PREFACE

Proteins that bind to intermediate filaments come in hundreds of sizes and "flavors." Some are structural giants that interlink the cytoskeleton. Others reside at the inner nuclear membrane, holding court with lamins and silent genes. Many more remain hidden from view, their potential contributions to cells or disease unknown. Studying such proteins requires a cutting-edge toolkit of methods and expert advice, both featured in this volume of *Methods in Enzymology*.

Roux and colleagues (Chapter 1) detail a powerful and broadly applicable method, biotinylation identification (BioID), to identify proteins located within 10 nm of a BioID-fusion protein in living cells. These "proximal" proteins include direct *in vivo* partners or associated protein complexes, as demonstrated for the nuclear intermediate protein, lamin A. Gräf and colleagues successfully modified the BioID toolkit for use in *Dictyostelium*, now enabling studies of intermediate filament-associated proteins in this evolutionarily diverse organism (Chapter 2). Taking "lamin association" to an entirely new level, Harr and Reddy detail their state-of-the-art new TCIS method to study how specific segments of DNA or chromatin-associated proteins drive intranuclear movement to lamins and the nuclear lamina (Chapter 21).

Proteins that bind lamins are exceedingly diverse. Schirmer and colleagues provide insights into the many biochemical and functional features that should be considered when expressing an emerging spectrum of lamin-binding nuclear membrane proteins as recombinant polypeptides in bacteria (Chapter 5). Successful methods for purification and structural analysis are provided for specific lamin-binding proteins. These include SUN-domain and KASH-domain proteins, which form LINC complexes that transduce force across the nuclear envelope (Schwartz and colleagues, Chapter 4), as well as *L*AP2, *e*merin and *M*AN1, which share the eponymous LEM-domain fold and also have unstructured regions (Zinn-Justin and colleagues, Chapter 3). Beyond proteins, Coen and colleagues describe how to purify entire "nuclear egress" complexes formed by the human cytomegalovirus, which include lamin-associated proteins (Chapter 25).

Advances in the purification and structural analysis of challengingly large cytoplasmic proteins are provided for plectin and BPAG1e (de Pereda and colleagues, Chapter 10) and desmoplakin (Choi and Weis, Chapter 11).

Functional assays are providing significant insight into the biological roles of cytoplasmic intermediate filament binding proteins, including molecular chaperones (Salas and colleagues, Chapter 8; Quinlan and colleagues, Chapter 9) and gigaxonin, an E3 ubiquitin ligase involved in the degradation of intermediate filament proteins (Bomont, Chapter 12). Borradori and colleagues describe a new GFP-based binding assay to study plakin association with intermediate filaments (Chapter 7). Green and colleagues use a 3D culture model to advance the functional interrogation of the intermediate filament-desmosome scaffold during keratinocyte differentiation (Chapter 15). Boczonadi and Määttä discuss general approaches to study the function of periplakin and envoplakin in cell culture models and gene-targeted mice (Chapter 16). The functional diversity of lamin-associated proteins is reflected in protocols to separate functionally distinct populations of lamin-binding proteins (Berk and Wilson, Chapter 6), identify transcription factors relevant to prelamin A pathology (Infante and Rodríguez, Chapter 23), and identify novel protein–protein interactions at the nuclear envelope via chemical cross-linking (Hallberg and colleagues, Chapter 24).

Genetic methods are increasingly important to understand intermediate filament biology and are expanding beyond mice. This volume of *Methods in Enzymology* includes state-of-the-art protocols to study lamin-binding proteins in *Caenorhabditis elegans*, which has one lamin gene (Gruenbaum and colleagues, Chapter 22), and to study the complex functions of the single spectraplakin locus in either *C. elegans* (Labouesse and colleagues, Chapter 20) or *Drosophila* (Prokop and colleagues, Chapter 19). Other chapters describe increasingly sophisticated use of mouse genetics to study the roles of plectin in skin and muscle (Wiche and colleagues, Chapter 13), epiplakin in epithelial cells (Fuchs and colleagues, Chapter 14), BPAG1 in neurons (Lynch-Godrei and Kothary, Chapter 18), and microtubule-actin cross-linking factor 1 in the skin, heart, nervous system, and intestinal epithelia (Goryunov and Liem, Chapter 17). We sincerely hope these protocols will help your research advance.

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