(Pluri)potent acid

By Benjamin Boettner, Associate Editor

Academics and companies alike are scrambling—and thus far failing—to reproduce a surprisingly simple method for generating pluripotent mouse stem cells that uses an external stress stimulus to trigger reprogramming. Whether the method simply does not work or just faces initial hurdles similar to those experienced by other induced pluripotent stem cell–generating technologies remains to be seen.

If it does work, the technique offers minimal invasiveness without the need for genetic reprogramming factors, nuclear transfer or small molecules.

A team from the **Brigham and Women's Hospital** and **RIKEN Center for Developmental Biology** reported the generation of pluripotent mouse stem cells by simply exposing somatic cells to low pH.[1]

The state of the art for making induced pluripotent stem (iPS) cells is to introduce pluripotency-promoting genetic elements or combinations of small molecules into differentiated cells.[2,3] In both cases, the transition to iPS cells takes at least two weeks.

The group, led by Charles Vacanti, took genetic manipulation out of the equation and sped up culture times. Based on observations that differentiated plant cells can revert to a stem cell state under stress,[4] the group hypothesized that a similar phenomenon could occur in differentiated animal cells.

The researchers started with hematopoietic cells from newborn mice carrying an *Oct4-GFP* reporter transgene and monitored its expression following different stresses. Oct4 is of one of the core pluripotency factors.

The most effective stressor turned out to be an acidic environment. Mouse hematopoietic cells that were shocked for 30 minutes by low pH and then cultured in neutral medium needed only 7 days to reprogram and activate *Oct4-GFP*.

Other pluripotency markers also were induced, suggesting that the hematopoietic cells had indeed reverted to an embryonic stem cell (ESC)-like state. The group named the resulting cells stimulus-triggered acquisition of pluripotency (STAP) cells.

In vitro differentiation and *in vivo* teratoma formation assays showed that STAP cells gave rise to cell types representing all three germ layers, one of the hallmarks of pluripotency. When injected into early embryonic blastocysts, STAP cells turned into all tissues of the developing chimeric animals and the germ line.

One initial caveat of using STAP cells was their limited self-renewal and proliferation capacity. As a remedy, Vacanti's team added adrenocorticotropic hormone (ACTH) and leukemia inhibitory factor (LIF) to STAP cell cultures. ACTH and LIF help propagate mouse ESCs; indeed, adding the two molecules enabled expansion of STAP cell–derived cell lines.

The findings were reported in *Nature*.

Some of the same authors published a companion article describing how STAP cells cultured in medium containing fibroblast growth factor 4 (FGF4) instead of ACTH and LIF produced cells with the characteristics of placental tissues *in vivo*.[5]

"Reprogramming seems to be part of a physiological response to damage, probably to initiate tissue regeneration," said Manuel Serrano, a group leader at the **Spanish National Cancer Research Centre** (CNIO).

Serrano's group recently reprogrammed adult cells to pluripotent cells in living mice.[6]

"Our findings may mimic Mother Nature's approach to repairing injured tissue," said Vacanti, who is a professor at **Harvard Medical School** and chair of the Department of Anesthesiology, Perioperative and Pain Medicine at Brigham and Women's Hospital.

Ian Wilmut, professor emeritus and chairman of the **MRC Centre for Regenerative Medicine**, said, "Assuming that the results can be replicated, the authors will have created very important new opportunities in research to understand the molecular basis of cell fate. In the longer term, the findings may also herald new approaches to cell therapy."

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Play it again

Shortly after publication, other researchers raised questions related to the data reported in the papers and the overall reproducibility of the method of generating STAP cells. RIKEN and *Nature* have launched investigations.

"There has been a significant amount of interest, speculation and scrutiny since our STAP cell papers were published in *Nature*," said Vacanti. "I understand that questions have been raised around certain images that were used in the publication. I believe that these concerns are a result of minor errors that occurred in the manuscript editing process and do not affect the overall content of the published reports, the scientific data or the conclusions."

Jacob Hanna, a principal investigator at the **Weizmann Institute of Science** who is focused on pluripotency, said that his team has been trying without success to reproduce the method. However, he added that if replicated by his or another lab, the findings would be extremely exciting for the stem cell field.

