PREFACE

Fluorescence microscopy plays an essential role in cell biology by allowing visualization of specific molecular components of cells. The initial implementation through immunofluorescence techniques transformed the cytoplasm from a gelatinous bag of enzymes into the highly organized cytoarchitecture that we appreciate today. Methods for directly labeling proteins and successfully reintroducing them into cells added a dynamic element, allowing specific chemical reactions, such as polmerization/depolymerization, intracellular transport, and ligand binding, to be examined in living cells by time-lapse microscopy. The discovery of GFP, the green fluorescent protein of the jellyfish *Aequorea victoria,* has revolutionized experimental analysis of the dynamic molecular organization of cells by reducing the methods required for fluorescently labeling proteins to the techniques of molecular biology.

The beauty of GFP, aside from its brilliant green color, is that it has established a universal method for introducing a fluorescent tag into nearly any biological structure. GFP fusion protein technology is applicable to essentially any protein, beginning with a gene, to visualize it in living cells. At the cellular level, specific biological structures—membranes, spindles, chromosomes, organelles—can be imaged using nonperturbing GFP-based labels. Above the cellular level, GFP alone or as a fusion protein can be used to mark populations of cells for experimental manipulation, to visualize tissue organization, and to dissect cell lineage relationships. In short, GFP has proven to be a tremendously versatile biological tool that has worked its way into nearly all aspects of experimental biology in the scant four years since its introduction.

This volume of *Methods in Cell Biology* contains a set of protocols for work with GFP at several levels. Chapters 1–3 deal with GFP biophysics, GFP variants, and quantitative imaging of GFP and provide a foundation for thinking about GFP fluorescence and for building probes. The remainder of the volume, Chapters 4–16, provide specific examples of GFP expression in a variety of cell systems, including yeasts, plants, insects, and animal cells. Many chapters focus on development and use of specific biological assay systems and include details of the design and implementation of GFP-based probes. Thus, the rationale for fusion protein design and cloning methods, techniques for expression, microscopy and data collection, data analysis, and other methods are discussed in several contexts. Other chapters detail specific experimental techniques such as single-molecule fluorescence analysis, fluorescence resonance energy transfer, and flow cytometric applications of GFP. It is our hope that this volume will serve as a source of specific protocols for

researchers wishing to implement the methods described here, as well as a guidebook that can help generate ideas on how to use GFP to solve new experimental problems.

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