Ann. Rev. Nutr. 1988. 8:461–82

PANTOTHENIC ACID AND COENZYME A IN CELLULAR MODIFICATION OF PROTEINS

Nora Plesofsky-Vig and Robert Brambl

Department of Genetics and Cell Biology, Department of Plant Pathology, and Plant Molecular Genetics Institute, 495 Borlaug Hall, The University of Minnesota, Saint Paul, Minnesota 55108

CONTENTS

INTRODUCTION

The involvement of pantothenic acid in cell metabolism through coenzyme-A-mediated and related reactions was once the subject of intense research, occupying the interests of some of the most vigorous investigators in major biochemistry laboratories. More recently, discoveries of modification of a number of proteins through acetylation and acylation steps that likely involve coenzyme A once again bring this vitamin and its role in metabolism into intense research investigation. This review considers the more recent findings

0199-9885/88/0715-0461$02.00

of the modification of proteins through processes that are mediated by derivatives of pantothenic acid, either as coenzyme A or as acyl carrier protein. In most cases the biochemical mechanisms involved in these modification reactions have yet to be explored, and the biological functions of these modifications are unknown. We hope that this review will help stimulate these needed investigations.

Pantothenic acid is a B vitamin whose nutritional essentiality has been demonstrated in all vertebrates that have been investigated. A human requirement for this vitamin has been deduced from results of experiments in which human volunteers provided a pantothenate-deficient diet and an analogue, ω-methyl-pantothenate, were observed to develop experimentally reversible peripheral neuropathy and adrenal cortical insufficiency, symptoms with parallels in experimental animals (56). Pantothenic acid is widely distributed in tissues of plants and especially animals, which thereby makes pantothenate deficiency in humans exceptionally rare.

The history of the discovery of pantothenic acid and its chemical characterization have been detailed elsewhere (86, 114). It was research into essential nutrients of the yeast *Saccharomyces cerevisiae* that led also to the isolation of the vitamin from liver. Pantothenate was copurified with pyridoxol from yeast and liver, and the distinct activities of these two vitamins were revealed by their separation via adsorption chromatography (26). Other research groups independently determined that pantothenic acid was an essential factor for lactic acid bacteria (47, 65) and that it was effective in stimulating growth and preventing dermatitis in chicks (42, 124). Several years later, a search for a biological equivalent of laboratory acetylation reagents—"active acetate"—led Lipmann and colleagues to the identification of coenzyme A as an essential cofactor for acetylation reactions (66). Subsequent degradation analyses showed that coenzyme A contains pantothenic acid, along with adenosine, phosphate, and a sulfur-containing moiety later shown to be 2-mercaptoethylamine (5).

An analogue of coenzyme A, the acyl carrier protein that is involved in synthesis of fatty acids, replaces the adenosine of the coenzyme with a peptide anchor, and the 4'-phosphopantetheine is linked through a phosphodiester bond to a serine residue of the acyl carrier protein (115). There are a few other examples in which pantothenate is bound to a peptide in the form of 4'-phosphopantetheine. These include a direct bond to the fatty acid synthetase of eukaryotes (106) or a bond through a second ribose moiety in the citrate lyase of anaerobically grown bacteria (73, 87). The bacterial nonribosomal enzyme systems responsible for synthesis of the peptide antibiotics such as tyrocidine and gramicidin S contain multiple subunits in addition to a 17–20-kDa peptide that contains a pantothenate derivative (52). In fatty acid synthesis, the pantetheine functions centrally as a thiol-carrying arm that bears a

polymer undergoing elongation through alternate steps of condensation and transthiolation. The proposed mechanism for peptide antibiotic synthesis is analogous to the multifunctional enzyme system for fatty acid biosynthesis, and it has been suggested that the nonribosomal antibiotic biosynthetic system is evolutionarily parallel to and intermediate between fatty acid biosynthesis and the much more complicated ribosomal polypeptide synthesis (55).

One interesting role of coenzyme A that is currently being studied is its direct involvement in the modification of peptides by the donation of acetyl groups and long-chain fatty acyl groups. Both acetylation and fatty acylation occur at two different types of location within proteins, either at the amino terminal residue or at an internal amino acid.

AMINO-TERMINAL ACETYLATION

It has been estimated that 80% of the soluble proteins of Ehrlich ascites cells are acetylated at their amino termini (10). This acetylation, which occurs cotranslationally, is usually on the amino acids alanine and serine after cleavage of the terminal methionine; methionine is the third most common *N*-α-acetylated amino acid (25, 77). Other amino acids that commonly appear within the first 10 residues of *N*-acetylated proteins include acidic residues at positions 2 and 4 and a centrally located lysine (77).

An important function of *N*-terminal acetylation may be to protect proteins from degradation. In *Dictyostelium discoideum* a minor unacetylated form of actin was found to be degraded more rapidly than the major acetylated form (89). It has been reported that terminally acetylated proteins are specifically resistant to ubiquitin-dependent proteolysis, in which ubiquitin covalently marks proteins for degradation. Cytochrome *c* and the glycolytic enzyme enolase, which are both acetylated in vertebrates but unacetylated in yeast (122), were subjected in vitro to ubiquitin-dependent degradation. The acetylated forms of enolase and cytochrome *c* resisted ubiquitin-mediated degradation, whereas unacetylated forms of these proteins were degraded at an accelerated rate (37). Experimental modification of free α-amino groups also made proteins resistant to ubiquitin-mediated proteolysis (37).

A restricted group of proteins that includes actin and certain mammalian hormones becomes *N*-terminally acetylated posttranslationally. The actin of *D. discoideum* becomes acetylated at amino-terminal methionine cotranslationally, but the acetylated methionine is cleaved after protein synthesis and the newly exposed aspartate becomes acetylated (84). If methionine acetylation is blocked during actin synthesis, the terminal methionine is not removed. The actin of *Drosophila melanogaster* has a cysteine intervening between methionine and aspartate, and the cysteine, which becomes acetylated, is removed before the aspartate is posttranslationally acetylated (90).

The processing of mammalian peptide hormones from polyprotein precursors is accompanied by amino-terminal acetylation of the products. Pro-opiomelanocortin is a polyprotein precursor that through cleavage gives rise to adrenocorticotropin (ACTH), which is a steroidogenic hormone, and to β-lipotropin, which is a lipolytic hormone. ACTH, in turn, is processed to α-melanocyte-stimulating hormone (MSH), and β-lipotropin is processed to β-endorphin, which is an opioid (36). The precursor pro-opiomelanocortin is present in both the anterior and intermediate lobes of the pituitary gland and in several places in the brain, including the hypothalamus (36). Both α-MSH and β-endorphin become amino-terminal acetylated in the intermediate pituitary (98, 101), but neither hormone becomes acetylated in the anterior lobe of the pituitary glands of rat (19), nor is β-endorphin acetylated in brain (27, 125). The biological effects of this modification on α-MSH and β-endorphin are very different. α-MSH activity is stimulated by acetylation (68); in contrast, acetylation inactivates β-endorphin, which becomes unable to bind to opioid receptors (101). Acetylation, therefore, may provide a mechanism for differentially activating two products of a single precursor molecule.

N-acetyltransferase activity with high affinity for ACTH and β-endorphin has been identified in the secretory granule fraction of the intermediate lobe, but not the anterior lobe, of rat and bovine pituitary (31). One enzyme may be responsible for the acetylation of both hormones, since α-*N*-acetyl-β-endorphin competitively inhibited the acetylation of ACTH (31). The regulation of this acetyltransferase would then be crucial for determining the relative concentrations of α-MSH/ACTH and *N*-acetyl-β-endorphin/β-endorphin. That a single enzyme in the intermediate lobe of the rat pituitary acetylates α-MSH and β-endorphin is also indicated by activity measurements and target size analysis, in which exposure to radiation inactivated both acetylating activities at the same rate (19).

ACETYLATION OF INTERNAL AMINO ACIDS

Within proteins, acetyl groups are added via an amide bond to the ε-amino group of lysines. Only a few proteins are known to be modified by internal acetylation, including histones, other DNA-binding proteins, and α-tubulin. Acetylation alters the positive charges of modified lysine residues, influencing the organization or stability of the higher order structures in which the proteins participate: chromatin for histones and microtubles for α-tubulin.

