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Mutations in *src* homology regions 2 and 3 of activated chicken c-*src* that result in preferential transformation of mouse or chicken cells

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ABSTRACT ***src* homology regions 2 and 3 (SH2 and SH3) of proteins encoded by *src* and closely related genes are conserved domains believed to modulate the protein-tyrosine kinase activity of this class of proteins, perhaps through interactions with other proteins. To explore the possibility of using *src* mutants as probes for such interactions, we have compared mouse NIH 3T3 cells with chicken embryo fibroblasts as host cells for 24 previously described substitution and deletion mutants with lesions in the SH2- and SH3-encoding regions of a transformation-competent allele of chicken c-*src*. Although several of these mutants are equally competent or equally defective for transformation of the two cell types, four mutants (three of which map within SH3) preferentially transform NIH 3T3 cells, and seven mutants (all of which map within SH2) preferentially transform chicken cells. Some of the SH2 mutants least able to transform mouse cells exhibit augmented transforming activity in chicken cells. In general, the *in vitro* protein-tyrosine kinase activities of the mutants correlated with transforming activities. Thus, in many cases, the catalytic activity of a mutant protein depended upon the host cell in which the protein was made. Such host-dependent mutants may be especially useful reagents for biochemical and genetic studies of the *src* gene family.**

The product of the cellular protooncogene c-*src* is a 60-kDa phosphoprotein ($pp60^{src}$) that displays a protein kinase activity specific for tyrosine residues (for reviews, see refs. 1–4). Several experimental approaches, including site-directed mutagenesis, protein biochemistry, and sequence comparisons among members of the *src* gene family, have produced a tentative map of functional domains of $pp60^{src}$ (Fig. 1 *Upper*; refs. 1–4). Nevertheless, little is known about the cellular proteins that regulate the activity of the *src* kinase, the physiologically significant targets for phosphorylation, or the factors that localize $pp60^{src}$ within the cell.

Several types of evidence indicate that significant interactions between $pp60^{src}$ and other proteins may be mediated through the SH2 and SH3 regions of $pp60^{src}$, regions that reside outside the kinase domain but are highly conserved among the protein-tyrosine kinases lacking transmembrane domains. Sequences related to SH2 and SH3 are also found in components of the signal-transduction system otherwise unrelated to $pp60^{src}$: phospholipase Cγ, GTPase activator protein, and the viral *crk* oncoprotein (5–7). We and others have made or isolated numerous mutants with lesions in SH2- and SH3-encoding regions of *src* and closely related genes and have observed complex effects on the oncogenic and catalytic properties of the resulting proteins (8–14). A few of these mutants have shown remarkably different phenotypes in cells from different species (11, 12, 15), implying that the mutant proteins may be altered in their ability to interact with significant host factors in one cell type as opposed to another.

Because mutants with such host-range differences may help to identify host components essential for the normal and oncogenic functions of the cytoplasmic tyrosine kinases, we have made an extensive survey of our recently described collection of SH2 and SH3 mutants of chicken c-*src* for those that behave differently in chicken embryo fibroblasts (CEFs) and mouse NIH 3T3 cells. We show here that several of these mutants are indeed host-dependent: some are more active in mouse than in chicken cells, and some are more active in chicken cells. All of the latter group map within SH2, and all but one of the former group map within SH3. Because the mouse cells are well-suited for further genetic manipulation, those mutants that fail to transform NIH 3T3 cells, despite production of substantial amounts of $pp60^{src}$, are promising reagents for attempts to identify the cellular factors responsible for the host-dependent phenotypes.

