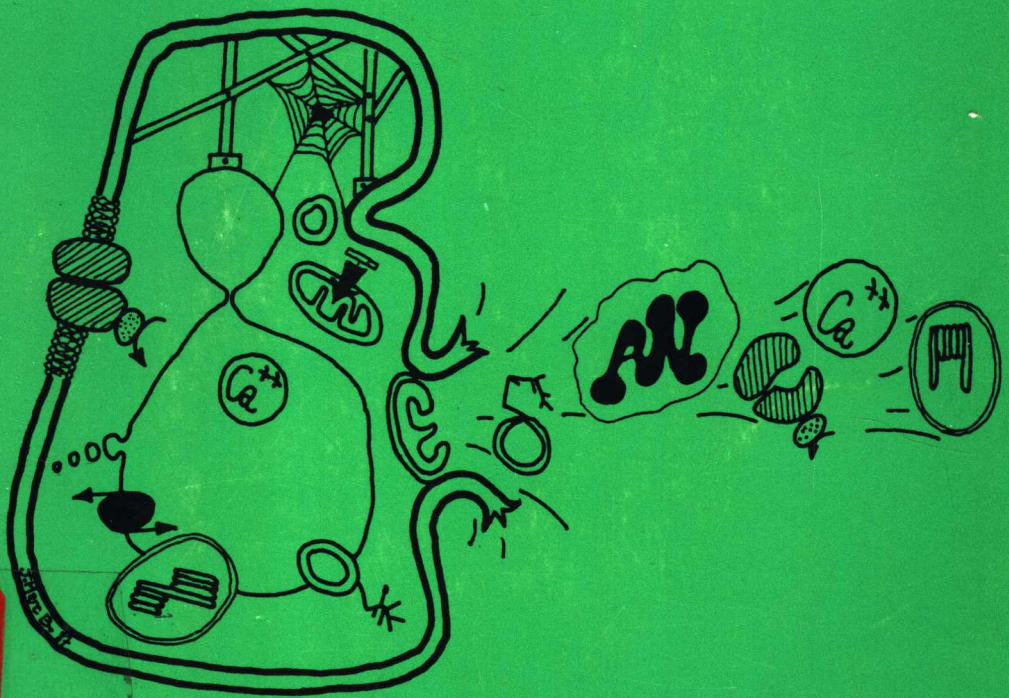
Dynamics of Membrane Proteins and Cellular Energetics

Edited by
Norbert Latruffe · Yves Gaudemer
Pierre Vignais · Angelo Azzi



Springer-Verlag

Dynamics of Membrane Proteins and Cellular Energetics

Edited by
Norbert Latruffe, Yves Gaudemer,
Pierre Vignais, Angelo Azzi

With 30 Figures

Springer-Verlag
Berlin Heidelberg New York
London Paris Tokyo

Professor Norbert Latruffe
Université de Franche-Comté
Laboratoire de Biochimie et Biologie Moléculaire
UA CNRS 531
25030 Besançon Cedex – France

Professor Yves Gaudemer
Université de Franche-Comté
Laboratoire de Biochimie et Biologie Moléculaire
UA CNRS 531
25030 Besançon Cedex – France

Professor Pierre Vignais
CENG – DRF Biochimie
Laboratoire de Biochimie
UA CNRS 1130, BP 85 X
38041 Grenoble Cedex – France

Professor Angelo Azzi
Universität Bern
Institut für Biochemie und Molekularbiologie
Bühlstrasse 28
3012 Bern – Switzerland

ISBN 3-540-50047-2 Springer-Verlag Berlin Heidelberg New York
ISBN 0-387-50047-2 Springer-Verlag New York Berlin Heidelberg

Printing and binding: Druckhaus Beltz, Hemsbach/Bergstr.
2131/3130-543210 – Printed on acid-free paper

Preface

This manual on "Dynamics of Membrane Proteins and Cellular Energetics" is the result of a FEBS-CNRS Course held in Grenoble and Besançon in September 1987. It appears to be, after the first, published in 1979 the fifth of the series. After focussing on the "Biochemistry of Membranes" (1979) it was the turn of "Membrane Proteins" (1981) and of Enzymes, Receptors and Carriers of Biological Membranes (1983), followed by "Membrane Proteins : Isolation and Characterization" (1986). Although the central issue has always been the biological membrane, its components and its functions, each manual has put the accent on somewhat different issues corresponding to the most innovative, interesting research in the field.

