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Activation of the transforming potential of p60$^{c\text{-}src}$ by a single amino acid change

(*src* oncogene/Rous sarcoma virus)

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ABSTRACT **Previous work showed that overexpression of the cellular *src* (c-*src*) gene does not cause transformation of chicken cells in culture. However, viral stocks isolated from cells transfected with Rous sarcoma virus DNA containing the c-*src* gene in place of the viral *src* gene did occasionally produce foci. Virus obtained from these foci were highly transforming and appeared to arise via spontaneous mutation in the c-*src*-containing viral populations. The p60 proteins of the transforming mutant *src* viruses were found to have higher levels of *in vitro* tyrosine kinase activity than the levels observed with the parental viruses. In this study, we have molecularly cloned the *src* DNA sequences of two transforming mutant *src* viruses. When compared to the DNA sequence of the parental c-*src* viruses, the mutant viruses contain single point mutations that result in single amino acid changes in the *src* gene products (p60 proteins). Both amino acid changes reside in the tyrosine kinase domain of the protein. The mutation detected in one virus involves replacement of the normal Glu-378 in p60$^{c\text{-}src}$ by Gly, whereas the p60 of the other transforming virus has Phe instead of the normal Ile-441. Our data indicate that when p60$^{c\text{-}src}$ is expressed at elevated levels in a retroviral context, a single amino acid change in its primary sequence can activate the kinase activity of this protein and cause cellular transformation.**

A cellular homolog of the viral *src* (v-*src*) transforming gene of Rous sarcoma virus (RSV) has been identified in the genome of many vertebrates (1, 2). The cellular *src* gene (c-*src*) encodes a protein (p60$^{c\text{-}src}$) that is expressed in normal cells (3) and that, like p60$^{v\text{-}src}$, is a tyrosine-specific protein kinase (4–6). Molecular clones have been isolated from a chicken genomic library that contain c-*src* sequences (7–9). DNA sequence analysis revealed a strong similarity between the cellular sequence coding for p60$^{c\text{-}src}$ (10) and the v-*src* gene (11–14). With respect to the Schmidt–Ruppin A strain of RSV (SRA-RSV), multiple single-base changes resulting in amino acid substitutions are dispersed throughout the v-*src* gene in addition to a divergence at the COOH-terminal end of the proteins, where the last 19 amino acids of p60$^{c\text{-}src}$ are replaced by 12 different amino acids in p60$^{v\text{-}src}$ (10).

Previous studies on transformation-defective RSV mutants (15) have shown that the majority of the *src* sequence of RSV can be replaced by the cellular *src* sequence to generate a fully transforming recovered avian sarcoma virus (rASV) (11, 16). In order to determine more precisely whether any of the structural differences between the coding sequences of v-*src* and c-*src* are needed for transformation, chimeric plasmids have been constructed by replacing portions of c-*src* with the corresponding portions of v-*src* (17). These variants are actively transforming. However, constructs containing the entire c-*src* gene in place of the v-*src* sequence were unable to transform cells even though p60$^{c\text{-}src}$ was overproduced (17–19). Further studies demonstrated that the overproduced p60$^{c\text{-}src}$ has low levels of tyrosine-specific protein kinase activity relative to wild-type p60$^{v\text{-}src}$ (20). In these studies it was found that low titers of transforming virus were generated from the nontransforming c-*src* virus, presumably by spontaneous mutation (17). The transforming mutant *src* viruses were shown to possess higher levels of tyrosine-specific kinase activity when compared to the parental c-*src* viruses (20). In this study, we have molecularly cloned the *src* genes of two isolates of the mutant viruses in order to identify the mutation(s) responsible for activating the transforming potential of p60$^{c\text{-}src}$.

MATERIALS AND METHODS

Cells and Virus. The conditions for preparing and maintaining cultures of primary and secondary chicken embryo fibroblasts (CEF) have been described (21). Infected cultures of CEF were kept either in liquid medium or under soft agar medium (0.4% agar). They were transferred once to achieve full infection before DNA isolation and protein analyses were performed. Isolation and biological and biochemical characterizations of the viruses NY501T7 and NYHB5T9 have been reported (20). NYN4, containing the v-*src* sequence of SRA-RSV, and NY501 and NYHB5, containing the c-*src* sequence, have been described (20).