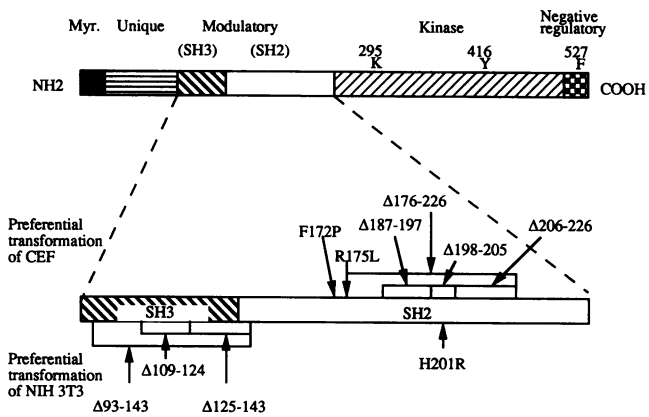

FIG. 1. Domains of $pp60^{c\text{-}src}$ and location of alterations that induce host dependence. (*Upper*) Map of activated chicken $pp60^{src}$ (533 amino acids) with regions implicated in myristoylation (Myr.), *src* family member-specific functions (Unique), modulation of activity [*src* homology regions 3 and 2 (SH3, SH2)], protein-tyrosine kinase, and negative regulation of the kinase. Also indicated are the Lys^{295} (K) residue at the ATP-binding site, the autophosphorylation site [Tyr^{416} (Y)], and the substitution of phenylalanine (F) for Tyr^{527} to activate the biological and kinase activities of $pp60^{c\text{-}src}$. (*Lower*) SH3 and SH2 alterations that result in preferential transformation of CEFs or NIH 3T3 cells as presented in Table 1. SH3 contains residues 88–139; SH2 contains residues 140–250.

MATERIALS AND METHODS

Construction of Mutant Plasmids. Twenty point mutations and 14 deletion mutations were introduced into the transfor-

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Abbreviations: SH2 and SH3, *src* homology regions 2 and 3; CEF, chicken embryo fibroblast; SRA, Schmidt–Ruppin strain of Rous sarcoma virus subgroup A; MLV, murine leukemia virus; TCA, trichloroacetic acid.

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mation-competent Y527F (Tyr^{527}→Phe) c-*src* gene (16) by oligonucleotide-directed mutagenesis (17). All resultant mutants were previously inserted into the proviral form of a helper-independent Rous sarcoma virus (RSV) vector, and biological and biochemical activities were analyzed in CEFs (17). In this study, normal c-*src*, parental Y527F c-*src*, v-*src* [Schmidt–Ruppin strain of RSV subgroup A (SRA)], 10 point mutants, and all 14 deletion mutants (see Table 1) were inserted into the *gag* position of a murine leukemia virus (MLV)-derived retroviral vector (ZAS) carrying a *gpt* marker gene (here called MLV-gpt). The vector was previously constructed by replacing the neomycin phosphotransferase (*neo*^{R}) gene of pZIP-neo with the *Escherichia coli* gene (*gpt*) coding for xanthine-guanine phosphoribosyltransferase (gift of R. Mulligan, Whitehead Institute, Massachusetts Institute of Technology).

Transfection and Biological Assays. ZAS plasmid DNAs were transfected into ψ-2 packaging cells with pSV2-neo DNA, and transfected cells were selected with medium containing G418 (GIBCO) at 400 μg/ml. Culture media were harvested as virus stocks. NIH 3T3 cells were infected with these viruses in the presence of Polybrene (4 μg/ml), and virus titers were determined by *gpt* selection in Dulbecco's modified Eagle's medium (DMEM) containing mycophenolic acid (20 μg/ml), xanthine (250 μg/ml), and hypoxanthine (14 μg/ml). NIH 3T3 cells (2×10^5) infected with virus stocks containing 200–500 Gpt$^+$ colony-forming units were assayed for the ability to form anchorage-independent colonies in 0.35% agar and for focus-forming activity in monolayer culture in nonselective medium. Colonies (0.5-mm diameter) and foci of morphologically altered cells were scored 2 weeks after plating. Transforming activities were calculated by correction for virus titers determined in selective medium. Clonal analyses were performed as follows. NIH 3T3 cells were infected with virus stocks and grown in *gpt*-selective DMEM. After 10 days, 10–15 colonies formed in each culture were cloned using cloning cylinders. Progeny of these clones were tested for growth in soft agar as described above and for levels of pp60^{src} as described below.

