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The First Seven Amino Acids Encoded by the v-*src* Oncogene Act as a Myristylation Signal: Lysine 7 Is a Critical Determinant

JOSHUA M. KAPLAN,[1,2,3]* GRAEME MARDON,[1]† J. MICHAEL BISHOP,[1,2,3] AND HAROLD E. VARMUS[1,3]

Departments of Microbiology and Immunology[1] and Biochemistry and Biophysics,[3] and The G. W. Hooper Research Foundation,[2] University of California, San Francisco, California 94143

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The transforming protein of Rous sarcoma virus, pp60$^{v\text{-}src}$, is covalently coupled to myristic acid by an amide linkage to glycine 2. Myristylation promotes the association of pp60$^{v\text{-}src}$ with cellular membranes, and this subcellular location is essential for transforming activity. The findings presented here, in conjunction with the previous reports of others, imply that the seventh amino acid encoded by v-*src* might be important in the myristylation reaction. Replacement of lysine 7 by asparagine greatly reduced the myristylation, membrane association, and transforming activity of pp60$^{v\text{-}src}$. In contrast, substitution of arginine at residue 7 had no effect on any of these properties of pp60$^{v\text{-}src}$. Addition of amino acids 1 to 7 encoded by v-*src* was sufficient to cause myristylation of a *src*-pyruvate kinase fusion protein. We conclude that the recognition sequence for myristylation of pp60$^{v\text{-}src}$ comprises amino acids 1 to 7 and that lysine 7 is a critical component of this sequence.

The v-*src* oncogene of Rous sarcoma virus (RSV) encodes a protein tyrosine kinase, pp60$^{v\text{-}src}$ (2). Association with membranes is among the properties of pp60$^{v\text{-}src}$ that are required for transforming activity (21). Variants of pp60$^{v\text{-}src}$ that are weakly associated with membranes have attenuated oncogenic activities (11, 20). Since the subcellular location of pp60$^{v\text{-}src}$ plays a pivotal role in transformation by RSV, we explored the mechanism by which pp60$^{v\text{-}src}$ associates with membranes.

Unlike most membrane proteins, pp60$^{v\text{-}src}$ is synthesized on soluble polyribosomes (22) and lacks a conventional hydrophobic signal sequence. These peculiarities are explained, in part, by the covalent coupling of pp60$^{v\text{-}src}$ to a 14-carbon saturated fatty acid, myristic acid (6, 38). Myristylation promotes the association of pp60$^{v\text{-}src}$ with membranes; nonmyristylated variants of pp60$^{v\text{-}src}$ are not associated with membranes and are consequently transformation defective (17, 33). Several other myristylated proteins have been described (1, 8, 9, 15, 25, 31, 34, 43), but pp60$^{v\text{-}src}$ is a rare example for which a function of the myristyl moiety is known. Myristylation is not, however, a sufficient explanation for the localization of pp60$^{v\text{-}src}$, because several unrelated myristylated proteins are cytosolic (28) and because myristylated yet cytosolic variants of pp60$^{v\text{-}src}$ have been described (6, 12).

Myristylation occurs during or immediately after synthesis of pp60$^{v\text{-}src}$ (6, 12). Upon removal of the N-terminal methionine residue from the nascent chain by an as yet uncharacterized amino peptidase (37), the resulting N-terminal glycine residue is coupled to myristic acid by a myristyl transferase, which has been partially purified and characterized by Towler et al. (47–50). This sequence of events has also been documented for other myristylated proteins (24, 27, 29, 53). The sequences required for myristylating pp60$^{v\text{-}src}$ are confined to its extreme N-terminus, the first 14 amino acids being sufficient (32). Of these 14 amino acids, only glycine 2 has been directly implicated in the myristylation process. *src* proteins containing glutamic acid 2 in place of glycine 2 are not cleaved by the amino-terminal peptidase, while those containing alanine 2 are cleaved but are not coupled to myristic acid by the transferase (7). Additional residues clearly play a role, because some *src* alleles, such as the NY316 v-*src* mutant, encode N-terminal Met-Gly, but the products are nonetheless poor substrates for the myristyl transferase (12). We report here that lysine 7 is an important component of the signal for myristylating pp60$^{v\text{-}src}$ and that the N-terminal seven amino acids of pp60$^{v\text{-}src}$ are sufficient to cause myristylation when transferred to a novel context.

