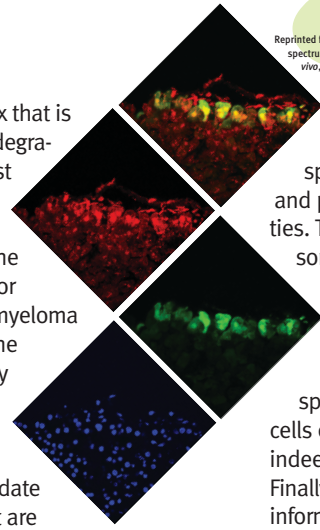
Spotlight

Proteasome Profiling



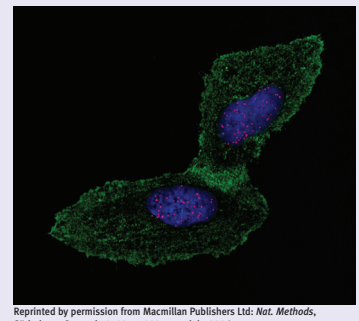
Reprinted from *Chem. Biol.*, *13*, Verdoes, M., *et al.*, A fluorescent broad-spectrum proteasome inhibitor for labeling proteasomes *in vitro* and *in vivo*, 1217–1226, Copyright 2006, with permission from Elsevier.

The proteasome, the multiprotein complex that is responsible for a large portion of protein degradation in the cell, has captured the interest of scientists from diverse areas, including evolutionary biology, the biochemistry of proteolysis, and drug discovery. Indeed, the recent approval of the proteasome inhibitor bortezomib for the treatment of multiple myeloma underscores the value of understanding the structure and function of this extraordinary protease. Activity-based inhibitors that become specifically and covalently attached to the proteasome have contributed greatly to its characterization, but to date no compounds have been developed that are simultaneously specific, irreversible, sensitive, and cell-permeable and that can enable visualization in live cells. Now, Verdoes *et al.* (*Chem. Biol.* 2006, *13*, 1217–1226) present the synthesis and biological characterization of MV151, a fluorescent, cell-permeable vinyl sulfone-based inhibitor that can label proteasomes both *in vitro* and *in vivo*.

MV151, synthesized with standard Fmoc-based solid-phase peptide chemistry, exhibited potent and specific activity against the trypsin-like, chymotrypsin-like, and peptidylglutamyl peptide hydrolytic proteasomal activities. The utility of MV151 was first demonstrated in proteasome profiling experiments in which cells were treated with known proteasome inhibitors, lysed, and then incubated with MV151. With a fluorescence scanner, direct in-gel visualization of proteaseome activity not blocked by the known inhibitors enabled elucidation of their specificity. Next, fluorescence microscopy analysis of live cells exposed to MV151 demonstrated that the inhibitor was indeed cell-permeable and colocalized with the proteasome. Finally, tissue analysis of mice treated with MV151 revealed information about the bioavailability of the inhibitor. The authors observed that MV151 accumulates in the liver and pancreas and that the proteasome was labeled as expected in these tissues. The ability of this new molecular tool to facilitate characterization of proteasome inhibitors for both biochemical and medical applications will contribute significantly to progress in proteasome research. **Eva J. Gordon, Ph.D.**

DNAbling Protein Partners

Mass spectrometry and proteomics approaches can generate a laundry list of factors present in a particular cell type, but a central question still remains on the molecular biologist's mind. What proteins interact with one another and work together within a cell? Common procedures that address this question have suffered from artifacts or insufficient resolution or sensitivity. Breaking open the cell and probing interactions within cellular extracts can also lead to misinterpretations. Now, a new procedure appears to take on the protein–protein interaction saga with a fresh set of twists. Drawing from the cell biologist's toolkit, Söderberg *et al.* (*Nat. Methods* 2006, *3*, 995–1000) start with antibodies directed against two candidate cellular proteins. *In lieu* of fluorescent dyes, the authors covalently attached small DNA oligonucleotides to each antibody. Incubation of the modified antibodies with fixed cells sent each DNA cargo to a particular spot in the cell. Then, a series of enzymes and extra oligonucleotides were added to assay the proximity of the DNAs found on the antibodies. If the two DNAs were close enough to template ligation, a polymerase could then replicate a circular DNA ligation product that formed at the site of ligation. After just 1 h, a DNA strand that included 1000 copies of that particular DNA circle was present where the two proteins were interacting with one another. The amplification products were then detected by hybridization of a fluorescent DNA complementary to the amplification product. This methods paper demonstrates the "proximity ligation" technique on cell lines and tissue sections using a known set of interacting proteins. Importantly, this method appears sensitive enough to visualize endogenous protein interactions without overexpression or other manipulations. With such a robust amplification, single-molecule interactions may be observable with the right conditions. **Jason Underwood, Ph.D.**