Immunoprecipitation of pp60^{src}. Infected NIH 3T3 cells were incubated for 12 hr in 2 ml of methionine-free DMEM containing 10% dialyzed fetal bovine serum and 400 μCi of [^{35}S]methionine (ICN; >1000 Ci/mmol; 1 Ci = 37 GBq). Labeled cells were lysed in 150 mM NaCl/20 mM Tris, pH 7.2/0.1% SDS/1% sodium deoxycholate/1% Triton X-100. Immunoprecipitation of pp60$^{c\text{-}src}$ from lysates containing equal amounts of trichloroacetic acid (TCA)-precipitable radioactivity was performed using an excess of monoclonal antibody 2-17 (Microbiological Associates), which recognizes residues 2–17 of pp60$^{c\text{-}src}$. After adsorption to formalin-fixed *Staphylococcus aureus* (Pansorbin; Calbiochem–Behring), labeled proteins in immune complexes were resolved by SDS/10% PAGE and autoradiography.

***In Vitro* Protein Kinase Assay.** Immune complexes from lysates containing equal amounts of TCA-precipitable radioactivity obtained as above were incubated in 20 mM Tris, pH 7.2/10 mM $MgCl_2$/0.1% Triton X-100 for 10 min at room temperature with 2 μg of acid-denatured rabbit muscle enolase (Sigma) and 1 μCi of [γ-^{32}P]ATP (3000 Ci/mmol) and subjected to SDS/10% PAGE.

RESULTS

Strategy for Characterization of Mutant Proteins. The 24 mutant alleles of Y527F c-*src*, positive and negative control alleles [A (Y527F c-*src*) and N (c-*src*)], and v-*src* were placed in MLV-gpt vector and tested for a variety of biological and biochemical properties. Results from three independent experiments are summarized in Table 1. Since relative colony-forming abilities were nearly identical to focus-forming activities in all assays, only the data for focus formation are shown. Although the labeling protocol can lead to modest underestimates of the concentration of *src* proteins with a short half-life, reduced amounts of labeled *src* protein in CEFs correlate well with reduced steady-state concentrations (as determined by Western blotting) and with reduced half-lives (as measured by pulse–chase experiments) (17).

Table 1. Biological and biochemical activities of mutant *src* proteins in infected NIH 3T3 cells

src allele	Mutation(s)	Focus-forming activity		Level of pp60^{src}		Total kinase activity	
		3T3*	CEF	3T3†	CEF	3T3‡	CEF
—§	—	<0.1	<0.1	0.1	<0.1	<0.1	<0.1
SR-A	v-*src*¶	4.2	1.9	0.8	1.0	1.2	2.4
N	c-*src*	<0.1	<0.1	2.0	2.1	0.2	0.2
A	Y527F	1.0	1.0	1.0	1.0	1.0	1.0
Class 1 mutants							
M1	W119Y	0.9	1.0	1.5	1.0	1.3	0.9
M2	W119A	0.7	1.2	1.1	1.0	0.8	0.9
M4	W148E	1.5	0.6	1.4	1.2	1.5	1.7
X1	Y92L	1.1	0.7	1.7	1.0	1.4	0.9
X9	R205L, K206E	0.8	0.6	1.0	2.1	0.8	1.5
D1	Δ93–108	0.8	1.1	1.1	1.0	0.7	0.7
Class 2 mutants							
X2	Q109E	<0.1	0.1	0.2	0.2	<0.1	0.1
X3	T125E	0.2	<0.1	0.7	0.6	0.2	0.2
D4	Δ144–156	0.1	0.1	0.8	0.3	0.2	0.1
D6	Δ166–175	0.1	0.4	0.6	0.6	<0.1	0.8
D7	Δ176–186	0.1	0.4	0.7	0.8	0.1	0.9
D12	Δ144–175	<0.1	<0.1	0.6	0.2	0.2	0.4
D14	Δ93–226	<0.1	<0.1	0.2	0.1	0.1	0.3
Class 3 mutants							
M7	H201R	1.1	0.3	1.1	0.8	1.0	0.7
D2	Δ109–124	0.8	0.1	1.3	1.0	1.1	0.2
D3	Δ125–143	0.7	0.2	1.3	0.8	1.3	0.3
D11	Δ93–143	0.8	0.1	1.0	0.3	1.3	0.3
Class 4 mutants							
M6	F172P	<0.1	1.7	0.3	2.6	0.1	4.7
M9	R175L	<0.1	2.1	0.9	4.0	0.2	4.8
D5	Δ157–165	0.2	0.6	0.7	0.8	0.1	1.2
D8	Δ187–197	0.2	1.0	0.5	1.3	0.2	3.0
D9	Δ198–205	<0.1	1.5	0.8	2.2	0.3	5.7
D10	Δ206–226	<0.1	1.3	0.7	1.9	0.3	4.9
D13	Δ176–226	0.2	0.7	0.7	0.8	0.9	2.4