MATERIALS AND METHODS

Materials. Cultures of primary chicken embryo fibroblasts (CEF) were prepared and propagated as described (52). Monoclonal anti-pp60$^{v\text{-}src}$ antibody 127 was provided by J. Brugge (23). Rabbit anti-chicken M1 pyruvate kinase (PK) antibody was provided by B. Roberts. Anti-pp60^{src} tumor-bearing rabbit (TBR) sera were prepared as described (3). A circularly permuted clone of the Prague A strain of RSV, pSL102, was provided by T. Parsons (4). A simian virus 40 (SV40) expression vector containing the chicken M1 PK cDNA, RL142PK10X, was provided by B. Roberts (16). Protease inhibitors were purchased from Sigma Chemical Co. [γ-^{32}P]ATP and L-[^{35}S]methionine were purchased from ICN. [^{3}H]myristic acid (22.4 Ci/mmol; 50 mCi/ml in dimethyl sulfoxide [DMSO]) was purchased from New England Nuclear Corp.

Metabolic labeling, immunoprecipitation, and subcellular fractionation. COS7 cells, Rat-1 cells, and CEF were labeled with L-[^{35}S]methionine for 18 h in Dulbecco modified Eagle medium containing 10% of the normal concentration of L-methionine, 10% dialyzed fetal calf serum, and 200 μCi of L-[^{35}S]methionine per ml. COS7 cells, Rat-1 cells, and CEF were labeled with [^{3}H]myristic acid for 18 h in medium with 10% fetal calf serum, 1% DMSO, and 500 μCi of [^{3}H]myristic acid per ml. Labeled cell extracts were prepared with lysis buffer (20 mM Tris chloride [pH 8.0], 150 mM NaCl, 1% Nonidet P-40, and 50 μg of soybean trypsin inhibitor, 50 μg of aprotinin, 20 μg of leupeptin, and 1 mg of fraction V bovine serum albumin per ml). Immunoprecipitations with TBR, polyclonal rabbit sera, and monoclonal antibodies were performed as described (19) with extracts

* Corresponding author.
† Present address: Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

containing equal amounts of trichloroacetic acid-precipitable radioactivity. Immune complex kinase reactions were performed as described (19). When these reactions were quantitated, the immune complexes were washed with lysis buffer supplemented with 0.2% sodium dodecyl sulfate and 300 mM NaCl and then counted in a Beckman LS7800 scintillation counter. Crude cell extracts, prepared by Dounce homogenization of hypotonically swelled cells, were fractionated into cytosol and membrane fractions by differential centrifugation, as described previously (19).

Oligonucleotide-directed mutagenesis. Codon 7 of v-*src* from the B77 strain of RSV was mutated by the method of Seeburg et al. (40). Nucleotide coordinates of RSV are as described by Schwartz et al. (39). Synthetic oligonucleotides partially complementary to nucleotides 7135 to 7155 of RSV were used to convert lysine 7 to either asparagine (AGCAA GAGCAATCCTAAGGAC) or arginine (AGCAAGAGC AGGCCTAAGGAC), the latter creating a novel *Stu*I restriction site at nucleotide (nt) 7138.

Isolation of the E3 *src* gene. The E3 *src* gene was isolated by selecting transforming back-mutants of a transformation-defective v-*src* allele, the E *src* gene (derived from the tdSF/LO302 strain of RSV), which we have previously described (30, 51). The DNA sequences of the E and E3 *src* genes reveal a single nucleotide deletion in codon 28 of E *src* and a compensating 89-nt deletion in E3 *src* that restores the reading frame (unpublished data of G. Mardon). The sequences deleted in E3 *src* normally encode amino acids 8 to 37 of pp60$^{v\text{-}src}$.