After almost a decade this new manual appears, which stresses the aspect of the integration of membrane research at a cellular level. Such a novel emphasis is the consequence of the common interest of cell biology and biochemistry to understand the results of the biochemical analysis of membrane proteins in the context of the cell complexity. Consequently most of the experimental protocols are dealing with cellular models, but with clear reference to the function and structure of isolated membrane proteins.

The present manual is divided into two sections. Part one is meant as a theoretical introduction to part two, the experimental section. The theoretical part has been kept very limited not to change the dominating practical character of the manual but it has, in any case, been introduced in order to give the interested scientist not only some simple, well described protocols, but some general background (in form of examples) on the field of membrane proteins and cellular events.

Who should be interested in having this manual in his bookshelf ? Everyone who likes to set up experiments using cellular models but lacks previous experience in the field. He can be the specialist as well, who likes to leave in the hands of his students a simple, reliable set of protocols, or he can be the teacher, who likes to run a practical course.

This manual is not encyclopaedic and does not want to compete with other publications which have this character. Rather than completeness we have chosen simplicity, rather than giving principles we have chosen to give examples.

A. Azzi
(for the editors)

Contributors

ADAMI Pascale, Université de Franche-Comté, Laboratoire de Biochimie et Biologie Moléculaire, UA CNRS 531, 25030 BESANCON Cedex - FRANCE

AZZI Angelo, University of Bern, Institute of Biochemistry and Molecular Biology, Bühlstrasse 28, CH 3012 BERN - SWITZERLAND

BAGGIOLINI Marco, Theodor Kocher Institute, University of Bern, Freiestrasse 1, CH 3000 BERN 9 - SWITZERLAND

BAILLY Anne, Université de Franche-Comté, Laboratoire de Biochimie et Biologie Moléculaire, UA CNRS 531, 25030 BESANCON Cedex - FRANCE

BERREZ Jean-Marc, Université de Franche-Comté, Laboratoire de Biochimie et Biologie Moléculaire, UA CNRS 531, 25030 BESANCON Cedex - FRANCE

BOF Mireille, CENG Laboratoire de Biochimie, DRF/Biochimie, BP 85 X, 38041 GRENOBLE Cedex - FRANCE

CHERKAOUI MALKI Mustapha, Université de Franche-Comté, Laboratoire de Biochimie et Biologie Moléculaire, UA CNRS 531, 25030 BESANCON Cedex - FRANCE

DELAAGE Michel, Immunotech Luminy, Case 915, 13288 MARSEILLE Cedex 9 - FRANCE

DERANLEAU D.A., Theodor Kocher Institute, University of Bern, Freiestrasse 1, CH 3000 BERN 9 - SWITZERLAND

DE VEYRAC Béatrice, Immunotech Luminy, Case 915, 13288 MARSEILLE Cedex 9 - FRANCE

DEWALD Béatrice, Theodor Kocher Institute, University of Bern, Freiestrasse 1, CH 3000 BERN 9 - SWITZERLAND

DOUCE Roland, CENG, Laboratoire de Physiologie Cellulaire Végétale, BP 85 X, 38041 GRENOBLE Cedex - FRANCE

DOUSSIERE Jacques, CENG Laboratoire de Biochimie, DRF/Biochimie, BP 85 X, 38041 GRENOBLE Cedex - FRANCE

GONZALEZ C., CENG Laboratoire de Biochimie, DRF/Biochimie, BP 85 X, 38041 GRENOBLE Cedex - FRANCE

JOB Didier, CENG INSERM U 244, LBio/DRF, BP 85 X, 38041 GRENOBLE Cedex - FRANCE

JOYARD Jacques, CENG, Laboratoire de Physiologie Cellulaire Végétale, BP 85 X, 38041 GRENOBLE Cedex - FRANCE

KANTE Arlette, Université de Franche-Comté, Laboratoire de Biochimie et Biologie Moléculaire, UA CNRS 531, 25030 BESANCON Cedex - FRANCE

KLEIN Gérard, CENG Laboratoire de Biochimie, DRF/Biochimie, BP 85 X, 38041 GRENOBLE Cedex - FRANCE

KURKDJIAN Armen, Laboratoire de Physiologie Végétale, CNRS - Avenue de la terrasse, BP 1, 91190 GIF/YVETTE - FRANCE

LATRUFFE Norbert, Université de Franche-Comté, Laboratoire de Biochimie et Biologie Moléculaire, UA CNRS 531, 25030 BESANCON Cedex - FRANCE

LE QUÔC Danielle, Université de Franche-Comté, Laboratoire de Biochimie et Biologie Moléculaire, UA CNRS 531, 25030 BESANCON Cedex - FRANCE

