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myc and *src* Oncogenes Have Complementary Effects on Cell Proliferation and Expression of Specific Extracellular Matrix Components in Definitive Chondroblasts

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The effects of the avian viral oncogenes *src* and *myc* were compared for their ability to alter the differentiated phenotype and the proliferative capacity of definitive chondroblasts. As previously demonstrated, viruses carrying the *src* oncogene suppressed the synthesis of the chondroblast-specific products, type II collagen and cartilage-specific sulfated proteoglycan. In contrast, infection with MC29 and HB1 viruses, which carry the *myc* oncogene, did not suppress the synthesis of these normal differentiated cell products, but the infected cells exhibited an increased proliferative potential. The MH2 virus, which carries both the *myc* and *mil* oncogenes, both induced the suppression of these chondroblast-specific products and increased cell proliferation. The implications of these results for cooperation between oncogenes and the multi-oncogene models for neoplastic transformation are discussed.

Recent experiments have demonstrated that the transformation of primary cells with oncogenes can require the cooperative effects of more than one oncogene (15). The observed effects of oncogene-driven transformation, both in vitro and in vivo, involve alterations in cell morphology and in cell proliferative potential. The alterations in cell morphology are correlated with, and probably caused by, an altered ability to synthesize particular differentiated cell products (8, 19). In particular, transformed fibroblasts and chondroblasts exhibit changes in the synthesis of the extracellular matrix and cytoskeletal proteins (1; E. Allebach, D. Boettiger, M. Pacifici, and S. Adams, submitted for publication). These structural proteins are important in determining cell morphology. Individual oncogenes may have their primary cellular effect on only one of these processes. However, since proliferation and differentiation are linked in the normal cellular developmental program, they are not easily separable. To examine the possibility that differentiation and proliferation may be affected separately by different oncogenes, we used the primary chicken embryo chondroblast system. It has been previously demonstrated, with temperature-sensitive mutants, that the expression of the viral *src* suppresses the synthesis of type II collagen and the cartilage-type sulfated proteoglycan (1, 10, 19). Furthermore, this suppression appears to be controlled primarily at the level of the mRNA (Allebach et al., submitted for publication) and is not mediated by differences in the cell proliferation rate (19). Synthesis of the definitive cell products by chondroblasts is not incompatible with continued cell proliferation, although cell proliferation does decrease as the extracellular proteoglycan-collagen matrix accumulates. Thus, it may be possible to use this system to separate the effects of oncogenes on cell proliferation from their effects on cell differentiation.

There is increasing evidence in support of the proposition that *myc* has its primary cellular effect on the cell cycle (2, 23). In hematopoietic cell cultures, the MC29 virus carrying the *myc* gene induces the transformation of macrophages. These transformed macrophages differ from their normal counterparts chiefly on the basis of increased proliferation; they are still able to produce substantial levels of the normal differentiated macrophage products (8). The experiments described below were undertaken to elucidate the effects of the *myc* oncogene on the expression of the differentiated cells program and proliferative potential of chondroblasts and to compare these with the effects of *src*. Three different *myc*-containing viruses were used: (i) the prototype strain MC29, (ii) a variant of MC29 which contains a replacement of some *v-myc* sequences with *c-myc* sequences (9, 25), and (iii) MH2 which expresses *myc* from a spliced mRNA and not as a *gag-myc* fusion product but contains in addition a *mil* oncogene (13).

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MATERIALS AND METHODS

Cells and viruses. Chondroblasts were prepared from 11-day-old chicken embryo vertebral cartilage as previously described (19). The cells were cultured on plastic dishes (Corning Glass Works) in Dulbecco minimal essential medium containing 10% fetal calf serum. The use of the ts-LA24A strain of Rous sarcoma virus (RSV) in this system has been described previously (1, 19, 28). Stocks of MC29 (RAV-1), HB1(RAV-1), and MH2(RAV-1) were prepared by superinfection of quail nonproducer cells (kindly provided by M. Hayman [London]) with RAV-1.

Soft-agar colonies. Agar colony assays were performed on third-passage control and infected chondroblasts. The cells were trypsinized, suspended in Dulbecco minimal essential medium containing 20% fetal calf serum and 0.4% agar, (Difco Laboratories), and plated at a density of 10^3 and 10^4 cells per plate on a base containing 0.8% agar. Cultures were incubated at 35 or 41°C and scored after 10 days.

Immunofluorescence microscopy. The following antibodies were used in this study: (i) monoclonal antibody IG-10 (immunoglobulin G), which is specific to the avian retrovirus P19 and also reacts with *gag* fusion proteins (M. Olsen and D. Boettiger, manuscript in preparation), or (ii) rabbit antiserum, A1, which is specific for the chicken cartilage-type

