Plenary 21

GPS: A Genomic Approach for Measuring Regulated Protein Turnover

Hsueh-Chi Sherry Yen

Department of Genetics, Harvard Medical School, USA
Department of Medicine, Division of Genetics, Brigham and Women's Hospital, USA

My research project aims at developing and applying high throughput genomic approaches to the problem of protein degradation regulation and ubiquitin ligase target identification. I have established a fluorescence-based system that allows us to monitor the stability of any protein of interest in single life cells. In this system, the expression cassette contains a single promoter (CMV) that, with an internal ribosome entry site (IRES), permits the translation of two spectrally separated fluorescent proteins from one mRNA transcript. The first fluorescent protein (DsRed) is expressed as an intact protein and serves as an internal control, while the second fluorescent protein (EGFP) is expressed as a fusion protein with the protein of interest (EGFP-X). Upon integration into the genome of cells, DsRed and EGFP-X should be produced at a constant ratio since they are derived from the same mRNA, although the stability of the individual proteins might differ. Events that selectively affect the protein stability of EGFP-X would be expected to lead to a change in the abundance of EGFP-X, which should be reflected in an alteration of the EGFP/DsRed ratio. I have coupled this method with cDNA libraries and microarray deconvolution to allow global protein stability profiling (GPS) of over 8,000 proteins in human cells. In addition, I applied the GPS approach to the isolation of SCF ubiquitin ligase substrates. I recovered most known SCF targets and identified many novel substrates involved in cell cycle, apoptosis and signaling pathways. The GPS approach is generally applicable to studying the regulation of the stability of any protein of interest and to identifying the targets of any ubiquitin ligase. Besides its use in studying ubiquitin-mediated proteolysis, this strategy can be further generalized to detect proteins whose stabilities increase/decrease in response to various stimuli such as cytokine stimulation, irradiation, and heat shock.