All values are averages from three independent experiments with pools of infected cells as described in *Materials and Methods*. Focus-forming activity, level of pp60^{src}, and total kinase activity are normalized to those of A (Y527F c-*src*). Data for CEFs are cited from ref. 17.

*Normalized according to the number of Gpt$^+$ clones. See text for discussion of low activities observed in NIH 3T3 cells with class 2 and class 4 mutants.

†[^{35}S]methionine radiolabel in pp60^{src} immunoprecipitates from lysates containing an equal amount of TCA-precipitable radioactivity following a 12-hr labeling of infected NIH 3T3 cells (see Fig. 2 *Upper*).

‡Total immune-complex kinase activity of pp60^{src} from the lysates containing an equal amount of TCA-precipitable radioactivity following a 12-hr labeling of infected NIH 3T3 cells with [^{35}S]-methionine. Total kinase activity was measured with enolase as substrate (see Fig. 2 *Lower*).

§Uninfected NIH 3T3 cells.

¶The multiple differences between c-*src* and SR-A v-*src* are presented in ref. 18.

Classification of Mutants by Biological Activity. By comparing the results of the biological assays in mouse NIH 3T3 cells with our previous results in CEFs (17), we were able to place the mutants into four classes: 1, mutants with no significant loss of transforming activity from parental Y527F