Histones are very basic, highly conserved proteins around which DNA is organized into nucleosomes, the subunit structure of chromatin. All four of the nucleosomal core histones, H4, H3, H2A, and H2B, contain lysines within their amino-terminal regions that become acetylated; only H1, the internucleosomal histone, has no internal acetylation sites (62). The pattern of lysine acetylation of the core histones varies according to whether the chroma-

tin is transcriptionally active or undergoing DNA replication and histone deposition. These two activities of chromatin, transcription and replication, have been experimentally dissected in examinations of histone acetylation in *Tetrahymena thermophila* and *Physarum polycephalum*.

The ciliated protozoan *T. thermophila* contains two kinds of nuclei that are active in DNA replication, macronuclei and micronuclei, of which only the macronuclei are transcriptionally active. This functional difference between the two kinds of nuclei has been exploited to separate transcriptionally related histone acetylation (113), which occurs only in macronuclei, from deposition-related histone acetylation (3). To detect acetylation of newly synthesized histones that associate with replicating DNA, it was necessary to pulse label cells, since the acetyl groups added during histone deposition are transient, being removed by a deacetylase activity (3). Steady-state labeling with acetate revealed only transcription-related acetylation (21, 113).

Four acetylation sites have been identified in histone H4; for *Tetrahymena* these are lysines 4, 7, 11, and 15 (21). The diacetylated form of histone H4 predominated both in connection with chromatin assembly and during active transcription, but the sites of acetylation differed between the two activities. Histone H4 of transcribing chromatin became diacetylated on lysines 4 and 7, whereas newly synthesized H4 was diacetylated on lysines 4 and 11 (21). Histones H3 and H4 displayed a specific order in which lysines became acetylated during transcription. H4 was monoacetylated exclusively on lysine 7 before becoming diacetylated on lysines 4 and 7. Histone H3, which also contains four possible acetylation sites, lysines 9, 14, 18, and 23, was diacetylated on lysines 9 and 14 and triacetylated on 9, 14, and 18 (21).

A histone acetyltransferase activity has been extracted from *Tetrahymena* macronuclei, whose acetylation site preference in vitro reflects in vivo acetylation patterns (20). Free histones provided as substrate in vitro were assumed to correspond to newly synthesized histones, and chromatin-bound histones in mononucleosomes represented a postdeposition, transcriptional stage. With free histones as substrate for the acetyltransferase, only H3 and H4 became acetylated, whereas in chromatin-bound form all the core histones, H2A, H2B, H3, and H4, became acetylated (20). Furthermore, free histone H4 became acetylated on lysines 4 and 11, whereas mononucleosomal H4 became acetylated on lysines 4 and 7. Both deposition- and transcription-related acetylation may be performed by a single acetyltransferase enzyme, because these activities had similar heat inactivation profiles and the two substrates were competitive when enzyme was limiting (20). A single histone acetyltransferase activity has also been extracted from yeast chromatin (112) and from mammalian nuclei (46). Other evidence, however, suggests that there are multiple histone acetylating enzymes in mammalian tissue (108),

and *Drosophila* has a cytoplasmic histone acetyltransferase that is specific for histone H4 (118).

Histone acetylation in *P. polycephalum* has been examined at specific stages during the cell cycle, which is synchronized in this plasmodial fungus. Gene transcription is most active during two stages of the cycle, S and G2, whereas DNA replication and histone synthesis occur only in S phase. Correlated with the high level of transcription in S and G2 was an increase in the tetraacetylated form of H4 (18). During S phase there was also a transient increase in diacetylated H4 (18), probably related to the deposition of newly synthesized histones onto replicated DNA. G2- and S-phase cells also differed in the turnover of histone acetyl groups. The transcriptionally active G2 cells turned over acetyl groups on the highly acetylated forms of histones H3 and H4. Superimposed on this pattern, S-phase cells turned over acetyl groups on the mono- and diacetylated forms of all four core histones (116). At a specific residue of H4, lysine 5, acetate turned over very slowly during G2, but rapidly during S phase as newly synthesized and acetylated H4 became deacetylated during chromatin assembly (78).

Histone acetylation has also been examined in isolated chromatin that was transcriptionally active. Nucleoli were isolated from *Physarum* during G2, and transcribed chromatin was separated from nontranscribed spacer sequences by sedimentation fractionation following nuclease or restriction enzyme digestion of the chromatin. Chromatin structure was probed with a fluorescent sulfhydryl reagent, iodoacetamidofluorescein, which reacts with the cysteine residue of histone H3. Open chromatin that allowed H3 labeling was found to correspond to transcribed rDNA sequences (83). Histone H3 was also derivatized with a radioactive label, 2-iodo[2-^{3}H]acetate, and its acetylation state was analyzed by gel electrophoresis. Radiolabeled histone H3, from transcribed chromatin, was found to consist of three different forms, mono-, tri-, and tetraacetylated; unacetylated and diacetylated H3 were apparently not associated with transcribed chromatin (41).

The acetylation sites of all four core histones are within their polar amino-terminal regions, which appear to be less tightly bound to nucleosomal DNA than are their central hydrophobic regions (17). It has been proposed that these amino-terminal regions of histones are involved in internucleosomal interactions or higher order chromatin structure. Since histone acetylation is associated with active and accessible chromatin, one of the functions of histone acetylation might be to disorder the chromatin, unfolding it into a more open configuration. The effects of histone acetylation on chromatin structure have been examined by inducing cellular hyperacetylation of histones with butyrate, which inhibits histone deacetylation (15). Comparison of butyrate-treated with untreated cells showed a small change in the circular dichroism spectra of core particles (4, 100), a slight decrease in the

sedimentation coefficient of highly acetylated particles (4), and a small decrease in the transition temperature for thermal denaturation of the mononucleosomes (100). Otherwise resistant sites in hyperacetylated mononucleosomes became susceptible to nuclease digestion (4, 100). By these criteria, histone acetylation affects the interactions of histones with DNA within the core particle, destabilizing them to a small degree. However, butyrate treatment increased the susceptibility of unfractionated nuclear chromatin to DNase I-digestion more than could be accounted for by increased mononucleosome digestion (100), which lends support to the proposal that acetylation of core histones ultimately affects higher order chromatin structure.

The assembled α-tubulin of a variety of organisms has recently been found to be modified, like the histones, by acetylation of the ϵ-amino group of a lysine residue, identified as lysine 40 (51). In contrast to histone acetylation, the acetylation of α-tubulin appears to stabilize the structures containing it. This modification, by altering the isoelectric point of the protein, was first observed in α-tubulin isolated from flagellar axonemes of *Chlamydomonas rheinhardtii* (53). Pools of unassembled subunits, from which *Chlamydomonas* flagella can be assembled, did not contain acetylated α-tubulin (53), and the cellular resorption of flagella led to deacetylation of the α-tubulin (54).

The development of monoclonal antibodies that recognize only the acetylated form of α-tubulin (79) has provided a sensitive means for detecting the presence of acetylated α-tubulin. These antibodies were raised against tubulin from the axonemes of sea urchin sperm flagella, but they cross-react with acetylated α-tubulin from a variety of organisms, including the cilia of sea urchin blastulae and *Tetrahymena,* as well as sperm of *Drosophila* and humans (79). Both acetylated and unacetylated α-tubulin were present in the axonemes of sea urchin sperm, and the acetylated form was not restricted to one type of axonemal microtubule but was equally represented in the central pair and in each of the outer pair of microtubules (79). Some nonaxonemal microtubules also were found to contain acetylated α-tubulin, including microtubules in the basal bodies and a subset of cytoplasmic microtubules of *Chlamydomonas* (50), the subpellicular singlet microtubules of *Trypanosoma brucei* (96), the neurites of spinal cord primary culture from chick embryo, and the midbodies and centrioles of mammalian cells (80).

Certain kinds of microtubules do not contain acetylated α-tubulin. Immunofluorescence microscopy and immunoblotting showed *P. polycephalum* flagellates and amoebae had both acetylated and unacetylated α-tubulin in their microtubules, but the plasmodial stage microtubules had no acetylated α-tubulin (23, 91). Furthermore, cytoplasmic as well as axonemal microtubules of the flagellate contained acetylated α-tubulin, whereas the cytoplasmic microtubules of amoebae were unacetylated. Acetylated α-tubulin was

detected in two human cell lines, HeLa and 3T3, but not in PtK_2 cells (80). However, no acetylated α-tubulin was detected in leading-edge microtubules of migrating 3T3 cells. In rat cerebellum sections, acetylated α-tubulin was enriched in axons compared with dendrites (14). In general, microtubules that contain acetylated α-tubulin are present in stable, cross-linked structures that are highly ordered.

