REVIEW ARTICLE

Acylation of viral and eukaryotic proteins

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INTRODUCTION

Post-translational modification has long been recognized as a way in which the properties of protein molecules may be subtly altered after synthesis of the polypeptide chain is complete. Amongst the moieties most commonly encountered covalently attached to proteins are acetyl and formyl groups, phosphate groups and carbohydrates. These modifications have been a source of study for many years, but in the last decade it has become apparent that the binding of lipids to proteins is also widespread and certainly of great importance to the correct functioning of the organism.

The first protein reported to contain covalently attached fatty acid was purified on the basis of its solubility in organic solvents and has been termed myelin proteolipid (Folch & Lees, 1951). It was considered for some time that this phenomenon was restricted to the unusual circumstances pertaining to the myelin sheath. However, over the past decade it has become apparent that this is not the case and acyl proteins are widespread both in eukaryotic cell membranes and in viruses. It is my intention to review here the current state of our knowledge of these systems. This seems a particularly appropriate time as the area of research has taken on an exciting new aspect with the realization that acylation is a dynamic process which may be as much akin to phosphorylation as it is to glycosylation, in so far as the lipid group may be only transiently attached to the polypeptide chain in response to an extracellular stimulus. This article will concentrate on acylation in viral systems where both structural and transforming proteins have covalently bound fatty acids. Since the enzymes involved in this process are present in the host cell, where they are involved in the modification of eukaryotic proteins, some discussion of these will also be included. However, limitations of space preclude a consideration of acylation of prokaryotic proteins (briefly reviewed by Schmidt, 1983) or of those polypeptide chains which have fatty acids linked through phosphatidylinositol to the C-terminal carboxyl group (reviewed in detail by Low *et al.*, 1986; Cross, 1987).

The linkage of palmitic acid and myristic acid to proteins

The addition of lipid to proteins normally occurs through one of two mutually exclusive pathways giving rise to the two major fatty acids detected in proteins, palmitic acid (n-hexadecanoic acid, $\text{CH}_3[\text{CH}_2]_{14}\text{CO}_2\text{H}$) or myristic acid (n-tetradecanoic acid, $\text{CH}_3[\text{CH}_2]_{12}\text{CO}_2\text{H}$). Whilst the former of these is a common metabolite in eukaryotic cells, the latter is present in only small amounts [appreciably less than

Fig. 1. Chemical structures of myristate and palmitate linked to amino acids in acylated polypeptide chains

stearic acid (C_{18})], suggesting a special role for myristoylation which would not be served by the addition of any other lipid.

When myristate has been detected covalently bound to protein molecules it is almost always linked through an amide bond to an N-terminal glycine residue (Fig. 1), whereas palmitate is linked through an ester or thioester bond to serine (or threonine) or cysteine respectively

Abbreviations used: VSV, vesicular stomatitis virus; MuLV, murine leukaemia virus; RSV, Rous sarcoma virus; SV40, simian virus 40; SFV, Semliki Forest virus.

Table 1. Proteins of viral origin containing covalently bound palmitate

Protein	Source	Glycosylated	Reference
RNA viruses			
E1, E2 glycoprotein	Sindbis virus (togavirus)	+	Schmidt <i>et al.</i> , 1979; Schmidt, 1982
HA ₂	Influenza virus (orthomyxovirus)	+	Schmidt, 1982; Schmidt & Lambrecht, 1985
G glycoprotein	Vesicular stomatitis virus (rhabdovirus)	+	Schmidt & Schlesinger, 1979
E1 and E2 glycoproteins	Semliki Forest virus (alphavirus)	+	Schmidt, 1982; Berger & Schmidt, 1984b
F ₀ and F ₁ fusion proteins	Newcastle disease virus (paramyxovirus)	+	Chatis & Morrison, 1982
E2 glycoprotein gp 150 precursor protein	Mouse hepatitis virus (coronavirus)	+	Niemann & Klenk, 1981; Schmidt, 1982; van Berlo <i>et al.</i> , 1987
Retroviruses			
p21 ^{ras}	Rat sarcoma virus	—	Sefton <i>et al.</i> , 1982, and numerous other references mentioned in the text
DNA viruses			
T antigen	Simian virus 40 (papovavirus)	—	Klockmann & Deppert, 1983a,b
Hexon	Adenovirus 12	—	R. J. A. Grand & P. H. Gallimore (unpublished work)
E1B 19 kDa tumour antigen	Adenovirus 12 and adenovirus 5	—	Grand <i>et al.</i> , 1985; McGlade <i>et al.</i> , 1987

(Fig. 1). The ester and thioester linkages are labile in the presence of base (e.g. 0.1 M-KOH in methanol) and neutral 1 M-hydroxylamine (Magee *et al.*, 1984), whereas the amide bonds are much more stable.

Acylation of viral structural proteins

RNA viruses. The first observations that certain viral proteins had a covalently attached lipid component were made by Schmidt *et al.* (1979) and Schmidt & Schlesinger (1979, 1980). In these studies virally infected cells grown in tissue culture were metabolically labelled with [9,10(n)-³H]palmitate and then subjected to polyacrylamide-gel electrophoresis. Using this technique, which has since provided many of the data in this area of research, it was shown that certain glycoproteins present in the enveloped RNA Sindbis and vesicular stomatitis viruses (VSV) contained covalently attached lipid. Only the E1 and E2 proteins in Sindbis virus and the G glycoprotein in VSV were radiolabelled and these to differing extents. The E1 and G glycoproteins contained between 1 and 2 mol of fatty acid per mol of protein (Schmidt *et al.*, 1979; Schmidt & Schlesinger, 1980) whereas the E2 spike glycoprotein contained between 5 and 6 mol of lipid (Schmidt *et al.*, 1979). The radioactivity could not be released by extraction with detergents or organic solvents (for example chloroform, methanol, acetone, hexane and acetone) but could be cleaved from the polypeptide chain by incubation at 23 °C for 30 min in 0.1 M-KOH in methanol. G.l.c. analysis showed that most of the fatty acid was recovered as methyl palmitate, although small amounts of methyl oleate and methyl stearate were also present (Schmidt *et al.*, 1979; Schmidt & Schlesinger, 1980). The acylation of glycoproteins has been observed subsequently in all other enveloped RNA viruses which have been examined (see Table 1). For example, the E1

and E2 glycoproteins of Semliki Forest virus (SFV) contain comparable amounts of fatty acid to those in Sindbis virus, which is perhaps to be expected since both fall into the general classification of togaviruses (Schmidt, 1982). Not all of the spike glycoproteins in the enveloped RNA viruses have a lipid component; for example, in various strains of influenza virus only the small subunit of haemagglutinin (HA₂) contains fatty acid whilst the larger haemagglutinin subunit (HA₁), the neuraminidase and the matrix (M) protein do not (Schmidt, 1982; Schmidt & Lambrecht, 1985).

