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Expression of v-*src* in Embryonic Neural Retina Alters Cell Adhesion, Inhibits Histogenesis, and Prevents Induction of Glutamine Synthetase

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Using Rous sarcoma virus as the vector, v-*src* or c-*src* genes were introduced into 6-day chicken embryo retina tissue in organ culture and their effects on retina development were investigated. Overexpression of c-*src* in many of the cells had no noticeable effect on retina development. In contrast, infection with v-*src* resulted in abnormal histogenesis and inhibition of differentiation. Although only a portion of the cells in infected tissue expressed the oncogene and displayed the transformation phenotype, the other cells were also hindered from becoming normally positioned and organized. Therefore, presence of oncogene-transformed cells within the tissue hindered organization and development of adjacent nontransformed cells. Failure of normal cell relationships impeded induction by cortisol of glutamine synthetase in Muller glia, which requires contact associations of the glia cells with neurons. The transformed cells tended to assemble into chaotic clusters, suggesting that their adhesiveness and contact affinities had become altered. This was confirmed by aggregation experiments with dissociated cells which showed that adhesiveness of transformed cells was greatly reduced and that they had lost the ability to cohere with nontransformed cells. In binary mixtures of transformed and nontransformed cells, the two sorted out into separate aggregates. Transformed cells formed loose clusters devoid of tissue architecture; aggregates of nontransformed cells became organized into retinotypic structures, and glutamine synthetase was inducible. Our findings suggest that the mechanisms of cell adhesion and cell affinities are a key target of v-*src* activity in infected cells and that modification of the cell surface may be a leading factor in other cellular changes characteristic of the v-*src* transformation phenotype.

In addition to cell growth and morphology, oncogene expression can modify cell differentiation. Infection with v-*src*, the transforming gene of Rous sarcoma virus (RSV), can modify normal differentiation in a variety of cell types, including myoblasts (18, 25, 50), chondroblasts (1, 52), retina pigment cells (6), and lens cells (37) and can result both in reduction (7, 24, 56) and increase (2, 14, 20, 21, 51) of expression of various cellular genes. It is unlikely that v-*src* directly modifies the activities of these diverse genes; more probably, it triggers a cascade of events which, depending on cell type and state of development, alter regulatory aspects of cell differentiation.

Investigation of v-*src* effects on developmental processes requires experimental systems accessible to viral infection and suitable for morphological and biochemical analyses of differentiation. The neural retina of chicken embryos is known to be especially advantageous for studying developmental-regulatory mechanisms (45, 53, 58, 61–63). It can be cleanly isolated at different embryonic ages. When cultured in vitro as intact tissue (organ culture), the neural retina continues to develop similarly to its development in the embryo. Its cells can be dissociated and reaggregated in vitro into multicellular aggregates; they can restore histological relationships and tissue architecture and continue to differentiate (42, 43, 47).

Induction and expression of the glutamine synthetase (GS) gene is an important differentiation feature in the retina. GS is a key enzyme for recycling the neurotransmitter glutamate; it is localized exclusively in the Muller glia cells (33, 34), and its developmental regulation in chicken embryo retina has been investigated in detail (43, 47, 53, 61, 62). During early embryonic stages, GS enzyme and mRNA levels are very low and begin to increase rapidly by day 15 to 16 of development. However, GS gene expression can be induced precociously in Muller glia cells as early as day 8, by prematurely supplying cortisol to the retina (33, 44, 46, 53). The hormone promptly stimulates GS gene transcription, resulting in rapid accumulation of GS mRNA and GS enzyme (63, 64). Crucial requirements for this induction are cell contact interactions between Muller glia and neurons. In separated cells GS cannot be induced; if the cells are reaggregated and reform tissue associations, inducibility is restored; if development of normal cell contacts is impeded, GS is not inducible (33, 34, 40, 48, 61).

In this study, v-*src* and its cellular counterpart c-*src* were introduced into embryonic retina tissue in organ culture and into dissociated retina cells and their effects on retina development and GS induction were investigated.

MATERIALS AND METHODS

Viral vectors. Chicken embryo fibroblasts were prepared from 11-day embryos (SPAFAS, Inc., Norwich, Conn.) and grown in Dulbecco's modified Eagle's medium containing penicillin and streptomycin, 5% fetal calf serum, and 0.3% (wt/vol) tryptose phosphate broth. Stocks of replication-

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competent constructs of RSV subgroup A were obtained by $CaPO_4$ transfection of viral DNA into chicken embryo fibroblasts (19). Plasmids carrying viral constructs of RSV that lack the v-*src* gene (RSV^-) or that contain v-*src* ($RSV^{v\text{-}src}$) were obtained from S. Hughes (National Cancer Institute, Frederick, Md.) (26). A virus stock of RSV that lacks v-*src* but carries c-*src* ($RSV^{c\text{-}src}$) (28) was provided by H. Hanafusa (The Rockefeller University, New York, N.Y.).

Culture methods and GS induction. Neural retina tissue was isolated under sterile conditions from 6-day chicken embryos (SPAFAS). Retinas were organ cultured in Erlenmeyer flasks in medium 199 with penicillin–streptomycin–10% fetal calf serum on a gyratory shaker at 37°C (33, 46).

Retina tissue was dissociated into cell suspension by trypsinization (41). Cell aggregates were prepared by dispensing 1.5×10^7 dissociated cells into 25-ml Erlenmeyer flasks with 3 ml of medium 199 that contained penicillin, streptomycin, and 10% fetal calf serum (33). The flasks were gassed with a 5% CO_2–95% air mixture and were incubated (37°C) on a gyratory shaker (72 rpm).

