Citations

Be More Positive

Fuse a bit of HIV-1 Tat protein or *Drosophila* antennapedia to your protein of interest, apply to cells, and by the magic of protein transduction, a once-exogenous protein is happily at home within the cell, all without the hassle of transfecting a DNA expression construct. Unfortunately, transduction success often depends upon high concentrations of recombinant protein, and matters only get more challenging with *in vivo* delivery. For these reasons, improving protein transduction remains an important area of technical development. Recently, a paper in *ACS Chemical Biology* from Cronican et al. brought some good news to the field. The authors' work grew out of previous research on improving the resilience of proteins by "supercharging" them, which is mutating solvent-exposed residues to charged amino acids. One of the proteins modified in this way was GFP, in which 29 amino acids were changed to lysine or arginine, resulting in a change in net charge from -7 to a predicted +36. Supercharged GFP proved to be robust against heat- or chemical-induced denaturation, and, in subsequent studies, succeeded in delivering siRNA and plasmid DNA into cells via an endocytotic pathway. In the new work, Cronican et al. compared the efficacy of supercharged GFP

for protein transduction with the well-established transduction domains of Tat, oligoarginine, and penetratin (a peptide from antennapedia). As tested in 5 cell lines, supercharged GFP delivered 10- to 100-fold more cargo than Tat or oligoarginine, and showed a 6- to 20-fold improvement over penetratin. Transduction in the presence of 50% serum halved but did not abolish transduction. Additional experiments verified that the cargo could access the cytosol or nucleus, as appropriate. In the most demanding test, a fusion of Cre recombinase with supercharged GFP was injected into the subretinal space of mice that were transgenic for a Cre-dependent reporter. Compared with a Tat-Cre fusion, supercharged GFP-Cre generated twice as many recombination-positive retinal cells. Researchers looking to supercharge delivery of protein cargos in tissue culture or living organisms should find the new GFP-based transduction domain a highly positive development.

Cronican et al. 2010. Potent delivery of functional proteins into mammalian cells *in vitro* and *in vivo* using a supercharged protein. *ACS Chem Biol.* 5:747-752.

TILLING New Ground

Targeting induced local lesions in genomes (or TILLING) is a popular technique for reverse genetic screens in plants. Since there are now TILLING centers with catalogs of DNA samples and seeds available for fee-for-service screens, obtaining mutant plants is as simple as sending a request to a resource center and awaiting delivery of the seeds. Despite their convenience, such centers only offer mutants on standard genetic backgrounds. Until recently, researchers wanting to screen specialized genetic backgrounds faced the prospect of plowing vast financial resources and labor into the infrastructure needed for traditional TILLING. Now, a paper in *Plant Physiology* from Bush and Krysan introduces iTILLING (that is, individualized TILLING), which allows a lone scientist to go from idea to mutant seedling in just 4 months. At first, iTILLING follows the standard procedure: seeds are exposed to a chemical mutagen and then allowed to germinate and self-pollinate. However, iTILLING avoids storing individual plants or DNA samples. Instead, the procedure uses a clever workaround called "Ice-Cap." The seedlings to be screened are grown in agar plugs housed in a 96-well spin column plate, which is

Seedlings grown in a 96-well plate awaiting mutation screening by iTILLING. Reprinted with permission © 2010 ACS.

stacked on a plate filled with water. After three weeks, the roots grow to reach the water and the lower plate is snap-frozen and removed. The root tips become raw material for DNA extraction, and the seedlings are refrigerated in the dark until mutation detection is complete. The screen for iTILLING is a high-resolution melting curve assay for heteroduplexes, based on PCR amplification of the target gene. If one seedling has been placed per well, the screen will only detect heterozygotes; alternatively,

seedlings can be doubled up in each well to screen for homozygotic mutations (the two mutant seedlings act as sources of wild-type DNA for each other). As proof-of-principle, the authors surveyed *Arabidopsis* plants containing a homozygous *mekk1* mutation for polymorphisms in the adjacent *mekk2* and *mekk3*. By screening 8000 plants, the authors identified 11 unique mutations in *mekk2* and *mekk3*. Analyzing the phenotypes of these mutants would entail retrieving the corresponding seedlings; the remainder of the seedlings and DNA samples are then discarded. All told, iTILLING yielded 1 mutation per 415 kb analyzed; a result that the authors say could be improved with higher-resolution melting curve analysis equipment. They see a valuable niche for iTILLING for screening ecotypes not covered by traditional TILLING, and for producing plants with multiple mutations in tandem gene families. And, provided that the seeds fit into the wells of the screening plate, any plant species should be compatible with iTILLING.

