Data (Government Printing Office, Washington, DC, 1981), vol. 1.
9. R. W. Eppley, E. H. Renger, W. G. Harrison, *Limnol. Oceanogr.* **24**, 483 (1979); F. D. King and A. H. Devol, *ibid.*, p. 65 (1979); C. Garside, *Deep Sea Res.* **32**, 723 (1985).
10. W. G. Harrison, in *Nitrogen in the Marine Environment*, E. J. Carpenter and D. G. Capone, Eds. (Academic Press, New York, 1983).
11. M. C. Gregg, C. S. Cox, P. W. Hacker, *J. Phys. Oceanogr.* **3**, 458 (1973); A. E. Gargett, *ibid.* **6**, 139 (1976); M. C. Gregg and T. B. Sanford, *ibid.* **10**, 105 (1980). M. C. Gregg *et al.*, *Nature (London)* **318**, 140 (1985). R. Lueck and T. Osborn, *J. Geophys. Res.* **91**, 803 (1986).
12. T. R. Osborn, *J. Phys. Oceanogr.* **10**, 83 (1980).
13. N. S. Oakey, *ibid.* **12**, 256 (1982). N. S. Oakey, *ibid.* **15**, 1662 (1985).
14. R. Schmitt, *ibid.* **11**, 1015 (1981).
15. P. Klein and B. Coste, *Deep Sea Res.* **31**, 21 (1984).
16. E. Shulenberger and J. L. Reid, *ibid.* **28**, 901 (1981). W. J. Jenkins, *Nature (London)* **300**, 246 (1982); W. J. Jenkins and J. C. Goldman, *J. Mar. Res.* **43**, 465 (1985); W. J. Jenkins, *J. Phys. Oceangr.*, in press. The required nitrogen flux of 2.5 mmol m^{-2} day^{-1} is calculated from data of W. J. Jenkins and J. C. Goldman [*J. Mar. Res.* **43**, 465 (1985)] with a new production rate of 4 mol of O_2 per meter squared per 150 days and an O_2:N ratio of 10.75 [T. Takahashi, W. S. Broecker, S. Langer, *J. Geophys. Res.* **90**, 6907 (1985)].
17. W. H. Munk, *Deep Sea Res.* **13**, 707 (1966); C. J. R. Garrett, *Dyn. Atm. Oceans* **3**, 239 (1979).
18. T. Platt, M. Lewis, R. Geider, in *Flows of Energy and Materials in Marine Ecosystems*, M. J. R. Fasham, Ed. (Plenum, New York, 1984), pp. 49–84; T. Platt, *Deep Sea Res.* **31**, 1311 (1984); ——— and W. G. Harrison, *ibid.*, in press.
19. J. Waterbury, *Can. J. Fish Aquatic Sci.*, in press.
20. L. A. Martinez, M. W. Silver, J. M. King, A. L. Alldredge, *Science* **221**, 152 (1983).
21. F. P. Chavez and R. T. Barber, *Eos* **66**, 1292 (1985).
22. O. B. Brown *et al.*, *Science* **229**, 163 (1985); National Academy of Sciences, *Global Ocean Flux Study* (National Academy Press, Washington, DC, 1984).
23. We thank A. Waite for assistance at sea, R. Palmer for chemical analyses, and C. J. R. Garrett for critical comments. Supported by operating grants from the Natural Sciences and Engineering Research Council (Canada) and by the Dalhousie University Research Development Fund.

9 June 1986; accepted 5 September 1986

Induction of Altered c-*src* Product During Neural Differentiation of Embryonal Carcinoma Cells

SALLY A. LYNCH, JOAN S. BRUGGE, J. M. LEVINE

The expression of the cellular *src* gene product pp60$^{c\text{-}src}$ was examined in an embryonal carcinoma cell line that differentiates in vitro into neuronlike cells after being treated with retinoic acid. Quantitative and qualitative changes in c-*src* expression accompanied the events associated with neuronal differentiation. The levels of pp60$^{c\text{-}src}$ increased 8- to 20-fold during the period when the cells elaborated neuritic processes and expressed neuron-specific proteins. The electrophoretic mobility of pp60$^{c\text{-}src}$ induced in these cells was retarded in comparison with that in untreated cells or in treated cells before neurite elaboration. The shift in electrophoretic mobility was due to an alteration in the amino terminal 16,000 daltons of pp60$^{c\text{-}src}$ and similar to an alteration of c-*src* protein found in neural tissues and in pure primary cultures of neuronal cells. These results indicate that expression of pp60$^{c\text{-}src}$ induced by retinoic acid in these embryonal carcinoma cells mimics the expression of c-*src* in developing neurons. Therefore, this embryonal carcinoma cell line provides a model system to investigate the function of the *src* protein in neuronal differentiation.

