*Asim K. Duttaroy and Friedrich Spener (Eds.)*

# Cellular Proteins and Their Fatty Acids in Health and Disease


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**Editors:**

***Professor Dr. Asim K. Duttaroy***
Institute for Nutrition Research
University of Oslo
POB 1046 Blindern
N-0316 Oslo
Norway

***Professor Dr. Friedrich Spener***
Institut für Biochemie
Universität Münster
Wilhelm-Klemm-Str. 2
48149 Münster
Germany

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**Library of Congress Card No.: applied for**

**British Library Cataloguing-in-Publication Data**
A catalogue record for this book is available from the British Library.

**Bibliographic information published by Die Deutsche Bibliothek**
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at <http://dnb.ddb.de>

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Printed in the Federal Republic of Germany
Printed on acid-free paper

**Composition** K+V Fotosatz GmbH, Beerfelden
**Printing** Druckhaus Darmstadt GmbH, Darmstadt
**Bookbinding** Buchbinderei Schaumann, Darmstadt

**ISBN** 3-527-30437-1

## Preface

Long-chain fatty acids, in addition to providing the cell with energy, are substrates for membrane biogenesis and act as signalling molecules. These fatty acids and their derivatives directly or indirectly regulate cellular processes such as differentiation, development and gene expression as well as the activities of enzymes, membrane receptors and ion channels. Dietary fats have profound effects on gene expression and fatty acid-activated transcription factors (nuclear receptors) may have a fundamental role in regulating energy balance through their sensing of fatty acid flux in metabolically active tissues. Because of the functional roles of these fatty acids and of their structural features and physico-chemical properties, it is important to understand the mechanisms that evolved for uptake and retention of these molecules. The picture emerging is that the cell has multiple binding proteins in the membranes as well as in the aqueous compartments that assure adequate uptake and intracellular movement of long-chain fatty acids and their regulatory action.

This book covers the various aspects of intracellular binding proteins (FABPs, ACBP, SCP-2), such as structure-function, ligand specificity, delivery of ligands by membrane-protein and protein-protein interaction, as well as their expression and roles pertaining to nutrition, health, and disease. Regulation and expression of membrane fatty acid transporters such as FABPpm, FAT, FATP, and ABC transporters are treated in further chapters. In addition, transcription factors PPARs, RXRs, RARs, LXR, and HNF4 which bind fatty acids or their derivatives are also dealt with in depth. They play a central role in regulating the storage and catabolism of dietary fats and essentially all major metabolic paths of lipids appear to be under control of one or more genes regulated by these transcription factors. Their roles in inflammatory disorders, obesity, cancers, and atherosclerosis are also discussed. Since these transcription factors require fatty acids or their derivatives as ligands, FABPs may play important roles in transporting these ligands.

Many leading investigators have contributed their most recent developments to this book. We believe that it will prove to be an invaluable reference text for both those familiar with and those new to the exciting, and ever changing world of cellular proteins whose common denominator is binding of fatty acids.

Finally, we would like to express our thanks to all contributors to this book and to the reviewers for their competent advice. The untiring support and patience of Ines Chyla and the staff at Wiley-VCH is gratefully acknowledged.

Oslo and Münster, February 2003

Asim K. Duttaroy, Oslo
Friedrich Spener, Münster

# Contents

## List of Contributors

Philippe Amouyel
INSERM U508
Institut Pasteur de Lille
1 rue du professeur Calmette
BP 245
59019 Lille Cedex
France

Arend Bonen
Department of Kinesiology
University of Waterloo
Waterloo, ON N2L 3G1
Canada

Nigel J. Brunskill
Department of Cell Physiology
and Pharmacology
Department of Nephrology
University of Leicester
Medical Sciences Building
University Road
Leicester, LE1 9HN
UK

Chris T. Coburn
and Nada A. Abumrad
Department of Physiology
and Biophysics
Stony Brook University
Stony Brook, NY 11794-8661
USA

