Fusion of Biological Membranes and Related Problems

Subcellular Biochemistry
Volume 34

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Subcellular Biochemistry Volume 34

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Kluwer Academic Publishers
New York, Boston, Dordrecht, London, Moscow

eBook ISBN: 0-306-46824-7
Print ISBN: 0-306-46313-X

Created in the United States of America

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Preface

Membrane fusion and targetting processes are tightly regulated and coordinated. Dozens of proteins, originating from both the cytoplasm and membranes are involved. The discovery of homologous proteins from yeast to neurons validates a unified view.

Although much is known about the interfering proteins, the events occurring when two lipid bilayers actually fuse are less clear. One cannot exclude that lipid bilayers behave like soap-bubbles fusing when meeting each other. In this respect interfering proteins should be considered as preventing undesirable and unnecessary fusion and eventually directing the biological membrane fusion process (when, where, how and overcoming the activation energy).

In this volume of the series *Subcellular Biochemistry* some aspects of fusion of biological membranes as well as related problems are presented. Although not complete, one will find a lot of recent information including on virus-induced membrane fusion. The contributors of the chapters are all among the researchers who performed many of the pioneering studies in the field.

A first glance to the subject is found in *Chapter 1,* written by Cardula Harter and Constanze Reinhard. They give an overview of the route of a protein along the secretory pathway and summarize the progress that was made within the last decades. They discuss the current models for the formation and fusion of vesicular carriers with a major focus on the mechanism underlying budding of a COPI-coated vesicle.

In *Chapter 2* Michal Linial describes how neurotoxins (α-Latroxin and Clostridial toxins) can be used as tools in dissecting the exocytic machinery. The mode of action of α-Latroxin (inducing massive and uncontrolled vesicular release of neurotransmitters and neuropeptides) is still not

known. The effect of Clostridial toxins (blocking neurotransmitter release causing neuroparalysis) on exocytosis can be explained by their activities on the SNAREs. According to the author, structural information on SNARE and on their associated proteins should be evaluated in view of the biophysics and kinetic properties of fusion. Therapeutic applications are described.

The role of annexins (a distinct family of Ca^{2+}-binding proteins) in vesicular traffic and membrane fusion events in exocytosis and endocytosis is discussed by Helmut Kubista, Sandra Sacre and Stephen Moss in *Chapter 3*. The authors state that many of the properties involved in NSF, SNAPs, SNAREs-dependent vesicle transport and membrane fusion machinery are shared by annexins. It is possible that annexins provide a specialized exocytotic pathway that works in parallel to other mechanisms, such as NSF-dependent exocytosis. Alternatively, some data suggest that annexins may function together with the NSF, SNAPs and SNARE-machinery. Some annexins are membrane fusogens, others may be involved in transport to and aggregation of vesicles at fusion sites, in regulation of the cytoskeleton at these sites, in organization of fusion membranes, in regulation of other annexins and in Ca^{2+}signaling.

In *Chapter 4* Martin Götte *et al.* show how the entire information of baker's yeast *Saccharomyces cerevisiae* genome is now known and freely accessible. All membrane fusion in eukaryotic cells is governed by a class of monomeric GTP-binding proteins, called Rab in mammals and Ypt in yeast. After a brief introduction to the Ras-superfamily the reader is familiarized with the structural features and posttranslational modifications common to all Ypt proteins. The functional cycling and the various states of Ypt proteins as well as the proteins known to interact with the Ypt GTPases are discussed. Current models of the function of every single member of the Ypt proteins in the vesicular transport are reviewed (Ypt1p, Yp31p, Ypt32p, Sec4p for exocytic and Ypt51p, Ypt52p, Ypt53p, Ypt7p for vacuolar and endocytic trafficking). The authors conclude that three Ypt GTPases (Ypt1p, Ypt3p, Sec4p) suffice in regulating trafficking from the ER to the plasma membrane, while three proteins (Ypt51p, Ypt6p, Ypt7p) mediate vesicular transport on the endocytic pathway.

In *Chapter 5* Nils Faergeman *et al.* highlight the numerous effects of long-chain fatty acyl-CoA esters (through partioning into membranes, acyl-CoA-dependent remodeling in vesicle trafficking or reversible acylation of proteins) as cofactors for the fission and fusion of biomembranes. The significance of these effects is discussed in relation to the physiological concentration of long-chain fatty acyl-CoA esters and their binding proteins.