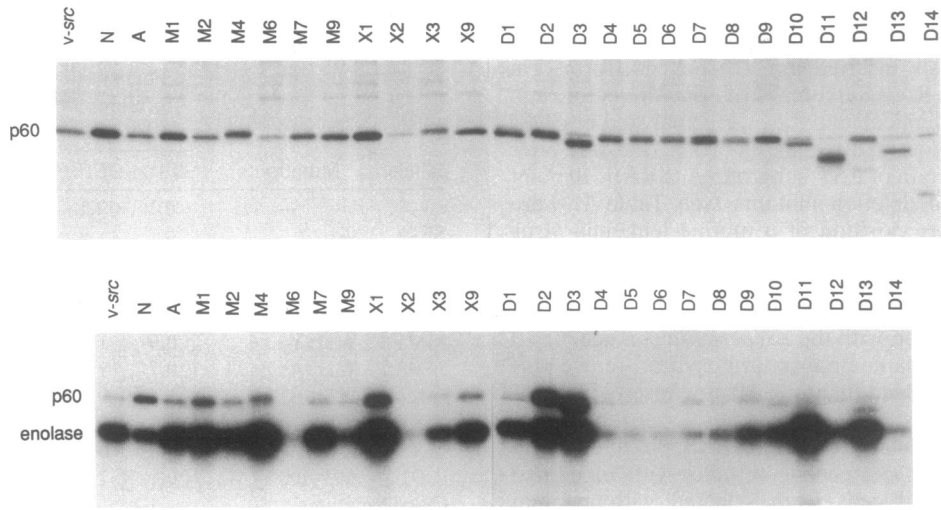

FIG. 2. The size, abundance, and kinase activities of *src* proteins encoded by SH2 and SH3 mutants expressed in NIH 3T3 cells. Pools of at least 100 independent Gpt^+ colonies were produced by infection of NIH 3T3 cells with MLV-gpt containing the indicated alleles of c-*src*. (*Upper*) *src* proteins were immunoprecipitated from lysates containing equal amounts of TCA-precipitable radioactivity with monoclonal antibody 2-17 and subjected to PAGE. The amount of endogenous mouse $pp60^{c\text{-}src}$ in these lanes can be discerned from the bands in the 60-kDa region in the lanes containing shorter proteins encoded by several deletion mutants (e.g., D11, D13, and D14). (*Lower*) An amount of immunoprecipitated protein equal to that shown in *Upper* was also tested for protein kinase activity by addition of [γ-^{32}P]ATP and acid-denatured enolase; the products of autophosphorylation and of phosphorylation of the exogenous substrate were then separated by PAGE and cut from the gel for liquid scintillation counting to provide the numbers presented in Table 1.

c-*src* in either cell type; 2, mutants with reduced transforming activity in both cell types; 3, mutants with transforming activity similar to that of Y527F c-*src* in NIH 3T3, but with impaired transforming activity in CEFs; and 4, mutants with reduced transforming activity in NIH 3T3, but with transforming activity similar to that of Y527F c-*src* in CEFs. Classes 3 and 4 represent the host-dependent mutants of primary interest here.

Class 1 mutants: Near-wild-type transforming activity in both cell types. Normalized values for transformation functions not less than half the activity of Y527F c-*src* were considered to be indistinguishable from wild type. All types of mutations produced this class of alleles, including some point mutations at highly conserved residues (M1, M2, and M4), mutations at less conserved sites (X1 and X9), and a single deletion mutation (D1). All of these mutations have minor effects on the level of $pp60^{src}$, total kinase activity, and specific kinase activity. In addition, the relative transforming activity of M4 in NIH 3T3 cells was >2-fold that in CEFs, although it transformed CEFs at 60% the efficiency of activated c-*src*.

Class 2 mutants: Reduced transforming activity in both cell types. Several mutants showed decreased transforming activity (relative activity <0.5) in both mammalian and avian cells. The lesions in these mutants also reduced the total kinase activity of $pp60^{src}$, supporting the claim that the protein kinase activity is an important determinant of oncogenic potency. In two cases (D6 and D7), the impairment of both biological and enzymatic activities was more severe in mouse than in chicken cells, where the kinase activities were near normal; thus these mutants, which map in the N-terminal region of SH2, could be considered modestly host-dependent.

Class 3 mutants: Impaired transforming activity only in CEFs. Four mutants transform CEFs significantly less efficiently than does activated c-*src*, but without a notable loss of capacity to transform NIH 3T3 cells. Three of these (D2, D3, and D11) are deletion mutants that map in the SH3 domain and display an impairment of kinase as well as biological activity. The fourth mutant, M7, with a single amino acid change in SH2, retains near-normal kinase activity in CEF, despite impairment of biological activity.

Class 4 mutants: Impaired transforming activity only in NIH 3T3 cells. All seven mutants in this class have lesions that map within the SH2 domain, including two point mutations (M6 and M9) that change single residues in the especially highly conserved FLVRES sequence (residues 172–177). Interestingly, five of the seven mutants were previously shown to exhibit augmented transforming and kinase activities in CEF; the SH2 alterations in these alleles were also able to confer CEF-transforming activity upon an otherwise normal c-*src* gene (17). In addition, four of the five mutants appeared to be completely devoid of transforming activity in mouse cells. Although the same five mutants produce proteins that are more stable in CEFs than in NIH 3T3 cells, only the M6-encoded protein is significantly less abundant than activated $pp60^{src}$ in mouse cells. All but one of the mutant proteins in this class displayed a reduction in total *in vitro* kinase activity after synthesis in mouse cells, but at least one of these mutants (M9) induced near-wild-type levels of phosphotyrosine-containing proteins in mouse cells (unpublished data).