Organ cultures and cell suspensions were infected for 2 h with the different viral constructs at a multiplicity of infection of about 1 focus-forming unit of virus per cell. The cultures were maintained for 6 days; the medium was changed daily.

GS was induced by adding cortisol (Sigma) to the cultures for the final 24 h, to a concentration of 0.33 μg/ml. The specific GS enzyme activity was determined in cell sonicates by the colorimetric assay (46) with some modifications.

RNA preparation and analysis. RNA was prepared by the guanidinium isothiocyanate-CsCl method (11). Poly(A)$^+$ RNA was selected by using an oligo(dT)-cellulose column (type III; Collaborative Research), was denatured by heating at 55°C in 0.8 M formaldehyde–1 M sodium chloride–30 mM sodium phosphate buffer, pH 7.4, and was blotted at different dilutions on nitrocellulose filters by using the BioDot microfiltration apparatus (BioRad). The filters were hybridized with nick-translated DNA probes and visualized by autoradiography.

Plasmid pGS116-9 is a subclone of the chicken GS gene (61). The cloned viral oncogene v-*src*, pEcoRI B (15), was obtained from the American Type Culture Collection. The cloned chicken H3.3 replacement histone gene, pCH3dR1 (17) was provided by J. D. Engel (Northwestern University, Chicago, Ill.).

Immunoprecipitation, protein kinase assay, and immunoblotting. For immunoprecipitation, equal volumes of cellular lysates (2.5 mg/ml) in RIPA buffer (0.1% sodium dodecyl sulfate [SDS], 1% Triton X-100, 1% sodium deoxycholate, 150 mM NaCl, 10 mM Tris [pH 7.2], 5 mM EDTA) were immunoprecipitated with an excess of the anti-*src* monoclonal antibody (MAb) LA 074 using formalin-fixed *Staphylococcus aureus* as the immunoabsorbent (35). This MAb was prepared against a synthetic peptide of the *src* protein (residues 2 to 17 which are common for both v-*src* and c-*src* proteins) (Microbiological Associates, Inc.). The immunoprecipitates were suspended in SDS sample buffer, subjected to electrophoresis on SDS–7.5% polyacrylamide gels, and blotted on nitrocellulose filters. Blots were incubated for 3 h with anti-*src* MAb LA 074 and with 1 μCi of ^{125}I-labeled goat anti-mouse immunoglobulin G (ICN) for 45 min (9). The bands were visualized by autoradiography. In vitro kinase activity of pp60^{src} was examined by measuring autophosphorylation in the immune complex. The assay was performed after immunoprecipitation of pp60^{src} (with MAb LA 074) by the addition of 10 μCi of [γ-^{32}P]ATP (ICN; 7,000 Ci/mmol)–20 mM Tris-HCl (pH 7.2)–5 mM $MgCl_2$–5 μM ATP for 15 min at 22°C. The proteins were resolved by electrophoresis on SDS–7.5% polyacrylamide gels, dried, exposed to X-ray film, and enhanced with intensifying screens.

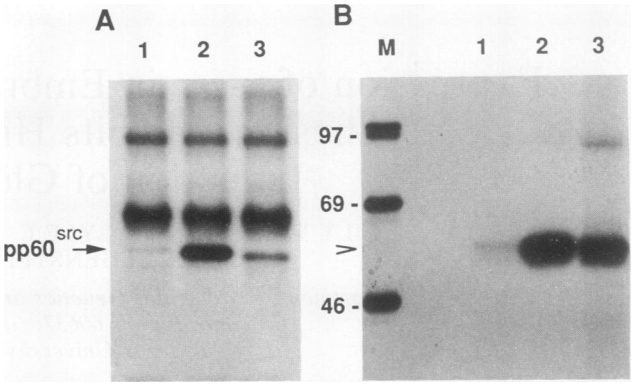

FIG. 1. Detection of pp60^{src} in uninfected (lane 1), $RSV^{c\text{-}src}$-infected (lane 2), and $RSV^{v\text{-}src}$-infected (lane 3) embryonic retina tissue. Cell lysates containing 600 μg of total proteins from uninfected and infected retina were immunoprecipitated with MAb LA 074. Half of each sample was assayed for pp60^{src} protein by an immunoblot assay (A), and the other half was assayed for pp60^{src}-specific kinase activity (B). (A) Immunoprecipitated proteins were electrophoresed on SDS–7.5% polyacrylamide gels, transferred onto nitrocellulose filter, reacted with MAb LA 074 and ^{125}I-labeled goat anti-mouse antibodies, and visualized by autoradiography. (B) For autophosphorylation of pp60^{src}, the immunoprecipitates were incubated with [γ-^{32}P]ATP, and the reaction products were separated by electrophoresis on SDS–7.5% polyacrylamide gels and visualized by autoradiography. In both experiments, the immunoglobulin G heavy and light chains are unreduced and migrate behind pp60^{src}. Lane M, molecular weight standards.

Immunostaining. Samples of organ cultures and cell aggregates were fixed in Carnoy's fixative, embedded in paraffin, and sectioned at 5 μm. Deparaffinized sections were immunostained with the following antibodies: anti-*src* monoclonal antibody MAb LA 074 (Microbiological Associates, Inc.) and GS-specific antiserum raised in rabbits (33). After treatment with antibody, sections were treated with appropriate conjugate of gamma globulin and fluorescein isothiocyanate (Boehringer). Antibody binding was detected by immunofluorescence, using epi-illumination from a UV source (33).

