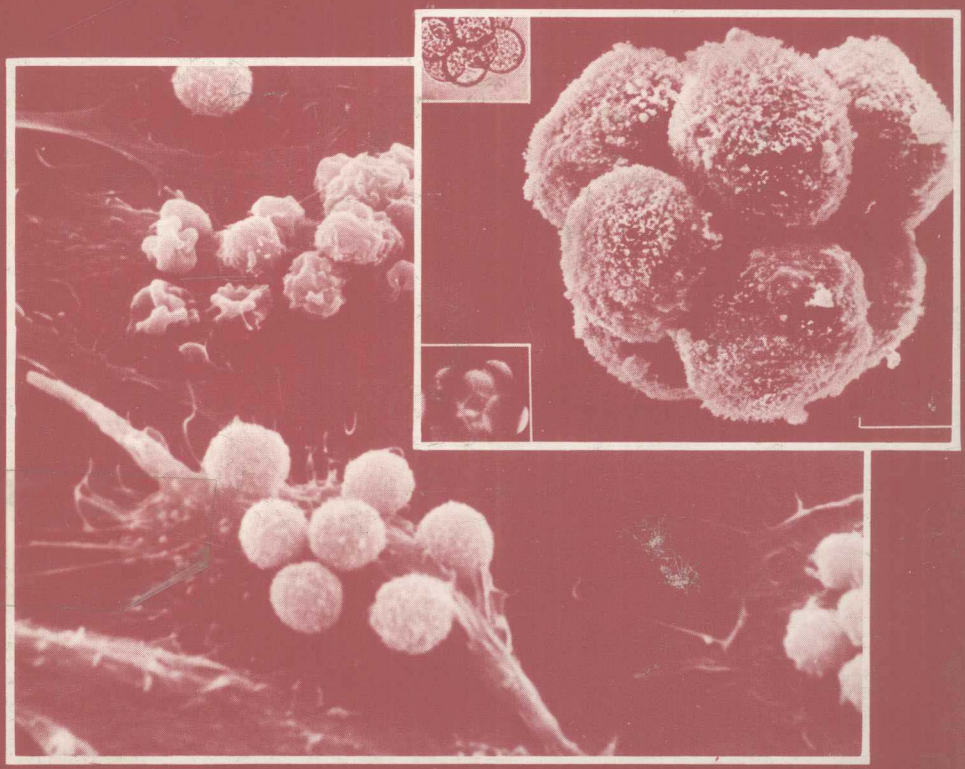
The Biology of Glycoproteins

Edited by

Raymond J. Ivatt



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Preface

This book is in many ways a sequel to *The Biochemistry of Glycoproteins and Proteoglycans*. The enormous recent progress in understanding the biological roles of glycoproteins has prompted the present volume. The reasons for studying glycoproteins have multiplied, and in the present volume the roles played by glycoproteins are explored in a variety of biological situations. The first two chapters describe molecules involved in cell–substratum and cell–cell interactions in a broad sense, and also focus on recent progress in identifying specific attachment molecules. Our understanding of how normal processes, such as cellular differentiation and tissue organization, are regulated is dependent on understanding how cells interact with the extracellular matrix. When these processes go awry the consequences can be tragic, for example, when manifest as birth defects and cancer. Our ability to devise appropriate therapies is in many cases limited by our understanding of such cell–matrix interactions. The third chapter explores the roles by glycoproteins during early mammalian development. The carbohydrate portions clearly play very important roles in presenting information during early embryogenesis, and an unusual tumor stem cell, the embryonal carcinoma, looks very promising in providing an experimental system for understanding how the expression of these complex carbohydrate determinants is regulated. The next three chapters explore the biology of glycoproteins in distinct situations: in the immune system, in the nervous system, and during erythropoiesis. Each chapter presents a wealth of information regarding the programmed expression of glycoproteins and emphasizes their functional involvement in various biological processes. The last chapter describes the life cycle of an unusual organism—the cellular slime mold, which can exist both as a unicellular vegetative ameba and as a multicellular sporulating or-

ganism—and summarizes the roles played by cell surface glycoproteins in regulating the complex cellular interactions that occur during its life cycle.

I have enjoyed interacting with these authors and have learned an enormous amount from each of them. I thank them for the conscientious efforts they have made to make their chapters concise, comprehensive, and yet still very readable.

