Topic Introduction

Elementary Protein Analysis in *Schizosaccharomyces pombe*

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Biochemical monitoring and interrogation of protein function is a critical component of most fission yeast studies. In particular, its small proteome size, high conservation of core molecular cell biology, and genetic malleability make *Schizosaccharomyces pombe* an excellent model organism in which to use mass spectrometry to conduct proteome-wide approaches. Here we discuss issues encountered during the analysis of fission yeast protein preparations.

BARRIERS TO BIOCHEMICAL ANALYSES

Although the ability to grow cultures of genetically modified strains at any scale in highly defined media makes *Schizosaccharomyces pombe* an attractive organism for biochemical studies, the limited application of biochemical approaches in *S. pombe* means that many of the protocols and practices are derived from the much larger budding yeast community. The size of the budding yeast community and its long history of commercial production of higher eukaryotic proteins means that far more avenues have been explored with *Saccharomyces cerevisiae* biochemistry than with *S. pombe*. This makes a survey of the budding yeast literature for tips and guidance a must for anyone embarking on a specialized biochemical assay in fission yeast (e.g., see Introduction: **Protein Complex Purification by Affinity Capture** [LaCava et al. 2016]). Indeed, a classic review of the challenges of protein analysis in budding yeast serves as an excellent introduction to frame the challenges of biochemical analyses in either yeast (Pringle 1975). Two major issues set biochemical analyses in either yeast apart from analogous studies with most higher eukaryotes: the cell wall and the aggressive proteases within the lysosomal vacuoles that are released during cell disruption to capture cell content.

The Cell Wall

Enzymatic, chemical, mechanical, and pressure-based approaches have all been used to overcome the considerable mechanical strength and elasticity of the yeast cell wall to capture cell content for biochemical analyses. These vary widely in their ability to yield representative protein extracts for analyses or purification steps. Enzymatic cell wall digestion can be achieved either by adding enzyme preparations or by autolytic methods in which endogenous enzymes are liberated and used to break down the cell wall. However, the long incubation times required for enzymatic digestion generate considerable issues with the newly released proteolytic activities and make these approaches less attractive than mechanical methods that rapidly destroy integrity at temperatures at which proteases are largely ineffective. The scale of the analysis dictates which mechanical method is most suitable:

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From the Fission Yeast collection, edited by Iain M. Hagan, Antony M. Carr, Agnes Grallert, and Paul Nurse.

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Cite this introduction as *Cold Spring Harb Protoc*; doi:10.1101/pdb.top079806

agitation with zirconia or glass beads for volumes of <1 mL, pulverization in a mortar and pestle or milling (for volumes up to 100 g), or high-pressure homogenizers (e.g., French presses) for larger volumes.

Vacuolar Proteases

The biggest challenge to biochemical analyses in fission yeast is proteolytic degradation during sample preparation. Traditionally, these activities were counteracted by the individual addition of pepstatin, leupeptin, aprotinin, and phenylmethylsulfonyl fluoride (PMSF) to extraction buffers, but the addition of commercial tablet “inhibitor cocktails” alongside freshly prepared PMSF to buffers immediately before use is easier and more effective. Furthermore, induction of sexual differentiation by nitrogen starvation promotes autophagy to recycle proteins that are no longer required as cells exit logarithmic phase growth to generate amino acids for synthesis of proteins required for the new state of sexual differentiation (Kohda et al. 2007). Although this enhancement of proteolytic activities presents particular challenges when analyzing starving or sexually differentiating cultures, these largely can be met by adding 1 mM PMSF directly to the cell culture 1 min before, or at the time of, harvesting.

Different proteins will have different sensitivities to the spectrum of proteases released by vacuolar disruption. If proteolysis continues to be an issue despite the addition of an armory of inhibitors, changing the pH of the buffer to compromise the activity of the resilient protease can be effective (Pringle 1975). Alternatively, genetic removal of the protease in question will be more reliable. Indeed, the TM101 protease-deficient strain isolated by Mitsuhiro Yanagida’s laboratory has proved useful in several studies (Selinger et al. 1994; Sirotkin et al. 2005; Beltzner and Pollard 2008; Nolen and Pollard 2008). More recently, the impact of systematic deletion of proteases has been assessed in the context of the integrity of secreted exogenously produced proteins rather than the preservation of native molecules (Idiris et al. 2006). These candidates would form a good starting point from which to identify a protease that is causing a particular issue with proteolysis of endogenous molecules.

EFFECTIVE METHODS FOR PROTEIN ISOLATION

The accompanying protocols describe various approaches for isolating proteins from fission yeast that take into account the issues described above. To generate total protein extracts for electrophoretic analyses, see Protocol: **Preparation of Protein Extracts from *Schizosaccharomyces pombe* Using Trichloroacetic Acid Precipitation** (Grallert and Hagan 2016a). For the isolation of specific proteins by immunoprecipitation at small and large scales, see Protocol: **Small-Scale Immunoprecipitation from Fission Yeast Cell Extracts** (Grallert and Hagan 2016b) and Protocol: **Large-Scale Immunoprecipitation from Fission Yeast Cell Extracts** (Grallert and Hagan 2016c), respectively. Protocol: **Improved Tandem Affinity Purification Tag and Methods for Isolation of Proteins and Protein Complexes from *Schizosaccharomyces pombe*** (Zilio and Boddy 2016) provides a variation on the classic tandem affinity purification protocol that exploits sequential high-affinity binding to two different resins via motifs that have been fused to the gene encoding a protein of interest (Puig et al. 2001; Tasto et al. 2001; Gould et al. 2004). Protocol: **Large-Scale Purification of Small Ubiquitin-Like Modifier (SUMO)-Modified Proteins from *Schizosaccharomyces pombe*** (Nie and Boddy 2016) presents a method for isolating proteins to which the small (as well as other) ubiquitin-like modifiers have been coupled.

ACKNOWLEDGMENTS

Funding was provided by Cancer Research UK (CRUK) C147/A6058.

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