The role of acylation of viral spike glycoproteins is not clear. However, a number of studies, particularly of the VSV G protein, have provided some relevant information. G protein from the Indiana strain of VSV comprises 511 amino acids (including a 16-residue signal peptide) (Rose & Gallione, 1981) and has two *N*-glycosylation sites (Asp-178 and -338) and an acylation site (Cys-489) (Rose *et al.*, 1984). The protein is orientated in the lipid bilayer of the virus so that a hydrophilic 29-residue *C*-terminal peptide to which the lipid is attached protrudes into the inside of the viral membrane, whilst the majority of the polypeptide chain forms the spike on the outside of the virus; a 20-residue hydrophobic transmembrane region joins the two (Fig. 2) (reviewed by Pal *et al.*, 1987).

Using pulse-labelling techniques it has been shown that protein acylation of G protein is a post-translational event, occurring approx. 15 min after completion of synthesis of the polypeptide chain (Schmidt & Schlesinger, 1980). It also occurs about 5–10 min before the carbohydrate moiety is converted to an endo- β -*N*-acetylglucosamidase-resistant form (i.e. concomitantly with glycosylation). Since both processes seem to occur at the same time it has been suggested that acylation of viral

Fig. 2. Examples of the integration of acylated proteins into cell membranes

Amino acid sequences around the sites of acylation are shown in the single letter code. Numbers indicate the relevant residue numbers, with the *N*-terminal amino acid taken as 1. The following proteins are shown: (a) p21^{N-ras} (Taparowsky *et al.*, 1983); (b) VSV G protein (Rose & Gallione, 1981); (c) the human transferrin receptor (Schneider *et al.*, 1984); (d) p60^{src} (Takeya *et al.*, 1982); (e) the catalytic subunit of cyclic AMP-dependent protein kinase (Carr *et al.*, 1982).

proteins takes place in the Golgi complex where glycosylation is presumed to occur (Schmidt & Schlesinger, 1980) (this is discussed in greater detail below).

Whilst it is tempting to suggest that the lipid moiety is essential for the transport and localization of protein in the membrane and thus for viral viability, it has been noted that G proteins of other VSV serotypes are not acylated (Gallione & Rose, 1983; Kotwal & Ghosh, 1984) and therefore it is probable that this post-translational modification is not the sole factor determining membrane constitution but perhaps plays a rather more subtle role, possibly within the host cell.

The widespread acylation of one or more membrane proteins in enveloped RNA viruses has encouraged workers to investigate the structural polypeptides in other virus types. The VP4 capsid protein in all four genera of the picornavirus family has been shown to have myristate covalently bound through an amide linkage to the glycine residue at the *N*-terminus (see below and Table 2). The binding of lipid to the protein was initially indicated by metabolic labelling of poliovirus-infected Hela cells with [³H]myristate or [³H]palmitate followed by SDS/polyacrylamide-gel electrophoresis of the purified virus or protein immunoprecipitated with specific

antibodies (Chow *et al.*, 1987). However, crystallographic studies of the Mahoney strain of type 1 poliovirus have confirmed myristoylation at the *N*-terminus of VP4 and suggested how it is involved in stabilization of the virus particle. In the viral protein shell, five copies of the *N*-terminal portion of VP3 molecules intertwine to produce a parallel β -structured tube. On the inner surface of the shell this is juxtaposed to five segments of β -sheet formed by the *N*-termini of VP4 proteins. The carbon chains of the myristate moieties form a 'cradle' to support the *N*-termini of the VP3 molecules, interacting with amino acid side chains of both VP3 and VP4 (Chow *et al.*, 1987).

Retroviruses. In retroviruses the *gag* genes encode structural proteins present in the viral core, which are believed to play a role in virus budding and maturation. The precursor polypeptide, Pr65^{gag} of mouse type C retroviruses (e.g. murine leukaemia virus, MuLV) is myristoylated at the *N*-terminus with lipid attached to the *N*-terminal glycine residue through an amide bond (Henderson *et al.*, 1983; Schultz & Oroszlan, 1983). The protein (p15^{gag}) obtained by proteolysis of the precursor retains the same group. Similarly, modified unglycosylated *gag* polypeptides have been shown to be present in type B and D retroviruses (summarized in Table 2) but not in an avian retrovirus (Rous sarcoma virus, RSV) (Schultz and Orošzlan, 1983). Loss of the *N*-terminal glycine residue, either through deletion or mutations (to alanine) of the MuLV Pr65^{gag} or p15^{gag}, gives rise to proteins which neither associate with the host cell membrane nor allow the production of virus particles (Rein *et al.*, 1986). It is reasonable to suppose that in the

early stages of virion assembly the modified Pr65^{gag} molecules are transported to and concentrated on the inner face of the cell membrane where they are then possibly involved in orientating other viral structural proteins. The widespread occurrence of myristoylation in unglycosylated *gag* polypeptides is given further credence by the detection of similar modifications in four *gag-onc* fusion proteins, p29^{gag-ras} of rat sarcoma virus, p85^{gag-fes} of feline sarcoma virus and p120^{gag-abl} of Abelson leukaemia virus (Schultz & Oroszlan, 1984) and p85^{gag-mos} (Gallick & Arlinghaus, 1984).

DNA viruses. Acylation of structural proteins in DNA viruses has also been observed. VP2 polypeptides of polyoma and SV40 (both members of the papova virus family) have covalently bound myristate which is attached at the *N*-terminal glycine residue (Streuli & Griffin, 1987; B. Griffin, personal communication). Similarly the pre S1 hepatitis B surface antigen has an *N*-terminal myristic acid when expressed in Cos cells or when present in frog oocytes (Table 2) (Persing *et al.*, 1987). Whether these modifications have structural implications, as is the case for VP4 in the picornaviruses, or are more important in the formation of the virus within the host cell is not clear at present (see below).

Acylation of transforming proteins of cellular and viral origin

As stated above, several retroviral *gag-onc* fusion proteins are acylated at their *N*-termini due to retention of a portion of the viral *gag* gene. However, a number of other tumour antigens have also been isolated containing myristate (p60^{src}) or palmitate (p21^{ras}, adenovirus E1B

Table 2. Proteins of viral origin containing covalently bound myristate

Protein	Virus	Reference
RNA viruses		
VP4 capsid protein	Poliovirus, bovine enterovirus, foot and mouth disease virus, encephalomyocarditis virus, human (picornavirus family)	Chow <i>et al.</i> , 1987; Paul <i>et al.</i> , 1987
VP0 and P1 precursor proteins		
Retroviruses		
p15 ^{gag}	Rauscher and Moloney murine leukaemia viruses	Henderson <i>et al.</i> , 1983; Schultz & Oroszlan, 1983
Pr65 ^{gag} precursor proteins	Murine leukaemia virus, feline leukaemia virus, baboon endogenous virus	Schultz & Oroszlan, 1983; Rein <i>et al.</i> , 1986
Pr77 ^{gag} precursor proteins	Mouse mammary tumour virus, Mason-Pfizer monkey virus	Schultz & Oroszlan, 1983; Rhee & Hunter, 1987
p27 (F) protein	HTLV-III/LAV	Allan <i>et al.</i> , 1985
p120 ^{gag-abl}	Abelson leukaemia virus	Schultz & Oroszlan, 1984
p29 ^{gag-ras}	Rat sarcoma virus	Schultz & Oroszlan, 1984
p110 ^{gag-fes}	Feline sarcoma virus	Schultz & Oroszlan, 1984
p85 ^{gag-fos}	Moloney sarcoma virus	Gallick & Arlinghaus, 1984
p60 ^{src}	Rous sarcoma virus	Sefton <i>et al.</i> , 1982, and numerous other references mentioned in the text
DNA viruses		
Pre S1 protein	Hepatitis B	Persing <i>et al.</i> , 1987
VP2 protein	Polyoma virus, Simian virus 40 (papova virus)	Streuli & Griffin, 1987

19 K protein and SV40 T antigen). Whilst the retroviral oncogene products are of cellular origin it is convenient to discuss them here with the DNA virus tumour antigens as, together, they form the most carefully and deeply studied group of acylated proteins. (It should be borne in mind that the observations made regarding the acylation of *v-oncs* apply equally to *c-oncs*.)

