



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

BBAREV 85359

Fatty acylation of proteins

Michael F.G. Schmidt

Kuwait University, Faculty of Medicine, Kuwait (Arabian Gulf)

(Received 30 November 1988)

Contents

I. Introduction	411
II. Ester-type palmitoylation	412
A. Identification of palmitoylated proteins of viral and cellular origin	412
B. The acyl linkage sites in palmitoylated proteins	414
C. Biochemistry of palmitoylation	415
1. Location of palmitoylating activity	415
2. Dynamic nature of palmitoylation	416
3. Towards the purification of a protein fatty acyltransferase (PAT)	416
4. Specificity of lipid substrates for ester-type fatty acylation of proteins	417
III. N-terminal myristoylation	417
A. Identification of myristoylated proteins	417
B. Biochemistry of myristoylation	418
1. Substrate specificity and intracellular location	419
IV. The function of protein bound fatty acids	419
A. Palmitoylation	419
1. Involvement in membrane fusion	419
2. Morphogenesis and protein interaction	420
3. Involvement in proteolysis protection, transport, cell transformation and signal transduction	420
4. Comparison of acylated and fatty acid-free peptides	421
B. Functions of myristoylation	422
1. Anchor function of myristoylation	422
2. Myristoylation in morphogenesis and virus entry	423
V. Concluding remarks	423
Acknowledgements	423
References	423

I. Introduction

Many proteins are modified during or after their synthesis. Some of these modifications have been known

for many years, as for instance glycosylation, phosphorylation and proteolytic cleavage of precursor polypeptides. These examples have been extensively covered in a number of review articles and in biochemical textbooks over the years.

Another type of protein modification discovered more recently is the covalent attachment of lipid molecules like phospholipid, diacylglycerol and various species of long chain fatty acids. Such binding of lipid molecules is expected to change the physical properties of the respective entity quite dramatically, because largely hydrophilic residues are converted into very hydrophobic

Abbreviations: VSV, vesicular stomatitis virus; SFV, Semliki Forest Virus; ER, endoplasmic reticulum; PAT, protein fatty acyltransferase; NMT, *N*-myristoyltransferase.

Correspondence: M.F.G. Schmidt, Kuwait University, Faculty of Medicine, P.O. Box 24923/Code 13110 Safat, Kuwait, Arabian Gulf.

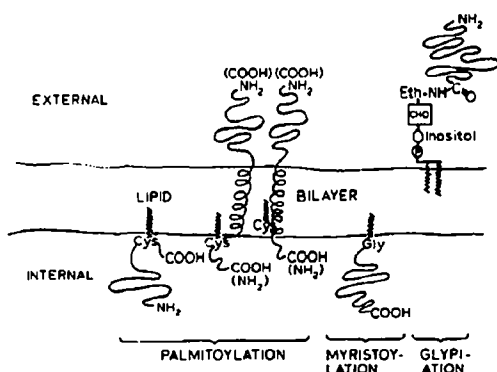


Fig. 1. Schematic diagram of hydrophobic modifications of proteins: palmitoylation, myristoylation and glypiation. Cys, cysteine; Gly, glycine; CHO, carbohydrate; Eth-NH₂, ethanolamine. Threonine has been identified as the palmitoylation site in one case only, with bovine brain myelin proteolipid apoprotein [90]. All other palmitate linkage sites chemically identified are cysteine residues (compare Table II).

ones. This will, of course influence the interactions between such modified proteins and other molecules present in their vicinity, be it other proteins, lipids or even nucleic acid. Likewise, intermolecular processes may be influenced, such as oligomerisation of polypeptides during biosynthesis or the co-operativity between subunits of a multimeric protein.

The present review aims at summarizing developments in the field of hydrophobic modifications of proteins since their discovery. Special emphasis will be placed on the acylation of viral and eukaryotic polypeptides with long chain fatty acids. This modification yields what could be regarded as a new class of proteins, which by analogy with glyco- or phosphoproteins, I suggest by termed 'acylproteins'. Two types of acylprotein are presently distinguished in the literature, those which contain exclusively the fourteen carbon myristic acid (tetradecanoic acid) in an amide-linkage, and those which are predominantly modified with palmitic-, stearic- and oleic acid (hexa- and octadecanoic acids) in ester- or thioester-type linkages. The various biochemical and cell biological aspects of palmitoylation and myristoylation will be discussed.

Another hydrophobic modification of proteins, the covalent attachment of a glycolipid tail to some membrane proteins (glypiation), will not be described here. Current reviews cover this area, which the reader is referred to [1-4]. Fig. 1 presents a schematic representation of the above-mentioned hydrophobic modifications of proteins.

II. Ester-type palmitoylation

Even before the term palmitoylation became widely used in the literature, a few protein species had been

previously identified that contained fatty acids as part of their covalent structure. These were the proteolipid apoprotein isolated from the myelin membrane, which is also termed lipophilin [5,6], the proteolipid component of preparations of the sarcoplasmic Ca²⁺/Mg²⁺-ATPase [7] and the bacterial lipoproteins [8,9]. The fatty acid species in these above-mentioned proteins more or less reflected the acyl patterns of the lipids of the membrane from which they had been isolated. In all these cases palmitic-, stearic- and oleic acid were the predominant acyl species. Although these proteins, due to their solubility in organic solvents, had initially been termed proteolipids, they can be classified as true acylproteins because of their covalently bound fatty acid residues. It should be mentioned that some other proteolipid species have been described. Despite their lack of any covalently bound fatty acid, these are soluble in organic solvents, but insoluble in water [10].

II-A. Identification of palmitoylated proteins of viral and cellular origin

At the time unaware of the above-mentioned acylated proteolipids, Schmidt and Schlesinger [11,12] observed that typical membrane glycoproteins can be labeled with tritiated long chain fatty acids. Although viral membrane glycoproteins were utilized initially, the same authors reported that fatty acylation also occurred with proteins of non-infected cells [13]. The first cellular membrane glycoprotein to be identified as fatty acylation was the transferrin receptor [14]. Many other species of acylproteins of viral or cellular origin with covalently bound fatty acids were subsequently identified (Table I).

In most of the proteins listed in Table I acylation was detected after labeling the protein with [³H]palmitic acid in the appropriate system followed by identification via immunoprecipitation, PAGE-analysis, chromatographic procedures, or combinations thereof. Although not examined in all acylproteins listed, in nearly all cases in which labeled fatty acids were analysed, palmitic acid was found to represent the major species bound to the acylprotein with stearic- and oleic acid comprising the balance of total radioactive fatty acid recovered from the protein under study. The same results have also been reported from analysis of acyl chains released from non-labeled acylproteins purified in large scale (for references refer to Table I). It should be noted that proteins modified by a glycolipid will incorporate ³H-labeled fatty acids during metabolic labeling, just like the acylproteins. In order to differentiate such glypiated proteins from acylproteins, labeling with [³H]ethanolamine or [³H]inositol should be applied. Only if such label is incorporated, is the protein most likely glypiated [3,4].

TABLE I

Palmitoylated proteins of eukaryotic and viral origin

Protein species	Origin of protein	Ref.
Cellular acylproteins		
Transferrin receptor	human leukemic T-cells	14
Acetylcholine receptor *	muscle cell line	15
Insulin receptor *	human lymphocytes (IM-9), Hep G2	16,17
Insulin like growth factor I - receptor		
	Hep G2, MDCK	18
IgE receptor	leukemic T-cells	19
Rhodopsin	bovine retina	20
Interphotoreceptor	bovine retina	21
Na ⁺ channel, α -subunit	rat neurons	22
Interleukin-2 receptor	human T-cell line (MT-1)	23
Sialo-gp 2,3 and gp3Pr	erythroblasts	24
HLA B7, DR	lymphoblastoid cell line	25
HLA-D/invariant chain	various human lymphoma cells	26
p41	p41 transfected cell	27
Ia alpha and beta	mouse spleen cells	28
Galactosyltransferase	Hela cells	29
Golgi mannosidase II	mouse 3T3 and other cells	30
Cardiac 51 kDa protein	mouse	31
Mucus glycoproteins	sublingual salivary glands	32
Mucus glycoproteins	gastric mucosal cells	33
TGF-alpha precursors	TGF-gene transfected cells	34
Butyrophilin	milk fat globule membrane (goat)	35
Xanthine oxidase	milk fat globule membrane (goat)	35
Lipophilin	myelin membrane (bovine)	5,5
DM-20	myelin membrane (rat)	36
PO-protein	myelin of peripheral nerve (rat)	37
Ca ²⁺ -ATPase	sarcoplasmic reticulum	7,38
Fibronectin	human fibroblasts	39
Ankyrin	human erythrocytes	40
Band 4.1 protein	human erythrocytes	40
Vinculin *	chicken embryo fibroblasts	41,42
Lens membrane proteins	intact ocular lens (rat)	43
Ligatin	ileal enterocytes (rat)	44
ras-proteins	various human cell lines	45
Apolipoproteins (AI,E)	human hepatoma cells (Hep-G2)	46
Apolipoprotein B	human LDL	47
Folate binding protein *	human KB-cells	48
Developmental gps	sea urchin embryo	49
RAS 1 and RAS 2	yeast cells	50
YPT1	yeast cells	51
Alpha factor	yeast cells	52,53
Membrane glycoproteins	yeast cells	54
Actin (sub population)	slime mold	55,56
Microtubule-binding protein	<i>Trypanosoma brucei</i>	57
Light harvesting protein *	duckweed plant	58
Rib 1,5-P ₂ -carboxylase *	duckweed plant	58
Six proteins (major 22 kDa)	Tetrahymena mimbres	59
Surfactant protein (SAP35)	alveolar epithelial cells	60
Human tissue factor	human lung fibroblast	61
CD9 surface gp	human platelets	62

TABLE I (continued)

Protein species	Origin of protein	Ref.
Viral structural acylproteins		
G-protein	rhabdoviruses (VSV)	11
E1.E2	togaviruses (Sindbis, SFV)	11,63
HA, HA ₂	avian/human influenza A viruses	63
HA	influenza B virus	^b
HEF I, HEF II	influenza C virus	^b
F ₀ , F ₁	Newcastle disease viruses	63
F, HN	mumps virus	64
HN	SV5	65
E2	mouse hepatitis virus	66
E2	bovine coronavirus L9	63
G1, G2	bunya viruses (La Crosse)	67
gp 35	Rous sarcoma virus	68
gp 65	spleen focus forming virus	69
p37 k	vaccinia virus	70
gE	herpes simplex virus (type 1)	71
Viral non-structural acylproteins		
E1b 18 kD	Adenovirus 12	72
E1b 19 kD	Adenovirus 1	73
T-antigen (large)	SV40	74
ras-protein	Harvey murine sarcoma virus	75

^a The fatty acid linkage in these proteins is partially or totally resistant to treatment with mild alkali or hydroxylamine.

^b Veit, M., Herrler, G. and Schmidt, M.F.G., unpublished data.