A.K. Duttaroy
Institute for Nutrition Research
University of Oslo
P.O. Box 1046 Blindern
0316 Oslo
Norway

Steve Farmer
Boston University of School
of Medicine
Department of Biochemistry
715 Albany St.
Boston, MA 02118
USA

Jason H. Gill
Molecular Pathology
Cancer Research Unit
University of Bradford
All Saints Road
Bradford BD7 1DP
UK

Jan F.C. Glatz, Joost J.F.P. Luiken
and Ger J. van der Vusse
Department of Physiology, CARIM
Maastricht University
P.O. Box 616
6200 MD Maastricht
The Netherlands

Luis H. Gutiérréz-González
Institut für Organische Chemie
und Biochemie II
Technische Universität München
Lichtenbergstr. 4
85747 Garching
Germany

Rachel Hertz and Jacob Bar-Tana
Department of Human Nutrition
and Metabolism
Hebrew University Medical School
91120 Jerusalem
Israel

Chris A. Jolly
Division of Nutritional Sciences
University of Texas at Austin
Gearing Hall 117
Austin, TX 78712
USA

Lene K. Juvet and Hilde I. Nebb
Institute for Nutrition Research
University of Oslo
P.O. Box 1046 Blindern
0316 Oslo
Norway

Claus Kerkhoff
Institut für Experimentelle
Dermatologie
Universität Münster
Von Esmarch Straße 56
48149 Münster
Germany

Christian Lücke
and James A. Hamilton
Department of Physiology
and Biophysics
Boston University School of Medicine
Boston, MA 02118
USA

Susanne Mandrup, Nils J. Færgeman
and Jens Knudsen
Department of Biochemistry
and Molecular Biology
University of Southern Denmark
Campusvej 55
5230 Odense M
Denmark

Alexander Mata de Urquiza
and Thomas Perlmann
Ludwig Institute for Cancer Research
Karolinska Institute
P.O. Box 240
17177 Stockholm
Sweden

Lindsay McDermott
Department of Chemistry
University of Glasgow
Glasgow G12 8QQ
UK

Aline Meirhaeghe
Clinical Chemistry
Addenbrooke's Hospital – Level 4
Hills Road
Cambridge CB2 2QR
UK

Liliane Michalik, Nguan Soon Tan,
Walter Wahli
and Béatrice Desvergne
Institut de Biologie Animale
Bâtiment de Biologie
Université de Lausanne
1015 Lausanne
Switzerland

Eric J. Murphy
Department of Pharmacology,
Physiology and Therapeutics
University of North Dakota
501 N. Columbia Rd., Room 3700
Grand Forks, ND 58203
USA

Isabelle Niot and Philippe Bresnard
Laboratoire de Physiologie
de la Nutrition (ENSBANA)
Université de Bourgogne
1, Esplanade Erasme
21000 Dijon
France

Yuji Owada and Hisatake Kondo
Division of Histology
Department of Cell Biology
Graduate School of Medical Science
Tokohu University
2-1 Seiryo-cho, Aoba-ku
Sendai 981-8575
Japan

Colin N.A. Palmer
Biomedical Research Centre
Nienewells Hospitals and Medical
School
University of Dundee
Dundee DD1 9SY
UK

Jorge Plutzky
Cardiovascular Division
Department of Medicine
Brigham and Women's Hospital
Harvard Medical School
221 Longwood Ave., LMRC 307
Boston, MA 02115
USA

Olof Radmark
Department of Medical Biochemistry
and Biophysics
Division of Physiological Chemistry II
Karolinska Institutet
17177 Stockholm
Sweden

Ruth A. Roberts
Aventis Pharma SA
Centre de Recherche de Paris
94400 Vitry sur Seine
France

Jean E. Schaffer
Departments of Internal Medicine
Molecular Biology and Pharmacology
Washington University School
of Medicine
660 South Euclid Ave
Box 8086
St. Louis, MO 63110-1010
USA