RESULTS

Expression of v-*src* and c-*src* genes in retina tissue in culture. c-*src* and v-*src* genes were introduced into organ cultures of 6-day embryo retina tissue, using RSV as the vector. Three constructs were investigated: (i) RSV^-, which lacks the transforming gene v-*src* (26); (ii) $RSV^{c\text{-}src}$, which lacks v-*src* but contains the c-*src* gene (28); and (iii) $RSV^{v\text{-}src}$, which contains the v-*src* gene (26). RSV proliferation was monitored by the activity of the viral reverse transcriptase (60). Activity increased during the first 4 days (data not shown), providing evidence of RSV infection and proliferation in cultured retina tissue.

$RSV^{v\text{-}src}$ and $RSV^{c\text{-}src}$-infected retina tissue expressed the *src* gene product pp60^{src}. Immunoblots (Fig. 1A) showed that the pp60^{src} level was much higher in infected than in uninfected retina (Fig. 1A, lanes 1, 2, and 3) and was highest in retina infected with $RSV^{c\text{-}src}$. The finding of a low level of

$pp60^{src}$ in the uninfected tissue (Fig. 1A, lane 1) was not unexpected, since it is known that the endogenous c-*src* gene is normally expressed in embryonic retina (58, 63). Also, the tyrosine kinase activity assay (12, 54) showed that $pp60^{src}$ was significantly higher in infected retinas (Fig. 1B). The differences between protein and autophosphorylation levels of v-*src* and c-*src* are consistent with the notion that tyrosine kinase specific activity of v-*src* in vitro is significantly higher than that of c-*src* (28). Thus, the results of both the accumulation and the autophosphorylation assays demonstrated expression of v-*src* and c-*src* genes introduced by the RSV vector into cultured retina tissue.

Infection with v-*src* prevents normal cell organization in the retina. Between days 6 and 12 of embryonic development, cells in the retina become organized in strata, their differentiation progresses, and their replication decreases (49). These changes take place also in organ cultures of retina tissue (Fig. 2A).

Infection with $RSV^{c\text{-}src}$ (Fig. 2C) or RSV^{-} (not shown) had no noticeable effects on structural development of the tissue; RSV proliferation and c-*src* overexpression did not interfere with cell organization and histogenesis. However, in retina infected with $RSV^{v\text{-}src}$, histogenesis was abnormal because the cells had failed to become organized into the characteristic stratified architecture (Fig. 2B). $RSV^{v\text{-}src}$-infected tissue contained two different kinds of cells: (i) numerous small cells localized predominantly in the outer region of the tissue and (ii) abnormally large, polymorphic, motile cells which expressed the oncogene (see below) and were identified as transformed; they were scattered throughout the tissue, but most were assembled into chaotic masses at the inner periphery.

To identify in which cells *src* was expressed, tissue sections of infected and control retinas were immunostained with the anti-*src* MAb and were examined for specific immunofluorescence (as explained in Materials and Methods, the anti-*src* MAb LA 074 is directed against an epitope shared by both the c-*src* and v-*src* peptides). In uninfected controls, very weak immunofluorescence was detected only in the outer layer (photoreceptor layer), attributable to the endogenous c-*src* product in this region (Fig. 2D). In retina infected with $RSV^{c\text{-}src}$, numerous cells throughout the tissue immunostained (Fig. 2F), but the staining did not seem restricted to any particular cell type. Despite the widespread expression of c-*src*, there was no discernible change in tissue architecture.

In $RSV^{v\text{-}src}$-infected retina, the two kinds of cells referred to above differed sharply in immunostaining with the anti-*src* MAb (Fig. 2E). The small cells did not immunostain, i.e., did not express the oncogene, and therefore were not transformed despite their failure to become normally organized and differentiated. The large cells in the chaotic masses and those dispersed among the small cells immunostained very intensely, i.e., they were transformed (Fig. 2E). The v-*src*-expressing cells constituted only about 28% of the cells in the tissue. This might be due to the fact that only dividing cells can be productively infected by retroviruses (38); thus, the nontransformed cells in the infected tissue might have been postmitotic at the time of infection. In any case, although only a portion of the cells in the tissue were transformed, overall histogenesis was inhibited and nontransformed cells failed to become normally organized. The shape and orientation of the transformed cells indicated migration toward the periphery of the tissue and segregation from the nontransformed cells; results obtained with cell aggregates (described below) were consistent with this interpretation.

Cell aggregates. When dissociated embryonic retina cells are reaggregated by rotation, they assemble into multicellular spheroids. At first, the various types of cells adhere randomly, but they progressively assort, become arranged into tissue structures, and form stratified retina rosettes and neuronal processes, as shown in Fig. 3A and C (31, 41, 45, 48). Cell aggregation provides a direct test of cell affinities and the capability of cells to associate and interact morphogenetically (41, 42). Using cell aggregation, we investigated the effects of viral infection on these cell properties.

$RSV^{c\text{-}src}$ or $RSV^{v\text{-}src}$ was added to suspensions of dissociated retina cells from 6-day embryos; controls were not infected. The cells were reaggregated by rotation, and the aggregates were examined histologically after 6 days in culture. There were no noticeable differences between aggregates of uninfected cells (Fig. 3A) and cells infected with $RSV^{c\text{-}src}$ (Fig. 3C); in both, there was histological organization into stratified rosettes and continued differentiation. Therefore, consistent with the finding in whole tissue, c-*src* infection and overexpression did not adversely affect cell positioning or the formation of histogenic cell associations.

The result of exposure of dissociated cells to $RSV^{v\text{-}src}$ was very different. The cells produced a mixture of two different kinds of aggregates (Fig. 3B). One kind consisted of large, loosely clustered cells devoid of histological organization which resembled the masses of transformed cells in the retina (Fig. 2B). The other kind of aggregates consisted predominantly of nontransformed cells; they were compact and contained histological structures, mostly small rosettes (Fig. 3B). Thus, under these conditions, transformed and nontransformed cells had segregated into separate groupings; furthermore, the nontransformed cells were capable of becoming histologically organized, whereas in the tissue this was hindered by the close presence of transformed cells.

