MOLECULAR AND CELLULAR BIOLOGY, Dec. 1986, p. 4155–4160
0270-7306/86/124155-06$02.00/0

Vol. 6, No. 12

Amino Acid Substitutions Sufficient To Convert the Nontransforming p60$^{c\text{-}src}$ Protein to a Transforming Protein

JUN-YA KATO,[1] TATSUO TAKEYA,[1]* CARLA GRANDORI,[2] HIDEO IBA,[2]† JOAN B. LEVY,[2]‡ AND HIDESABURO HANAFUSA[2]

Institute for Chemical Research, Kyoto University, Kyoto-fu 611, Japan,[1] and The Rockefeller University, New York, New York 10021[2]

Received 16 June 1986/Accepted 8 August 1986

We have previously shown that Rous sarcoma virus variants that carry the cellular homolog (c-*src*) of the viral *src* gene (v-*src*) do not transform chicken embryo fibroblasts. We also have shown that replacement of sequences upstream or downstream from the *BglI* site of the cellular *src* gene with the corresponding regions of v-*src* restored transforming activity to the hybrid genes. Since there are only six amino acid changes between p60$^{c\text{-}src}$ and p60$^{v\text{-}src}$ within the sequences upstream from *BglI*, we constructed chimeric molecules involving v-*src* and c-*src* to determine the effect of each amino acid substitution on the biological activities of the gene product. We found that the change from Thr to Ile at position 338 or the replacement of a fragment of c-*src* containing Gly-63, Arg-95, and Thr-96 with a corresponding fragment of v-*src* containing Asp-63, Trp-95, and Ile-96 converted p60$^{c\text{-}src}$ into a transforming protein by the criteria of focus formation, anchorage-independent growth, and tumor formation in newborn chickens. These mutations also resulted in elevation of the protein kinase activity of p60$^{c\text{-}src}$.

Previous studies have indicated that the cellular *src* gene (c-*src*) and the viral *src* gene (v-*src*) are functionally different. Overexpressed c-*src* in a retrovirus vector is unable to transform chicken embryo fibroblasts (CEF) in culture (15, 17, 33). Similar conclusions have been derived from studies of the mammalian system with a pSV2 vector containing c-*src* (23, 25). Further analysis showed that the gene product of c-*src*, p60$^{c\text{-}src}$, has lower protein kinase activity than p60$^{v\text{-}src}$, possibly accounting for the lack of transforming activity of the cellular gene product (6, 16).

DNA sequence analysis of v-*src* of the Schmidt-Ruppin (SR) strain of Rous sarcoma virus (RSV) (10, 24, 31) and c-*src* of chickens (30) revealed that eight single-base changes scattered across the v-*src* gene result in amino acid substitutions. This analysis also showed that the last 19 amino acids of p60$^{c\text{-}src}$ had been replaced by a completely new set of 12 amino acids derived from sequences present downstream from the c-*src* coding region (30). In our previous studies we reported that replacement of amino acids 1 to 432 or 432 to 533 of p60$^{c\text{-}src}$ with the corresponding sequences from p60$^{v\text{-}src}$ (using the *BglI* site at position +1294 of the *src* gene as the point of recombination) results in hybrid proteins with transforming activity (16). Both of the hybrid proteins have high levels of protein kinase activity (17). Since there are six amino acid substitutions between p60$^{v\text{-}src}$ and p60$^{c\text{-}src}$ upstream of the *BglI* site, we sought to determine which of these mutations is sufficient to confer transforming activity on the c-*src* gene product.

MATERIALS AND METHODS

Cells and viruses. CEF were prepared from 11-day-old embryos as described previously (14). Cell culture conditions and virus assays have also been described previously (14).

