Platelets and Megakaryocytes
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Volume 2
Perspectives and Techniques

Edited by

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Preface

The average human body has on the order of $10^{12}$ circulating platelets. They are crucial for hemostasis, and yet excessive platelet activation is a major cause of morbidity and mortality in Western societies. It is therefore not surprising that platelets have become one of the most extensively investigated biological cell types. We are, however, far from understanding precisely how platelets become activated under physiological and pathophysiological conditions. In addition, there are large gaps in our knowledge of platelet production from their giant precursor cell, the megakaryocyte. Understanding megakaryocyte biology will be crucial for the development of platelet gene targeting. The aim of *Platelets and Megakaryocytes* is therefore to bring together established and recently developed techniques to provide a comprehensive guide to the study of both the platelet and the megakaryocyte. It consists of five sections split between two volumes. The more functional assays appear in Volume 1, whereas Volume 2 includes signaling techniques, postgenomic methods, and a number of key perspectives chapters.

Part I of Volume 1, *Platelets and Megakaryocytes: Functional Assays*, describes many well-established approaches to the study of platelet function, including aggregometry, secretion, arachidonic acid metabolism, procoagulant responses, platelet adhesion under static or flow conditions, flow cytometry, and production of microparticles. Although one would ideally wish to perform experiments with human platelets, studies within the circulation using intravital microscopy require the use of animal models, which are described in Chapter 16, vol. 1. These approaches are becoming increasingly important in our understanding of how platelet responses contribute to the complex formation of thrombi within the circulation. Although naturally occurring genetic mutations can indicate the importance of specific proteins, these are limited in frequency and scope and thus many laboratories are using transgenic animals to delete or upregulate individual gene products (see Chapter 2, vol. 2). Consequently, the application of platelet techniques to murine models has become a focus of many labs in recent years (e.g., Chapters 2, 16, 20, vol. 1). In addition to basic and advanced approaches to study platelet function, several chapters in this section (particularly 1 and 2, vol. 1) focus on the long-standing issue of the effects of different anticoagulants and procedures to prepare platelets. The experimenter has a choice of studying platelets within the blood, in plasma, or in an artificial medium. In whole blood, potential interactions with other cell types and plasma proteins are included, which is in many ways the most physiological in vitro approach (see Chapter 6, vol. 1), however this is a complex situation and interpretation can be difficult. In studies within plasma, other cells are removed, but the clotting cascade is retained (see Chapter 5, vol. 1). Frequently, however, platelets are studied in isolation from other cells and plasma following their resuspension in an artificial medium. The preparation of platelets from human and other species is not a trivial matter and great care is required.
to ensure that the method of preparation does not adversely affect subsequent analysis (see Chapter 2, vol. 1).

Part II of Volume 1 focuses on approaches used to study megakaryocyte function, including the development of specialized structures for future production of platelets (e.g., the demarcation membrane system), the appearance of platelet-specific surface receptors, and the increase in ploidy. The source of megakaryocytes is often a complex issue facing many researchers owing to the extremely low density (<1%) of this cell type in its primary location, the marrow. Techniques to purify megakaryocytes from marrow based on their unique size and surface markers are described in Chapter 22, vol. 1, along with approaches to maintain these cells in culture and monitor formation of platelet-generating proplatelet structures. An alternative approach to generating megakaryocytes is to grow them in culture from precursor cells as detailed in Chapter 23, vol. 1. This requires the presence of thrombopoietin (Chapter 26, vol. 1) acting through its receptor, c-Mpl, and normally other cytokines. The availability of systems to generate megakaryocytes in vitro provides a promising avenue to generate genetically modified platelets. Although there is no doubt that continuous megakaryocyte cell lines are useful for some studies of signaling in these cells, they have their limitations and the pros and cons are discussed in Chapter 27, vol. 1.

Many basic and advanced techniques for the general study of cell signaling have been applied in studies to characterize the mechanisms of regulation of platelet function. These include ligand-binding assays, the study of protein and lipid kinases and phosphatases, the analysis of lipid rafts in the regulation of cell signaling, the measurement of intracellular calcium levels, electrophysiological techniques, nitric oxide signaling, the use of venom proteins, and the internalization of proteins into platelets through permeabilization. These techniques and more are presented in Part II of Volume 2. In many respects, the megakaryocyte is a giant platelet. Differences do occur in the arrangement of cellular organelles and cytoskeleton in the two cells, however, megakaryocytes respond to platelet agonists such as ADP with full downstream functional responses (discussed in Chapters 1 and 16, vol. 2). Therefore, despite differences in ultrastructure, the megakaryocyte has earned its place as a sufficient, if not comparable model of platelet signaling. Many of the signaling techniques are therefore beginning to be applied to the megakaryocyte, which, because of its size, is proving to be an extremely interesting model for platelet signaling, particularly using single cell approaches such as imaging and electrophysiology (see Chapters 16 and 17, vol. 2).

Part III of Volume 2 is dedicated to recent advances in molecular techniques and post-genomic techniques and how they may be applied to the study of platelets and megakaryocytes. This section includes descriptions of how retroviruses may be used to express genes in primary megakaryocytes, the use of GFP-fusion proteins to study signaling in live cells, two-dimensional electrophoresis for platelet proteomics, the production of platelet cDNA libraries and the use of gene array technology.

Although the main aim of the book is to include practical approaches to the study of platelets and megakaryocytes, a series of perspectives chapters are included (Part I, vol. 2). These chapters review the current understanding of platelet and megakaryo-
cyte biology in addition to their discussions of important new developments and experimental strategies. Many of the methods chapters also include further discussion and background on specific techniques.

This book has only been made possible by the efforts of many international experts in the field. We are grateful to them for their willingness to contribute their knowledge, in particular their tricks of the trade, which have resulted from many years of dedicated hands-on work. We also wish to thank our colleagues within the Department of Physiology at Cambridge and the School of Animal and Microbial Sciences at Reading for helpful discussion during the course of the editing work, in particular Peter Wooding on electron microscopy and Gwen Tolhurst on molecular techniques. We are also grateful to Margaret Bardy and Karen Parr for considerable secretarial assistance. We are also grateful to the following companies for supporting the cost of color reproduction: Eli Lilly and Company, Cairn Research Ltd., Bio Rad Laboratories Ltd., and Sysmex UK Ltd.

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Color Plates

Color Plates 1–5 appear as an insert following p. 300.

PLATE 1  Schematic representation of basic retroviral structure and the process of infection. (See full caption on p. 37, Chapter 2.)

PLATE 2  Megakaryocyte-specific retroviral infection through the TVA receptor. (See full caption on p. 39, Chapter 2.)

PLATE 3  Ribbon diagram representation of the crystal structure of wild-type green fluorescent protein from *Aequoria victoria*. (See full caption on p. 408, Chapter 25.)

PLATE 4  Comparison of GFP images obtained with laser scanning and spinning disk confocal microscopes. (See full caption on p. 413, Chapter 25.)

PLATE 5  Summary of the microarray process. (See full caption on p. 481, Chapter 29.)