LE QUÔC Khanh, Université de Franche-Comté, Laboratoire de Biochimie et Biologie Moléculaire, UA CNRS 531, 25030 BESANCON Cedex - FRANCE

LUTHY Roland, University of Bern, Institute of Biochemistry and Molecular Biology, Bühlstrasse 28, CH 3012 BERN - SWITZERLAND

MAHONEY Charles, University of Bern, Institute of Biochemistry and Molecular Biology, Bühlstrasse 28, CH 3012 BERN - SWITZERLAND

MIDOUX Patrick, Centre de Biophysique Moléculaire du CNRS, 1, rue Haute, 45071 ORLEANS Cedex - FRANCE

MILANI Daria, University of Padova, Institute di Patologia Generale, Via Loredan, 35131 PADOVA - ITALY

MONSIGNY Michel, Centre de Biophysique Moléculaire du CNRS, 1, rue Haute, 45071 ORLEANS Cedex - FRANCE

MOREL Anne, Immunotech Luminy, Case 915, 13288 MARSEILLE Cedex 9 - FRANCE

MOREL Françoise, CENG Laboratoire de Biochimie, DRF/Biochimie BP 85 X, 38041 GRENOBLE Cedex - FRANCE

NEUBURGER Michel, CENG, Laboratoire de Physiologie Cellulaire Végétale, BP 85 X, 38041 GRENOBLE Cedex - FRANCE

PIROLLET Fabienne, CENG INSERM U 244, LBio/DRF, BP 85 X, 38041 GRENOBLE Cedex - FRANCE

POZZAN Tulio, University of Ferrara, Institute di Patologia Generale, FERRARA - ITALY

PUGIN Alain, Université de Franche-Comté, Laboratoire de Biochimie , UA CNRS 531, 25030 BESANCON Cedex - FRANCE

ROLS Marie-Pierre, Centre de Recherches en Biochimie et Génétique Cellulaires du CNRS, 118, route de Narbonne, 31062 TOULOUSE Cedex - FRANCE

SATRE Michel, CENG Laboratoire de Biochimie, DRF/Biochimie, BP 85 X, 38041 GRENOBLE Cedex - FRANCE

SEMENZA Giorgio, ETH-Zentrum, Laboratorium für Biochemie, CH-8092 ZURICH - SWITZERLAND

TEISSIE Justin, Centre de Recherches en Biochimie et Génétique Cellulaires du CNRS, 118, route de Narbonne, 31062 TOULOUSE Cedex - FRANCE

THELEN M., Theodor Kocher Institute, University of Bern, Freiestrasse 1, CH 3000 BERN 9 - SWITZERLAND

Von TSCHARNER V., Theodor Kocher Institute, University of Bern,
Freiestrasse 1, CH 3000 BERN 9 - SWITZERLAND

TREVES Susan, University of Padova, Institute di Patologia Generale, Via Loredan, 35131 PADOVA - ITALY

VIGNAIS Pierre, CENG Laboratoire de Biochimie, DRF/Biochimie, BP 85 X, 38041 GRENOBLE Cedex - FRANCE

WYMANN M.P., Theodor Kocher Institute, University of Bern, Freiestrasse 1, CH 3000 BERN 9 - SWITZERLAND

Contents

PART ONE : THEORETICAL INTRODUCTION

THE STRUCTURE AND FUNCTION OF Ca^{+2} - AND PHOSPHOLIPID-DEPENDENT PROTEIN KINASE (PROTEIN KINASE C), A TRANS-MEMBRANE SIGNAL TRANSDUCER

C.W. MAHONEY & A. AZZI

Abbreviations used : PKC, protein kinase c ; PS, phosphatidylserine ; PDB, phorbol 12, 13 dibutyrate ; DAG, diacylglycerol ; M_r, apparent molecular weight ; kDa, kilo-dalton.