Molecular Cloning and Subcloning of the *src*-Containing DNA Fragments of NY501T7 and NYHB5T9. Total cellular DNA was extracted from CEF infected with NY501T7 and NYHB5T9 viruses as described previously (22). The *src*-containing fragments of integrated proviral DNA were prepared by first extensively digesting the chicken chromosomal DNA with *Eco*RI and then fractionating the digested products by 0.8% agarose gel electrophoresis to obtain the 3.1-kilobase (kb) *Eco*RI fragment containing all of the *src* gene and part of the *env* gene (9). The region of the gel corresponding to 2.8–3.5 kb was excised, and the DNA was recovered by electroelution (23). This DNA was ligated to λgtWES.λB *Eco*RI arms (24) and then packaged *in vitro* into λ phage particles (25). Phages containing *src* DNA fragments (Fig. 1*A*) were identified by using the *in situ* plaque-transfer procedures of Benton and Davis (26) and hybridization conditions specified by Thomas (27). The probe used was a ^{32}P-labeled nick-translated *Nco* I–*Bgl* II fragment of plasmid pHB5 (Fig. 1*B*). This fragment contains the entire *src* gene of the pHB5 plasmid (17). Positive recombinants containing the *src* fragments of either NYHB5T9 or NY501T7 were purified

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Abbreviations: RSV, Rous sarcoma virus; SRA-RSV, Schmidt–Ruppin A strain of RSV; rASV, recovered avian sarcoma virus; CEF, chicken embryo fibroblast(s); kb, kilobase(s); ffu, focus-forming unit(s).

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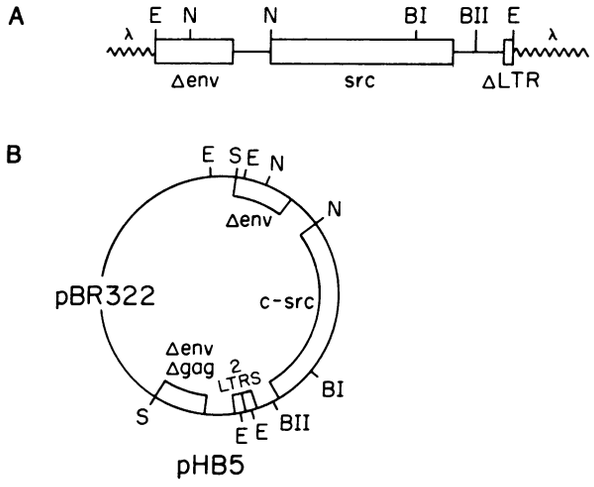

FIG. 1. Cloning and subcloning of the *src*-containing fragment of the viruses NYHB5T9 and NY501T7. (*A*) CEF were infected with the viruses NYHB5T9 and NY501T7 and total cellular DNA was extracted. The 3.1-kb *Eco*RI fragment containing all of the *src* gene and part of the *env* gene was ligated to λgtWES.λB *Eco*RI arms. The λ clones containing the *src* sequences of the two mutant viruses are λHB5T9 and λ501T7. (*B*) The construction of pHB5 has been described (17). The c-*src* gene of pHB5 was replaced with the *src* genes of λHB5T9 and λ501T7. Restriction enzyme sites: *Eco*RI (E), *Nco* I (N), *Bgl* I (BI), *Bgl* II (BII), and *Sal* I (S). LTR, long terminal repeat.

by three rounds of plaque purification. They are called λHB5T9 and λ501T7, respectively, and are illustrated in Fig. 1*A*. DNA from λHB5T9 and λ501T7 was extracted and fully digested with the restriction enzymes *Nco* I and *Bgl* II. Plasmid pHB5 (Fig. 1*B*) was first fully digested with *Bgl* II and then partially digested with *Nco* I. A 7.5-kb fragment was produced that lacked the c-*src* present in pHB5. This fragment was then ligated with the *src*-containing *Nco* I–*Bgl* II fragment of either λHB5T9 or λ501T7. The newly constructed plasmids are called pHB5T9 and p501T7 and contain the molecularly cloned *src* genes of λHB5T9 and λ501T7, respectively, in the corresponding region of the c-*src* gene of pHB5. Procedures for the ligation reaction, nick-translation reaction, and phage and plasmid DNA isolation were essentially as described (23).

Transfection Procedures. Plasmids containing *src* sequences were digested with *Sal* I and ligated to a *Sal* I digest of pSR-REP (28). This pSR-REP plasmid contained the *pol* sequence derived from the Bryan strain of RSV and has been tested in previous experiments (17). Ligated DNA was introduced into CEF by the calcium phosphate transfection method (29).

