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Transformation by the *src* Oncogene Alters Glucose Transport into Rat and Chicken Cells by Different Mechanisms

MARTYN K. WHITE AND MICHAEL J. WEBER*

Department of Microbiology and Cancer Center, Box 441, University of Virginia School of Medicine, Charlottesville, Virginia 22908

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Transformation of both rat and chicken fibroblasts by the *src* oncogene leads to a four- to fivefold increase in the rate of glucose transport and in the level of the glucose transporter protein. We have previously shown that, with chicken embryo fibroblasts, transformation leads to a reduction in the rate of degradation of the transporter, with little or no increase in the rate of its biosynthesis. We now show that, with the rat-1 cell line, the opposite result was obtained. *src*-induced transformation led to an increase in transporter biosynthesis, with little effect on turnover. A *src*-induced increase in transporter mRNA entirely accounted for the increase in biosynthesis of the protein. By contrast, in chicken embryo fibroblasts, the level of transporter mRNA was low and was not induced to rise by *src* transformation. Thus, *src* induced an increase in the level of the glucose transport protein by fundamentally different mechanisms in chicken embryo fibroblasts and rat-1 cells. To test whether this difference was due to rat-1 cells being an immortalized cell line, we measured transporter mRNA levels in primary fibroblast cultures from rat embryos and in parallel cultures transformed by *src*. Transporter mRNA was inducible by *src* in these cells. Thus, the difference in mRNA inducibility between chicken and rat cells is not due to immortalization.

Malignant transformation of chicken and rat embryo fibroblasts by Rous sarcoma virus (RSV) results in a marked increase in the rate of hexose transport across the plasma membrane (11, 14, 15, 17, 26, 27). In cells transformed by RSV mutants in which the transforming protein $pp60^{v\text{-}src}$ is temperature sensitive, the increase in transport is also temperature sensitive, indicating that the activity of $pp60^{v\text{-}src}$ is necessary for both establishing and maintaining the transport alteration (14, 15, 17). It is generally accepted that the enhancement of transport is due to an increased number of hexose transporter molecules at the cell membrane, rather than a modification of existing carriers, for the following reasons. (i) Kinetic evidence indicates that the V_{max} for transport is increased (7, 14, 15, 26, 27). (ii) Glucose-inhibitable binding of [^{3}H]cytochalasin B (which represents specific binding to the glucose transporter) is increased in chick cells transformed by RSV (21). (iii) Antiserum raised against purified human erythrocyte glucose transporter precipitates larger amounts of transport protein from RSV-transformed cells than from untransformed cells (20).

There are several levels at which $pp60^{v\text{-}src}$ could act to increase the number of transporters. It might act to increase transcription of the gene that encodes the transporter or translation of its mRNA, or it might alter the rate of degradation of the transporter.

Recently we have shown that, with chicken embryo fibroblasts, transformation by *src* results in a reduction in the rate of turnover of the transporter (22). Cells transformed by a temperature-sensitive mutation of *src* displayed markedly slower degradation of the transporter protein at the permissive temperature than at the nonpermissive temperature. However, a comparison of the rates of transporter protein biosynthesis showed that there was little or no difference between normal and transformed cells (22).

In contrast, results obtained by others using rodent cells indicate that *src* transformation induces increased glucose transport by elevating the level of the mRNA for the transporter (8). To resolve this apparent discrepancy, we measured the effects of *src* transformation on the levels of transporter mRNA, transporter biosynthesis, and transporter degradation in chicken embryo fibroblasts, rat-1 cells, and primary cultures of fibroblasts explanted from a rat embryo. We concluded that *src* increases the level of transporter by mechanisms that differ between rat and chicken cells. In the former, *src* elevates transporter mRNA levels and biosynthesis, whereas in the latter this is not the case and *src* acts to inhibit transporter turnover.

MATERIALS AND METHODS

Cells and cell culture. Experiments on chicken and rat embryo fibroblasts were performed with passage 3 or 4 cells prepared by standard techniques. rat-1 cells were obtained from John Wyke (Imperial Cancer Research Fund, London, United Kingdom). Cells were grown in high-glucose Dulbecco modified Eagle medium (DME; GIBCO Laboratories, Grand Island, N.Y.) plus 10% fetal bovine serum.