Segregation of v-*src*-expressing and non-v-*src*-expressing cells. The cell aggregates were further characterized by immunostaining with the anti-*src* MAb. In aggregates of uninfected control cells (Fig. 3D), there was weak immunofluorescence in the photoreceptor layer of rosettes, consistent with normal presence of the endogenous c-*src* product (see above). Aggregates of $RSV^{c\text{-}src}$-infected cells showed scattered immunostaining in numerous cells (Fig. 3F), consistent with c-*src* overexpression.

The two kinds of aggregates produced by $RSV^{v\text{-}src}$-treated cells differed markedly in immunostaining for the *src* product (Fig. 3E). The compact aggregates consisted predominantly of nonimmunostaining, i.e., nontransformed cells. In the loose cell clusters, virtually all the cells immunostained intensely, strongest in the cell membrane, confirming that they were transformed. These results demonstrated a striking propensity of transformed retina cells to separate and segregate from the nontransformed cells.

Next, the formation of these two kinds of aggregates was followed by tracing cell distribution at different times. When 2-day aggregates of cells treated with $RSV^{v\text{-}src}$ were immunostained with the anti-*src* MAb, the aggregates were found to contain both transformed (immunostained) and nontransformed cells randomly interspersed (Fig. 4A). The shape and orientation of the transformed cells suggested migration toward the periphery of the aggregate. Indeed, the two kinds of cells became sorted out and, by the 4th day (Fig. 4B), the aggregates contained distinct regions consisting predominantly of either transformed or nontransformed cells. By the 6th day, the two kinds of cells had become completely

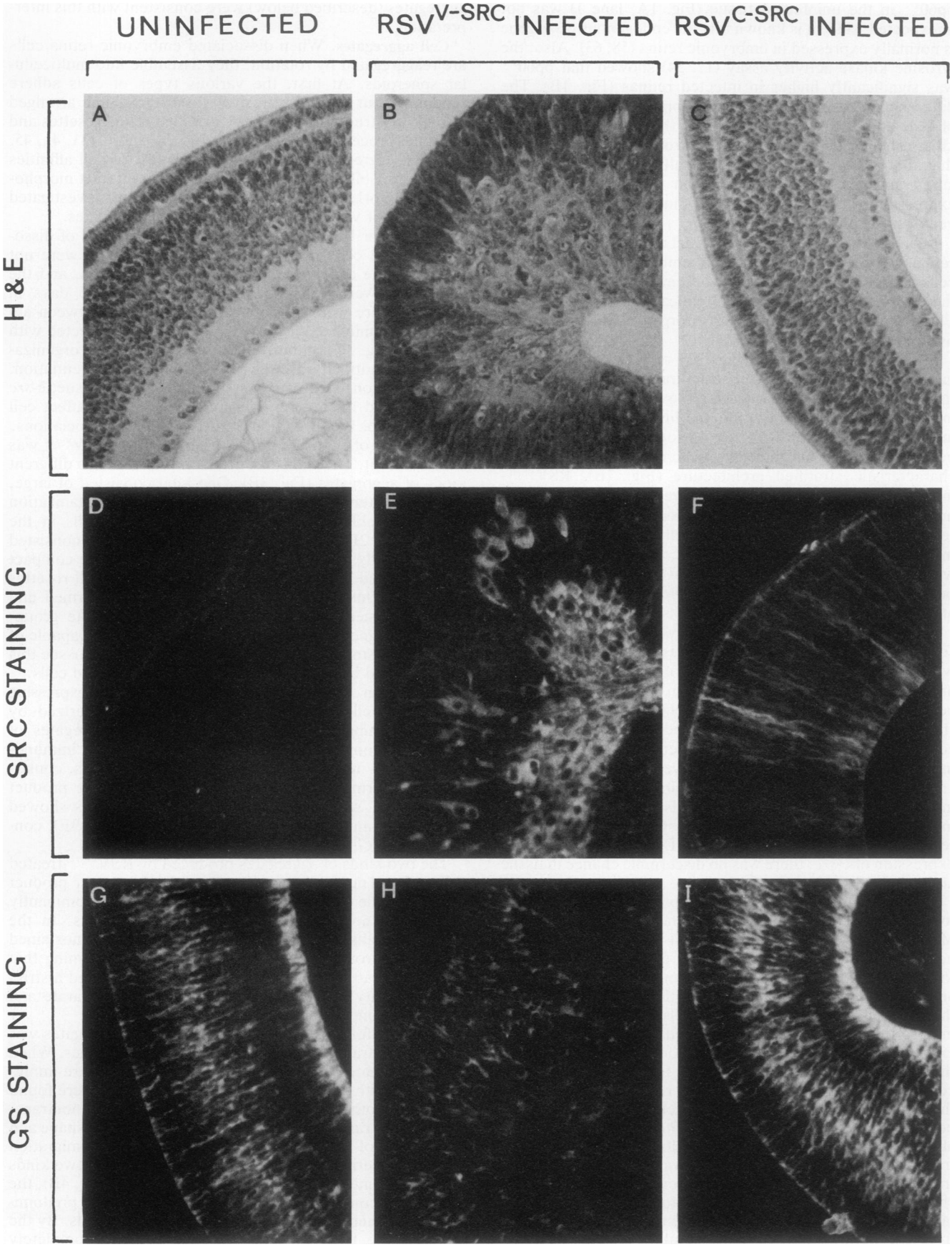
UNINFECTED
RSV V-SRC INFECTED
RSV C-SRC INFECTED
H&E
SRC STAINING
GS STAINING
A
B
C
D
E
F
G
H
I