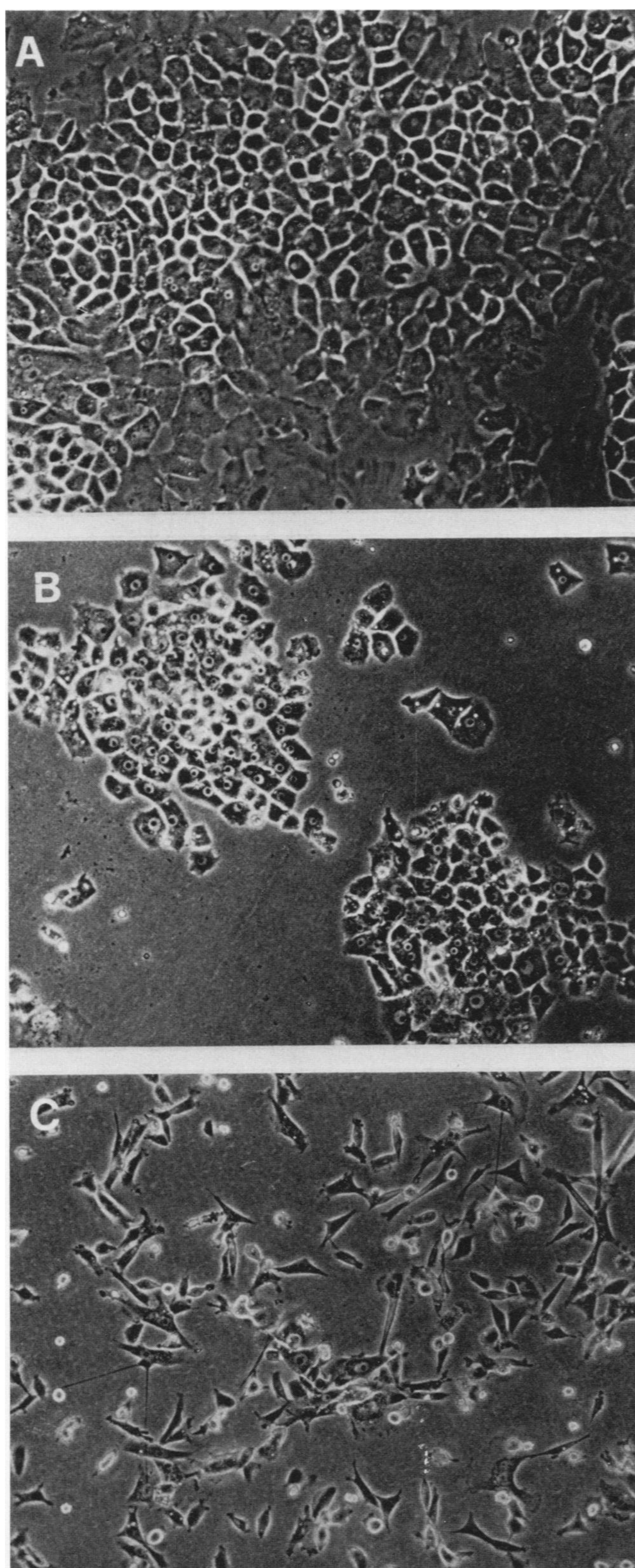

FIG. 1. Cell morphology: phase micrographs of living third-passage uninfected (A), HB1-infected (B), and MH2-infected (C) chondroblasts. Note the prominent nucleoli in B and C.

sulfated proteoglycan (20; a gift from M. Pacifici). Cell monolayers grown on plastic were incubated with or without hyaluronidase (20 μg/ml) at 37°C for 2 h, rinsed with phosphate-buffered saline, fixed with 70% ethanol at room temperature for 5 min, and air dried. Fixed cells were incubated with primary antisera: a 1:100 dilution of the A1 antiserum or a 1:1 dilution of the anti-p19 hybridoma supernatant at 37°C for 60 to 90 min. The second antiserum was either a 1:50 dilution of fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G or rhodamine-conjugated anti-mouse immunoglobulin G. Fluorescent micrographs were taken with a Zeiss photomicroscope.

Radioactive cell labeling, immunoprecipitation, and electrophoresis. Control and infected chondroblasts grown on plastic dishes were washed twice with Earle balanced salt solution and then incubated for 15 or 30 min in the same solution containing either 350 μCi of [^{35}S]methionine per ml or 100 μCi of [^{3}H]proline (New England Nuclear Corp.) per ml, respectively. Labeled cultures were washed twice with phosphate-buffered saline and frozen in situ at −85°C. Cells were solubilized in sodium dodecyl sulfate sample buffer, and equal trichloroacetic acid-precipitable counts were electrophoresed on 7.5% sodium dodecyl sulfate-polyacrylamide gels (14). For immunoprecipitation, labeled cells were harvested and lysed in RIPA buffer (2) containing 0.1% Trasylol, 0.1 mM phenylmethylsulfonyl fluoride, and 5 mM EDTA. The A1 antiserum (20) for the proteoglycan, or an anti-chick fibronectin antiserum (17), was added to the clarified extracts, and the mixtures were incubated on ice for 60 min. *Staphylococcus aureus* was added, and incubation was carried out for an additional 30 min. Immunoprecipitates were collected and washed three times with RIPA buffer by centrifugation at 4°C. Final pellets were disrupted by boiling for 2 min in sodium dodecyl sulfate-sample buffer, and proteins were resolved by electrophoresis on 7.5% polyacrylamide gels (14).

