Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 4424–4428, July 1984
Cell Biology

Rous sarcoma virus variants that carry the cellular *src* gene instead of the viral *src* gene cannot transform chicken embryo fibroblasts

(proto-oncogene/transfection/transformation)

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Communicated by Igor Tamm, April 8, 1984

ABSTRACT **The transforming activity of the cellular *src* (c-*src*) gene as well as of hybrid genes between viral and cellular *src* was tested by constructing derivatives of Rous sarcoma virus DNA in which all or part of the viral *src* gene (v-*src*) was replaced by the corresponding portion of the c-*src* gene. After these derivatives were introduced into chicken embryo fibroblasts by transfection, replication-competent virus was recovered, which induced the expression of $p60^{src}$ at a level equivalent to $p60^{v\text{-}src}$ expression in cells infected with Rous sarcoma virus wild type. Replacement of the portion of the v-*src* gene, either upstream or downstream of the *Bgl* I site, with the homologous portion of the c-*src* gene resulted in fully transforming viruses. On the other hand, the virus stock obtained from cells transfected with Rous sarcoma virus DNA containing the entire c-*src* gene had a very low titer of focus-forming virus, while it contained a high titer of infectious virus. We present evidence that the rare small foci are formed by mutant viruses generated from the original c-*src*-containing virus. These results indicate that overproduction of the c-*src* gene product does not cause cell transformation, and that this proto-oncogene is subject to a relatively high rate of mutation when incorporated in a retrovirus genome, resulting in the acquisition of transforming capacity.**

Some retroviruses contain genes (oncogenes) responsible for their oncogenicity (1). It is now widely accepted that certain host cell genes (proto-oncogenes) were captured by retroviruses in their replication cycle and have evolved into these oncogenes (2, 3). Whether a proto-oncogene itself can function as a transforming gene or whether some genetic change is essential for cellular transformation remains to be established for many oncogenes.

Rous sarcoma virus (RSV) contains such an oncogene, designated the viral *src* gene (v-*src*), which encodes a M_r 60,000 tyrosine-specific protein kinase ($p60^{src}$) (4, 5). Its cellular counterpart, the cellular *src* gene (c-*src*), is expressed in uninfected chicken embryo fibroblasts (CEF) at a level of 1%–2% of the expression of $p60^{v\text{-}src}$ in RSV-infected CEF (6–8). The chicken cellular sequence coding for $p60^{c\text{-}src}$ (9–11) consists of 11 exons (12), and the vast majority of it is quite similar to the v-*src* gene (12–16). In the case of the Schmidt–Ruppin A strain of RSV (SR-RSV), only eight single base changes scattered across the v-*src* gene result in amino acid substitutions (12). However, in addition to these single amino acid changes, a clear difference exists in the COOH-terminal regions: the last 19 amino acids in $p60^{c\text{-}src}$ are replaced by another sequence of 12 amino acids in $p60^{v\text{-}src}$ (12). The sequence encoding most of the COOH-terminal sequence of $p60^{v\text{-}src}$ is found 0.9 kilobase (kb) downstream from the termination codon of the c-*src* gene, suggesting that this sequence was incorporated into the v-*src* sequence when RSV was formed (12, 17).

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In studies on the formation of recovered avian sarcoma virus (rASV) in chickens infected with RSV mutants containing partial deletions of the *src* gene (3, 8, 18, 19), it has been shown that the majority of v-*src* can be substituted by c-*src* to encode an active transforming protein. We constructed recombinant RSV DNAs in which various portions of the v-*src* sequence were replaced by the corresponding portions of c-*src*, and we determined whether overproduced c-src protein, as well as chimeric proteins between v- and c-src protein, can transform CEF. A preliminary account of some of these findings has been presented (20).

MATERIALS AND METHODS

Cells and Virus. Primary and secondary CEF were prepared as described (21). Cell culture conditions and virus assays were also those of Hanafusa (21).