Construction, transfection, and assays of replicating recombinant RSV viruses. A circularly permuted clone of the Prague A strain of RSV, pSL102, was used to construct two plasmids, pPR-REP and pPRΔ*Bam*. pPR-REP is a pBR322 clone containing the 7-kilobase (kb) *Pvu*II (nt 8671)-*Sal*I (nt 6031) fragment of pSL102. pPRΔ*Bam* is a pBR322 clone containing the 4-kb *Sal*I (nt 6031)-*Bam*HI (nt 530) fragment of pSL102. pPRΔ*Bam*N7 and pPRΔ*Bam*R7 are identical to pPRΔ*Bam* except for the mutation introduced in codon 7 of v-*src*. The pPRΔ*Bam* subclones and pPR-REP were linearized with *Sal*I and ligated (200 μg/ml, 20 min, 25°C). The resulting ligation products were ethanol precipitated and then used to transfect CEF as described (14). Viral stocks were harvested 10 days after transfection. To confirm that the transfections had worked equivalently, the extent to which the cultures were infected was determined by the pp60$^{v\text{-}src}$ immune complex kinase assay. Viral stocks derived from transfections with pPRΔ*Bam*, pPRΔ*Bam*N7, and pPRΔ*Bam*R7 are referred to as wild-type, N7, and R7 RSV viruses, respectively. Viral stock titers were determined either by focus assays, as described previously (46), or by relative kinase titers. Relative kinase titers were determined by infecting equal numbers of CEF with equal volumes of virus-free medium or medium conditioned by cells infected with wild-type RSV, N7 RSV, or R7 RSV; at 48 h postinfection, before viral spread had occurred, infected cultures were lysed in lysis buffer, subjected to immune complex kinase assays, and quantitated as described above. Acute infection with wild-type RSV resulted in a 5- to 10-fold increase in pp60$^{v\text{-}src}$ kinase activity over uninfected controls.

Construction and transfection of a recombinant R7-PK gene. Nucleotides 7093 to 7146 of R7 v-*src* were subcloned into pUC9 between the *Bam*HI and *Eco*RI sites. The 100-nt *Hin*dIII-*Eco*RI fragment of the resulting plasmid was recombined with the 5.4-kb *Hin*dIII-*Eco*RI fragment of RL142PK10X. The resulting plasmid, pR7-PK, was used to transfect COS7 cells as described (14). COS7 cells were exposed to calcium phosphate precipitates containing 20 μg of either RL142PK10X or pR7-PK for 18 h and then subjected to shock with Tris-buffered saline (0.8% NaCl, 0.1% glucose, 0.038% KCl, 0.2% Tris chloride, 0.06% Tris base, and 4.5 mg of phenol red per liter) containing 25% DMSO for 2 min. Transfected COS7 cells were labeled for 18 h with either L-[^{35}S]methionine or [^{3}H]myristic acid starting 36 h after the transfection had begun.

TABLE 1. Amino-terminal sequence[a] and myristylation of various protein tyrosine kinases related to pp60$^{v\text{-}src}$

Protein tyrosine kinase	Amino acid sequence										Myristylation
Wild-type v-*src*	M	G	S	S	K	S	K	P	K	D	+
E3 *src*	M	G	S	S	K	S	K	T	A	A	+
NY316 *src*	M	G	S	S	K	S	N	R	S	G	−
N7 *src*	M	G	S	S	K	S	N	P	K	D	−
R7 *src*	M	G	S	S	K	S	R	P	K	D	+
R7-PK	M	G	S	S	K	S	R	G	I	N	+
lck	M	G	C	V	C	S	S	N	P	E	+
fyn	M	G	C	V	Q	C	K	D	K	E	?
hck	M	G	C	M	K	S	K	F	L	Q	?
yes	M	G	C	I	K	S	K	E	N	K	?
abl-1b	M	G	Q	Q	P	G	K	V	L	G	+
c-*fes*	M	G	F	S	S	E	L	C	S	P	?
lyn	M	G	C	I	K	S	K	G	K	D	?
fgr	M	G	C	V	F	C	K	K	L	E	?

[a] The sequence of amino acids 1 to 10 of the wild-type (38), E3 (our unpublished data), and NY316 (32) *src* proteins and the R7-PK, mouse *lck* (26), human *fyn* (41), human *hck* (35), human *yes* (44), human *abl*-1b (41; personal communication from P. Jackson), feline c-*fes* (36), human *lyn* (54), and human *fgr* (18) proteins are given in the single-letter amino acid code. Underlining indicates conservation of glycine 2 and lysine 7.

