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Myristic Acid, a Rare Fatty Acid, Is the Lipid Attached to the Transforming Protein of Rous Sarcoma Virus and Its Cellular Homolog

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The lipid bound to $p60^{src}$, the transforming protein of Rous sarcoma virus, has been identified by gas and thin-layer chromatography as the 14-carbon saturated fatty acid, myristic acid. The protein can be labeled biosynthetically with either [^{3}H]myristic acid or [^{3}H]palmitic acid. Incorporation of [^{3}H]myristic acid was noticeably greater than incorporation of [^{3}H]palmitic acid. All of the [^{3}H]myristic acid-derived label in $p60^{src}$ was present as myristic acid. In contrast, none of the radioactivity derived from [^{3}H]palmitic acid was recovered as palmitic acid. Instead, all ^{3}H incorporated into $p60^{src}$ from [^{3}H]palmitic acid arose by metabolism to myristic acid. The cellular tyrosine kinase, $p60^{c\text{-}src}$ also contains myristic acid. By comparison of the extent of myristylation of $p60^{v\text{-}src}$ with that of the Moloney murine leukemia virus structural protein precursor, $Pr65^{gag}$, we estimate that greater than 80% of the molecules of $p60^{v\text{-}src}$ contain one molecule of this fatty acid. Myristylation is a rare form of protein modification. $p60^{v\text{-}src}$ contains 10 to 40% of the myristic acid bound to protein in cells transformed by Rous sarcoma virus and is easily identified in total cell lysates when [^{3}H]myristic acid-labeled proteins are separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Comparison of the amount of [^{3}H]myristic acid-labeled $p60^{src}$ in total cell lysates and in immunoprecipitates suggests that immunoprecipitation with rabbit anti-Rous sarcoma virus tumor sera detects ca. 25% of the $p60^{src}$ present in cells.

Eucaryotic proteins undergo at least two distinct types of lipid modification. A number of transmembrane cell surface glycoproteins contain the 16-carbon saturated fatty acid, palmitic acid (16, 18, 19, 24). Palmitic acid is found within the domains of these proteins, which interact with the lipid bilayer, and is attached through a linkage which has the chemical stability of an ester bond. In the G protein of vesicular stomatitis virus, the palmityl moiety is probably linked through a thioester bond to cysteine (15, 17).

In contrast, at least two cytoplasmic cellular enzymes, the catalytic subunit of cyclic AMP (cAMP)-dependent protein kinase (5) and the protein phosphatase calcineurin B (1), and the $p15^{gag}$ internal structural proteins of all of the mammalian leukemia viruses (13, 21) contain the 14-carbon saturated fatty acid, myristic acid. This lipid is attached through an amide bond to the alpha-amino group of the amino-terminal glycine residues in these proteins (1, 5, 13).

The transforming protein of Rous sarcoma virus (RSV), $p60^{src}$, also contains covalently attached lipid (24). It was initially observed that $p60^{src}$ could be labeled biosynthetically with [^{3}H]palmitic acid (12, 24). However, the [^{3}H]palmitic acid-derived label differed from that in other proteins which contain palmitic acid in that it remained attached to the protein after treatment with mild alkali (24). This suggested that the lipid was linked to the protein through an amide bond and in this sense resembled protein-bound myristic acid. Here we demonstrate that the fatty acid which modifies $p60^{src}$ is in fact myristic acid and characterize in detail the lipid present in the viral and cellular forms of this tyrosine-specific protein kinase.

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MATERIALS AND METHODS

Cells, viruses, and antisera. The preparation of chicken embryo cell cultures and transformation by Schmidt-Ruppin RSV of subgroups A and D (SR-RSV-A and SR-RSV-D) and Prague RSV of subgroup C (PR-RSV-C) have been described previously (22). $p60^{c\text{-}src}$ and $p60^{v\text{-}src}$ were isolated with rabbit anti-RSV-tumor serum (22). Goat anti-$p15^{gag}$ serum was used to isolate the viral precursor protein, $Pr65^{gag}$.