Plasmid Construction. All the plasmids constructed here are derivatives of pSR-XD2, which contains the v-*src* gene of SR-RSV (22). The structure of *Sal* I inserts of the plasmids are schematically shown in Fig. 1 with their molecular weights and the sites of restriction enzymes used for the construction. (*i*) pN4 was constructed by a *Bgl* II linker insertion into pSR-XD2 at an *Nru* I site located 95 base pairs (bp) downstream from the termination codon of v-*src*. (*ii*) pBB4 was constructed by replacing the 3′ region of v-*src* of pN4 (*Bgl* I/*Bgl* II fragment) with the corresponding portion of c-*src*, a *Bgl* I/*Sac* I fragment of pRW10, which is a subclone of the c-*src* locus (19). The *Sac* I site of the latter fragment is 13 bp downstream from the termination codon of c-*src* (12) and was changed into a *Bgl* II site by a *Bgl* II linker ligation after conversion into a flush end by T4 DNA polymerase (23). (*iii*) pPB5 is a chimeric plasmid similar to pBB4, but a *Pst* I site rather than the *Bgl* I site was used for the exchange of the 3′ terminal region of v- and c-*src*. (*iv*) pTT701 was constructed by substitution of the 5′ region of v-*src* with the corresponding region of c-*src*. From one derivative of pSR-XD2, pSR-XDR65, in which an *Rsa* I site between *env* and *src* (Fig. 1) was replaced by a *Bgl* II linker (unpublished data), the *Bgl* II/*Bgl* I fragment containing all the pBR322 was purified and ligated with two other fragments. One fragment (*Bam*HI/*Nco* I) was obtained from pTT107, a subclone of SR-RSV (11) after changing an *Eco*RI site into a *Bam*HI site by a linker ligation; the other fragment (*Nco* I/*Bgl* I) was isolated from λRCS3, a clone of the chicken c-*src* locus (11). (*v*) pTT501 was constructed by exchanging the *Bgl* I/*Sac* I fragment of pTT701 with the equivalent fragment from pBB4 to encode the entire c-*src* gene. (*vi*) pHB5, another plasmid encoding the entire c-*src* sequence, was constructed by exchanging the *Hga* I/*Bgl* I fragment of pBB4 with the equivalent fragment from pTT108, which is a subclone containing the *src* gene of rASV1441 (11).

Abbreviations: RSV, Rous sarcoma virus; SR-RSV, Schmidt–Ruppin A strain of RSV; rASV, recovered avian sarcoma virus; bp, base pair(s); kb, kilobase(s); CEF, chicken embryo fibroblasts; TBR, tumor-bearing rabbit.

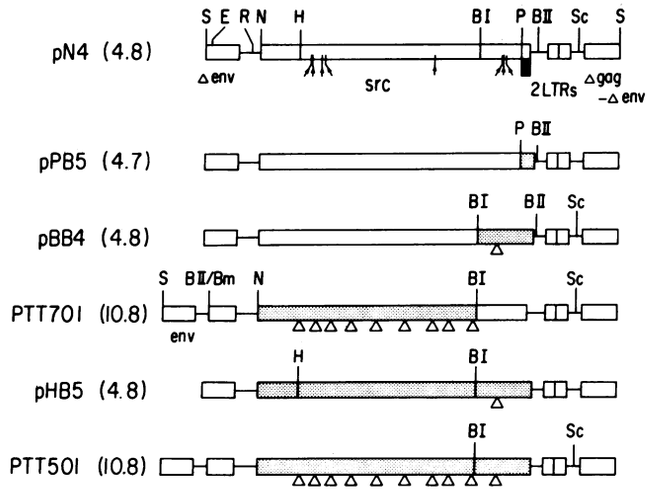

FIG. 1. The structure of constructed pSR-XD2 derivatives. The *Sal* I fragment inserted into pBR322 is shown together with the size of insertion shown in parentheses (kb) and restriction enzyme sites used for the plasmid construction. The abbreviations for restriction enzymes are as follows: *Sal* I (S), *Eco*RI (E), *Rsa* I (R), *Nco* I (N), *Hga* I (H), *Bgl* I (BI), *Pst* I (P), *Bgl* II (BII), *Sac* I (Sc), and *Bam*HI (Bm). The figure is not drawn to scale. In the diagram of the *src* gene, white boxes and shaded boxes indicate the v-*src* sequence and the c-*src* coding sequence, respectively. The sites of introns present in the corresponding nucleotide sequence of c-*src* are indicated by triangles (△). The positions of 8 amino acid differences between v-*src* and c-*src* (located 95, 96, 117, 124, 338, 467, 469, and 474 amino acid residues from the NH_2 terminus) (12, 15) are shown by arrows under the v-*src* in pN4. The black box shows the location of the COOH-terminal 12 amino acids of v-*src*, which are different from the COOH-terminal 19 amino acids of c-*src*.

