Citations

Reawakening Sleeping Beauty

A dozen years ago, a reconstituted fish transposon was shown to be active in mammalian cells. Though called *Sleeping Beauty* (SB) to reflect its reemergence, this transposon retained some somnolent features, most notably poor results in primary cells. Writing in *Nature Genetics*, Mátés et al. reawaken the transposase component by creating a hyperactive mutant, SB100X. In HeLa cells, SB100X was 4–100× more potent than the original and outperformed an optimized *piggyBac* transposon. Crucially, SB100X did best when transposon copies were limited, including when mobilizing a chromosomally housed element. When plasmid-borne SB transposon was coinjected into mouse oocytes with SB100X transposase mRNA, 37% of resultant mice generated transgenic offspring. In human hematopoietic stem cells transfected with SB transposon and the SB100X expression plasmid, 70–90% of transfected cells underwent transposition. After in vitro differentiation, 33–45% of cells of the resulting hematopoietic lineages were positive for the transposed marker. A related experiment using human cord blood stem cells engrafted into immunodeficient mice showed transposon integration and persistence throughout the reconstituted hematopoietic system. After in vivo transfer of a liver-directed construct in mice and partial hepatectomy, expression levels were restored after recovery, implying that the transposon was stably integrated and perpetuated through cell proliferation. Together, these findings suggest SB100X is a promising new tool for transgenesis and, potentially, therapeutic gene transfer. For the former, SB100X offers higher insertion frequencies without the drawback of concatemeric integration. For the latter, SB100X opens the door to efficient transposon-based transfer in primary cells, and, unlike viral vectors, integration preferentially occurs in intergenic regions, presumably reducing the risk of insertion oncogenesis. Hence, SB100X may reawaken interest in SB for gene transfer applications.

Mátés et al. Molecular evolution of a novel hyperactive *Sleeping Beauty* transposase enables robust stable gene transfer in vertebrates. *Nat Genet* [Epub ahead of print, May 3, 2009; doi: 10.1038/ng.343].

A Polarizing Influence

Polarizing environments don't bring out the best in adult brains, but in neurodevelopment, polarization—here an increase in the magnitude of a cell's membrane potential—is a critical step. In the neonatal brain, expression changes in chloride transporters diminish this anion's intracellular concentration over time. Under high intracellular chloride, activation of GABA_A receptors produces a depolarization, but later, when the chloride balance has shifted, these receptors lead to hyperpolarization. Unfortunately, reproducing these steps while differentiating progenitors in vitro is difficult, and so cultured neurons tend to have a depolarized resting membrane potential. Extrapolating from studies in *Drosophila* and writing in the *Journal of Neurochemistry*, Schaarschmidt et al. hypothesized that shifting extracellular ion concentrations in the culture medium could get neurons on the right track. Thus, the authors took human neural progenitor cells and, instead of using standard differentiation medium, turned to high-potassium medium for one week (depolarizing conditions), followed by high-sodium (hyperpolarizing) medium the following week. Compared with cells exposed to standard differentiation medium, cultures treated with the modified regimen showed an increased number of neuronal cells and a decrease in glial cells. In addition, the modified procedure significantly upregulated neurite growth. Importantly, the changes were not confined to morphology. Most notably, the modified procedure produced neurons consistently capable of firing action potentials; by contrast, only about two-thirds of

neurons grown in standard conditions managed this, and often only after receiving stronger stimulation. Although there was evidence of diminished viability over time, researchers with an open mind about trying the new protocol will likely find the more authentic neuronal responses ideal for short-term experiments.

Schaarschmidt et al. 2009. A new culturing strategy improves functional neuronal development of human neural progenitor cells. *J Neurochem* 109(1): 238–47.

Network News

Sticking to nothing but the facts is vital to reliably mapping gene regulation networks. However, in silico prediction of putative transcription factor binding sites has outpaced methods to identify the responsible regulatory factors. Closing this gap is the goal of a

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technique published by Freckleton et al. in *PLoS Genetics*. The authors call their approach MaPS, for microarray-based profiling of phage-display selection. In the first step, a DNA containing a predicted transcription factor binding sequence is immobilized and exposed to yeast genomic DNA phage display library. Binders are eluted, amplified, and then

reselected. Next, DNA is prepared from the selected phage, fluorescently labeled, and hybridized to a yeast whole-genome ORF microarray. If all goes well, the highest-ranking microarray hits should be factors that bind the input DNA sequence, a hypothesis that can be tested with gel shifts, chromatin immunoprecipitation, and other traditional DNA-protein binding assays. The process works well: the authors readily identified the binding factor of a computationally predicted motif that had eluded previous validation efforts. As a combination of well-established techniques, MaPS is readily implementable in almost any lab. This is a contrast to DNA affinity chromatography, which requires considerable technical skill. Moreover, MaPS is not limited to detecting just one DNA-binding protein at a time, and, unlike chromatography, does not require large quantities of starting material. Similarly, MaPS avoids the artifacts associated with yeast one-hybrid approaches, and there is no need to express purified proteins as required by protein-binding microarray methods. In short, MaPS offers a straightforward, global method to survey the proteome for binders of a specific DNA sequence, a development that should be welcome news to anyone seeking to identify all the players in a transcriptional network.

Freckleton et al. 2009. Microarray profiling of phage-display selections for rapid mapping of transcription factor–DNA interactions. *PLoS Genet* 5(4): e1000449.

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