Preface

The second edition of *Adhesion Protein Protocols* combines traditional techniques with cutting-edge and novel techniques that can be easily adapted to different molecules and cell types. These protocols are suited for both novice and expert scientists and will hopefully be used to gain further insight into the complex and incompletely understood processes that are involved in cellular adhesion. The book begins with two chapters covering novel techniques for studying cell–cell adhesion, which traditionally has been less straightforward to examine. The first chapter by Nelson et al. describes their novel technique for studying cell–cell adhesion using bowtie-shaped microwells. This technique not only allows one to control the degree to which the cells spread, but also allows manipulation of cell–substratum interactions. In the second chapter, Vogelmann and Nelson describe the use of differential centrifugation to isolate cell–cell adhesion complexes as a useful starting point to further examine adhesion protein interactions.

Chapter 3 by Nuzzi and co-workers describes the analysis of neutrophil chemotaxis, and includes methods for retroviral infection of these difficult-to-transfect cell types and time-lapse video microscopy. In Chapter 4, McGettrick and colleagues describe several in vitro assays used to study leukocyte migration through monolayers of cultured endothelial cells. Cell motility requires the formation of pseudopodia, and in Chapter 5, Wang and Klemke describe a novel technique to purify pseudopodia from migratory cells. This technique allows the physical separation of the pseudopodia from the cell body, which allows a detailed analysis of the components of pseudopodia in migratory cells while providing a novel method to study the signaling processes involved in cell migration.

The next few chapters deal with the study of cell–matrix interaction. Petroll deals with the study of cell–matrix interactions in three-dimensional culture, using the experimental model system he has developed whereby changes in focal adhesion reorganisation can be correlated with mechanical deformation of a collagen matrix. Gallant and García describe a comprehensive set of methods to quantify cellular adhesion strength. The first of these can be used to determine initial as well as long-term adhesion strength; furthermore, two biochemical assays are described that can be used to quantitate proteins involved in adhesion.

In Chapter 8, Griffith details the use of RNA interference, now widespread in its use, to study the effects of knocking down an adhesion protein, and in

Chapter 9, Carragher provides a detailed description of how to monitor the activity of calpain, an important protease that regulates cell adhesion and signaling.

Both microarray and proteomics techniques are becoming increasingly used in the laboratory and Chapters 10 (Dalby and Yarwood) and 11 (Boyd and colleagues) describe the application of these techniques to study adhesion proteins. Bioinformatics is in constant use in the laboratory but can often be overlooked as a general laboratory protocol. Adams and Engel have provided a detailed and interesting overview of how to embark on bioinformatic analysis using adhesion proteins as examples. They cover all the major methods, such as the identification of sequence homologies, examination of sequence relationships and domain organization, as well as how to use comparative genomics.

The final chapters deal with three important techniques used in the study of adhesion molecules. In Chapter 13, Wehrle-Haller provides an excellent review of the use of fluorescence recovery after photobleaching. Beningo and Wang have designed a novel system to study cells in three-dimensional culture and in Chapter 14 describe how to employ this simple system using hydrogel coated with defined matrix proteins as substrates. In the final chapter, Zuchero provides an overview of actin purification as well as how to perform in vitro actin assembly assays, two techniques commonly employed to investigate the roles of actin-binding proteins in the kinetics and morphology of actin assembly.

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