To examine the relationship between α-tubulin acetylation and microtubule structure, specific inhibitors of microtubule polymerization were used. The depolymerizing activities of nocodazole and colchicine were ineffective against the cytoplasmic microtubules that contained acetylated α-tubulin. The microtubule stabilizer taxol, in turn, induced acetylation of previously unacetylated microtubular α-tubulin (80). These data indicate a strong correlation between microtubule stability and α-tubulin acetylation; however, it is not yet known whether stabilized microtubules are more readily acetylated or whether α-tubulin acetylation produces stability. The kinetics of microtubule assembly and disassembly in vitro were the same with unacetylated α-tubulin as with α-tubulin acetylated in vitro (61).

An acetylase activity has been extracted from the flagella of *Chlamydomonas*, and in vitro it specifically acetylated α-tubulin of *Chlamydomonas* and mammalian brain by using [^{3}H]acetyl-CoA (61). The polymerized microtubule was a better substrate for acetylation than the tubulin dimer; histones proved to be poor substrates. The acetylase activity was masked in cytoplasmic extracts of *Chlamydomonas* by a deacetylase activity and an inhibitor of acetylation, which were not present in flagellar extracts. The compartmentation of these activities may contribute to the differential acetylation of α-tubulin in flagellar microtubules compared with unassembled subunits in the cytoplasm. α-Tubulin deacetylase activity in mammalian cells was not inhibited by butyrate (80).

FATTY ACID ACYLATION OF PEPTIDES

Within the last few years, a peptide modification has been discovered in which long-chain fatty acyl groups are added to eukaryotic proteins (59, 97, 123). The covalent attachment of lipid to peptides was earlier observed in bacterial proteins, including the outer membrane lipoprotein of *Escherichia coli* (34). The attachment of fatty acids to eukaryotic proteins was first investigated in viruses, but the discovery has been extended to a variety of cellular proteins. Three general patterns of acylation have been observed, in one of which the peptide is linked indirectly to glycerol-esterified fatty acids through a complex, glycolipid (97). In the other two patterns, which we discuss below, fatty acids are directly attached to the peptides, either by an amide linkage at the amino terminus or by ester linkage to an internal residue. Myristate, a 14-carbon saturated fatty acid, has been identified as the amino-

terminal amide-linked fatty acid, and palmitate, a 16-carbon saturated fatty acid, has been the fatty acid commonly found in ester linkage.

After the discovery of fatty acyl modification of viral structural and transforming proteins, fatty acylation of proteins in noninfected cells received attention. In several studies (58, 60, 64, 70, 71, 92, 119), cultured avian cells or mammalian cells were provided with radiolabeled palmitate or myristate and the labeled polypeptides were analyzed by gel electrophoresis. Different classes of proteins became radiolabeled with the two fatty acids. The type of linkage, ester or amide, was determined by conditions of hydrolysis. Ester bonds were cleaved by hydroxylamine or alkaline methanol, whereas amide bonds required acid hydrolysis. The released fatty acid derivatives were analyzed by thin-layer chromatography (92) and by high performance liquid chromatography (64, 70, 71). Treatment of the cells with cycloheximide (58, 64) showed that protein modification with myristate depended on protein synthesis, but that attachment of palmitate continued in the absence of protein synthesis. Pulse-chase experiments also indicated that myristate addition was likely cotranslational, while palmitate was added posttranslationally (70); furthermore, palmitate, unlike myristate, turned over rapidly (64).

Fatty acylation of proteins may be related to their localization within the cell, and most identified acylated proteins appear to be membrane associated. However, cell fractionation studies indicate that there are differences between the distribution of myristate-modified proteins and those modified with palmitate. When membrane and cytosolic fractions were prepared from cultured cells, all the proteins acylated with palmitate were found in the membrane fraction, but myristate-containing proteins were present in either of the two cell fractions (58, 71). Furthermore, subfractionation of cellular membranes indicated that most palmitate-containing proteins were restricted to the plasma membrane fraction, while myristate-modified proteins were associated with other membrane fractions as well (119). These studies have also shown that only a small percentage of the acylated cellular proteins are glycosylated (58, 70, 119).

PEPTIDE ACYLATION AT THE AMINO TERMINUS

Structural proteins of several transforming viruses have been found to have myristate attached to their amino termini. The *gag* gene of murine leukemia virus, a type C retrovirus, encodes a polyprotein capsid precursor, $Pr65^{gag}$, which is myristylated at its amino-terminal glycine (35). One of the four capsid proteins derived from it, p15, also bears the terminal myristate and is the most hydrophobic of the four *gag* proteins. The covalently attached fatty acid may serve to anchor the $Pr65^{gag}$-RNA complex in the cellular plasma membrane at the early stages of viral assembly; after processing, the myristate of p15 may help to stabilize the viral membrane (35). In vitro mutagenesis to

delete the peptide's *N*-terminal glycine or replace it with alanine blocked myristylation of the *gag* proteins. The unacylated peptides did not associate with membranes, and virus particles did not form (85).

This modification is not sufficient, however, for determining localization of the *gag* proteins, nor is it consistently present on retroviral capsid proteins. Avian type C retroviruses, which also assemble at the plasma membrane, lack myristate (85), and myristylated *gag* proteins are found in retroviruses that assemble within the cell, rather than at the plasma membrane (85). The DNA tumor viruses, polyoma virus and simian virus 40, also have myristate attached to their structural protein, VP2, which is located in cell nuclei (107).

The RNA-containing picornaviruses, which are not enveloped viruses, have a myristylated capsid protein, VP4, whose precursors, VP0 and P1, are also myristylated. Myristate seems to form an integral part of the protein shell of the virion, where it interacts with amino acid side chains of VP4 and VP3 (22). Among the four classes of picornavirus there are two methods for exposing the *N*-terminal glycine residue for myristate attachment. In the enteroviruses and rhinoviruses, the glycine is exposed by removal of the initiating methionine, but in the cardioviruses and aphthoviruses the glycine becomes exposed by removal of a leader peptide (22).

Myristylation of the tyrosine kinase of Rous sarcoma virus or $p60^{src}$, which is responsible for cell transformation, has been intensively studied. Myristate attachment to $p60^{src}$ occurs during or soon after synthesis, and myristylated $p60^{src}$ is found first in a soluble complex with cellular proteins p50 and p90 and later, within 20 min of synthesis, it becomes membrane bound (11, 30). Myristate attachment is not required for association or dissociation of $p60^{src}$ from the soluble protein complex, but the modification is required for its tight association with the cytoplasmic surface of the plasma membrane (30). Mutant proteins that did not become myristylated, due to mutations within the first 10 amino acids, remained in the cytosol fraction (75). In turn, the first 14 amino acids of $p60^{src}$, fused to either a viral gene or an α-globin gene, directed the myristylation of the fusion protein and its association with the plasma membrane (76).

The modification of $p60^{src}$ with myristate is required for cellular transformation (43, 75), probably through its effect on membrane localization. Substitution of only the terminal glycine residue with an alanine or glutamic acid residue blocked myristylation and cell transformation, despite the mutant proteins' unaffected tyrosine kinase activity (43). Eight identified substrates of $p60^{src}$, including vinculin, enolase, phosphoglycerate mutase, and lactate dehydrogenase, continued to be phosphorylated by the mutant proteins, which suggests that the unidentified substrates whose phosphorylation is crucial for transformation only become phosphorylated when $p60^{src}$ is tightly associated with the plasma membrane (44).

Several cellular proteins have been identified that contain amino-terminal

myristate. In addition to the cellular homolog of $p60^{src}$ (12), they include cAMP-dependent protein kinase (16), calcineurin B (2), and cytochrome b_5 reductase (74). The attached fatty acid was identified by gas chromatography–mass spectrometry, and the linkage of the acyl group and sequence of the amino-terminal peptide were determined by fast atom bombardment (2, 16, 74) and direct chemical ionization mass spectrometry (16, 74). Myristate attachment may serve functions for these proteins other than directing them to membranes. cAMP-dependent protein kinase, despite its location on the inner surface of the plasma membrane, dissociates from the membrane when it is displaced from its regulatory subunit (16). NADH cytochrome b_5 reductase is bound to the endoplasmic reticulum, or microsomal membrane, where it participates in the desaturation of fatty acids. The enzyme consists of both a hydrophilic, catalytic domain and a membrane-protected hydrophobic region at the amino terminus. Since the amino acids of the amino-terminal region are predominantly hydrophobic by themselves, it is unlikely that myristate attachment to this region is responsible for anchoring the protein in the membrane (74). It is possible that myristate serves, instead, to orient the protein with respect to other proteins in the electron transport chain. Protein interactions may also be influenced by myristylation of calcineurin B, which, in a complex with calcineurin A, acts as a calmodulin-dependent protein phosphatase (2).