RESULTS

Morphological changes in chicken chondroblasts induced by temperature-sensitive RSV, MC29, HB1, and MH2. Floater chondroblasts from primary cultures were trypsinized, infected, passaged twice to allow spreading of the viral infection, and plated at low density to promote attachment to the plastic. Control cultures exhibited the pavementlike packing typical of chondroblasts in monolayer culture (6, 19) (Fig. 1A). Cells infected by the *myc*-containing viruses MC29 and HB1 produced similar pavement-packed colonies except that the cells tended to be smaller and the cells in the center of the colonies were round, more refractile, and tended to detach from the monolayer (Fig. 1B). These cells resembled the more highly proliferating cells present in primary chondroblast cultures. MH2-infected cells acquired a heterogeneous fibroblastoid morphology and grew in a disordered manner (Fig. 1C). All *myc*-infected cells had prominent nucleoli, as previously reported (21), and tended to be shed into the medium, a property which was particularly evident for MH2-infected cells. Chondroblasts infected by RSV and incubated at the permissive temperature exhibited the typical refractile, bipolar morphology as previously described (19) (data not shown).

The growth properties of the control chondroblasts and chondroblasts infected with the different viruses were examined by using agar suspension cultures. Normal chondroblasts produce an extracellular matrix which enables them to grow in agar suspension and to produce disperse-type colonies (D. Boettiger, M. Pacifici, manuscript in

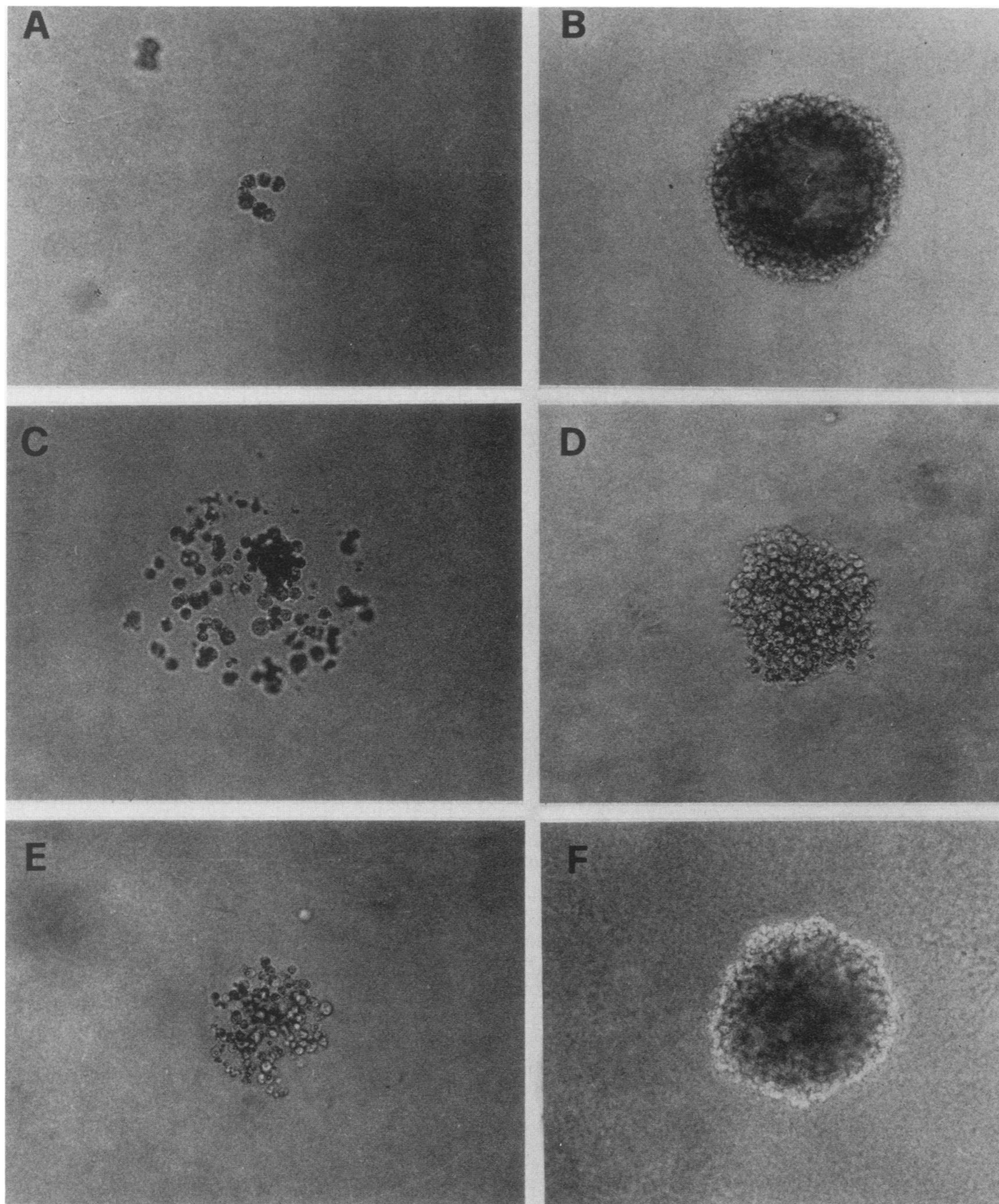

FIG. 2. Chondroblast agar colonies. Third-passage infected or uninfected chondroblasts were seeded in soft agar and photographed after 12 days at 36°C. Chondroblasts: uninfected (A), MH2 infected (B), MC29 infected (C), HB1 infected (D and E), and RSV infected (F).