I would like to take this opportunity to acknowledge the debt I owe to the following scientists from whom I have learned so much: Michael Rosemeyer, Charles Gilvarg, Phillips Robbins, Richard Hynes, and Garth Nicolson.

Raymond J. Ivatt

Contents

Chapter 3

Role of Glycoproteins during Early Mammalian Embryogenesis

Raymond J. Ivatt

Chapter 4

Cell Surface Glycoproteins and Carbohydrate Antigens in Development and Differentiation of Human Erythroid Cells

Minoru Fukuda and Michiko N. Fukuda

Chapter 7

The Role of Glycoproteins in the Life Cycle of the Cellular Slime Mold Dictyostelium discoideum

Ellen J. Henderson

1

Integral Membrane Glycoproteins in Cell–Cell and Cell–Substratum Adhesion

Caroline H. Damsky, Karen A. Knudsen, and Clayton A. Buck

1. INTRODUCTION

The focus of this chapter is on the integral membrane glycoproteins of the animal cell involved in cell–cell and cell–substratum adhesion. The approach to the subject, both intellectually and experimentally, reflects not only the prejudices of the authors but also the ideas and concepts gleaned from the literature and from conversations with colleagues. For the free interchange of ideas, we are grateful; for misinterpretations or oversights, we apologize. We have focused attention on that body of experimental data that has used both biochemical and biological approaches in an attempt to understand the adhesion process. Immunology, with its potential for specificity, has played a particularly crucial role in the discovery of adhesion-related glycoproteins and, with the increased use of monoclonal antibody technology, will play an increasingly important role in exploring this area. It is a tool that must be used with caution, however, as will be noted throughout the chapter.

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From our point of view, surface membrane glycoproteins may be viewed as decoders of extracellular information. As such, when a cell interacts with a particular substratum (fibronectin, laminin, the collagens, proteoglycans, etc.) or with ligands on the surface of another cell, the information as to whether to form an adhesive complex or not must rest in the molecular architecture of these glycoproteins and be transmitted to elements of the cytoskeleton and to other membrane proteins. If the correct combination of molecular signals is present, the cell will begin to organize an adhesive complex. Ultrastructural studies and a careful examination of the adhesion process in culture show that there are several types of adhesive interactions, and that adhesion is a stepwise process. According to this idea, the cell initially forms loose attachments to its substratum or to a neighboring cell. This attachment triggers a series of molecular events in which more glycoproteins are recruited into the attachment areas, resulting in a stabilization of adhesion and perhaps eventually in the formation of adhesive plaques or well-defined junctional complexes. This is a complicated series of events that could be disrupted (and perhaps controlled) at any point. Thus, it will be difficult both to prove whether a particular glycoprotein is involved directly or indirectly with the adhesion process, and to establish precisely what role the glycoprotein plays in the series of events leading to cellular adhesion. Such proof will require carefully correlated biochemical, immunological, biological, and ultrastructural studies. While no molecule has as yet been subjected to the rigors of this combined approach, there are several candidates that must be considered as major participants in the adhesion process. We will begin by examining recent developments in the study of cell–cell adhesion and then move on to the subject of cell–substratum adhesion.

2. CELL–CELL ADHESION

The process whereby cells recognize and adhere to each other is obviously one of the most crucial and complicated events in biology. When this process goes well as in normal embryonic development, the result is absolutely fascinating—the organization of cells into fully functional tissues and organs and the establishment of the intricate circuitry of the brain and peripheral nervous system. But when this process fails, the results are disastrous: terribly malformed individuals on one extreme or rampant metastasis on the other. The information for establishing and maintaining the correct contacts must lie in the expression and organization of molecules on the cell surface. Therefore, an understanding of the molecular basis of this drama requires an identification of the participating characters and a knowledge of how they move, respond, and in-

teract with each other. As will be seen below, the cast of molecular characters thought to be involved is large, diverse, and will undoubtedly continue to grow. In some cases, our biochemical casting will be imperfect and certain characters will be lost by the wayside due more than likely to the uncritical eye or excessive enthusiasm of their biochemist casting agents, whereas other molecules will be placed on the scene as new approaches and new systems are developed for further investigation. The purpose of this section is to enumerate the various glycoproteins implicated in the adhesion process and to see if any meaningful generalizations as to their various roles can be made. The focus will be on those glycoproteins that have been biochemically identified on the basis of some biologically functional parameters either as related to *in vitro* cellular behavior or to some *in vivo* tissue organizational phenomenon. We will deal with those molecules more likely involved in the early and perhaps more transitional events of intercellular adhesion rather than those involved in the later organization of more permanent junctional complexes such as desmosomes, gap junctions, and tight junctions.