An enzyme activity that attaches myristate to the *N*-terminal glycine residues of analogs of myristylated peptides was identified and characterized in yeast (111) and murine muscle cells (110). The *N*-myristyltransferase showed a strict specificity for myristyl-CoA as the acyl donor and strong selectivity for the sequence of the acceptor peptide. It has been highly purified from yeast extracts, and the sequence requirements for attaching myristate to a peptide were tested with the purified enzyme (110). In addition to the terminal glycine, an uncharged subterminal residue was found to be important (110). In all known examples of myristylated proteins, the fifth residue is the next most conserved after the terminal glycine, being either serine or threonine (22); an exception occurs in the cAMP-dependent protein kinase, which has alanine in the fifth position. A synthetic peptide containing alanine in the fifth position was bound less strongly in vitro by the *N*-myristyltransferase than was a similar synthetic peptide containing serine in that position (110).

PEPTIDE ACYLATION AT INTERNAL SITES

Another type of fatty acid modification that appears to be more common for cellular peptides involves the 16-carbon, saturated fatty acid palmitate. This modification was first observed in two small lipophilic cellular proteins, the proteolipid subunit of brain myelin (1, 105) and a subunit of the Ca^{2+}, Mg^{2+}-ATPase from sarcoplasmic reticulum (57). The covalent attachment of

palmitic acid has been examined in structural glycoproteins of two enveloped RNA viruses, vesicular stomatitis virus (VSV) (94) and Sindbis virus (93). These studies indicate that palmitate was added to the glycoproteins 15 to 20 min after translation but before maturation of oligosaccharides attached to the VSV G protein. Acylation likely occurs in the Golgi complex (95), and processing mutants of the G protein that were blocked from entering the Golgi did not become acylated (126).

The molecular site of palmitate attachment to the VSV G protein was determined, by deletion mutagenesis, to be a cysteine residue within a part of the carboxy-terminal cytoplasmic domain that adjoins a hydrophobic transmembrane region (88). Replacement of this cysteine (23 residues from the carboxy end) with a serine residue abolished acylation. Despite the absence of palmitate from the mutant G protein, it was glycosylated and transported to the cell surface (88). Therefore, a function for this modification of viral glycoproteins has not yet been determined.

A group of viral proteins that do not become glycosylated or Golgi associated have been found to contain covalently linked palmitate. These include the large tumor antigen of simian virus 40 (SV40) (48), the transforming p21 proteins encoded by *ras* genes of Harvey murine and Kirsten sarcoma viruses, and cellular *ras* proteins (13, 29). The palmitylated forms of the large T antigen and the *ras* proteins, which constitute only a subset of these proteins, were found associated with the plasma membrane. The majority of the large T antigen of SV40 is unacylated and located in the cell nucleus (48). All mature forms of the *ras* proteins appear to be membrane bound, but less than 15% of the nonphosphorylated, mature forms of p21 of Ha-MuSV are palmitylated (13).

Deletion mutagenesis of v-ras^H indicated that the six carboxy-terminal amino acids contained the site(s) required for acylation (120), and cysteine 186 (out of 189 residues) was established by point mutations as the likely site for palmitate attachment (121). Mutant proteins that did not become acylated remained cytosolic rather than associating with membranes (121), and cells transfected with such viral mutants did not become transformed (120). These studies suggest that palmitate modification of the *ras* proteins is required for their localization at the plasma membrane and for their role in cellular transformation, like myristate attachment to $p60^{src}$ discussed above.

Saccharomyces cerevisiae has two *ras*-encoded proteins that are homologous to the mammalian *ras* proteins, and like them they bind GTP and GDP and hydrolyze GTP. In *S. cerevisiae,* these proteins have been shown to modulate adenylate cyclase activity, and yeast cells that are defective in both *ras* proteins do not grow (29). Palmitylation of cellular proteins has been examined in a yeast mutant that is unable to acylate *ras* proteins and other cellular proteins (28, 82). This mutant, *ram* or *dprl,* is also unable to acylate

mammalian *ras* proteins produced from introduced genes, whereas wild-type yeast cells process mammalian *ras* proteins normally. An additional characteristic of the mutant is its inability to secrete the peptide mating hormone *a*-factor (28, 82), whose secretion is independent of the Golgi-mediated pathway. The *ras* proteins and yeast *a*-factor share a common sequence at their carboxy termini, C-A-A-X, in which cysteine is the residue to which palmitate is attached, followed by two aliphatic residues and the carboxy-terminal amino acid (82, 121).

The cellular membranes of yeast cells contain five major proteins that are labeled with [^{3}H]palmitate in ester linkage; the acylation of only three of these was blocked in the *ram* mutant (82). Therefore, another acylation mechanism must exist in yeast cells. It is likely that the cellular mechanism for acylating glycosylated peptides that traverse the Golgi differs from *ram*-mediated acylation. Yeast secretory mutants have been reported to accumulate several fatty acylated proteins (117), but not the *ras* proteins (29).

Glycoproteins that are integral to the plasma membrane are prominent among cellular proteins whose palmitylation has been characterized. These include the mammalian receptor for the iron transport protein transferrin (72); the sialoglycoproteins of murine erythrocytes, gp2 and gp3 (24); the heavy chains of the major histocompatibility antigens HLA-B and HLA-DR (45); the contact site A glycoprotein of *D. discoideum,* which is involved in intercellular adhesion (102); and rhodopsin, the photoreceptor of retinal rod cells (67). Also palmitylated is ankyrin, a peripheral plasma membrane protein of erythrocytes that links cytoskeletal components to the membrane (104). Palmitylated proteins not clearly membrane associated include actin of *D. discoideum,* which is a major cytoskeletal component found in the cytosol (103); the plasma apolipoprotein A-I, which is a secreted protein (38); and transforming growth factor, which is secreted by transformed cells and binds to the receptor for epidermal growth factor, inducing a mitogenic response (9).

Palmitate addition to cellular proteins is not coupled to protein synthesis and occurs late in protein processing and assembly. Acylation of ankyrin occurred in the presence of protein synthesis inhibitors and also in adult avian erythrocytes that do not actively synthesize proteins (104), which indicates a high turnover of palmitate on ankyrin or continual palmitylation of a small fraction of ankyrin molecules. Rhodopsin, composed of the protein opsin and its vitamin A chromophore, assembles in the rod outer segments to which the protein is transported after synthesis in the inner segments; palmitate attachment to rhodopsin occurred, after transport, in rod outer segments that were prepared from bovine retinas (67). The kinetics of acylation for the transferrin receptor, which is a glycoprotein homodimer, differs from the acylation pathway of viral structural glycoproteins. Unlike palmitate attachment to the

glycoproteins of VSV (94) and Sindbis virus (93), oligosaccharide processing on the transferrin receptor preceded acylation and only the mature form of the transferrin receptor became palmitylated (72). Pulse-chase experiments showed that the palmitate moiety turned over five times more rapidly than the receptor peptide itself, which suggests that palmitate is added and removed repeatedly from the same molecules (72).

The sites of acylation have been determined for some palmitate-modified cellular peptides, and they do not conform to the consensus palmitylation site of *ras* and other peptides, discussed above. One of the first acylated cellular proteins to be identified, the proteolipid subunit of brain myelin, was found to have a hydroxyester linkage to palmitate, along with some stearate and oleate, at threonine 198 (105). The proteolipid subunit, consisting of 276 amino acids, is a hydrophobic integral membrane protein, but the acylation site is within a hydrophilic segment (105). Other palmitylated proteins have a thioester link via cysteine to the fatty acid. Cysteine 318 of the human HLA-B heavy chain appears to be the palmitate-linked residue; this cysteine is found at the end of a transmembrane hydrophobic region, adjacent to the peptide's carboxy-terminal cytoplasmic domain (45). The 760-residue human transferrin receptor is thioesterified to palmitate at cysteine 62, as determined by site-directed mutagenesis (40). This residue is also located at the juncture of a cytoplasmic region, here amino terminal, and a hydrophobic transmembrane region. However, the murine transferrin receptor, which is also acylated, does not have a cysteine in this position and must, therefore, be acylated at a different site (40). The transferrin receptor encoded by the malarial parasite *Plasmodium falciparum* and expressed in infected human erythrocytes is covalently modified with myristic acid in ester linkage via a diacylglycerol bridge (33), differing from the mammalian receptor both in mechanism of acylation and in modifying fatty acid.