Labeling with [^{3}H]palmitic acid, [^{3}H]myristic acid, and [^{35}S]methionine. 9,10-[^{3}H]palmitic acid (23.5 Ci/mmol) in toluene (New England Nuclear Corp.) or 9,10-[^{3}H]myristic acid (12.9 Ci/mmol) in ethanol (very generously provided by A. Schultz and S. Oroszlan) were dried in sterile air and dissolved in dimethyl sulfoxide. The complete medium for biosynthetic labeling with these fatty acids was as described previously (24). Cells were labeled with 0.1 mCi of [^{35}S]methionine per ml in methionine-free Dulbecco-Vogt modified Eagle medium containing 10% dialyzed calf serum.

Immunoprecipitation and gel electrophoresis. Lysis and immunoprecipitation of $p60^{src}$ from chicken cells in RIPA buffer was as described previously (22). Lysates of cells labeled with [^{3}H]myristic acid were prepared by the addition of electrophoresis sample buffer directly to a washed cell monolayer. Samples were passed through a 27-gauge needle to shear DNA and then loaded onto 15% polyacrylamide gels (22).

Partial proteolytic mapping. Partial proteolytic mapping was performed in gel pieces with 25 ng of *Staphylococcus aureus* V8 protease as described previously (6, 24).

Gas chromatography. $p60^{src}$ were eluted from a preparative sodium dodecyl sulfate (SDS)-polyacrylamide gel as described before (2). The protein and 50 μg of carrier immunoglobulin G were precipitated with 15% trichloroacetic acid and washed with ethanol and ethanol-ethyl ether

(1:1). The dried precipitate was allowed to reflux for 5 h at 90°C in 2 M HCl in 83% methanol (24). Released ^{3}H-methyl esters of fatty acids were extracted into petroleum ether, dried, and mixed in hexane with the methyl esters of myristic and palmitic acids. Samples were analyzed in a Hewlett-Packard 5711A chromatograph equipped with a gas effluent splitter. Separation was achieved with an ethylene glycol succinate column at 140°C, with a nitrogen flow rate of 40 cm^3/min. Fractions to be analyzed for radioactivity were collected at 1-min intervals by condensation of the gas effluent in Pasteur pipettes and counted in Aquasol.

Analysis of the fatty acid derived from [^{3}H]palmitic acid. p60^{src} labeled with [^{3}H]palmitic acid was isolated by immunoprecipitation and purified by SDS-polyacrylamide gel electrophoresis (24). The protein was eluted from the gel, precipitated with trichloroacetic acid, and washed as described above. Fatty acids were then released by hydrolysis in 6 N HCl for 16 h at 110°C under N_2. The hydrolysate was extracted twice with toluene, and the organic phase was dried with N_2. The extracted fatty acids were dissolved in toluene and analyzed by ascending thin-layer chromatography on KC_{18} reversed phase plates (Whatman, Inc.) in acetic acid-acetonitrile (1:1) (20). Commercial [^{3}H]palmitic acid and [^{3}H]myristic acid were used as markers. The developed plate was dried, dipped in 2-methylnaphthalene containing 0.4% diphenyloxazole, and exposed to presensitized film at −70°C.

RESULTS

Myristic acid is the fatty acid attached to p60^{src}. RSV-transformed cells were labeled for 2 h with either 1 mCi of [^{3}H]palmitic acid or 0.2 mCi of [^{3}H]myristic acid (Fig. 1), and p60^{src} was isolated by immunoprecipitation. The incorporation of [^{3}H]myristic acid into p60^{src} was ca. 100-fold greater than the incorporation of [^{3}H]palmitic acid. The serum used to isolate p60^{src} also recognizes gp37^{env}, the small structural glycoprotein of RSV. gp37^{env} was labeled with [^{3}H]palmitic acid but was not labeled detectably with [^{3}H]myristic acid. The great differences in incorporation of the two fatty acids suggested that p60^{src} might contain a myristyl rather than a palmityl group. We therefore examined directly the identity of the lipid in p60^{src}.