Chick cells were transformed by infection with the Schmidt-Ruppin strain of RSV, subgroup A, or with the temperature-conditional derivative of this virus, tsNY68 (13). Rat embryo fibroblasts and rat-1 cells were transformed by infection with an amphotropic murine leukemia virus (MuLV) vector carrying the *src* gene (designated MuLV-*src*) (1). rat-1 cells carrying the temperature-conditional *src* of RSV LA29 (23) were constructed by infection with a subgroup D pseudotype, followed by single-cell cloning. All cultures were subconfluent or just confluent at the time of experimentation.

To label cells with [^{35}S]methionine, the culture medium

* Corresponding author.

was aspirated and replaced with medium containing 3 mg of methionine plus 150 μCi (turnover experiments) or 500 μCi (biosynthesis experiments) of [^{35}S]methionine (New England Nuclear Corp., Boston, Mass.) per ml supplemented with 10% dialyzed fetal bovine serum.

Immunoprecipitation. Labeled cultures of cells were lysed in ice-cold RIPA buffer (1% sodium deoxycholate, 1% Nonidet P-40, 0.1% sodium dodecyl sulfate, 0.15 M NaCl, 50 mM Tris, pH 7.2) and stored at −20°C. Lysates were cleared by centrifugation at 30,000 rpm (Beckman L5-50 ultracentrifuge) for 30 min and then further clarified by incubation of the supernatant with Pansorbin (Calbiochem-Behring, La Jolla, Calif.) for 30 min, followed by centrifugation at 12,000 × *g* in an Eppendorf microcentrifuge for 1 min.

The supernatant was incubated for 30 min with excess antiserum raised against the human erythrocyte glucose transporter (22). Excess Pansorbin was then added, followed by another 30 min of incubation. The immune complexes were collected by microcentrifugation (12,000 × *g*, 1 min) and washed three times with RIPA, with 1 M NaCl, and then three times with RIPA.

The immune complexes were released from the Pansorbin by 5 min of boiling in 2× Laemmli sample buffer without 2-mercaptoethanol. After the Pansorbin was centrifuged out, the samples were electrophoresed on a sodium dodecyl sulfate-polyacrylamide gel. 2-Mercaptoethanol was omitted, since unreduced immunoglobulin G runs near the top of the gel and does not interfere with the transporter band.

Measurement of transporter mRNA level. Total RNA was extracted from cells with guanidine isothiocyanate and isolated by centrifugation through a solution of cesium chloride as described by Chirgwin et al. (4). Usually this RNA was electrophoresed without further purification, but in some cases poly(A)$^+$ RNA was selected on oligo(dT) columns (type III; Collaborative Research, Inc., Waltham, Mass.). In all cases, 15 μg of RNA was loaded for total RNA and 10 μg was loaded for poly(A)$^+$-selected RNA.

RNA was electrophoresed on 1.2% agarose–formaldehyde gels and subject to Northern (RNA) blot analysis using as a probe the 880-base-pair *Eco*RI-*Nco*I fragment from plasmid pGT25L (19). This fragment has a sequence from within the coding region of human glucose transporter mRNA. Plasmid pGT25L was isolated from a cDNA library derived from the HepG2 human hepatoma cell line by screening with the same antiserum against the human erythrocyte glucose transport protein used in the experiments reported here (19). HepG2 cells express very high levels of transporter mRNA, and RNA from these cells was used as a positive control in all of the Northern analyses. Hybridizations were carried out at 42°C in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) with 40% formamide, and filters were washed several times in 0.2× SSC at 62°C.

RESULTS

Transporter mRNA levels. The level of mRNA for the glucose transporter was determined by electrophoresing total or poly(A)$^+$ RNA on formaldehyde-agarose gels, followed by Northern blot analysis with a high-specific-activity nick-translated cDNA fragment corresponding to the coding region of the human glucose transporter message (19). The amount of radioactive probe bound to Northern blotted nitrocellulose filters was determined by autoradiography. Video densitometry was used to measure the relative intensities of bands on the autoradiographs.