Many of the palmitoylated proteins listed in Table I are glycosylated and represent membrane components with widely diverse biological functions. Among these are polypeptides which span the membrane once or multiple times. Usually the palmitoylated membrane proteins are oriented with their carboxyterminus towards the cytoplasmic side of the membrane or homologously towards the inside of the enveloped virus particles listed. However, reversely oriented proteins may also be palmitoylated, e.g., the hemagglutinin-neuraminidase protein (HN) of mumps virus and of paramyxovirus SV5 [64,65] as well as the transferrin receptor [14]. Some of the membrane proteins listed occur as monomers [20], others as homo-oligomers [11,63] or as hetero-oligomers. An example for the latter is the insulin receptor, in which only the β -subunit is palmitoylated [17,18]. Beside the plasma membrane, internal membranes may also contain proteins with covalently linked fatty acid, e.g., the Golgi-located mannosidase II and galactosyltransferase [29,30] as well as the proteolipid component of sarcoplasmic Ca²⁺-ATPase [7,38]. Recent more generalized studies of the intracellular location of palmitoylated proteins showed that most of them are membrane bound [76-78]. However, the phenomenon is more complex in a functional sense, since a small group of palmitoylated proteins are secreted by the cells [21,33,34,46,47] and a somewhat larger group of acylproteins comprises components of the cellular cytoskeletal elements [39,40,42,55]. Consid-

ering palmitoylated proteins of viral origin it can be stated that with the exception of two serotypes of vesicular stomatitis virus (VSV) [79] and of Sendai virus [65] (see below) all RNA- and DNA-viruses with a lipid envelope analysed so far contain at least one *structural* protein with covalently bound palmitic acid. These are all located in the viral envelope, and except for p 37 k of vaccinia virus [70], are also glycosylated. It is noteworthy that viral spike proteins with a known low pH-dependent fusogenic activity are palmitoylated (see below).

With viral *non-structural* proteins palmitoylation is not as common as myristoylation (see below). However, cells transformed by the non-enveloped viruses Simian virus 40, adenovirus and with the enveloped Harvey murine sarcoma virus express early proteins which become acylated and are located in the plasma membrane of the infected cells [72-75], where they may play a role in the process of cell transformation (see subsection IV-B).

As is apparent from Table I, palmitoylated acylproteins are found in organisms of different levels of complexity. In their evolutionary range of occurrence they reach from the fungi *Dictyostelium* and yeast, across the angiosperma (*Spirodela olgorrhiza*), across the non-vertebrates (sea urchin) up to the vertebrates, mammals and human. As mentioned above, viruses from a variety of hosts as well as prokaryotes contain acylproteins. The latter group will not be dealt with, since it has been covered in a recent review by Wu and Tokunaga [80]. Although the list of palmitoylated proteins is extensive at this point, more acylated species will probably have to be added once the numerous acylproteins detectable after [³H]palmitoylation in vivo of various vertebrate cells have been identified [13,81-83].

II-B. Acyl linkage sites in palmitoylated proteins

From the early studies of myelin proteolipid [5,6] and of the acylproteins present in the viral envelope [11,12,63,84], it is clear that fatty acids are bound by ester-type linkage, since they can be released by mild alkali and hydroxylamine treatment. Stability studies utilizing these agents have since been applied routinely to identify ester-type acylation of most of the viral or cellular palmitoylated proteins listed in Table I. However, direct structural analysis of the palmitate linkage site proved to be a formidable task. Due to their unusual and unpredictable properties, peptides with covalently bound fatty acid moieties derived from palmitoylated proteins were extremely difficult to purify [12,85]. More indirect studies, as for instance limited proteolysis pointed to a location of the fatty acid linkage site close to the membrane spanning segment of acylated membrane proteins [14,63,85-88]. However, despite com-

parative analysis of the stability of the fatty acid linkage it was initially not even possible, to decide conclusively between an oxygen- and a thioester linkage of the fatty acid to serine or cysteine. For instance, fatty acids linked to Semliki Forest virus (SFV) E1-protein were released with hydroxylamine under the same conditions which cleaved the acetyl-group from *o*-acetyl serine, suggesting an *o*-ester linkage between fatty acid and E1 [89]. However, direct structural analysis has recently revealed a cysteine as the linkage site, and thus a thioester linkage between protein and fatty acid (see below and Ref. 91).

The first direct identification of one of the two palmitoyl linkage sites present in myelin lipoprotein by sequence analysis came from Stoffel and co-workers, who had virtually unlimited supplies of this acylprotein from bovine brain. Their results showed that fatty acid is bound to a threonine residue located in the extracytosolic domain of one of the hydrophilic loops between two of the five membrane segments of lipophilin [90].

A similar protein chemistry approach was also utilized with three other palmitoylated acylproteins, HLA-D associated invariant (ii) chain, VSV G-protein and SFV E1-protein. In all three cases palmitic acid was found to be bound to cysteine in thioester linkage. The fatty acylated cysteine of the two viral acylproteins was located on the 'cytoplasmic' (internal) face of the lipid bilayer with SFV-E1 [91] and at the base of the carboxyterminal cytoplasmic tail with the VSV G-protein [92] (compare schematic diagram in Fig. 1). Prior to the biochemical identification of fatty acylation sites, other authors had turned to recombinant DNA technology to locate fatty acids within the primary structure of acylproteins. Replacement of specific cysteine residues suspected to represent the linkage site with serine after site-directed mutagenesis led to a loss of the covalent attachment of fatty acid during in vivo labeling experiments with the mutated *ras*-protein [93,94], the *ras*-like YPT1 from yeast [51], VSV G-protein [95] and the transferrin receptor [97]. The lack of fatty acylation clearly indicated an involvement of the mutated cysteine residues with fatty acid binding. However, it could not be stated with certainty, that those cysteines were the actual linkage sites. Fortunately, the hypothesized Cys residues in *ras* [98] and the G-protein [92] have since been confirmed as fatty acid linkage sites by direct biochemical methods.

The results of the various analyses of fatty acid attachment sites in different palmitoylated proteins are summarized in Table II. Mainly from comparison of amino acid sequences around suspected acylation sites of the *ras* family proteins, a consensus sequence for palmitoylation has been proposed recently [99]. However, this so called CAAX-box (C for Cys, A for aliphatic and X for any amino acid) at the C-terminus is by

TABLE II

Cysteine residues as palmitoylation sites and their topography^a

Palmitoylation sites are marked by asterisks.

Integral membrane proteins		
VSV-G		
NH ₂	GLFLVL	RVGIHLK*IKLK
SFV-E1		
NH ₂	VVVC*IGL	RR-COOH
Transferrin-receptor		
HOOC	GYC*ISGSC*	KKPKLVNAK
Bovine rhodopsin		
NH ₂	IYIMM	NKQFRNCMVTTLC*C*G
HLA-D associated invariant chain Ii		
HOOC	QYL	YLAG
RSC*K		NH ₂
Peripheral membrane proteins		
ras-protein		
		HOOC-SLVC*KC
YPT1-protein		
		HOOC-C*C*GGGTN
(external)	(membrane segment)	(cytoplasmic)

^a Sequences are from Refs. 92,95,97,101,26,94 and 51 in this order from top to bottom.

definition a rather unprecise recognition sequence. It may be valid for the few palmitoylated ras-type proteins only. When the primary structures of other acylated and non-acylated membrane glycoproteins are compared, due to the wide variability of amino acids around the putative palmitoylation sites, no recognition sequence can be deduced. The only structural theme common to the palmitoylated species is the occurrence of at least one cysteine residue usually within about four residues from the putative border between the inner leaflet of the lipid bilayer and the cytoplasm [65,100]. With the VSV G-protein the palmitoylated cysteine residue was located seven residues into the cytoplasm [92]. In a recent report Ovchinnikov and co-workers identified the acylation sites of bovine rhodopsin. The two fatty acylated cysteine residues were located 13 and 14 amino acid residues away from the membrane border into the cytoplasm. These authors suggest that using its fatty acids as anchors, this region of the protein loops back into the lipid bilayer of the photoreceptor membrane [101].

II-C. Biochemistry of palmitoylation

II-C1. Location of palmitoylating activity

From early studies of palmitoylation of viral acylproteins in vivo, it has been apparent that this modification is an event which in the presence of ongoing protein synthesis can be detected by metabolic short pulse labeling [102]. In order to define the intracellular location of palmitoylation, fatty acylation was related to the various stages of oligosaccharide processing, and to the timing of proteolytic processing and intracellular

transport. The results of such experiments revealed that palmitoylation of VSV G-protein and Sindbis E1 and E2 occurs shortly after translation and just prior to the acquisition of Endo H resistance [102]. While various trimming inhibitors (swainsonin, deoxynojirimycin, castanospermin) either had no effect on palmitoylation (McDowell, W. and Schmidt, M.F.G., unpublished results) or led to a stimulation [103], the effect of tunicamycin depended on the glycoprotein under study. While tunicamycin had no effect on the acylation of the HLA invariant-chain [26], coronavirus E2 [104,105] and mannosidase II [30], it completely abolished palmitoylation of VSV G-protein [12] and of the sodium channel [22]. Thus, it seems that the influence of tunicamycin on acylation is an indirect one, perhaps by preventing transport of the glycoprotein to the acylation site [106]. This indicates, that glycosylation per se is not required for fatty acylation. Also other experiments pointed to a crucial intracellular location for palmitoylation to operate. Ts-mutants of the G-protein defective in transport between the ER and Golgi could not be acylated at nonpermissive temperatures [12], whereas blocking of transport between the Golgi and the plasma membrane with monensin had no influence on fatty acylation [107]. From pulse-chase experiments with [³H]palmitic acid Dunphy et al. [108] and Quinn et al. [109] reported the *cis*-Golgi to be the intracellular location at which palmitoylation occurs. Subsequently, short pulse labeling in vivo of acylproteins with [³H]palmitic acid was utilized frequently as a marker for the *cis*-Golgi compartment (e.g., Ref. 110). However, more recent data proved that palmitic acid binding begins at an even earlier stage. By cell fractionation after extremely short

pulses (20 s) with [³H]palmitic acid in SFV infected baby hamster kidney cells (BHK), most of the [³H]palmitoylated F-protein was found in the rough ER fraction [111]. After establishing an in vitro system of acylation, which utilizes deacylated exogenous SFV E1-acceptor protein [54], particular membrane fractions could be tested for protein fatty acyltransferase (PAT). Studies with such a cell-free system using viral acceptors revealed the highest specific activity of PAT in the rough ER fraction [111]. The use of transport mutants revealed that early acylation occurs also with yeast cell glycoproteins [112] and different Ts-mutants of the influenza hemagglutinin (Veit, M., Schmidt, M.F.G. and Klenk, H.D., unpublished results). Recently a chimeric construct between a fragment of the influenza hemagglutinin joined to the C-terminus of nearly complete rat growth hormone was shown to be palmitoylated. Since this hybrid protein failed to be transported to the Golgi apparatus, the authors concluded that fatty acid addition occurs in a fraction of the ER which they termed smooth ER cisternae [113].

Although the evidence for early acylation of the above proteins seems quite solid, contrasting results were obtained with other cellular acylproteins, as for instance mucus glycoproteins, myelin apolipoproteins and the SV40 large T-antigen. Utilizing an in vitro assay for palmitoylating mucus glycoproteins from gastric mucosa, Slomiany and co-workers reported the Golgi-membrane to be the location of palmitoylating activity [114-116]. However, the membrane fractions in that study were rather crude and had not been tested for marker enzymes. More recent reports on the detection of nascent peptidyl-tRNA with bound fatty acids by the same group, in contrast to their earlier reports, favor a cotranslational acylation of O-glycosylated mucus glycoproteins [117].