EMBRYONAL CARCINOMA (EC) CELL lines provide experimental models of the molecular events involved in cellular differentiation (*1*). EC cell lines develop from malignant stem cells present in teratocarcinomas and display morphological, biochemical, and biological properties of pluripotent cells of the early embryo (*2*). Murine EC cells can differentiate into a variety of cell types, depending on the conditions of cell culture. The nature of the cells that develop from stem cells is generally monitored on the basis of the morphology of the cells and of the expression of macromolecular markers specifically expressed in the differentiated cells (*3*). Although these markers are useful for classifying the differentiating cells, the analyses of these molecules do not provide insight into the events triggering cellular differentiation. Several lines of evidence implicate the normal cellular homologs of retroviral oncogenes (proto-oncogenes) as genes that might regulate cellular differentiation. Certain proto-oncogenes display patterns of expression specific to cell type; some actually encode growth factors or the receptors for growth factors that induce the proliferation of specific cells (*4*). In addition, experimental manipulation of the expression of the oncogenic homologs of these genes either stimulates differentiation of immature cells (*5, 6*) or interferes with the normal pathway of differentiation in cell culture (*7*).

One such proto-oncogene is c-*src*, the cellular homolog of the transforming gene of Rous sarcoma virus (RSV) (*8*). This proto-oncogene is one of several that encode tyrosine-specific protein kinases (*9*). Analysis of the c-*src* (pp60$^{c\text{-}src}$) protein during embryonic development has revealed both qualitative and quantitative differences in its pattern of tissue-specific expression (*10*). All neural tissues from developing embryos and adult animals contain high levels of a structurally distinct form of pp60$^{c\text{-}src}$ containing an alteration within the amino terminal region of the molecule (*11*). This novel form of pp60$^{c\text{-}src}$ was specifically expressed in primary cultures of pure neuronal cells and displayed a higher tyrosine kinase–specific activity than the protein expressed in astrocytic or fibroblastic cells (*12*). This pattern of expression suggests that the c-*src* protein may be important in either the differentiation of neurons or the events that take place in mature neurons. However, in the absence of a genetic system that provides the means to manipulate the expression of pp60$^{c\text{-}src}$, it is difficult to assess the role of pp60$^{c\text{-}src}$ in these events. Alemà and co-workers have shown that introducing the viral *src* protein into rat pheochromocytoma (PC12) cells stimulated the expression of a neuronal phenotype similar to that induced by nerve growth factor (NGF) (*5*). Although these results suggest a potential function for c-*src* in stimulating neuronal differentiation, it is difficult to correlate the functional behavior of the RSV pp60$^{v\text{-}src}$ protein with that of pp60$^{c\text{-}src}$ (*13*). Furthermore, PC12 cells may not be a useful system for analyzing the role of pp60$^{c\text{-}src}$ in normal differentiation, since no significant differences have been detected in the quantitative levels of pp60$^{c\text{-}src}$ or in its electrophoretic mobility after differentiation induced by NGF or cyclic adenosine monophosphate (*14*).

We have analyzed the expression of pp60$^{c\text{-}src}$ in an EC cell line, p19S18O1A1 (O1A1), that can be induced to differentiate into neuronlike cells under the influence of retinoic acid (*15*). We induced the O1A1 cells to differentiate by growing them as aggregates in retinoic acid, dissociating the aggregates into a single cell suspension, and plating the dissociated cells onto tissue culture plates coated with poly-L-lysine. Within 24 hours after plating, the cells began to elaborate neuritic processes, and after 3 to 5 days more than 80% of the cells could be labeled with antibodies to the neurofilament triplet polypeptides. This in vitro differentiation parallels the in vivo differentiation of

S. A. Lynch and J. S. Brugge, Department of Microbiology, State University of New York, Stony Brook, NY 11794.
J. M. Levine, Department of Neurobiology and Behavior, State University of New York, Stony Brook, NY 11794.