Proteins within organelles also become covalently modified with fatty acids. An examination of chloroplast proteins in the aquatic plant, *Spirodela oligorrhiza,* showed that four chloroplast proteins became radiolabeled with [^{3}H]palmitate (63), including the chloroplast acyl carrier protein (ACP) and the large subunit of ribulose bisphosphate carboxylase, which are soluble proteins. The other two acylated proteins, the 32-kDa herbicide-binding protein of photosystem II and the chlorophyll a/b-binding protein of the light-harvesting complex, are membrane associated. Unlike other palmitylations that have been described, the fatty acid was not esterified to any of these chloroplast proteins except for ACP, but was attached by a more stable bond (63). The site of acylation on the 32-kDa herbicide-binding protein lies within the protein's membrane-associated amino-terminal region (63).

The precursor of the herbicide-binding protein, which is synthesized in the chloroplast, is processed to the mature 32-kDa protein in the unstacked

stromal lamellae, and the mature protein is subsequently translocated to the stacked granal lamellae of the thylakoid membrane, where it was found to contain palmitate (63). Deacylation also occurred rapidly in the stacked granal lamellae. Acylation may play a role in translocation of the peptide from stroma to grana or in promoting the peptide's proper assembly in the photosystem II complex (63).

The reactions in which palmitic acid is added to viral and cellular proteins are more diverse than the characterized addition of myristate to the amino-terminal glycine of peptides. In addition to the subset of proteins that become palmitylated at cysteine of the consensus sequence C-A-A-X, proteins become acylated on internal cysteines and even threonine at sites that vary among peptides in location and sequence. An acylating activity has been extracted in microsomal membranes that adds palmitate from palmityl-CoA to the E1 glycoprotein of Semliki Forest virus (SFV) (6). However, this activity was not specific for palmitate, contrasting with the high fatty acid specificity shown by extracted myristyltransferase (111), discussed above. A deacylating activity has also been extracted in microsomal membranes that releases palmitate from the acylated E2 glycoprotein of SFV (7).

INVOLVEMENT OF PANTOTHENIC ACID IN MEMBRANE ASSEMBLY AND TRANSPORT

In addition to these structurally well-characterized examples of acetylation and fatty acylation of peptides, there are other cases in which acylation or a novel activity involving pantothenate influences cellular metabolic function. Although these relationships are not yet understood at the structural level, they reveal functional aspects of pantothenate-dependent activities that may also apply to the cases of peptide modification already discussed.

One possible function of peptide acylation is to regulate the assembly of multisubunit peptide complexes at membranes. The nicotinic acetylcholine receptor is an integral membrane glycoprotein complex composed of five subunits, four of which are distinct. In a muscle-like cell line provided with radiolabeled palmitate, two subunits of the acetylcholine receptor (α and β) became acylated by a bond that is probably not an ester (69). Treatment of the cells with cerulenin, which inhibits fatty acylation of glycoproteins, decreased by 50% the appearance of functional acetylcholine receptors on the cell surface (69); this decrease suggests that fatty acylation is required for proper membrane assembly of the acetylcholine receptor complex.

The assembly of two respiratory enzyme complexes in the mitochondrial inner membrane of the fungus *Neurospora crassa* was found to depend on pantothenate. In immunoprecipitation assays, unassembled subunits of both cytochrome *c* oxidase and mitochondrial ATPase-ATP synthase were closely

associated with [^{3}H]pantothenate-labeled peptides (81), but the association with pantothenate was transient, being lost during subunit assembly (8). As a test for pantothenate function, enzyme complex assembly was measured in an unsupplemented pantothenate auxotroph of *N. crassa;* structural criteria of assembly, including intersubunit association and ^{55}Fe incorporation, were applied, as well as functional criteria of enzyme activity (8). Assembly of the 7-subunit cytochrome *c* oxidase and the 12-subunit mitochondrial ATPase was specifically inhibited in pantothenate-deprived cells, relative to their assembly in pantothenate-supplemented cells (8). These results suggest that pantothenate plays an important role in the assembly of enzymes in the mitochondrial inner membrane.

It remains to be determined whether pantothenate is covalently attached to enzyme subunit peptides with which it comigrates electrophoretically (81), or whether it is attached to a mitochondrial acyl carrier protein that appears in different acylation states (49). In either case, the association of pantothenate with unassembled subunits may be part of a mechanism to fatty acylate subunit peptides, and thereby to mediate their association with one another or with the mitochondrial membrane. Radioactive myristate, but not palmitate, when provided to *N. crassa* cells labeled two peptides that were immunoprecipitated by cytochrome *c* oxidase antiserum (E. Nixon, N. Plesofsky-Vig, and R. Brambl, unpublished results). The identities of these peptides, one of which comigrated with the immunoprecipitated pantothenate-containing peptide and the other of which had a slower mobility, remain under investigation.

A study of glycoprotein transport through the Golgi cisternae unexpectedly revealed that coenzyme A was a necessary component (32). In a cell-free system whose membrane components had been inactivated by the sulfhydryl reagent *N*-ethylmaleimide, restoration of transport depended on two components. One factor (NSF) was proteinaceous and could be extracted from untreated Golgi membranes. The other factor initially seemed to be coenzyme A, which acted synergistically with NSF to restore transport. However, palmityl-CoA and other fatty acyl CoAs were eight times more effective than reduced CoA in stimulating transport through the Golgi cisternae (32). One hypothesis is that acyl CoA donates a fatty acid to a protein or other molecule whose acylation activates transport and whose deacylation recycles the molecule; NSF would be involved in this cycle of acylation and deacylation that regulates transport through the Golgi cisternae (32).

A novel involvement in oligosaccharide synthesis has been described for the acyl carrier protein (ACP) of *E. coli* (109). Membrane-derived oligosaccharides, located in the periplasmic space of Gram-negative bacteria, consist of modified β-glucan chains. The synthesis of these β-glucan chains requires both a membrane enzyme fraction and a soluble protein called transglucosyla-

tion factor, which has been identified as ACP. The identification of transglucosylation factor is based on electrophoretic migration, substitution of ACP in assays of transglucosylation, immunological reactivity, and inhibition of transglucosylation activity by ACP antibody (109). Furthermore, the nodulation genes, *nodF* of *Rhizobium leguminosarum* (99) and *hsnA* of *R. meliloti* (39), encode proteins that resemble ACP, particularly at the phosphopantetheine attachment site. It has been suggested that these ACP-like proteins may affect host nodulation through a role in glucan or carbohydrate synthesis, similar to that of ACP (109). Although the role of ACP in oligosaccharide synthesis is unclear, it could regulate the activity of other molecules by participating in acylation and deacylation reactions, as suggested for coenzyme-A-mediated Golgi transport.

CONCLUSION

The cellular functions served by molecules carrying pantothenate in the form of pantetheine are extremely diverse. In addition to the many reactions involving molecular synthesis and oxidative degradation that have been well established, it is now clear that another type of activity based on the modification of preexisting molecules, frequently proteins, is common. By donating acetate or long-chain fatty acids to these proteins, coenzyme A and acyl carrier protein appear to influence especially the proper localization and assembly of the modified peptide; they also affect the stability and activity of the larger structure to which the acylated peptide contributes. Many aspects of this type of peptide modification remain to be explored. The prevalence of reversibility for these modifications, except for *N*-terminal peptide myristylation, suggests the existence of cycles of acylation and deacylation, with the implication of regulatory significance.

ACKNOWLEDGMENT

Research from the authors' laboratory was supported by NIH research grant GM 19398 from the National Institute of General Medical Sciences.

Literature Cited

1. Agrawal, H. C., Randle, C. L., Agrawal, D. 1982. In vivo acylation of rat brain myelin proteolipid protein. *J. Biol. Chem.* 257:4588–92
2. Aitken, A., Cohen, P., Santikam, S., Williams, D. H., Calder, A. G., et al. 1982. Identification of the NH_2-terminal blocking group of calcineurin B as myristic acid. *FEBS Lett.* 150:314–18
3. Allis, C. D., Chicoine, L. G., Richman, R., Schulman, I. G. 1985. Deposition-related histone acetylation in micronuclei of conjugating *Tetrahymena*. *Proc. Natl. Acad. Sci. USA* 82:8048–52
4. Ausio, J., van Holde, K. E. 1986. Histone hyperacetylation: its effects on nucleosome conformation and stability. *Biochemistry* 25:1421–28
5. Baddiley, J., Thain, E. M., Novelli, G. D., Lipmann, F. 1953. Structure of coenzyme A. *Nature* 171:76
6. Berger, M., Schmidt, M. F. G. 1984.