preparation) (Fig. 2A). RSV-transformed chondroblasts produce larger, compact colonies under the same conditions (3, 19) (Fig. 2F). The colonies produced by the MC29- and HB1-infected chondroblasts exhibited the more disperse morphology of the normal-type colonies but were considerably larger than the parallel control colonies (Fig. 2C, D, and E). Normal chondroblasts derived from limb buds or from earlier-passage vertebral chondroblast cultures produce large colonies more similar to the MC29 and HB1 colonies shown here. These colonies do not appear to be different in kind from that produced by normal chondroblasts, but they appear to show enhanced proliferation relative to the parallel control. MH2, like RSV, produced compact, large colonies (Fig. 2B).

Synthesis of extracellular matrix components by virus-infected chondroblasts. Primary chondroblasts were infected with ts-LA24A, MC29, HB1, and MH2 and passaged three times as above. For the experiments in this section, both normal and virus-infected chondroblasts were grown as adherent cells. This deviation from our usual protocol (1) permits a better visualization of the effects of the *myc* gene on the expression of the chondrogenic phenotype. The cultures were labeled with [^{3}H]proline, which selectively labels the collagens, or [^{35}S]methionine, and total extracts

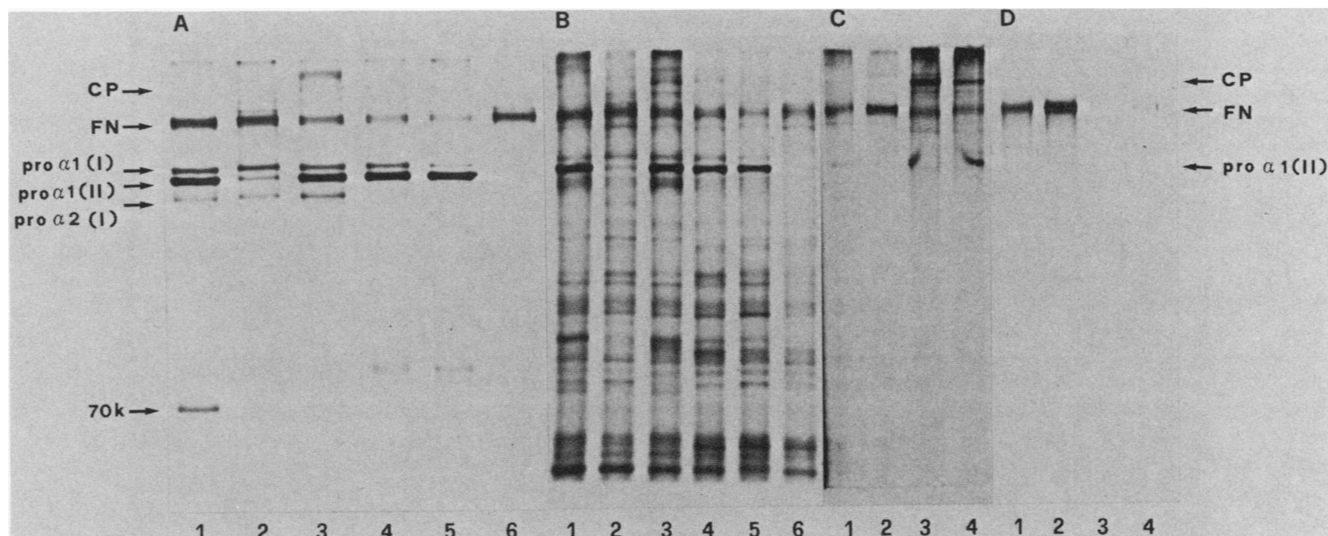

FIG. 3. Synthesis of chondroblast-specific proteins. Third-passage uninfected and infected chondroblasts were maintained at 36°C or 41°C for 5 days and labeled for 15 to 30 min with [^{3}H]proline or [^{35}S]methionine, and radioactive proteins were subjected to electrophoresis on 7.5% sodium dodecyl sulfate-polyacrylamide gels. (A) [^{3}H]proline extracts; (B) [^{35}S]methionine extracts. Lanes: 1, uninfected chondroblasts grown at 35°C; 2, ts-LA24A-infected chondroblasts grown at 36°C; 3, ts-LA24A-infected chondroblasts grown at 41°C; 4, MC29-infected chondroblasts; 5, HB1-infected chondroblasts; 6, MH2-infected chondroblasts. Immunoprecipitations of [^{35}S]methionine-labeled proteins with (C) A1 antiproteoglycan serum (C) or antifibronectin serum (D). Lanes: 1, ts-LA24A-infected chondroblasts grown at 35°C; 2, MH2-infected chondroblasts; 3, MC29-infected chondroblasts; 4, HB1-infected chondroblasts. CP, Core protein for the cartilage-specific proteoglycan; FN, fibronectin band; k, kilodalton. Magnification, ×100. proα1(I) and proα2(I), Procollagen chains for type I collagen; proα1(II), procollagen chain for type. II collagen.