To obtain sufficient ^{3}H-labeled p60^{src} for this analysis, we made use of a fortuitous observation. Comparison of the [^{3}H]myristic acid-labeled proteins in total cellular lysates from uninfected chicken cells and chicken cells infected with either SR-RSV-A or PR-RSV-C revealed a prominent ^{3}H-labeled 60-kilodalton (kDa) protein present in the infected

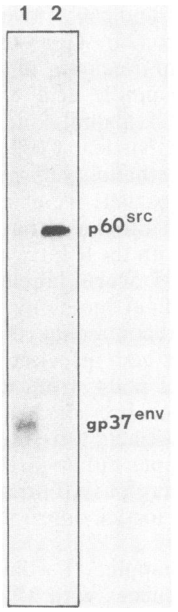

FIG. 1. Comparison of the incorporation of [^{3}H]palmitic and [^{3}H]myristic acid into p60^{src}. p60^{src} was isolated by immunoprecipitation from biosynthetically labeled cells, analyzed by SDS-polyacrylamide gel electrophoresis, and visualized by fluorography. Exposure time was 28 days. Lanes: 1, [^{3}H]palmitic acid-labeled cells; 2, [^{3}H]myristic acid-labeled cells.

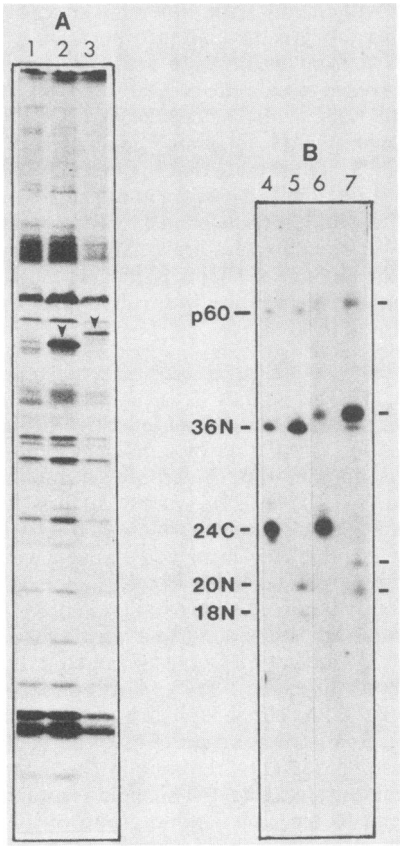

FIG. 2. (A) [^{3}H]myristic acid-labeled proteins in cell lysates. Cells labeled with [^{3}H]myristic acid were lysed directly in electrophoresis sample buffer and analyzed by SDS-polyacrylamide gel electrophoresis. Labeled proteins were visualized by fluorography. Exposure time was 7 days. The 60-kDa proteins are indicated with arrows. Lanes: 1, uninfected cells; 2, SR-RSV-A-infected cells; 3, PR-RSV-C-infected cells. (B) Comparison by partial proteolysis of [^{35}S]methionine-labeled p60^{src} and the [^{3}H]myristic acid-labeled 60-kDa protein from cell lysates. Authentic [^{35}S]methionine-labeled p60^{src} was isolated by immunoprecipitation. Duplicate cultures were labeled with [^{3}H]myristic acid and lysed as in A. Proteins were digested with 25 ng of *S. aureus* protease, and radioactive peptides were visualized by fluorographic exposure for 30 days. The strain-specific 36-, 20-, and 18-kDa fragments which contain the N terminus of p60^{src} and the common C-terminal 24-kDa peptide are indicated. Lanes: 4, SR-RSV-A-infected cells, [^{35}S]methionine-labeled p60^{src}; 5, SR-RSV-A-infected cells, [^{3}H]myristic acid-labeled 60-kDa protein; 6, PR-RSV-A-infected cells, [^{35}S]methionine-labeled p60^{src}; 7, PR-RSV-A-infected cells, [^{3}H]myristic acid-labeled 60-kDa protein.