Construction of plasmids. The numbering of both nucleotides and amino acids starts at the initiator AUG codon of *src* unless otherwise noted. pBB4 and pHB5 were constructed and described in a previous work (17). pBB4 consists of v-*src* from the SR-A strain up to the *BglI* site (amino acids 1 to 432), with its C-terminal region (downstream from the *BglI* site) replaced with the corresponding region of c-*src* (amino acids 432 to 533) (Fig. 1). pHB5, derived from pBB4, has an *HgaI-BglI* fragment (amino acids 79 to 432) replaced with c-*src* from a recovered avian sarcoma virus (rASV1441) (31). Since the biological activity of pHB5 is the same as that of pTT501, which contains authentic chicken c-*src* obtained from chicken genomic DNA (17), we used pHB5 in subsequent constructions for reasons of convenience (pHB5 lacks most of the intron sequences present in pTT501). pTT801 was obtained by replacing the *MluI-MluI* fragment (amino acids 259 to 533) of pBB4 with the corresponding fragment of pHB5. pTT801 differs from pHB5 in carrying mutations at amino acid positions 95, 96, 117, and 124 (Fig. 1). pTT901 was obtained by reciprocal exchange and therefore differs from pHB5 only at amino acid position 338 (Fig. 1). More recently we found that pHB5 encodes the entire c-*src* sequence except for one point mutation at nucleotide 188, which results in the change from Gly to Asp at amino acid position 63 (20a). To exclude the possibility of interaction with the mutation present at amino acid position 63, pTT951 was constructed. It was derived from pTT901 by replacing the *NcoI-MluI* fragment (amino acids 1 to 258) with authentic c-*src* sequences from plasmid p5H, described elsewhere (20a). pTT951 encodes the entire c-*src* sequence with only one mutation at amino acid position 338 (Fig. 1). pTT851 was constructed by replacing the *NcoI-HincII* fragment (amino acids 1 to 111) of pHB5 with the corresponding portion of pBB4. This chimeric molecule contains v-*src* sequences up to amino acid 111 and differs from pHB5 by the mutations at amino acids 95 and 96 (Fig. 1).

* Corresponding author.
† Present address: Department of Biophysics and Biochemistry, University of Tokyo, Tokyo, Japan.
‡ Present address: Department of Microbiology, State University of New York, Stony Brook, NY 11794.

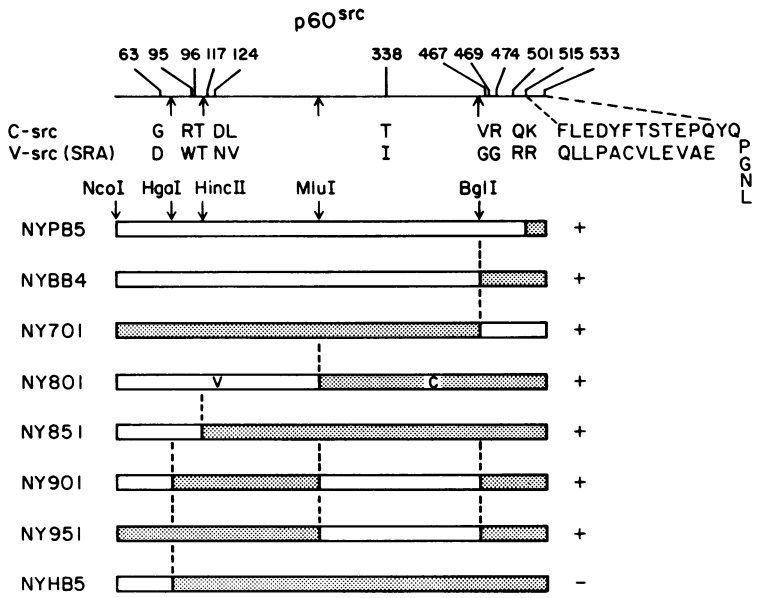

FIG. 1. Structure of the variant *src* genes. The derivatives used in the previous work (15, 17) are also shown for comparison. All the variants are derivatives of pSR-XD2 (8), and only the p60 coding region of the recombinant molecules is shown. The shaded and open boxes indicate DNA fragments derived from c-*src* and v-*src*, respectively. The positions of the nine amino acid differences between v-*src* and c-*src* are shown at the top. Transforming ability (+, positive; −, negative) is also indicated.

Transfection procedure. The *src*-encoding plasmids were cleaved with *Sal*I, ligated to a *Sal*I digest of a derivative of pSR-REP in which the *pol* gene of SR-A has been replaced by the *pol* gene of Bryan high-titer RSV (9, 27), and transfected into CEF by the calcium phosphate method (9, 32). In some experiments a *Sal*I digest of pRAV2-REP instead of pSR-REP was used. pRAV2-REP is a plasmid containing the entire Rous-associated virus 2 (RAV2) genome cloned at the *Sal*I site of pBR322. In the latter case one *Sal*I site was destroyed by a repair reaction so that a linearized molecule can supply most of the replication genes of RSV (9). At 8 to 10 days after transfection, virus was collected and used in focus formation and anchorage-independent growth assays on CEF.