Ca^{+2}- and phospholipid- dependent protein kinase (protein kinase c), first discovered by Nishizuka's group as a protease-activated kinase in 1977 (Takai et al., 1977 ; Inoue et al., 1977), has generated tremendous interest in the biochemical community in the last 11 years because of its implication in numerous biological processes including tumor promotion (Hecker & Schmidt, 1979), membrane transporter and channel modulation (Sigel et al., 1988 ; Costa & Catterall, 1985 ; Liles et al., 1986 ; Witters et al., 1985), differentiation (Morin et al., 1987 ; Pahlman et al., 1983), muscle contraction (Ikeba et al., 1985 ; Nishikawa et al., 1984 & 1985), neural synaptic communication (Tanaka et al., 1986), secretion (Ieyasu et al., 1982 ; Kajikawa et al., 1983), the respiratory burst (Mahoney & Azzi, in press, 1988 ; Lüthy & Azzi, 1987 ; Mahoney et al., 1986 ; Serhan et al., 1983 ; Fujita et al., 1984), the immune response (Patel et al., 1987), growth (Cooper et al., 1982 ; Cochet et al., 1984 ; McCaffrey et al., 1984 ; Davis & Czech, 1985) and platelet aggregation (Froscio et al., 1988 ; Mahoney & Azzi, in press ; Naka et al., 1983 ; Sano et al., 1983 ; Watson et al., 1988 ; Mahoney et al., this volume) (for reviews see Nishizuka 1984 & 1986). This paper will focus on the basic structural features of protein kinase c and some of the most recent developments in the elucidation of its structure and function.

Protein kinase c (PKC) consists of a single polypeptide with an apparent molecular weight (M_r) of 84,000 kDa and

is the only protein kinase examined to date that requires Ca^{+2} and phospholipid for activation, in contrast to the other well characterized cAMP-activated (for review see Beavo & Mumby, 1982), cGMP-activated (for review see Kua & Shoji, 1982) and the Ca^{+2} - calmodulin-activated protein kinases (for review see Schulman, 1982). An additional feature that distinguishes protein kinase c from the other protein kinases is its activation by tumor promoters (e.g. the phorbol 12, 13 diesters) and the neutral lipid diacylglycerol (DAG), both of which can lower the Ca^{+2} requirement for enzyme activation to the sub-micromolar level (Takai et al., 1979 ; Castagna et al., 1982). PKC is a cytosolic protein in the cell resting state (≃ 80 % cytosolic, ≃ 20 % membrane associated) (Ashendel et al., 1983) and upon cell stimulation by a variety of extracellular ligands (e.g. hormones, neurotransmitters, the phorbol 12, 13 diesters or DAG) most of the PKC becomes cell membrane associated (≃ 80 % membrane associated, ≃ 20 % cytosolic) (Kraft & Anderson, 1983 ; Kraft et al., 1982). A rise in internal Ca^{+2} alone (Melloni et al., 1985 ; Wolf et al., 1985 b) can cause this redistribution and activation of PKC as can phorbol diester or DAG (Gopalakrishna et al., 1986 ; Wolf et al., 1985 b). Physiologically, in many cases, when an extracellular ligand (as above) binds to its cell surface receptor, a phospholipase C is activated through a coupling with G protein(s) thereby resulting in the hydrolysis of phosphatidylinositoldiphosphate (PIP2) to inositoltriphosphate (IP3) and DAG. The water soluble IP3 can indirectly activate PKC by inducing a release of intracellularly stored Ca^{+2} (non-mitochondrial) (Berridge, 1984 ; Michell, 1983) and DAG can directly induce a redistribution and activation of the enzyme (Gopalakrishna et al., 1986 ; Wolf et al., 1985 b). Binding of radioactive phorbol 12, 13 diester can be competitively inhibited by the addition of DAG suggesting that both compounds have overlapping binding sites on the enzyme-phospholipid complex (Sharkey & Blumberg, 1985 ; Sharkey et al., 1984).

The Catalytic and Regulatory Domains

PKC is readily cleaved by a Ca^{+2} activated intra-cellular protease into a 50 kDa catalytically active (Ca^{+2} - phospholipid - independent) and a 30 kDa M_r regulatory fragment (Inoue et al., 1977 ; Melloni et al., 1985 ; Nakadate et al., 1987). There is some evidence to suggest that proteolysis is the mechanism of down regulation of protein kinase c (Young et al., 1987 ; Woodgett & Hunter, 1987). The 50 and 30 kDa M_r catalytic and regulatory fragments can also be generated in vitro by limited tryptic digestion (Lee & Bell, 1986 ; Mochly-Rosen & Koshland, 1987 ; Huang & Huang, 1986) which has been useful in elucidating the function of these domains. Recently, Lee & Bell (1986) have presented evidence demonstrating that the Ca^{+2}, phosphatidylserine (PS), and PDB (Huang & Huang, 1986) binding sites are located on the regulatory fragment. The 30 and the 50 kDa regulatory and catalytic domains have been localized to the N and C terminal regions of the protein respectively (Parker et al., 1986). Since most of the regulators of PKC activity are hydrophobic compounds which compete for PDB or PS binding it is likely that these compounds also modulate the activity of PKC by binding in the 30 kDA M_r domain. Current knowledge suggests that the active site of resting PKC located in the 50 kDa domain is blocked by the 30 kDa regulatory domàin and that on the binding of Ca^{+2}, PS, and PDB or DAG in this domain the active site becomes reactive to the substrates MgATP and protein acceptor.