Figure 1 shows the results obtained with RNA from rat-1 cells and with four independently cloned derivatives transformed by *src* as a result of infection with the MuLV-*src* virus (1), which carries the *src* gene (lanes a, b, c, and d). Transformation by *src* elevated the level of the transporter message three- to sixfold, which was about the same as the increase in 2-deoxyglucose transport.

When rat-1 cells were transformed by the temperature-sensitive *src* mutant LA29 (23), the level of transporter message at the permissive temperature (35°C) was 5- to 20-fold higher than in the same cells at the nonpermissive temperature or in untransformed rat-1 cells. The results of a temperature shift-down experiment (to the permissive temperature for transformation) are shown in Fig. 2A. Cells growing at 39.5°C were shifted to 35°C at time zero. The message level was increased 20-fold 12 h after the shift. This increase was larger than that induced by wild-type *src* transformation in the clones examined, as was the increase in the rate of 2-deoxyglucose transport. The results of the reverse shift (35 to 39.5°C) is shown in Fig. 2B. The message level dropped over threefold within 3 h.

The time courses of these shifts are shown in Fig. 2C. The shift to the nonpermissive temperature led to a rapid (less than 3 h) drop in message level, whereas there was a 6-h lag after the shift to the permissive temperature before the message level became detectably elevated. The time courses of alterations of 2-deoxyglucose uptake in temperature-shifted rat-1–LA29 cells correlate well with those of the changes in mRNA level. There was a lag of at least 6 h before a transport rate increase was seen in temperature shift-down experiments.

By contrast, in chicken embryo fibroblasts, the level of the transporter message was low and was not changed when the cells were transformed by *src* (Fig. 3). Note that the *src*-transformed chicken cells used in this experiment were highly morphologically transformed and exhibited 2-deoxyglucose transport rates around fivefold higher than those of normal controls. The intensity of the transporter mRNA band in the chicken cells was around 50-fold lower than that in HepG2 cells. Poly(A)$^+$ selection was necessary to clearly visualize the band above background. The low signal in chicken cells could be due to low homology between the human probe and the chicken RNA, a low level of the

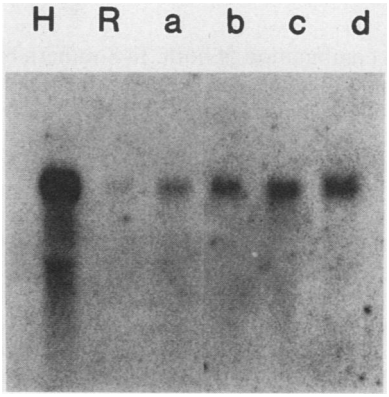

FIG. 1. Increased levels of glucose transporter mRNA in rat-1 cells transformed by *src*. RNA was extracted from rat-1 cells (lane R) and from four rat-1 clonal derivatives transformed by the MuLV-*src* virus (1) designated a, b, c, and d. This RNA was subject to Northern blot analysis with human glucose transporter cDNA as a probe (19). RNA from HepG2 human hepatoma cells was used as a positive control (lane H). The position of the transporter band in all of the lanes corresponds to a molecular size of 2.7 kb.

