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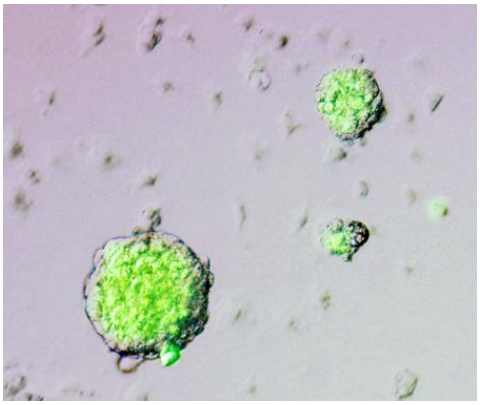
STAP cells overturn the pluripotency paradigm

Jan 30, 2014 – The diversity of the cells in the body results from the process of differentiation, in which cells sequentially express subsets of their genetic code and take on specialized functional and morphological characteristics. In development, this process traces back to pluripotent cells in the early embryo, which are capable of giving rise to any type of cell in the body. Embryonic stem cells are perhaps the best-known example of cellular pluripotency, but in recent years a number of techniques, such as somatic cell nuclear transfer (SCNT) and the transfer of pluripotency-associated genes, have been developed to reprogram cells to a pluripotent state. But such methods tend to show poor efficiency, and involve technically demanding and time-consuming techniques.

Now, a new pair of reports by Haruko Obokata (Unit Leader, Laboratory for Cellular Reprogramming) describes a naturally occurring process that the authors have dubbed stimulus-triggered acquisition of pluripotency (STAP), which converts differentiated mouse cells shocked by external stress into a remarkably pluripotent state. These breakthrough findings, made in collaboration with Charles Vacanti's lab at Brigham and Women's Hospital, Harvard University, and colleagues at the CDB laboratories for Genomic Reprogramming (Teruhiko Wakayama, Team Leader; now at Yamanashi University), Pluripotent Stem Cells (Hitoshi Niwa, Project Leader), and Organogenesis and Neurogenesis (Yoshiki Sasai, Group Director), stand to revolutionize not only the study of pluripotency and cell reprogramming, but our understanding of the stability and irrevocability of differentiation itself.



Lymphocytes exposed to low-pH conditions are reprogrammed to a pluripotent state within 3 days. GFP shows expression of pluripotency marker Oct4, which is activated within about 2 days.

Previous reports by Vacanti's and other groups had suggested that such cells might be present in trace amounts in the adult body, but these findings remained controversial. The new work in *Nature*, however, now shows that the acquisition of pluripotency is the result of a process of stress-triggered acquisition of pluripotency, rather than an endogenous cellular phenomenon. In this years-long long undertaking, Obokata et al. set out to rigorously test observations of functionally pluripotent cells obtained from differentiated tissue.

At the Vacanti lab in Harvard BWH as a visiting student from the Waseda University–Tokyo Women's Medical University joint graduate school program, Obokata was engaged in a hunt for endogenous pluripotent cells, using various methods to screen cells harvested from adult tissues. She published initial findings with Vacanti and colleagues on their observations of what seemed to be just such a pluripotent population in 2011, but the mechanism behind their appearance remained unclear.

Having noticed that cells screened by trituration, which involves forcing them through a tiny capillary, formed pluripotent clusters, she began to suspect that the technique she was using to *select for* pluripotent cells might instead be *creating* them. "A big part of the discovery process was in trying to work out a better explanation for what at the time was an unexplainable observation," says Obokata. "I'm not sure if there was a single 'Eureka!' moment, but as I kept working with and thinking about these cells, there increasingly seemed to be only one way to account for what I was seeing."

Returning to Japan to investigate this serendipitous finding on a more stringent basis, Obokata and

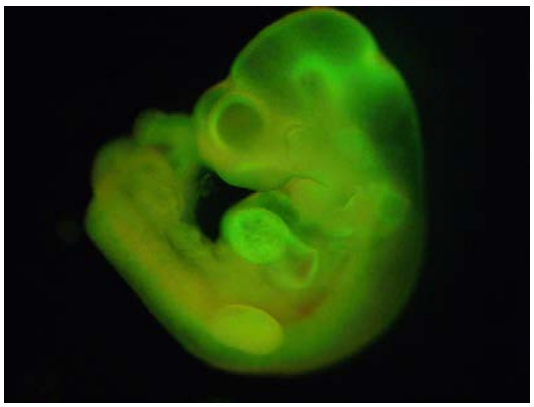
colleagues at RIKEN CDB and Harvard next began collaboratively testing how lymphocytes, which undergo natural genetic rearrangements that enable reliable identification of their progeny, respond to various sublethal chemical and physical stresses. Screening for expression of the hallmark pluripotency gene, *oct4*, she found that following a brief exposure to low pH conditions, a significant percentage of cells began to shrink, lose their 'memory' of lymphocyte differentiation, and express *oct4*. Tests with cells from other neonatal tissues, including skin, brain, muscle, fat, and bone marrow, showed that a wide range of differentiated somatic cells can similarly undergo STAP conversion.