p60 analysis. Infected CEF were labeled with [^{3}H]leucine for 4 h as described previously (8, 9). p60 proteins were analyzed by immunoprecipitation of cellular lysates in RIPA buffer (24) with monoclonal antibody (MAb) 327 (provided by J. Brugge) (21), followed by electrophoresis on sodium dodecyl sulfate (SDS)-polyacrylamide gels. Pulse chase analysis for determination of the half-life of p60^{src} was performed with a 1-h labeling time with [^{35}S]methionine followed by washing and further incubation in complete medium containing cold methionine. Cell extracts were then immunoprecipitated with MAb 327 and electrophoresed. Quantitation of the intensity of gel bands was done by densitometric scanning of the M_r 60,000 (60K) region of the autoradiogram.

p60 kinase activity. p60 immunoprecipitates, described above, were split in two parts: electrophoresis sample buffer was added to half for analysis of [^{3}H]leucine-labeled p60, and the other half was assayed for enolase phosphorylating activity (11). The radioactivity of the band corresponding to enolase was estimated by densitometric scanning of the autoradiogram. Equivalent amounts of cell lysates from $^{32}P_i$-labeled CEF were immunoprecipitated with anti-34K protein serum (provided by M. Greenberg) and analyzed by SDS-polyacrylamide gel electrophoresis. Replica samples were run in a parallel gel which was then subjected to alkali treatment at high temperature (4) to selectively detect phosphorylation of tyrosine residues in the 34K band.

RESULTS

Construction of *src* hybrid genes and transfection on CEF. We constructed hybrid plasmids with v-*src* and c-*src* DNA (Fig. 1) as described in Materials and Methods. These pSR-XD2 derivatives were cut at a *Sal*I site and ligated to pSR-REP, which contains most of the replication genes of RSV, as described previously (9). CEF transfected with the ligated DNAs subsequently produced replication-competent virus.

Biological characterization of the RSV variants carrying hybrid *src* genes. CEF transfected with the plasmids described above were subcultured once and overlaid with agar, and 8 to 10 days later transfection foci were detected (Table 1). An assay for reverse transcriptase in the culture medium revealed that the activity reached maximum levels at 9 to 10 days after transfection. Virus collected from the transfected cultures was assayed by focus formation and by soft-agar colony formation on CEF (Table 1). Viruses were designated by the prefix NY followed by the number of the corresponding plasmid. NY801, NY851, NY901, and NY951 titers were somewhat lower than that obtained with the plasmids containing wild-type v-*src* (NYN4). The morphology of representative foci produced by each virus is shown in Fig. 2. Foci produced by NYN4, NYBB4, and NY701 showed the characteristic morphology of wild-type-virus-transformed cells as described previously (17). NY801, NY851, NY901, and NY951 foci were not as distinct as those induced by wild-type virus, and their appearance occurred 2 or 3 days later than wild-type foci. The soft-agar colony assay at 37°C showed that NY801, NY851, NY901, and NY951 were able to confer anchorage-independent growth to CEF at a frequency similar to that at which they induced foci (Table 1). As a control we confirmed that NYHB5 produced both foci

TABLE 1. Focus formation by transfected CEF and titer of transforming virus produced

Plasmids	Foci[a] on transfected CEF	Virus titer				Tumor formation
		FFU/ml[b]		CFU/ml[c]		
		Expt 1	Expt 2	Expt 1	Expt 2	
pN4 and pREP	TMTC	3×10^6	1×10^7	9×10^5	5×10^5	+
pBB4 and pREP	TMTC	3×10^6	7×10^6	2×10^6	2×10^6	+
pTT801 and pREP	TMTC	7×10^5	2×10^6	1×10^5	2×10^5	+
pTT851 and pREP	TMTC	5×10^5	4×10^5	4×10^5	2×10^5	+
pTT901 and pREP	TMTC	6×10^5	6×10^5	3×10^5	1×10^5	+
pTT951 and pREP	TMTC	1×10^6	2×10^6	ND[d]	2×10^5	+
pHB5 and pREP	0	280	5×10^3	2×10^3	1×10^3	−
pREP only	0	ND	ND	ND	ND	ND

[a] Foci in hard agar overlaid cultures counted 8 to 10 days after transfection. TMTC, Too many to count.
[b] Focus formation assay on CEF infected with virus stock collected 8 to 13 days after transfection.
[c] CFU of virus stock derived from transfected culture fluid in soft agar.
[d] ND, Not determined.

in a monolayer and colonies in soft agar with a 100- to 1,000-fold-lower efficiency than the transforming viruses, and these activities are probably due to the generation of mutants as described before (15, 17).