Clonal Analysis to Determine Levels of $pp60^{src}$ Required for Transformation. In our earlier report (17) of the behavior of SH2 and SH3 mutants in CEFs, we proposed that quantitative changes in transforming efficiency, often reproducibly <2-fold, could be explained if the mutations altered the amount of $pp60^{src}$ required for transformation. Since the cells in a mass culture of infected CEFs might have a wide range of $pp60^{src}$ levels, the apparent transformation frequency would be determined by the threshold for transformation. It is, however, difficult to test this proposal in CEFs, since the infected cells cannot be readily subjected to clonal analysis.

The established mouse line NIH 3T3, in contrast, is easily subcloned. We therefore isolated 10–14 representative clones of Gpt^+ mouse cells after infection with MLV-gpt vectors carrying 20 of the mutant alleles described above. As anticipated, clones infected with viruses carrying the same *src* allele showed up to a 30-fold range of $pp60^{src}$ levels (Table 2), and there was an excellent correlation between the $pp60^{src}$ concentration and the phenotype (Fig. 3). Using Y527F c-*src* as the standard, an arbitary value of 1.0 was established as that amount of $pp60^{src}$ required for transformation (as defined by growth of >10 colonies from 10^3 cells plated in soft agar). The v-*src* allele is clearly more potent than activated c-*src* by

Table 2. Threshold levels of $pp60^{src}$ for transformation of NIH 3T3 cells

src allele	Number of clones analyzed	Range of $pp60^{src}$ level	$pp60^{src}$ level required for transformation*
SR-A	14	0.2–2.7	0.3
N	10	0.6–3.0	>3.0
A	14	0.2–3.0	1.0
Class 1			
M4	10	0.4–1.9	0.7
X9	14	0.2–1.4	1.0
D1	10	0.2–1.4	1.2
M1	10	0.2–2.1	1.3
M2	12	0.2–2.3	1.8
Class 2			
X2	10	0.1–0.5	>0.5
D7	11	0.1–0.9	>0.9
D4	12	0.1–1.0	>1.0
D14	12	0.1–1.0	>1.0
X3	10	0.1–1.4	>1.4
D12	11	0.1–1.5	>1.5
Class 3			
M7	11	0.5–1.1	0.8
D2	12	0.5–1.5	1.2
D3	11	0.2–1.5	1.3
D11	14	0.3–2.0	1.4
Class 4			
M6	12	0.1–0.5	>0.5
D9	10	0.1–1.0	>1.0
D10	10	0.1–1.2	>1.2
D13	12	0.1–1.6	>1.6
M9	10	0.5–3.0	>3.0

Multiple clones of NIH 3T3 cells infected with MLV-gpt vector containing the indicated *src* allele were tested for levels of $pp60^{src}$ as in Table 1 and Fig. 2 *Upper* and for ability to grow in soft agar (as illustrated for several alleles in Fig. 3).

*Relative level of *src* protein products in clones that produce at least 10 colonies per 10^3 cells grown in soft agar (see Fig. 3). With normal c-*src* (N) and the 11 mutants in classes 2 and 4, no anchorage-independent clones were obtained, and the threshold value is therefore indicated as greater than the highest observed level of $pp60^{src}$.

this criterion, since <0.3 unit of $pp60^{v\text{-}src}$ is required for transformation. In contrast, cells producing as much as 3.0 units of $pp60^{src}$ encoded by normal c-*src* remain untransformed, with only background numbers of colonies in soft agar (<5 per 10^3 cells).

Analysis of cell clones infected with mutant alleles from classes 1 and 3 produced patterns consistent with the results obtained with mass cultures of NIH 3T3 cells (Tables 1 and 2). At least two mutants (M4 and M7) that are slightly more efficient than the parental Y527F c-*src* allele in transformation of NIH 3T3 cells showed a slightly reduced threshold for transformation in the clonal analysis. Likewise, several mutants with reduced transformation efficiencies manifested a higher threshold for transformation. Alleles with higher thresholds also tended to induce fewer agar colonies at super-threshold levels (e.g., contrast M4 with D11 in Fig. 3).