Palmitoylation in the vicinity of the late Golgi or the plasma membrane was reported for the large T-antigen [118], the transferrin receptor [119,120,121], myelin lipophilin [122,123] and bovine rhodopsin [20]. However, with the two latter acylproteins autoacylation was observed [124,125,125a], so that in these cases a different mechanism of fatty acid attachment may operate. Some non-enzymatic or autocatalytic acylation has also been observed with E2-protein in SFV-infected cells [112]. Such a process may not depend on cellular enzymes, but rather on the availability of acyl-CoA and a receptive conformation of the protein acceptor. The plasma membrane as a possible location for palmitoylation of cytoskeletal proteins resulted from studies with ankyrin in rabbit erythrocytes, which are devoid of nuclei and internal membranes [40,126]. With plant cells, another compartment, the chloroplast, must have palmitoylating activity, since a number of thylakoidal proteins are acylated in that location both in vivo and in vitro (Ref. 58 and Mattoo, personal communication). Only a slight

degree of palmitoylation was detected in the fungus *Physarium polycephalum*, but the location of the event was not identified. Interestingly, in this fungus most of the acylated proteins turned out to be myristoylated in amide linkages [127].

Although a definitive general statement on the intracellular location of protein acyltransferase is not yet possible, most data available indicate that enzymatic palmitoylation occurs soon after translation. Most likely this modification of proteins operates in an area which could be designated 'late ER', whatever such a location's cell biological features may be. One could speculate that fatty acylation may occur in the first set of vesicles leaving the ER with a *cis*-Golgi destination. This would fit with the hypothesis that fatty acylation may enhance vesicular transport which depends on fusion and fission of lipid membranes (see subsection IV-A1).

II-C2. Dynamic nature of palmitoylation

Fatty acids bound to acylproteins by ester-type linkage are metabolically dynamic. Omary and Trowbridge [14,119] observed that the transferrin receptor could be labeled with ³H-fatty acid 48 h after its translation, and that the protein bound fatty acids 'turned over' more rapidly than the protein portion of the receptor. Similar observations were also reported for bovine rhodopsin [20,126], mannosidase II [30], ankyrin [128], ras-protein [129] and a number of cellular acylproteins with unknown identity [81]. Recently, different half lives were reported for the acyl groups of different proteins of red blood cells [129a]. In accordance with those observations, Berger and Schmidt [130] detected an enzymatic activity in microsomal membranes of diverse origin, which specifically released fatty acids from exogenously added viral acylproteins. This protein fatty acylesterase requires mild detergents, is completely inhibited at 4° or 60°C and by sodium dodecylsulfate, and is clearly distinct from the acylating enzyme protein fatty acyltransferase [130].

The fact that acyl chains can be metabolically released from palmitoylated proteins may be of some importance, because it allows the regulation of this hydrophobic modification. Therefore, whatever biological function a given palmitoylated protein may have, it can be switched on and off by fatty acylation or fatty acid release (deacylation by esterase), respectively. In addition to directly modulating biological functions of an acylprotein, specific intracellular locations of an acylprotein could potentially be controlled by acylation and deacylation. Thus, fatty acylation of proteins could have the same regulatory potential as the well known phosphorylation of polypeptides.

II-C3. Towards the purification of protein fatty acyltransferase (PAT)

Palmitoylating activity has been measured in various

cell-free systems utilizing exogenous or endogenous acceptor proteins [111,112,115,116,123-125,126,128,131,132]. However, purification of a palmitoylating protein fatty acyltransferase (PAT) has not yet been achieved. Published data on the enrichment of this enzyme are scarce. Partial purification of PAT by cell fractionation and chromatography on hydroxylapatite has been reported, but no information with regard to structure of the PAT proteins was given [111,130]. In the same reports a PAT assay was used, which required exposure times of several weeks for the detection of [³H]palmitoylated E1 during SDS-PAGE analysis and fluorography [111,130].

By improving the cell-free assay for PAT in our laboratory to 500-fold increased sensitivity, fractionation of this activity on various chromatographic columns is now possible [132a]. The key features of this new assay are threefold. (1) High specific radioactivity [³H]palmitoyl-CoA prepared by enzymatic synthesis with microbial acyl-CoA synthetase was utilized as lipid substrate. (2) Purified deacylated and hydroxylamine free E1-protein from Senziki Forest virus was used as exogenous acceptor for fatty acid. (3) Microsomal membranes from human placental tissue were identified as a rich source of protein fatty acyltransferase (PAT) (132b). Utilizing this powerful assay, the enzymological characterization of PAT as well as its protein chemical characterization are presently in progress. Initial results show that PAT is an enzyme of complex structure, which is bound to membranes of the rough ER (Schmidt, M.F.G. and Burns, G.R., unpublished data).

II-C4. Specificity of lipid substrates for ester-type fatty acylation of proteins

Despite this early state of analysis of ester type acylation of proteins, some information is available on its substrate requirements. It has been established that the activated form of fatty acid, acyl-coenzyme A, needs to be available to allow the acyltransfer onto protein. Alternatively, free fatty acid can also be transferred in the presence of ATP, Mg²⁺ and coenzyme A, provided the source of PAT has intrinsic acyl-CoA synthetase activity [115,116,123,133,134]. Since a variety of acyl species have been identified in different 'palmitoylated' acylproteins [65,83,135], fatty acylation into ester-type linkage was not expected to be very specific with regard to the fatty acyl species. Nevertheless, comparison of acyl-CoA substrates with different chain lengths in cell-free acylation revealed a preference for palmitoyl-CoA [135]. Studies in our laboratory with enriched preparations of PAT revealed that palmitic acid (16:0) is preferred over shorter as well as longer acyl chains. Likewise, unsaturated acyl species are less well incorporated into E1 acceptor protein when compared to the saturated species of the same chain length (Schmidt, M.F.G., Qanbar, R. and Burns, G.R., unpublished data).

Similar results were recently reported for the acylation of the transferrin receptor. In a study of acylation of reticulocyte proteins *in vivo* and *in vitro*, Adam et al. [121] demonstrated a clear preference for palmitic acid over myristic acid in the transfer onto the receptor protein. Another recent study indicates that acylation may not be completely restricted to physiological acyl-CoA substrates. Hertz and Bar-Tana [136] were able to incorporate xenobiotic amphipathic carboxylic acids (e.g., nafenopin, bezafibrate) into hepatocyte proteins. However, full linkage analyses have not been provided for these acylproteins.

During several studies of acyl specificity of ester-type acylation of proteins by metabolic labeling, it has become apparent that the fatty acids utilized are subject to interconversion into other fatty acid species or to amino acids [65,83,135,137]. Thus, if radioactivity from [³H]palmitic acid used for labeling is eventually detected in a given protein, this does not necessarily imply that *palmitic acid* is actually bound. A warning example for this feature is provided by the first report on the acylation of the RSV transforming protein pp60^{src} with 'palmitic acid' [138]. This acylation was later found to represent the highly specific myristoylation. Thus, the tiny fraction of myristic acid arising from interconversion [135] must have been selectively utilized for incorporation into pp60^{src} [139,140]. Likewise, various viral or cellular acylproteins were labeled when normal and virus-infected cells were grown in the presence of [³H]myristic acid. Yet, when the purified proteins were analysed for fatty acid [65,135], they contained mainly [³H]palmitic and [³H]stearic acid. The extent of elongation and/or desaturation of radioactive fatty acids during the labeling period was observed to vary considerably when different cell types were compared [83]. Likewise, the spike glycoproteins of influenza C virus, after labeling with [³H]palmitic acid, were found to contain predominantly stearic acid (Veit, M., Herrler, G. and Schmidt, M.F.G., unpublished data. It will be of interest to determine whether the structure of these proteins specifies selection for one acyl species (18:0) or whether these particular proteins follow a different route of maturation. On such a new pathway they might encounter a new type of protein fatty acyltransferase. Alternatively, only restricted pools of acyl-CoA substrate may be available. Further development of cell free assay systems for palmitoylation and their application with different protein acceptors will help clarify such issues.

III. N-Terminal myristoylation

III-A. Identification of myristoylated proteins

The covalent binding of myristic acid to the N-terminus of a protein was first detected with cAMP-de-

pendent protein kinase. When Carr et al. [141] sequenced the catalytic subunit of this kinase they found a blocking group at the N-terminus. Gas chromatographic and fast atom bombardment (FAB)-mass spectrometric analysis of peptides derived from the N-terminus revealed the presence of myristic acid in amide linkage to N-terminal glycine [141]. Shortly thereafter, and employing similar analytical procedures, two further proteins were found to contain N-terminal myristic acid, calcineurin (a Ca^{2+} - and calmodulin-dependent protein phosphatase [142]) and the rough ER-located NADH-cytochrome- β_5 reductase [143]. With the above-mentioned cellular acyl proteins and with the retroviral coded gag-protein p15 [144,145], an amide linkage between myristic acid and N-terminal glycine was established by analyzing non-labeled peptide material with a combination of biochemical [146] and the above-mentioned physical techniques. In all other and subsequent reports on myristoylated proteins (see below) a less sophisticated method was employed for identification. This involved metabolic labeling with [^3H]myristic acid, PAGE analysis of cell lysates after immunoprecipitation, and (unfortunately not in all cases) fatty acid analysis after HCl-hydrolysis of the gel purified acylprotein.

Table III lists all myristoylated proteins reported to date. It reveals that many of these polypeptides are

functionally related to the regulation of cell growth and/or signal transduction. Particular examples for this are the cellular and viral oncogene products, pp60^{c-src} [82,147], p56 [148], p36 [149], pp60^{v-src} [138,140] and p28 coded by 3'orf of the AIDS virus [150,151], as well as particular α -subunits of GTP-binding proteins [152,153].