Transfection Procedure. The *src*-encoding plasmids were cut with *Sal* I, ligated to a *Sal* I digest of a derivative of pSR-REP (22), and introduced into CEF by the calcium phosphate transfection method essentially as described (22, 24). The derivative of pSR-REP used in this study contains the *pol* sequence derived from the Bryan strain of RSV and is more efficient in virus production after transfection (25). For convenience, this derivative will be called pREP in this paper.

DNA Analysis. Agarose gel electrophoresis, Southern blotting analysis, and nick-translation for preparing labeled probes are essentially the same as described (23).

Protein Analysis. CEF were labeled with [^{3}H]leucine as described (26). p60 was analyzed by immunoprecipitation of cellular extracts (26) with antiserum from a rabbit immunized against $p60^{v\text{-}src}$ produced in *Escherichia coli* (anti-p60) (27) (supplied by R. Erikson) or with a tumor-bearing rabbit (TBR) serum (supplied by J. Brugge), followed by electrophoresis on sodium dodecyl sulfate polyacrylamide slab gels. The *in vitro* protein kinase assay was performed with immunoprecipitates as described (26).

RESULTS

Construction of Plasmids and Transfection of CEF. It was shown that foci can be formed in CEF by transfection with RSV DNA only when infectious virus is produced (28). We reported previously the construction of two plasmids, pSR-XD2, containing v-*src* of SR-RSV, and pSR-REP, containing most of the replication genes of RSV (22). When ligated together, these two plasmids form a proviral structure of RSV that produces replication-competent RSV after transfection, although neither plasmid alone can produce virus (22). We have constructed recombinant plasmids between pSR-XD2 and cellular *src* DNA (Fig. 1). pN4, a derivative of pSR-XD2, which has only one *Bgl* II site inserted downstream of the v-*src* termination codon, was used as the starting material of recombinant plasmid construction and was also used as the wild-type RSV plasmid in transfection experiments. In pPB5, a segment of the COOH-terminal region of v-*src* was replaced by the corresponding c-*src* fragment, which codes for all the c-*src*-specific 19 amino acid sequence. pBB4 is similar to pPB5, but its product contains three additional c-*src*-specific amino acids (Fig. 1). pTT701 is exactly the reciprocal construct to pBB4 in terms of v- and c-*src* order. pTT501, constructed by recombination between pBB4 and pTT701, contains the complete c-*src* sequence with 10 introns. Since both the efficiency of splicing of introns of the c-*src* gene in transfected cultures and the efficiency of packaging of unspliced RNA species were unknown, we also constructed another plasmid, pHB5, whose *src* gene consists of the v-*src* of SR-RSV (*Nco* I/*Hga* I), the *src* gene of rASV1441 (*Hga* I/*Bgl* I) and c-*src* (*Bgl* I/*Bgl* II) (Fig. 1). Since the *Hga* I/*Bgl* I region of rASV as well as the remaining v-*src* sequence in the 5′ region encode an amino acid sequence identical to that of c-*src* (Fig. 1) (12, 14), pHB5 should encode a protein completely identical to c-*src* with only one small intron in the *src* locus (Fig. 1) (12).

Analysis of Focus Formation on Transfected CEF and Recovery of Virus. CEF were transfected with the plasmids described above after ligation with pREP. Four days later, transfected cultures were subcultured in two plates. One was kept under soft agar for detection of foci, and the other was kept in fluid medium and was used for the assay of reverse transcriptase activity and for harvesting virus. Beginning 6–7 days after transfection, foci were detectable in CEF transfected with pPB5, pBB4, pTT701, and pN4. Nine days after transfection, these cultures kept in liquid medium became completely transformed, and large numbers of foci were observed in the soft agar-overlaid cultures (Table 1). The foci formed by pPB5 and pBB4 transfection (Fig. 2 *B* and *C*) were similar in size and appearance to those made by wild-type pN4 (Fig. 2*A*), but they tended to diffuse to a larger area and individual cells were a little more elongated. The foci formed by pTT701 (Fig. 2*D*) were smaller than the wild type and the cells assumed a smaller and rounder morphology, similar to

Table 1. Focus formation on transfected CEF and titer of transforming virus produced