Rous sarcoma virus p60^{src}. The viral and cellular *src* gene products (*v-src* and *c-src*) are tyrosine protein kinases of 526 and 530 amino acids respectively (reviewed by Krueger *et al.*, 1983; Hunter, 1987) and each contains one molecule of myristic acid linked to the *N*-terminus of the polypeptide chain (Sefton *et al.*, 1982; Garber *et al.*, 1983, 1985; Buss & Sefton, 1985). Acylation involves loss of the initiating *N*-terminal methionine residue followed by covalent attachment of fatty acid to glycine (Kamps *et al.*, 1985; Garber *et al.*, 1985; Schultz *et al.*, 1985). Studies in which p60^{src} has been mutated so that myristoylation does not occur have shown that acylation is essential for membrane localization. In cells infected with mutant RSV less than 12 % of the non-myristoylated p60^{src} was found in the membrane fraction whereas over 80 % of the wild type protein was membrane bound (Buss *et al.*, 1986). In those viruses where mutation was limited to the *N*-terminus, loss of the acylation site was accompanied by loss of transforming ability (Cross *et al.*, 1984; Kamps *et al.*, 1985, 1986). However, no loss of the protein's ability to bind to the cellular polypeptides p50 and p90 (Garber *et al.*, 1985) nor of tyrosine kinase activity was observed, although some differences in the extent of phosphate incorporation into substrates could be detected (Buss *et al.*, 1986; Kamps *et al.*, 1986). For example, vinculin, enolase, lactate dehydrogenase, p36, p50 and p81 are all considered to be possible substrates for p60^{src} *in vivo* and all are phosphorylated to a greater or lesser extent by the mutant kinase (Buss *et al.*, 1986). It might be expected that those substrates associated with the membranes would be phosphorylated to a lesser extent by the cytosolic mutant p60^{src}. This is not invariably the case however, since the phosphorylation of vinculin (present on the plasma membrane) is appreciably increased in cells containing the mutant kinase whereas the phosphorylation of cytoplasmic lactate dehydrogenase is decreased. Buss *et al.* (1986) have argued that the difference between transforming and non-transforming p60^{src} could lie in the ability of the former to phosphorylate a so-far-undetected membrane-bound protein substrate, this ability being lost when the mutant polypeptide remains in the cytoplasm. However, the recent observations (reviewed by Hunter, 1987) that the position of tyrosine phosphorylation (residues 416 or 527) rather than whether or not the protein possesses phosphotyrosine at all is of importance in determining the ability of p60^{src} to transform. Thus it is possible that phosphorylations by cytoplasmic or membrane-bound p60^{src} could appear very similar when analysed by polyacrylamide-gel electrophoresis even though they might be occurring at different sites in the target proteins. Also the disruptive effect of the introduction of a novel protein (myristoylated p60^{src}) into the cell membrane could have an appreciable effect on the morphology of the infected cell.

Murine sarcoma virus p21^{ras}. Like p60^{src}, but unlike the products of many other *v-onc* genes, the *ras* p21 poly-

peptides are not fusion proteins and therefore do not contain parts of the viral structural (*gag*) proteins; they do, however, contain covalently bound fatty acid. *ras* genes are ubiquitous in eukaryotes comprising a family of three functional members (*H-ras*, *K-ras* and *N-ras*) (reviewed by Lowy & Willumsen, 1986; Barbacid, 1987). They encode polypeptide chains of 188 or 189 amino acids; to each chain is bound one molecule of palmitate (Sefton *et al.*, 1982; Chen *et al.*, 1985; Buss & Sefton, 1986). The mammalian and viral p21 proteins (*c-ras* and *v-ras*) are highly conserved throughout much of their amino acid sequence with the only appreciable divergence occurring in the *C*-terminal 20 residues. However, in all *ras* genes for which sequence data are available a cysteine at amino acid 186 has been conserved and this has been shown to be the site of lipid binding (Willumsen *et al.*, 1984a,b). Mutation of residue 186 leads to a loss of palmitate binding ability, and loss of membrane localization (Willumsen *et al.*, 1984a,b), with the p21 remaining in the cytosol after subcellular fractionation. Interestingly, mutants which are not acylated are defective for transformation of NIH 3T3 cells, failing to induce morphological change.

Proteins homologous to the mammalian *ras* p21 polypeptides are expressed in yeast (*RAS* 1, *RAS* 2 and *YPT1*) and are required for cell viability and the initiation of the mitotic cell cycle (reviewed by Laporte, 1985). Sequence analysis has shown the presence of two adjacent cysteine residues close to the *C*-termini of these proteins and recent data suggest that the retention of at least one of them is essential for protein palmitoylation, membrane location and optimal growth of the organism (Deschenes & Broach, 1987; Molenaar *et al.*, 1988). In the case of all of the *ras* p21 species the palmitoylated cysteine residue is followed by two hydrophobic amino acids (valine, leucine or isoleucine). These might be important recognition signals for the acyltransferase involved in the acylation reaction. Alternatively, they may represent the very short area of amino acid sequence which is incorporated into the lipid bilayer (Fig. 2).

Several lines of evidence suggest that *ras* p21, like a number of other acylated polypeptides, is not a typical membrane protein. Firstly, it is relatively hydrophilic with no sequence of hydrophobic amino acids which can serve as a transmembrane region. Secondly, the crystal structure of *H-ras* p21 reveals it to be a globular molecule with the *C*-terminal α -helix projecting slightly (de Voos *et al.*, 1988). Thirdly, electron-micrographic (Willingham *et al.*, 1980) and biochemical (Grand *et al.*, 1987) data have shown the protein to be located on the cytoplasmic side of the plasma membrane with almost all of its structure available to the cytoplasm. It appears therefore that in the case of p21, as with some of the myristoylated proteins discussed above, acylation is a method by which a hydrophilic, essentially cytoplasmic, enzyme may be juxtaposed to the cell membrane where it is most advantageously placed to perform its function. A recent report (Lacal *et al.*, 1988) has presented data which have shown that the substitution of an *N*-terminal myristate moiety for the *C*-terminal palmitate has no appreciable effect on the biological activity of p21. Whether this means that acylation, in this case, is of importance only for the addition of a hydrophobic group anywhere on the protein molecule is not clear at present but it certainly suggests a lack of specificity in the lipid requirement for the functioning of *ras* p21.