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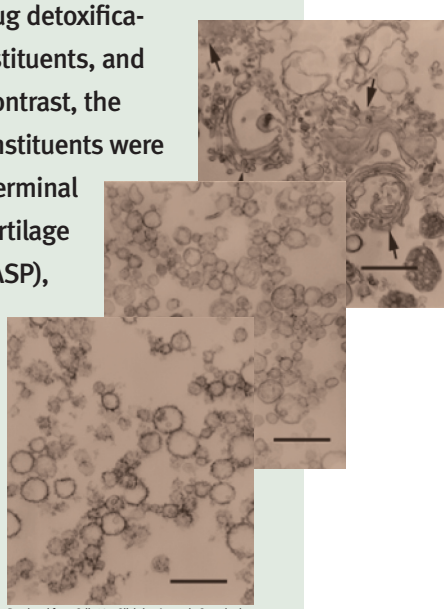
The Secrets of Secretion

The endoplasmic reticulum (ER) and the Golgi apparatus are key elements of the secretory pathway of the cell. Recent advances in proteomics have enabled quantitative profiling of the proteomes of various subcellular compartments, but a complete and accurate map of the secretory pathway has not yet been elucidated. Now, Gilchrist *et al.* (*Cell* 2006, *127*, 1265–1281) describe a quantitative proteomic map of the rough ER, the smooth ER, and the Golgi apparatus.

Using ER and Golgi fractions isolated from rat liver homogenates and tandem mass spectrometry, the authors extensively characterized the ER and Golgi proteomes. A method referred to as redundant peptide counting was employed to quantify the relative abundance of the proteins in the organelles of interest and also provided a means to approximate the amount of contamination present from other organelles. Investigation of the Golgi proteome was extended to include coatomer protein complex I (COPI) vesicles, which have been speculated to be involved in protein transport between the ER and Golgi compartments. Notably, 1430 proteins were found to be components of the ER and Golgi apparatus. Of these, 832 were found only in the ER, 193 were present solely in the Golgi/COPI vesicles, and 405 were found in both organelles. Functional analysis revealed that the proteins unique to the ER were largely represented by ribosomal proteins, translocon constituents, molecular chaperones, proteins involved in lipid oxidation and drug detoxification, proteasome constituents, and ubiquitin ligases. In contrast, the Golgi/COPI vesicle constituents were composed mainly of terminal sugar transferases, cartilage associated protein (CASP), Golgin, soluble *N*-ethylmaleimide-sensitive factor attached receptor proteins (SNAREs), and a subset of Ras-related in brain proteins (Rabs) and conserved oligomeric Golgi proteins (COGs). Additional analysis indicated that 28% of the proteins were integral membrane proteins, and 345 proteins were of unknown function. This impressive portrayal of the ER and Golgi proteomes helps to expose the mechanisms that direct the secretory pathway machinery, such as cisternal maturation. Importantly, the combination of rigorous biochemical fractionation and random peptide counting used in this study can also be applied toward the characterization of other proteomes or, indeed, applied directly in medical studies to define biomarkers in disease. Eva J. Gordon, Ph.D.



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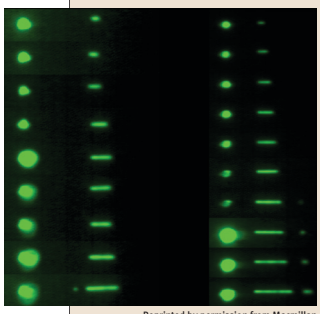
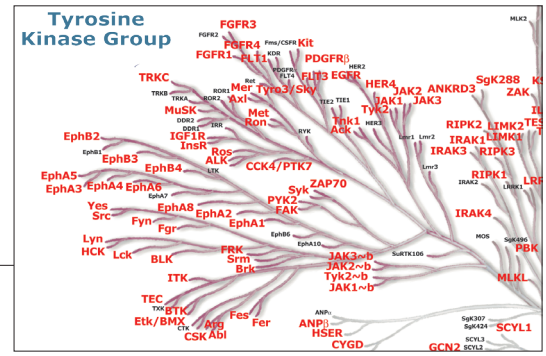
Filaments under the Flashlight

