

the regenerative effect of PTEN deficiency. Genetic ablation of a negative mTOR regulator, tuberous sclerosis complex 1 (TSC1), partially but not completely mimicked the effects of PTEN deficiency on axon regeneration, indicating that other PTEN-regulated pathways such as glycogen synthesis kinase-3 (GSK-3) could be involved in controlling axon growth.

It is uncertain whether the intrinsic regenerative mechanism observed by Park *et al.* is vigorous enough to overcome a hostile extrinsic environment. For example, an optic nerve crush produces much less inflammation and glial scarring than a contusion injury that damages the spinal cord. It will be important to determine the extent to which the findings of Park *et al.* generalize to other neuronal populations such as corticospinal axons and to understand why mTOR activity

is reduced during development and after axon injury.

Will the studies by Atwal *et al.* and Park *et al.* lead to advances in treating human spinal cord injuries? Prior work with myelin-inhibitory proteins has initiated exploratory clinical trials. The identification of LILRB2 as a human receptor for myelin-inhibitory proteins should stimulate new thinking in this area. However, work with primates, which more adequately model human injuries than rodents, is just beginning (19). It is unclear whether therapeutic approaches centered around PTEN inhibition could be developed, and whether PTEN inhibitors can mimic the positive effects of deleting the PTEN gene on axon regeneration. Nevertheless, the idea of enhancing protein synthesis to promote long-distance axon growth after

injury is an appealing possibility.

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CELL BIOLOGY

Going Global on Ubiquitin

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Our vision of protein control has for many years been viewed from a transcriptional or activity-based perspective. More recently, protein stability and regulated degradation have emerged as equally important issues to address. On pages 918 and 923 of this issue, Elledge and colleagues describe a new technology to analyze global protein stability (GPS). The studies introduce the new approach (1) and illustrate how it can be applied to identify substrates of a specific enzyme (ubiquitin ligase) (2).

In addition to protein degradation that occurs in the lysosomal compartment of cells, degradation by a cellular shredding machine known as the proteasome is another major route to eliminate proteins. Targeting to the proteasome is preceded by the addition of ubiquitin chains to the selected substrates, an event catalyzed by the sequential action of activating, conjugating, and ligating enzymes (E1, E2, and E3, respectively). The specificity of substrate recognition is mediated mainly by divergent use of the estimated ~617 ubiquitin ligases encoded by the human

genome (3). E3 ligases fall into different classes, based on structural composition and mechanism of action. In some cases, a group of proteins comes together to form multisubunit ubiquitin ligases, as is the case for the SCF (Skp1-cullin-F-box) complex where Skp1, Cul1, Rbx1, and an F-box protein form the core of the ligase (4).

Despite extensive efforts to map substrate targeting by individual ligases, the methods used have been laborious and the results far from complete. Most studies have used direct substrate-ligase interaction as a basis for substrate identification and have thus been biased toward strongly interacting targets (5). The GPS approach offers a new mode to navigate the ubiquitin-proteasome system and identify substrates for a given E3 ligase or, in general, to investigate how a chemical or physical stimulus affects the stability of a given protein. The system evaluates protein abundance at a global level in living cells, with accuracy comparable to conventional time-consuming experiments that analyze only a few proteins at a time. Eight thousand distinct complementary DNAs were used to generate a library of cultured human cells in which each cell expresses a common stable red fluorescent protein (DsRed) together with a variable fusion protein composed of enhanced green fluorescent protein (EGFP) and a unique

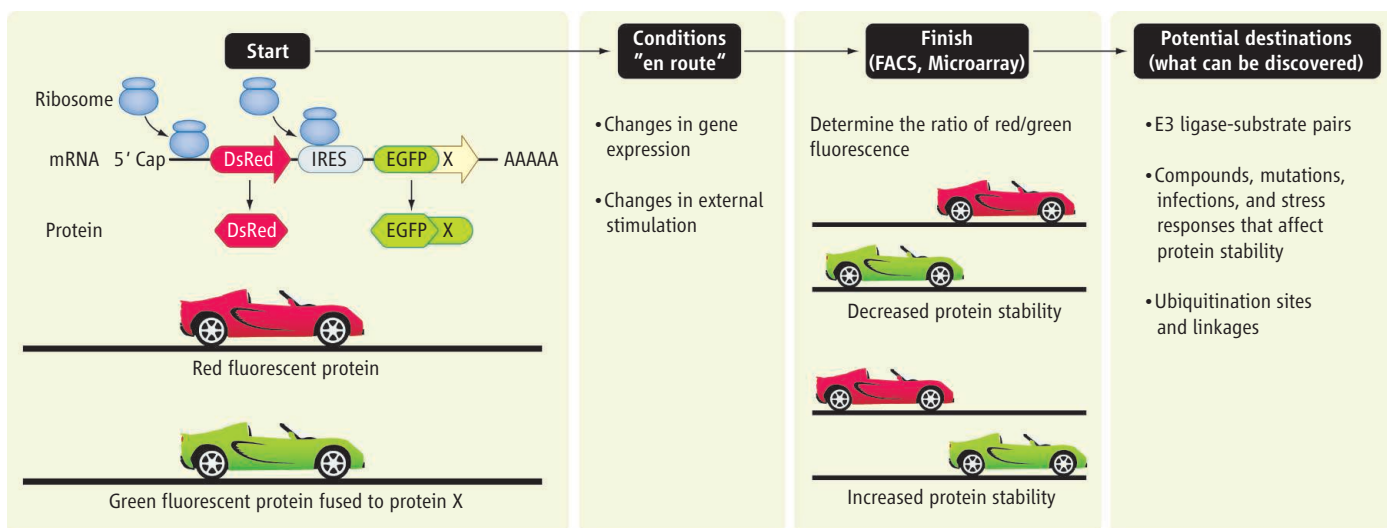
A new technique that profiles protein stability provides a powerful platform in which high-throughput screening can be performed in real time with single-cell resolution.

open reading frame (ORF), produced from the same transcript.