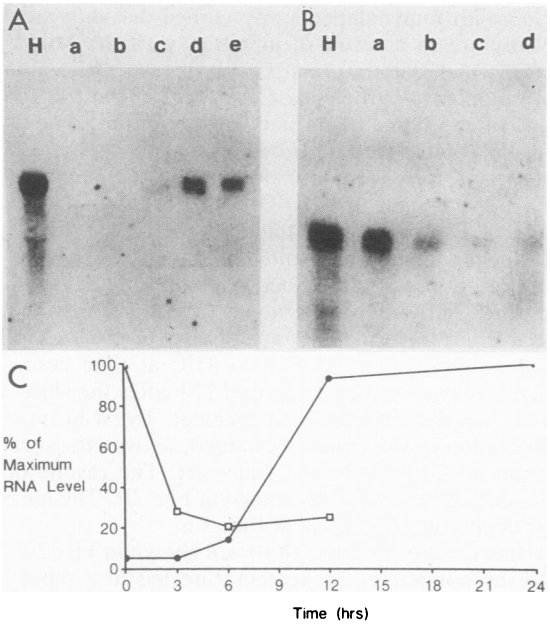

FIG. 2. Changes in glucose transporter mRNA levels observed in shift-up and shift-down experiments with the LA29 temperature-sensitive *src* mutant. rat-1 cells transformed by the temperature-sensitive LA29 *src* mutant were (A) grown for 24 h at the nonpermissive temperature (39.5°C) and shifted at time zero to the permissive temperature (35°C) or (B) grown for 24 h at the permissive temperature (35°C) and shifted at time zero to the nonpermissive temperature (39.5°C). RNA was extracted at 0, 3, 6, and 12 h (lanes a, b, c, and d) and analyzed by Northern blotting. HepG2 RNA served as a positive control (lanes H). (C) The autoradiographs shown in panels A and B were scanned with a video densitometer, and the relative intensity of each band was calculated. Since the shift-up and shift-down data represent two different Northern blot analyses, i.e., band intensities cannot be compared between the experiments, the data were normalized by expression of the intensity of each band as a percentage of the maximum-intensity band found in each experiment. Thus, 100% represents the maximal glucose transporter mRNA expression in each experiment, i.e., at time zero, when cells were shifted from the permissive temperature and at $t = 24$ h, when cells were shifted to the permissive temperature.

message, or a combination of both. In Southern blot analysis of total genomic chicken and human DNAs, a single band was found for both species and the intensity of the band was only around twofold lower in the chicken than in the human DNA when hybridization and washing were done under the same stringency as for the Northern blots (data not shown). However, this is not definitive proof of good homology, since in Southern blots the cDNA probe binds to both strands and the melting temperatures of hybrids formed to the sense and antisense strands may differ. Also, RNA hybrids formed on nitrocellulose may exhibit lower stability than predicted (25). To resolve this question, we are attempting to clone the chicken transporter cDNA. In any case, it is clear that the level of transporter mRNA is not altered by *src* transformation. The molecular weight of the chicken glucose transporter mRNA is slightly higher (around 3.4 kilobases [kb]) than those of humans and rats (around 2.7 kb).

Since rat-1 cells are unlike chicken embryo cells in that they are an established immortalized cell line, we derived primary cultures of fibroblasts from rat embryos and transformed them with MuLV-*src*. Figure 4 shows Northern blot

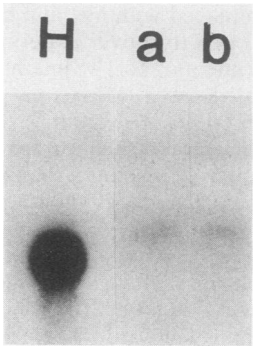

FIG. 3. Levels of glucose transporter mRNA in normal and RSV-transformed chicken embryo fibroblasts. Primary cultures of fibroblasts were prepared from chicken embryos and infected with Schmidt-Ruppin strain RSV, subgroup A. When the cells became highly transformed (at around two passages after infection), RNAs were extracted from them (lane b) and from parallel uninfected cultures (lane a). The message level in these cells was very low, and long exposure times were required for autoradiography. For this reason, poly(A)$^+$ selection of the RNA was done on oligo(dT)-cellulose before the Northern gel was run. The chick message ran slightly higher (3.4 kb) than the rat and human messages (2.7 kb). RNA from HepG2 cells (lane H) was not poly(A)$^+$ selected.

analysis of RNAs from transformed and normal rat embryo fibroblasts. Transformation led to a 2.5-fold induction of transporter message. RNAs from rat-1 cells and *src*-transformed rat-1 cells were run on the same Northern blot (Fig. 4). Rat embryo fibroblasts gave results qualitatively similar to those obtained with rat-1 cells. Note that the transformed rat embryo fibroblasts were not a clonal population and may have contained some untransformed cells.