FIG. 2. Infection of embryonic retina tissue with RSV$^{v\text{-}src}$ and RSV$^{c\text{-}src}$. Histological development (A, B, and C), *src* expression (D, E, and F), and GS induction (G, H, and I) are shown. Retina tissue from 6-day chicken embryos was infected and organ cultured for 6 days. Control cultures were not infected. Sections of uninfected (A, D, and G), RSV$^{v\text{-}src}$-infected (B, E, and H), and RSV$^{c\text{-}src}$-infected (C, F, and I) tissues were stained with hematoxylin and eosin (H&E) (A, B, and C) or were immunostained with MAb LA 074 (D, E, and F) which binds to *src* protein. Anti-GS rabbit antiserum was used for immunostaining and detection of GS in cortisol-induced tissues (G, H, and I). Magnification, ×323.

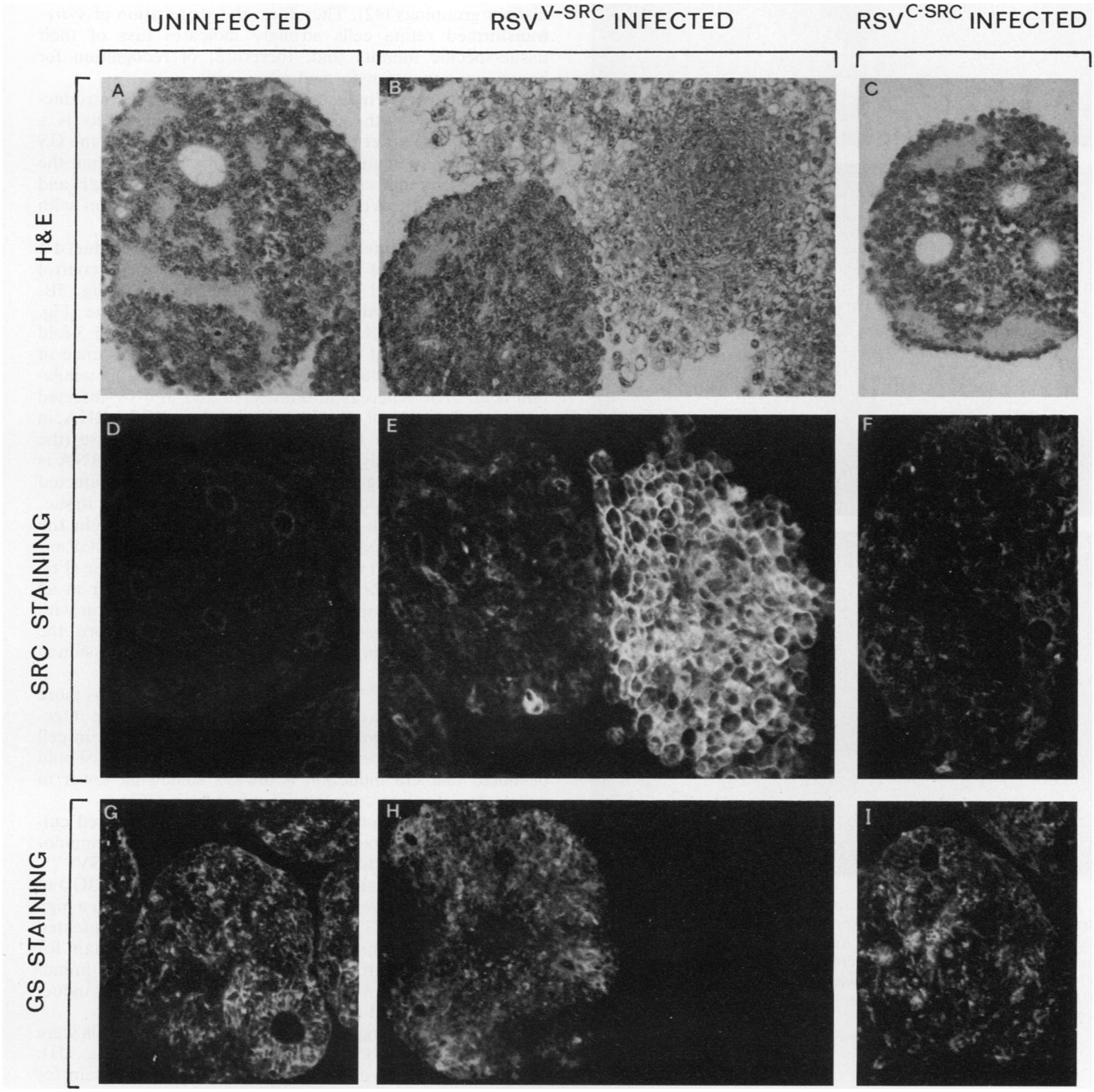

FIG. 3. Aggregates of retina cells, uninfected and infected with RSV$^{v\text{-}src}$ and with RSV$^{c\text{-}src}$. Histological development (A, B, and C), *src* expression (D, E, and F), and GS induction (G, H, and I) are shown. Dissociated retina cells from embryos at day 6 of development were infected with RSV$^{v\text{-}src}$ or RSV$^{c\text{-}src}$ and were reaggregated by rotation on a gyratory shaker for 6 days. Control cultures were uninfected. Sections of aggregates were stained with hematoxylin and eosin (H&E) (panels A, B, and C), immunostained with MAb LA 074 (panels D, E, and F) to detect the *src* protein, or immunostained with anti-GS antiserum (G, H, I) to detect cortisol-induced GS. Magnification, ×241.

