus, he calls them “beta-like.”
Groups led by Douglas Melton at Harvard University and Timothy Kieffer at the University of British Columbia have had some success on that front, however. Kieffer described a seven-step protocol that could turn an IPS cell into what he called S7 cells in about six weeks. These cells, he writes in a recent publication, are similar to beta cells “but also [exhibit] notable differences,” including in the kinetics of glucose response. Nevertheless, they reversed diabetes in a mouse model of the disease. Melton’s team reported a 34-day, six-step method of its own for “large-scale production of functional human β cells from [human pluripotent stem cells] in vitro.”

Because each cell line represents an individual with a distinct genetic background, comparing phenotypes across lines can be complicated. “You have to ask yourself, is [the phenotype] due to the variation you have anyway between different IPS cells … or is it due to the disease-relevant mutation you want to study,” Jaenisch says.

Increasingly, researchers address this problem using so-called isogenic controls—cell lines that differ by a single mutation. Typically, researchers use gene-editing tools, such as CRISPR/Cas9, to either repair or insert a key mutation in a cell line. Then, by comparing the modified line against the parental line, “you can compare apples with apples,” Jaenisch explains. His team has applied this approach to Parkinson’s disease, identifying subtle differences between normal and mutant cells, as well as pharmaceuticals that could negate those differences.

Going 3-D

While researchers often can drive cells towards their desired endpoint, they often stumble near the goal line. “What we get from IPS are very immature cells, whether it’s an islet cell or a heart cell or a neuron,” says Svendsen. “They don’t seem to mature to the extent they mature in the human body.”

For instance, it takes 90 days to turn IPS cells into RPE, Tsang says, and 200 days to make photoreceptors. Yet even then, the cells aren’t quite right: instead of hyperpolarizing in response to light, most are nonresponsive, or even depolarize.

In part, that could be a function of cellular age itself—cells may need to physically age to better mimic adult-onset diseases. (“Maybe if we grow [photoreceptors] for 300 days, then they’ll be right,” Tsang quips.) But, it also could be because the cells are maturing not in the human body, with all its environmental cues, but in 2-D sheets on plastic dishes.

Likewise, James Wells, director of the Pluripotent Stem Cell Facility at the Cincinnati Children’s Hospital Medical Center, has turned ES and IPS cells into gastric organoids (i.e., “mini-stomachs”) by delivering a precise set of growth factors in a defined sequence that models human development. “We’ve identified signaling pathways that we think are used in the developing embryo to induce this transition from two to three dimensions,” Wells explains. The result is a “primitive gut tube,” from which other gastrointestinal organs arise. Using that system, his team was able, among other things, to model Helicobacter pylori infection.

Wells now is extending the work to iPS cells from patients with such gastrointestinal maladies as cystic fibrosis, malabsorption syndromes, and Hirschsprung’s disease. “We can actually study embryonic development in a dish and find out what goes wrong developmentally in these congenital syndromes.” And at the end of the day, he adds, “it will also allow us in the future to correct the genetic defect and then generate healthy tissue.”

But don’t look for artificial stomachs any time soon, he says. Though researchers have had success generating relatively simple structures in a dish, the stomach and intestines are far more complex, with vasculature, neural networks, absorptive and secretory cells, and so on. Such structures could require “a Manhattan Project-level of investment in bioengineering [and] tissue engineering.”

It will take years to bridge that gap, if ever. But considering how far the research community has already come since 2006, anything is possible. Says Wells, “The therapeutic use of pluripotent stem cells is no longer science fiction. It’s current reality.”

Jeffrey M. Perkel is a freelance science writer based in Pocatello, Idaho.

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