The tumorigenic potential of the recombinant viruses was tested in newborn chickens (Table 1). About 10^5 FFU were injected subcutaneously in both wing webs, and tumor formation was scored 2 weeks later. NY801, NY851, NY901, and NY951 induced tumors of about 1 to 2 cm, while those induced by NYBB4 were about 3 cm. No tumors were detected with NYHB5.

Analysis of p60^{src} kinase activity. We analyzed p60^{src} kinase activity with the virus stocks obtained from the transfected cultures. MAb 327 was used to immunoprecipitate [^{3}H]leucine-labeled p60 coded by all the recombinant viruses as well as wild-type p60$^{v\text{-}src}$ coded by NYN4 and p60$^{c\text{-}src}$ of NYHB5 (Fig. 3A). Since the labeling time with [^{3}H]leucine (4 h) was shorter than the half-lives of the p60s (8 h for p60$^{v\text{-}src}$, 24 h for p60$^{c\text{-}src}$) (16), the amount of radioactive p60 was almost proportional to the synthesis rate (16). We examined the half-lives of the chimeric p60s and found no significant differences between NY801 (8.5 h), NY851 (8 h), or NY901 (11 h) and wild-type NYN4 (8.5 h). The similar half-lives of the p60 variants allowed a comparison of their in vitro kinase activities. The protein kinase activity in cell extracts was assayed in immune complexes with enolase as the exogenous substrate (Fig. 3B). The extent of phosphorylation of enolase was quantitated by densitometic scanning and normalized to the [^{3}H]leucine-labeled p60 immunoprecipitated from an equivalent amount of cell lysates. The p60s of transformed cultures were 10- to 20-fold more active in enolase phosphorylation than p60$^{c\text{-}src}$ and similar to wild-type p60$^{v\text{-}src}$ (Table 2). Increased kinase activity of the transforming proteins was also detected when casein was used as the substrate (data not shown). We examined the level of phosphorylation of the 34K protein, one of the major cellular substrates of p60$^{v\text{-}src}$ (12), in vivo. Infected CEF were labeled for 4 h with $^{32}P_i$, and the 34K protein was

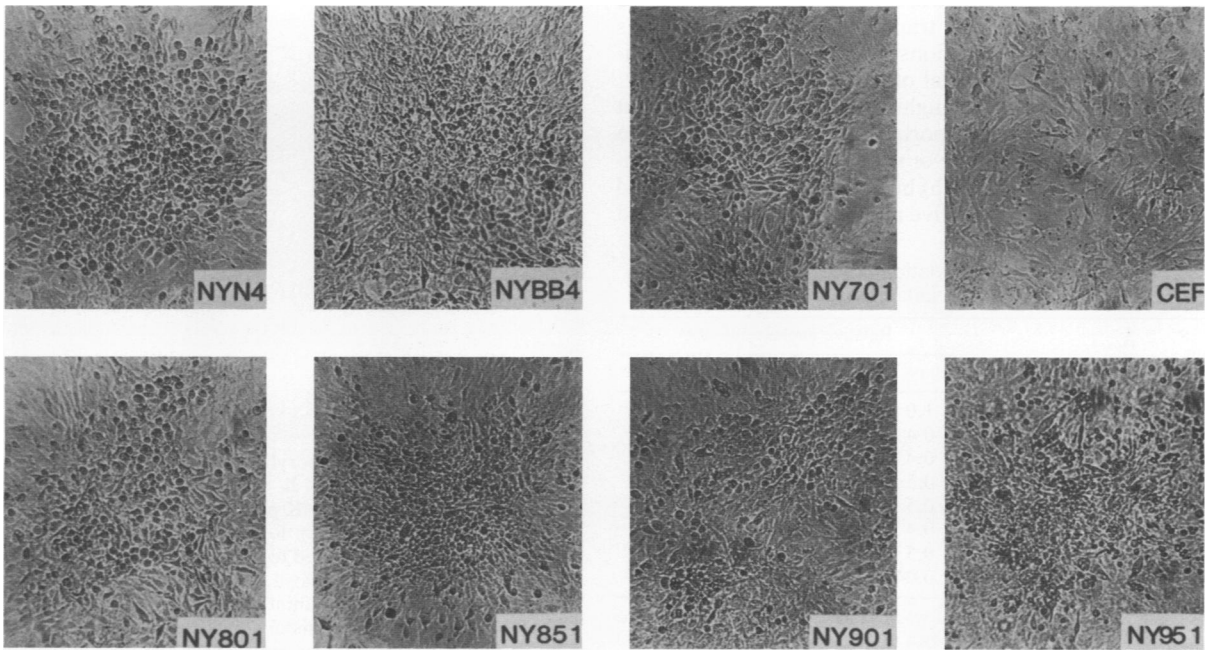

FIG. 2. Morphology of foci in infected CEF by the recombinant viruses 8 to 9 days after infection. NYN4 is equivalent to wild-type RSV. CEF, Uninfected CEF.