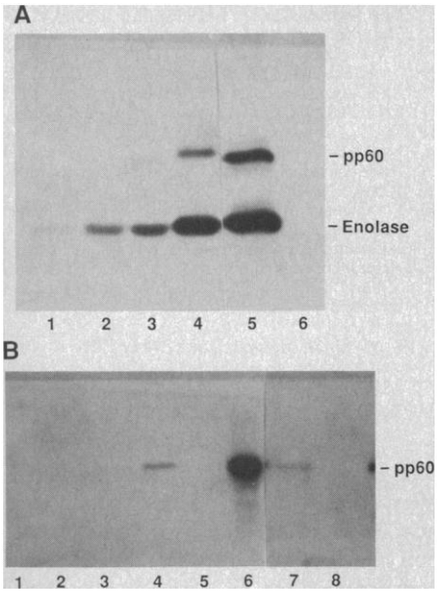

Fig. 1. Analysis of pp60$^{c\text{-}src}$ during retinoic acid–induced differentiation. Neural differentiation of O1A1 cells was induced (*15, 16*) by aggregating the cells in the presence of 5×10^{-7} *M* retinoic acid (Sigma) for 48 hours (day 3). After an additional 48 hours without retinoic acid (day 5), the aggregates were harvested, dissociated with 0.25% trypsin in calcium-free phosphate-buffered saline into a single-cell suspension and plated on poly-L-lysine–coated tissue culture dishes in N2 media (*22*) + fibronectin (5 μg/ml). Lysates (*23*) were prepared from the undifferentiated cells (U), 3-day aggregates (3-A), 5-day aggregates (5-A) and cells incubated for 5 days after dissociation and plating on poly-L-lysine (5-D) (*23*). Cell lysate (2 ml) (*23*) containing 2 mg of total cell protein were incubated with Formalin-fixed *Staphylococcus aureus* and either 2 μl of a 1:1000 dilution of ascites fluid from hybridoma (MAb327) cells and 2 μg of rabbit antiserum to mouse Ig or rabbit anti-mouse Ig alone (*24*). Half of the sample was assayed for pp60$^{c\text{-}src}$-specific kinase activity (A), and the other half for protein by an immunoblot assay (B). (A) Immunoprecipitates were incubated with 2 μg of acid-treated enolase as an exogenous substrate (*25*), and [γ-^{32}P]ATP (*24*). The reaction products were then separated by electrophoresis on 7.5% SDS-polyacrylamide gels (*26*) and quantitated (*27*). Lanes 1 to 5 contained MAb327 + rabbit anti-mouse Ig; lane 6 contained only rabbit anti-mouse antibody. (Lane 1) Undifferentiated (U); (lane 2) 3-day aggregated (3-A); (lane 3) 5-A; (lane 4) 5-day differentiated (5-D); (lane 5) RSV-transformed NIH-3T3 cells (RSV-3T3); (lane 6) RSV-3T3. (B) After polyacrylamide gel electrophoresis of the immunoprecipitates, the 60-kD region was excised, electroblotted onto nitrocellulose paper (*28*), and probed with MAb327 followed by ^{125}I-labeled anti-mouse Ig. All monoclonal antibody reactions were in 50 m*M* tris-HCl, 150 m*M* NaCl, and 0.5% Tween 20. (Lane 1) U cells, MAb327; (lane 2) 3-A, MAb327; (lane 3) 5-A, MAb327; (lane 4) 5-D, MAb327; (lane 5) 5-D anti-mouse control; (lane 6) pp60$^{v\text{-}src}$ expressed in *Escherichia coli*; (lane 7) RSV-3T3, MAb327; and (lane 8) RSV-3T3 anti-mouse Ig.

neuroectoderm in regard to the expression of cell surface carbohydrate antigens and intracellular intermediate filament protein antigens (*16*). To analyze the c-*src* protein, we prepared lysates from these cell cultures at various stages during the differentiation process. The lysates were normalized for protein concentration, and pp60$^{c\text{-}src}$ was immunoprecipitated from the lysates with a monoclonal antibody (MAb327) directed against this protein.

Low levels of c-*src* protein and tyrosine kinase activity (measured as enolase phosphorylation) were detected in the pluripotent (undifferentiated) O1A1 cells (Fig. 1A, lane 1). A slight elevation (two- to three-fold) in pp60$^{c\text{-}src}$ kinase activity was detected in the aggregates treated with retinoic acid (Fig. 1A, lanes 2 and 3); however, a more significant increase (8- to 20-fold) in both the level of pp60$^{c\text{-}src}$ protein and enolase phosphorylation activity was observed after attachment to the substratum and elaboration of neuritic processes (Fig. 1, A and B, lanes 4). The amount of pp60$^{c\text{-}src}$ detectable in the differentiated cells was similar to that of the viral *src* protein present in the RSV-transformed cells that were used as a reference cell line (Fig. 1B, lane 7). The amount of pp60$^{c\text{-}src}$ in cultured neurons is similar to that found in RSV-transformed cells and 15 to 20 times as high as that in fibroblasts (*12*).