Plasmid	Foci on transfected CEF* Exp. 1	Exp. 2	Virus	Titer of transforming virus in culture fluid† Exp. 1	Exp. 2
pN4 and pREP‡	1800	2200	NYN4	1.2×10^7	ND
pPB5 and pREP	800	ND§	NYPB5	5.0×10^6	ND
pBB4 and pREP	1100	2300	NYBB4	8.7×10^6	5.4×10^5
pTT701 and pREP	ND	570	NY701	ND	4.0×10^5
pHB5 and pREP	0	4	NYHB5	1.3×10^3	ND
pTT501 and pREP	0	0	NY501	4.0×10^2	2.0×10^2
pREP alone	0	0	None		

*The number of foci in soft agar-overlaid cultures (a 60-mm plate) was counted 9 days after transfection.

†Nine days after transfection, the virus stock was collected from transfected CEF kept in liquid medium and titrated on fresh CEF. Data are expressed in focus forming units per ml.

‡For the transfection, 1 μg of *Sal* I-cut pN4 was ligated with 1 μg of *Sal* I-cut pREP. In other *src*-encoding plasmids, equivalent molecular amounts to 1 μg of pN4 were ligated with *Sal* I-cut pREP (1 μg) after *Sal* I digestion.

§ND, not determined.

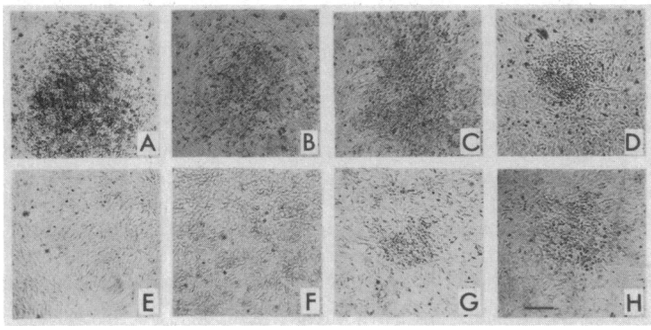

FIG. 2. Morphology of infected CEF. Cultures were kept in soft agar after transfection with pN4 (*A*), pPB5 (*B*), pBB4 (*C*), pTT701 (*D*), pTT501 (*E*), and no DNA (F). The small foci detected at a low frequency in CEF transfected with pHB5 (*G*) and pTT501 (*H*) are also shown. (Bar = 0.2 mm.)

that of rASV-infected CEF (3). On the other hand, CEF transfected with pHB5 or pTT501 formed very few foci, if any, at this stage, whether they were kept in liquid medium or in soft agar (Table 1). Examples of foci occasionally detectable in these cultures are shown in Fig. 2 *G* and *H*.

The success of cotransfection of each DNA with pREP was evaluated by the assay of reverse transcriptase activity in the culture medium. The activity became detectable at 6 days and reached a maximum at 9 days after transfection in all cultures, including pHB5 and pTT501. This result indicated that virus was produced and spread at a similar rate in all the transfected cultures. The titers of transforming virus in the culture medium collected 9 days after transfection are shown in Table 1. The titers in the stocks of NYN4, NYPB5, NYBB4, and NY701 (the viruses are designated after the name of plasmids, as shown in Table 1) were in the range of the titers of usual transforming viruses. The virus stocks of NY501 and NYHB5, on the other hand, contained low titers of transforming virus, although the amounts of total virus detectable by reverse transcriptase activity were comparable to that of NYN4.

Proviral Analysis of Transfected CEF. Shimotohno and Temin reported that introns of some cellular genes are not efficiently spliced out when they are located in a retrovirus genome (29). Since the c-*src*-encoding plasmid pTT501 contains 10 introns derived from c-*src*, we analyzed cellular DNA in cells at 9 days post-transfection to determine whether the introns of this plasmid were successfully processed and whether the c-*src* sequence is integrated efficiently in the transfected cells. Cellular DNA samples from pN4, pHB5, and pTT501-transfected CEF and uninfected CEF were digested with *Sal* I and *Bgl* II and hybridized with the v-*src* probe (Fig. 3). Since unintegrated viral DNA was undetectable in these cellular DNA samples (data not shown), the majority of viral DNA was integrated into the chromosome at this stage. Every sample had a 14.0-kb band that was derived from the endogenous c-*src* sequence (11). As shown in Fig. 3, the fragment derived from the original pTT501 plasmid was much larger (8.9 kb) than that of pHB5 (2.9 kb). Compared with DNA fragments of the original plasmid, the fragment obtained from proviral DNA in pHB5-transfected cells was shorter (2.8 kb), indicating that the small intron present in pHB5 (≈80 bp) had been removed. This was further confirmed by other Southern blotting analyses examining smaller fragments (data not shown). DNA from pTT501-transfected cells had a band of the same size (2.8 kb) as that of DNA from pHB5-transfected cells. This result, together with other Southern blotting analyses (data not shown), shows that all introns as well as one copy of the *env* sequences, which were duplicated in the original plasmid in the process of construction (Fig. 1), were removed from the proviral DNA. Furthermore, by comparing the density of the bands, we can conclude that essentially the same amounts of proviral DNA were present in pTT501, pHB5, and pN4-transfected CEF.