RESULTS

Codons 8 to 37 of v-*src* are not required for myristylation and transforming activity of pp60$^{v\text{-}src}$. We have isolated a naturally occurring variant of B77 v-*src*, E3 *src*, which has suffered an in-frame deletion of codons 8 to 37 (unpublished data of G. Mardon), creating the novel amino-terminal sequence depicted in Table 1. The deletion in E3 *src* overlapped the deletions in several mutants described previously by Pellman et al. (32). The most closely related of these mutants was NY316 *src*, in which codons 7 to 15 were replaced by four unrelated amino acids, creating the amino-terminal sequence shown in Table 1. The NY316 *src* protein is recognized by the amino peptidase but not by the myristyl transferase (12). Therefore, we expected that the E3 *src* gene would also encode a nonmyristylated and consequently transformation-defective *src* protein.

We tested the biological potency of E3 *src* by incorporating it into a Moloney murine leukemia virus expression vector and using the resulting plasmid to transfect Rat-1 fibroblasts. Rat-1 cells expressing the E3 *src* protein were morphologically transformed (data not shown) and were anchorage independent for growth (1% cloning efficiency in soft agar). Since virtually all known biologically active alleles of v-*src* encode myristylated *src* proteins, we expected that the E3 *src* protein would also be efficiently acylated. This was tested by comparing the incorporation of methionine (Fig. 1, lanes 1 to 3) and myristic acid (lanes 4 and 5) into the wild-type and E3 *src* proteins. The E3 and wild type *src* proteins were myristylated to the same extent.

We conclude from these experiments that amino acids 8 to 37 are not required for proper acylation and transforming

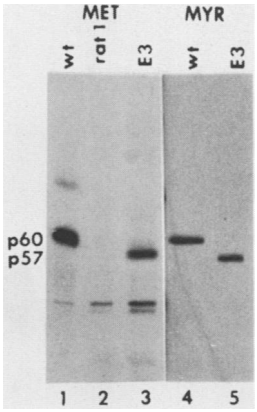

FIG. 1. Myristylation of the E3 *src* protein. Rat-1 cells expressing either the wild-type (wt, lanes 1 and 4) or the E3 (lanes 3 and 5) *src* protein were labeled for 18 h with either L-[^{35}S]methionine (MET, lanes 1 to 3) or [^{3}H]myristic acid (MYR, lanes 4 and 5). Labeled cells were solubilized in lysis buffer, and *src* proteins were immunoprecipitated with TBR serum as described in the text.

activity of pp60$^{v\text{-}src}$. Furthermore, the simplest explanation for the behavior of the NY316 *src* protein is that amino acid 7 is a critical component of the recognition sequence for the myristyl transferase.

Transforming activity of the N7 and R7 *src* proteins. We directly tested the role of lysine 7 in myristylation of pp60$^{v\text{-}src}$ by replacing it with either an asparagine (N7) or an arginine (R7) (Table 1). These mutant *src* genes were incorporated into an RSV vector, and virus was produced by transfecting CEF with these viral DNAs. After 10 days to allow virus spread, CEF infected with either the wild-type (Fig. 2B) or the R7 (Fig. 2C) RSV viruses were morphologically transformed, while CEF infected with the N7 (Fig. 2D) RSV virus were indistinguishable from uninfected CEF (Fig. 2A). Viral stocks derived from these cultures were assayed for their ability to induce transformed foci on monolayers of CEF and to produce pp60$^{v\text{-}src}$ protein kinase activity in acutely infected CEF (Table 2). Although the N7 virus stock was at least 1,000-fold less active than either the R7 or wild-type viruses in the focus assay, it produced equivalent pp60$^{v\text{-}src}$ kinase activity in acutely infected cells. These experiments demonstrate that the N7 *src* gene encodes an