were analyzed on polyacrylamide gels (Fig. 3A and B). The uninfected chondroblasts produced a predominant band of about 165 kilodaltons (Fig. 3A, lane 1) which has been identified as the α1 chain for the cartilage-specific type II collagen (1). This band was reduced in the ts-LA24A-transformed cells (lane 2) and undetectable in the MH2-infected cells (lane 6) (Fig. 3A). In contrast, there was no reduction type II collagen synthesis in the ts-LA24A-infected cells maintained at a nonpermissive temperature (lane 3) or in the MC29- and HB1-infected chondroblasts (lanes 4 and 5) (Fig. 3A). Since these chondroblasts were grown as attached cells, there is also a prominent fibronectin band which labeled efficiently with proline. This band was reduced in the MC29- and HB1-infected chondroblasts. The low level of fibronectin synthesis of the MC29- and HB1-infected chondroblasts compared with that of the MH2- and RSV-transformed chondroblasts was confirmed by immunoprecipitation (Fig. 3D).

The additional [^{3}H]proline-labeled bands on either side of the α1(II) collagen band in Fig. 3A represent the two preprocollagen chains for type I collagen, the α1(I) and the α2(I), since they comigrated with similar bands from chicken embryo fibroblasts (data not shown) (1). The synthesis of these type I collagen chains was low in the MC29- and HB-1 infected chondroblasts (lanes 4 and 5) as compared with the uninfected chondroblasts and was virtually absent in the MH2-infected chondroblasts (lane 6) (Fig. 3A). The core protein for the cartilage-specific proteoglycan labeled inefficiently with the [^{3}H]proline, but the relative synthesis of this protein can be seen in the immunoprecipitation studies (Fig. 3C). The synthesis of the core protein was reduced in the RSV- and the MH2-transformed chondroblasts (lanes 1 and 2), but no reduction was observed for the MC29- and HB1-infected chondroblasts (lanes 3 and 4) (Fig. 3C).

These conclusions were supported by the parallel gel containing the total [^{35}S]methionine-labeled cell extract for each of the samples (Fig. 3B). The synthesis of the cartilage-specific extracellular matrix components, type II collagen and the core protein of the cartilage-type proteoglycan, was reduced sharply by either the *src* oncogene (as previously reported [19; Allebach et al., submitted for publication]) or a combination of the *myc* and *mil* oncogenes, whereas the *myc* oncogene alone gave no suppression of these products and even appears to suppress the synthesis of type I collagen and fibronectin, which may be synthesized by chondroblasts in response to transformation or degeneration of the differentiated phenotype in culture (5, 6, 22).

Localization of cartilage-specific proteoglycan in infected chondroblasts. Since normal chondroblast products were detected in the MC29- and HB1-infected cultures, it might be argued that this synthetic pattern resulted from contaminating, uninfected chondroblasts. The level of infection of cells by these viruses was monitored by indirect immunofluorescence to the viral p19 portion of the *gag-myc* fusion protein (11, 25, 26). To obtain selective staining of the fusion protein, the viral p19 was removed by treating the cells with a Triton buffer. This treatment removed all staining from the RSV-infected cells but left a typical nuclear staining of the MC29- and HB1-infected cells (7, 11). These experiments revealed that >95% of the cells were infected with the *myc*-containing viruses (data not shown). In addition, double-immunofluorescence studies were performed to test for the simultaneous presence of both the *gag-myc* fusion protein and the chondroblast-specific sulfated proteoglycan in the same cell (Fig. 4). Ethanol was used as a fixative, which is not a good fixative for the *gag-myc* protein but is necessary to demonstrate the proteoglycan (20). Because the proteoglycan is a secreted protein, the cultures were pretreated with hyaluronidase to remove the extracellular proteoglycan (20). Figure 4A shows the typical nuclear staining