Protein Analysis. Cells were labeled with [^{3}H]leucine and then lysed in RIPA buffer according to conditions described previously (20, 30). Immunoprecipitations were performed with the monoclonal antibody 327, provided by J. Brugge (31), as previously described (20). A portion of the immunocomplex was suspended in 30 μl of 20 mM Tris·HCl (pH 7.2) containing 5 mM $MgCl_2$, 0.1 μM [γ-^{32}P]ATP (3000 Ci/mmol; 1 Ci = 37 GBq), 5 μM unlabeled ATP, and 5 μg of enolase and was incubated at 23°C for 10 min. The labeled proteins were analyzed by $NaDodSO_4$/10% PAGE as described (4).

DNA Sequence Analysis. The 3.1-kb *Eco*RI fragment containing the entire *src* sequence was isolated from p501T7 and pHB5T9 and then digested with *Pst* I. Of the seven fragments generated as a result of these digestions (11), only five contained *src* information. Four *Pst* I–*Pst* I fragments, of 402, 345, 255, and 612 base pairs, were subcloned into the *Pst* I site of phage M13 mp8; the remaining *Pst* I–*Eco*RI fragment, measuring 456 base pairs and containing the 3′ end of *src*, was subcloned between the *Pst* I and *Eco*RI sites of M13 mp8 (32). The DNA sequence was determined by the Sanger chain-terminating procedure (33) using [α-[^{35}S]thio]dATP (New England Nuclear).

RESULTS

Molecular Cloning of the *src*-Containing Fragments of NY501T7 and NYHB5T9. The 3.1-kb *src*-containing fragment was purified from *Eco*RI-digested chromosomal DNA isolated from CEF infected with the viruses NYHB5T9 and NY501T7. A λ phage containing the *src* sequence of either mutant after molecular cloning of the 3.1-kb *Eco*RI fragment into λgtWES.λB is diagrammed in Fig. 1*A*. The *src* DNA sequences contained in λ501T7 and λHB5T9 were then subcloned in plasmid pHB5 (Fig. 1*B*) by replacing the c-*src* sequences of pHB5 with the *src* fragments contained in λ501T7 and λHB5T9 to generate plasmids p501T7 and pHB5T9, respectively.

Biological Activity of pHB5T9 and p501T7 DNAs. CEF were transfected with *Sal* I-digested pHB5T9 and p501T7 DNAs after ligation with *Sal* I-cut pSR-REP. In addition, pN4 and pHB5 DNAs, containing the v-*src* and c-*src* genes, respectively (17), were used as controls. Transfected cultures were maintained as described (17). Five days after transfection, foci were detected in CEF transfected with p501T7, pHB5T9, and pN4 DNAs. The foci produced in cultures transfected with pHB5T9 and p501T7 DNAs were similar morphologically to those formed after transfection by wild-type pN4 DNA (data not shown). As described previously (17), pHB5 formed no foci at this stage. At 8 and 9 days after transfection, viral stocks were harvested from the liquid cultures. Viral stocks from cultures transfected with pHB5T9 and p501T7 DNAs (designated as NYHB5T9-m and NY501T7-m) had high titers of transforming virus [10^6–10^7 focus-forming units (ffu) per ml] comparable to the levels of transforming virus obtained from pN4-transfected cultures (data not shown). These viruses were also tumorigenic when inoculated into the wing webs of 11-day-old chicks, although the initial appearance of tumors was 1 day later than in those inoculated with wild-type N4 virus. All tumors progressively increased in size for 2 weeks (data not shown), at which point the chickens were sacrificed.

$p60^{c\text{-}src}$ Production in CEF Infected with NYHB5T9-m and NY501T7-m Virus. The levels of p60 production in cells infected with NYHB5T9-m and NY501T7-m virus stocks obtained from the transfected cultures were determined by immunoprecipitation with monoclonal antibody 327, which can react with both $p60^{v\text{-}src}$ and $p60^{c\text{-}src}$ (31). The [^{3}H]leucine-labeled proteins immunoprecipitated are shown in Fig. 2*A*. The NYHB5T9-m and NY501T7-m p60 proteins were identical in size to the p60 proteins made by cells infected with NYHB5 and NY501, the two c-*src*-containing parental viruses (17), and to the p60 proteins produced by cells infected with the originally isolated NYHB5T9 and NY501T7 viruses. In addition, the p60 proteins of the molecularly cloned mutant viruses were expressed at levels similar to those of the parental viruses NYHB5 and NY501 (Fig. 2*A*). Therefore, as described previously (20), the transforming activity of the mutant *src* viruses was not due to changes in the level of p60 expression.