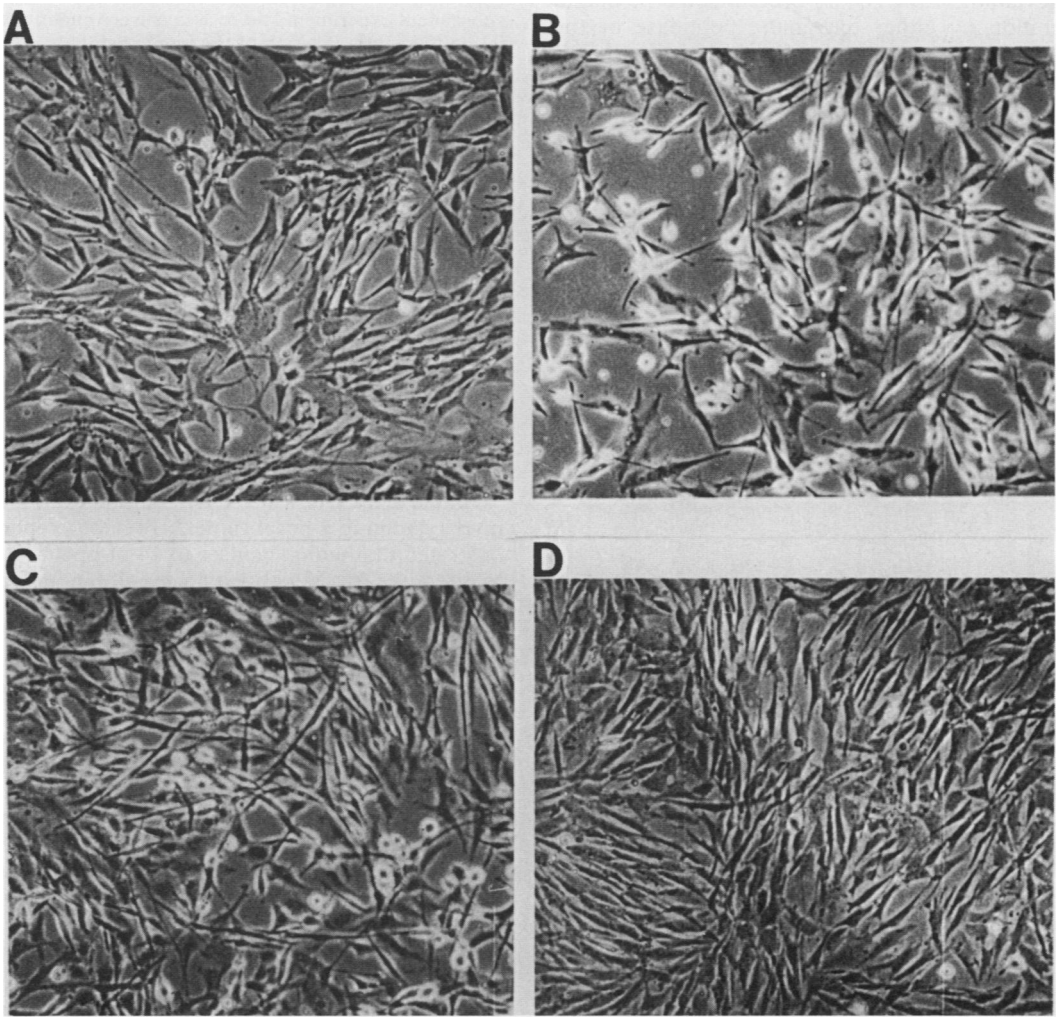

FIG. 2. Morphology of CEF infected with wild-type, N7, and R7 RSVs. Untransfected CEF (A) or CEF transfected 10 days previously with DNA encoding wild-type (B), R7 (C), or N7 (D) RSV as described in the text. Viral stocks derived from these cultures had equivalent relative kinase titers.

TABLE 2. Titers of mutant RSVs

Virus	Titer (FFU/ml)[a]	Relative kinase titer[b]
Wild type	2×10^5	1.00
R7	5×10^5	1.25
N7	$<1 \times 10^2$	1.12

[a] CEF were infected with dilutions of virus and overlaid with agar. Foci of transformed cells were counted after 14 days.

[b] Relative kinase titers were determined as described in the text. These results represent the average of triplicate cultures.

active protein kinase that is defective for morphological transformation of chicken cells.

Myristylation of the N7 and R7 *src* proteins. We next asked whether the attenuated transforming activity of N7 reflected an underlying defect in the myristylation of the N7 *src* protein. The extent to which the wild-type, R7, and N7 *src* proteins are acylated was determined by comparing the incorporation of methionine (Fig. 3A) and myristic acid (Fig. 3B) into these proteins. The N7 *src* protein (lanes 3) was apparently myristylated 80 to 90% less well than either the wild-type (lanes 2) or the R7 (lanes 4) *src* proteins, in general agreement with their respective transforming activities.