ras p21 has appreciable sequence homology with the α subunits of guanine nucleotide binding proteins (G proteins) (Masters *et al.*, 1986) and like them is capable of binding and hydrolysing guanine nucleotide. This similarity is particularly marked in the nucleotide binding sites but is virtually non-existent in the C-terminal 40 amino acids of the protein, including the acylation site at residue 186. However, it has recently been shown that some of the G protein α subunits have myristate present at the N-terminus (see below) (Schultz *et al.*, 1987; Buss *et al.*, 1987). Thus in the two sets of membrane-bound polypeptides (*ras* p21 and G protein α subunit) there appears to have been both amino acid conservation to produce a guanine nucleotide binding domain and divergence to produce membrane-anchoring sites, in the case of p21 a C-terminal palmitate acylation site and in the case of α subunits an N-terminal myristate acylation site. Buss *et al.* (1987) have suggested that in the GDP-bound state the G protein α subunit interacts with the β and γ subunits, the former of which functions as an integral membrane protein. On hydrolysis of the nucleotide triphosphate to GDP (in response to a signal from an acceptor protein) the α subunit dissociates from β and γ but is retained close to the membrane by the N-terminal myristate. Dissociation of the complex could then trigger a cytoplasmic effector molecule. It is possible that *ras* p21 may behave in a similar fashion except that the interaction sites for receptor and effector proteins are present within the single polypeptide chain (Grand *et al.*, 1987; Trahey & McCormack, 1987; Grand, 1988). It is interesting to consider what functional differences in the two sets of proteins exist which have required the adoption of two different modes of membrane localization. Certainly, there is evidence to suggest that a significant proportion of p21 within the cell exists in an unacylated cytoplasmic form at any particular time (see section below) and that there is a relatively rapid turnover of bound lipid. Such a dynamic mode of action might necessitate membrane binding through palmitate rather than myristate. On the whole, however, this seems unlikely since rapid myristoylation occurs with a number of other proteins (see below). An alternative explanation would be that the particular fatty acid bound determines the site of membrane incorporation and the palmitate attached to p21 is required for interaction with the membrane and/or a membrane-bound receptor protein.

It is not clear what effect the presence or absence of the fatty acid moiety has on the nucleotide binding and hydrolysis properties of p21, since removal of the lipid obviously removes the protein from its environment close to the membrane. However, Calés *et al.* (1988) have shown that the GTPase activity of H-*ras* p21 carrying a Gly→Val mutation at position 12 is similar in the acylated and unacylated forms, whereas the GTPase activity of the membrane-bound wild type H-*ras* p21 is about five times that of unacylated protein. The significance of this observation is not clear, although it does suggest that the presence of the lipid moiety has importance for enzymic activity as well as for subcellular localization.

Simian virus 40 T antigen. SV40 T antigen is a multifunctional protein of M_r about 90000 comprising 708 amino acids. It is located mainly in the nucleus in transformed cells (over 95%) where it performs most of its functions although a small proportion is present in the plasma membrane and on the cell surface (Deppert &

Walter, 1976, 1982; reviewed by Butel & Jarvis, 1986). Metabolic labelling studies have indicated that the membrane-bound large T is acylated with palmitate attached through a hydroxylamine-sensitive ester or thioester bond (Klockmann & Deppert, 1983a,b; Henning & Lange-Mutschler, 1983). Fractionation of plasma membranes prepared from SV40 transformed cells has shown that the acylated T antigen is associated with the NP-40-resistant plasma membrane lamina from which it can only be released by rigorous treatment (Klockmann & Deppert, 1983b). The small proportion of large T which is NP-40 soluble is not acylated. Two regions of the polypeptide chain are important for membrane localization. Firstly, it has been suggested that the 28 kDa C-terminal fragment, which is not acylated, may become associated with internal and plasma membranes due to the presence of a 15-amino-acid hydrophobic sequence (residues 568–582) [or possibly through the scheme of Sharma *et al.* (1985) outlined below]. Secondly, larger peptides of 42 and 56 kDa (stretching towards the N-terminus of the T antigen) are also present in the membrane but are acylated (Klockmann *et al.*, 1984). That portion of the SV40 T antigen which has covalently bound lipid behaves as a transmembrane protein with a proportion of its sequence present on the outside of the cell (Klockmann & Deppert, 1985). Presumably, as a result of this the plasma membrane large T is responsible for the induction of SV40 tumour-specific transplantation immunity (see Rigby & Lane, 1983, and Tevethia, 1986, for review).

One problem posed by the distribution of SV40 antigen is what factor(s) determine the relative proportions of protein transported to the nucleus and the plasma membrane. Nuclear localization seems to be governed by the presence of an amino acid signal sequence comprising residues 127–134 (Kalderon *et al.*, 1984). However, peptide mapping studies indicate very few if any structural differences between the nuclear and membrane species of large T (reviewed by Butel & Jarvis, 1986). It is unlikely that palmitoylation is the signal responsible for plasma membrane location, since the modification occurs some time after protein synthesis (Magee & Courtneidge, 1985; McIlhinney *et al.*, 1985; see below). Sharma *et al.* (1985) have suggested that all of the protein is synthesized on free polyribosomes in the cytoplasm (Butel & Jarvis, 1986) and subsequently transported to the nucleus under the influence of the nuclear recognition signal. A small proportion of T antigen is then associated with the nuclear membrane. During cell division this forms a number of small vesicles, which, after cytokinesis, fuse with various vesicular structures or the plasma membrane of the daughter cells. Acylation is suggested to occur just prior to, or following, fusion of the nuclear vesicles with plasma membrane lamina.

Adenovirus early region 1B 19 kDa protein. The transformation of rodent and human cells in tissue culture by DNA encoding the early region 1 (E1) of adenovirus 2 (5) and 12 is now very well documented (reviewed by Bernards & van der Eb, 1984; Gallimore *et al.*, 1985). Four major polypeptides are encoded by E1, the two related E1A products and two unrelated E1B proteins of molecular weights 19 and 54–60 kDa (depending on virus serotype) (reviewed by Grand, 1987). The smaller E1B polypeptide has been shown to be localized in the cell membrane (Persson *et al.*, 1982; Rowe *et al.*, 1983;

Grand & Gallimore, 1984) of virus-infected and transformed cells and to have lipid covalently bound in both adenovirus 12 (Grand *et al.*, 1985) and adenovirus 5 (McGlade *et al.*, 1987). However, there appear to be some differences between the acylation pathways in the two virus serotypes. Incorporation of both palmitate and myristate seems to take place (Grand *et al.*, 1985; McGlade *et al.*, 1987; R. J. A. Grand, unpublished work), but whilst the linkage in the adenovirus 12 protein is through an alkali-labile ester bond, that for the adenovirus 5 is through a stable amide bond. Reasons for this discrepancy are not clear at present but if the results obtained for the adenovirus 5 protein (i.e. palmitate covalently bound via an amide linkage; McGlade *et al.*, 1987) prove to be correct this will represent a novel pathway not observed in other systems. Similarly the presence of myristate which is not linked to an *N*-terminal glycine has been observed only rarely.