We measured protein kinase activity in immunocomplexes formed by the monoclonal antibody 327 (31), using enolase as an exogenous substrate (34). The p60 proteins of the two molecularly cloned transforming viruses, NYHB5T9-m and NY501T7-m, contained higher levels of kinase activity with respect to both p60 autophosphorylation and enolase phosphorylation when compared to the levels observed with the p60 proteins of NYHB5 and NY501 (Fig. 2*B*). The kinase

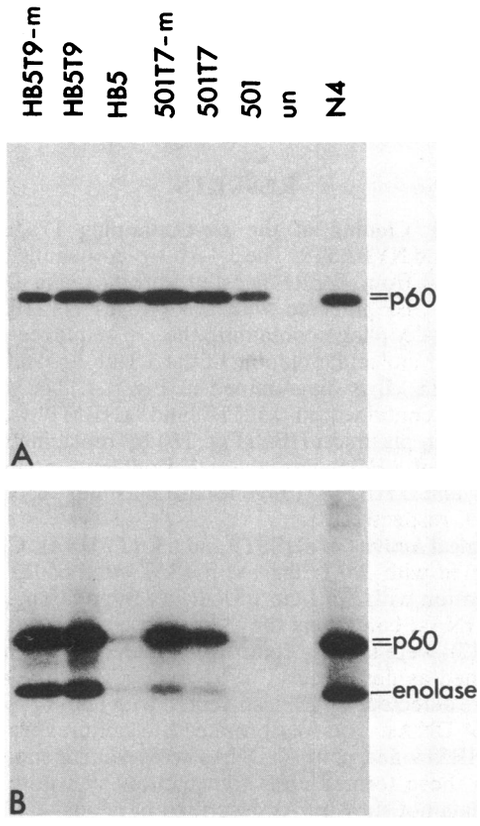

FIG. 2. Analysis of p60 proteins produced in the infected cells and their protein kinase activity. (*A*) Uninfected (un) and virus-infected cells were labeled with [^{3}H]leucine for 4 hr. p60 proteins were immunoprecipitated from aliquots of cell lysates with monoclonal antibody 327. Half of the immunoprecipitated material was analyzed by $NaDodSO_4$/10% PAGE and fluorography. (*B*) The remaining half of the immunoprecipitated material was used in a protein kinase assay with enolase included as an exogenous substrate. The reaction products of the kinase assay were separated by electrophoresis and detected by autoradiography.

activities of the *src* proteins of NYHB5T9-m and NY501T7-m were comparable to the activities of the p60 proteins of the original viral stocks, NYHB5T9 and NY501T7 (Fig. 2*B*).

DNA Sequence of the *src* Fragments of pHB5T9 and p501T7. Restriction maps of the *src* genes in pHB5T9 and p501T7 were identical to those predicted from the c-*src* sequence (10), indicating that the mutations in their *src* genes did not involve large deletions or insertions (data not shown). The DNA sequences of the coding regions of the entire *src* genes of both plasmids were determined to identify mutation(s) in these viruses. Compared with the c-*src* sequence (10), pHB5T9 contains point mutations at nucleotide numbers 188, 1133, and 1502 that result in a change of amino acids Gly-63, Glu-378, and Arg-501 in c-*src* to Asp, Gly, and Lys in NYHB5T9-m, respectively. Sequence analysis of p501T7 revealed three point mutations, at nucleotides 270, 1321, and 1502. The first point mutation is a third-base change at nucleotide 270, which does not result in a change at the amino acid level. The second and third mutations result in a change in amino acid 441 from Ile in c-*src* to Phe in NY501T7-m, and in amino acid 501 from Arg in c-*src* to Lys in NY501T7-m. The locations of the mutations in the two viruses are summarized in Table 1.