Defective acylation of the N7 *src* protein could be due to altered recognition by either the amino peptidase or the myristyl transferase. These two possibilities can be distinguished by the presence or absence of Met-1 in the mature N7 *src* protein. We tested this by asking whether we could detect [^{35}S]methionine-labeled amino-terminal 18- and 16-kilodalton V8 proteolytic fragments of pp60$^{v\text{-}src}$, since these fragments contain methionine only if the amino peptidase fails to remove Met-1. We determined that methionine-1 was not present in the mature N7 *src* protein, which implies that asparagine 7 affects recognition by the myristyl transferase but not by the amino peptidase (data not shown).

Membrane association of the N7 and R7 *src* proteins. The extent to which these proteins were myristylated also correlates well with their association with total cellular membranes in subcellular fractionations (Fig. 4). Crude membrane (lanes 4, 8, and 12) and cytosolic (lanes 3, 7, and 11) fractions were isolated by differential centrifugation, and the amount of pp60$^{v\text{-}src}$ in these fractions was determined by immune complex kinase reactions. In agreement with previous reports (10, 19), 70 to 80% of the wild-type (lane 4) and R7 (lane 12) pp60$^{v\text{-}src}$ kinase activity was recovered with total cellular membranes; however, only 10% of the N7 pp60$^{v\text{-}src}$ kinase activity (lane 8) was recovered with total cellular membranes. These experiments demonstrate that amino acid 7 can determine whether pp60$^{v\text{-}src}$ is myristylated and whether it is associated with cellular membranes. These results also provide an explanation for the behavior of the NY316 *src* protein, which also contains asparagine-7.

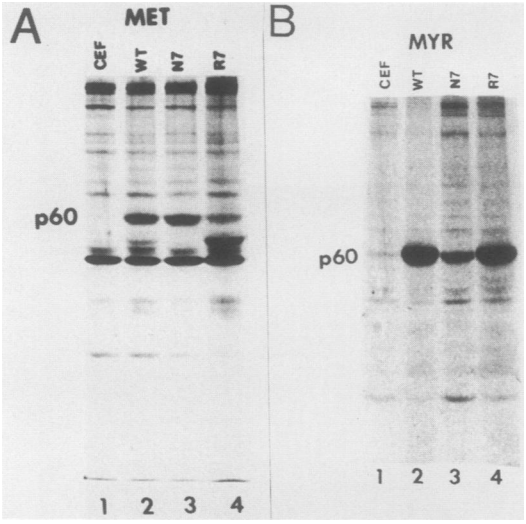

FIG. 3. Myristylation of the N7 and R7 *src* proteins. Infected CEF were labeled for 18 h with either L-[^{35}S]methionine (MET) (A) or [^{3}H]myristic acid (MYR) (B). Labeled cells were solubilized in lysis buffer, and *src* proteins were immunoprecipitated with monoclonal antibody 127 (anti-pp60$^{v\text{-}src}$). Panel A was exposed for 20 h, and panel B was exposed for 30 days. CEF had previously been infected with wild-type (WT, lane 2), N7 (lane 3), or R7 (lane 4) RSV.

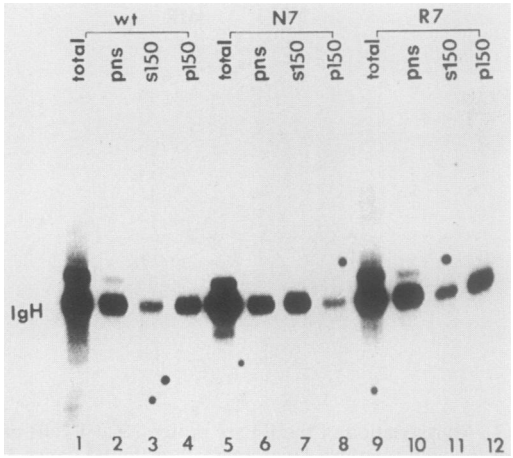

FIG. 4. Membrane association of the N7 and R7 *src* proteins. CEF infected with wild-type (wt, lanes 1 to 4), N7 (lanes 5 to 8), or R7 (lanes 9 to 12) RSV were subjected to subcellular fractionation as described in the text. Equivalent amounts of the total extract (lanes 1, 5, and 9), the supernatant of a 1,000 × *g* spin (pns, lanes 2, 6, and 10), the supernatant of a 150,000 × *g* spin (s150, lanes 3, 7, and 11), and the pellet of a 150,000 × *g* spin (p150, lanes 4, 8, and 12) were analyzed by immune complex kinase assays with TBR sera. In subsequent experiments these assays were quantitated as described in the text. IgH, Position of the immunoglobulin heavy chain.