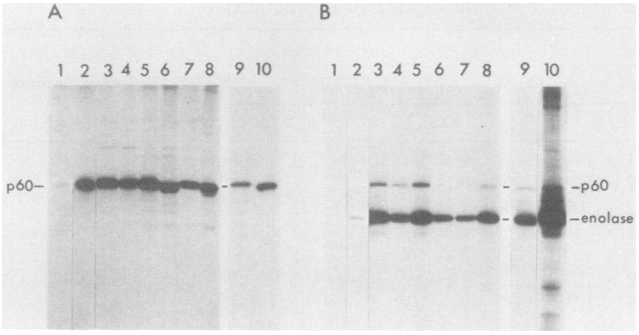

FIG. 3. Synthesis of chimeric p60s and their in vitro kinase activities. [^{3}H]leucine-labeled cell extracts were immunoprecipitated with MAb 327; immunoprecipitates were run on an SDS-polyacrylamide gel (A) or assayed for phosphotransferase activity in the presence of enolase as the exogenous substrate (B). Lanes: 1, uninfected CEF; 2, NYHB5; 3, NY801; 4, NY851; 5, NY901; 6, NY701; 7, NYBB4; 8, NYN4; 9, NY951; 10, NYN4.

immunoprecipitated from cell lysates with anti-34K protein serum (Fig. 4). To compare tyrosine-specific phosphorylation, the gel was treated with alkali as described previously (4) (Fig. 4B). All the transforming viruses induced higher levels of phosphorylation of the 34K protein than in NYHB5 or uninfected CEF. Overall there was good correlation among in vitro kinase activity, in vivo phosphorylation of the 34K protein, and transforming activity of the recombinant viruses.

DISCUSSION

Using constructed variants of RSV in which the entire v-*src* sequence was replaced by the c-*src* sequence (15, 17) we showed previously that simple overproduction of $p60^{c\text{-}src}$ does not cause cell transformation. Replacement of the portion of the v-*src* gene either upstream or downstream of the *BglI* site (which corresponds to amino acid 432) with the homologous portion of the c-*src* gene generates chimeric p60s capable of causing cell transformation. These results already suggested that mutations at multiple sites can activate the transforming potential of the c-*src* gene product.

In the present study we sought to determine which point mutations in the N-terminal portion of $p60^{v\text{-}src}$ (from amino acids 1 to 432) are responsible or sufficient for the transforming activity of NYBB4. New hybrid genes were constructed to determine the effect of the five amino acid changes located at positions 95, 96, 117, 124, and 338. After these hybrid genes were constructed, we discovered that the c-*src* sequence of pHB5 used in the constructions contains a mutation at amino acid 63 relative to authentic c-*src* (20a). The results indicate that the hybrid genes encoded proteins in which amino acid Thr-338 was replaced with Ile or the three amino acids Gly-63, Arg-95, and Thr-96 were replaced with Asp, Trp, and Ile, respectively, acquired transforming activity. However, the foci induced by these viruses were somewhat less distinct than those of the wild-type virus. In addition, all of the viruses induced tumors with a latent period and frequency similar to those of the wild-type virus. Therefore, the substitution at position 338 is sufficient to convert $p60^{c\text{-}src}$ to an actively transforming protein. The fact that a single point mutation is sufficient to confer transforming activity was also observed with spontaneous mutants in which the glutamic acid (amino acid 378) of c-*src* is replaced with glycine in one virus and Ile-441 of c-*src* is replaced with phenylalanine in another virus (20a). These findings are consistent with the relatively high frequency of generation of transforming viruses after only a few passages of c-*src*-containing virus (17). The lesions at positions 63, 95, and 96 were also sufficient to activate the transforming potential of $p60^{c\text{-}src}$. Interestingly, when all of the mutations were simultaneously present (NYBB4), a synergistic effect was observed on the transformed morphology of cells. This suggests that even though one mutation is sufficient to activate $p60^{c\text{-}src}$, the additional mutations have been selected for because they enhance the transforming ability of the viral protein.