Brefeldin A (BFA) is known to prevent association of ARF to Golgi membranes and to inhibit secretion. In *Chapter 6* Catherine Jackson

discusses the early work describing the effects of BFA at both morphological and molecular levels. Next she introduces the family of Sec7 domain ARF exchange factors, and discusses the studies showing that they are major targets of BFA both *in vitro* and *in vivo*.The implications of the recently elucidated mechanism of action of BFA on Sec7 domain ARF exchange factors in transport through the ER-Golgi system are reviewed. Finally the author places ARF and its regulators into a model for ER-through-Golgi transport that takes into consideration the diverse *in vivo* effects of BFA.

The membrane fusion events during nuclear envelope assembly is reviewed by Philippe Collas and Dominic Poccia in *Chapter 7.* Several issues are addressed, including the multistep assembly of the nuclear envelope (from nuclear vesicles), the biochemical requirements for fusion of nuclear vesicles, evidence for the involvement of small GTPases in nuclear vesicle fusion, analogies between nuclear vesicle fusion and fusion events in intracellular membrane trafficking, the controversial role of Ca^{2+} in nuclear envelope assembly and evidence that some nuclear vesicles may harbor specific fusogenic elements.

A genetic approach to study the various processes involved in the maintenance, biosynthesis and proliferation of peroxisomes, creating a wealth of new ideas and information, experimental tools and mutants are discussed in *Chapter 8* by Ben Distel *et al.*

Neurons, chromaffin cells and membrane fusion are the subject of *Chapter 9,* the result of the colaboration between three different research groups (Hilde De Busser *et al.;* Peter Partoens *et al.;* Peter Vaughan). The authors limit themselves to a description of the membrane composition of the large dense-cored vesicles (LDVs)/secretory granules from which it can be concluded that the crucial actors governing small synaptic vesicles (SSVs) exocytosis are also responsible for LDV exocytosis. Subsequently they emphasize the role of the cytoskeleton in the exocytosis of LDVs and the role of GTP-binding proteins. Finally the related isoprenylating and carboxymethylating mechanisms are highlighted.

Yves Gaudin describes in *Chapter 10* how rhabdovirus-induced membrane fusion is an exception to the rule that the fusogenic protein in most cases of virus–plasma membrane fusion is synthetized in a metastable conformation, using the energy released during the fusogenic structural transition to achieve the energetically expensive membrane-fusion reaction. For rhabdoviruses the low pH-induced structural transition is absolutely reversible. A plausible role for the fusion inactive state is to avoid undesirable fusion in the acidic Golgi vesicles during the transport of fusogenic glycoprotein. The reversibility would be necessary for the protein being incorporated in a native functional conformation in neosynthetized virions.

The specific roles for lipids in virus fusion and exit is discussed by Margaret Kielian in *Chapter 11* using alphaviruses as an example. An efficient alphavirus exit requires the virus spike and nucleocapsid, the specific interaction of the E2 tail with nucleocapsid, the expression of 6K and correct lateral spike proteins interactions. In order to understand the roles played by cholesterol (entry, exit) and sphingolipids (hemifusion, complete fusion) in the alphavirus lifecycle (by interacting with viral proteins promoting fusogenic conformational changes) the current molecular understanding of alphavirus membrane fusion is discussed in detail. At the end of the article the author considers the available data suggesting a specific role of lipids in the entry and exit of other viruses and pathogens.

Chapter 12 is devoted to the fusion mediated by the HIV-1 envelope protein. In this chapter Carrie McManus and Robert Doms discuss the current understanding of the viral (Env, Gp120, Gp41) and cellular components (CD4, major and alternative HIV-1 coreceptors) necessary for the viral entry process. The identification of the HIV coreceptors coupled with detailed structural information on CD4, pg120 and gp41 has suggested several new anti-virai approaches. In addition to the coreceptors, Env itself can be targeted. Triggered or partialy triggered Env proteins may elicit antibodies to highly conserved but poorly immunogenic domains that are competent to neutralize diverse virus isolates.

In the last chapter (*Chapter 13*) David Sanders shows how sulfhydryl groups are involved in fusion mechanisms. Cysteine is a special residue because it is sensitive to the oxidation/reduction potential of its environment. The author states that proteins promoting intracellular membrane fusion reside in the reducing environment of the cytoplasm, whereas those participating in membrane fusion between enveloped viruses and cells or between two or more cells are exposed to an oxidizing milieu. Following discussion of protein thiols in cellular membrane fusion and in viral-glycoprotein-mediated membrane fusion and virus entry, the author reconsiders the alphavirus entry, incorporating both acidified endosomes and thiol-disulfide exchange.

Finally, we would like to thank Paul Van Dyck for assisting in preparation of this book.

We wish all of you much enthusiasm and pleasure in reading the book.

Antwerp, Belgium Herwig Hilderson
Heidelberg, Germany Steve Fuller

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