Amino acids 1 to 7 of the R7 *src* gene are sufficient to cause myristylation in a novel context. Previous reports have demonstrated that amino acids 1 to 14 of pp60$^{v\text{-}src}$ are sufficient to direct the myristylation of a *src*-globin fusion protein (32). The simplest explanation for the behavior of the E3 *src* protein, however, is that amino acids 1 to 7 constitute a sufficient myristylation signal. We tested the role of sequences C-terminal to amino acid 7 by fusing codons 1 to 7 of the R7 *src* gene to a gene encoding a normally nonmyristylated protein, the chicken muscle PK gene. The resulting fusion gene, R7-PK (Table 1), was incorporated into an SV40 expression vector and used to transfect COS7 cells (Fig. 5). The R7-PK fusion protein (Fig. 5, lanes 3 and 6) incorporated myristic acid, while the native PK protein (lanes 2 and 4) did not. These results demonstrate that amino acids 1 to 7 constitute a sufficient myristylation signal.

DISCUSSION

As a first step toward elucidating the mechanism of the membrane localization of pp60$^{v\text{-}src}$, we identified a new determinant of the myristylation signal, lysine 7, and we have shown that amino acids 1 to 7 of pp60$^{v\text{-}src}$ are sufficient to cause myristylation in a novel context.

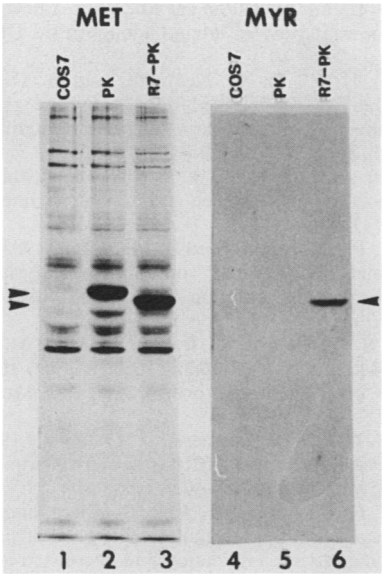

FIG. 5. Myristylation of a hybrid *src*-PK protein. Untransfected COS7 cells (lanes 1 and 4) or COS7 cells transfected with either RL142PK10X encoding native PK (lanes 2 and 5) or pR7-PK encoding the R7-PK fusion protein (lanes 3 and 6), as described in the text, were labeled with either L-[^{35}S]methionine (MET, lanes 1 to 3) or [^{3}H]myristic acid (MYR, lanes 4 to 6). Labeled cells were solubilized with lysis buffer, and PK proteins were immunoprecipitated with rabbit anti-chicken M1 PK antibody. Lanes 1 to 3 were exposed for 20 h; lanes 4 to 6 were exposed for 30 days. Arrows indicate the positions of the PK and R7-PK proteins.

What is the role of lysine 7 in the myristylation of pp60$^{v\text{-}src}$? Replacing lysine 7 with asparagine largely abolished myristylation of pp60$^{v\text{-}src}$. It is formally possible that the residual labeling of the N7 *src* protein by myristic acid represents metabolic conversion of the label into amino acid precursors. We think this is unlikely, because 10 to 20% of the N7 *src* protein pelleted with microsomal membranes, which is consistent with residual myristylation.

Our conclusions are also supported by in vitro myristylation studies. A myristyl transferase partially purified from yeast acylates the octapeptide Gly-Ser-Ser-Lys-Ser-Asn-Pro-Lys (K_m, 1,000 μM; V_{max}, 8%), derived from the N-terminal sequence of the N7 *src* protein, substantially less well than Gly-Ser-Ser-Lys-Ser-Lys-Pro-Lys (K_m, 40 μM; V_{max}, 43%), derived from the wild-type *src* protein (49). This result demonstrates that asparagine 7 directly affects recognition by myristyl transferase. Furthermore, the octapeptide derived from the amino-terminal sequence of the N7 *src* protein has substantial residual activity as a substrate for myristyl transferase, which is consistent with the residual myristylation that we saw in vivo. Since asparagine 7 had no effect on recognition of either the N7 or the NY316 *src* protein by the amino peptidase (12; unpublished observations), we conclude that lysine 7 is a critical determinant of recognition by the myristyl transferase.