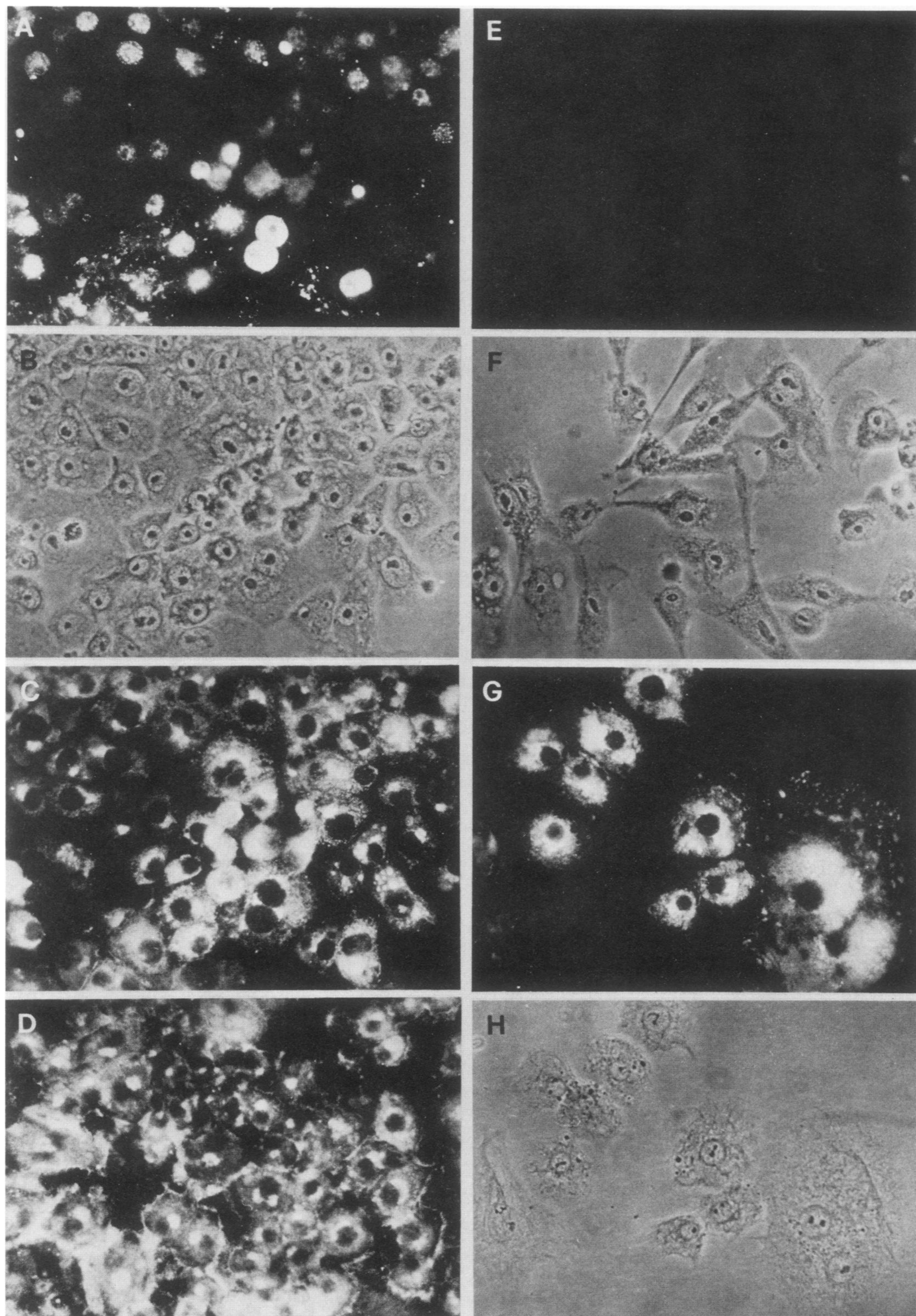

FIG. 4. Localization of cartilage-specific proteoglycan in chondroblasts. (A to C) The same field of MC29-infected chondroblasts were double stained for both *gag*- and cartilage-specific proteoglycan: A, anti-P19-*gag* monoclonal antibody; B, phase contrast; C, antiproteoglycan serum after a 2-h treatment with hyaluronidase. (D) Antiproteoglycan staining of HB1-infected cells without prior hyaluronidase treatment; both intra- and intercellular staining can be seen. (E and F) Antiproteoglycan staining of MH2-infected chondroblasts: fluorescence (E) and phase contrast (F) micrographs of the same field; note the complete absence of staining. (G and H) Uninfected chondroblasts stained with antiproteoglycan serum after 2 h of treatment with hyaluronidase: fluorescence (G) and phase contrast micrographs of the same field. Magnification, ×400.

of the *gag-myc* fusion protein; Fig. 4C shows the staining of the same field for intracellular cartilage-specific proteoglycan which localizes to the golgi apparatus (20) (seen here as a perinuclear region, and similarly in the uninfected chondroblasts [Fig. 4G and H]).

Proteoglycan staining without prior treatment with hyaluronidase demonstrated that HB1-infected cells deposited a normal extracellular matrix (Fig. 4D). In contrast to the lack of an effect on the synthesis of the chondroblast-specific proteoglycan in the cells infected by HB1 and MC29, the cells infected with MH2 showed no staining with this proteoglycan antiserum (Fig. 4E and F). A similar result was obtained with the ts-LA24A-transformed cells (data not shown). These immunofluorescence data corroborate the synthetic data shown in Fig. 3.

DISCUSSION

The objective of this study was to contrast the previously described effects of the *src* oncogene both on the synthesis of the normal chondroblast differentiation products and on the proliferation of chondroblasts with the effects of the *myc* oncogene. The data presented here demonstrate that the expression of the *v-myc* oncogene in chondroblasts is compatible with the continued expression of the chondroblast-specific products, type II collagen and the cartilage-type sulfated proteoglycan. The double immunofluorescence of *gag-myc* and the cartilage-type sulfated proteoglycan clearly demonstrates that the same cell can expresses both products. The infection process resulted in the appearance of smaller, less-adherent cells with prominent nucleoli. The altered nucleolar morphology distinguished the MC29-, HB1-, and MH2-infected chondroblasts from the uninfected chondroblasts and was similar to that previously observed in fibroblasts transformed by *myc*-containing viruses (21). The agar colony assays demonstrate that the MC29- and HB1-infected chondroblasts had a higher proliferative potential than the uninfected chondroblasts. These results suggest that *myc* is affecting primarily the cell proliferation without altering the differentiated cell phenotype.

