

Preface

Over the past several decades the range and diversity of experimental approaches used by biologists has expanded dramatically. Today, cell-based analyses of biological processes are often linked intimately with the use of molecular biology, biochemistry, proteomics and genomics. The research goals of modern cell biology have been facilitated tremendously by advances in laboratory techniques and the imaginative application of a broad spectrum of experimental approaches. The relative ease with which these approaches can now be used has led to an evolution of scientists across varied disciplines from being specialists to jacks-of-all-trades. While extremely exciting, this has also raised the bar with respect to cross-disciplinary approaches that can be used, and are often expected, when addressing the ever-growing and complex biological questions posed by researchers around the globe. Thus proficiency in, or at least a thorough understanding of, such diverse approaches has become an attribute necessary to the modern biologist. The chapters included in this volume, taken from *Cell Biology: A Laboratory Handbook, 3rd edition*, describe a variety of methods for identification of proteins of interest, detection and analysis of these proteins, determination of protein-protein interactions and DNA-protein interactions. In combination with cell-based experiments, each may factor critically in studies aimed at elucidating detailed molecular mechanisms as to the inner-workings of cells.

The methods described in these chapters can be broadly separated into five groups. In the first, incorporation of radioactive amino acids,

ions and phosphates or chemical modification with fluorophores to label known or unknown proteins are outlined. Sensitive methods for detecting proteins with silver and fluorescent stains are also described. The second group of methods lays out procedures for the separation and detection of proteins by isoelectric focusing and mobility in 2D gels for proteomic analysis. The third section includes chapters describing the use of immunoprecipitation, with and without chemical cross-linking, affinity binding of soluble proteins to others immobilized on solid supports, yeast-2-hybrid, and co-transport assays in cells to identify and characterize protein-protein interactions. In the fourth group of chapters, methods to identify interactions of proteins with DNA using chromatin immunoprecipitation, electrophoretic mobility shift assays and oligonucleotide trapping are described. And finally, an extensive set of mass spectrometry approaches are described in detail for the identification of proteins and protein post-translational modifications.

Using experimental examples, the chapters in this volume provide detailed methods for a wide range of techniques that can be applied in most laboratories. Some are relatively straightforward, require little special equipment and can be adapted to accommodate commonly available tools and supplies. The chapters describing mass spectrometry methods also enunciate important considerations regarding the usefulness and applicability of each technique for assessing specific questions related to protein identification and characterization. This is particularly helpful

for researchers that aim to use mass spectrometry on occasion, rather than routinely. Together, the step-by-step instructions with detailed discussion of each technique make this laboratory handbook an essential resource. In this era of

cell biology, technical breadth serves an enabling function, allowing researchers to address, at a molecular level, the many questions associated with complex biological events.