Transporter biosynthesis. The rates of biosynthesis of the glucose transporter protein in different cells were determined by labeling of the cells for increasing periods of time, lysing of the cells in RIPA buffer, and immunoprecipitation of the transporter. Figure 5 shows an autoradiograph of a sodium dodecyl sulfate-polyacrylamide gel of immunoprecipitates from rat-1 cells and rat-1 cells transformed by the LA29 *src* mutant. Both cell types were grown at 35°C, at which temperature the latter cell type is highly transformed and exhibits 2-deoxyglucose transport rates five- to sevenfold higher than those of untransformed rat-1 cells. Evidently more label was incorporated into the transporter in

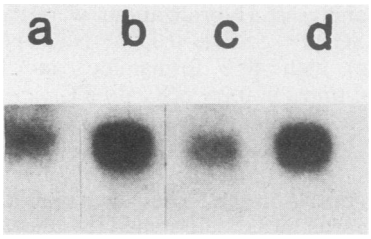

FIG. 4. Increased levels of glucose transporter mRNA in rat embryo fibroblasts transformed by *src*. Primary cultures of fibroblasts were prepared from rat embryos by the standard method. On passage 2, cells were infected with the MuLV-*src* virus. At 2 weeks later, when these cells were highly transformed morphologically and showed a threefold elevation in 2-deoxyglucose transport, RNA was extracted from them (lane b) and from uninfected controls (lane a). RNAs from rat-1 cells (lane c) and the rat-1 *src*-transformed clone b (lane d), which has a threefold higher 2-deoxyglucose transport rate than rat-1, were included in the same Northern blot.

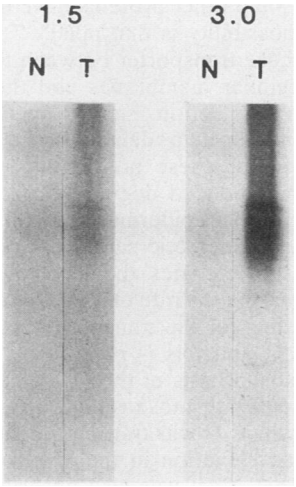

FIG. 5. Increased rate of glucose transporter biosynthesis in rat-1 cells transformed by *src*. rat-1 cells (lanes N) and rat-1 cells transformed by the LA29 temperature-sensitive *src* mutant (Rat-1–LA29; lanes T) were grown at the permissive temperature (35°C), at which rat-1–LA29 cells show a highly transformed morphology and exhibit a five- to sevenfold higher transport rate for 2-deoxyglucose than do rat-1 cells. Cells were washed, and the fluid was changed to DME with 500 μCi of [^{35}S]methionine per ml and no cold methionine at time zero. Cells were lysed at 1.5 and 3 h, and the transporter was immunoprecipitated. Immunoprecipitation was done from an equal amount of trichloroacetic acid-precipitable label for each sample.

transformed cells than in normal cells, even at the earliest labeling periods. When the intensities of these bands were measured with a video densitometer, it was found that (i) incorporation of label into the transporter increased for 3 h, (ii) at both 1.5 and 3 h, fivefold more label was incorporated into the transporter of transformed cells than into that of normal cells.

Thus, the rate of transporter biosynthesis in rat-1 cells was increased fivefold by *src* transformation. This increase is sufficient to account for most of the difference in transport between the two cell types.

A similar experiment performed with chicken cells and *src* transformants revealed very little difference in the rate of transporter biosynthesis caused by *src* (22), in sharp contrast to the results with rat cells. In three separate biosynthesis experiments with chick cells, the intensity of the transporter band at the earliest time points was slightly less for the transformants in two experiments and slightly higher in one. We concluded that *src* does not significantly alter transporter biosynthesis in chicken embryo fibroblasts.