As normal chondroblasts differentiate in vitro as nonadherent cells, the initial cultures consist primarily of small cells which increase in size and decrease in proliferation rate as the cultures progress (D. Boettiger and M. Pacifici, manuscript in preparation). The smaller cell size, increased growth producing dispersed colonies in agar, and less synthesis of fibronectin and type I collagen are properties associated with earlier passage chondroblasts or chondroblasts derived from earlier embryonic tissue such as limb buds (D. Boettiger, unpublished data). The association of these properties with the MC29- and HB1-infected chondroblasts suggests that the *myc*-infected cells retain their normal differentiated phenotype better than do the uninfected cells. This pattern of higher proliferation rates associated with smaller cells which nevertheless expressed the markers of terminal differentiation also has been observed for chicken embryo macrophages infected with MC29 (8).

The results presented here are complemented by similar experiments with these viruses in the myoblast system. The link between expression of the differentiated phenotype and cell proliferation is fundamentally different in the chondroblast and myoblast lineages. Myoblasts withdraw from the cell cycle before synthesis of their differentiated products and fusion into myotubes (12). Myoblasts infected with MC29 or HB1 did not withdraw from the cell cycle, and the expression of the differentiated cell products was completely blocked (G. Falcone, F. Tatò, and S. Alemà, manuscript in preparation).

In contrast to *myc*, which appears to affect primarily cell proliferation and only alters the course of cell differentiation as a consequence of effects on cell proliferation, *src* appears to have an effect on the synthesis of the differentiated cell products which is independent of its effects on cell proliferation. In chondroblasts infected with ts-LA24A strain of RSV, there was a dramatic increase in the synthesis of chondroblast-specific products in cultures shifted to the nonpermissive temperature for transformation even though the chondroblasts retained the same proliferation rate as their transformed counterparts retained at the permissive temperature (19). Also, the induction of *src* expression in mature myotubes by shifting cultures infected with ts-LA24A to permissive temperature suppressed the synthesis of myogenic products even though the cells are incapable of being induced to cycle (27). It could be argued that the effects of *src* on cell proliferation are indeed secondary to the effects on cell differentiation. For example, fibroblasts and chondroblasts decrease their proliferation rate as they accumulate extracellular matrix in culture; *src* prevents the synthesis of these products and hence their accumulation. Thus, the *src*-transformed cells may continue to proliferate after the controls have stopped.

The combined effect of *myc* and *mil* in the MH2 virus-infected cells provided a stronger suppression of the chondrogenic phenotype than has been previously observed even for *src*. No chondroblast products could be detected in the MH2-infected cells, and their morphology was completely distinct from that of normal chondroblasts. An unusual aspect of this suppression is the effect on type I collagen synthesis. Both the control and the ts-LA24A-transformed cells synthesized a small amount of type I collagen; however, none was detected in the MH2-transformed chondroblasts. The complete absence of collagen synthesis in these cells suggests that the mechanism of suppression of the differentiated phenotype by MH2 is different from RSV. Since the expression of *myc* alone in the MC29- and HB1-infected chondroblasts did not suppress the synthesis of the chondroblast-specific products, it is tempting to speculate that the observed suppression by MH2 is due to the action of *mil*. However, MH2 differs from MC29 and HB1 in that *myc* is expressed as a separate gene rather than as part of a *gag* fusion protein (4, 18). It is possible that the presence of *gag* determinants in the protein modify its transforming effects, as has been reported for the transformation of hematopoietic cells by Abelson virus (24). In quail tumors initiated either by MH2 or an MH2 variant lacking the *mil* gene, the presence of *mil* was found to have no effect (16; P. Linial, personal communication). Hence, additional experiments will be required to determine whether the suppression of the chondrogenic phenotype by MH2 is caused by its particular *myc* gene, cooperative effect of *myc* and *mil*, or the *mil* gene alone.

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LITERATURE CITED

1. **Adams, S. L., D. Boettiger, R. J. Focht, H. Holtzer, and M. Pacifici.** 1982. Regulation of the synthesis of extracellular matrix

components in chrondroblasts transformed by a temperature-sensitive mutant of Rous sarcoma virus. Cell **30**:737–384.