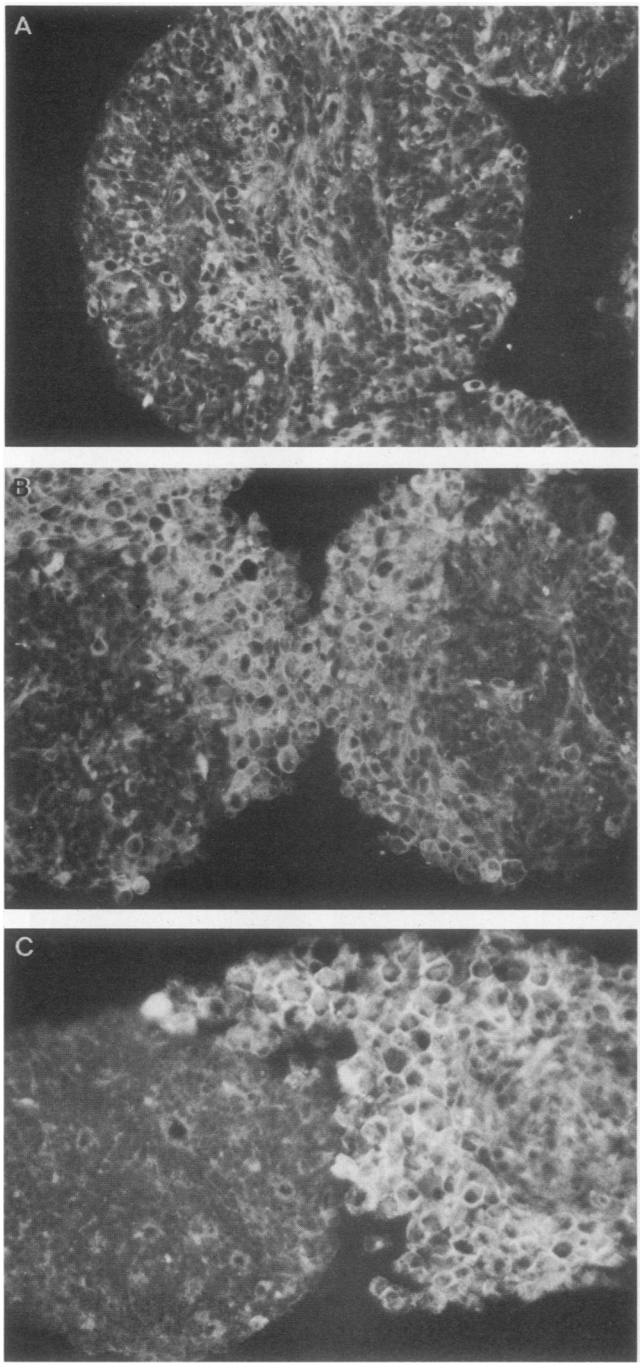

FIG. 4. Segregation into separate aggregates of v-*src*-expressing (transformed) and non-v-*src*-expressing cells. Dissociated retina cells (from 6-day embryos) were infected with $RSV^{v\text{-}src}$ and reaggregated by rotation. Aggregates were examined after 2 (A), 4 (B), and 6 (C) days in culture. Sections were immunostained with MAb LA 074 to identify and localize *src*-expressing cells. Magnification, ×242. See text for discussion.

separated into the two different kinds of aggregates (Fig. 4C). These findings demonstrated that the transformed cells were unable to cohere with the nontransformed cells; they no longer displayed compatible recognition and contact affinities and therefore segregated and separated. The very loose clustering of the transformed cells clearly implied that their mutual adhesiveness also was greatly reduced.

We interpret these findings as indicating that transformation with v-*src* modifies the cell surface and alters cell adhesiveness; the transformed cells lose their tissue-specific contact affinity and, consequently, separate from nontransformed cells. It should be recalled that a strikingly similar cell segregation typically occurs in aggregates composed of cells from different tissues; the different cells sort out according to tissue-specific identities and affinities and form distinct groupings (42). Therefore, the segregation of v-*src*-transformed retina cells strongly indicates loss of their tissue-specific identity and, therefore, of recognition for homologous, nontransformed retina cells.

Suppression of GS induction. As described in the Introduction, induction of the GS gene in Muller glia cells is a differentiation marker in the retina. Transcription of the GS gene can be prematurely induced with cortisol, but the induction also requires cell contacts between Muller glia and neurons (43, 61). We investigated whether infection with $RSV^{c\text{-}src}$ or $RSV^{v\text{-}src}$ affected GS induction.

In retina tissue infected with $RSV^{c\text{-}src}$, cortisol induced a multifold increase of GS enzyme similar to that in control tissue, about 14-fold higher than the basal level (Fig. 5B, lanes 3 to 6). In contrast, in $RSV^{v\text{-}src}$-infected tissue (Fig. 5B, lanes 1 and 2) GS increased only slightly, about 1.5-fold above the somewhat elevated basal level. This absence of induction was reflected in the lack of GS mRNA accumulation (Fig. 5A). Whereas in uninfected and in c-*src*-infected retinal tissue, cortisol elicited accumulation of GS mRNA, in v-*src*-infected tissue there was only a small increase (the reason for the slightly elevated basal level of GS mRNA is not known). It is significant that GS induction in infected tissue was 10-fold lower than that in uninfected tissue, despite the fact that only about 28% of the cells in the infected tissue expressed v-*src*. As expected, the level of *src* mRNA was higher in infected than in uninfected tissue (Fig. 5C). The fact that histone H3.3 mRNA was similar in all cases (Fig. 5D) is important in showing that infection with v-*src* did not suppress histone gene activity; therefore, the failure of GS induction was not due to general suppression of transcription.