Gene expression alone, however, is insufficient to demonstrate bona fide pluripotency, so Obokata next embarked on a series of tests of the function of these apparently reprogrammed cells. Epigenetic tests revealed demethylation of pluripotency genes, while in vitro differentiation assays showed these *oct4*$^{+}$ STAP cells were able to give rise to derivative cells representing all three germ layers. Interestingly, STAP cells show only limited capacity for self-renewal and colony formation when compared to ES cells, suggesting a disconnect between pluripotency and these other definitive 'stemness' traits.

Building on these exciting in vitro findings, Obokata turned to in vivo assays, the gold standard tests of pluripotency. Working with Teru Wakayama, then a CDB team leader, she found that STAP cells injected into mouse blastocysts contributed efficiently to the formation of healthy, genetically normal chimeric mice, and were capable of germline transmission. Using a technique known as tetraploid complementation, which enables pluripotent cells injected into a blastocyst to give rise to all of the cells in the resulting individual, she found that lymphocyte-derived STAP cells could in fact give rise to an entire embryo.

Even more remarkably, STAP cells were capable of contributing not only to all embryonic lineages, but to extraembryonic tissues, such as placenta and fetal membrane, as well. This represents a degree of differentiative capacity that exceeds that of even embryonic stem cells, which can give rise only to embryonic somatic lineages, not extra-embryonic ones.

Obokata next turned to the question of whether these extraordinarily pluripotent cells could be induced to take on a more stem cell-like fate. She found that by adding ACTH (adrenocorticotropic hormone) to mouse ESC culture medium, she could prompt STAP cells to form colonies capable of exponential long-term expansion in vitro, and even the formation of colonies from isolated individual cells, while retaining their pluripotency. She called these ESC-like cells, STAP stem cells (STAP-SCs), in recognition of their combination of pluripotency and capacity for self-renewal. Like their STAP cell antecedents, STAP-SCs were able to contribute to pluripotent in vitro differentiation and chimera formation, but showed differences in gene expression and epigenetic regulation, suggesting they had assumed a state more closely resembling that of embryonic stem cells.



On injection into a mouse blastocyst, STAP cells contribute to embryonic development. Using tetraploid complementation, it is possible to generate chimeric mice in which 100% of the embryo's somatic tissues are derived from STAP cells.

In a final test of the developmental potency of STAP cells, Obokata examined whether they could also

give rise to trophoblast stem (TS) cell-like cells as well. She found that by adding FGF4 to STAP cell culture, she could trigger their differentiation into a TS cell-like fate. These FGF4-induced stem cells (FI-SCs) closely resemble mouse trophoblast stem cells in their expression of trophoblast marker proteins, capacity for in vitro expansion, and the ability to contribute to placental tissue. The STAP-derived FI-SCs do exhibit some differences from ordinary trophoblast stem cells, in that they retain *oct4* expression and express lower levels of Cdx2 (a protein marker of TS cells), and contribute to both embryonic and extraembryonic lineages—something TS cells do not ordinarily do. Intriguingly, when cultured in embryonic stem cell culture medium, FI-SCs showed the ability to give rise secondarily to ESC-like cells expressing pluripotency genes and capable of teratoma formation, indicating the remarkable extent of these STAP-derived cells' plasticity.

These new reports of a stress-induced process whereby differentiated cells can shed their cellular identity and convert to a previously unknown 'super-pluripotent' state capable of giving rise both to embryonic and extra-embryonic lineages have startling and far-reaching implications for our understanding of cell differentiation, pluripotency, and reprogramming. "This research would not have been possible without the significant contributions of every author involved and the international collaboration between Brigham and Women's Hospital and the RIKEN CDB," says Charles Vacanti, chairman of the Department of Anesthesiology, Perioperative and Pain Medicine and Director of the Laboratory for Tissue Engineering and Regenerative Medicine at BWH.

"I think it is no exaggeration to say this represents something of a Copernican revolution for developmental biology," continues CDB Deputy Director Sasai. "More than just the ability to generate pluripotent cells from a somatic source, this is a fundamental challenge to some of the core principles of cell differentiation and plasticity, principles upon which the entire field is based."

Obokata adds, "It's exciting to think about the new possibilities these findings open up, not only in areas like regenerative medicine, but perhaps in the study of cellular senescence and cancer as well. But the greatest challenge for me going forward will be to dig deeper into the underlying mechanisms, so that we can gain a deeper understanding of how differentiated cells can convert to such an extraordinarily pluripotent state."

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