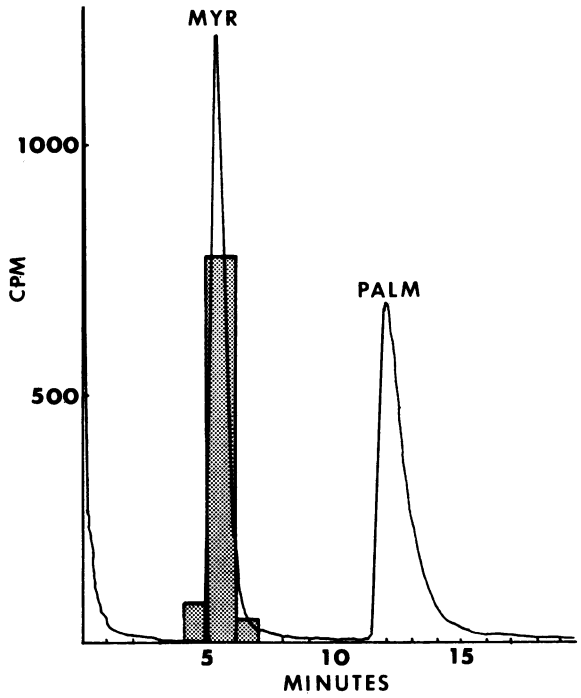

FIG. 3. Analysis of the ^{3}H-lipid released from p60^{src} by gas chromatography. p60^{src} isolated from [^{3}H]myristic acid-labeled cell lysates was hydrolyzed and analyzed by gas chromatography as described in the text. Added authentic methyl myristate and methyl palmitate were detected by flame ionization (solid line). Radioactivity (hatched bars) was quantified by scintillation counting of samples collected at 1-min intervals. A background of 32 cpm has been subtracted.

cells (Fig. 2A). The ^{3}H-labeled 60-kDa proteins comigrated precisely with the p60^{src} proteins that are encoded by these two strains of RSV and which differ slightly in apparent molecular weight (data not shown; 3). This suggested that the 60-kDa proteins might be p60^{src}. Partial proteolytic digestion revealed that the fragments from the prominent myristate-labeled 60-kDa bands isolated directly from lysates of cells transformed by SR- or PR-RSV matched exactly the fragments containing the amino terminus from authentic p60^{src} labeled with either [^{35}S]methionine (Fig. 2B) or [^{3}H]myristic acid (data not shown) isolated by immunoprecipitation. The absence of ^{3}H in the 24-kDa carboxy-terminal fragments demonstrated that the observed radioactivity resulted from incorporation of fatty acid and not from label which had been degraded and reutilized as amino acids. No other significant ^{3}H-labeled fragments were observed. We therefore concluded that the 60-kDa band in the lysates was p60^{src} and that contamination of it with other cellular myristate-containing proteins was minimal.

^{3}H-labeled p60^{src} was isolated by simple fractionation of [^{3}H]myristic acid-labeled cell lysates on an SDS-polyacrylamide gel. The protein was eluted from the gel and hydrolyzed with methanolic HCl. Gas chromatography revealed that essentially 100% of the radioactivity released from [^{3}H]myristic acid-labeled p60^{src} coeluted with methyl myristate and not with methyl palmitate (Fig. 3).