The pp60$^{c\text{-}src}$ protein was also phosphorylated within the immune complexes and the increase in the autophosphorylation correlated with the elevation of enolase phosphorylation. Neither enolase nor 60-kD protein was phosphorylated in immunopre-

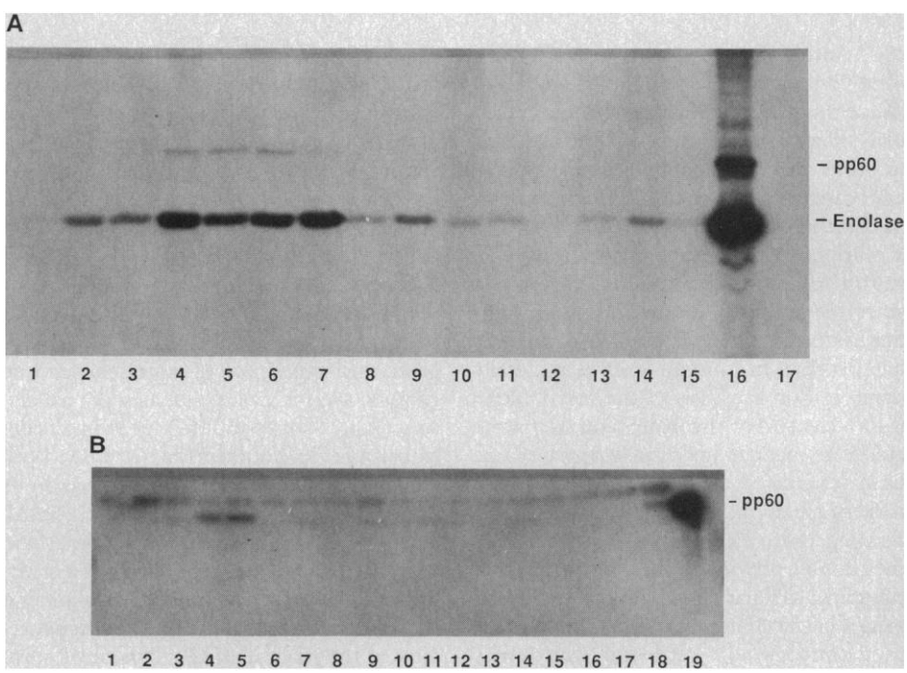

Fig. 2. Analysis of pp60$^{c\text{-}src}$ expression in O1A1 cells grown under various conditions. One set of cells was aggregated in the presence of retinoic acid (RA), dissociated and grown as described for Fig. 1. For muscle differentiation, a second set of cells was aggregated in 1% DMSO as described (*18*). A third set of cells was grown in the absence of any drugs. Cells of the last two groups were dissociated and grown in either DMEM + 10% FCS or N2 media. Cell lysate (1 ml) containing 500 μg of total cell protein was assayed for in vitro kinase activity (A) and pp60$^{c\text{-}src}$ protein levels (B) as described for Fig. 1. All lanes contain lysates incubated with MAb327 and anti-mouse Ig except as noted. (A) (Lane 1) undifferentiated (U) cells; (lane 2) 3-day aggregated (3-A) cells, RA; (lane 3) 5-A, RA; (lane 4) 3-day differentiated, RA; (lane 5) 5-D, RA; (lane 6) 7-D, RA; (lane 7) 12-D, RA; (lane 8) 3-A, DMSO; (lane 9) 5-A, DMSO; (lane 10) 3-D, DMSO in DMEM + 10% FCS; (lane 11) 3-D, DMSO in N2; (lane 12) untreated 3-A in DMEM + FCS; (lane 13) untreated 5-A in DMEM + 10% FCS; (lane 14) 3 days after dissociation of untreated 5-A in N2 media; (lane 15) same as lane 14 except in DMEM + 10% FCS; (lane 16) RSV-3T3; (lane 17) RSV-3T3, anti-mouse Ig control. (B) (Lane 1) U cells; (lane 2) 3-A, RA; (lane 3) 5-A, RA; (lane 4) 3-D, RA; (lane 5) 5-D, RA; (lane 6) 3-A, DMSO; (lane 7) 5-A, DMSO; (lane 8) 3-D, DMSO in N2; (lane 9) 3-D, DMSO, DMEM + 10% FCS; (lane 10) untreated 3-A in N2; (lane 11) untreated 5-A in N2; (lane 12) 3 days after dissociation of untreated 5-A in N2 media; (lane 13) same as lane 12 except in DMEM + FCS; (lane 14) RSV-3T3; (lane 15) RSV-3T3 anti-mouse Ig control; (lane 16) 5-A, RA anti-mouse Ig control; (lane 17) 5-D, RA anti-mouse Ig control; (lane 18) 7-D, RA; (lane 19) pp60$^{v\text{-}src}$ expressed in *E. coli* (*29*).