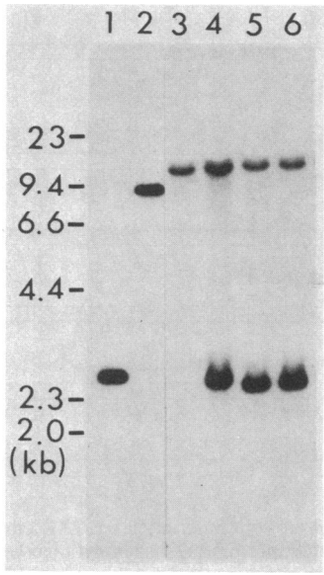

FIG. 3. Analysis of proviral DNA in transfected cultures. DNA samples were completely digested with both *Sal* I and *Bgl* I, separated on a 0.8% agarose gel, and analyzed by Southern blot hybridization. The probe was a ^{32}P-labeled 0.87-kb *Pvu* II DNA fragment, which covers ≈60% of the v-*src* sequence. DNA of plasmid pHB5 and pTT501 (lanes 1 and 2), uninfected CEF DNA (15 μg) (lane 3), DNA from CEF 9 days after transfection with pN4, pHB5, and pTT501 (15 μg each) (lanes 4–6). The numbers on the left indicate the size of *Hin*dIII-cut DNA fragments.

p60^{src} Production in CEF Infected with RSV Variants. We analyzed p60^{src} in both the initial transfected cultures and in cultures infected with virus stocks obtained from the transfected cultures. Results from these two sets were essentially the same. By screening of several available antisera, we found that one lot of TBR antiserum (from J. Brugge) and one lot of anti-p60 antiserum (from R. Erikson) were useful for precipitating p60$^{c\text{-}src}$ and all the chimeric p60^{src}, as well as the original immunogen p60$^{v\text{-}src}$. The proteins immunoprecipitated by anti-p60 antiserum from infected CEF are shown in Fig. 4*A*. The size of p60 in CEF infected with NYHB5, NY501, NYPB5, and NYBB4, all of which contain the COOH-terminal sequence of c-*src*, was identical to that of endogenous c-src protein. The p60s of N4 and NY701 were slightly smaller than p60$^{c\text{-}src}$, as expected from the size difference in COOH-terminal amino acid sequence (12). Furthermore, by the immunoprecipitation of these lysates with antiserum raised against a v-*src*-specific COOH-terminal peptide (30) (supplied by B. Sefton), we confirmed that only p60s produced by NYN4 or NY701 have the v-*src*-specific COOH-terminal region (data not shown). Quantification of the radioactivity in gel bands showed that the amounts of p60 in virus-infected cells were similar to each other, and they were more than 30 times the amount of the endogenous p60$^{c\text{-}src}$ expressed in the uninfected cell.

We analyzed protein kinase activity in the immune complex formed by anti-p60 antiserum (Fig. 4*B*). In this immune complex, p60 phosphorylates itself but not IgG heavy chain (27). Phosphorylation of p60s was observed with all variants, although the phosphorylation by extracts of NY701, NYHB5, and NY501 was lower than that of the other three by approximately a factor of 10. When the TBR antiserum, which can also precipitate all of the p60s, was used for the protein kinase reaction in immune complex, we were able to