2. **Armelin, H. A., M. C. Armelin, K. Kelly, T. Stewart, P. Leder, B. H. Cochran, and C. D. Stiles.** 1984. Functional role for *c-myc* in mitogenic response to platelet derived growth factor. Nature (London) **310**:655–660.
3. **Boettiger, D., and E. M. Durban.** 1980. Progenitor-cell populations can be infected by RNA tumor viruses, but transformation is dependent on the expression of specific differentiated functions. Cold Spring Harbor Symp. Quant. Biol. **44**:1249–1254.
4. **Bunte, T., I. Grieser-Wilke, and K. Moelling.** 1983. The transforming protein of MC29 related virus CMIII is a nuclear DNA binding protein whereas MH2 codes for a cytoplasmic RNA-DNA binding polyprotein. EMBO J. **2**:1087–1092.
5. **Chacko, S., J. Abbott, S. Holtzer, and H. Holtzer.** 1969. The loss of phenotypic traits by differentiating cells. VI. Behavior of the progeny of a single chondrocyte. J. Exp. Med. **130**:417–442.
6. **Dessau, W., J. Sasse, R. Timpl, F. Jelik, and K. von der Mark.** 1978. Synthesis and extracellular deposition of fibronectin in chondrocyte cultures. J. Cell Biol. **79**:342–355.
7. **Donner, P., I. Greiser-Wilke, and K. Moelling.** 1982. Nuclear localization and DNA binding of the transforming gene product of avian myelocytomatosis virus. Nature (London) **296**:262–266.
8. **Durban, E. M., and D. Boettiger.** 1981. Differential effects of transforming avian RNA tumour viruses on avian macrophages. Proc. Natl. Acad. Sci. U.S.A. **78**:3600–3604.
9. **Enrietto, P. J., L. N. Payne, and M. J. Hayman.** 1983. A recovered avian myelocytomatosis virus that induces lymphomas in chickens: pathogenic properties and their molecular basis. Cell **35**:369–379.
10. **Gionti, E., O. Capasso, and R. Cancedda.** 1983. The culture of chick embryo chondrocytes and the control of their differentiated functions in vitro. J. Biol. Chem. **258**:7190–7194.
11. **Hann, S. R., H. D. Abrams, L. R. Rohrschneider, and R. N. Eisenman.** 1983. Proteins encoded by v-*myc* and c-*myc* oncogenes: identification and localization in acute leukemia virus transformants and bursal lymphoma cell lines. Cell **34**:789–798.
12. **Holtzer, H., N. Rubinstein, S. Fellini, G. Yeoh, J. Chi, J. Brinbaum, and M. Okayama.** 1975. Lineages, quantal cell cycles, and the generation of cell diversity. Q. Rev. Biophys. **8**:4–34.
13. **Jansen, H. W., B. Ruckert, R. Lurz, and K. Bister.** 1983. Two unrelated cell-derived sequences in the genome of avian leukemia and carcinoma inducing retrovirus MH2. EMBO J. **2**:1969–1975.
14. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **277**:680–685.
15. **Land, H., L. F. Parada, and R. A. Weinberg.** 1983. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature (London) **304**:596–602.
16. **Linial, M.** 1982. Two retroviruses with similar transforming genes exhibit differences in transforming potential. Virology **119**:382–391.
17. **Menko, A. S., Y. Toyama, D. Boettiger, and H. Holtzer.** 1983. Altered cell spreading in cytochalasin B: a possible role for intermediate filaments. Mol. Cell. Biol. **3**:113–125.
18. **Pachl, C., B. Biegalke, and M. Linial.** 1983. RNA and protein encoded by MH2 virus: evidence for subgenomic expression of *v-myc*. J. Virol. **45**:133–139.
19. **Pacifici, M., D. Boettiger, K. Roby, and H. Holtzer.** 1977. Transformation of chondroblasts by Rous sarcoma virus and synthesis of the sulfated proteoglycan matrix. Cell **11**:891–899.
20. **Pacifici, M., R. Soltesz, G. Thal, D. J. Shanley, D. Boettiger, and H. Holtzer.** 1983. Immunological characterization of the major chick cartilage proteoglycan and its intracellular localization in cultured chrondroblasts: a comparison with Type II procollagen. J. Cell Biol. **97**:1724–1736.
21. **Palmieri, S., P. Kahn, and T. Graf.** 1983. Quail embryo fibroblasts transformed by four v-myc virus isolates show enhanced proliferation but are not tumorigenic. EMBO J. **2**:2385–2389.
22. **Pawlowski, P. J., G. T. Brierley, and L. N. Lukens.** 1981. Changes in the type II and type I collagen messenger RNA population during growth of chondrocytes in 5-bromo-2-deoxyuridine. J. Biol. Chem. **256**:7695–7698.
23. **Pfeifer-Ohlsson, S., A. S. Goustin, J. Rydnert, T. Wahlstrom, L. Bjersing, D. Stehelin, and R. Ohlsson.** 1984. Spatial and temporal pattern of cellular *myc* oncogene expression in developing human placenta: implications for embryonic cell proliferation. Cell **38**:585–596.
24. **Prywes, R., J. G. Foulkes, N. Rosenberg, and D. Baltimore.** 1983. Sequences of the A-MuLV protein needed for fibroblast and lymphoid cell transformation. Cell **34**:569–579.
25. **Ramsay, G. M., P. J. Enrietto, T. Graf, and M. J. Hayman.** 1982. Recovery of *myc*-specific sequences by a partially transformation defective mutant of avian myelocytomatosis virus, MC29, correlates with the restoration of transforming activity. Proc. Natl. Acad. Sci. U.S.A. **79**:6885–6889.
26. **Ramsay, G. M., T. Graf, and M. J. Hayman.** 1980. Mutants of avian myelocytomatosis virus MC29 with smaller *gag*-gene related proteins have an altered transforming ability. Nature (London) **288**:170–172.
27. **West, C., and D. Boettiger.** 1983. Selective effect of Rous sarcoma virus *src* gene expression on contractile protein synthesis in chick embryo myotubes. Cancer Res. **43**:2042–2046.
28. **Wyke, J. A., and M. Lineal.** 1973. Temperature-sensitive avian sarcoma viruses: a physiological comparison of twenty mutants. Virology **53**:152–161.