cipitates formed with only antibodies to mouse immunoglobulin (Ig) used as a negative control (Fig. 1A, lane 6) (*17*).

We cultured the O1A1 cells under a variety of conditions to examine whether the elevation in pp60$^{c\text{-}src}$ expression specifically correlated with differentiation along the neuronal pathway. The expression of pp60$^{c\text{-}src}$ remained high in retinoic acid–differentiated cells maintained in culture as long as 12 days after dissociation of these cells from 5-day aggregates (Fig. 2A, lanes 1–7; Fig. 2B, lanes 1–5). Edwards *et al.* have shown that incubation of O1A1 aggregates with dimethyl sulfoxide (DMSO) induces the differentiation of cardiac and skeletal muscle cells, but few (if any) neurons or glial cells (*18*). A slight increase in pp60$^{c\text{-}src}$ kinase activity was detected in lysates from cells aggregated in the presence of DMSO (Fig. 2A, lanes 8 and 9); however, no further increases in pp60$^{c\text{-}src}$ phosphorylating activity were observed upon dissociation of these aggregates and their subsequent differentiation into muscle cells (Fig. 2A, lanes 10 and 11). Immunoprecipitates from cell lysates that were aggregated without any additives (Fig. 2A, lanes 12 and 13) also displayed a slight increase in enolase phosphorylation, which suggests that this marginal activity may be attributed to the process of aggregation and not to either drug treatment.

Cells that had been aggregated in DMSO or spontaneously aggregated without drugs were grown in either defined medium (N2) or Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) to examine whether use of either medium affected pp60$^{c\text{-}src}$ expression. Cells grown in either N2 media or DMEM + 10% FCS (Fig. 2A, lanes 14 and 15) showed a small increase in phosphorylation of enolase compared with untreated O1A1 cells; however, this increase was insignificant compared with the increase (8- to 20-fold) observed in retinoic acid–treated cultures. In addition, treatment of mouse embryo fibroblasts with retinoic acid did not increase pp60$^{c\text{-}src}$ expression (*17*). These results indicate that the induction of pp60$^{c\text{-}src}$ protein kinase activity in retinoic acid–treated O1A1 cells correlates with the acquisition of the neuronal properties of O1A1 cells. The relative levels of pp60$^{c\text{-}src}$-specific protein kinase activity in immunoprecipitates from all cultures in Fig. 2A were comparable to the relative levels of pp60$^{c\text{-}src}$ protein detected in the comparable samples subjected to an immunoblot assay (Fig. 2B). This result indicates that the increase in pp60$^{c\text{-}src}$ kinase activity detected in the retinoic acid–treated O1A1 cultures was due (at least in part) to an increase in the level of pp60$^{c\text{-}src}$ protein. The slight increase in pp60$^{c\text{-}src}$-mediated enolase phosphorylation in all aggregated cultures also seems to reflect an increase in pp60$^{c\text{-}src}$ expression.

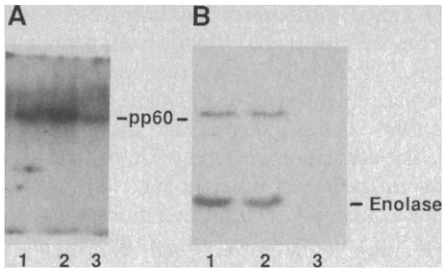

Fig. 3. Comparison of pp60$^{c\text{-}src}$ expression in neurally differentiated O1A1 cells and in pure primary cultures of neurons. The pp60$^{c\text{-}src}$ protein was immunoprecipitated from 5-day monolayer O1A1 cultures differentiated in retinoic acid as described for Fig. 1 (lanes 1); pure primary cultures of neurons were prepared as described by Kaufman (*30*) (lanes 2); NIH 3T3 cells transfected with a plasmid carrying the c-*src* gene (*31*) was included for comparison (lanes 3). Immunoprecipitates were analyzed by immunoblot assay (A) and by in vitro kinase activity (B) as described for Fig. 1.