Although some of the adenovirus E1B 19 kDa protein has been located in the plasma membrane of both infected and transformed cells a significant proportion appears to be of nuclear origin (Grand & Gallimore, 1984; White *et al.*, 1984; McGlade *et al.*, 1987), mainly present in the nuclear envelope (White *et al.*, 1984). A number of adenovirus mutants have been isolated with lesions in the E1B 19 kDa region which on infection gives rise to the degradation of chromosomal DNA and enhanced cytopathic effects (large plaque, *lp*, mutants). White *et al.* (1984) have shown a correlation between the degradation of DNA and the absence of the 19 kDa protein from the nuclear envelope in mutant-virus-infected cells. It is interesting to note that there are superficial similarities between the cellular location of SV40 T antigen and adenovirus E1B 19 kDa protein in that both are nuclear and membrane proteins with a small amount of each present on the outside surface of the cell (Grand *et al.*, 1985; K. J. Smith & R. J. A. Grand, unpublished work). It is not clear whether that portion of the E1B protein which is in the nucleus is acylated or is unmodified as is the case for the SV40 T antigen. Certainly both polypeptides seem to exert their main influence on DNA within the nucleus and no function for the membrane component is obvious at present.

Mechanisms of protein acylation

As with viral proteins, acylation in eukaryotic cells

appears to take place via two quite distinct pathways, giving rise to polypeptide chains which have either ester-linked alkali-labile palmitate or amide-linked myristate (Olson *et al.*, 1985; Magee & Courtneidge, 1985; McIlhinney *et al.*, 1985). This is obviously to be expected since viruses possess no genes encoding acyl transferases and rely exclusively on the host cell enzymes for acylation.

Myristoylation. The first eukaryotic protein shown to contain covalently bound myristate was the catalytic subunit of cyclic AMP-dependent protein kinase prepared from bovine cardiac muscle (Carr *et al.*, 1982); the structure of this lipid-protein linkage is similar to that in almost all myristoylated derivatives which have since been demonstrated (a list of myristoylated eukaryotic proteins is provided in Table 3). The fatty acid is bound through a hydroxylamine-stable amide link to an *N*-terminal glycine residue, rendering the protein refractory to amino acid sequencing. From a consideration of the primary structures of known myristoylated proteins and an examination of the rates of acylation of synthetic peptides a consensus recognition sequence has been suggested which is required for acylation of proteins by myristoyl CoA:protein *N*-myristoyltransferase. The enzyme has a specific requirement for myristoyl-CoA (Towler & Glaser, 1986), produced from myristate by acyl-CoA synthetase in the presence of ATP. Before myristoylation can take place the initiator methionine at the *N*-terminus of most newly synthesized apomyristoyl proteins has to be removed (Towler *et al.*, 1987a). The myristoyltransferase, however, is not responsible for this cleavage reaction. On the basis of results obtained from experiments using a number of synthetic peptide substrates, Towler *et al.* (1987b) have shown that the enzyme has an absolute requirement for *N*-terminal glycine and a preference for substrates where the second residue is Asn, Gln, Ser, Val, Ala or Leu. A serine at amino acid 5 is essential for very high affinity binding of the substrate to the enzyme (Towler *et al.*, 1987a). Towler *et al.* (1988) have thus defined a core hexapeptide sequence (Gly-Asn-Ala-Ala-Ser-Ala) possessing a low K_m (2 μ M) which is indicative of high-affinity binding. There are few restrictions on amino acids occupying positions 3 and 4 but the serine at position 5 is probably hydrogen-bonded to a negative charge on the enzyme (Towler *et al.*, 1988). Restrictions on the amino acid sequence of the myristoyl-

Table 3. Eukaryotic cell proteins with covalently bound myristate

Protein	Source	Glycosylated	Reference
Insulin receptor α and β subunits	Human lymphocytes	+	Hedo <i>et al.</i> , 1987
G _s (stimulatory) protein α subunits	Human astrocytoma cell line	—	Buss <i>et al.</i> , 1987
Immunoglobulin heavy chain	Mouse B cell line	+	Pillai & Baltimore, 1987
NADH-cytochrome <i>b₅</i> reductase	Bovine liver	—	Ozols <i>et al.</i> , 1984
Vinculin	Chick embryo fibroblasts	—	Kellie & Wigglesworth, 1987; Burn & Burger, 1987
G _o protein α subunit	Bovine brain	—	Schultz <i>et al.</i> , 1987
p56 tyrosine protein kinase	LSTRA cells	—	Marchildon <i>et al.</i> , 1984; Voronova <i>et al.</i> , 1984
Catalytic subunit of cyclic AMP-dependent protein kinase	Bovine cardiac muscle	—	Carr <i>et al.</i> , 1982
Calcineurin B	Bovine brain	—	Aitken <i>et al.</i> , 1982

transferase substrate may be imposed outside the *N*-terminal hexapeptide however, since Towler *et al.* (1988) have noted the addition of basic amino acids to the *C*-terminus of the consensus sequence increases the affinity of binding to the enzyme whilst acidic residues destabilize the enzyme:substrate complex. These workers have also reported that a 'structural punctuation' around residues 10–12 is common to all the sequences of known myristoylated proteins when analysed by computer programme. Also inhibition of rat myristoyltransferase with short peptides has been considered indicative of an enzyme specificity past residue 9 (Glover *et al.*, 1988).

Protein myristoyltransferase has been purified to homogeneity from yeast (Towler *et al.*, 1987a) and partially purified from rat tissue (Towler *et al.*, 1988; Glover *et al.*, 1988). The former native enzyme is a single polypeptide chain of molecular mass 55 kDa. It has a pH optimum of 7.5–8.0, exhibits no requirement for metal cations and probably has a blocked *N*-terminus (Towler *et al.*, 1987a). A comparison of the myristoylation properties of the yeast and rat liver enzymes suggests that, although they are similar, they have slightly different specificities as evidenced by variations in K_m values obtained for the myristoylation of synthetic peptides (Towler *et al.*, 1988). These results probably reflect different requirements imposed by the substrates encountered *in vivo* in lower and higher eukaryotes. It is apparent from the ability of the purified yeast and partially purified rat and wheat germ enzymes to myristoylate a range of peptide substrates, albeit at different rates, that enzymes specific for each substrate protein are not present within the cell. This conclusion is supported by the fact that during the relatively straightforward purification procedure used only a single activity was detected at any stage (Towler *et al.*, 1987a, 1988).

Myristoyltransferase activity appears to be associated with cytoplasmic and membrane fractions in both yeast and mammalian cells (Towler & Glaser, 1986; Towler *et al.*, 1987a, 1988; Glover *et al.*, 1988). This is consistent with the view that myristoylation occurs on soluble polysomes during or immediately after protein synthesis (Buss *et al.*, 1984). A number of groups have shown that the addition of protein synthesis inhibitors to cell cultures immediately reduces the incorporation of myristate into proteins whereas there is an appreciable time lag before any effect on palmitoylation is observed (see below), indicating a significant temporal difference between the two forms of acylation (Buss *et al.*, 1984; McHinnery *et al.*, 1985; Magee & Courtneidge, 1985; Olson & Spizz, 1986). It is quite possible that myristoylation could occur before the completion of protein synthesis on the ribosome immediately after cleavage of the initiator methionine. This latter event has been shown to take place after the synthesis of the first 30–40 amino acids whilst the polypeptide is still ribosomally attached (Palmiter *et al.*, 1978). It is possible therefore that myristoylation could be triggered by the exposure of an *N*-terminal glycine, if it is followed by a sequence which can be recognized by the myristoyltransferase.