Gerd Schmitz
and Thomas Langmann
Institut für Klinische Chemie
und Laboratoriumsmedizin
Universität Regensburg
Franz-Josef-Strauß-Allee 1
93042 Regensburg
Germany

Udo Seedorf
Institut für Arteriosklerosеforschung
Domagkstr. 3
48149 Münster
Germany

Friedrich Spener
Institut für Biochemie
Universität Münster
Wilhelm-Klemm-Str. 2
48149 Münster
Germany

Jack M. Stewart
Biochemistry Program
Department of Biology
Mount Allison University
Sackville, NB E4L 1G7
Canada

Judith Storch
Department of Nutritional Sciences
Rutgers University
New Brunswick, NJ 08901
USA

Christian Wolfrum
Rockefeller University
1230 Yorck Ave.
New York, NY 10021
USA

# Part 1
# The Molecular Basis of Protein-Lipid Interaction and Functional Consequences

# 1
# Structure-Function of CD36 and Evidence for its Role in Facilitating Membrane Fatty Acid Transport

Chris T. Coburn and Nada A. Abumrad

## 1.1
## Introduction

CD36 (also known as GPIV, GPIIIb, PAS IV, and FAT) is an integral membrane glycoprotein with a wide cellular distribution. It has been identified on the surface of megakaryocytes, erythroid precursors, platelets, monocytes, dendritic cells, adipocytes, myocytes, retinal and mammary epithelial cells, and endothelial cells of the microvasculature and small intestine. Depending upon the cellular context, CD36 may perform its primary role as a mediator of fatty acid (FA) uptake, a cell adhesion molecule, or a class B scavenger receptor. This remarkable versatility may reflect both the diverse tissue distribution of CD36 and its ability to interact with a wide variety of ligands.

CD36 binds long-chain FA with high affinity and is a major facilitator of FA uptake in muscle and adipose tissues. As a receptor for the extracellular matrix proteins thrombospondin 1 (TSP-1) and collagens type I and IV, CD36 acts as an adhesion molecule modulating platelet aggregation and the cell–cell interactions important for recruitment and trafficking of monocytes to damaged tissues. In a pathological context, CD36 is the receptor in the microvasculature for the *Plasmodium falciparum* protein expressed on the surface of malaria-infected erythrocytes and as a result contributes to the virulence of this form of malaria. In macrophages and dendritic cells, CD36 is a scavenger receptor important to recognition and phagocytosis of apoptotic cells. On macrophages it is also the major receptor mediating binding and internalization of oxidized low-density lipoproteins (oxLDL), a role reflecting its ability to bind anionic phospholipids as well as lipids or proteins modified by lipid peroxidation.

Because of the varied roles of CD36, a great deal has been discovered about this protein. With functions impacting on lipid metabolism, atherogenesis and thrombosis, inflammation, platelet function, the pathogenesis of malaria and even angiogenesis, further study of the physiology and molecular interactions of CD36 will no doubt continue to progress at a rapid pace. This chapter presents an overview of current knowledge with particular emphasis given to the role of CD36 in lipid metabolism and metabolic homeostasis.

## 1.2 Primary Structure

CD36 is the founder member of a gene family of structurally related glycoproteins expressed at the cell surface and within lysosomes. Known members include, in addition to CD36, the high-density lipoprotein receptor SR-B1 (also CLA-1), *Drosophila* plasma membrane proteins emp and croquemort, the ubiquitously expressed lysosomal integral membrane protein LIMPII, and the recently identified amoeboid endolysosomal proteins LmpA, LmpB, and LmpC. All are believed to share a "hairpin" topology defined by two transmembrane domains with both termini in the cytoplasm (Fig. 1.1). In this configuration, the intervening amino acids localize either to the cell surface (CD36) or to the lumen of lysosomal vesicles (LIMPII). This arrangement is exceedingly rare among membrane proteins but has been experimentally confirmed for both CD36 and SR-B1 [1, 2].