Transporter degradation. The effect of *src* transformation on transporter turnover in rat-1 cells is shown in Fig. 6. rat-1 cells and LA29-transformed rat-1 cells were labeled for 16 h with [^{35}S]methionine and chased with cold methionine at 35°C. At time zero, about five- to sevenfold higher steady-state incorporation into the transporter was observed for transformed cells than for untransformed rat-1 cells, which is comparable to the difference observed in 2-deoxyglucose transport rates. However, analysis of scans of the autoradiographs in Fig. 6 gave half-lives for transporter turnover of 17 h for transformed cells and 13 h for nontransformed cells. This 33% increase in transporter half-life on transformation is close to the limit of experimental variability and is too small to account for more than a small part of the five- to sevenfold difference in steady-state transporter protein levels. In this experiment, the transporter was also immunoprecipitated from cells that had been labeled in the presence of tunicamycin. The unglycosylated transporter appears as a sharp band of apparent molecular weight 38,000, compared with a broader band of apparent molecular weight 45,000 to 50,000 in immunoprecipitations from untreated cells. This is in agreement with the data of Haspel et al. (10). Since these data also demonstrate that the antiserum recognizes unglycosylated transporter, they show that alterations in glycosylation cannot affect the results obtained in biosynthesis and degradation experiments.

The effect of *src* transformation on transporter turnover in chicken embryo fibroblasts is shown in Fig. 7. Labeled cell cultures infected with the temperature-sensitive *src* mutant tsNY68 (13) were chased at the permissive (36°C) and nonpermissive (42°C) temperatures. An approximately fourfold increase in half-life was observed at the permissive temperature; the half-lives were 3 to 6 h at the nonpermissive temperature and 12 to 24 h at the permissive temperature. These data demonstrate that the increase in transporter half-life can largely account for the elevation in the steady-state transporter in *src*-transformed chicken embryo fibroblasts as we reported earlier (22). The turnover of total protein is the same at both temperatures, and the effect is not observed with wild-type temperature-insensitive *src* (22).

DISCUSSION

Both chicken and rat fibroblasts undergo an increase in the level of the glucose transporter protein when they are malignantly transformed by the *src* oncogene. However, it is apparent from the studies presented here that different

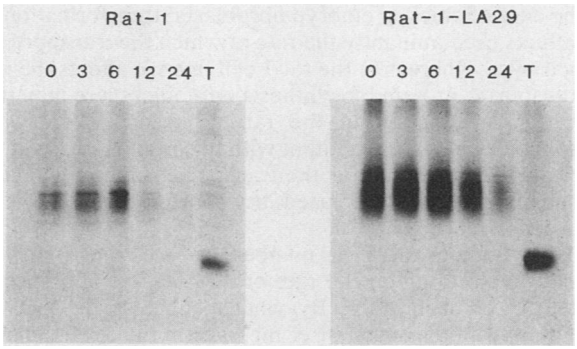

FIG. 6. Turnover of the glucose transporter in untransformed and *src*-transformed rat-1 cells. rat-1 cells and rat-1 cells transformed by the LA29 temperature-sensitive *src* mutant (rat-1–LA29) were grown at the permissive temperature (35°C), at which rat-1–LA29 cells show a highly transformed morphology and exhibit a five- to sevenfold higher transport rate for 2-deoxyglucose than do rat-1 cells. Cells were labeled with 200 μCi of [^{35}S]methionine for 16 h in DME containing 10% of the normal amount of cold methionine and then washed, and the fluid was changed to DME with the normal content of cold methionine (30 mg/liter) at time zero. Cells were lysed at 0, 3, 6, 12, and 24 h, and the glucose transporter was immunoprecipitated. The volume of lysate used in the immunoprecipitation of each sample was normalized between rat-1 and rat-1–LA29 according to the total amount of trichloroacetic acid-precipitable label incorporated into each cell type at time zero. The rate of turnover of total protein was the same in both cell types. In addition, cells were labeled in the presence of 10 μg of tunicamycin per ml and lysed at time zero (lanes T). Comparison with a lane of molecular weight standards showed that the transporter band ran at an apparent molecular weight of 45,000 to 50,000 in untreated cells and 38,000 in tunicamycin-treated cells.