The effect of $RSV^{v\text{-}src}$ infection on GS induction was more moderate in cell aggregates. Whereas in intact tissue infection resulted in a 10-fold decline in GS inducibility, in cell aggregates the decline was only 1.8-fold (Table 1). It should be noted that GS induction is always somewhat lower in aggregates of retina cells than in intact tissue (61).

Immunodetection of GS. Sections of cortisol-treated cultures of retina tissue and cell aggregates were immunostained with GS antiserum. In uninfected and in $RSV^{c\text{-}src}$-infected tissue and cell aggregates (Fig. 2G and I and 3G and I), Muller glia cells immunofluoresced strongly due to a high level of induced GS (33, 61). In contrast, in $RSV^{v\text{-}src}$-infected tissue (Fig. 2H), transformed cells did not immunostain for GS; only a few nontransformed cells showed weak immunostaining, consistent with the virtual absence of GS induction in v-*src*-infected retina.

The two kinds of aggregates of $RSV^{v\text{-}src}$-treated cells were immunostained for GS after exposure to cortisol (Fig. 3H). The clusters of transformed cells did not immunostain for GS. However, aggregates of nontransformed cells which formed retina rosettes contained numerous immunostained cells, i.e., when associated in separate aggregates the nontransformed cells were capable of histological organization and GS induction, whereas within the infected tissue their

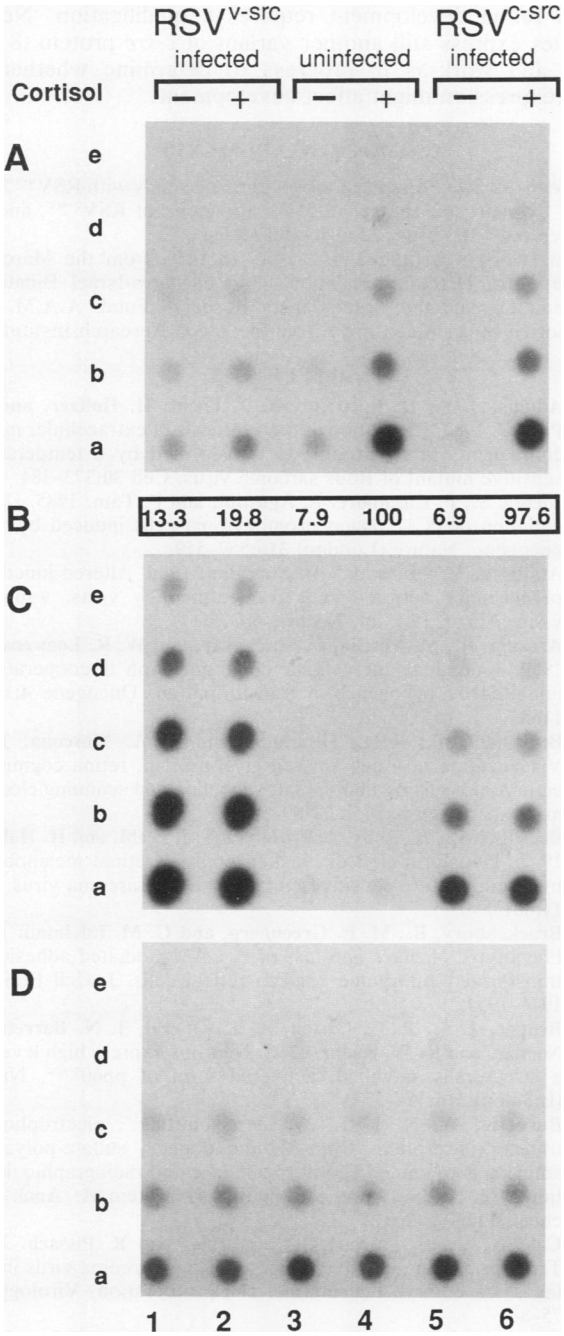

FIG. 5. Expression of v-*src* prevents the hormonal induction of GS. Uninfected (lanes 3 and 4), RSV$^{v\text{-}src}$-infected (lanes 1 and 2), and RSV$^{c\text{-}src}$-infected (lanes 5 and 6) retina tissue (from day 6 embryos) was organ cultured for 6 days. Cortisol was added for the last 24 h (lanes 2, 4, and 6). Poly(A)$^+$ RNA was prepared and analyzed by dot blotting. Rows: a, undiluted samples (2.5 μg); b to e, samples diluted 1:2, 1:4, 1:8, and 1:16. Samples were dot blotted in triplicate on nitrocellulose filter. One filter (panel A) was hybridized with the ^{32}P-labeled clone of the chicken GS gene, pGS116-9 (61). The second filter (panel C) was hybridized with the ^{32}P-labeled clone of the *src* gene, pEcoRI B (15). The third filter (panel D) was hybridized with the ^{32}P-labeled clone of the chicken H3.3 replacement histone gene, pCH3dR1 (17), which provides an internal standard. GS enzyme activity is shown in panel B; it was determined as micromoles of gamma-glutamylhydroxamate per milligram of protein per hour and is presented in arbitrary units; highest levels were assigned a value of 100.

TABLE 1. GS induction ratios in intact retina tissue and in cell aggregates[a]

Culture type	Uninfected	RSV$^{v\text{-}src}$ infected	Uninfected/ infected ratio
Intact tissue	14.2	1.4	10.1
Cell aggregates	4.1	2.2	1.8

[a] Uninfected or RSV$^{v\text{-}src}$-infected retina tissue and cell aggregates were cultured for 6 days. Cortisol was added for the final 24 h to induce GS. Induction was measured (46), and the ratio of GS enzyme activity in cortisol-treated cultures to that in untreated cultures was determined.

organization and inducibility were hindered by the transformed cells.