III-B. Biochemistry of myristoylation

The biochemistry of myristoylation has been studied in great detail by Gordon, Glaser, Towler and co-workers. These authors have recently achieved the purification of an enzyme from yeast cells, which transfers myristic acid onto synthetic octapeptide acceptors [163-165]. They designated this enzyme *N*-myristoyl transferase (NMT). It has been known for some time, that non-acylated proteins can be myristoylated *in vivo* once they contain the N-terminal region of myristoylated proteins fused onto their N-terminus [166-169]. By systematically altering the sequence of synthetic peptides homologous to the N-terminus of myristoylated cAMP-dependent protein kinase, it was possible to get some information on the substrate specificity of NMT *in vitro*. From their own results [170], and from those reported by others [171-174], Towler and colleagues defined the structural requirements for *N*-myristoylation in a recent review article. According to these data, a glycine residue at position 1 is an absolute requirement for the peptide to function as lipid acceptor. Proline or amino acids with bulky or charged side chains are not tolerated at positions 2 and 5, whereas a broad spectrum of amino acids is permitted at positions 3 and 4. Position 5 is occupied by serine in almost all myristoylated proteins identified so far. While Buss et al. [175] recently reported evidence that the six amino-terminal amino acids of p60^{src} are sufficient for myristoylation, Kaplan et al. [176] find the requirement for a seventh amino acid with the same protein. They report that the seventh position should preferably be a lysine and must not be asparagine [176]. Both these reports utilize fusion proteins in which a short amino-terminal peptide from p60^{src} extends the N-terminus of p21^{ras} [175] or pyruvate kinase [176]. These data indicate that not all proteins with an N-terminal glycine will be subject to myristoylation. In addition, there is evidence that even proteins with a 'good' recognition region for myristoylation may not be acylated. A prominent example for this is the GTP-binding protein transducin. Its N-terminus fulfills all the structural requirements described above and yet it fails to be myristoylated *in vivo* [153]. Surprisingly, synthetic peptides of this region of transducin become myristoylated *in vitro* [177]. It should be noted that prior to the attachment of myristate to the N-terminal glycine of a protein, the initiating methionine has to be cleaved. This cleavage

TABLE III

Myristoylated proteins of eukaryotic and viral origin

Protein	Origin	Refs.
Cellular acylproteins		
cAMP-dependent protein kinase	bovine heart	141
Calcineurin	bovine brain	142
Cytochrome- β_5 reductase	bovine liver	143
pp56	LSTRA-cells	61
pp60 ^{c-src}	chicken cells	82,147
G-protein α_0, α_1	bovine brain	152
G-protein $\alpha_{39,40,41}$	astrocytoma cells	153
p36 ^a	chicken cells	149
IgM ^a	B-lymphocyte cell line	154
Various undefined	<i>Physarium polycephalum</i>	127
GAP-43	neuronal growth cones (rat)	154a
Viral acylproteins		
VP 2	SV40 and polyoma virus	155
VP 2	BFDV virus	156
VP 4	picorna virus	151,158
p15 ^{src} , Pr65 ^{src}	mammalian retroviruses	144,145
pre S1 antigen	hepatitis B virus	159
pp60 ^{v-src}	Rous-Sarcoma virus	138,140
orf-protein (p28)	HIV I	150
different gag-onc fusion proteins	retroviruses	139,160,161
p15 ^{src}	HTLV I	162
P23 ^{src}	HTLV II	162

^a The precise location of the acylated amino acid is not known.

occurs while the growing chain is still attached to the ribosome. It has been shown that NMT reveals no such proteolytic activity [165], thus two enzymes must operate in concert to yield the myristoylated protein.

III-B1. Substrate specificity and intracellular location

A number of recent studies have addressed the question of specificity for the lipid substrate of myristoylation. Utilizing the cell free system described above, Towler and Glaser revealed that the coenzyme A thioester of myristate (14:0), but not of palmitate (16:0) operates as a lipid donor [163]. This observation is in agreement with former studies on the chemical analysis of amide-linked fatty acids [133,141-143,147] and reveals a remarkable specificity of NMT for this rare fatty acid. In a recent comprehensive study Glover et al. [178] confirmed this exclusive specificity for tetradecanoic acid during transfer *in vitro*. However, in the same report palmitoyl-CoA, was found to bind to NMT from various rat tissues [178]. This competition in binding to NMT may have regulatory implications.

Similar enzymes with only slightly different substrate specificities have been partially purified from rat liver and plant cells [151,177]. Thus, it seems that this enzyme is highly conserved throughout evolution, which may indicate a crucial biological function for myristoylation. It should be noted that a myristoylating activity is also present in wheat germ and reticulocyte lysates, which are used for *in vitro* translation experiments [151].

Immunocytochemical studies regarding the intracellular location of NMT are not available at present. However, after cell fractionation the enzyme is distributed between the soluble and crude membrane fraction [163]. Thus, NMT may be only loosely attached to cellular membranes or, more likely, is associated with ribosomes. The later assumption is supported by the observation that nascent polypeptide chains isolated from ribosomes already contain myristic acid [179]. This latter finding is compatible with the cotranslational attachment of myristic acid after cleavage of the initiator methionine [180]. Myristoylation during translation had originally been shown by experiments in which myristoylation stops rapidly after addition of inhibitors of protein synthesis [10,82,83,181]. From all these data it appears that myristoylation is dependent on ongoing protein translation. Consequently, the 14:0 fatty acid at the N-terminus cannot be expected to 'turn-over', but instead remains stably attached to the polypeptide.

Recently, it has been suggested that in macrophages a major substrate of protein kinase C becomes myristoylated after external stimulus with bacterial lipopolysaccharide (LPS) [182,183]. This implies that transfer of myristic acid to the 68 kDa target of protein kinase C occurs post-translationally, which is in clear contrast to the above-mentioned results. On the other hand, one of

the reports by Aderem et al. [183] contains data indicating that treatment with inhibitors of protein synthesis prevents myristoylation of this protein. It seems, therefore, that LPS induces *de novo* synthesis of a myristoylated protein rather than the transfer of myristic acid onto a polypeptide, which has been translated before the stimulus. Alternatively, myristoylation of this particular target protein may be different from the well established N-terminal myristoylation. Future analysis of this substrate of protein kinase C will have to clarify whether myristic acid is really linked to the N-terminus or to some internal lysine or arginine.

It should be mentioned at this point that some proteins do contain amide-bound fatty acids, which are not linked to the N-terminus of the polypeptide chain. This was first shown for the acetylcholine receptor [15]. More recently, the same was found to be true for the insulin receptor [16], the heavy and light chain of immunoglobulin M [154], and other cellular proteins [48,149,184]. The fatty acids bound to these proteins have been analysed and revealed myristate [154], palmitate [15,184] or both acyl species [16]. An ϵ -amino group of a lysine residue located near the membrane has been suspected to be the fatty acid binding site in the acetylcholine receptor [15]. N-Amidical acylation of IgM occurs post-translationally during transit of this protein to the plasma membrane [154], which is in contrast to the co-translational myristoylation of N-terminal glycine. More information on the biochemistry of this third probable type of acylation will be necessary before its biological significance can be assessed.

IV. The function of protein bound fatty acids

Although many acylated polypeptides have been identified, experimental studies concerning the function of protein bound fatty acids are still rare. The functional heterogeneity of the acylproteins occurring in phylogenetically distant organisms makes it near to impossible to deduce one or few biological functions of protein bound fatty acids, particularly for the palmitoylated species. Nevertheless, in almost each report on the acylproteins listed in Tables I and III, potential functions are offered and discussed. However, at this early stage of research on hydrophobic modifications of proteins, most of the biological functions of acylation suggested, are still hypothetical, since they are often not sufficiently backed by experimental data. The most frequently recurring hypotheses for the biological function of fatty acylation of proteins will be discussed separately for palmitoylation and myristoylation.

IV-A. Palmitoylation

IV-A1. Involvement in membrane fusion

Addressing the function of palmitoylated spike glycoproteins of envelope viruses, it has been suggested

that protein bound fatty acid may be required for the fusion process between viral and cellular membranes [185] and for the release of newly synthesized virus particles from the infected cell [186]. The first experimental data in support of the former hypothesis comes from studies by Lambrecht and Schmidt, who compared the biological activities of acylated and deacylated influenza hemagglutinin [89,187]. Fusion activity of the glycoprotein was drastically impaired after release of protein bound fatty acids with hydroxylamine, whereas hemagglutinating activity and other properties of the viral surface glycoproteins were not affected. Although control experiments gave no indication of other effects of hydroxylamine than fatty acid release [89,187], it should be borne in mind that this agent has been used to cleave peptide bonds [188]. Involvement of protein bound fatty acid in the induction of membrane fusion is also supported by the fact that most acylated protein species found in enveloped RNA or DNA viruses are well known for their fusion-inducing property [189]. On the other hand, there are a few enveloped viruses which lack fatty acylated surface proteins and which are still able to enter their host cells by membrane fusion. Therefore acylproteins cannot be the only possible mediators of fusion [65,79,100]. To test the above hypothesis further, we are presently following an approach that utilizes the expression of mutated clones of the influenza hemagglutinin and of a paramyxovirus fusion protein both of which lack their fatty acid linkage sites (Veit, M., Klenk, H.-D. and Schmidt, M.F.G., unpublished data).

Following our idea about the participation of protein bound fatty acids in triggering membrane fusion [185], Glick and Rothman suggested an involvement of fatty acylation in the fusion-driven transport of glycoproteins along the secretory pathway [190]. These authors investigated the transfer of VSV G-protein between Golgi vesicles *in vitro*. They observed that addition of palmitoyl-CoA to their cell-free transport assay had a highly stimulatory effect on the rate of glycoprotein transport from *cis*- to medial Golgi-vesicles. Glick and Rothman, therefore, hypothesize that cycles of acylation and deacylation of an as yet unknown protein may facilitate protein transport by enhancing membrane fusion between the different populations of transport vesicles [190].

IV-A2. Morphogenesis and protein interaction

A potential contribution of acylation to the morphogenesis of virus particles has been drawn from experiments by Schlesinger and Malfer [186] in which vesicular stomatitis virus (VSV) infected cells were treated with cerulenin [191], a putative inhibitor of protein fatty acyltransferase (PAT). The release of progeny virus particles was prevented by this antibiotic, although fatty acid-free glycoprotein was still transported to the cell

surface, the location where new VSV particles assemble [87,189].

It is interesting to note that the maturation of viral spike glycoproteins includes an oligomerization step which yields, for instance, trimers in the case of the influenza hemagglutinin. It has recently been shown, by site-directed mutagenesis experiments, that this process requires the full membrane spanning segments of the respective glycoprotein (VSV G-protein, influenza HA) down to the carboxyterminus [192,193]. A contribution, by hydrophobic interaction of fatty acids present in that area, to this process of trimerization is a likelihood. Experiments are under way to test this hypothesis (Veit, M., Schmidt, M.F.G. and Klenk, H.-D., unpublished; J. Kruppa, personal communication). Results which point to fatty acid involvement in protein-protein interactions have also been obtained with cellular acylproteins. When nicotinic acetylcholine receptor was synthesized without its fatty acids (i.e., in the presence of cerulenin) its five subunits failed to assemble to the heterooligomeric functional receptor [15].

While these above cases concern non-covalent interactions between polypeptides, Koch and Haemmerling [26] found that fatty acylation of the invariant chain (I_I) prevents it from forming S-S linked dimers. This may be important for the essential non-covalent association of the I_I-polypeptide with MHC class II antigens (I_a in the mouse and HLA-D in human).

Although potential links between acylation and morphogenetic events based on protein-protein interactions are now well documented, some reservation with studies involving Cerulenin as an inhibitor of acylation are due. Since this drug has an inhibitory influence not only on *de novo* synthesis of fatty acids, but also on the synthesis of lipids, RNA and proteins (Refs. 186 and 194 and Schmidt, M.F.G., unpublished observation), its effects on fatty acylation are difficult to interpret. Because of this lack of specificity of cerulenin, alternative ways of generating fatty acid free acylproteins for functional studies *in vivo* will have to be developed.