Analysis of the radioactive fatty acid in p60^{src} from cells labeled with [^{3}H]palmitic acid. p60^{src} can be labeled, albeit with low apparent efficiency, by [^{3}H]palmitic acid. Even though it was clear that all of the radioactivity present in p60^{src} isolated from cells labeled with [^{3}H]myristic acid was in fact myristic acid, it remained possible that p60^{src} contained both myristic acid and palmitic acid. We therefore examined whether the incorporation of [^{3}H]palmitic acid into p60^{src} reflected the presence of a palmityl group in the protein or arose simply from catabolism of the palmitic acid to myristic acid and subsequent incorporation of the metabolized precursor. p60^{src} is difficult to distinguish among the many labeled proteins present in lysates from [^{3}H]palmitic acid-labeled cells (data not shown). p60^{src} of SR-RSV-A therefore was isolated by immunoprecipitation from cells labeled overnight with [^{3}H]palmitic acid. The protein was excised from a preparative gel and hydrolyzed for 16 h at 110°C with 6 N HCl, and the released fatty acids were analyzed by thin-layer chromatography (Fig. 4). All of the incorporated radioactivity comigrated with a bona fide [^{3}H]myristic acid marker. None comigrated with a [^{3}H]palmitic acid marker. We conclude that all of the labeling of p60^{src} with [^{3}H]palmitic acid in chicken cells arises from conversion of the precursor to [^{3}H]myristic acid and that the protein does not contain palmitic acid.

Most molecules of p60^{src} contain myristic acid. We determined the amount of myristate in p60^{src} by comparing the amount of radioactive lipid incorporated into p60^{src} with the amount of lipid incorporated into the precursor to the p15^{gag} protein of Moloney murine leukemia virus (MLV), Pr65^{gag}. Pr65^{gag} was selected because its extent of myristylation was known. p15^{gag}, which is derived from Pr65^{gag}, contains 1 mol of myristic acid per mol of protein (13, 21). Because we wished to compare p60^{src} and Pr65^{gag} synthesized within the same cell, we superinfected mouse cells already transformed by RSV (SR-3T3 cells) with Moloney MLV. These cells were labeled for 5 h with [^{35}S]methionine or [^{3}H]myristic acid, and p60^{src} and Pr65^{gag} were isolated from the same cellular lysate (Fig. 5). When the incorporated radioactivity was corrected to reflect the fact that Pr65^{gag} contains 3 methionine residues (25), whereas p60^{src} contains 11 (10, 26), p60^{src} was found to contain 84% as much [^{3}H]myristic acid as did Pr65^{gag}. We predict that there is one site for myristate attachment to p60^{src} and that most p60^{src} molecules possess this modification.

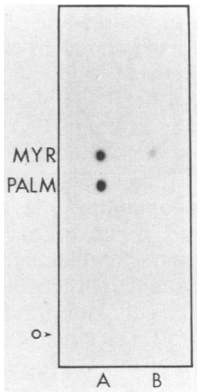

FIG. 4. Analysis of radioactive fatty acids in p60^{src} labeled biosynthetically with [^{3}H]palmitic acid. p60^{src} labeled with [^{3}H]palmitic acid was isolated and hydrolyzed with HCl as described in the text. The released fatty acids were analyzed by ascending chromotography on reversed phase thin-layer plates. [^{3}H]palmitic acid (PALM) and [^{3}H]myristic acid (MYR) standards and the fatty acid in p60^{src} were detected by fluorography. Exposure was for 10 days. Lanes: A, [^{3}H]palmitic acid and [^{3}H]myristic acid standards; B, fatty acid released from p60^{src}.