Whilst most cellular palmitoylated proteins are found in the membrane this is not the case for those with covalently bound myristate, a number of which are readily soluble and cytoplasmic (Magee & Courtneidge, 1985; McHinnery *et al.*, 1985; Olson *et al.*, 1985). Even those polypeptides which are membrane-associated generally do not have typical hydrophobic trans-

membrane regions (for example the α subunits of the G proteins). It appears therefore that myristoylation is a process whereby essentially cytoplasmic proteins/enzymes can become membrane-bound, thus locating them at their site of action.

Palmitoylation. The incorporation of palmitate molecules into polypeptide chains has not been studied to the same extent as myristoylation. However, a number of general points can be made. Firstly, there seems to be considerably less restriction placed on the site of acylation since both ester and thioester linkages have been reported and no consensus sequences have been observed. Secondly, the process is energy-dependent with a requirement for ATP. This is probably involved in the production of palmitoyl-CoA, which has been identified as the acyl donor species for palmitoylation (Berger & Schmidt, 1984a; Mack *et al.*, 1987). Thirdly, most of the proteins containing covalently bound palmitate are strongly anchored in the cell membrane (Olson & Spizz, 1986) (most of these proteins are listed in Table 4).

The lipid binding site on the VSV glycoprotein has been characterized in some detail (see above and Fig. 2) (Rose *et al.*, 1984). Studies of the acylation sites in the HLA-B and DR heavy chains (Kaufman *et al.*, 1984), the transferrin receptor (Jing & Trowbridge, 1987) and the HLA-D-associated invariant chain (Koch & Hammerling, 1986) suggest that in these eukaryotic systems a similar polypeptide sequence is involved. In all cases palmitate is covalently linked to a cysteine residue on the cytoplasmic side of the transmembrane region. The acylated cysteine is normally three to six amino acids away from the start of the hydrophobic domain, but with so few well-characterized proteins it is not possible to say what elements of the surrounding amino acid sequence are required for recognition by the fatty acyltransferase.

In an elegant study of the transferrin receptor Jing & Trowbridge (1987), using a site-directed mutagenesis, have identified Cys-62 as the major site of acylation. Interestingly, the replacement of this amino acid by a serine results in no incorporation of lipid, although transport to the cell membrane takes place in a manner similar to wild type. If serine can act as an acceptor amino acid in certain proteins (although this has never been unequivocally demonstrated), it suggests that there must exist in the cell acyltransferases specific for serine and cysteine residues. A fatty acyltransferase, capable of adding palmitic acid moieties onto SFV E1 and E2 glycoproteins, has been shown to be present in the rough endoplasmic reticulum (Berger & Schmidt, 1985); unfortunately, further characterization of the enzyme has not been undertaken to resolve whether it can utilize serine and/or cysteine residues as acceptor amino acids.

At least two acyl proteins appear to have autocatalytic properties such that incubation of the apo-polypeptide chain with acyl-CoA results in incorporation of lipid into the protein. When purified myelin proteolipid or bovine rhodopsin has been deacylated and then treated with palmitoyl-CoA in the absence of any added cellular extract, palmitate was incorporated into the protein, the substrate saturation curves for palmitoyl-CoA following typical Michaelis–Menten kinetics (Bizzozero *et al.*, 1987a; O'Brien *et al.*, 1987). Loss of catalytic activity was observed after treatment with proteases and the reaction was shown to be time-, temperature- and protein concentration-dependent. Acylation of myelin proteolipid is

Table 4. Eukaryotic proteins with covalently bound palmitate

Abbreviation: ND, not determined.

Protein	Source	Binding site	Molecules of lipid bound	Glycosylated	Reference
Myelin proteolipid protein	Rat brain	ND	Approx. 2	-	Folch & Lees, 1951
Myelin DM20	Rat brain	ND	ND	-	Agrawal <i>et al.</i> , 1982
Transferrin receptor	Human cell lines	Cys-62	Probably 1	+	Omary & Trowbridge, 1981 <i>a,b</i> ; Jing & Trowbridge, 1987
Nictonic acetylcholine receptor, α and β subunits	BC ₃ H1 muscle cell line	ND	ND	+	Olson <i>et al.</i> , 1984
Insulin receptor, α and β subunits	Human lymphocytes Human hepatoma cell line	ND	ND	+	Hedo <i>et al.</i> , 1987; Magee & Siddle, 1988
HLA-B and HLA-DR heavy chains	Human lymphoblastoid cell line	Cys close to the transmembrane region (residue 318 in the B7 heavy chain)	ND	+	Kaufman <i>et al.</i> , 1984
Ia-associated invariant chain	Mouse cell line	Cys-28	Probably 1	+	Koch & Hammerling, 1985
HLA-D-associated invariant chain	Human lymphoblastoid cell line	Cys-28	Probably 1	+	Koch & Hammerling, 1986
Gastric mucus glycoprotein	Rat mucus secretion	ND	> 20	+	Slomiany <i>et al.</i> , 1984
Sodium channel, α subunit	Rat brain cells	ND	ND	+	Schmidt & Catterall, 1987
Rhodopsin	Bovine retina	ND	ND	+	O'Brien & Zatz, 1984
Fibronectin	Human fibroblast cell lines	ND	ND	+	Lange-Mutschler, 1986
Ankyrin	Chicken embryo erythroid cells	ND	ND	-	Staufenbiel & Lazarides, 1986
Actin	<i>Dictyostelium</i>	ND	ND	-	Stadler <i>et al.</i> , 1985
Contact site A glycoprotein	<i>Dictyostelium</i>	ND	ND	+	Stadler <i>et al.</i> , 1984
Ligatin	Rat intestine	ND	1.4-1.7	-	Jakoi <i>et al.</i> , 1987
32 kDa herbicide-binding protein	<i>Spirodela</i>	ND	ND	-	Mattio & Edelman, 1987

relatively unspecific with regard to acyl donor species, with similar values being obtained for apparent K_m and V_{max} (41 μM and 115 pmol/min per mg of protein respectively) for palmitoyl-, stearoyl- and oleoyl-CoAs. When myristoyl-CoA has been used, however, lower rates of fatty acid incorporation into the protein were observed (Bizzozero *et al.*, 1987b). A similar autoacylation appears to take place with SFV protein E2 although extent of deacylation did not alter lipid binding (Berger & Schmidt, 1984b).

Palmitoylation may be considered a classic post-translational event (compared with myristoylation) since there is an appreciable time lag (up to 20 min) between the end of protein synthesis and the addition of fatty acid to the acceptor protein (Schmidt & Schlesinger, 1980). Not surprisingly the acylation step can occur for some time in the absence of protein synthesis (for example, Magee & Courtneidge, 1985; McIlhinney *et al.*, 1985; Magee *et al.*, 1987), although this may be more indicative of palmitate turnover than of a large pool of unacylated protein in the cell. Certainly the observation of Omary & Trowbridge (1981a) that palmitoylation of the transferrin receptor can take place up to 48 h after the addition of a protein synthesis inhibitor suggests a dynamic aspect to fatty acid attachment (see below).