Mutants from classes 2 and 4, which are deficient in transforming potency in mouse cells (Table 1), were unable to induce transformation in any of the clones, even at the highest levels of expression achieved. In some cases (e.g., those in which relative levels of $pp60^{src}$ did not exceed 1.0), it was not possible to determine the degree of impairment of the mutant protein. The absence of transformed clones suggested that the impairment in class 2 and 4 mutants might be more profound than implied by the 10–20% of wild-type activity observed for some mutants in mass cultures (Table 1). In accord with this suggestion, when 10–15 foci or agar colonies from mass cultures infected with the class 2 mutant X3 or the class 4 mutants D5, D8, and D13 were picked and replated, they failed to show evidence of transformation by morphological criteria or focus or agar colony formation (data not shown). In contrast, cells regrown from foci or colonies formed by infection with class 1 or class 3 mutants (M1, M2, M7, and D11) were transformed by all criteria. Thus some of the class 2 and 4 mutants appear to increase the already substantial background of focus and agar colony formation of uninfected NIH 3T3 cells, without causing the stable transformation characteristics of class 1 and class 3 alleles.

DISCUSSION

We have transferred into mouse NIH 3T3 cells 24 of our SH2 and SH3 mutants of activated c-*src*, which were previously characterized in CEFs, and found that 11 of these alleles were host-dependent for transformation—i.e., able to transform one, but not both, of these host cell types with efficiencies similar to that of the parental allele, Y527F c-*src*. The high frequency of host-dependent mutants is consistent with the

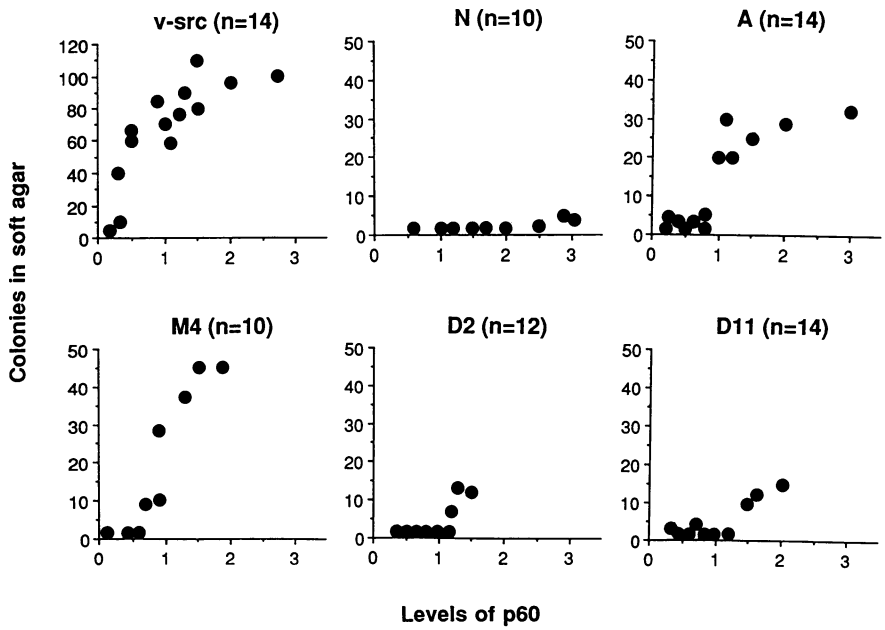

FIG. 3. Determination of the level of *src* proteins required for transformation of NIH 3T3 cells. Multiple Gpt^+ colonies (n = 10–14, as in each panel) were isolated after infection of NIH 3T3 cells with MLV-gpt carrying the indicated alleles of chicken c-*src*. After passage, the relative levels of $p60^{c\text{-}src}$ were measured for each clone as described for the pooled culture in Fig. 2. Each clone was tested for anchorage-independent growth by seeding 10^3 cells into 0.35% agar in a 60-mm dish, and the numbers of agar colonies (>0.5 mm) observed after 14 days were plotted against relative levels of $pp60^{src}$. Levels required for significant anchorage-independent growth (defined as 10 colonies per 10^3 plated cells) are listed in Table 2.

proposal that the SH2 and SH3 domains mediate significant interactions with other cellular proteins that modulate the activity of pp60^{src}; SH2 and SH3 mutants that reveal the species differences between such cellular proteins may be especially useful regents for further study of the interactions.