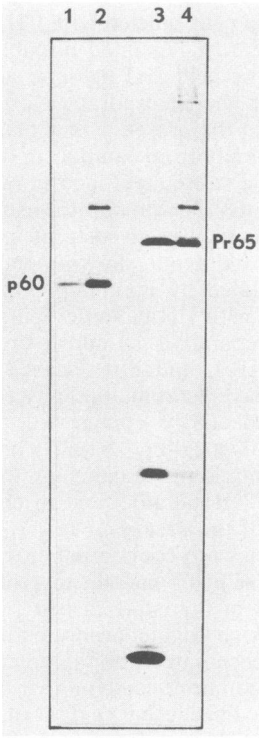

FIG. 5. Comparison of incorporation of [^{3}H]myristic acid into p60^{src} and Pr65^{gag}. SR-3T3 cells infected with Moloney MLV were labeled for 5 h with [^{3}H]myristic acid or [^{35}S]methionine, and p60^{src} and Pr65^{gag} were isolated by immunoprecipitation. The fluorograph was exposed for 3 days. [^{35}S]methionine-labeled p60^{src} and Pr65^{gag} contained 307 and 342 cpm, respectively. [^{3}H]myristic acid-labeled p60^{src} contained 137 cpm, and Pr65^{gag} contained 666 cpm. Lanes: 1, [^{3}H]myristic acid-labeled cells, antitumor serum; 2, [^{35}S]methionine-labeled cells, antitumor serum; 3, [^{3}H]myristic acid-labeled cells, anti-p15^{gag} serum; 4, [^{35}S]methionine-labeled cells, anti-p15^{gag} serum.

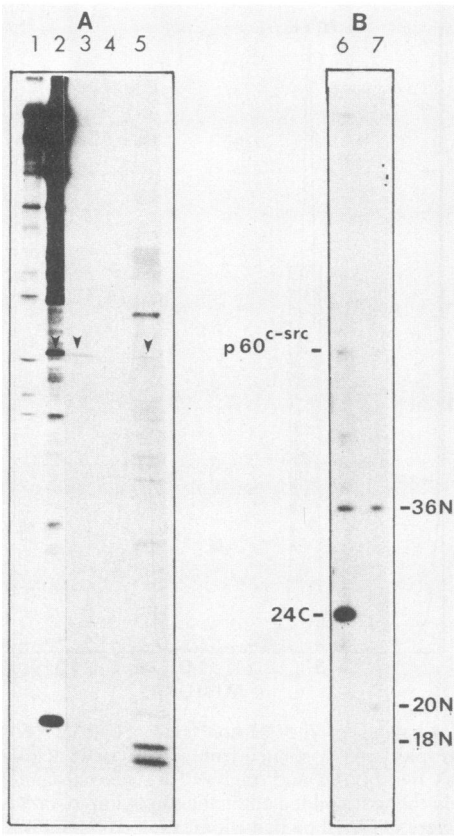

FIG. 6. (A) p60$^{c\text{-}src}$ labeled with [^{3}H]myristic acid. p60$^{c\text{-}src}$ was isolated with rabbit anti-RSV tumor serum. The fluorograph was exposed for 1 day for [^{35}S]methionine-labeled proteins and for 7 days for [^{3}H]myristic acid-labeled proteins. p60$^{c\text{-}src}$ is indicated by an arrowhead. Lanes: 1, [^{35}S]methionine-labeled cells, normal serum; 2, [^{35}S]methionine-labeled cells, antitumor serum; 3, [^{3}H]myristic acid-labeled cells, antitumor serum; 4, [^{3}H]myristic acid-labeled cells, normal serum; 5, [^{3}H]myristic acid-labeled cells lysed in RIPA buffer. (B) Partial proteolysis of [^{35}S]methionine- and [^{3}H]myristic acid-labeled p60$^{c\text{-}src}$. Digestion was with *S. aureus* protease, and the fragments were analyzed by gel electrophoresis. The fluorograph was exposed for 30 days. The amino-terminal 36-, 20-, 18-kDa peptides and the 24-kDa carboxy-terminal peptides are indicated. Lanes: 6, [^{35}S]methionine-labeled p60$^{c\text{-}src}$; 7, [^{3}H]myristic acid-labeled p60$^{c\text{-}src}$.