In all organisms, double-stranded lesions in the DNA are repaired by homologous recombination. A critical protein for this process in bacteria, RecA, polymerizes on single-stranded DNA to form nucleoprotein filaments and signal exchange with the proper double-stranded DNA. The formation of RecA filaments requires binding of a nucleotide cofactor but not hydrolysis. Many labs have investigated RecA's structure and the sequence requirements for proper function, but assay limitations have made studying the polymerization phenomenon difficult. Now, a new single-molecule technique illuminates RecA filament formation for the eye to see. Galletto *et al.* (*Nature* 2006, *443*, 875–878) took advantage of a fluorescently labeled RecA protein and optically trapped phage DNA. Using a laminar flow cell, the authors effectively dipped the DNA in and out of the protein solution under different salt and nucleotide conditions. Using a fluorescence microscope, they took snapshots at various time points to visualize both the formation of new RecA nuclei and the subsequent polymerization of RecA on the DNA. The authors show that the nucleation event is exquisitely sensitive to protein and salt concentrations and requires a nucleoside triphosphate cofactor but not its hydrolysis. Once the RecA seed was planted, the polymerization rate was not affected by the nucleotide cofactor, and two to seven RecA molecules were added per second. Also, this unique assay revealed that while filaments mostly grow in a 5′ to 3′ direction, they can also grow in the opposite direction from an initial RecA nucleus. The parameters uncovered by this technique indicate that the rate-limiting step is formation of the initial nucleus of four to five RecA molecules. The authors comment that a similar protein, RAD51, is found in eukaryotes, so this technique will be applicable to other similar phenomena that lack compelling mechanistic data. Such an assay might also be useful for small-molecule screens that take aim at inhibiting DNA repair. **Jason Underwood, Ph.D.**





Characterizing the Kinome

If the role of kinases in countless cellular processes is not reason enough, the recent success of two anticancer drugs that target protein kinases, Gleevec and Iressa, is ample evidence that kinases are tractable and enticing drug targets, despite some inherently challenging characteristics. The sheer number of existing kinases (>500 in the human proteome), coupled with post-translational regulation of their activity, certainly complicates functional characterization and drug design efforts. Moreover, highly conserved ATP-binding sites serve as the target for most drugs and drug candidates, and this makes potent and selective inhibitors quite elusive creatures. Now, Patricelli *et al.* (*Biochemistry* 2007, *46*, 350–358) present a clever, effective method for characterizing the kinome and profiling kinase and other ATP-binding protein inhibitor selectivity.

The method is based on the strategic design of a trifunctional activity-based probe. The probe contains an ADP- or ATP-based recognition element that targets the molecule to the ATP-binding site, a reactive acyl phosphate group that forms a covalent attachment to the kinase through a lysine residue in the active site, and a biotin that enables detection and quantification of the target proteins. Incubation of >100 different human, mouse, rat, and dog proteomes with the ATP-based probe, followed by digestion with trypsin, purification with streptavidin–agarose beads, and mass spectrometry analysis, enabled the identification of 394 distinct kinases, an indication that these probes can effectively pull out a majority of the kinase needles from the proteome haystack. The probes were further employed to identify and quantitatively analyze the kinases from a set of 10 human cancer cell lines, a sign of their utility in kinase profiling. Additionally, the probes were used to determine the target identities and potencies of staurosporine, a broad-spectrum kinase inhibitor, and this validates their ability to facilitate kinase inhibitor profiling as well. These remarkable molecular tools establish a pioneering method for characterizing the kinome. **Eva J. Gordon, Ph.D.**

A Cerebral Look at Cerebellar Neurons

The cerebellum is the part of the brain that helps us control our movements, integrating spatiotemporal information with motor function. It consists of two major components, the cerebellar nuclei and the overlying cerebellar cortex. The cellular infrastructure that defines these components and connects them to the surrounding tissue consists of three types of neurons: the Purkinje cell and the granule cell of the cerebellar cortex and the neurons of cerebellar nuclei. These mature cerebellar neurons are derived from neuronal progenitor cells, but the molecular mechanisms that guide the progenitors to their fate are not well understood. Morales *et al.* (*J. Neurosci.* 2006, *26*, 12,226–12,236) now identify patterns of gene expression in cerebellar progenitors that provide insight into the development of the cerebellar system.

Using immunohistochemistry, RNA *in situ* hybridization, and transgenic mice, the authors examined the expression characteristics of several key transcription factors throughout cerebellar development in both the mouse and the chick. They observed that differential gene expression patterns of members of the TALE, LIM, and basic helix–loop–helix transcription factor families guide the step-wise generation of the three types of cerebellar neurons from neuronal progenitors. In addition, they found that coordinated migration of these neuronal populations leads to formation of the cerebellar nuclei and the cerebellar cortex. Intriguingly, gene expression patterns identified in this study linked progenitors for both the cerebellar nuclei and the cerebellar cortex to a part of the hindbrain termed the rhombic lip. The molecular markers uncovered in this study flag the pathways involved in the cerebellar circuitry and will help delineate the mechanisms behind cerebellar development and function.

Eva J. Gordon, Ph.D.

iHOP Not Just for Pancakes

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Take your favorite protein with its confusing nomenclature and orthologous proteins in other organisms. Add the known interacting proteins, four mutations, three diseases, and a small-molecule inhibitor. Sound familiar? A web-based search tool hopes to bring more clarity out of the clutter. Using iHOP (information hyperlinked over proteins), scientists can create a spider web with their favorite gene linked to other genes. The tool combs the literature for sentences containing your gene and any other condition, protein partner, or chemical compound. Then, links are available to the citation, or a link can be marked as a node to generate the spider web. The tool is freely available at www.ihop-net.org.

Jason Underwood, Ph.D.