BioSpotlight

Catching more proteins from the POND

Analyzing the dynamics of replication fork progression is critical for understanding DNA replication and repair as well as epigenetic regulation involving the deposition of histones and other chromatin proteins. Recently, isolation of protein on nascent DNA (iPOND) was developed to purify proteins found at the replication fork. Nascent DNA at the replication fork is labeled through the incorporation of a brief pulse of the thymidine analog 5-ethynyl-2'-deoxyuridine (EdU), followed by formaldehyde crosslinking of chromatin proteins to DNA and the covalent linkage of biotin azide to the alkyne group of EdU through a copper-catalyzed “click” reaction. After sonication of the chromatin, biotinylated nascent DNA and associated proteins are isolated using streptavidin-coated beads and proteins are analyzed by Western blotting after the reversal of the formaldehyde crosslinking. iPOND, however, has drawbacks, including modest protein yields and the need for formaldehyde crosslinking that can affect Western blotting and mass spectrometry protein analysis. In this issue, R. Bremner and colleagues at the University of Toronto (Ontario, Canada) describe their faster and simpler adaptation of iPOND, which they call accelerated native iPOND (aniPOND). aniPOND allows for the isolation of proteins un-

der native conditions with a 5- to 20-fold improvement in yield. After initial EdU incorporation, a key modification in aniPOND is to combine the cell harvesting, lysis, extraction of soluble cytoplasmic and nuclear proteins, and collection of nuclei all into a single step by adding nuclei extraction buffer directly to cells in the flask. This reduction in sample manipulation likely accounts for the enhanced protein recovery observed when using aniPOND. After the click reaction, chromatin is sonicated in a 1% NP40 buffer instead of the SDS buffer used for iPOND to maintain the protein complexes in their native state. Two single sonication/centrifugation steps are then performed to remove non-chromatin proteins, followed by extensive sonication to thoroughly shear and solubilize the chromatin. Isolation of the biotin-labeled replication forks using streptavidin beads is done as in iPOND, except the crosslinking reversal step is eliminated since aniPOND does not utilize formaldehyde crosslinking. Using aniPOND, the authors demonstrate increased capture of abundant replication fork proteins as well as detection of rarer proteins.

See “A rapid and efficient method to purify proteins at replication forks under native conditions” on page 204.

Chasing protein turnover

When talking about protein interactions, how long a protein remains active in the cell is an important part of the story. Protein half-lives are often determined by pulse-chase experiments with radiolabeling or by treating cells with the chemical cycloheximide (CHX) to inhibit protein synthesis, followed by immunoblotting. While CHX treatment is less laborious and lower in cost than pulse-chase, it is also toxic to cells and lacks specificity, indiscriminately inhibiting synthesis of all transcripts. Neither method allows measurement of proteins in single cells, experiments where fluorescently labeled proteins are ideal. With fluorescence loss in photobleaching (FLIP) or fluorescence recovery after photobleaching (FRAP) approaches, fluorescently tagged proteins can be photobleached, allowing researchers to monitor fluorescence restoration in the bleached area. These techniques offer information on protein trafficking and diffusion, but tagged proteins are continuously made and degraded, so measuring protein turnover and temporal expression of GFP-labeled proteins is

Dendra2 in combination with RNAi can be used to monitor protein turnover in cells following photobleaching.

often impossible. Moreover, in many instances, GFP fusion proteins are expressed in addition to the endogenous native form of the protein, resulting in overexpression that further complicates protein turnover measurements. In the current issue of *BioTechniques*, Tell et al. from the University of Udine (Udine, Italy) detail their development of a method to overcome such drawbacks by using the photoconvertible fluorescent protein (PCFP) Dendra2, which changes from green to red fluorescence in response to ultraviolet or blue light irradiation. Using Dendra2 fused to

APE1, an endonuclease important for base excision repair, in combination with RNAi-mediated knockdown of endogenous APE1, the authors were able to successfully determine the APE1 half-life at physiological levels. Red and green fluorescence signals were quantified before and after photoconversion with confocal microscopy and then used to calculate the rate of APE1 turnover. This new approach proved to be more accurate than CHX methods, avoided artifacts that readily occur with immunolabeling approaches, and should be adaptable to a wide range of proteins in the future.

See “Combining RNAi and in vivo confocal microscopy analysis of the photoconvertible fluorescent protein Dendra2 to study a DNA repair protein” on page 198.

Selected and written by Patrick C. H. Lo, Ph.D. and Kristie Nybo, Ph.D.

BioTechniques 55:159 (October 2013)
doi 10.2144/000114082