Bush and Krysan. 2010. iTILLING: a personalized approach to the identification of induced mutations in *Arabidopsis*. *Plant Physiol.* 154:25-35.

Lift-off

Synapses are the dedicated sites for signaling and communication between neurons. Alteration or damage to the architecture of a synapse can result in neuronal disorders and diseases. While recent studies have greatly increased our understanding of the molecular composition, assembly, and function of synapses, translating this knowledge into new methods for restoring function to damaged nerve cells remains challenging. This deficiency and barrier to therapeutic development prompted Lucido et al. to devise a method for isolating presynaptic complexes from in vitro neuronal cultures. Their technique, which is published in *ACS Chemical Neuroscience*, takes advantage of the authors' previous fabrication and development of surface-modified latex and silica beads that can induce specific cellular responses when co-cultured with cells. In particular, polystyrene sulfonate beads coated in poly-D-lysine and supported lipid bilayer promoted the formation of functional presynaptic boutons when co-cultured with neuronal cells. But before these beads can be used to develop therapies for damaged CNS neurons, the artificially induced synapses need to be carefully and thoroughly characterized. To enable this, the authors developed the "sandwich/lift-off" method. In this strategy, primary rat hippocampal neuronal cultures are established and grown on coverslips prior to the addition of the coated beads, which are dropped into the culture and remain with the cells until isolation of the complexes. After induction of the presynaptic assemblies at the points where the beads contact the axons, a second coverslip coated with poly-D-lysine is pressed on top of the culture and then lifted off, carrying with it the isolated beads and presynaptic boutons containing presynaptic proteins and functional synaptic vesicles capable of releasing neurotransmitter, but leaving behind neuronal cell bodies and postsyn-

Isolated bead-axonal membrane complexes labeled with Dil. Reprinted with permission © 2010 ACS.

aptic factors. The method is simple and versatile allowing experiments to be carried out under ambient conditions for further understanding of the function of presynaptic boutons. These preparations may be particularly valuable for studying the molecular composition of presynaptic endings, the kinetics of synaptic vesicle cycling, and the molecular triggers of presynapse formation. Another benefit of the technique is the ability to perform mass spectrometry-based analysis of the synaptic components recruited to the beads, thus providing a technique for studying synaptogenesis and potentially aiding in the development of regenerative therapeutic approaches.

Lucido et al. 2010. Isolation of functional presynaptic complexes from CNS neurons: A cell-free preparation for the study of presynaptic compartments in vitro. *ACS Chem Neurosci*. 1:535-541.

Sight Unscreen

While generation of hybridomas is the traditional technique for producing monoclonal antibodies, methods such as B-cell immortalization or yeast and phage display of synthetic antibody libraries have been advanced in recent years. These newer methods, however, require high-throughput screens to identify the desired monoclonal antibody, which is often a complex and lengthy process. However, in a recent *Nature Biotechnology* paper, Reddy et al. describe a simple and rapid method for monoclonal antibody isolation without the need for screening. The key to this new approach is the use of high-throughput DNA sequencing and bioinformatics to characterize the V_L and V_H gene repertoires expressed in the mRNA of the antibody-secreting bone marrow plasma cells (BMPCs). These plasma cells—which cannot be immortalized—are longer-lived than plasma cells derived from secondary lymphoid organs and are responsible for generating the stable

population of circulating antibodies that are the most critical for the humoral immune response. To test their method, the authors injected pairs of mice with one of three different antigens. Total RNA was then extracted from the purified BMPCs of each mouse and reverse transcribed into first-strand cDNA. Using degenerate V gene primers, V_L and V_H mRNA sequences were amplified and subjected to high-throughput sequencing. Bioinformatics analysis identified the most abundant V_L and V_H gene sequences expressed in each immunized mouse, which were then paired with each other based on similar rank-order frequencies of expression. The assumption is that V_L and V_H genes expressed at the same frequency in each mouse were likely to be derived from the same plasma cell and therefore naturally pair with each other. Candidate antibody genes were next assembled using automated gene synthesis techniques and expressed as single-chain variable

fragments (scFv) in bacteria and as full-length IgG in mammalian cells. Of the 28 antibodies produced from the six mice, 21 were antigen-specific when tested by ELISA. Although only demonstrated in mice, this method should be easily adapted to primates, including humans, and the authors believe that these monoclonal antibodies could be especially suitable for therapeutic applications given their derivation from BMPCs.

Reddy et al. 2010. Monoclonal antibodies isolated without screening by analyzing the variable-gene repertoire of plasma cells. *Nat Biotechnol*. 28:965-969.

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