Estimation of the abundance of p60^{src} from [^{3}H]myristic acid-labeled cell lysates. Detection of [^{3}H]myristic acid-labeled p60^{src} directly in cell lysates provided an opportunity to determine for the first time the efficiency with which p60^{src} can be immunoprecipitated by anti-RSV tumor sera. Equal samples of [^{3}H]myristic acid-labeled cell lysates were either mixed directly with gel sample buffer or immunoprecipitated with an excess of anti-RSV tumor serum, and analyzed by SDS-polyacrylamide gel electrophoresis. By using two different antitumor sera, immunoprecipitates prepared from a lysate containing 162 cpm of [^{3}H]myristic acid-labeled p60^{src} were found to contain an average of 38 cpm (n = 3) of [^{3}H]myristic acid-labeled p60^{src}. Immunoprecipitation with these two antisera thus detects ca. 25% of the total p60^{src} present in the cell. Our previous determination of the abundance of p60^{src} (23) was therefore an underestimate. We now calculate that p60^{src} represents 0.06 to 0.08% of cellular protein.

The cellular homologue of p60^{src} contains myristic acid. Uninfected chicken cells contained a 60-kDa [^{3}H]myristic acid-labeled protein that was precipitated specifically by antisera which recognize p60$^{c\text{-}src}$ (Fig. 6A). This 60-kDa protein comigrated precisely with [^{35}S]methionine-labeled p60$^{c\text{-}src}$, and partial proteolysis produced the 36-, 20-, and 18-kDa myristylated fragments expected from p60$^{c\text{-}src}$ (24) (Fig. 6B). Careful inspection of lysates from [^{3}H]myristate-labeled uninfected cells also revealed a protein which comigrated with p60$^{c\text{-}src}$ (Fig. 6A, lane 5) and which yielded 36-, 20-, and 18-kDa fragments upon partial proteolysis (data not shown). The cellular tyrosine protein kinase, p60$^{c\text{-}src}$, is therefore one of the small number of myristylated proteins present in normal chicken cells.

DISCUSSION

A remarkably small number of cellular proteins contain a myristyl group. Although p60^{src} is a minor component of total cellular protein in RSV-transformed chicken cells (23), it is easily visible among the limited number of labeled proteins when total cell lysates from RSV-transformed cells labeled biosynthetically with [^{3}H]myristic acid are analyzed by gel electrophoresis. Even the cellular form of p60^{src} could be detected in [^{3}H]myristic acid-labeled cell lysates. Pr65^{gag}, P120$^{gag\text{-}abl}$, and P70$^{gag\text{-}fgr}$, the proteins encoded by the *gag* and fused *gag-onc* transforming genes of Moloney MLV,

Abelson MLV, and Gardner-Rasheed feline sarcoma virus, all contain a myristyl group (13, 20, 21; Buss, unpublished data) and are also readily detected in lysates from myristic acid-labeled infected cells (Buss, unpublished data). The ability to detect these proteins without precipitation is of significant value in studying their subcellular location (4).

Detection of [^{3}H]myristic acid-labeled p60^{src} in unfractionated cell lysates allowed us to determine directly that immunoprecipitation by rabbit anti-RSV tumor sera detects ca. 25% of the p60^{src} in cells. We can now correct our previous estimate of the abundance of p60^{src} in the cell (23) and calculate that p60^{src} constitutes between 0.06 and 0.08% of total cellular protein. The incomplete immunoprecipitation of p60^{src} by the rabbit anti-RSV tumor sera may be a reflection of (i) a low affinity of the antibody for the protein, (ii) inefficient solublization of the protein in RIPA buffer, or (iii) the existence of a subpopulation of p60^{src} molecules in which the determinants recognized by these sera are inaccessible.