Studies of in vitro-generated deletion mutants of RSV (3, 7, 8, 9) have defined amino-terminal and kinase domains in $p60^{v\text{-}src}$. Amino acids 1 to 14 are essential for $p60^{v\text{-}src}$ myristylation, membrane association, and transformation (8). $p60^{c\text{-}src}$ is also myristylated and membrane associated, fulfilling two of the requisites for $p60^{src}$-mediated cell transformation (16). The carboxy-terminal 30 kilodaltons of $p60^{src}$ constitutes a tyrosine kinase domain (2, 20). Also, a high

TABLE 2. In vitro phosphorylation of exogenous enolase by MAb 327 immunoprecipitates of p60 proteins

Virus	Relative enolase activity[a]	
	Expt 1	Expt 2
NYN4	1.0	1.0
NYBB4	0.4	0.8
NY701	0.4	0.4
NY801	0.5	0.8
NY851	0.5	0.6
NY901	0.4	0.7
NY951	0.5	ND[b]
NYHB5	0.04	0.05

[a] Radioactivity in the enolase band was estimated and normalized to the amount of [^{3}H]leucine-labeled p60 shown in Fig. 3A. The activity of NYN4 was given a value of 1.0, and the activities of the recombinant viruses are shown relative to that of NYN4.

[b] ND, Not determined.

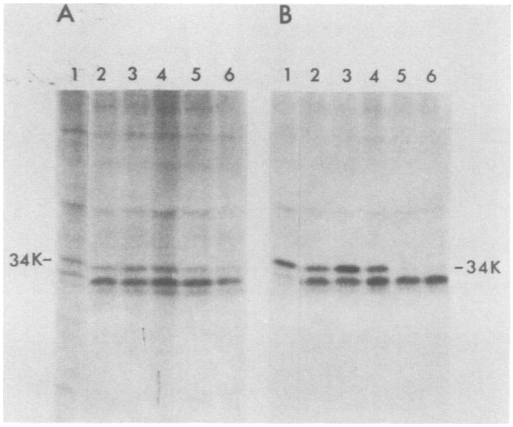

FIG. 4. In vivo phosphorylation of the 34K protein. Infected CEF were labeled for 4 h with $^{32}P_i$; cell extracts were immunoprecipitated by anti-34K protein antiserum and analyzed on an SDS-polyacrylamide gel (A). Replica samples were run in a parallel gel which was then subjected to alkali treatment at high temperature prior to drying (B). Lanes: 1, NYN4; 2, NY901; 3, NY801; 4, NY851; 5, NYHB5; 6, uninfected CEF. An additional lower-molecular-weight alkali-resistant band was detected with the anti-34K protein serum. It was present in uninfected, infected, and transformed cells and might be nonspecifically recognized by the particular batch of serum used in these experiments.

degree of homology among tyrosine kinases encoded by different oncogenes is present in the same region (22). The substitution of Thr for Ile at position 338 in NY901 and NY951 falls into the kinase domain. The increased kinase activity produced by the mutation at position 338 may be due to a local structural change within this domain or to an altered conformation of the whole protein.

The finding that the mutations at positions 63, 95, and 96 also increased the kinase activity suggests that in p60$^{c\text{-}src}$ an interaction takes place between the N-terminal region and the kinase domain. A specific involvement of amino acid residues 95 and 96 in this interaction or an overall conformational change due to the presence of N-terminal mutations may be the cause of the observed constitutive activation of the tyrosine kinase in NY801 and NY851 p60. The activation of p60$^{c\text{-}src}$ kinase through modification of its N-terminal region has also been recently implicated in transformation by polyomavirus (5). The activated p60$^{c\text{-}src}$ is associated with middle T antigen and is phosphorylated in vitro and possibly in vivo on tyrosine residues within its N-terminal region (34). Moreover, studies of activated p60$^{c\text{-}src}$ in neuronal cell cultures also demonstrated a modification of the N-terminal domain (1). While the nature of this N-terminal modification is not understood, the point mutations present in NY851 and NY801 might be related to the effect of the N-terminal changes present in the activated p60$^{c\text{-}src}$ in neurons or in the middle T-associated p60$^{c\text{-}src}$. Somewhat similar activation of protein kinase by a modification of the amino termini of cellular oncogenes has also been seen with c-*fps* (13) and c-*abl* (18, 26).

The inability of p60$^{c\text{-}src}$ to transform has been attributed to low levels of tyrosine kinase activity (16). All the recombinant hybrids between c-*src* and v-*src* previously studied (16) and the ones described here, which were transforming, show increased levels of tyrosine kinase activity both in vitro and in vivo. These observations substantiate the notion that tyrosine phosphorylation of a target cellullar protein(s) by p60$^{v\text{-}src}$ is required for cell transformation.