Cell-free fatty acid acylation of Semliki Forest viral polypeptides with microsomal membranes from eukaryotic cells. *J. Biol. Chem.* 259:7245–52

7. Berger, M., Schmidt, M. F. G. 1986. Characterization of a protein fatty acylesterase present in microsomal membranes of diverse origin. *J. Biol. Chem.* 261:14912–18
8. Brambl, R., Plesofsky-Vig, N. 1986. Pantothenate is required in *Neurospora crassa* for assembly of subunit peptides of cytochrome *c* oxidase and ATPase/ATP synthase. *Proc. Natl. Acad. Sci. USA* 83:3644–48
9. Bringman, T. S., Lindquist, P. B., Derynck, R. 1987. Different transforming growth factor-α species are derived from a glycosylated and palmitoylated transmembrane precursor. *Cell* 48:429–40
10. Brown, J. L., Roberts, W. K. 1976. Evidence that approximately eighty percent of the soluble proteins from Ehrlich ascites cells are *N*-α-acetylated. *J. Biol. Chem.* 251:1009–14
11. Buss, J. E., Kamps, M. P., Sefton, B. M. 1984. Myristic acid is attached to the transforming protein of Rous sarcoma virus during or immediately after synthesis and is present in both soluble and membrane-bound forms of the protein. *Mol. Cell. Biol.* 4:2697–2704
12. Buss, J. E., Sefton, B. M. 1985. Myristic acid, a rare fatty acid, is the lipid attached to the transforming protein of Rous sarcoma virus and its cellular homolog. *J. Virol.* 53:7–12
13. Buss, J. E., Sefton, B. M. 1986. Direct identification of palmitic acid as the lipid attached to $p21^{ras}$. *Mol. Cell. Biol.* 6:116–22
14. Cambray-Deakin, M. A., Burgoyne, R. D. 1987. Posttranslational modifications of α-tubulin. *J. Cell Biol.* 104:1569–74
15. Candido, E. P. M., Reeves, R., Davie, J. R. 1978. Sodium butyrate inhibits histone deacetylation in cultured cells. *Cell* 14:105–13
16. Carr, S. A., Biemann, K., Shoji, S., Parmelee, D. C., Titani, K. 1982. *n*-Tetradecanoyl is the NH_2-terminal blocking group of the catalytic subunit of cyclic AMP-dependent protein kinase from bovine cardiac muscle. *Proc. Natl. Acad. Sci. USA* 79:6128–31
17. Cary, P. D., Moss, T., Bradbury, E. M. 1978. High-resolution proton-magnetic-resonance studies of chromatin core particles. *Eur. J. Biochem.* 89:475–82
18. Chahal, S. S., Matthews, H. R., Bradbury, E. M. 1980. Acetylation of histone H4 and its role in chromatin structure and function. *Nature* 287:76–79
19. Chappell, M. C., O'Donohue, T. L., Millington, W. R., Kempner, E. S. 1986. The size of enzymes acetylating α-melanocyte-stimulating hormone and β-endorphin. *J. Biol. Chem.* 261:1088–91
20. Chicoine, L. G., Richman, R., Cook, R. G., Gorovsky, M. A., Allis, C. D. 1987. A single histone acetyltransferase from *Tetrahymena* macronuclei catalyzes deposition-related acetylation of free histones and transcription-related acetylation of nucleosomal histones. *J. Cell. Biol.* 105:127–35
21. Chicoine, L. G., Schulman, I. G., Richman, R., Cook, R. G., Allis, C. D. 1986. Nonrandom utilization of acetylation sites in histones isolated from *Tetrahymena*. *J. Biol. Chem.* 261:1071–76
22. Chow, M., Newman, J. F. E., Filman, D., Hogle, J. M., Rowlands, D. J., et al. 1987. Myristylation of picornavirus capsid protein VP4 and its structural significance. *Nature* 327:482–86
23. Diggins, M. A., Dove, W. F. 1987. Distribution of acetylated alpha-tubulin in *Physarum polycephalum*. *J. Cell Biol.* 104:303–9
24. Dolci, E. D., Palade, G. E. 1985. The biosynthesis and fatty acid acylation of the murine erythrocyte sialoglycoproteins. *J. Biol. Chem.* 260:10728–35
25. Driessen, H. P. C., de Jong, W. W., Tesser, G. I., Bloemendal, H. 1985. The mechanism of *N*-terminal acetylation of proteins. *CRC Crit. Rev. Biochem.* 18:281–306
26. Elvehjem, C. A., Koehn, C. J. Jr. 1935. Studies on vitamin B_2 (G). The non-identity of vitamin B_2 and flavins. *J. Biol. Chem.* 108:709–28
27. Evans, C. J., Erdelyi, E., Weber, E., Barchas, J. D. 1983. Identification of pro-opiomelanocortin-derived peptides in the human adrenal medulla. *Science* 221:957–60
28. Fujiyama, A., Matsumoto, K., Tamanoi, F. 1987. A novel yeast mutant defective in the processing of *ras* proteins. *EMBO J.* 6:223–28
29. Fujiyama, A., Tamanoi, F. 1986. Processing and fatty acid acylation of *RAS1* and *RAS2* proteins in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 83:1266–70
30. Garber, E. A., Cross, F. R., Hanafusa, H. 1985. Processing of $p60^{v\text{-}src}$ to its myristylated membrane-bound form. *Mol. Cell. Biol.* 5:2781–88
31. Glembotski, C. C. 1982. Characterization of the peptide acetyltransferase activity in bovine and rat intermediate pituitaries responsible for the acetylation