To exclude the possibility that the mutations identified in the two viruses were actually present in the c-*src* parental viruses, NYHB5 and NY501, we sequenced fragments of the parental DNA corresponding to amino acid positions 63, 378, 441, and 501. Sequence analysis of a 3′ fragment of genomic

Table 1. Location of mutations in the *src* genes of the two mutant viruses relative to c-*src*

Nucleotide (amino acid)*	501T7	HB5T9	c-*src*†
270 (90)	TAT (Tyr)	TAC (Tyr)	TAC (Tyr)
1321 (441)	TTC (Phe)	ATC (Ile)	ATC (Ile)
1502 (501)	AAG (Lys)	AAG (Lys)	AGG (Arg)‡
188 (63)	GGC (Gly)	GAC (Asp)	GGC (Gly)
1133 (378)	GAG (Glu)	GGG (Gly)	GAG (Glu)

*Numbering of nucleotides and amino acids (in parentheses) begins with the first nucleotide or amino acid in the coding region of c-*src* (10).
†c-*src* sequences are from ref. 10.
‡Reexamination of the c-*src* sequence revealed an AAG at this codon, corresponding to Lys (see text).

c-*src* revealed that amino acid 501 is Lys instead of the previously reported Arg (10). Therefore, Lys-501 encoded in the two mutant viruses does not result from a mutation of c-*src*. The sequence of this fragment of c-*src* also revealed an Ile at amino acid 441, thereby indicating that a true mutation had occurred at this position in NY501T7-m. Thus, it appears that the primary sequence of p60 in the mutant virus NY501T7-m differs from the $p60^{c\text{-}src}$ by a single amino acid change at position 441. A similar sequence analysis was performed with fragments containing nucleotides 188 and 1133, corresponding to amino acids 63 and 378 of the virus NYHB5, the parental c-*src* virus of NYHB5T9. pHB5 was originally constructed as a chimeric molecule containing 5′ sequences derived from the SF strain of SRA-RSV, combined with an internal portion derived from the rASV 1441 genome and 3′ sequences isolated from genomic c-*src* (17). The reported amino acid sequence of the fragments of v-*src* of the SF strain of SRA-RSV (13) and rASV (11) used in this construction was identical to the amino acid sequence of c-*src* in the same regions (10). Our DNA sequence analysis of a fragment of pHB5 that codes for amino acid 63 revealed that the parental NYHB5 virus, like the mutant virus NYHB5T9-m, has Asp at this position. The Gly predicted at this position by the original sequence of the viral fragment used in this construction (13) is actually an Asp in both the parental NYHB5 and mutant NYHB5T9-m viruses. The fragment of pHB5 containing the mutation detected in pHB5T9-m at nucleotide 1133 (amino acid 378) was also sequenced. This fragment of pHB5 contains sequences derived from rASV. In agreement with the reported sequence of rASV (11), amino acid 378 is Glu. Therefore, the p60 of NYHB5T9-m contains two amino acid differences relative to $p60^{c\text{-}src}$, one at amino acid 63 and the other at amino acid 378. The difference at amino acid 63, however, is also encoded in the parental virus NYHB5.

Construction of c-*src* Plasmid p5H. The mutation in p501T7-m is located 3′ of a *Bgl* I restriction site that is present in c-*src* (10). The two mutations in pHB5T9-m are located 5′ of this *Bgl* I site. By utilizing this convenient restriction site, a new c-*src*-containing plasmid, p5H, was constructed by ligating the portions of the two plasmids that were identical in sequence to c-*src* (Fig. 3). The two original c-*src* containing plasmids, pHB5 and p501, contain 1 and 10 introns, respectively (17). Previous experiments have shown that the introns were efficiently spliced out upon generation of NYHB5 and NY501 virus (17). It was further confirmed by the present DNA sequence analysis of NY501-T7 and NYHB5-T9 DNA (data not shown). Unlike pHB5 and p501, construct p5H would have only c-*src* coding information. In addition, p5H has the Gly-63 codon normally present in c-*src*, because this portion of the construct is derived from p501T7-m. Transfection of CEF with p5H DNA that had been ligated to pSR-REP produced no foci in cultures maintained in liquid