Figure 3 shows a direct comparison of the specific activity of pp60$^{c\text{-}src}$ expressed in the differentiated O1A1 cultures and in the previously characterized cultures of neuronal cells from embryonic rat brain (*12*). Similar amounts of pp60$^{c\text{-}src}$ protein were detected in lysates from the two neuronal cultures (Fig. 3A), and the levels of enolase phosphorylation detected in the immune complexes were almost identical (Fig. 3B). These results indicate that the specific activity of pp60$^{c\text{-}src}$ expressed in the differentiated O1A1 cells resembles that of pp60$^{c\text{-}src}$ from the cultured embryonic neurons. It was not possible to determine the specific activity of pp60$^{c\text{-}src}$ in O1A1 stock cells and in the aggregates because the levels of pp60$^{c\text{-}src}$ protein detected in the immunoblots were so low that accurate comparisons were not feasible.

We have shown that the electrophoretic mobility of the pp60$^{c\text{-}src}$ protein isolated from primary cultures of pure neuronal cells is slower than that of pp60$^{c\text{-}src}$ from other cell types (*12*). The region of pp60$^{c\text{-}src}$ that

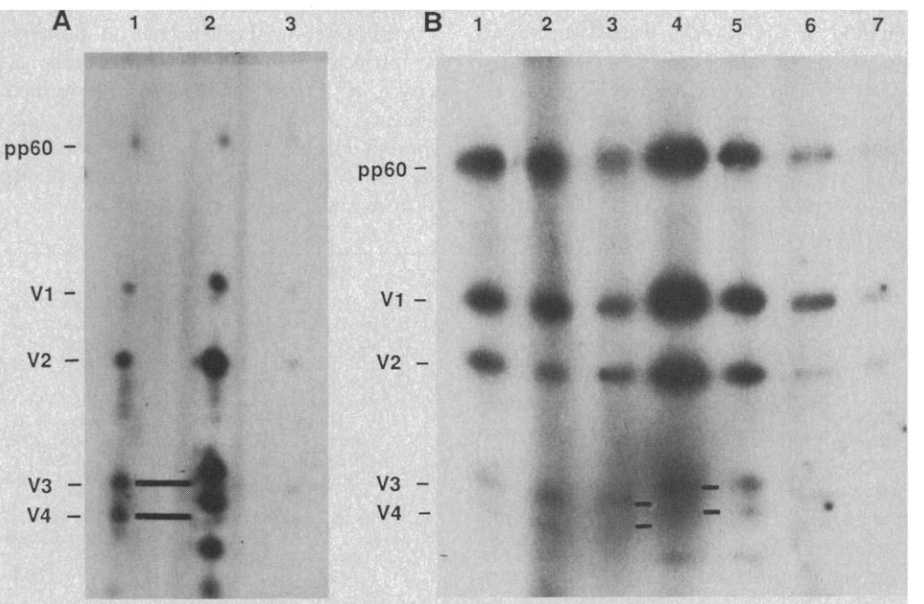

Fig. 4. Partial proteolytic peptide mapping of pp60$^{c\text{-}src}$. Cell cultures were labeled with [^{32}P]orthophosphate (1mCi/ml) for 4 hours, and pp60$^{c\text{-}src}$ was immunoprecipitated as described for Fig. 1. The 60,000-dalton phosphorylated (P) proteins were excised from a 7.5% polyacrylamide gel and subjected to partial proteolytic peptide mapping with *S. aureus* V8 protease (*32*). After electrophoresis, samples were autoradiographed with XAR-5 (Kodak) film and a Lightning intensifying screen. (A) (Lane 1) Undifferentiated (U) O1A1; (lane 2) 5-day differentiated (D) O1A1; (lane 3) 5-day aggregated (A) O1A1 +RA. (B) (Lane 1) U O1A1; (lane 2) 5-A O1A1, −RA; (lane 3) 5-A O1A1, +RA; (lane 4) 5-D O1A1; (lane 5) primary cultures of pure neuronal cells; (lane 6) primary cultures of pure glial (astrocytic) cells; and (lane 7) a cell line (pmc-src302A) derived from NIH 3T3 cells transfected with a plasmid carrying the c-*src* gene (*31*). (C) *Staphylococcus aureus* V8 protease cleavage map of pp60$^{c\text{-}src}$. The cells in lanes 5 and 6 were prepared as described (*30*).