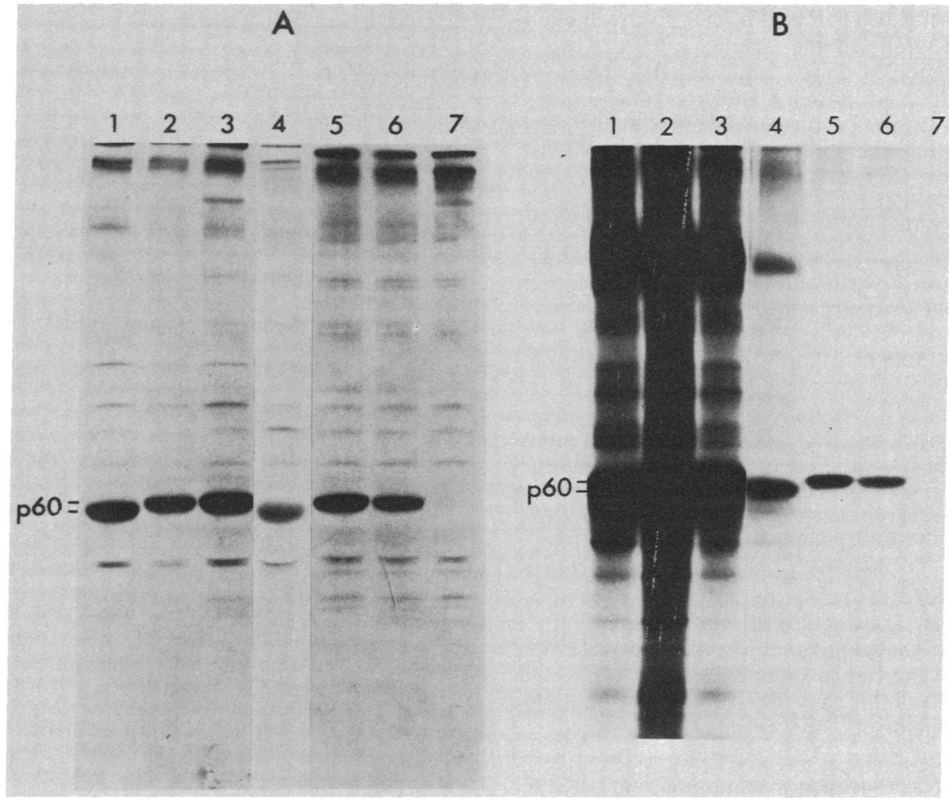

FIG. 4. Analysis of p60s in the infected cells and their protein kinase activity. (*A*) Cells infected with NYN4 (lane 1), NYPB5 (lane 2), NYBB4 (lane 3), NY701 (lane 4), NYHB5 (lane 5), and NY501 (lane 6) as well as uninfected cells (lane 7), were labeled with [^{3}H]leucine. p60s were immunoprecipitated from an amount of each cell lysate containing 2.5×10^7 cpm of trichloroacetic acid-insoluble ^{3}H radioactivity using anti-p60 antiserum, and were analyzed by sodium dodecyl sulfate polyacrylamide (8.5%) gel electrophoresis and detected by fluorography. (*B*) The same amount of the cell lysates used in lanes 1–7 of *A* were immunoprecipitated under the same conditions as in *A*, and the reaction products of protein kinase assay were separated on a gel and detected by autoradiography.

detect roughly the same amount of heavy chain phosphorylation by the p60s of all variants (20).

These results indicate that all infected cultures, including those infected with NYHB5 and NY501 that did not show detectable morphological transformation, produced at least 30 times more abundant enzymatically active p60 than uninfected CEF.

Virological Analysis of Foci Detected in Cultures Transfected with pHB5 and pTT501. As described above, we detected occasional small foci in cultures transfected with pHB5 and pTT501. The virus stocks isolated from these cultures had a low titer of transforming virus, although the assay of reverse transcriptase activity indicated that the stocks contained high titers of viral particles. Furthermore, when fresh CEF were infected with 0.1 ml of serial 10-fold dilutions of the stocks of NYHB5 and NY501, and subcultured, cultures inoculated with a dilution up to 10^{-6} formed 10–300 foci of transformed cells and developed interference with wild-type RSV (a sign of the replication of retrovirus). Thus, stocks of NYHB5 and NY501 contained 10^7 infectious virus per ml that were unable to cause immediate transformation but had the potential to cause a low level of transformation when these viruses multiplied to a high infectious titer.