of β-endorphin and α-melanotropin. *J. Biol. Chem.* 257:10501–9
32. Glick, B. S., Rothman, J. E. 1987. Possible role for fatty acyl-coenzyme A in intracellular protein transport. *Nature* 326:309–12
33. Haldar, K., Henderson, C. L., Cross, G. A. M. 1986. Identification of the parasite transferrin receptor of *Plasmodium falciparum*–infected erythrocytes and its acylation via 1,2-diacyl-sn-glycerol. *Proc. Natl. Acad. Sci. USA* 83:8565–69
34. Hantke, K., Braun, V. 1973. Covalent binding of lipid to protein. *Eur. J. Biochem.* 34:284–96
35. Henderson, L. E., Krutzsch, H. C., Oroszlan, S. 1983. Myristyl amino-terminal acylation of murine retrovirus proteins. *Proc. Natl. Acad. Sci. USA* 80:339–43
36. Herbert, E., Uhler, M. 1982. Biosynthesis of polyprotein precursors to regulatory peptides. *Cell* 30:1–2
37. Hershko, A., Heller, H., Eytan, E., Kaklij, G., Rose, I. A. 1984. Role of the α-amino group of protein in ubiquitin-mediated protein breakdown. *Proc. Natl. Acad. Sci. USA* 81:7021–25
38. Hoeg, J. M., Meng, M. S., Ronan, R., Fairwell, T., Brewer, H. B. Jr. 1986. Human apolipoprotein A-I: post-translational modification by fatty acid acylation. *J. Biol. Chem.* 261:3911–14
39. Horvath, B., Kondorosi, E., John, M., Schmidt, J., Torok, I., et al. 1986. Organization, structure and symbiotic function of *Rhizobium meliloti* nodulation genes determining host specificity for alfalfa. *Cell* 46:335–43
40. Jing, S., Trowbridge, I. S. 1987. Identification of the intermolecular disulfide bonds of the human transferrin receptor and its lipid-attachment site. *EMBO J.* 6:327–31
41. Johnson, E. M., Sterner, R., Allfrey, V. G. 1987. Altered nucleosomes of active nucleolar chromatin contain accessible histone H3 in its hyperacetylated forms. *J. Biol. Chem.* 262:6943–46
42. Jukes, T. H. 1939. The pantothenic acid requirement of the chick. *J. Biol. Chem.* 129:225–31
43. Kamps, M. P., Buss, J. E., Sefton, B. M. 1985. Mutation of NH_2-terminal glycine of $p60^{src}$ prevents both myristoylation and morphological transformation. *Proc. Natl. Acad. Sci. USA* 82:4625–28
44. Kamps, M. P., Buss, J. E., Sefton, B. M. 1986. Rous sarcoma virus transforming protein lacking myristic acid phosphorylates known polypeptide substrates without inducing transformation. *Cell* 45:105–12
45. Kaufman, J. F., Krangel, M. S., Strominger, J. L. 1984. Cysteines in the transmembrane region of major histocompatibility complex antigens are fatty acylated via thioester bonds. *J. Biol. Chem.* 259:7230–38
46. Kelner, D. N., McCarty, K. S. 1984. Porcine liver nuclear histone acetyltransferase. *J. Biol. Chem.* 259:3413–18
47. King, T. E., Fels, I. G., Cheldelin, V. H. 1949. Pantothenic acid studies. VI. A biologically active conjugate of pantothenic acid. *J. Am. Chem. Soc.* 71:131–35
48. Klockmann, U., Deppert, W. 1983. Acylated simian virus 40 large T-antigen. *EMBO J.* 2:1151–57
49. Lakin-Thomas, P. L., Brody, S. 1986. *Neurospora crassa* mitochondria contain two forms of a 4'-phosphopantetheine-modified protein. *J. Biol. Chem.* 261:4785–88
50. LeDizet, M., Piperno, G. 1986. Cytoplasmic microtubules containing acetylated α-tubulin in *Chlamydomonas reinhardtii:* spatial arrangement and properties. *J. Cell Biol.* 103:13–22
51. LeDizet, M., Piperno, G. 1987. Identification of an acetylation site of *Chlamydomonas* α-tubulin. *Proc. Natl. Acad. Sci. USA* 84:5720–24
52. Lee, S. G., Lipmann, F. 1977. Isolation of amino acid activating subunit-pantetheine protein complexes: their role in chain elongation in tyrocidine synthesis. *Proc. Natl. Acad. Sci. USA* 74:2343–47
53. L'Hernault, S. W., Rosenbaum, J. L. 1983. *Chlamydomonas* α-tubulin is posttranslationally modified in the flagella during flagellar assembly. *J. Cell Biol.* 97:258–63
54. L'Hernault, S. W., Rosenbaum, J. L. 1985. Reversal of the posttranslational modification on *Chlamydomonas* flagellar α-tubulin occurs during flagellar resorption. *J. Cell Biol.* 100:457–62
55. Lipmann, F. 1971. Attempts to map a process: evolution of peptide biosynthesis. *Science* 173:875–84
56. Lubin, R., Daum, K. A., Bean, W. B. 1956. Studies on pantothenic acid metabolism. *Am. J. Clin. Nutr.* 4:420–30
57. MacLennan, D. H. 1975. Resolution of the calcium transport system of sarcoplasmic reticulum. *Can. J. Biochem.* 53:251–61
58. Magee, A. I., Courtneidge, S. A. 1985. Two classes of fatty acid acylated pro-

teins exist in eukaryotic cells. *EMBO J.* 4:1137–44
59. Magee, A. I., Schlesinger, M. J. 1982. Fatty acid acylation of eukaryotic cell membrane proteins. *Biochim. Biophys. Acta* 694:279–89
60. Marinetti, G. V., Cattieu, K. 1982. Tightly (covalently) bound fatty acids in cell membrane proteins. *Biochim. Biophys. Acta* 685:109–16
61. Maruta, H., Greer, K., Rosenbaum, J. L. 1986. The acetylation of alpha-tubulin and its relationship to the assembly and disassembly of microtubules. *J. Cell Biol.* 103:571–79
62. Matthews, H. R., Waterborg, J. H. 1985. Reversible modifications of nuclear proteins and their significance. In *The Enzymology of Post-Translational Modification of Proteins,* ed. R. B. Freedman, H. C. Hawkins, 2:125–85. New York: Academic
63. Mattoo, A. K., Edelman, M. 1987. Intramembrane translocation and post-translational palmitoylation of the chloroplast 32-kDa herbicide-binding protein. *Proc. Natl. Acad. Sci. USA* 84:1497–1501
64. McIlhinney, R. A. J., Pelly, S. J., Chadwick, J. K., Cowley, G. P. 1985. Studies on the attachment of myristic and palmitic acid to cell proteins in human squamous carcinoma cell lines. *EMBO J.* 4:1145–52
65. Neilands, J. B., Higgins, H., King, T. E., Handschumacher, R. E., Strong, F. M. 1950. Concentration of bound pantothenic acid. *J. Biol. Chem.* 185:335–46
66. Novelli, G. D., Lipmann, F. 1947. The involvement of coenzyme A in acetate oxidation in yeast. *J. Biol. Chem.* 171:833–34
67. O'Brien, P. J., Zatz, M. 1984. Acylation of bovine rhodopsin by [^{3}H]palmitic acid. *J. Biol. Chem.* 259:5054–57
68. O'Donohue, T. L., Handelmann, G. E., Miller, R. L., Jacobowitz, D. M. 1982. *N*-acetylation regulates the behavioral activity of α-melanotropin in a multineurotransmitter neuron. *Science* 215:1125–27
69. Olson, E. N., Glaser, L., Merlie, J. P. 1984. α-and β-subunits of the nicotinic acetylcholine receptor contain covalently bound lipid. *J. Biol. Chem.* 259:5364–67
70. Olson, E. N., Spizz, G. 1986. Fatty acylation of cellular proteins. *J. Biol. Chem.* 261:2458–66
71. Olson, E. N., Towler, D. A., Glaser, L. 1985. Specificity of fatty acid acylation of cellular proteins. *J. Biol. Chem.* 260:3784–90
72. Omary, M. B., Trowbridge, I. S. 1981. Biosynthesis of the human transferrin receptor in cultured cells. *J. Biol. Chem.* 256:12888–92
73. Oppenheimer, N. J., Singh, M., Sweeley, C. C., Sung, S.-J., Srere, P. A. 1979. The configuration and location of the ribosidic linkage in the prosthetic group of citrate lyase *(Klebsiella aerogenes). J. Biol. Chem.* 254:1000–2
74. Ozols, J., Carr, S. A., Strittmatter, P. 1984. Identification of the NH_2-terminal blocking group of NADH-cytochrome b_5 reductase as myristic acid and the complete amino acid sequence of the membrane-binding domain. *J. Biol. Chem.* 259:13349–54
75. Pellman, D., Garber, E. A., Cross, F. R., Hanafusa, H. 1985. Fine structural mapping of a critical NH_2-terminal region of $p60^{src}$. *Proc. Natl. Acad. Sci. USA* 82:1623–27
76. Pellman, D., Garber, E. A., Cross, F. R., Hanafusa, H. 1985. An *N*-terminal peptide from $p60^{src}$ can direct myristylation and plasma membrane localization when fused to heterologous proteins. *Nature* 314:374–77
77. Persson, B., Flinta, C., von Heijne, G., Jornvall, H. 1985. Structures of *N*-terminally acetylated proteins. *Eur. J. Biochem.* 152:523–27
78. Pesis, K. H., Matthews, H. R. 1986. Histone acetylation in replication and transcription: turnover at specific acetylation sites in histone H4 from *Physarum polycephalum. Arch. Biochem. Biophys.* 251:665–73
79. Piperno, G., Fuller, M. T. 1985. Monoclonal antibodies specific for an acetylated form of α-tubulin recognize the antigen in cilia and flagella from a variety of organisms. *J. Cell Biol.* 101:2085–94
80. Piperno, G., LeDizet, M., Chang, X.-J. 1987. Microtubules containing acetylated α-tubulin in mammalian cells in culture. *J. Cell Biol.* 104:289–302
81. Plesofsky-Vig, N., Brambl, R. 1984. Three subunit proteins of membrane enzymes in mitochondria of *Neurospora crassa* contain a pantothenate derivative. *J. Biol. Chem.* 259:10660–63
82. Powers, S., Michaelis, S., Broek, D., Santa Anna-A., S., Field, J., et al. 1986. *RAM,* a gene of yeast required for a functional modification of *RAS* proteins and for production of mating pheromone *a*-factor. *Cell* 47:413–22
83. Prior, C. P., Cantor, C. R., Johnson, E.