p60^{src} contains a hydroxylamine-stable, presumably amide-linked myristic acid. Where on the polypeptide is this lipid attached? The catalytic subunit of the cAMP-dependent protein kinase, calcineurin, and p15^{gag} all contain a myristyl group linked to the alpha-amino group of an N-terminal glycine (1, 5, 13). We know that all of the fatty acid of p60^{src} is located within an amino-terminal 18-kDa domain (24) and that the amino terminus of p60^{src} is blocked to Edman degradation (K. Beemon, personal communication). The predicted amino acid sequences for p60$^{v\text{-}src}$ and p60$^{c\text{-}src}$ begin Met-Gly-Ser-Ser- (10, 27). The glycine at position 2 is likely to be the amino-terminal residue of mature p60^{src} because [^{35}S]methionine is not present in the 18-kDa fragment of p60^{src} which contains the N terminus of the mature protein (Fig. 2) (7). It is probable, given the precedents of the catalytic subunit of the cAMP-dependent protein kinase, calcineurin, and p15^{gag}, that the myristyl group is attached to the alpha-amino group of the N-terminal glycine of p60^{src}.

The lipid of the catalytic subunit of the cAMP-dependent protein kinase and p15^{gag} proteins has been analyzed chemically and found to consist exclusively of myristic acid (5, 13). Although p60^{src} can be labeled biosynthetically with both [^{3}H]palmitic acid and [^{3}H]myristic acid, the small amount of radioactivity incorporated into the protein in transformed chicken cells labeled with [^{3}H]palmitic acid results exclusively from metabolism of the palmitic acid to myristic acid. Thus, even though we have not characterized the lipid present in p60^{src} chemically, we consider it extremely likely that myristic acid is the only fatty acid present in the polypeptide. Because the catalytic subunit, p15^{gag}, calcineurin, and p60^{src} all appear to contain only myristic acid, a minor component of cellular fatty acids, rather than an assortment of long-chain acyl groups more representative of the composition of total cellular fatty acids, it would appear that the enzymes responsible for N-terminal acylation have a strict specificity for myristyl groups.

Like p60$^{v\text{-}src}$, p60$^{c\text{-}src}$ also contains a myristyl group. Myristylation therefore is not a unique aspect of the viral form of this enzyme. These two tyrosine protein kinases are thus modified by the same lipid as at least two other enzymes involved in protein phosphorylation and dephosphorylation, the cAMP-dependent protein kinase and calcineurin B. In light of the rarity of myristyl groups in protein, we doubt that this is coincidential. The myristic acid attached to the amino terminus of these enzymes may play some as yet unanticipated role in protein phosphorylation. Myristylation of the cAMP-dependent protein kinase and p60^{src} is not simply a reflection of sequence homology between these two protein kinases. Although both proteins contain homologous catalytic domains, they have no sequence homology in their amino-terminal regions, save for the N-terminal glycine. Not all protein kinases, however, contain myristic acid. Another cellular tyrosine kinase, the receptor for epidermal growth factor, contains neither myristic acid nor palmitic acid (24; our unpublished data).

Because almost every molecule of p60^{src} contains a myristyl group, this modification would appear to be important. A major fraction of p60^{src} is found in association with the plasma membrane (8, 14). For membrane-bound forms of p60^{src}, the myristyl group may stabilize this interaction of the protein with the lipid bilayer. Such stabilization may be important to enable the kinase to phosphorylate substrates crucial to cell transformation. The p60^{src} proteins of several variant avian sarcoma viruses appear to lack myristic acid, interact poorly with the plasma membrane, and produce only a partial cellular transformation (9, 11). However, stabilization of membrane-protein interactions is probably not the only function of this unusual type of protein modification. We have found that not all myristate-modified proteins are anchored to membranes (4). This is perhaps not surprising. The first protein found to contain a myristyl group, the catalytic subunit of the cAMP-dependent protein kinase, is a soluble protein after dissociation from the regulatory subunits of the enzyme (5). Clearly, cellular membranes are not the only site where myristate-containing proteins may be found. For soluble proteins, myristylation could play a role in protection of the protein from degradation, in interactions with other proteins, or in the formation of a hydrophobic nucleus around which the protein can fold in either a soluble or membranous environment.

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