contains the alteration responsible for this shifted mobility is within the amino terminal 16 kD of pp60$^{c\text{-}src}$ (*12*). The electrophoretic mobility of the pp60$^{c\text{-}src}$ protein isolated from neurally differentiated O1A1 cells also shifted (Fig. 1A, lane 4; Fig. 2B, lanes 4 and 5). To determine whether the more slowly migrating form of pp60$^{c\text{-}src}$ present in neuronal O1A1 cells was similarly altered, we labeled cells metabolically with ^{32}P orthophosphate, and the 60-kD proteins immunoprecipitated from the labeled cell lysates were subjected to partial proteolysis with staphylococcus V8 protease (Fig. 4). The V1 (34-kD) and V2 (26-kD) peptides result from cleavage at a single site near the center of the molecule (Fig. 4C). The V1 fragment is subject to further cleavage to generate either the V3 (18-kD) or V4 (16-kD) peptides containing the amino terminus of pp60$^{c\text{-}src}$. The V1, V3, and V4 peptides contain the major sites of serine phosphorylation of the pp60$^{c\text{-}src}$ (*19*); the V2 peptide contains the site of tyrosine phosphorylation (*20*). The cleavage patterns of the pp60$^{c\text{-}src}$ molecules extracted from undifferentiated cells (Fig. 4, A and B, lanes 1), untreated aggregates (Fig. 4B, lane 2), or the retinoic acid–treated aggregates (Fig. 4, A and B, lanes 3) of O1A1 cells were identical to those of the fibroblastic or astrocytic form of pp60$^{c\text{-}src}$. The mobility of the V3 and V4 peptides from pp60$^{c\text{-}src}$ expressed in the retinoic acid–differentiated O1A1 cells (Fig. 4A, lane 2; Fig. 4B, lane 4) was similar to that observed in the V3 and V4 peptides from the cultured rat neurons (Fig. 4B, lane 5).

The structural alteration associated with the neuronal form of the c-*src* protein is not known. We have not detected any unique phosphorylated sites within the neuron-specific V3 or V4 peptides (Fig. 4), and pulse-labeling experiments have not provided evidence for a posttranslational alteration of c-*src* in neurons (*21*). We have found that the c-*src* protein translated in rabbit reticulocyte lysates containing embryonic brain RNA migrated more slowly than the product programmed with embryonic limb RNA (*21*). These results suggest that neurons contain a novel messenger RNA that encodes the modified neuronal c-*src* protein.

Our results indicate that the pp60$^{c\text{-}src}$ molecules expressed after retinoic acid–induced neuronal differentiation of O1A1 cells contain a structural alteration similar to that specifically found in postmitotic neurons and in neural tissues from chicken or mammalian embryos or adult animals. In addition, the pp60$^{c\text{-}src}$ present in the O1A1 cells increased 8- to 20-fold during the process of differentiation into neuronlike cells, reaching a level similar to that previously detected in cultured rat neurons or in RSV-transformed cells. Thus, on the basis of the two criteria that distinguish the expression of pp60$^{c\text{-}src}$ in neuronal cells, the pattern of expression of pp60$^{c\text{-}src}$ in O1A1 cells treated with retinoic acid mimics that of neuronal cells in culture or in vivo. The O1A1 cell line may provide a useful system for determining whether the c-*src* gene product is required for the induction of neuronal differentiation or for specific functions of mature, postmitotic neurons. The expression of pp60$^{c\text{-}src}$ could be manipulated experimentally at different stages during the process of neuronal differentiation, possibly by interfering with pp60$^{c\text{-}src}$ synthesis or activity or by the introduction of purified neuronal pp60$^{c\text{-}src}$ into immature cells. These questions are not easily accessible to analysis with neuronal germinal cells isolated from embryonic tissues.