Wilmut said that the media reports of irreproducibility were "disappointing because it seems to be such a simple procedure, but those of us with experience of laboratory work with cells know that it is actually quite common. There may be small differences in the way in which the protocol is applied which prejudice the outcome."

Chris Parker, VP and CCO at **Cellular Dynamics International Inc.**, and Matthew Vincent, director of business development at **Advanced Cell Technology Inc.**, said that their respective companies are going to test the STAP cell–generating methodology.

"It will be important to find out whether this can be done with human cells. Mouse cells are very malleable, and human cells are often more difficult to manipulate."
—Chris Parker, Cellular Dynamics International Inc.

Parker noted that there originally were problems in reproducing the first iPS cell–generating procedures. He thinks that STAP cell generation could get over this initial technical hurdle once the protocol's details are worked out.

Firming up the biology

Even if the reproducibility issue is laid to rest, there needs to be more research on the molecular and cellular mechanisms that initiate the transition to STAP cells and on the epigenetic state of the STAP cells.

In addition, Parker said that "it will be important to find out whether this can be done with human cells. Mouse cells are very malleable, and human cells are often more difficult to manipulate."

Cellular Dynamics produces iPS cell–derived cells for disease modeling, drug discovery and regenerative medicine.

Both Serrano and Hanna wanted to see a comparison between the transcriptional and epigenetic changes during STAP cell generation and those seen during iPS cell generation.

"The findings raise the question from what cellular context pluripotency in STAP cells arises," said Hanna. "We always think of transcription factors acting upstream, but these data say this state can also be induced without ectopic expression of transcription factors and beg the question of how cellular stresses affect chromatin and epigenetic imprinting."

"Given that nothing is known about the early events that are initiated by low pH treatment, coming up with informative measurements will be much more difficult than the analysis of defined transcriptional and genomic changes after the activation of pluripotency factors. The analytical net has to be broadened," said Parker.

Hans Keirstead, president and CEO of **California Stem Cell Inc.**, agreed that the underlying biology of STAP cells needs to be fleshed out. He also wanted to know more about the batch-to-batch consistency of STAP cells.

"Often, stem cells in the lab have great heterogeneity in proliferation rates and differentiation biases. A lot of reprogrammed cells are stable short term, but in longer-term, specific culture conditions [they] halt in their differentiation paths, lose lineage identity or even become neoplastic," he said. "These problems are pronounced in larger-scale cultures as part of manufacturing processes that require batch-to-batch consistency."

Vincent said that once sufficiently validated, STAP cells might help establish additional animal models that are hard to efficiently generate, such as dogs.

Vacanti acknowledged that generating STAP cells is unlikely to be a cookie-cutter approach. "In newborn mouse lymphocytes, low pH was most effective at creating STAP cells. However, I believe that the efficacy of different stresses in causing this reversion in mature, fully differentiated cells will vary with the specific cell type and species," he said. "A combination of sublethal stressful treatments that seriously injure specific mature cell types could ultimately be the most effective for causing them to revert to stemness."

Brigham and Women's Hospital has filed for a patent covering the technology. The IP is unlicensed.

Note added in proof: At press time, Vacanti told SciBX *that one of the coauthors on the* Nature *paper who had originally done a lot of the work with the* Oct4-GFP *mice also seemed to have trouble replicating the findings. Vacanti's scientific director met with the coauthor to review the technique he was using and generate STAP cells in the coauthor's laboratory.*

"We learned that there are some very specific facets to the technique that evidently are not perfectly obvious to the reader," said Vacanti. "If our protocol is strictly followed, the STAP cells can easily be generated. If, however, some seemingly minor steps are omitted or not performed precisely, STAP cells generating Oct4 *are not generated. Consequently, we are in the process of setting up a 'Vacanti Lab/STAP cells' Web site that contains the precise protocols in sufficient detail for anyone to generate* $Oct4^+$ *STAP cells from mature cells acquired from young animals as well as from fully mature adults."*

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COMPANIES AND INSTITUTIONS MENTIONED

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