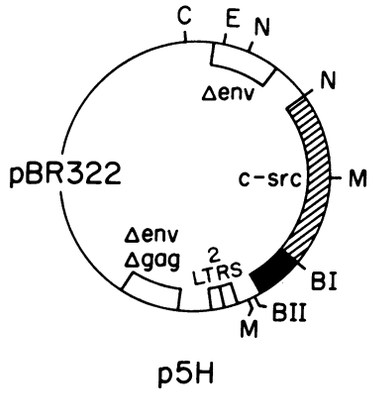

FIG. 3. Construction of the c-*src*-containing plasmid p5H. Plasmid p5H was constructed by a three-part ligation that involved the following fragments: a 3.1-kb *Cla* I–*Bgl* I fragment of p501T7 containing Δ*env* and *src* sequences, a 0.38-kb *Bgl* I–*Bgl* II fragment of pHB5T9 containing *src* sequences, and a 5.7-kb *Bgl* II–*Cla* I fragment of pN4 including the two long terminal repeats (LTRs), Δ*env* Δ*gag*, and pBR322 sequences. The hatched area represents *src* sequences derived from p501T7. The solid black area includes *src* sequences from pHB5T9. Restriction enzyme sites: *Cla* I (C), *Eco*RI (E), *Nco* I (N), *Mlu* I (M), *Bgl* I (BI), and *Bgl* II (BII).

medium (data not shown). Control cultures that had been transfected with pN4 DNA showed foci within 4–5 days after transfection. Viral stocks harvested from cultures transfected with p5H DNA were found to contain low levels of transforming virus (7×10^2 ffu/ml). This titer is comparable to those detected with viral stocks isolated from pHB5- and p501-transfected cultures (17). These viral stocks were also used to examine tumor formation in chickens. A 0.1-ml aliquot of undiluted virus was injected into the wing web of 11-day-old chicks. Small tumors appeared with a latency of approximately 12–14 days after inoculation. In contrast, animals injected with NYN4 (10^5 and 10^6 ffu/ml) showed signs of tumors 4–5 days after injection. The delayed tumor formation by NY5H virus is most likely due to the combination of the low level of transforming virus present in the original viral population and continuing spontaneous mutations arisen in c-*src* of NY5H in chickens. The level of p60 produced in cells transfected with p5H DNA was similar to that of pHB5-transfected cultures (Fig. 4*A*). Both autophosphorylation of p60 and enolase phosphorylation, measured in the *in vitro* kinase reaction, were extremely low in p5H-transfected cultures relative to the levels observed in wild-type pN4-transfected cultures (Fig. 4*B*). The low levels were comparable to those observed in CEF transfected with pHB5 DNA (Fig. 4*B*).

A Single Point Mutation at Amino Acid 378 also Activates the Transforming Potential of c-*src*. The p60 of the transforming virus NYHB5T9-m differs from $p60^{c\text{-}src}$ at positions 63 and 378. The mutation at amino acid 63 that was present in the p60 of the parental NYHB5 virus could not by itself activate the transforming potential of this p60. A question still remained, however, as to whether NYHB5T9-m had acquired transforming activity by the presence of a single point mutation located at amino acid 378 in its p60 or whether the mutations at amino acids 63 and 378 were acting in a cooperative manner. We replaced the 0.9-kb *Mlu* I fragment of p5H that contains amino acid 378 with the equivalent fragment from pHB5T9 (see Fig. 3). The new construct, pCHB, contains a *src* sequence identical to that of p5H except for the substitution of a Gly codon for the Glu-378 codon present in c-*src*. Plasmids pCHB-3 and pCHB-11, isolated from two separate bacterial colonies transformed by the pCHB construct, were ligated to pSR-REP and transfected into CEF. In cultures transfected with either pCHB-3 or pCHB-11, foci appeared at

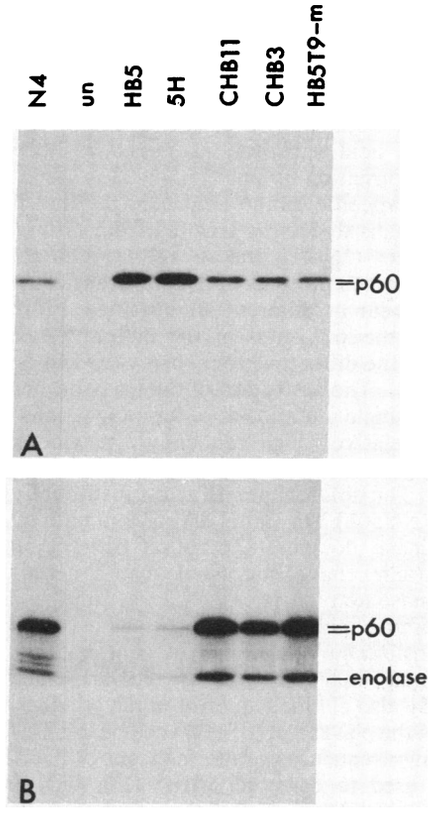

FIG. 4. p60 production and reaction products of the protein kinase assay. (*A*) CEF transfected with the various DNAs, as well as uninfected (un) cells, were labeled with [^{3}H]leucine. The p60 proteins were immunoprecipitated and analyzed as described for Fig. 2*A*. (*B*) Products of the *in vitro* kinase reaction utilizing enolase as an exogenous substrate.