IV-A3. Involvement in proteolysis protection, transport, cell transformation and signal transduction

As stated above, some palmitoylated proteins are not membrane bound, but occur in the cytoplasm or in the extracellular space. As an example for secretory palmitoylated proteins Slomiany and co-workers reported that covalently linked fatty acids protect human gastric mucus glycoprotein from proteolytic digestion [33,195]. Interestingly, the stoichiometric amount of covalently bound fatty acids in mucus glycoproteins is 2- or 3-fold higher in patients with cystic fibrosis compared to healthy individuals [196]. The authors conclude that the increased fatty acid content may prevent normal turnover of these glycoproteins. Accumulation of such viscous and poorly soluble proteins may therefore lead

to an obstruction of the various secretory glands, which is a characteristic clinical feature of cystic fibrosis [196]. Recently, the presence of fatty acids in mucus glycoproteins reported by Slomiany and co-workers [114–117] has been challenged. Hansson et al. were not able to detect any stoichiometric fatty acid in highly purified preparations of porcine and human mucus glycoproteins [196a]. It will be important to clarify whether the latter group might have cleaved the fatty acids during their elaborate purification procedure or whether Slomiany's group was operating with preparations of mucus glycoproteins which still contained other fatty acylated proteins.

Recently there is debate about the participation of GTP-binding proteins in the transport of glycoproteins along the secretory pathway [197]. For instance, the yeast ras-like YPT 1 protein, which plays a role in transport phenomena, is palmitoylated [51]. Furthermore, acylation and deacylation of a retroviral ras-protein has been shown [129]. However, the question of whether or not this modification of ras (and/or the myristoylated GTP-binding proteins, see below) is actually involved in the transport process, has not yet been addressed. Since quite a number of cell surface receptors and the Na⁺ channel [198] are palmitoylated, a regulatory involvement of protein-bound acyl chains in signal transduction can be regarded as a serious possibility. Impairment of coupling to the adenylate cyclase system has recently been reported for non-palmitoylated Cys⁻-mutants of the human β_2 -adrenergic receptor [219].

For the ras-oncogene product it has been proposed that thioester-linked palmitate may serve the function of binding this peripheral membrane protein to the inner surface of the plasma membrane, where it may initiate the process of cell transformation. After changing the fatty acid binding cysteine residue (which is located near the terminal end of the polypeptide and conserved in all viral, mammalian and yeast ras proteins, see Table II) to serine by site-directed mutagenesis, Willumsen and co-workers observed that the mutated protein is neither palmitoylated nor associated with membranes. Furthermore, the mutated ras-protein had lost its cell-transforming abilities [92,94].

In contrast to the hydrophilic ras-protein, palmitoylated integral membrane glycoproteins do not require covalently bound fatty acid for membrane attachment. Further, the transferrin receptor and VSV G-protein, do not require fatty acylation for intracellular transport. After replacing the acylation site by site-directed mutagenesis, these fatty acid-free glycoproteins are still transported to and stably attached to the plasma membrane [95,97,199]. The only report with a positive correlation between fatty acylation and transport of a protein from the Golgi apparatus to the cell surface was by Zilberstein et al. [200], who utilized mutant strains of

VSV in VERO-cells. In this study the possibility has not been fully excluded, that lack of transport was due to other defects of the mutant protein than deficient acylation.

IV-A4. Comparison of acylated and fatty acid-free peptides

The above ideas on functions of protein-linked fatty acids can only be valid if the acyl chains have the physical potential to interact with components in their environment rather than being packed inside the protein. A number of physical experiments reported in the literature indicate that this is indeed the case. In an early biophysical study, Petri et al. [201] labeled VSV G-protein with 16-(9-anthroxylxy)palmitate and reconstituted it into phospholipid vesicles. Fluorescence anisotropy measurements revealed that the labeled fatty acid is not buried inside the molecule, but is located in a phospholipid domain which does not undergo temperature-dependent phase transition. Thus, with this viral acylprotein, covalently bound fatty acids can influence their micro environment. In such an exposed position, acyl chains will also have the potential to interact with heterologous membranes in close proximity, or with any other hydrophobic surface.

Recently, Joseph and Nagaraj [202] compared a 13 amino acid synthetic peptide which constitutes part of the membrane spanning segment of VSV G-protein before and after chemical palmitoylation. They find no direct modulation of peptide conformation with circular dichroism (CD) spectroscopy, but fatty acylation apparently promotes self-association and association of the acylpeptide with micelles [202]. Unfortunately, palmitic acid was bound amidically to the ϵ -amino group of a lysine located towards the N-terminus of the membrane segment, and not to the physiological cysteine linkage site just outside the lipid bilayer towards the C-terminus (Ref. 92; compare also Table II). Thus, the peptide studied [202] may not allow any valid conclusion for the segment of G which is naturally fatty acylated.

A different approach was followed by Bizzozero and Lees [203] who deacylated myelin proteolipid proteins by treatment with hydroxylamine for spectroscopic comparison with the fatty acylated form. While the conformation of the acylated and deacylated form of lipoprotein in organic solution was about the same, clear differences in folding emerged during spectroscopic analysis in an aqueous milieu. Although no definitive anchor function could be proven, the authors hypothesize from experiments with liposomes, that fatty acids attached to one of the external loops of this protein [90] could be important for maintenance of the lamellar structure of the normal myelin sheath [203].

If acylation affects the conformation of a protein, it should also affect its biological activity. This was the case with fusogenic spike proteins of viruses. These

proteins lost their fusion activity upon deacylation by hydroxylamine treatment of complete virus and of solubilized spikes although the overall conformation of the proteins was not affected to any detectable extent [187,204]. Likewise, with deacylated mucus glycoproteins, sensitivity towards proteolytic digestion was much increased [33,115,195]. This latter phenomenon has also been observed with bee venom phospholipase A₂. Utilizing an approach the reverse of that with virus spikes and mucus glycoproteins, McLeod and colleagues [205] chemically acylated phospholipase A₂. By this well controlled modification, they rendered this enzyme much more stable against treatment with thiol and proteinases when compared to the fatty acid-free counterpart. Along with this protective effect resulting from covalent acylation, the authors found an activation of enzymatic activity [205,206].

It seems reasonable that a potentially exposed fatty acid in covalent linkage would promote interaction of an acylprotein with a hydrophobic environment in its vicinity. Experimental data emphasize this view. By chemically acylating IgG, Huang et al. [207] were able to stably anchor this usually water-soluble protein into liposomes, a technique of potential use in drug targeting. Likewise, the hydrophobic protein α -bungarotoxin was partitioned into a lipid bilayer by chemical addition of a single palmitoyl residue [208]. Not only that, 'artificial' acylation of this toxin reduced its receptor affinity to one twentieth of that of the non-acylated (natural) counterpart [208]. This implicated the potential of acylation and deacylation to modulate the biological activity of any given acylprotein (see subsection II-C2).

Water-soluble enzymes may acquire the ability not only for binding to vesicles, but also for complete translocation across lipid membranes. This has recently been shown elegantly by Levashov et al. [209] for stearoylated proteinases. The authors found that only after chemical stearoylation could trypsin or chymotrypsin reach a substrate enclosed in multilamellar liposomes [210]. This finding indicates the potential of protein-bound fatty acids, to aid in the translocation of proteins across lipid bilayers. Although all these latter data may point to an anchor function of protein bound acyl chains (see next subsection) or to similar protein-lipid interactions, one should bear in mind that many palmitoylated proteins (unlike the glypiated polypeptides [1-4]) already have a membrane spanning segment to keep them inserted in the ER membrane during synthesis. Nevertheless, additional fatty acids inside or close to this hydrophobic segment may still have an influence on the structure and biological activity of these limited regions. Furthermore, any structural changes in these areas may well be relevant to the overall three-dimensional structure of the respective acylprotein.

IV-B. Functions of myristoylation

IV-B1. Anchor function of myristoylation

From the previous subsection it has become apparent that fatty acids do modify the physical properties of a protein. Therefore it seems reasonable to expect that an otherwise quite hydrophilic protein develops a general affinity for membranes once it is myristoylated. However, the surprise comes with the fact, that a number of myristoylated proteins are associated specifically with the cytoplasmic face of the plasma membrane only, and not with any of the other intracellular membranes. The crucial involvement of N-terminal myristic acid in this specific interaction comes from a series of studies mainly with the transforming retroviral src-kinase (p60^{src}). By replacing or deleting the amino-terminal glycine residue of this protein Kamps, Buss, Sefton and others were able to show that the mutated proteins are neither myristoylated nor membrane-bound. Furthermore, they show that the mutated molecules have lost their cell-transforming abilities, although the protein's kinase activity is not affected [171,172,166-168].

The ability of N-terminal myristic acid to bind otherwise soluble proteins to the inner surface of the plasma membranes has also been confirmed for the gag-protein precursor (Pr65^{gag}) of mammalian retroviruses. In this case the recombinant mutant proteins failed to be transported to the membrane, and as a result the release of progeny virus particles was prevented [176,177]. In related experiments, Pellman and co-workers showed that a 14 amino acid peptide derived from the N-terminus of the src-kinase can direct myristoylation and membrane binding of the non-myristoylated and soluble protein product of the fps-gene [169]. Analogous results have also been reported for hybrids between the myristoylated N-terminus of p60^{src} and both p21^{ras} [175] and pyruvate kinase [176]. On the basis of such data, it has been speculated that amide-bound myristic acid is a transport signal, which can target polypeptides to the inner surface of the plasma membrane [173,211]. At that location the myristoylated protein (e.g., p60^{src}) is believed to interact with putative receptors for N-terminal myristic acid. Due to the specific topological positioning of this protein, its intrinsic kinase activity will ensure phosphorylation of the 'right' target protein [140,147]. Although this attractive hypothesis may be valid for myristoylated retroviral src-kinases, it is difficult to reconcile with the fact that some myristoylated proteins are either soluble (e.g., cAMP-dependent protein kinase, [141]), bound to the ER (e.g., cytochrome reductase, [147]) or accumulate in the cell's nucleus (e.g., VP2 of polyoma viruses, [155]). Two more exceptions need to be mentioned. While the myristoylated src-gene products of the transformation-defective mutants ts NY 68 [147] and NY 18-3 occur in the cytoplasm [212], the avian src-gene product is not

myristoylated at all, but is nevertheless located on the cytoplasmic side of the plasma membrane [213].

IV-B2. Myristoylation in morphogenesis and virus entry

The use of site-directed mutagenesis allowed assay for the involvement of myristoylation in processes other than membrane anchorage. On the basis of such comparative studies, myristoylation of structural proteins of tumor and other non-enveloped viruses was suggested to be important for intracellular transport of retroviral gag-proteins [174] and for the assembly process of different viruses [166,119,120,170,214]. In this respect, the report by Streuli and Griffin [155] is noteworthy. These authors found myristoylated VP2 protein in the nucleus of cells infected with polio and SV40 virus. They suggest that myristoyl-VP2, while anchored in the nuclear membrane, could position the newly made viral DNA for nucleation of capsid assembly [155]. Involvement in capsid packaging was also proposed for myristoyl chains bound to VP4 protein of the RNA containing polio and foot and mouth disease virus [157,158]. Although the myristoylated polio virus VP4 is packaged inside the capsid, it possibly participates in the entry of virus particles into the host cell. Since a cellular membrane has to be passed during this process, myristic acid linked to the amino terminus of VP4 could well serve a function similar to the one suggested for palmitoylated fusogenic proteins of enveloped viruses [215]. VP4 is the first protein to vanish during entry of this virus into the cell [216-218], which may be indicative of its prime involvement in this crucial process. However, only future research will clarify whether a general mechanism for fusion-mediated entry of viruses into host cells operates in nature, which is enhanced by protein-bound fatty acids.