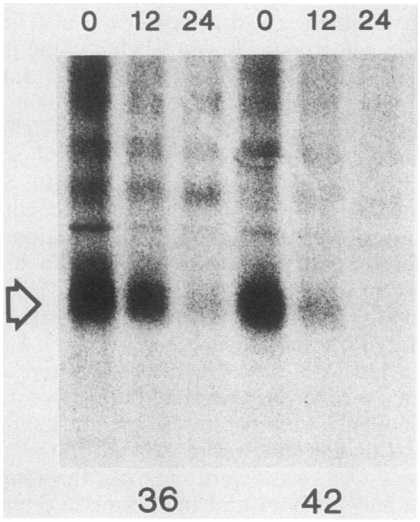

FIG. 7. Turnover of the glucose transporter in chicken embryo fibroblasts transformed by a temperature-sensitive *src* mutant. Cultures of chicken embryo fibroblasts infected with the temperature-sensitive *src* mutant tsNY68 were labeled with [^{35}S]methionine in DME containing 10% of the normal amount of methionine for 10 h at the permissive temperature (36°C). The cultures were washed, the fluid was changed to DME containing the full amount of cold methionine, and half of the cultures were shifted to the nonpermissive temperature (42°C). Cultures were lysed at 0, 12, and 24 h, and the transporter was immunoprecipitated. The volume of lysate used in each immunoprecipitation was adjusted according to the amount of trichloroacetic acid-precipitable counts at time zero. The overall rate of protein degradation was the same at both temperatures.

mechanisms underlie this common effect in these cell types. In the case of chicken embryo fibroblasts, transformation by *src* affects predominantly the rate at which the transporter is turned over, whereas in the rat-1 cell line *src* affects the rate of transporter protein biosynthesis (although there may be a very small reduction in the rate of transporter protein turnover as well). Concomitant with this is an increase in the level of mRNA coding for the transporter (and presumably responsible for the increased transporter biosynthesis in rat-1 cells).

These findings raise a number of questions. How is pp60$^{v\text{-}src}$ able to affect the rate of degradation of a specific protein in chicken cells? By what mechanism is pp60$^{v\text{-}src}$ able to elevate the transporter message in rat cells? Finally, why does pp60$^{v\text{-}src}$ have different effects in different cell types?

The finding that transformation of chicken embryo fibroblasts by *src* acts predominantly through an effect on protein degradation is consistent with reports that *src*-induced elevation in hexose transport is insensitive to inhibitors of RNA synthesis in this system (13–16). This effect is specific for the transporter (or for a small group of cellular proteins, including the transporter), since transformation does not alter the rate of total protein turnover whether measured by trichloroacetic acid precipitation or by electrophoresis of total cell lysates (22).

The mechanism whereby pp60$^{v\text{-}src}$ acts to stabilize the glucose transporter is not known. It is clear from immunoprecipitation experiments with lysates of cells labeled with $^{32}PO_4$ that the glucose transporter is not a substrate for in vivo tyrosine phosphorylation by pp60$^{v\text{-}src}$ (22). It is possible that pp60$^{v\text{-}src}$ stabilizes the transporter by forming a complex with it without phosphorylating it, and such complexes between pp60$^{v\text{-}src}$ and other proteins have been reported (3, 6, 18). Another possibility is that pp60$^{v\text{-}src}$ alters the distribution of the glucose transporter between the plasma membrane and intracellular membranes and thereby renders it less accessible to degradation. Finally, pp60$^{v\text{-}src}$ might act to regulate a specific protein degradation system. We are currently investigating these possibilities. It has recently been reported that reduced degradation is responsible for elevated levels of the epidermal growth factor receptor protein in a human pancreatic carcinoma cell line (9).