Numerous studies have contributed to the development of a detailed model for the primary structure of CD36 (Fig. 1.1). The human cDNA predicts a sequence of 472 amino acids with an N-terminal signal peptide directing transcription to the endoplasmic reticulum (residues 1–30). Limited N-terminal sequencing of purified CD36 shows that the signal peptide is uncleaved but the initiating methio-

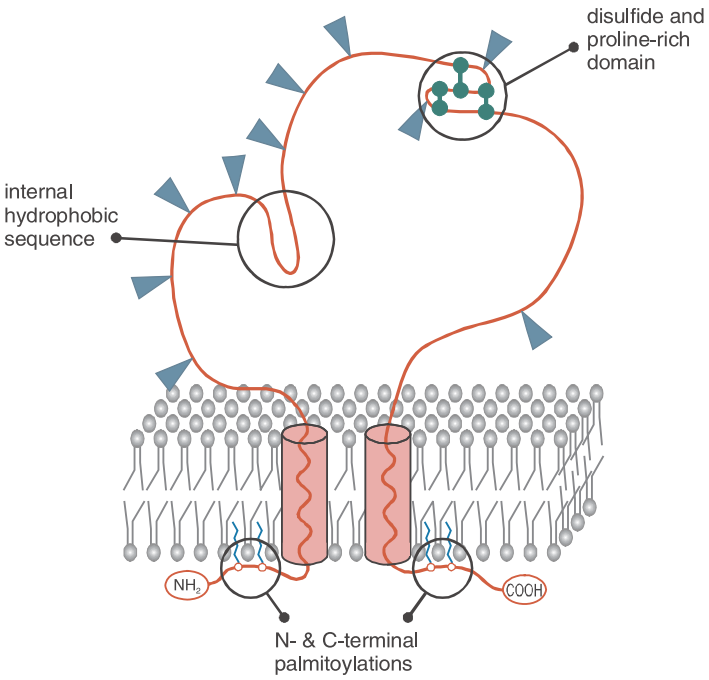


**Fig. 1.1** Cartoon of CD36 in the membrane, highlighting the major structural features. *N*-linked glycosylations are shown as triangles. Disulfide bonds are shown in green.

nine is removed. Residues 2–7 are in the cytoplasm while 8–30 form the N-terminal membrane-spanning domain. The second transmembrane domain (440–463) is near the C-terminus. An uninterrupted hydrophobic segment (186–204) is centrally located but not long enough to span the bilayer. This segment, which may form a hydrophobic pocket or may be associated with the outer leaflet, is not conserved in other known members of the CD36/LIMPII family.

The cytoplasmic domain of CD36 consists of only 15 amino acids (6 at the N-terminus and 9 at the C-terminus). It contains four cysteines (N residues 3 and 7 and C residues 464 and 466), which were shown to undergo palmitoylation, confirming the membrane topology [1]. From examples of other acylated proteins, it can be speculated that palmitoylation of the cytoplasmic domain may play an important role in modulating interactions with other proteins and/or membrane localization. Since palmitoylation is reversible, a variable palmitoylation state may also serve to acutely regulate CD36 function. In this respect, in isolated rat adipocytes, insulin or energy depletion with 2,4-dinitrophenol was shown to rapidly increase CD36 palmitoylation by about 3- and 12-fold, respectively [3].