The site of palmitoylation (or the palmitoyl acyltransferase) is still somewhat open to question. Most studies to resolve this point have been carried out using virally infected cells (in particular with VSV), so it is not clear to what extent the results obtained can be extrapolated to eukaryotic proteins in normal cells. It does, however, seem probable that in general terms similar sites of modification are involved. Both viral and membrane glycoproteins are translated on membrane-bound ribosomes, with glycosylation commencing in the endoplasmic reticulum, where, at least in the case of VSV G protein, high-mannose oligosaccharides are transferred onto the polypeptide chain as it is inserted into the endoplasmic reticulum membrane. Initial stages of oligosaccharide trimming take place in the endoplasmic reticulum (Grinna & Robbins, 1979; Bischoff & Kornfeld, 1983) with the addition of palmitate occurring just before the final stages of oligosaccharide processing (Schmidt & Schlesinger, 1980). If this were the case it would be reasonable to conclude that acylation takes place in the *cis* or medial part of the Golgi stack (Dunphy *et al.*, 1981; Featherstone *et al.*, 1985). Similar conclusions have been reached on the basis of a study of the infection of baby hamster kidney cells with SFV. However, more recent studies, using very short labelling times, have indicated that acylation occurs in regions of the rough endoplasmic reticulum before translocation to the Golgi stack for completion of oligosaccharide trimming (Berger & Schmidt, 1985). The occurrence of palmitoylation in the endoplasmic reticulum is certainly more consistent with the location of a fatty acid CoA ligase, required for the synthesis of palmitoyl-CoA, which is known to be present in the endoplasmic reticulum (Bell & Coleman, 1980).

The temporal and spatial relationship of palmitoylation to other post-translational modifications has been studied in a number of systems. As discussed above, the addition of oligosaccharide occurs concomitantly with palmitoylation in viral proteins and unglycosylated polypeptide chains do not become acylated (Schmidt *et al.*, 1979). Post-translational proteolytic cleavage, however,

takes place at relatively late times, certainly after addition of fatty acid and sugar moieties. For example, palmitoylation of the p62 precursor protein of SFV occurs before it is cleaved to produce the acylated E2 and E1 polypeptides (Berger & Schmidt, 1984a). Similarly, fatty acid is incorporated into the Sindbis virus glycoprotein precursor PE2 a few minutes before cleavage to E2 (Schmidt & Schlesinger, 1980). Whether palmitoylation of cellular (as opposed to viral) polypeptides also takes place in the rough endoplasmic reticulum is not clear, but it seems likely that the virus utilizes the enzymes present in the host cell which would normally be involved in processing cellular polypeptides. Acylation of the *ras* p21 occurs through the palmitoyl-CoA intermediate, as is the case for the viral polypeptides (Grand *et al.*, 1987) and the acyltransferase has been shown to be present in a crude membrane fraction although more precise localization has not been undertaken (Grand *et al.*, 1987). From studies comparing the relationship of palmitoylation to glycosylation it has been concluded that acylation takes place either in the endoplasmic reticulum or *cis* region of the Golgi stack, certainly for the α subunit of the sodium channel (Schmidt & Catterall, 1987) and the 1a-associated invariant chain (Koch & Hammerling, 1985), such that the addition of fatty acid occurs after the initiation of glycosylation but before its completion (Koch & Hammerling, 1985). There appear to be some differences observed in the acylation of unglycosylated polypeptides in different systems. For example, blocking glycosylation with tunicamycin still allows palmitoylation to proceed for the HLA-D-associated invariant chain (Koch & Hammerling, 1986) but not for the α subunit of the sodium pump (Schmidt & Catterall, 1987). Similarly, the addition of fatty acid to the transferrin receptor can take place in the absence of glycosylation (Omary & Trowbridge, 1981a). It seems likely that these differences may be indicative of the particular behaviour of specific cellular proteins, for example polypeptides which may be involved in transport to and from the membrane (i.e. recycled like the transferrin receptor).

Dynamic aspects of protein acylation

Post-translational addition of lipids to polypeptide chains has been thought to be a process akin to glycosylation and one whose sole role was the permanent localization of the protein in a lipid bilayer in the cell. Whilst it seems likely that this may be the case for many of the acylated proteins listed in Tables 3 and 4, it appears that in other cases fatty acid addition is reversible, suggesting a dynamic aspect to this modification.

Initial observations that palmitoylation of certain proteins could occur many hours after the inhibition of protein synthesis originally suggested a very late post-translational modification (Omary & Trowbridge, 1981a), but it has become apparent that it is more probably indicative of a reversible modification taking place. Magee *et al.* (1987) have shown, using pulse-chase experiments, that the half-life of the palmitate moiety attached to N-*ras* p21 is very much shorter than that of the polypeptide chain. This, taken in conjunction with the presence of an appreciable pool of unacylated cytoplasmic p21, suggests that acylation may only take place in response to an external stimulus. Similar results have been obtained in studies of ankyrin in erythrocytes, where it was observed that the half-life of the lipid label was very much shorter than that of the polypeptide chain

(Staufenbiel, 1987). It was concluded that relatively rapid acylation-deacylation could be important in changing 'the strength and molecular geometry of the membrane-cytoskeleton linkage' (Staufenbiel, 1987). Interestingly, mature erythrocytes do not contain extensive endoplasmic reticulum or Golgi stacks and therefore it is likely that re-acylation would take place in the plasma membrane.

Rapid myristoylation has also been observed in a number of systems in response to external stimulus. For example, when macrophages were incubated in the presence of bacterial lipopolysaccharide, incorporation of myristate into proteins of approx. 68 kDa and 45 kDa was observed after 30 min (Aderem *et al.*, 1986). Treatment with phorbol esters produced a similar effect as well as the myristoylation of an additional protein of 50 kDa. The authors concluded that the acylation reaction was specific in two ways: firstly, only myristate was incorporated, and secondly different sets of proteins could be modified in response to different stimuli. The 68 kDa polypeptide has been identified as one of the major substrates for protein kinase C (Aderem *et al.*, 1988) and a sequence of events has been proposed to explain the observed distributions of acylated and phosphorylated 68 kDa polypeptide chains. Stimulation by lipopolysaccharide results in the relatively rapid incorporation of myristate into the protein which is translocated close to or into the membrane where it is proximal to protein kinase C which phosphorylates it. Release of the phosphorylated 68 kDa protein into the cytoplasm occurs possibly as a result of demyristoylation (Aderem *et al.*, 1988). It is considered that the myristoylation response to lipopolysaccharide may be involved in a subsequent transduction of a protein kinase C-dependent stimulus. It is possible that that 82 kDa polypeptide which is myristoylated in an HL60 cell line in response to phorbol ester treatment is identical to the protein kinase C substrate observed in macrophages, but this will have to await further investigation (Malvoisin *et al.*, 1987). Interestingly, of two HL60 lines treated with phorbol ester differentiation was induced in one together with protein myristoylation whilst treatment of the other, a Blast II variant, did not lead to differentiation or myristoylation.