The turnover of the EGFP-ORF fusion proteins can thus be monitored by flow cytometry (which counts, examines, and sorts whole cells) as a ratio of red to green fluorescence in each cell (see the figure). Cells in which the fluorescence ratio changes in response to a gene perturbation or stimulus can be sorted by the degree of change, and the identity of the ORFs they express can be easily identified by a polymerase chain reaction-based microarray approach. During the development of GPS profiling, a comparative analysis of all ORFs tested was used to assign each ORF a protein stability index value that roughly categorizes each corresponding protein as having a short, medium, long, or extra-long life span. An impressive power of the GPS method is the capacity of single-cell resolution, which is in contrast to other established methods that frequently generate population-averaged readouts. In addition, measurements can take place in live cells, in real time, and can be integrated with systems for automation to enable high-throughput studies.

The GPS approach is a major advance in the quest to gain a comprehensive understanding of protein turnover in cells and will be a valuable complement to the biophysical methods that have emerged in the past 5 years to analyze substrate ubiquitination (6). Large-

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Navigating the world of ubiquitin and protein degradation. In GPS profiling, the ratio between a constant factor (DsRed) and a variable factor (EGFP-X) is measured by a combinatorial approach of flow cytometry (FACS analysis) and microarray technology. This serves as a readout of protein abundance and stabil-

ity. By challenging the system with internal modifications or external stimuli, the responses can be monitored on a global scale. Many questions concerning the process of protein degradation, including E3 ligase specificity, routes to destruction, and importance of ubiquitin linkage, can be addressed.

scale analysis of ubiquitinated proteins has relied mainly on a combination of affinity purification and mass spectrometry analysis of proteins in yeast (7), human cell lines (8), and transgenic mice (9). To more specifically analyze ubiquitinated targets downstream of individual E3 ligases, Ota *et al.* used SILAC (stable isotope labeling with amino acids in cell culture) to quantify the overall change in protein ubiquitination after altering E3 ligase activity (10). Substrates of the E3 ligase Rsp5 were recently identified in a high-throughput assay in which all proteins expressed in yeast were spotted onto a nitrocellulose chip and directly tested for ubiquitination by Rsp5 *in vitro* (11).

The GPS technology moves the field closer toward global *in vivo* mapping of ligase-substrate pairs. Illustrating the feasibility of the system, Yen and Elledge used GPS profiling to identify substrates of the SCF ubiquitin ligase complex (2). Broadly outlined, in the background of the described GPS library, SCF function was abrogated by the expression of a dominant negative Cull1, whereupon the perturbed cells were analyzed by GPS profiling. An impressive number of 359 targets were postulated as putative SCF substrates, among which 66 were tested and 31 verified. In a majority of cases, verification of a specific protein was accomplished by analyzing individual samples by flow cytometry [fluorescence-activated cell sorting (FACS) analysis] as well as by biochemical detection in cell extracts with antibodies (immunoblotting). In the future, combining a GPS-based ORF library with methods that perturb gene expression on a global scale, such as genome-wide

RNA interference, leaves few limitations to the amount of knowledge that may be acquired with this innovative method.

A feature that is both a strength and a limitation is that the GPS technique does not monitor E3 substrates directly but rather the outcome of E3 activity. Thus, there is no discrimination between the direct and indirect effects of an E3 ligase. However, this could be resolved by following the kinetics of protein degradation. GPS profiling is also biased toward identifying ubiquitinated substrates that are destined for either degradation or stabilization. This excludes proteins that are functionally affected by the modification with regard to their enzymatic activity, localization in the cell, and ability to form complexes with other cellular constituents. Nevertheless, together with conventional proteomic approaches, the GPS system will provide a powerful means to distinguish the consequences of different types of ubiquitination, sorting the proteolytic events from those of regulatory nature (12).

In addition to assigning ligase-substrate pairs, GPS profiling has the potential to elucidate the essence of differential ubiquitin chain linkages, in particular how linkage affects the efficiency of proteasomal targeting. It may also help to identify degradation signals (degrons) encoded by amino acid sequences, to associate specific lysine residues that are modified by ubiquitin to functionality, and to explain why some proteins are directly routed to the proteasome whereas others require shuttle factors to ensure proper targeting. There is also the potential to transfer GPS profiling into model organisms such as *Drosophila melanogaster* and *Caenorhabditis*

elegans, creating ORF libraries that will enable substrate screening *in vivo*.

Given the importance of the ubiquitin-proteasome system for cellular functions, dysfunctions of the involved players clearly have the capacity to cause disease. Indeed, defective ubiquitination and protein degradation is implicated in the etiology of cancer and neurodegenerative disorders, among others (13, 14). In this context, the GPS method could be used to screen for compounds that counteract such deficiencies. Another interesting aspect would be to investigate how protein degradation is altered when cells are exposed to infectious agents or various stress situations. Overall, the GPS approach will likely become an important component of the integrated approaches needed to systematically map the mechanisms of regulated protein degradation.

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