Another possible interpretation of these data is that asparagine 7 is a substitution that uniquely alters recognition by the myristyl transferase. This interpretation implies that most substitutions for lysine 7 would be active and therefore that lysine 7 would not be conserved among myristylated proteins generally. There is very poor conservation of amino acid 7 among these proteins (not shown). However, if one compares the N-terminal sequences of protein tyrosine kinases related to pp60$^{v\text{-}src}$, lysine 7 is found in most of them (Table 1). Other than glycine 2, this is the most conserved of the first 10 amino acids of these proteins. Another surprising feature of these sequences is that none contains arginine 7, which we have shown to be active in pp60$^{v\text{-}src}$ and which we predict would be an active substitution in other proteins related to pp60$^{v\text{-}src}$.

In summary, it seems likely that lysine 7 promotes recognition of a family of proteins by the myristyl transferase. Alternatively, lysine 7 might be conserved if this domain performs some function other than recognition by the myristyl transferase that these proteins have in common. The dissimilarity between the amino-terminal sequence of pp60$^{v\text{-}src}$ and that of other known myristylated proteins might reflect recognition by distinct myristyl transferases.

What are the other sequence determinants of the myristylation signal? It seems very likely that amino acids 1 to 7 of pp60$^{v\text{-}src}$ constitute a sufficient myristylation signal. This putative signal works in three different sequence contexts, namely, the wild-type and E3 *src* proteins and the R7-PK protein. Furthermore, the sequences C-terminal to lysine 7 in pp60$^{v\text{-}src}$ bear no resemblance to those in its relatives. Thus, sequences C-terminal to lysine 7 play a minor role, if any, in the myristylation of pp60$^{v\text{-}src}$. Since methionine 1 must be removed and glycine 2 provides the site for joining to myristate, amino acids 3 to 6 are the remaining potential determinants of this signal. Towler et al. (47, 48) have reported that the yeast myristyl transferase is sensitive to amino acids 3 and 6 of its substrates, utilizing octapeptides derived from the catalytic subunit of the cyclic AMP-dependent protein kinase. Leucine, phenylalanine, tyrosine, and aspartate at position 3 (48) and aspartate at position 6 are inactivating substitutions, whereas serine at position 6 strongly favors binding of the octapeptides to the transferase (47). However, these substitutions have not been tested in octapeptides derived from pp60$^{v\text{-}src}$. It is interesting that the c-*fes* protein, which is at most loosely associated with membranes (55), contains phenylalanine 3 and glutamate 6, both of which should be inactive substitutions. It has not been reported whether the c-*fes* protein is myristylated.

What is the function of the myristyl moiety? The myristyl moiety has been hypothesized to promote the association of pp60$^{v\text{-}src}$ with membranes (7, 12). The behavior of the N7 *src* protein supports this hypothesis. The N7 *src* protein, like the previously reported nonmyristylated variants (17, 32), is enzymatically active, not associated with membranes, and biologically impotent. These similarities, which result from various alterations of the amino-terminal sequence, suggest a common underlying cause—the lack of myristylation. We cannot, however, conclude from these data that the myristyl moiety is sufficient, or even necessary, for the membrane association and biological activity of pp60$^{v\text{-}src}$. The transformation-defective tsNY68 (6) and NY18-3 (12) *src* proteins, like several myristylated proteins unrelated to *src*, are not associated with membranes despite their myristylation. This behavior implies the presence of a second prerequisite for localization, perhaps a tertiary or quaternary structure. On the other hand, the nonmyristylated *src* proteins of the recovered avian sarcoma virus strains 157 and 1702 are both biologically active and membrane associated, albeit weakly (20). This behavior implies that alternative amino-terminal sequences (derived in these cases from the signal sequence of gp85^{env}) can substitute functionally for the myristyl moiety. Thus, covalently bound myristate probably plays no part in other properties of pp60$^{v\text{-}src}$, such as concentration within adhesion plaques (37) and substrate recognition.

In summary, the myristyl moiety contributes to the membrane localization of pp60$^{v\text{-}src}$, which is a prerequisite for its transforming activity. Additional studies should reveal whether pp60$^{v\text{-}src}$ has a second signal that is required in conjunction with myristylation for membrane association and localization within membranes.

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