We and others have previously described mutants of *src* and related genes that display host-dependent phenotypes. The relevant lesions include small and large deletion mutations of v-*src* (12, 15) and linker insertion mutations of v-*fps* (10, 11) and v-*src* (12). Almost all of these mutations reside in SH2, and one of them deletes a single residue (F172) that is also the site of the amino acid change (F172P) in the host-dependent mutant M6 described in this study. Notably, this mutant and one other (M9) produce extremely host-dependent phenotypes, and their lesions are located within the most highly conserved region of SH2, the FLVRES sequence at positions 172–177. Since this sequence can be mutated or deleted without loss (and even with enhancement) of the catalytic and biological activities of c-*src*-encoded protein in avian cells (17) and can be mutated in v-*src* protein without significant consequence in mammalian or avian cells (12), it is probable that the region is not required for the constitutive activity of the kinase but instead is involved in mediating interactions with species-specific host proteins that modulate activity.

Host-dependent mutants have often been used to explore viral gene functions or the requirements for transformation. For example, the *hr-t* mutants of polyoma virus identify a replication function provided by certain host cells or by complementing virus genomes (19). Similarly, transformation-deficient mutants of the *E1A* and *E1B* genes of adenoviruses have been isolated by virtue of their dependence for growth upon host cells that supply missing functions in trans from resident subgenomic viral DNA (20, 21). Other examples of host dependence for transformation—e.g., the ability of v-*src* and certain alleles of v-*myc* to transform fibroblasts but not hematopoietic cells (22, 23)—may be closer in character to the mutants described here. Our host-dependent mutants of c-*src* presumably require for transformation functions missing in the cells they fail to transform; however, we have not excluded the possibility that host resistance to transformation by the relevant alleles is a dominant trait expressed by either cell type.

Several aspects of the findings reported here are especially provocative. (*i*) Four of our mutants (M7, D2, D3, and D11), three of which were generated by SH3 deletions, display preferential transformation of mammalian rather than avian cells. These mutants rule out simple models for host dependence that propose that the threshold for transformation is higher in mouse cells. (*ii*) Although most mutant proteins appear to have diminished protein kinase activity when measured *in vitro* after precipitation from cells that they transform inefficiently, a few show near-normal levels of activity, implying that affinities for crucial substrates may be affected in the resistant cell types. In work to be presented elsewhere, we have found that at least one mutant defective for transformation of mouse cells (M9) induces a pattern of phosphotyrosine-containing proteins in mouse cells similar to that induced by activated c-*src*. (*iii*) Those mutants with the most profound impairment of transforming activity in mouse cells show enhanced activity in CEFs and have lesions in SH2 (e.g., M6, M9, D9, and D10). This dramatic host dependence strongly suggests that the SH2 region interacts with host proteins that have sufficient species-specific differences to allow even single amino acid substitutions to serve as probes for the differences. Such mutants provide further evidence against the proposal that host dependence can be explained by different thresholds for transformation in different cell types. (*iv*) The clonal analysis of mutants in infected mouse cells (Fig. 3, Table 2) confirms the idea that a relatively precise threshold exists for the concentration of pp60^{src} required to induce transformation (24) and shows that SH2 and SH3 mutations may alter that threshold. Further, the ease of cloning NIH 3T3 cells simplifies the isolation of cells containing desired levels of mutant proteins and sets the stage for attempts to identify the cellular genes responsible for susceptibility to transformation by such mutants.

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