Two explanations for the poor focus formation by pHB5 and pTT501 seem plausible. First, the overproduction of $p60^{c\text{-}src}$ may not cause transformation of CEF, but the virus with mutations in $p60^{c\text{-}src}$ may emerge after replication and these mutants may be responsible for the foci. Alternatively, c-*src*-encoding virus may transform CEF with a low frequency. To discriminate between these two possibilities, CEF were infected with undiluted stocks of NYHB5 and NY501, and virus was isolated both from transformed foci and from nontransformed background areas. If the first hypothesis is correct, the titer of transforming virus from the focal area should be high, whereas the second hypothesis predicts that virus in focal and nonfocal areas should be identical with low titers of transforming virus like the original stock. Of 17 isolates of virus from independent foci of NYHB5 or NY501, 16 produced high titers ($>10^5$ focus-forming virus per ml) of transforming virus, whereas all 17 isolates from nonfocal areas produced low titers ($<10^3$ focus-forming virus per ml). There were some differences in the morphologies of foci produced by independent isolates. These results support the first hypothesis that stocks of both NY501 and NYHB5 contain mutant transforming viruses. The speed with which foci appeared in these cultures indicates that the rate of mutation is rather high.

DISCUSSION

We examined the effect of overproduction of $p60^{c\text{-}src}$ in chicken cells, the natural host of RSV, and the source of our c-*src* clones. Using recombinant DNA technology and subsequent transfection of CEF, we obtained RSV variants that encode $p60^{c\text{-}src}$ or recombinant proteins between $p60^{c\text{-}src}$ and $p60^{v\text{-}src}$. RSV variants that encode c-*src* expressed a large amount of $p60^{c\text{-}src}$ in CEF but failed to cause cell transformation. The differences in biological activities of various constructed DNAs were reproducible in four independent experiments. The results thus clearly indicate that there is some qualitative difference between v-*src* and c-*src* gene products in functions related to cell transformation. This result is in contrast with the reports on some other proto-oncogenes: c-*mos* (31, 32), c-H-*ras* (33) and, more recently, c-*fos*

(34). These proto-oncogenes can lead to cell transformation after being linked to viral promoter sequences.

The major divergence in amino acid sequence between $p60^{c\text{-}src}$ and $p60^{v\text{-}src}$ located in their COOH-terminal regions cannot explain the difference of transforming ability, because pPB5, which has the c-*src*-specific COOH-terminal region, can transform CEF quite well. The fact that both NY701 and NYBB4, which encode reciprocal chimeric p60s, can transform CEF, indicates that the v-*src* sequence of SR-RSV has at least two mutations critical for transformation, one upstream and one downstream of the *Bgl* I site. Either mutation is sufficient to convert $p60^{c\text{-}src}$ to a transforming protein.

We observed small foci in cultures infected with virus encoding $p60^{c\text{-}src}$ and showed that these foci were due to transforming virus generated by mutation of the original virus. Since every virus stock of NYHB5 and NY501 contains transforming virus at the ratio of 10^{-3} to 10^{-4} of the total infectious virus, we must assume the mutation rate is rather high. The high divergence in RNA sequences in retroviral genome RNA derived from a single cloned virus stock was reported previously (35), and this seems to be attributable to the low fidelity of reverse transcription (36–38). These results show that, after transduction of the c-*src* gene into a retrovirus, this proto-oncogene is subject to a relatively high rate of mutation and the transforming virus can be selected rather easily by virtue of this capacity. This fact may be relevant to the natural history of RSV formation.

We are greatly indebted to J. Brugge, R. Erikson, and B. Sefton for their gifts of sera against $p60^{src}$. We thank T. Lerner for supplying us with pREP and R. Williams for excellent technical assistance. We also thank L.-H. Wang and E. Garber for critically reading the manuscript. T.T. thanks M. Takanami for his support and encouragement. The work was supported by Public Health Service Grants CA14935 and CA18213 from the National Cancer Institute. F.R.C. is supported by Training Grant T32AI07233.