REFERENCES AND NOTES

1. G. B. Pierce, in *Current Topics in Developmental Biology*, A. Monroy and A. A. Moscona, Eds. (Academic Press, New York, 1967), vol. 2, pp. 223–246; L. C. Stevens, *Adv. Morphog.* **6**, 1 (1967).
2. G. R. Martin, *Science* **209**, 768 (1980).
3. S. Strickland and V. Mahdavi, *Cell* **15**, 393 (1978); M. W. McBurney, E. M. V. Jones-Villeneuve, M. K. S. Edwards, P. J. Anderson, *Nature (London)* **299**, 165 (1982); E. Jones-Villeneuve, M. W. McBurney, K. A. Rogers, V. I. Kalins, *J. Cell Biol.* **94**, 253 (1982).
4. T. Hunter, *Trends Biochem. Sci.* **10**, 275 (1985).
5. S. Alemà, P. Casalbore, E. Agostini, F. Tatò, *Nature (London)* **316**, 557 (1985).
6. D. Bar-Sagi and J. R. Feramisco, *Cell* **42**, 841 (1985); H. Holtzer, J. Biehl, G. Yeoh, R. Meganathan, A. Kaji, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4051 (1975); M. Fiszman and P. Fuchs, *Nature (London)* **254**, 429 (1975); S. L. Adams, D. Boettiger, R. J. Focht, H. Holtzer, M. Pacifici, *Cell* **30**, 373 (1982); R. W. Keane, L. Lipsich, J. S. Brugge, *Dev. Biol.* **103**, 38 (1984).
7. D. Boettiger, *BioEssays* **2**, 106 (1985).
8. D. Stehelin, H. E. Varmus, J. M. Bishop, P. K. Vogt, *Nature (London)* **260**, 170 (1976).
9. B. M. Sefton, in *Current Topics in Microbiology and Immunology*, P. K. Vogt and H. Koprowski, Eds. (Springer Verlag, Berlin, 1986), vol. 23, pp. 39–72.
10. J. H. P. Skene, *Trends Neurosci.* **6**, 353 (1983).
11. P. Cotton and J. S. Brugge, unpublished results.
12. J. S. Brugge *et al.*, *Nature (London)* **316**, 554 (1985).
13. H. Hanafusa, in *Oncogenes and Growth Control*, P. Kahn and T. Graf, Eds. (Springer-Verlag, Heidelberg, in press).
14. S. A. Lynch, J. S. Brugge, S. Halegoua, unpublished results.
15. J. K. S. Edwards and M. W. McBurney, *Dev. Biol.* **98**, 187 (1983).
16. J. M. Levine and P. Flynn, *J. Neurosci.*, in press.
17. S. A. Lynch, J. S. Brugge, J. Levine, unpublished data.
18. M. R. S. Edwards, J. F. Harris, M. W. McBurney, *Mol. Cell. Biol.* **3**, 2280 (1983).
19. M. S. Collett, E. Erikson, R. L. Erikson, *J. Virol.* **29**, 770 (1979).
20. J. E. Smart *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6013 (1981).
21. P. Cotton, P. Coussens, L. Lipsich, A. Queral-Lustig, J. S. Brugge, unpublished results.
22. M. Darmon, J. Bottenstein, G. Sato, *Dev. Biol.* **85**, 463 (1981).
23. Radioimmunoprecipitation assay (RIPA) buffer (1% sodium deoxycholate, 1% Triton X-100, 0.1% sodium dodecyl sulfate, 150 m*M* NaCl, 5*M* EDTA, 10 m*M* tris-HCl, *p*H 7.4).
24. L. A. Lipsich *et al.*, *J. Virol.* **48**, 352 (1983).
25. J. A. Cooper and T. Hunter, *J. Biol. Chem.* **258**,
26. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
27. Quantification of enolase phosphorylation and pp60$^{c\text{-}src}$ reactivity in immunoblots was determined by densitometric scanning of the 60-kD region of the autoradiogram with a Joyce-Loebbel densitometer in the Rascer scan mode and Cherenkov counting of the excised 60-kD radioactive bands.
28. H. Towbin, T. Staehelin, J. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350 (1979).
29. T. Gilmer and R. L. Erikson, *Nature (London)* **294**, 771 (1981).
30. L. M. Kaufman and J. N. Barret, *Science* **220**, 1394 (1983).
31. P. J. Johnson, P. M. Coussens, A. V. Danko, D. Shalloway, *Mol. Cell. Biol.* **5**, 1073 (1985).
32. D. W. Cleveland, S. G. Fischer, M. W. Kirschner, U. K. Laemmli, *J. Biol. Chem.* **252**, 1102 (1977).

33. We thank M. McBurney for providing us with O1A1 cells; S. Halegoua, S. Strickland, and M. Hayman for critical review of this manuscript; P. Flynn for technical assistance; K. Donnelly for help in the preparation of this manuscript; and R. Keane and J. Barrett for neuronal and astrocytic cultures. Supported by grant CA 28146 from the National Cancer Institute and grant NS 21198 from the National Institutes of Health.

18 April 1986; accepted 9 September 1986