LITERATURE CITED

1. **Brugge, J. S., P. C. Cotton, A. E. Queral, J. N. Barret, D. Nonner, and R. W. Keane.** 1985. Neurons express high levels of a structurally modified activated form of pp60$^{c\text{-}src}$. Nature (London) **316**:554–557.
2. **Brugge, J., and D. Darrow.** 1984. Analysis of the catalytic domain of the phosphotransferase activity of two avian sarcoma virus transforming proteins. J. Biol. Chem. **259**:4550–4557.
3. **Bryant, D., and T. Parsons.** 1982. Site-directed mutagenesis of the *src* gene of Rous sarcoma virus: construction and characterization of a deletion mutant temperature sensitive for transformation. J. Virol. **44**:683–691.
4. **Cooper, J. M., and T. Hunter.** 1981. Changes in protein phosphorylation in Rous sarcoma virus-transformed chicken embryo cells. Mol. Cell. Biol. **1**:165–178.
5. **Courtneidge, S., and A. E. Smith.** 1983. Polyoma virus transforming protein associates with the product of the c-src cellular gene. Nature (London) **303**:435–439.
6. **Coussens, P. M., J. A. Cooper, T. Hunter, and D. Shalloway.** 1985. Restriction of the in vitro and in vivo tyrosine protein kinase activities of pp60$^{c\text{-}src}$ relative to pp60$^{v\text{-}src}$. Mol. Cell. Biol. **5**:2753–2763.
7. **Cross, R. F., E. A. Garber, and H. Hanafusa.** 1985. N-terminal deletions in Rous sarcoma virus p60^{src}: effects on tyrosine kinase and biological activity and on recombination in tissue culture with the cellular *src* gene. Mol. Cell. Biol. **5**:2789–2795.
8. **Cross, F. R., E. A. Garber, D. Pellman, and H. Hanafusa.** 1984. A short sequence is required for p60^{src} myristylation and membrane association and for cell transformation. Mol. Cell. Biol. **4**:1834–1842.
9. **Cross, F. R., and H. Hanafusa.** 1983. Local mutagenesis of Rous sarcoma virus: the major sites of tyrosine and serine phosphorylation of p60^{src} are dispensable for transformation. Cell **34**:597–607.
10. **Czernilofsky, A. P., A. D. Levinson, H. E. Varmus, J. M. Bishop, E. Tisher, and H. M. Goodman.** 1983. Correction to the nucleotide sequence of the src gene of Rous sarcoma virus. Nature (London) **301**:736–738.
11. **Davis, R. L., J. B. Konopka, and O. N. Witte.** 1985. Activation of the c-*abl* oncogene by viral transduction generates altered c-*abl* proteins with similar in vitro kinase properties. Mol. Cell. Biol. **5**:204–213.
12. **Erikson, E., and R. L. Erickson.** 1980. Identification of a cellular protein substrate phosphorylated by the avian sarcoma virus transforming gene product. Cell **21**:829–836.
13. **Foster, D. A., M. Shibuya, and H. Hanafusa.** 1985. Activation of the transforming potential of the cellular *fps* gene. Cell **42**:105–115.
14. **Hanafusa, H.** 1969. Rapid transformation of cells by Rous sarcoma virus. Proc. Natl. Acad. Sci. USA **63**:318–325.
15. **Hanafusa, H., H. Iba, T. Takeya, and F. Cross.** 1984. The transforming activity of the c-*src* gene, p. 1–7. *In* G. F. Vande Woude, A. J. Levine, and J. D. Watson (ed.), Cancer cells, vol. 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Iba, H., F. R. Cross, E. A. Garber, and H. Hanafusa.** 1985. Low level of cellular protein phosphorylation by nontransforming overproduced p60$^{c\text{-}src}$. Mol. Cell. Biol. **5**:1058–1066.
17. **Iba, H., T. Takeya, F. R. Cross, T. Hanafusa, and H. Hanafusa.** 1984. Rous sarcoma virus variants which carry the cellular *src* gene instead of the viral *src* gene cannot transform chicken embryo fibroblasts. Proc. Natl. Acad. Sci. USA **81**:4424–4428.
18. **Konopka, J. B., S. M. Watanabe, and O. N. Witte.** 1984. An alteration of the human c-abl protein in K562 leukemia cells unmasks associated tyrosine kinase activity. Cell **37**:1035–1042.
19. **Lerner, T. L., and H. Hanafusa.** 1984. DNA sequence analysis of the Bryan high-titer strain of Rous sarcoma virus: extent of *env* deletion and possible genealogical relationship with other viral strains. J. Virol. **49**:549–556.
20. **Levinson, A. D., S. A. Courtneidge, and J. M. Bishop.** 1981. Structural and functional domains of the Rous sarcoma virus transforming protein (pp60^{c}). Proc. Natl. Acad. Sci. USA **78**:1624–1628.