V. Concluding remarks

Fatty acylation of proteins is a modification as diverse and complicated in many respects as glycosylation or phosphorylation. Owing to a number of distinct biochemical features, two major types of this hydrophobic modification can be distinguished, palmitoylation and myristoylation. Both are enzymatic processes which covalently modify proteins mainly on internal cysteine and N-terminal glycine residues. Both types of acylprotein occur in many different organisms as well as in many different intracellular locations. This fact and the scarcity of experiments which assess fatty acid function make it difficult at present to offer any unified theories on the biological function of palmitoylation or myristoylation. What emerges from the literature in this respect is perhaps that protein bound acyl chains contribute to a variety of different biological functions rather than to just one. The unifying concept in all of these are the interaction of the covalently linked fatty

acids with hydrophobic components present in their immediate environment. The dynamic nature of palmitoylation, which is mainly found in membrane glycoproteins, may mean that this modification plays a role in modulating the structure and function of the membrane spanning segments of such proteins, as for instance in signal transduction and membrane fusion. The 'once and for ever' myristoylation of a protein seems to be mainly responsible for anchoring this acylprotein to specific membrane locations, often to the cytoplasmic face of the plasma membrane. Only in such a topological orientation can the myristoylated protein exert its function, which may be, for instance, to phosphorylate particular target proteins to initiate a cascade leading to various biological consequences. This is not the place to discuss the many exceptions from these two 'major' hypothetical functions of palmitoylation and myristoylation. It seems, once again nature does not follow our simplifying ideas. However, with the recent purification of *N*-myristoyl transferase (NMT) and progress in the characterization of the palmitoylating enzyme, protein fatty acyltransferase (PAT), we may soon be able to gain a deeper insight into this hydrophobic modification of proteins.

Acknowledgement

Work by the author cited in this article was funded by Deutsche Forschungsgemeinschaft through SFB 47 (B7) and by Kuwait University grant MB 019. I wish to thank Gary R. Burns, Kuwait, for many discussions and critical reading of the manuscript. Contributions with regard to surveying the literature by Marion Schmidt, Michael Veit, Riad Qanbar and Basima M. Al-Khedery are gratefully acknowledged. I also thank Mary Samuel for typing the manuscript.

References

- 1 Low, M.G., Ferguson, M.A.J., Futerman, A.K. and Silman, I. (1986) *Trends Biochem. Sci.* 11, 212-215.
- 2 Cross, G.A.M. (1987) *Cell* 48, 179-181.
- 3 Low, M.G. (1987) *Biochem. J.* 244, 1-13.
- 4 Ferguson, M.A.J. and Williams, A.F. (1988) *Annu. Rev. Biochem.* 57, 285-320.
- 5 Folch, J. and Lees, M.B. (1951) *J. Biol. Chem.* 191, 807-817.
- 6 Stoffyn, P. and Folch, J. (1971) *Biochem. Biophys. Res. Commun.* 44, 157-161.
- 7 MacLennan, D.H., Ostwald, T.J. and Stewart, P.S. (1974) *Ann. N.Y. Acad. Sci.* 227, 527-536.
- 8 Hantke, K. and Brzun, V. (1973) *Eur. J. Biochem.* 34, 284-296.
- 9 Wu, H.C. and Tokunaga, M. (1986) *Curr. Top. Microbiol. Immunol.* 125, 127-157.
- 10 Schlesinger, M.J. (1981) *Annu. Rev. Biochem.* 50, 193-206.
- 11 Schmidt, M.F.G., Dracha, M. and Schlesinger, M.J. (1979) *Proc. Natl. Acad. Sci. USA* 76, 1687-1691.
- 12 Schmidt, M.F.G. and Schlesinger, M.J. (1979) *Cell* 17, 813-817.
- 13 Schlesinger, M.J., Magee, A.I. and Schmidt, M.F.G. (1980) *J. Biol. Chem.* 255, 10021-10024.

- 14 Omary, M.B. and Trowbridge, I.S. (1981) *J. Biol. Chem.* 256, 4715-4718.
- 15 Olson, E.N., Glaser, L. and Merlie, J.P. (1984) *J. Biol. Chem.* 259, 5364-5367.
- 16 Hedo, J.A., Collier, E. and Watkinson, A. (1987) *J. Biol. Chem.* 262, 954-957.
- 17 Magee, A.I. and Siddle, K. (1986) *Biochem. Soc. Trans.* 14, 1103-1104.
- 18 Magee, A.I. and Siddle, K. (1988) *J. Cell. Biochem.* 37, 347-357.
- 19 Kinet, J.P., Quarto, R., Perez-Monfort, R. and Metzger, H. (1985) *Biochemistry* 24, 7342-7348.
- 20 O'Brien, P.J. and Zatz, M. (1984) *J. Biol. Chem.* 259, 5054-5057.
- 21 Bazan, N.G., Reddy, T.S., Redmond, T.M., Wiggert, B. and Chader, G.J. (1985) *J. Biol. Chem.* 260, 13677-13680.
- 22 Schmidt, J.W. and Caterall, W.A. (1987) *J. Biol. Chem.* 262, 13713-13723.
- 23 Wano, Y., Uchiyama, T., Yodoi, J. and Uchino, H. (1985) *Microbiol. Immunol.* 29, 451-466.
- 24 Dolci, E.D. and Palade, G.E. (1985) *J. Biol. Chem.* 260, 10728-10735.
- 25 Kaufmann, J.F., Krangel, M.S. and Strominger, J.L. (1984) *J. Biol. Chem.* 259, 1-9.
- 26 Koch, N. and Haemmerling, G.J. (1986) *J. Biol. Chem.* 261, 3434-3440.
- 27 Koch, N. (1988) *Biochemistry* 27, 4097-4102.
- 28 Simonis, S. and Cullen, S. (1986) *J. Immunol.* 136, 2962-2967.
- 29 Strous, G.J., Van Kerkhof, P., Sloot, J.W. and Ceuze, H.J. (1985) *J. Cell Biol.* 101, 57a.
- 30 Morecni, K.W. and Touster, O. (1985) *J. Biol. Chem.* 260, 6654-6662.
- 31 Guertin, D., Grise-Miron, L. and Riendeau, D. (1986) *Biochem. Cell Biol.* 64, 1249-1255.
- 32 Liau, Y.H., Murty, V.L.N., Gwozdziński, K., Slomiany, A. and Slomiany, L. (1986) *Biochim. Biophys. Acta* 880, 108-116.
- 33 Slomiany, A., Witas, H., Aono, M. and Slomiany, B.L. (1983) *J. Biol. Chem.* 258, 8535-8538.
- 34 Bringman, T.S., Lindquist, P.B. and Dernyck, R. (1987) *Cell* 48, 429-440.
- 35 Keenan, T.W., Heid, H.W., Stadler, J., Jarasch, E.D. and Franke, W.W. (1982) *Eur. J. Cell Biol.* 26, 270-276.
- 36 Agrawal, H.C., Randle, C.L. and Agrawal, D. (1982) *J. Biol. Chem.* 257, 4588-4592.
- 37 Agrawal, H.C., Schmidh, R.W. and Agrawal, D. (1983) *J. Biol. Chem.* 258, 6556-6560.
- 38 MacLennan, D.H., Yip, C.C., Iles, G.H. and Seeman, P. (1972) *Cold Spring Harbour Symp. Quant. Biol.* 27, 469-477.
- 39 Lange-Mutschler, J. (1986) *FEBS Lett.* 201, 210-214.
- 40 Staufenbiel, M. and Larzarides, E. (1986) *Proc. Natl. Acad. Sci. USA* 83, 318-322.
- 41 Kellie, S. and Wigglerworth, N.M. (1987) *FEBS Lett.* 2, 428-432.
- 42 Burn, P. and Burger, M. (1987) *Science* 235, 476-479.
- 43 Cenedella, R.J. (1989) *Biochem. Biophys. Res. Commun.*, in press.
- 44 Jakoi, E.R., Ross, P.E., Ping-Ting-Beall, H., Kaufman, B. and Yanaman, T.C. (1987) *J. Biol. Chem.* 262, 1300-1304.
- 45 Finkel, T., Der, C.J. and Cooper, G.M. (1964) *Cell* 37, 151-158.
- 46 Hoeg, J.M., Meng, M.S., Ronan, R., Fairwell, T. and Brewer, H.B. (1986) *J. Biol. Chem.* 261, 3911-3914.
- 47 Huang, G., Lee, D.M. and Singh, S. (1988) *Biochemistry* 27, 1395-1400.
- 48 Luhrs, C.A., Pitiranggon, P., DaCosta, M., Rosenberg, J.P., Slomiany, B.L., Brink, L., Tous, G.I. and Stein, S. (1987) *Proc. Natl. Acad. Sci. USA* 84, 6546-6549.
- 49 Bolanowski, M.A., Earles, B.J. and Lennarz, W.T. (1984) *J. Biol. Chem.* 259, 4934-4940.
- 50 Fujiyama, A. and Tamanoi, F. (1986) *Proc. Natl. Acad. Sci. USA* 83, 1266-1270.
- 51 Molenaar, C.M.T., Prange, R. and Gallwitz, D. (1988) *EMBO-J.* 7, 971-976.
- 52 Betz, R., Crabb, J.W., Meyer, H.E., Wittig, R. and Duntze, W. (1987) *J. Biol. Chem.* 262, 546-548.
- 53 Becker, J.M., Marcus, S., Kundu, B., Shenbagamurthi, P. and Naider, F. (1987) *Mol. Cell Biol.* 7, 4122-4124.
- 54 Wen, D. and Schlesinger, M.J. (1984) *Mol. Cell. Biol.* 4, 688-694.
- 55 Stadler, J., Gerisch, G., Bauer, G. and Deppert, W. (1985) *EMBO J.* 4, 1153-1156.
- 56 Stadler, J., Bauer, G. and Gerisch, G. (1984) *FEBS Lett.* 172, 326-330.
- 57 Schneider, A., Eichenberger, W. and Seebeck, T. (1988) *J. Biol. Chem.* 263, 6472-6475.
- 58 Mattoc, A.K. and Edelman, M. (1987) *Proc. Natl. Acad. Sci. USA* 84, 1497-1501.
- 59 Ryals, P.E. and Thompson, G.A., Jr. (1988) *Arch. Biochem. Biophys.* 266, 408-415.
- 60 Weaver, T.E. and Whitsett, J.A. (1987) *J. Cell Biol.* 105, 79a.
- 61 Bach, R., Konigsberg, W.H. and Nemerson, Y. (1988) *Biochemistry* 27, 4227-4231.
- 62 Seehafer, J.G., Slupsky, J.R., Tang, S.-C. and Shaw, A.R.E. (1988) *Biochim. Biophys. Acta* 952, 92-100.
- 63 Schmidt, M.F.G. (1982) *Virology* 116, 327-338.
- 64 Merz, D.C. and Wolinski, J.S. (1983) *Virology* 131, 328-340.
- 65 Veit, M., Schmidt, M.F.G. and Rott, R. (1989) *Virology* 168, 173-176.
- 66 Niemann, H. and Klenk, H.-D. (1981) *J. Mol. Biol.* 153, 993-1010.
- 67 Madoff, D.H. and Lenard, J. (1982) *Cell* 28, 821-829.
- 68 Gebhardt, A., Bosch, J.V., Ziemiński, A. and Friis, R.R. (1984) *J. Mol. Biol.* 174, 297-317.
- 69 Srinivas, R.V. and Compans, R.W. (1983) *J. Biol. Chem.* 258, 14718-14724.
- 70 Hiller, G. and Weber, K. (1985) *J. Virol.* 55, 651-659.
- 71 Johnson, D.C. and Spear, P.G. (1983) *Cell* 32, 987-997.
- 72 Grand, R.J.A., Roberts, C. and Gallimore, P.H. (1985) *FEBS Lett.* 181, 229-235.
- 73 McGlade, C.J., Tremblay, N.L., Yee, S.P., Ross, R. and Branton, P.E. (1987) *J. Virol.* 61, 3227-3234.
- 74 Klockmann, U. and Deppert, W. (1983) *EMBO J.* 2, 1151-1157.
- 75 Sefton, B.B., Trowbridge, I.S., Cooper, J.A. and Scolnick, E.M. (1982) *Cell* 31, 465-474.
- 76 Olson, E.N. and Spizz, G. (1986) *J. Biol. Chem.* 261, 2458-2466.
- 77 McIlhinney, R.A.J., Chadwick, J.K. and Pelley, S.J. (1987) *Biochem. J.* 244, 109-115.
- 78 Wilcox, C.A. and Olson, E.N. (1987) *Biochemistry* 26, 1029-1036.
- 79 Kotwal, G.J. and Ghosh, H.P. (1984) *J. Biol. Chem.* 259, 4699-4701.
- 80 Wu, H.C. and Tokunaga, M. (1986) *Curr. Top. Microbiol. Immunol.* 125, 127-157.
- 81 McIlhinney, R.A.J., Pelley, S.J., Chadwick, J.K. and Cowley, G.P. (1985) *EMBO J.* 4, 1145-1152.
- 82 Magee, A.I. and Courtneidge, S.A. (1985) *EMBO J.* 4, 1137-1144.
- 83 Olson, E.N., Towler, D.A. and Glaser, L. (1985) *J. Biol. Chem.* 260, 3784-3790.
- 84 Magee, A.I. and Schlesinger, M.J. (1982) *Biochim. Biophys. Acta* 694, 279-289.
- 85 Rice, C.M., Bell, J.R., Hunkappiler, M.W., Strauss, E.G. and Strauss, J.H. (1982) *J. Mol. Biol.* 154, 355-378.
- 86 Petri, W.A. and Wagner, R.R. (1980) *Virology* 107, 543-547.
- 87 Schlesinger, M.J., Magee, A.I. and Schmidt, M.F.G. (1981) *The Replication of Negative Strand Viruses* (Bishop, D.H.L. and Compans, R.W., eds.), Elsevier, Amsterdam.
- 88 Capone, J., Toneguzzo, F. and Gosh, H.P. (1982) *J. Biol. Chem.* 257, 16-19.
- 89 Schmidt, M.F.G. and Lambrecht, B. (1985) *J. Gen. Virol.* 66, 2635-2647.