Functions of acylation

Although the addition of fatty acids to protein was first noted in 1951 (Folch & Lees, 1951) it is only in the last decade that it has been recognized how widespread and important such a modification is. However, specific functions for myristoylation and palmitoylation are still uncertain. Obviously the addition of a relatively large hydrophobic moiety to a protein will increase its overall hydrophobicity somewhat, and the hydrophobicity of the particular area to which it is attached appreciably. Based on this self-evident fact and the observation that most acylated proteins are membrane-bound it has been assumed that the acylation is simply a way for the cell to target polypeptide chains to the membrane. This is certainly true in a number of cases. For example, myristoylation is essential for membrane localization of p60^{src} and in the absence of this modification the protein remains in the cytoplasm. Indeed, when the first 14 amino acids of p60^{src} are fused to other, normally cytoplasmic, polypeptides (α globin and F36 of Fujinami sarcoma virus) the heterologous fusion proteins are

directed to the plasma membrane (Pellman *et al.*, 1985). As described above, the *N*-terminal region of the protein contains the recognition signal for the acyltransferase involved but is not likely itself to be involved in membrane binding. Therefore it seems likely that myristate is primarily responsible for localization. The fact that a relatively rare fatty acid (myristate) is involved in this modification rather than the much more common palmitate or stearate is obviously of significance and raises the possibility that myristate may be essential in determining which membrane is the target (the plasma membrane rather than other lipid bilayers present in the cell) or even which particular site on that membrane is the required location. If this were the case it would suggest that there would be recognition signals in the membrane (for example, particular proteins) which could serve as binding sites for myristoylated polypeptide chains. It is likely, however, that even this hypothesis is an oversimplification since it must be borne in mind that a number of proteins containing covalently bound *N*-terminal myristate are cytoplasmic (for example, the catalytic subunit of cyclic AMP-dependent protein kinase), which indicates that other factors besides the presence of the fatty acid determine membrane localization.

In the case of myristate bound to viral proteins the results of Chow *et al.* (1987) have shown that the fatty acid can play a purely structural role, being involved in the interaction of the myristoylated VP4 protein with VP3 within the virus capsid. However, it has been suggested that it could also be important in the initiation of capsid virus assembly, localizing the precursor P1 polypeptide in the membrane (Paul *et al.*, 1987) as is the case for the *gag* protein of Moloney murine leukaemia virus (Rein *et al.*, 1986). In viruses, therefore, it seems that myristate can play a role in mediating protein-protein interactions within the virus and in organizing, both temporally and spatially, some of the polypeptide chains during the later stages of infection. Again the specificity for myristate is noteworthy, suggesting that very demanding criteria are imposed on the choice of lipid bound.

The binding of palmitate to proteins poses even more problems with regard to function than does that of myristate. Whilst palmitoylation of *ras* p21 is relatively straightforward, being akin to myristoylation, in that binding of the fatty acid is obviously important in localizing and retaining an otherwise hydrophilic protein in the plasma membrane, fatty acid binding to other proteins, both viral and cellular, appears to have no obvious explanation. For example, the transferrin receptor has an acylation site very close to its hydrophobic transmembrane region. Mutation of that amino acid abolishes fatty acid binding but does not appear to effect transport to, or expression on, the surface of the cell (Jing & Trowbridge, 1987). As mentioned above, in most of the proteins for which data are available the site of palmitate attachment is very close to an existing hydrophobic region of the polypeptide chain. Reasons for this apparent duplication are not clear, although a number of explanations have been advanced (Kaufman *et al.*, 1984). Firstly, it is possible that palmitate is important in determining the specific site or strength of binding to the membrane; whilst this is possible for some proteins the observation that only a limited number of MHC species have covalently bound lipid would argue against this possibility. Secondly, it has been suggested

that acylation of cysteine side chains inhibits undesirable disulphide bond formation; again this seems unlikely since the studies of Jing & Trowbridge (1987) have demonstrated that Cys-62 is the site of acylation in the transferrin receptor but that Cys-67 is unmodified, and if disulphide bond formation were going to occur the latter residue seems to be as likely a candidate as the former. A third possibility is that acylation in eukaryotic cell proteins like the MHC antigens and the transferrin receptor is fortuitous and takes place because they have features in common with other polypeptides where palmitoylation does play a role. This also seems unlikely because of the common and presumably conserved structural feature, seen in a number of viral and eukaryotic proteins, of an acylated cysteine residue situated just a few amino acids on the cytoplasmic side of the transmembrane region. Fourthly, it is possible that palmitoylation may play a role during glycosylation either in the transport from the endoplasmic reticulum to the Golgi stack or within this latter organelle. In support of this argument is the observation of Koch & Hammerling (1985) that blocking of fatty acylation interferes with the later stages of carbohydrate processing of the Ia-associated invariant chain.

The VSV G protein also has palmitate bound to a cysteine residue on the cytoplasmic side of a membrane-spanning region, as has been observed for the eukaryotic proteins described above. Acylation is not necessary for glycosylation or for membrane localization, suggesting that the lipid plays a rather subtle role, possibly in viral assembly or in maintaining stability of the intact virus (Pal *et al.*, 1987). The fact that in only certain serotypes of VSV is the G protein acylated is rather difficult to rationalize at present.

Concluding remarks

As Sefton & Buss (1987) have observed, the binding of fatty acids to polypeptide chains is neither very rare nor very common. Appreciably fewer proteins are acylated than are glycosylated or phosphorylated, and yet at least one structural protein in most virus types has covalently attached lipid. Similarly, perhaps up to 100 cellular polypeptides can be distinguished containing covalently bound myristate or palmitate. One of the more puzzling aspects of the acylation phenomena with which one is faced, after even only a cursory examination of the published data, is the diversity of possible functions which this co-translational or post-translational modification might fulfil. It obviously plays a role in mediating protein-lipid interactions in targeting and binding polypeptide chains to different types of membrane, but it also seems to be important in mediating protein-protein interactions (certainly in virus capsids and most probably within the eukaryotic cell). The recent observations that acylation is a dynamic process is of particular importance because it may provide at least one relatively straightforward mechanism by which a protein can be relocated within the cell in response to an external (or internal) stimulus. This is particularly exciting since some of those proteins which have been shown to be acylated play central roles in the cellular second messenger system, such as the catalytic subunit of cyclic AMP-dependent protein kinase, *ras* p21, a major substrate for protein kinase C and the B subunit of calcineurin. The very elegant work of Towler and Glaser and their colleagues has gone a very long way towards elucidation of the

actual mechanism of myristoylation; it is hoped that a similar application of perspicacity and effort will demonstrate, in the near future, how acylation fits into the mechanism of actions of second messengers.

Powerful molecular genetic techniques are now available such that loci which may be the sites of lipid addition can be mutated. Complementary studies using cell biology should allow the placement of these mutated genes or proteins into different cell types or even sites within the cell. A combination of these methods should provide additional data that will allow us to define more precisely the importance of acylation to individual proteins.

I am most grateful to Dr. Phillip Gallimore (Department of Cancer Studies, University of Birmingham) for critical reading of this manuscript and to Anthony Davies (Department of Cancer Studies, University of Birmingham) for creating the computer graphics. I should also like to thank Deborah Williams for excellent secretarial assistance and the Cancer Research Campaign, London, for financial support.

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