Primary Structure, Properties, and Distribution of the α, β, and γ Forms of PKC

Recently the complete nucleic acid sequences for the 3 isozymic forms of PKC (α, β, γ) and their deduced amino acid sequences have been reported (Parker et al., 1986 ; Coussens et al., 1986) thereby facilitating further elucidation of the

mechanisms of activation and inhibition by various ligands. The primary sequences of the α, β, γ forms of PKC consist of 672, 673, and 693 amino acid residues and each contains an amino terminus cysteine-rich domain, a down stream predicted Ca^{+2} binding domain, and a conserved protein kinase active site domain in the carboxy terminus (Parker et al., 1986). The differential role of the three isozymes remains unclear yet recent studies have reported differences in the enzymatic properties and distributions of these isozymes. The α, β, γ forms of PKC, readily separated by hydroxylapatite chromatography (Ido et al., 1987 ; Huang, K. et al., 1986 b ; Huang F. et al., 1987 ; Shearman et al., 1987) are differentially activated by unsaturated free fatty acids (Sekiguchi et al., 1987). The γ isoform shows a slight activation (30-40 % relative to Ca^{+2}, PS, DAG activated) in the presence of arachidonic, oleic, or linoleic acid in the 25-100 μM concentration range, whereas the α isoform is activated by these 3 fatty acids (50-400 μM range) in the presence of Ca^{+2} to a similar degree as in the presence of Ca^{+2}, PS, and DAG. The β isoform shows intermediate activation properties by free fatty acid in the presence of Ca^{+2} (Sekiguchi et al., 1987). Although Sekiguchi et al. were able to find no significant difference in the Ca^{+2}, PS, DAG activation properties of the 3 isozymes, in contrast Huang K. et al. (1986 b) found similar Ca^{+2}, PS, DAG activation properties (> 15 fold) for α and β PKC, but only a 4-8 fold activation for the γ form. Ido et al. (1987) have provided preliminary evidence that the α PKC is able to phosphorylate the EGF receptor in membranes of epidermal carcinoma cells the most rapidly, β PKC at an intermediate rate, and γ with the lowest rate. Auto-phosphorylation of α and γ PKC occurs only at serine residues yet in the case of β PKC threonine residue(s) can be phosphorylated as well (Huang K. et al., 1986 b). Since only 1-2 moles Pi/mole PKC are autoincorporated this suggests that in the case of α and γ PKC two serine residues are autophosphorylated

whereas for β PKC a single serine and threonine are modified. The differential distribution of α, β, γ PKC isoforms within the brain has been reported by several groups (Huang F. et al., 1987 ; Shearman et al., 1987). Human neutrophils (Sekiguchi et al., 1987) and normal and ras-transformed 3T3 fibroblasts (McCaffrey et al., 1987) contain only α PKC. McCaffrey et al. (1987), in addition, were not able to find any differences between α PKC from normal and ras-transformed 3T3 fibroblasts.

Auto-phosphorylation

Protein kinase c can be auto-phosphorylated (Lepeuch et al., 1983 ; Huang K. et al., 1986 a ; Mochly-Rosen & Koshland, 1987) in both the regulatory and catalytic domains (Newton & Koshland, 1987 ; Huang K. et al., 1986 a) and enzymatic activity is stimulated as a result (Huang K. et al., 1986 a ; Mochly-Rosen & Koshland, 1987). Auto-phosphorylation resulted in an activation of PKC through a 2 fold decrease in the Km for histone (Mochly-Rosen & Koshland, 1987), a 4 fold lowering of the Ca^{+2} requirement for activation (7.5 vs. 31.6 μM), and a 2 fold lowering of the Kd for PDB (Huang K. et al., 1986 a). In addition it was found that the limited-tryptic generated 50 kDa fragment is not capable of auto-phosphorylation and that auto-phosphorylation is an intra-molecular process (Newton & Koshland, 1987 ; Mochly-Rosen & Koshland, 1987). Hence, it appears that auto-phosphorylation activates PKC, yet a report by Wolf et al. (1985 a) provides suggestive evidence that down regulation of PKC may occur through auto-phosphorylation by stimulating dissociation of the active membrane bound enzyme from the membrane. It is quite possible that both mechanisms are working in a temporal sequence of events in vivo.