- 90 Stoffel, W., Hillen, H., Schroeder, W. and Deutzmann, W. (1983) Hoppe Seyler's Z. Physiol. Chem. 364, 1455-1466.
- 91 Schmidt, M., Schmidt, M.F.G. and Rott, R. (1988) J. Biol. Chem. 263, 18635-18639.
- 92 Mack, D. and Kruppa, J. (1988) Biochem. J. 256, 1021-1027.
- 93 Willumsen, B.M., Christensen, A., Hubbert, N.L., Papageorge, A.G. and Lowy, D.R. (1984) Nature 310, 583-586.
- 94 Willumsen, B.M., Norris, K., Papageorge, A.G., Hubbert, N.L. and Lowry, D.R. (1984) EMBO J. 3, 2581-2585.
- 95 Rose, J.K., Adams, G.A. and Gallione, C.J. (1984) Proc. Natl. Acad. Sci. USA 81, 2050-2054.
- 96 Rose, J.K. and Gallione, C.J. (1981) J. Virol. 39, 519-528.
- 97 Jing, S. and Trowbridge, I.S. (1987) EMBO J. 6, 327-331.
- 98 Chen, Z.C., Ulsch, I.S., Dubois, G. and Shih, T.Y. (1985) J. Virol. 56, 607-612.
- 99 Magee, A.I. and Hanley, M. (1988) Nature 335, 114-115.
- 100 Veit, M. (1987) Diploma Thesis, Justus-Liebig-Universität Gießen, FRG.
- 101 Ovchinnikov, Yu. A., Abdulaev, N.G. and Bogachuk, A.S. (1988) FEBS Lett. 230, 1-5.
- 102 Schmidt, M.F.G. and Schlesinger, M.J. (1980) J. Biol. Chem. 255, 3334-3339.
- 103 Wellner, R.B., Ghosh, P.C., Roeklein, B. and Wu, H.C. (1987) J. Biol. Chem. 262, 13204-13211.
- 104 Repp, R., Tamura, T., Boschek, C.B., Wege, H., Schwarz, R.T. and Nieman, H. (1985) J. Biol. Chem. 260, 15873-15879.
- 105 Van Berlo, M.F., Van den Brink, W.J., Horzinek, M.C. and Van der Zeijst, B.A.M. (1987) Arch. Virol. 95, 123-128.
- 106 Gibson, R., Schlesinger, S. and Kornfeld, S. (1979) J. Biol. Chem. 254, 3600-3607.
- 107 Johnson, D.C. and Schlesinger, M.J. (1980) Virology 103, 407-424.
- 108 Dunphy, W.E., Fries, E., Urbani, L.J. and Rothman, J.E. (1981) Proc. Natl. Acad. Sci. USA 78, 7453-7457.
- 109 Quinn, P., Griffiths, G. and Warren, G. (1983) J. Cell. Biol. 96, 851-856.
- 110 Rothman, J.E. (1981) Science 213, 1212-1219.
- 111 Berger, M. and Schmidt, M.F.G. (1985) FEBS Lett. 187, 289-294.
- 112 Berger, M. and Schmidt, M.F.G. (1984) J. Biol. Chem. 259, 7245-7252.
- 113 Rizzolo, L.J. and Kornfeld, R. (1988) J. Biol. Chem. 263, 9520-9525.
- 114 Slomiany, B.L., Takagi, A., Liau, Y.H., Jozwiak, Z. and Slomiany, A. (1984) J. Biol. Chem. 259, 11997-12000.
- 115 Slomiany, A., Jozwiak, Z., Takagi, A. and Slomiany, B.L. (1984) Arch. Biochem. Biophys. 229, 560-567.
- 116 Slomiany, A., Liau, Y.H., Takagi, A. and Slomiany, B.L. (1984) J. Biol. Chem. 259, 13304-13308.
- 117 Slomiany, B.L., Tsukada, H. and Slomiany, A. (1986) Biochem. Biophys. Res. Commun. 141, 387-393.
- 118 Henning, R. and Lenge-Mutschler, J. (1983) Nature 305, 736-738.
- 119 Omary, M.B. and Trowbridge, I.S. (1981) J. Biol. Chem. 256, 12888-12892.
- 120 Adam, M., Rodriguez, A., Turbide, C., Larrick, J., Meighen, E. and Johnstone, R.M. (1984) J. Biol. Chem. 259, 15460-15463.
- 121 Adam, M., Turbide, C. and Johnstone, R.M. (1988) Arch. Biochem. Biophys. 264, 553-563.
- 122 Bizzozero, O.A. and Lees, M.B. (1986) J. Neurochem. 46, 630-636.
- 123 Bizzozero, O.A., McGarry, J.F. and Lees, M.B. (1987) J. Biol. Chem. 262, 2138-2145.
- 124 Bizzozero, O.A., McGarry, J.F. and Lees, M.B. (1987) J. Biol. Chem. 262, 13550-13557.
- 125 O'Brien, P.J., St. Jules, R.S., Reddy, T.S., Bazan, N.G. and Zatz, M. (1987) J. Biol. Chem. 262, 5210-5215.
a Ross, N.W. and Braun, P.E. (1988) J. Neurosci. Res. 21, 35-44.
- 126 St. Jules, R.S. and O'Brien, P.J. (1986) Exp. Eye Res. 43, 929-940.
- 127 Monteiro, M. and Magee, A.I. (1987) Cell Biol. Int. Rep. 11, 645-650.
- 128 Staufenbiel, M. (1987) Mol. Cell. Biol. 7, 2981-2984.
- 129 Magee, A.I., Gutierrez, L., McKay, I.A., Marshall, C.J. and Hall, A. (1987) EMBO J. 6, 3353-3357.
a Staufenbiel, M. (1988) J. Biol. Chem. 263, 13615-13622.
- 130 Berger, M. and Schmidt, M.F.G. (1986) J. Biol. Chem. 261, 14912-14918.
- 131 Bizzozero, O.A., McGarry, F. and Lees, M.B. (1987) J. Biol. Chem. 262, 13550-13557.
- 132 Yoshimura, T., Agrawal, D. and Agrawal, H.C. (1987) Biochem. J. 246, 611-617.
a Schmidt, M.F.G. and Burns, G.R. (1989) Biochem. Soc. Trans. 17, 625-626.
b Schmidt, M.F.G. and Burns, G.R. (1989) Biochem. Soc. Trans., in press.
- 133 Berger, M. and Schmidt, M.F.G. (1984) EMBO J. 3, 713-719.
- 134 Mack, D., Berger, M., Schmidt, M.F.G. and Kruppa, J. (1987) J. Biol. Chem. 262, 4297-4302.
- 135 Schmidt, M.F.G. (1984) EMBO J. 3, 2295-2300.
- 136 Hertz, R. and Bar-Tana, J. (1988) Biochem. J. 254, 39-44.
- 137 Bizzozero, O.A., McGarry, J.F. and Lees, M.B. (1986) J. Neurochem. 47, 772-778.
- 138 Sefton, B.M., Trowbridge, I.S., Cooper, J.A. and Scolnick, E.M. (1982) Cell 31, 465-474.
- 139 Schultz, A. and Oroszlan, S. (1984) Virology 133, 431-437.
- 140 Schultz, A.M., Henderson, L.E., Oroszlan, S., Garber, E.A. and Hanafusa, H. (1985) Science 227, 427-429.
- 141 Carr, A.A., Biemann, K., Shoji, S., Parmelee, D.C. and Titani, K. (1982) Proc. Natl. Acad. Sci. USA 79, 6128-6131.
- 142 Aitken, A., Cohen, P., Santikam, S., Williams, D.H., Calder, G.A., Smith, A. and Klee, C.B. (1982) FEBS Lett. 150, 314-318.
- 143 Ozols, J., Carr, S.A. and Strittmatter, P. (1984) J. Biol. Chem. 259, 13349-13354.
- 144 Henderson, L.E., Krutzsch, H.C. and Oroszlan, S. (1983) Proc. Natl. Acad. Sci. USA 80, 339-343.
- 145 Schultz, A. and Oroszlan, S. (1983) J. Virol. 46, 355-361.
- 146 Shoji, S., Hayashi, M., Funakoshi, T. and Kubota, Y. (1986) J. Chromatogr. 356, 179-185.
- 147 Buss, J.E. and Sefton, B.M. (1985) J. Virol. 53, 7-12.
- 148 Marchildon, G.A., Casnellie, J.E., Walsh, K.A. and Krebs, E.G. (1984) Proc. Natl. Acad. Sci. USA 81, 7679-7682.
- 149 Soric, J. and Gordon, J.A. (1985) Science 230, 563-566.
- 150 Guy, B., Kieny, M.P., Riviere, Y., Peuch, C.L., Dotz, K., Girard, M., Montagnier, L. and Lecocq, J.P. (1987) Nature 330, 266-269.
- 151 Heuckeroth, R.O., Towler, D.A., Adams, S.P., Glaser, L. and Gordon, J.I. (1988) J. Biol. Chem. 263, 2127-2133.
- 152 Schultz, A.M., Tsai, S., Kung, H., Oroszlan, S., Moss, J. and Vaughan, M. (1987) Biochem. Biophys. Res. Commun. 146, 1234-1239.
- 153 Buss, J.E., Mumby, S.M., Casey, P.J., Gilman, A.G. and Sefton, B.M. (1987) Proc. Natl. Acad. Sci. USA 84, 7493-7497.
- 154 Pilai, S. and Baltimore, D. (1987) Proc. Natl. Acad. Sci. USA 84, 7654-7658.
a Skene, J.H.P. and Virag, I. (1989) J. Cell Biol. 108, 613-624.
- 155 Streuli, C.H. and Griffin, B.E. (1987) Nature 326, 619-622.
- 156 Schmidt, M., Müller, H., Schmidt, M.F.G. and Rott, R. (1989) J. Virol. 63, 429-431.
- 157 Chow, M., Newman, J.F.E., Filman, D., Hogle, J.M., Rowlands, D.J. and Brown, F. (1987) Nature 327, 482-486.
- 158 Paul, A.V., Schultz, A., Pincus, S.E., Oroszlan, S. and Wimmer, E. (1987) Proc. Natl. Acad. Sci. USA 84, 7827-7831.
- 159 Persing, D.H., Varmus, H.E. and Garnem, D. (1987) J. Virol. 61, 1672-1677.
- 160 Schultz, A.M., Copeland, T.D., Mark, G.E., Rapp, U.R. and Oroszlan, S. (1985) Virology 136, 78-89.
- 161 Gallick, G.E. and Arlinghaus, R.B. (1984) Virology 133, 228-232.
- 162 Ootsuyama, Y., Shimotohno, K., Miwa, M., Oroszlan, S. and Sugimura, T. (1985) J. Cancer Res. 76, 1132-1135.