the same time that foci were observed in cultures transfected with pHB5T9-m and pN4 DNAs (data not shown). Levels of p60 produced in cultures transfected with pCHB-3 and pCHB-11 DNAs were comparable to those of cultures transfected with pHB5T9-m DNA, and their *in vitro* protein kinase activity was also similar (Fig. 4). These data show that a single point mutation at amino acid codon 378 in c-*src* can activate its transforming potential.

DISCUSSION

We have molecularly cloned the *src* sequences of two mutant *src*-containing viruses that, unlike their parental viruses, have acquired the ability to transform cells in culture. The results in this study show that the mutant viruses, NY501T7-m and NYHB5T9-m, contain one and two amino acid substitutions, respectively, when compared with the c-*src* sequence (10). DNA sequence analysis of the *src* gene of p501T7-m revealed a single point mutation at nucleotide 1321 that results in conversion of amino acid 441 from isoleucine in c-*src* to phenylalanine in the mutant. The other virus, NYHB5T9-m, encoding substitutions in the c-*src* sequence at amino acids 63 and 378, was shown to be different only at amino acid 378 when compared with the sequence of the parental NYHB5 virus. A new construct NYCHB, which is different from c-*src* only at codon 378 (Gly→Glu) was also actively transforming. Therefore we can conclude that single amino acid substitutions at different sites can activate the transforming potential of $p60^{c\text{-}src}$. It should be noted that the mutant p60 proteins were expressed at high levels because the *src* sequences were under the control of the strong viral long-terminal-repeat (LTR) promoter. A previous report (35)

demonstrated that cellular transformation by the v-*src* gene product is dependent on the amount of $p60^{v\text{-}src}$ present in the cells, with a specific threshold of $p60^{v\text{-}src}$ required to achieve morphological transformation and anchorage-independent growth. Thus, both high levels of expression of p60 and critical amino acid substitutions might be necessary for cellular transformation by $p60^{c\text{-}src}$.

Unlike the specific amino acid changes that have been identified in activated c-*ras* isolated from different human tumor cell lines (36–41), the substitutions in the primary structure of the p60 proteins of the two transforming mutant *src* viruses occur at different amino acids. Moreover, the mutations in the *src* genes of the mutant viruses do not coincide with the differences between v-*src* and c-*src* coding sequences (10). The activation of the transforming potential of c-*src* by a single mutation at different locations is consistent with the relatively high frequency with which transforming virus is generated from the c-*src*-containing viral populations (17). The different amino acid substitutions in the p60 proteins of NY501T7-m and NYHB5T9-m both reside in the tyrosine kinase domain (42) but at distinct sites. Since previous studies have suggested that a certain level of tyrosine kinase activity is essential for transformation (20), the single amino acid substitutions in the p60 proteins of the mutant transforming viruses might contribute to a conformational change in p60 that activates its kinase activity.

This study also clarified an error in the previously reported sequence of the plasmid pHB5 (17) and one in c-*src* (10). Based on the known sequences of the fragment of the SF strain of SRA-RSV used to construct pHB5 (13), this plasmid was thought to be identical in sequence to c-*src* (10). We have shown, however, that pHB5 encodes Asp at amino acid 63 instead of the Gly encoded at this position in c-*src*. In addition, we have corrected the original sequence at amino acid 501 in c-*src*, from the previously reported Arg to Lys (10).

A new plasmid, p5H, containing c-*src* coding information without introns, was generated by ligating portions of the two mutant viruses that are identical to the c-*src* sequence (10). This construct could be useful because its cDNA-like structure allows expression in bacteria and yeast vectors to generate large quantities of $p60^{c\text{-}src}$. The protein could be purified further and thereby aid in studies designed to elucidate the tertiary structure of $p60^{c\text{-}src}$ or be used for generating antibodies against $p60^{c\text{-}src}$.

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