DISCUSSION

The effects of infection with RSV$^{c\text{-}src}$ and RSV$^{v\text{-}src}$ on development of chicken embryo retina in culture were investigated. Neither the RSV vector (without the v-*src* gene) nor overexpression of c-*src* noticeably affected normal development. Infection with RSV$^{v\text{-}src}$ resulted in major abnormalities.

In tissue infected with RSV$^{v\text{-}src}$, only a portion of the cells expressed the oncogene and displayed the transformed phenotype; nevertheless, the overall architecture of the tissue was abnormal because the nontransformed cells failed to become organized and differentiated. The most likely interpretation of this finding is that the presence of transformed cells within the tissue interfered with positioning and organization of adjacent normal cells. Whether this interference was merely physical or involved other factors is not known. In any case, the noteworthy point, which may have important and broader implications, is that oncogene-transformed cells can adversely affect development and behavior of adjacent nontransformed cells. The transformed cells tended to progressively segregate at the periphery of the tissue into chaotic clusters (Fig. 2B and 2E), indicating that their adhesiveness had become altered and that they had lost contact affinity for the nontransformed cells. These assumptions were confirmed by cell aggregation experiments.

When dissociated retina cells in suspension were infected with RSV$^{v\text{-}src}$ and were aggregated by rotation, they gave rise to a mixture of two different kinds of aggregates (Fig. 3B and 4C). Some consisted solely of transformed cells that were loosely clustered and were devoid of tissue architecture; others consisted of nontransformed cells that were compact and were organized into retina tissue structures. Further examination revealed that at earlier stages each aggregate contained both transformed and nontransformed cells that were randomly interspersed and disorganized (Fig. 4A); however, the transformed cells gradually separated and segregated from the others and detached as separate clusters (Fig. 3B, 4B, and 4C). We conclude that v-*src*-transformed retina cells lose contact affinity and cohesiveness for nontransformed cells and that their mutual adhesivity also is greatly reduced, as evidenced by the looseness of their clusters.

Cell affinities and cell adhesion are determined by molecular mechanisms on the cell surface (5, 16, 23, 39, 42); reduction or loss of cell adhesiveness reflects changes in these mechanisms. It is known (7) that N-CAM (a widespread cell adhesion molecule) is reduced in cultured retina cells infected with RSV. It would be of interest to determine whether retina Cognin, a cell recognition-adhesion molecule

specific for retina cells (23), is lost in v-*src*-transformed cells. Other cell-binding membrane specializations, cell junctions (64), and gap junctions (3, 4, 27, 37) are affected in v-*src*-expressing cells. In fact, it appears that down regulation of specific cell adhesion is a key consequence of v-*src* expression. Since modification of the cell surface can be relayed into the cell's regulatory network (34, 45, 61), it is conceivable that loss of adhesiveness in oncogene-infected cells is a leading factor in bringing about changes of cell growth, gene expression, and differentiation (45) (growth stimulation and transformation may depend on different information expressed by virus in the cells [10]). According to this suggestion, the transformation cascade triggered by v-*src* infection and expression may commence (at least in part) at the cell surface, starting with loss of cell-cell recognition, cell deadhesion, and a failure of contact-dependent regulation of cell functions. Whether the oncogene alters cell adhesion mechanisms at the gene control level or at the level of function at the cell surface is a matter for future investigation.

Infection with v-*src* inhibited the induction by cortisol of GS in Muller glia cells. In uninfected retina, cortisol elicited a multifold increase in GS mRNA and GS enzyme levels; in $RSV^{v\text{-}src}$-infected retina the increase was, at best, very slight (Fig. 5). GS was never detected in transformed cells. Since it is not known whether the transformants were originally Muller glia cells, the reason for their noninducibility is not clear. A direct inhibitory effect of v-*src* on glucocorticoid-dependent gene transcription has been suggested by others in other systems (22, 29, 30, 55); however, this suggestion refers only to transformed cells, whereas in the retina nontransformed cells also were not inducible, which implies an indirect effect of the oncogene in this case. Our interpretation of this indirect effect is based on the fact that GS induction requires, in addition to the hormonal inducer, contact associations between glia cells and neurons (34, 47, 61) and on the present finding that cell organization in v-*src*-infected retina was disorderly, probably resulting in abnormal glia-neuron cell contacts. We suggest that the transformed cells impeded the formation (and persistence) of normal glia-neuron contact relationships and thereby indirectly prevented GS induction in nontransformed cells.

This interpretation is strongly supported by the findings in cell aggregates. After becoming separated and segregated from the transformed cells, the nontransformed cells established histological relationships and GS was inducible (Fig. 3H). Previous studies have shown that the presence of phenotypically modified nonneoplastic retina cells within a population of normal retina cells can interfere with histogenesis and suppress differentiation (48). The present evidence extends this concept to the effects of oncogene-transformed cells and raises important questions concerning the influence of neoplastic cells on the functions of normal cells. It is of interest to note that in the embryo, in ovo, v-*src* oncogenesis was found to be attenuated in mesenchymal and epithelial tissues but not in endothelia (59).

We detected no noticeable effects of c-*src* overexpression on retina development. Overexpression of c-*src* does not appear to influence retina cell proliferation (28). The endogenous c-*src* gene is normally expressed in chicken embryo retina, is developmentally regulated (13, 58, 63), and its activity increases concurrently with histological organization and cell differentiation (63). A similar pattern was detected also during neural tissue development in *Drosophila* species (57), suggesting that the evolutionarily conserved c-*src* gene may be important for neural differentiation. The present finding that c-*src* infection did not interfere with retina development requires a qualification. Neural tissues express still another variant of c-*src* protein (8, 32, 36), and work is in progress to determine whether its overexpression might affect development.

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