- 163 Towler, D.A. and Glaser, L. (1986) *Proc. Natl. Acad. Sci. USA* 83, 2812-2816.
- 164 Towler, D.A., Eubanks, S.R., Towery, D.S., Adams, S.P. and Glaser, L. (1987) *J. Biol. Chem.* 262, 1030-1036.
- 165 Towler, D.A., Adams, S.P., Eubanks, S.R., Towery, D.S., Jackson-Machelski, E., Glaser, L. and Gordon, J.I. (1987) *Proc. Natl. Acad. Sci. USA* 84, 2708-2712.
- 166 Kamps, M.P., Buss, J.E. and Sefton, B.M. (1986) *Cell* 45, 105-112.
- 167 Cross, F.R., Garber, E.A., Pellman, B. and Hanafusa, H. (1984) *Mol. Cell. Biol.* 4, 1834-1842.
- 168 Pellman, D., Garber, E.A., Cross, F.R. and Hanafusa, H. (1985) *Proc. Natl. Acad. Sci. USA* 82, 1623-1627.
- 169 Pellman, D., Garber, E.A., Cross, F.R. and Hanafusa, H. (1985) *Nature* 314, 374-376.
- 170 Towler, D.A., Gordon, J.I., Adams, S.P. and Glaser, L. (1988) *Annu. Rev. Biochem.* 57, 69-99.
- 171 Kamps, M.P., Buss, J.E. and Sefton, B.M. (1985) *Proc. Natl. Acad. Sci. USA* 82, 4625-4628.
- 172 Buss, J.E., Kamps, M.P., Gould, K. and Sefton, B.M. (1986) *J. Virol.* 58, 468-474.
- 173 Rein, A., McClure, M.R., Rice, N.R., Luftig, R.B. and Schultz, A.M. (1986) *Proc. Natl. Acad. Sci. USA* 83, 7246-7250.
- 174 Rhee, S.S. and Hunter, E. (1987) *J. Virol.* 61, 1045-1053.
- 175 Buss, J.E., Der, C.J. and Solski, P.A. (1988) *Mol. Cell. Biol.* 8, 3960-3963.
- 176 Kaplan, J.M., Mardon, G., Bishop, J.M. and Varmus, H.E. (1988) *Mol. Cell. Biol.* 8, 2435-2441.
- 177 Towler, D.A., Adams, S.P., Adams, S.P., Eubanks, S.R., Towery, D.S., Jackson-Machelski, E., Glaser, L. and Gordon, J.I. (1988) *J. Biol. Chem.* 263, 1784-1790.
- 178 Glover, C.J., Goddard, C. and Felsted, R.L. (1988) *Biochem. J.* 250, 485-491.
- 179 Wilcox, C.A., Hu, J.S. and Olson, E.N. (1987) *Science* 238, 1275-1278.
- 180 Palmiter, R.D., Gagnon, J. and Walsh, K.A. (1978) *Proc. Natl. Acad. Sci. USA* 75, 94-98.
- 181 Buss, J.E., Kamps, M.P. and Sefton, B.M. (1984) *Mol. Cell. Biol.* 4, 2697-2704.
- 182 Aderem, A.A., Keum, M.M., Pure, E. and Cohn, Z.A. (1986) *Proc. Natl. Acad. Sci. USA* 83, 5817-5821.
- 183 Aderem, A.A., Albert, K.A., Keum, M.M., Wang, J.K.T., Greengard, P. and Cohn, Z.A. (1988) *Nature* 332, 362-364.
- 184 Towler, D.A. and Glaser, L. (1986) *Biochemistry* 25, 878-881.
- 185 Schmidt, M.F.G. (1982) *Trends Biochem. Sci.* 7, 322-34.
- 186 Schmidt, M.F.G. (1983) *Curr. Top. Microbiol. Immunol.* 102, 101-129.
- 187 Lambrecht, B. and Schmidt, M.F.G. (1986) *FEBS Lett.* 202, 127-132.
- 188 Deselnicu, M., Lange, P.M. and Heidemann, E. (1973) *Hoppe Seylers Z. Physiol. Chem.* 354, 105-116.
- 189 Klenk, H.-D. and Rott, R. (1980) *Curr. Top. Microbiol. Immunol.* 90, 19-47.
- 190 Glick, B.S. and Rothman, J.E. (1987) *Nature* 326, 309-312.
- 191 Schlesinger, M.J. and Malfer, C. (1982) *J. Biol. Chem.* 257, 9887-9890.
- 192 Gething, M.-J., McCammon, K. and Sambrook, J. (1986) *Cell* 46, 939-950.
- 193 Kreis, T.E. and Lodish, H.F. (1986) *Cell* 46, 929-937.
- 194 Omura, S. (1976) *Bacteriol. Rev.* 40, 681-697.
- 195 Slomiany, B.L., Murty, V.L.N., Sarosiek, J., Piotrowski, J. and Slomiany, A. (1988) *Biochem. Biophys. Res. Commun.* 151, 1046-1053.
- 196 Slomiany, A., Slomiany, B.C., Witas, H., Aono, M. and Newman, L.J. (1983) *Biochem. Biophys. Res. Commun.* 113, 286-293.
- 197 a Hansson, G.C., Sheehan, J.K. and Carlstedt, I. (1988) *Arch. Biochem. Biophys.* 266, 197-200.
- 197 Bourne, H.R. (1988) *Cell* 53, 665-671.
- 198 Thornhill, W.B. and Levinson, S.R. (1987) *Biochemistry* 26, 4381-4388.
- 199 Adams, G.A. and Rose, J.K. (1985) *Cell* 41, 1007-1015.
- 200 Zilberstein, A., Snider, M.D., Porter, M. and Lodish, H.F. (1980) *Cell* 21, 417-427.
- 201 Petri, W.A., Pal, R., Barenholz, Y. and Wagner, R.R. (1981) *J. Biol. Chem.* 256, 2625-2627.
- 202 Joseph, M. and Nagaraj, R. (1987) *Biochim. Biophys. Acta* 911, 231-237.
- 203 Bizzozero, O.A. and Lees, M.B. (1986) *Biochemistry* 25, 6762-6768.
- 204 Lambrecht, B.L. (1986) PhD thesis, Justus-Liebig Universität, Giessen, FRG.
- 205 Diaz, R.E.C., Elansari, O., Lawrence, A.J., Lyall, F. and McLeod, W.A. (1985) *Biochim. Biophys. Acta* 830, 52-58.
- 206 Van der Wiele, F. Chr., Atsma, W., Roelofson, B., Van Linde, M., Van Binsbergen, J., Radvanyi, F., Raykova, D., Slotboom, A.J. and De Haas, G. (1988) *Biochemistry* 27, 1688-1694.
- 207 Huang, A., Huang, L. and Kennel, S.J. (1980) *J. Biol. Chem.* 255, 8015-8018.
- 208 Babbitt, B. and Huang, L. (1985) *Biochemistry* 24, 15-21.
- 209 Levashov, A.V., Kabanov, A.V., Nametkin, S.N., Martinek, K. and Berezin, I.V. (1985) *Dokl. Acad. Nauk SSSR* 284, 755-758.
- 210 Levashov, A.V., Kabanov, A.V. and Khmel'nitskii, Yu.L. (1984) *Dokl. Akad. Nauk SSSR* 278, 246-248.
- 211 Sefton, B.M. and Buss, J.E. (1987) *J. Cell Biol.* 104, 1449-1453.
- 212 Garber, E.A., Cross, F.R. and Hanafusa, H. (1985) *Mol. Cell. Biol.* 5, 2781-2788.
- 213 Krueger, J.G., Garber, E.A., Goldberg, A.R. and Hanafusa, H. (1982) *Cell* 28, 889-896.
- 214 Katoh, I., Yoshinaka, Y. and Luftig, R.B. (1986) *Virus Res.* 5, 265-276.
- 215 Schmidt, M.F.G. (1987) *Habilitationschrift, Justus-Liebig-Universität Giessen, FRG.*
- 216 Crowell, R.L. and Philipson, L. (1971) *J. Virol.* 8, 509-515.
- 217 DeSena, J. and Mandell, B. (1977) *Virology* 78, 554-566.
- 218 Guttman, N. and Baltimore, D. (1977) *Virology* 82, 25-36.
- 219 O'Dowd, B.F., Hnatowich, M., Caron, M.G., Lefkowitz, R.J. and Bouvier (1989) *J. Biol. Chem.* 264, 7564-7569.