Two general approaches have been taken in identifying the molecules involved in cell–cell adhesion. One has been to establish *in vitro* systems of cell–cell adhesion involving dispersed embryonic tissue or even cultured cell lines, and to identify factors that either promote or inhibit the particular cell–cell interaction being studied (see reviews by Moscona, 1974; Lilien *et al.*, 1979; Roth, 1982; Frazier and Glaser, 1979; Steinberg, 1978). A second approach, patterned after that used by Gerisch's group (Gerisch, 1977) to identify surface molecules involved in aggregation of individual slime mold amebae (see Henderson, this volume), consists of preparing a broad-spectrum antiserum capable of disrupting some form of cell–cell interaction, and then attempting to identify the antigen(s) involved. The work utilizing the latter approach will be dealt with in more detail here as it has been responsible for the latest surge of interest in the molecular basis of adhesion events.

The use of antibodies to dissect adhesion events has been fruitful, but the results must be interpreted with caution. The antiserum produced is only as good as the antigen injected, and the most immunogenic constituent of any injected material may not be quantitatively the major component. The final absolute identification of the cell surface molecule with which a particular antiserum is reacting to produce the desired biological effect requires careful, precise biochemical analysis and the eventual production of monospecific antibodies. The isolation of hybridomas producing monoclonal antibodies has proven invaluable in this regard. However, even these reagents must be used with extreme care and a wary eye for the existence of cross-reacting sequences involving either oligosaccharides or polypeptides on different proteins.

With these reservations in mind, let us examine the validity of using antibodies in a search for cell surface molecules involved in cell–cell adhesion. This approach makes different assumptions from those used previously to study cell–cell interactions by the phenomenological and biochemical dissection of *in vitro* aggregating systems (as discussed by Moscona, 1974; Lilien *et al.*, 1979; Steinberg, 1978) and hence may reveal molecules involved in other aspects of intercellular adhesion. It must be kept in mind that the principal antigen recognized by any antibody may not be the ligand directly involved in holding two cells together, but may instead represent another cell surface molecule somehow required to maintain the structure or organization of molecules at the site of adhesion. Such molecules, although not involved directly at the site of attachment, would have to be present and functioning for the adhesion process to work. Antibodies may well react with such molecules and thereby perturb cellular behavior. Obviously, in the grand lottery of antibody production (especially when complex immunogens are used), reagents will be prepared that interfere with the biology of adhesion in different ways leading to the discovery of many different antigens each involved in an important aspect of the adhesion process. As will be seen below, antibodies have already presented the biochemist and biologist with an interesting group of molecules whose precise roles in the adhesion process have yet to be determined.

2.1. Adhesion Glycoproteins Discovered Using Antibodies to Perturb the Adhesion Process

2.1.1. Cell Adhesion Molecules from Developing Nervous Tissue: N-CAM

2.1.1a. Early Studies on N-CAM. Probably the most thoroughly studied cell–cell adhesion glycoprotein to be discovered using antibodies is N-CAM (nerve-cell adhesion molecule) (see review by Edelman, 1983). This work extends over several years and will be discussed in some detail. In 1976, Rutishauser *et al.* published the results of preliminary studies in which antibodies were prepared whose Fab′ fragments could inhibit chick neural retinal cell or chick brain cell aggregation. Identification of the antigen with which the antibody reacted was complicated by the presence of proteolytic fragments in the cellular extracts (Edelman, 1983). Subsequently, a new broad-spectrum antiserum (anti-R10) was produced against 10-day neural retinal cells that had recovered from trypsinization prior to their injection into rabbits. Anti-R10 Fab interferred with the aggregation of chick neural retinal or brain cells (Brackenbury *et al.*, 1977). As nonspecific controls, it was shown that neither antibodies