The extracellular domain of CD36 contains 10 potential glycosylation sites and glycosylation increases the apparent protein mass from 53 kDa (non-glycosylated) to between 78 and 88 kDa, depending on the tissue source. Extensive glycosylation is a characteristic of the CD36/LIMPII family of proteins perhaps affording protection in the protease-rich environments of lysosomes or at sites of inflammation and tissue injury. Indeed, CD36 was initially identified in platelets based on its resistance to protease digestion [4] and deglycosylation with endoglycosidase F yields a protein that is susceptible to a range of proteases [5]. A cursory examination of the primary structure of CD36 shows a natural division between the N- and C-terminal halves of the extracellular domain. The N-terminal half contains 7 of the 10 potential *N*-linked glycosylation sites as well as the internal hydrophobic domain, while the C-terminal half is proline-rich and contains all of the extracellular cysteines and subsequent interchain disulphide bonds. Whether this may translate into separate functional domains remains to be determined.

In megakaryocytes and in CD36-transfected COS cells, Thr92, which fits within a protein kinase C consensus site, is constitutively phosphorylated during maturation of the protein in the Golgi apparatus [6]. The phosphorylation state of this residue appears to modulate the selectivity of CD36 on platelets for TSP-1 or collagen binding. A cAMP-dependent ectoprotein kinase A on the surface of platelets has also been shown to phosphorylated CD36 [7]. The phosphorylated residue was not determined but most likely occurs within a protein kinase A (PKA) consensus site around Ser237, though PKA phosphorylation of Thr92 cannot be ruled out. No functional change in CD36 activity has yet been attributed to this phosphorylation event.

## 1.3 Ligand Binding Domains

Monoclonal antibodies have been used to identify CD36 domains involved in ligand binding. Amino acids 155–183 define an immunodominant region shown to bind a number of anti-CD36 monoclonal antibodies, including the widely used OKM5 [8]. These antibodies block interactions of CD36 with TSP-1, OxLDL, malaria-infected red blood cells, apoptotic neutrophils, and phosphatidylserine [9–13]. Since antibody binding to CD36 might sterically hinder ligand interaction, synthetic and recombinant peptides have been used to more closely define the binding domains. These studies have shown that OxLDL and TSP-1 bind to sequences outside of the immunodominant domain. OxLDL binds with high affinity to CD36 amino acids 28–93 [14]. TSP-1 binds in a two-step process interacting first with CD36 residues 139–155. This induces a conformational change in TSP-1 unmasking a high-affinity site for amino acids 93–120 of CD36 [15]. Malaria-infected erythrocytes also bind within this region (residues 97–110) [6].

A possible site for long-chain FA binding in the extracellular domain of CD36 has been identified with an alignment comparing CD36 sequence with that of a representative member of the lipocalin family of cytosolic FA binding proteins [16]. Members of this family may exhibit as little as 20% sequence identity but share a common and distinct structural motif. The region comprising amino acids 127–279 of CD36 exhibits homology to human muscle FA binding protein (M-FABP) throughout 73% of its sequence, although identity is only 14.5%. Secondary structure predictions indicate this sequence may consist of a single $\alpha$-helical region interposed between regions of sheets similar to the known structure of M-FABP and other lipocalin family members. It is also of interest that of the amino acids conserved throughout the lipocalin family, Arg126 and Tyr128 of M-FABP, which interact with the FA carboxyl group and are necessary for FA binding, are conserved in this alignment (Arg272 and Tyr275 of CD36). It may be noteworthy that this region includes the hydrophobic domain of CD36 (186–204), thought to be membrane associated or to form a hydrophobic pocket.

## 1.4 Membrane Localization and Role in Cell Signaling

CD36 in many cells is associated with membrane microdomains rich in cholesterol and sphingolipid and known as rafts or caveolae. The long, largely saturated acyl chains of the sphingolipids favor tight packing with cholesterol and promote formation of small freely floating domains (hence rafts) within the membrane (reviewed in Ref. [17]). These detergent-resistant membranes (DRM), which can be biochemically isolated from the rest of the membrane by virtue of their insolubility in Triton X-100 at 4 °C, typically account for about 5% of the plasma membrane of mammalian cells. Although sphingolipid-rich rafts are mostly confined to the outer leaflet they are coupled to similar domains incorporating mono-un-