1. Bishop, J. M. & Varmus, H. (1982) in *RNA Tumor Viruses*, eds. Weiss, R., Teich, N. & Coffin, J. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 999–1108.
2. Stehelin, D., Varmus, H. E., Bishop, J. M. & Vogt, P. K. (1976) *Nature (London)* **260,** 170–173.
3. Hanafusa, H., Halpern, C. C., Buchhagen, D. L. & Kawai, S. (1977) *J. Exp. Med.* **146,** 1735–1747.
4. Brugge, J. S. & Erikson, R. L. (1977) *Nature (London)* **269,** 346–348.
5. Hunter, T. & Sefton, B. (1980) *Proc. Natl. Acad. Sci. USA* **64,** 191–195.
6. Collett, M. S., Brugge, J. S. & Erikson, R. L. (1978) *Cell* **15,** 1363–1369.
7. Oppermann, H., Levinson, A., Varmus, H. E., Levintow, L. & Bishop, J. M. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1804–1808.
8. Karess, R. E. & Hanafusa, H. (1981) *Cell* **24,** 155–164.
9. Shalloway, D., Zelenety, A. D. & Cooper, G. M. (1981) *Cell* **24,** 531–541.
10. Parker, R. C., Varmus, H. E. & Bishop, J. M. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 5842–5846.
11. Takeya, T., Hanafusa, H., Junghans, R. P., Ju, G. & Skalka, A. M. (1981) *Mol. Cell. Biol.* **1,** 1024–1037.
12. Takeya, T. & Hanafusa, H. (1983) *Cell* **32,** 881–890.
13. Czernilofsky, A. P., Levinson, A. D., Varmus, H. E., Bishop, J. M., Tischer, E. & Goodman, H. M. (1980) *Nature (London)* **287,** 198–203.
14. Takeya, T., Feldman, R. A. & Hanafusa, H. (1982) *J. Virol.* **44,** 1–11.
15. Czernilofsky, A. P., Levinson, A. D., Varmus, H. E., Bishop, J. M., Tischer, E. & Goodman, H. M. (1983) *Nature (London)* **301,** 736–738.
16. Schwartz, D. E., Tizard, R. & Gilbert, W. (1983) *Cell* **32,** 853–869.
17. Lerner, T. L. & Hanafusa, H. (1984) *J. Virol.* **49,** 549–556.
18. Wang, L.-H., Halpern, C. C., Nadel, M. & Hanafusa, H. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5812–5816.
19. Takeya, T. & Hanafusa, H. (1982) *J. Virol.* **44,** 12–18.
20. Hanafusa, H., Iba, H., Takeya, T. & Cross, F. R. (1984) in *Cancer Cells*, eds. Vande Woude, G. F., Levine, A. J., Topp, W. C. & Watson, J. D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Vol. 2, pp. 1–7.
21. Hanafusa, H. (1969) *Proc. Natl. Acad. Sci. USA* **63,** 318–325.
22. Cross, F. R. & Hanafusa, H. (1983) *Cell* **34,** 597–607.
23. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
24. Wigler, M., Pellicer, A., Silverstein, S., Axel, R., Urlaub, G. & Chasin, L. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1373–1376.
25. Lerner, T. L. (1983) Dissertation (Rockefeller Univ., New York).
26. Poirier, F., Calothy, G., Karess, R. E., Erikson, E. & Hanafusa, H. (1982) *J. Virol.* **42,** 780–789.
27. Gilmer, T. M. & Erikson, R. L. (1983) *J. Virol.* **45,** 462–465.
28. Cooper, G. M. & Okenquist, S. (1978) *J. Virol.* **28,** 45–52.
29. Shimotohno, K. & Temin, H. M. (1982) *Nature (London)* **299,** 265–268.
30. Sefton, B. M. & Walter, G. (1982) *J. Virol.* **44,** 467–474.
31. Oskarsson, M., McClements, W. L., Blair, D. G., Maizel, J. V. & Vande Woude, G. F. (1980) *Science* **207,** 1222–1224.
32. Blair, D. G., Oskarsson, M. K., Wood, T. G., McClements, W. L., Fischinger, P. J. & Vande Woude, G. F. (1981) *Science* **212,** 941–943.
33. Chang, E. H., Furth, M. E., Scolnick, E. M. & Lowy, D. R. (1982) *Nature (London)* **297,** 479–483.
34. Miller, A. D., Curran, T. & Verma, I. M. (1984) *Cell* **36,** 51–60.
35. Darlix, J.-L. & Spahr, P.-F. (1983) *Nucleic Acids Res.* **11,** 5953–5967.
36. Battula, N. & Loeb, L. A. (1974) *J. Biol. Chem.* **249,** 4086–4093.
37. Mizutani, S. & Temin, H. M. (1976) *Biochemistry* **15,** 1510–1516.
38. Shields, A., Witte, O. N., Rothenberd, E. & Baltimore, D. (1978) *Cell* **14,** 601–609.