In rat-1 cells, *src* elevates the level of the transporter primarily by increasing the rate of its biosynthesis. The level of the mRNA coding for the transporter is also elevated by *src* transformation, and this is presumably responsible for the increase in biosynthesis of this protein. These observations are consistent with those reported by Flier et al. (8). They found that when *src* was transfected into Fisher rat 3T3 cells there was an elevation in the level of glucose transporter mRNA. This effect was also observed when the *ras* oncogene was used but not when the *myc* oncogene was used (8). In principle, the elevation of transporter mRNA could be caused by an increase in transcription or by a decrease in message turnover. Birnbaum et al. have reported that transformation of 3Y1 rat fibroblasts by Fujinami sarcoma virus (which contains the *fps* oncogene) increases the level of transporter message and that this increase is preceded by an absolute increase in the level of glucose transporter gene transcription (2). Our suspicion is that *src* also acts to induce transcription of this gene. It has been reported (24) that *src* also induces transcription in chicken embryo fibroblasts of a gene designated 9E3, which codes for a mitogen-related protein. Thus, *src* appears capable of inducing expression of a number of genes associated with changes in growth and metabolism. However, as demonstrated by our findings with chicken cells, the ability of *src* to alter expression of a particular gene depends on the cell type and is possibly related to the presence of specific regulatory factors in the cells. It will be interesting to determine whether 9E3 is induced in other cells which are susceptible to *src* transformation.

In rat-1 cells transformed by the LA29 temperature-sensitive *src* mutant, the changes in transporter message levels produced by temperature shifts are especially dramatic. When cells are shifted up to the nonpermissive temperature, there is a rapid (within 3 h or less) drop in the level of the transporter message. Since the tyrosine kinase activity of the pp60$^{v\text{-}src}$ protein of LA29 is also rapidly inactivated at the nonpermissive temperature both in vivo and in vitro (23), these data indicate that pp60$^{v\text{-}src}$ activity is continuously required for elevated expression of this gene. When cells are shifted from the nonpermissive to the permissive temperature, there is a lag of approximately 6 h before the transporter message level is significantly elevated. The increase in the rate of 2-deoxyglucose transport into these cells shows a similar lag. These observations provide circumstantial evidence for involvement of the tyrosine kinase activity of pp60$^{v\text{-}src}$ in the elevation of the level of transporter mRNA.

Initially we thought that the differences observed between rat-1 cells and chicken embryo fibroblasts might be because the former are immortal, whereas the latter have a limited life-span. Thus, immortalization might render the transporter gene inducible to *src*. However, it is clear that the transporter is inducible, even in early-passage fibroblasts from a rat embryo. Thus, it appears that the difference in the control of the transporter is not dependent on immortaliza-

tion. It is possible that pp60$^{v\text{-}src}$ recognizes target proteins in rat cells that are not recognized in chicken cells and vice versa.

Another possibility is that the intracellular processes involved in transporter regulation differ between chickens and rats. For example, there may be more than one form of the transporter. Since the clone which we used to probe the Northern blots was derived by using the same antiserum which was used to immunoprecipitate the protein itself (19), it is clear that the mRNA and the protein which we measured are cognate. Also, we have Southern blot data which indicate that there is a single gene. It is not clear why the Northern blot signal in chickens is low. This may be due to poor homology. However, we cannot rule out the possibility that additional splice variants of the transporter message exist such that the protein product(s) remains recognizable to the antiserum but is sufficiently diverged as to be poorly detectable in Northern hybridizations with the human transporter probe under the conditions of stringency used by us. If this is the case, control of the glucose transporter would be more complex than had hitherto been envisioned, with different isozymes of the transporter being under different mechanisms of regulation. These may be related to tissue-specific differences in glucose transport and may have different biochemical properties and physiological roles; for example, it has been reported that there is biochemical and functional heterogeneity in rat adipocyte glucose transporters (12) and that there are biochemical and functional differences in the hepatic and adipocyte glucose transporters (5). Changes in the expression of glucose transporter isozymes might account for differences in the Michaelis constants for hexose transport associated with certain tumors (28, 29). We are currently cloning the cDNA for the chicken embryo fibroblast transporter message to gain a better understanding of the regulation of the chick transporter gene and to investigate this possibility.

Finally, one general point should be made concerning the differences between chicken and rat cells in their responses to *src*. The fact that *src* induces increased glucose transport in both cell types, but by fundamentally different mechanisms, raises the possibility that *src* can induce other transformation parameters, such as loss of growth control, by mechanisms which differ, depending on the genetic and phenotypic properties of the cell.

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