M., Littau, V. C., Allfrey, V. G. 1983. Reversible changes in nucleosome structure and histone H3 accessibility in transcriptionally active and inactive states of rDNA chromatin. *Cell* 34:1033–42

84. Redman, K. L., Rubenstein, P. A. 1984. Actin amino-terminal acetylation and processing in a rabbit reticulocyte lysate. *Methods Enzymol.* 106:179–92
85. Rein, A., McClure, M. R., Rice, N. R., Luftig, R. B., Schultz, A. M. 1986. Myristylation site in $Pr65^{gag}$ is essential for virus particle formation by Moloney murine leukemia virus. *Proc. Natl. Acad. Sci. USA* 83:7246–50
86. Robinson, F. A. 1966. *The vitamin cofactors of enzyme systems,* pp. 406–86. New York: Pergamon. 896 pp.
87. Robinson, J. B. Jr., Singh, M., Srere, P. A. 1976. Structure of the prosthetic group of *Klebsiella aerogenes* citrate (*pro*-3-*S*)-lyase. *Proc. Natl. Acad. Sci. USA* 73:1872–76
88. Rose, J. K., Adams, G. A., Gallione, C. J. 1984. The presence of cysteine in the cytoplasmic domain of the vesicular stomatitis virus glycoprotein is required for palmitate addition. *Proc. Natl. Acad. Sci. USA* 81:2050–54
89. Rubenstein, P., Deuchler, J. 1979. Acetylated and nonacetylated actins in *Dictyostelium discoideum*. *J. Biol. Chem.* 254:11142–47
90. Rubenstein, P. A., Martin, D. J. 1983. NH_2-terminal processing of *Drosophila melanogaster* actin. *J. Biol. Chem.* 258:11354–60
91. Sasse, R., Glyn, M. C. P., Birkett, C. R., Gull, K. 1987. Acetylated α-tubulin in *Physarum*. *J. Cell Biol.* 104:41–49
92. Schlesinger, M. J., Magee, A. I., Schmidt, M. F. G. 1980. Fatty acid acylation of proteins in cultured cells. *J. Biol. Chem.* 255:10021–24
93. Schmidt, M. F. G., Bracha, M., Schlesinger, M. J. 1979. Evidence for covalent attachment of fatty acids to Sindbis virus glycoproteins. *Proc. Natl. Acad. Sci. USA* 76:1687–91
94. Schmidt, M. F. G., Schlesinger, M. J. 1979. Fatty acid binding to vesicular stomatitis virus glycoprotein. *Cell* 17:813–19
95. Schmidt, M. F. G., Schlesinger, M. J. 1980. Relation of fatty acid attachment to the translation and maturation of vesicular stomatitis and Sindbis virus membrane glycoproteins. *J. Biol. Chem.* 255:3334–39
96. Schneider, A., Sherwin, T., Sasse, R., Russell, D. G., Gull, K., et al. 1987. Subpellicular and flagellar microtubules of *Trypanosoma brucei brucei* contain the same α-tubulin isoforms. *J. Cell Biol.* 104:431–38
97. Sefton, B. M., Buss, J. E. 1987. The covalent modification of eukaryotic proteins with lipid. *J. Cell Biol.* 104:1449–53
98. Seizinger, B. R., Hollt, V. 1980. In vitro biosynthesis and *N*-acetylation of β-endorphin in pars intermedia of the rat pituitary. *Biochem. Biophys. Res. Commun.* 96:535–43
99. Shearman, C. A., Rossen, L., Johnston, A. W. B., Downie, J. A. 1986. The *Rhizobium leguminosarum* nodulation gene *nodF* encodes a polypeptide similar to acyl-carrier protein and is regulated by *nodD* plus a factor in pea root exudate. *EMBO J.* 5:647–52
100. Simpson, R. T. 1978. Structure of chromatin containing extensively acetylated H3 and H4. *Cell* 13:691–99
101. Smyth, D. G., Massey, D. E., Zakarian, S., Finnie, M. D. A. 1979. Endorphins are stored in biologically active and inactive forms: isolation of α-*N*-acetyl peptides. *Nature* 279:252–54
102. Stadler, J., Bauer, G., Gerisch, G. 1984. Acylation in vivo of the contact site A glycoprotein and of other membrane proteins in *Dictyostelium discoideum*. *FEBS Lett.* 172:326–30
103. Stadler, J., Gerisch, G., Bauer, G., Deppert, W. 1985. In vivo acylation of *Dictyostelium* actin with palmitic acid. *EMBO J.* 4:1153–56
104. Staufenbiel, M., Lazarides, E. 1986. Ankyrin is fatty acid acylated in erythrocytes. *Proc. Natl. Acad. Sci. USA* 83:318–22
105. Stoffel, W., Hillen, H., Schroder, W., Deutzmann, R. 1983. The primary structure of bovine brain myelin lipophilin (proteolipid apoprotein). *Hoppe-Seyler's Z. Physiol. Chem.* 364:1455–66
106. Stoops, J. K., Wakil, S. J. 1981. Animal fatty acid synthetase. A novel arrangement of the β-ketoacyl synthetase sites comprising domains of the two subunits. *J. Biol. Chem.* 256:5128–33
107. Streuli, C. H., Griffin, B. E. 1987. Myristic acid is coupled to a structural protein of polyoma virus and SV40. *Nature* 326:619–21
108. Sures, I., Gallwitz, D. 1980. Histone-specific acetyltransferases from calf thymus. *Biochemistry* 19:943–51
109. Therisod, H., Weissborn, A. C., Kennedy, E. P. 1986. An essential function for acyl carrier protein in the biosyn-

thesis of membrane-derived oligosaccharides of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 83:7236–40
110. Towler, D. A., Adams, S. P., Eubanks, S. R., Towery, D. S., Jackson-Machelski, E., et al. 1987. Purification and characterization of yeast myristoyl CoA:protein *N*-myristoyltransferase. *Proc. Natl. Acad. Sci. USA* 84:2708–12
111. Towler, D., Glaser, L. 1986. Protein fatty acid acylation: enzymatic synthesis of an *N*-myristoylglycyl peptide. *Proc. Natl. Acad. Sci. USA* 83:2812–16
112. Travis, G. H., Colavito-Shepanski, M., Grunstein, M. 1984. Extensive purification and characterization of chromatin-bound histone acetyltransferase from *Saccharomyces cerevisiae J. Biol. Chem.* 259:14406–12
113. Vavra, K. J., Allis, C. D., Gorovsky, M. A. 1982. Regulation of histone acetylation in *Tetrahymena* macro- and micronuclei. *J. Biol. Chem.* 257:2591–98
114. Wagner, A. F., Folkers, K. 1964. *Vitamins and Coenzymes*, pp. 93–112. New York: Wiley-Interscience. 532 pp.
115. Wakil, S. J., Stoops, J. K., Joshi, V. C. 1983. Fatty acid synthesis and its regulation. *Ann. Rev. Biochem.* 52:537–79
116. Waterborg, J. H., Matthews, H. R. 1984. Patterns of histone acetylation in *Physarum polycephalum*. *Eur. J. Biochem.* 142:329–35
117. Wen, D., Schlesinger, M. J. 1984. Fatty acid-acylated proteins in secretory mutants of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 4:688–94
118. Wiegand, R. C., Brutlag, D. L. 1981. Histone acetylase from *Drosophila melanogaster* specific for H4. *J. Biol. Chem.* 256:4578–83
119. Wilcox, C. A., Olson, E. N. 1987. The majority of cellular fatty acid acylated proteins are localized to the cytoplasmic surface of the plasma membrane. *Biochemistry* 26:1029–36
120. Willumsen, B. M., Christensen, A., Hubbert, N. L., Papageorge, A. G., Lowy. D. R. 1984. The p21 *ras* C-terminus is required for transformation and membrane association. *Nature* 310:583–86
121. Willumsen, B. M., Norris, K., Papageorge, A. G., Hubbert, N. L., Lowy, D. R. 1984. Harvey murine sarcoma virus p21 *ras* protein: biological and biochemical significance of the cysteine nearest the carboxy terminus. *EMBO J.* 3:2581–85
122. Wold, F. 1981. In vivo chemical modification of proteins. *Ann. Rev. Biochem.* 50:783–814
123. Wold, F. 1986. Fatty acylation of proteins. *Trends Biochem. Sci.* 11:58–59
124. Woolley, D. W., Waisman, H. A., Elvehjem, C. A. 1939. Studies on the structure of the chicken antidermatitis factor. *J. Biol. Chem.* 129:673–79
125. Zakarian, S., Smyth, D. 1979. Distribution of active and inactive forms of endorphins in rat pituitary and brain. *Proc. Natl. Acad. Sci. USA* 76:5972–76
126. Zilberstein, A., Snider, M. D., Porter, M., Lodish, H. F. 1980. Mutants of vesicular stomatitis virus blocked at different stages in maturation of the viral glycoprotein. *Cell* 21:417–27