20a. **Levy, J. B., H. Iba, and H. Hanafusa.** 1986. Activation of the transforming potential of p60$^{c\text{-}src}$ by a single amino acid change. Proc. Natl. Acad. Sci. USA **83**:4228–4232.

21. **Lipsich, L. A., A. J. Lewis, and J. S. Brugge.** 1983. Isolation of monoclonal antibodies that recognize the transforming proteins of avian sarcoma viruses. J. Virol. **48**:352–360.
22. **Neckamayer, W. S., and L. H. Wang.** 1985. Nucleotide sequence of avian sarcoma virus UR2 and comparison of its transforming gene with other members of the tyrosine protein kinase oncogene family. J. Virol. **53**:879–884.
23. **Parker, R. C., H. E. Varmus, and J. M. Bishop.** 1984. Expression of v-*src* and chicken c-*src* in rat cells demonstrates qualitative differences between pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$. Cell **37**:131–139.
24. **Poirier, F., G. Calothy, R. E. Karess, E. Erikson, and H. Hanafusa.** 1982. Role of p60^{src} kinase activity in the induction of neuroretinal cell proliferation by Rous sarcoma virus. J. Virol. **42**:780–789.
25. **Shalloway, D., P. M. Coussens, and P. Yaciuk.** 1984. c-*src* and *src* homolog overexpression in mouse cells, p. 10–17. *In* G. F. Vande Woude, A. J. Levine, and J. D. Watson (ed.), Cancer cells, vol. 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Shtivelman, E., B. Lifshitz, R. P. Gale, and E. Canaani.** 1985. Fused transcript of abl and bcr genes in chronic myelogenous leukemia. Nature (London) **315**:550–554.
27. **Sudol, M., T. L. Lerner, and H. Hanafusa.** 1986. Polymerase-defective mutant of the Bryan high-titer strain of Rous sarcoma virus. Nucleic Acids Res. **14**:2391–2405.
28. **Takeya, T., R. A. Feldman, and H. Hanafusa.** 1982. DNA

sequence of the viral and cellular *src* of chicken. I. Complete nucleotide sequence of an *Eco*RI fragment of recovered avian sarcoma virus which codes for gp37 and pp60^{src}. J. Virol. **44**:1–11.

29. **Takeya, T., and H. Hanafusa.** 1982. DNA sequence of the viral and cellular *src* gene of chicken. II. Comparison of the *src* gene of two strains of avian sarcoma virus and of the cellular homolog. J. Virol. **44**:12–18.
30. **Takeya, T., and H. Hanafusa.** 1983. Structure and sequence of the cellular gene homologous to the RSV *src* gene and the mechanism for generating the transforming virus. Cell **32**:881–890.
31. **Takeya, T., H. Hanafusa, R. P. Junghans, G. Ju, and A. M. Skalka.** 1981. Comparison between the viral transforming gene (*src*) of recovered avian sarcoma virus and its cellular homolog. Mol. Cell. Biol. **1**:1024–1037.
32. **Wigler, M., A. Pellicer, S. Silverstein, R. Axel, G. Uriaub, and L. Chasin.** 1979. DNA-mediated transfer of the adenine phosphoribosyltransferase locus into mammalian cells. Proc. Natl. Acad. Sci. USA **76**:1373–1376.
33. **Wilhemsen, K. C., W. G. Tarpley, and H. M. Temin.** 1984. Identification of some of the parameters governing transformation by oncogenes in retroviruses, p. 303–308. *In* G. F. Vande Woude, A. J. Levine, and J. D. Watson (ed.), Cancer cells, vol. 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
34. **Yonemoto, W., M. Jarvis-Morar, J. S. Brugge, J. B. Bolen, and M. A. Israel.** 1985. Tyrosine phosphorylation within the amino-terminal domain of pp60$^{c\text{-}src}$ molecules associated with polyoma virus middle-sized tumor antigen. Proc. Natl. Acad. Sci. USA **82**:4568–4572.